



Circulating miR-29 and miR-320 as biomarkers of clinical compensation in ischaemic and non-ischaemic dilated cardiomyopathy

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Abstract

Background: Patients with dilated cardiomyopathy (DCM) may present as clinically compensated or decompensated despite similar degrees of ventricular dysfunction, suggesting that clinical compensation reflects distinct pathophysiological states that may differ across disease aetiologies. This study aimed to evaluate whether circulating miR-29 and miR-320 are associated with clinical compensation status in ischaemic dilated cardiomyopathy (IDCM) and non-ischaemic dilated cardiomyopathy (NIDCM), and to determine whether these associations demonstrate aetiology-specific patterns.

Methods: This study included 46 patients with IDCM, 44 with NIDCM and 30 controls. Clinical compensation was defined by New York Heart Association (NYHA) class, supported by evaluation of congestion signs. Plasma miR-29 and miR-320 were quantified by reverse transcription quantitative polymerase chain reaction (RT-qPCR) using miR-103 for normalisation, with aetiology-stratified comparisons performed using non-parametric analyses.

Results: Baseline microRNA (miRNAs) expression differed by aetiology. miR-29 was higher in IDCM than in NIDCM and controls ($p < 0.001$), whereas miR-320 was elevated in both IDCM and NIDCM relative to the controls ($p = 0.003$ and 0.009 , respectively). In IDCM, compensated patients exhibited higher miR-29 levels than decompensated patients ($p = 0.024$). NIDCM, decompensated patients showed higher miR-320 levels than compensated patients ($p = 0.027$). The alternate miRNA did not vary with the compensation status.

Conclusions: Circulating miR-29 and miR-320 demonstrate distinct, aetiology-dependent clinical significance in DCM. miR-29 was associated with clinical compensation specifically in IDCM, whereas miR-320 is associated with decompensation in NIDCM. These findings suggest potential aetiology-specific biological pathways underlying clinical stability and should be considered as hypothesis-generating, warranting confirmation in larger and longitudinal studies.

Keywords

Ischaemic dilated cardiomyopathy, non-ischemic dilated cardiomyopathy, clinical compensation, miR-29, miR-320

Introduction

Dilated cardiomyopathy (DCM) is a heterogeneous myocardial disorder characterised by left ventricular dilation and systolic dysfunction^[1,2]. Despite similar degrees of ventricular impairment, patients with DCM may present in clinically compensated or decompensated states, reflecting differences in symptoms, congestion and short-term stability^[3,4]. Clinical compensation represents a dynamic physiological state influenced by neurohormonal activation, perfusion and volume status, and does not necessarily parallel the extent of ventricular dysfunction alone^[1,3]. Clinical compensation is clinically important as it reflects short-term stability, guides treatment decisions and is closely associated with hospitalisation risk and patient outcomes in heart failure (HF)^[1,2]. Despite its central role in clinical assessment and management, the biological basis underlying clinical compensation in DCM remains poorly defined.

Aetiology plays a major role in determining these haemodynamic trajectories. Ischemic dilated cardiomyopathy (IDCM) is predominantly driven by scar formation, fibrosis and matrix-related remodeling^[5,6], whereas non-ischaemic dilated cardiomyopathy (NIDCM) more commonly involves metabolic dysregulation, oxidative stress, mitochondrial impairment and energetic failure^[7,8]. These divergent biological pathways may lead to distinct molecular signatures and help explain why patients with similar ventricular measurements may follow different clinical courses.

Circulating microRNAs (miRNAs) have emerged as promising biomarkers due to their stability, tissue specificity and regulatory involvement in key cardiac pathways^[9,10]. Among them, miR-29 plays a central role in extracellular matrix (ECM) regulation and fibrosis signalling^[11,12]. Experimental and clinical studies have associated altered miR-29 expression with fibrosis, hypertrophy and adverse remodelling^[13–15], suggesting potential relevance in ischaemic substrates characterised by chronic matrix activation.

By contrast, miR-320 is closely linked to metabolic and mitochondrial disturbances, including oxidative stress, impaired fatty-acid handling and energetic imbalance^[16–18]. Elevations in miR-320 have been reported in metabolic cardiomyopathy, diabetes-

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related cardiac injury and models of mitochondrial dysfunction, supporting its relevance in NIDCM, where metabolic destabilisation may contribute to loss of compensation^[19,20].

Several studies have described circulating miRNA signatures associated with DCM, heart-failure progression or differentiation between cardiomyopathy subtypes. However, the majority of these investigations have focused on long-term disease progression or prognostic outcomes, rather than the short-term physiological state of clinical compensation. Importantly, few studies have examined whether the relationship between circulating miRNAs and clinical status is modified by underlying disease aetiology, despite the well-established biological differences between IDCM and NIDCM^[21–23].

Prior findings on circulating miR-29 and miR-320 in cardiomyopathy have been heterogeneous, with studies linking miR-29 to both profibrotic and adaptive ECM responses and associating miR-320 with metabolic injury across variable clinical phenotypes^[11,12,19]. Such variability likely reflects differences in disease aetiology, disease stage, sampling timing and assay methodology.

Given these considerations, the present study was designed to examine circulating miR-29 and miR-320 in the context of clinical compensation. Specifically, we investigated whether these miRNAs demonstrate aetiology-specific associations with compensated and decompensated states in IDCM and NIDCM using a single, well-characterised cohort and standardised measurement approach. By focusing on clinical compensation as a biological phenotype and explicitly accounting for disease aetiology, this study aims to provide insight into biological heterogeneity underlying clinical stability in DCM.

Material and Methods

This study was performed to evaluate biological differences across clinically compensated and decompensated state by assessing circulating miR-29 and miR-320 profiles in patients with IDCM and NIDCM.

Study population

Participants were divided into three groups:

1. IDCM

Forty-six patients with LV ejection fraction (EF) <40% and echocardiographic evidence of chamber dilation (left ventricular end-diastolic diameter (LVEDD) >58 mm, together with angiographically confirmed obstructive coronary artery disease, were classified as IDCM. LV dilation was defined as LVEDD >58 mm, consistent with our laboratory reference limits. Although current guidelines recommend indexing ventricular dimensions to body surface area and considering sex-specific thresholds^[1,2], a fixed cutoff was used in this study to provide a uniform and pragmatic definition across the cohort and to avoid additional variability introduced by indexing, particularly in a relatively small sample.

2. NIDCM

Forty-four patients with EF <40% and LVEDD >58 mm together with the absence of significant coronary artery obstruction on angiography and without alternative identifiable causes of myocardial dysfunction.

3. Healthy Controls

The control group included 30 subjects and was frequency-matched to the overall patient cohort by age and sex and free of any medical diseases.

Exclusion criteria

Individuals with EF >40%, LVEDD <58 mm, significant valvular disease, hypertrophic or restrictive cardiomyopathy, cardiotoxic drug exposure, autoimmune or collagen vascular disease, hepatic or renal impairment or active inflammatory/infectious conditions were excluded. These criteria were applied to reduce confounding from alternative causes of myocardial dysfunction or systemic illness that could influence circulating miRNA levels.

Clinical assessment

A detailed medical history and physical examination were performed. Compensation status was defined using the New York Heart Association (NYHA) functional classification, with NYHA I–II considered clinically compensated and NYHA III–IV considered decompensated, consistent with contemporary HF guidelines^[1].

Bedside signs of congestion including orthopnoea, elevated jugular venous pressure (JVP), pulmonary rales and lower-limb oedema were recorded to provide clinical context and supportive descriptive data. Agreement between NYHA-based compensation status (I–II vs III–IV) and the presence of congestion signs was evaluated using Cohen's kappa statistic.

Cardiac evaluation

All participants underwent comprehensive transthoracic echocardiography and coronary angiography as part of their aetiological evaluation. Left ventricular systolic function was quantified using Simpson's biplane method, and study inclusion required an EF <40% and LVEDD >58 mm, consistent with DCM.

Aetiological classification was based on coronary angiography. IDCM was defined by the presence of significant obstructive coronary artery disease ($\geq 70\%$ stenosis in a major epicardial vessel or $\geq 50\%$ stenosis in the left main coronary artery). NIDCM was assigned in the absence of coronary lesions meeting these thresholds and in the absence of alternative identifiable causes of myocardial dysfunction.

Blood collection and sample processing

Venous blood (10 mL) was collected into ethylenediaminetetraacetic acid (EDTA) tubes for plasma and plain tubes for serum. After centrifugation, plasma aliquots were stored at -80°C until RNA extraction to preserve sample integrity. All samples were processed using the same collection and handling procedures. Blood samples were not collected under standardised fasting conditions. However, all samples were obtained under similar clinical conditions across groups, which may reduce the likelihood of systematic bias in group comparisons.

miRNA extraction and reverse transcription

Circulating microRNAs were isolated from plasma using the miRNeasy Serum/Plasma Kit (Qiagen, Germany). RNA purity was verified by spectrophotometric assessment, and only samples meeting the predetermined quality thresholds were processed further. Complementary deoxyribonucleic acid (cDNA) synthesis was performed using the miRCURY LNA cDNA Synthesis Kit (Qiagen). All samples were prepared in parallel to minimise technical variability.

Quantitative real-time PCR (qRT-PCR)

Circulating levels of miR-29 and miR-320 were quantified using SYBR-Green-based qRT-PCR on the Rotor-Gene Q platform. Each sample was analysed in duplicate; assays with replicate Ct differences >0.3 were repeated. Melt-curve analysis confirmed specificity of amplification products.

miR-29 and miR-320 expression levels were normalised to miR-103, which was selected as an endogenous reference based on previous studies reported it as a stable normalisation miRNA in cardiovascular conditions^[24]. In the present study, Ct values of miR-103 showed minimal variation across samples, supporting its stability and suitability for normalisation. Expression levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method^[25]. Due to skewed distribution, values were $\log_{10}$ -transformed prior to statistical analysis. Circulating miRNA quantification by qRT-PCR is known to exhibit wide inter-individual variability due to both biological and technical factors. To minimise this variability, all samples were processed under identical conditions using the same extraction and amplification protocols. Statistical analyses were performed on untransformed $2^{-\Delta\Delta\text{Ct}}$ values using non-parametric tests. All quantitative polymerase chain reaction (qPCR) procedures followed minimum information for publication of quantitative real-time PCR experiments (MIQE) recommendations^[26].

Bias control and quality assurance

Multiple measures were implemented to ensure analytical rigour:

- Laboratory personnel performing RNA extraction and qRT-PCR were blinded to clinical categories.
- All samples were processed using identical collection and extraction protocols.

- Hemolysed or poor-quality samples were excluded.
- Consecutive patient recruitment and age/sex matching of controls reduced selection bias.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27. Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of histograms. Because circulating miRNA expression values exhibited non-normal distributions, the results are presented as median (interquartile range [IQR]), and group comparisons were performed using the Mann–Whitney U test.

Associations between circulating miRNA levels and age were evaluated using Spearman's rank correlation. Relative miRNA expression was calculated using untransformed $2^{-\Delta\Delta\text{Ct}}$ values for statistical testing; $\log_{10}$ -transformed values were used only for graphical presentation. Statistical significance was defined as a two-tailed p -value <0.05 .

Results

1. Baseline characteristics

Baseline characteristics of the IDCM and NIDCM groups are presented in Table 1. Patients with IDCM were older and had a higher prevalence of hypertension (HTN), diabetes and smoking, consistent with the ischaemic aetiology and its associated risk-factor profile. These differences were reflected in higher systolic and diastolic blood pressure (BP) and slightly increased septal thickness in the IDCM group. Distribution of NYHA functional class is shown across all four categories (I–IV), and a clinically meaningful comparison of NYHA III–IV versus I–II demonstrated no significant difference between the two aetiologies.

2. Distribution of congestion findings according to NYHA Class

Across both aetiologies, congestion-related findings showed clear differences between the compensated and decompensated patients. Peripheral oedema and orthopnoea were the most frequent findings in NYHA III–IV, while elevated JVP and pulmonary rales were also more commonly observed in the decompensated group (Table 2). NYHA class demonstrated high agreement with the presence of congestion signs ($\kappa = 0.91$), supporting its validity as a marker of clinical compensation in this cohort.

3. Group comparisons of circulating miR-29 and miR-320

Baseline comparisons showed distinct distributions of circulating miRNAs across the study groups. For miR-29, IDCM demonstrated significantly higher levels than both NIDCM and controls, whereas NIDCM and controls displayed similar values. For miR-320, both ischaemic and non-ischaemic DCM groups had significantly higher levels than controls, while the difference between the two aetiologies did not reach statistical significance (Table 3, Figure 1).

Table 1 - Baseline characteristics of IDCM and NIDCM patients.

Variable	IDCM (n = 46)	NIDCM (n = 44)	p-value
Age (years), mean $\pm$ SD	58.61 $\pm$ 7.20	53.91 $\pm$ 8.93	0.007
Sex (male), n (%)	26 (56.5)	26 (59.1)	0.80
HTN, n (%)	34 (73.9)	3 (6.8)	<0.001
DM, n (%)	18 (39.1)	3 (6.8)	<0.001
Smoking, n (%)	30 (65.2)	6 (13.6)	<0.001
EF (%), mean $\pm$ SD	32.59 $\pm$ 4.57	32.05 $\pm$ 6.97	0.67
LVEDD (mm), mean $\pm$ SD	66.43 $\pm$ 5.80	65.95 $\pm$ 6.99	0.72
LVESD (mm), mean $\pm$ SD	56.54 $\pm$ 6.11	55.64 $\pm$ 11.18	0.64
IVS (cm), mean $\pm$ SD	0.89 $\pm$ 0.21	0.79 $\pm$ 0.11	0.006
Compensated status, n (%)	27 (58.7)	22 (50.0)	0.47
NYHA class, n (%)			
• I	19 (41.3)	15 (34.1)	—
• II	8 (17.4)	7 (15.9)	—
• III	9 (19.6)	16 (36.4)	—
• IV	10 (21.7)	6 (13.6)	—
NYHA III–IV, n (%)	19 (41.3)	22 (50.0)	0.31
Lower-limb oedema, n (%)	24 (52.2)	29 (65.9)	0.25
Pulmonary rales, n (%)	7 (15.2)	8 (18.2)	0.78
Elevated JVP, n (%)	16 (34.8)	14 (31.8)	0.81
Orthopnoea, n (%)	19 (41.3)	24 (54.5)	0.27
Heart rate (bpm), mean $\pm$ SD	81.22 $\pm$ 9.77	85.34 $\pm$ 11.70	0.074
Systolic BP (mmHg), mean $\pm$ SD	128.22 $\pm$ 12.09	114.77 $\pm$ 14.06	<0.001
Diastolic BP (mmHg), mean $\pm$ SD	80.26 $\pm$ 6.08	72.27 $\pm$ 6.77	<0.001

Values are presented as mean $\pm$ SD or n (%). Continuous variables were compared using independent-samples *t* test. Categorical variables were compared using chi-square test.

BP, blood pressure; bpm, beats per minute; DM, diabetes mellitus; EF, ejection fraction; HTN, hypertension; IDCM, ischaemic dilated cardiomyopathy; IVS, interventricular septum; JVP, Jugular venous pressure; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; NIDCM, non-ischaemic dilated cardiomyopathy; NYHA, New York Heart Association; SD, standard deviation.

4. Associations with compensation status

miR-29

In IDCM, compensated patients exhibited higher miR-29 levels than decompensated patients (median 42.81 vs 4.99; $p = 0.024$). No difference in compensation status was observed in NIDCM ($p = 0.664$).

miR-320

In NIDCM, decompensated patients had significantly higher miR-320 levels than compensated patients (median 4.17 vs 0.91; $p = 0.027$). No compensation-associated difference occurred in IDCM ($p = 0.184$) (Table 4, Figure 2).

The relatively wide IQRs observed for some miRNA measurements likely reflect biological variability and skewed distribution patterns commonly reported in circulating miRNA data, rather than isolated outliers or technical artefacts.

5. Association with cardiovascular risk factors

Neither miR-29 nor miR-320 demonstrated consistent or clinically meaningful associations with age, HTN, DM or smoking in either aetiology.

Apart from a single significant difference in miR-320 between hypertensive and non-hypertensive IDCM patients, no consistent associations were observed between circulating miRNA levels and traditional cardiovascular risk factors in univariate analyses. This isolated finding likely reflects statistical variability rather than a biologically meaningful effect (Table 5).

Discussion

This study investigated whether circulating miR-29 and miR-320 exhibit aetiology-specific associations with clinical compensation in DCM. Baseline expression levels differed across aetiologies with higher miR-29 in IDCM compared with NIDCM and controls and higher miR-320 in both cardiomyopathy groups compared with controls, providing aetiological context for subsequent compensation analyses. In IDCM, compensated patients exhibited higher circulating miR-29 levels compared with decompensated patients with no difference in the compensation status of NIDCM patients. Whereas in NIDCM, higher miR-320 levels were observed in decompensated compared with compensated states with no difference in the compensation

Table 2 - Distribution of congestion findings by NYHA class within each aetiology.

Parameter	IDCM (NYHA I–II)	IDCM (NYHA III–IV)	NIDCM NYHA I–II	NIDCM NYHA III–IV
LL oedema	11 (40.7%)	13 (68.4%)	10 (45.5%)	19 (86.4%)
Pulmonary rales	0 (0.0%)	7 (36.8%)	0 (0.0%)	8 (36.4%)
Elevated JVP	6 (22.2%)	10 (52.6%)	1 (4.5%)	13 (59.1%)
Orthopnoea	2 (7.4%)	17 (89.5%)	3 (13.6%)	21 (95.5%)

IDCM, ischaemic dilated cardiomyopathy; JVP, Jugular venous pressure; NIDCM, non-ischemic dilated cardiomyopathy; NYHA, New York Heart Association.

Table 3 - Comparison of circulating miR-29 and miR-320 levels across study groups.

miRNA	Controls(n = 30) Median (IQR)	IDCM (n = 46) Median (IQR)	NIDCM (n = 44) Median (IQR)	IDCM vs Controls (p-value)	NIDCM vs Controls (p-value)	IDCM vs NIDCM (p-value)
miR-29	2.96 (2.1–3.7)	14.72 (7.8–29.5)	1.48 (0.6–2.9)	<0.001	0.76	<0.001
miR-320	0.98 (0.6–1.5)	8.17 (3.2–17.4)	2.28 (1.2–3.8)	0.003	0.009	0.087

Continuous variables are presented as median (IQR). Comparisons between groups were performed using the Mann–Whitney U test. IDCM, ischaemic dilated cardiomyopathy; IQR, interquartile range; miR, microRNA; NIDCM, non-ischaemic dilated cardiomyopathy.

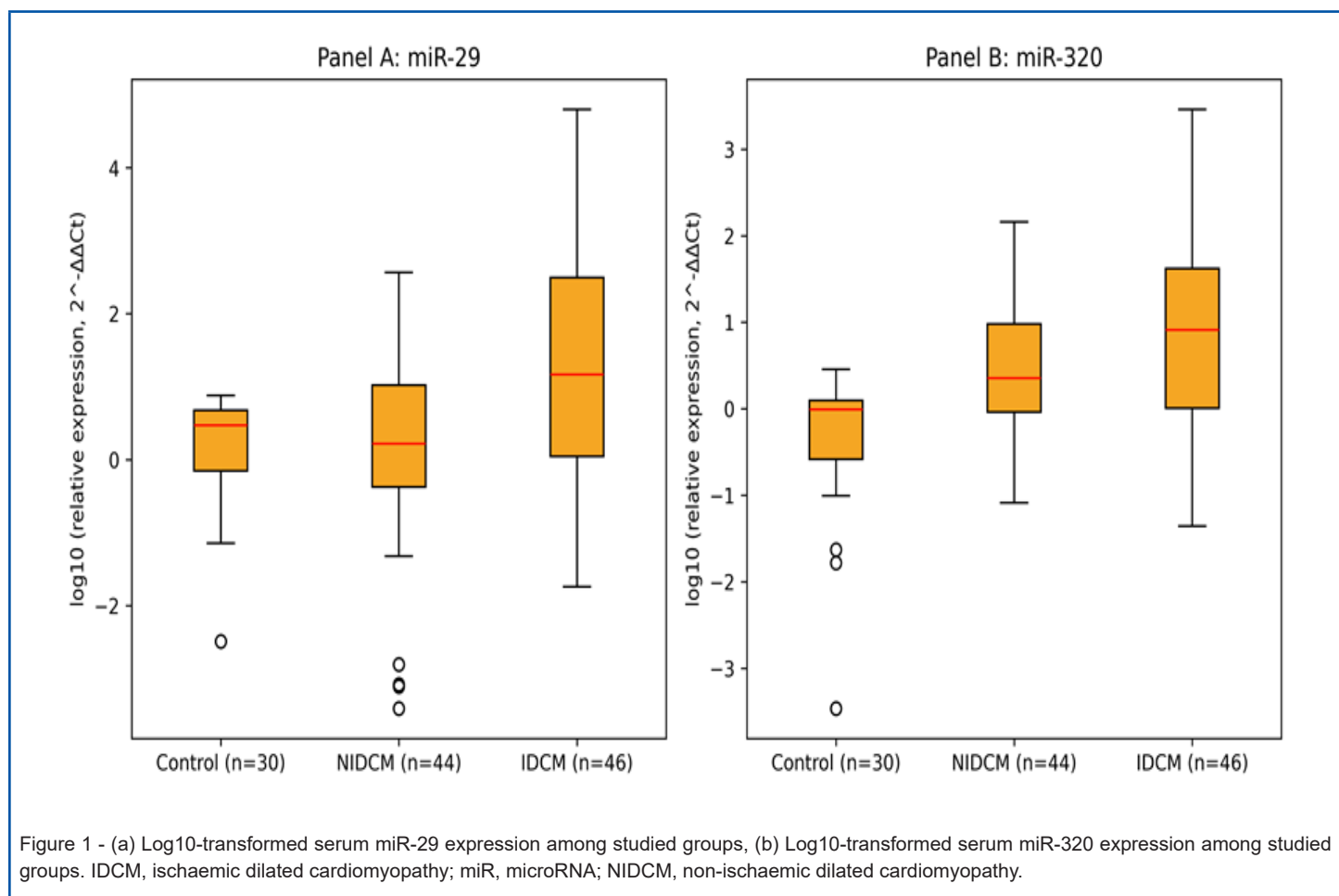


Table 4 - Comparison of miR-29 and miR-320 levels in compensated versus decompensated patients.

miRNA	Group	Compensated Median (IQR)	Decompensated Median (IQR)	p-value
miR-29	IDCM	42.81 (8.64–2429.96)	4.99 (0.86–13.09)	0.024
	NIDCM	2.14 (0.21–7.36)	1.62 (0.59–13.82)	0.664
miR-320	IDCM	13.64 (1.51–58.89)	8.17 (0.91–12.82)	0.184
	NIDCM	0.91 (0.80–3.58)	4.17 (1.19–16.28)	0.027

Continuous variables are presented as median (IQR). Comparisons between compensated and decompensated groups were performed using the Mann–Whitney U test.

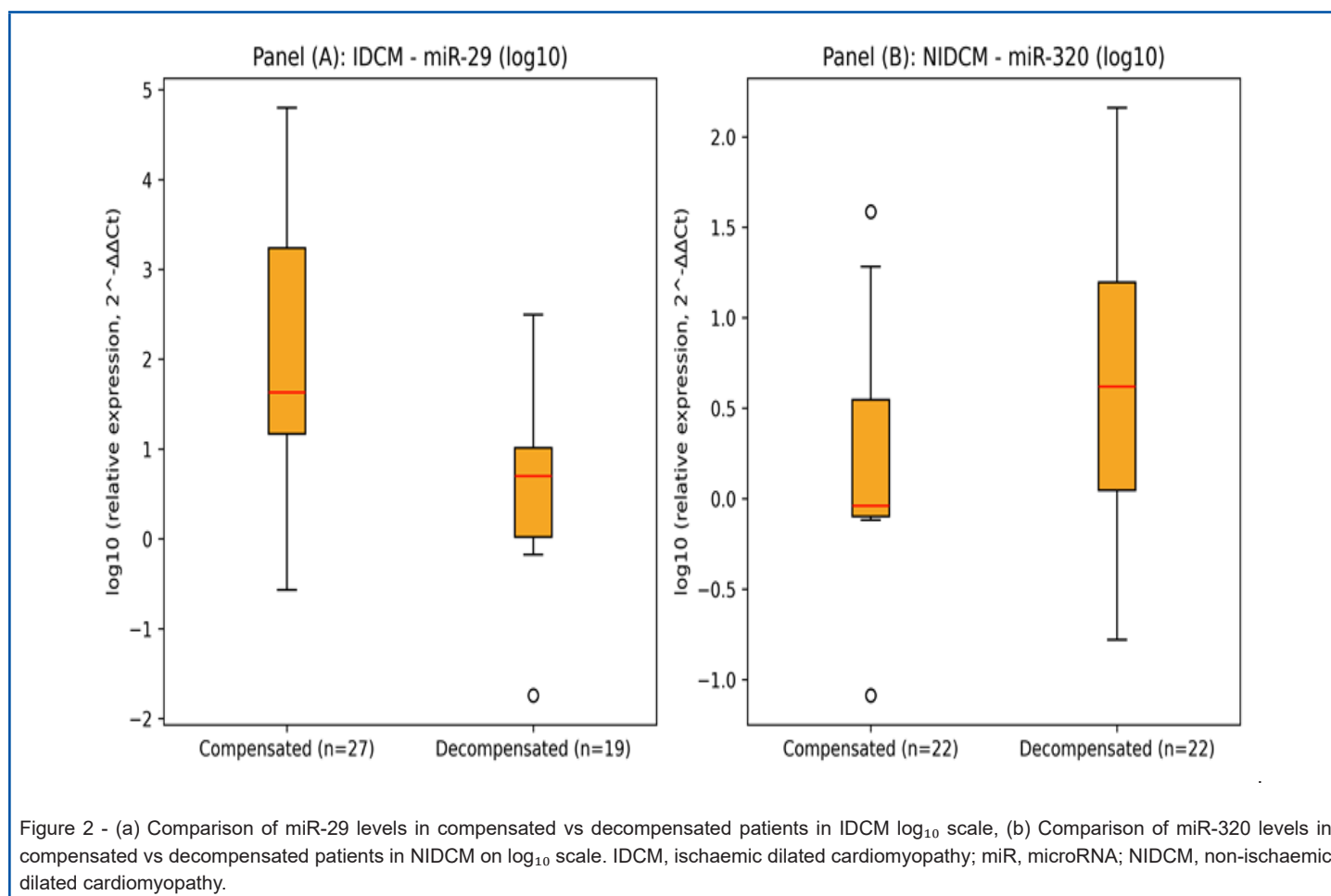
IDCM, ischaemic dilated cardiomyopathy; IQR, interquartile range; miR, microRNA; NIDCM, non-ischaemic dilated cardiomyopathy.

Table 5 - Correlation of miR-29 and miR-320 with age and cardiovascular risk factors.

Risk factor	IDCM miR-29	NIDCM miR-29	IDCM miR-320	NIDCM miR-320
Age (spearman)	$r = 0.090, p = 0.551$	$r = 0.005, p = 0.974$	$r = -0.146, p = 0.332$	$r = -0.127, p = 0.410$
HTN (median yes/no)	13.09/55.34, $p = 0.430$	0.96/1.52, $p = 0.560$	3.18/47.83, $p = 0.017$	0.91/2.55, $p = 0.058$
DM (median yes/no)	8.17/23.93, $p = 0.693$	1.80/1.43, $p = 0.926$	8.17/8.41, $p = 0.796$	4.17/2.01, $p = 0.907$
Smoker (median yes/no)	14.72/25.49, $p = 0.217$	2.38/1.47, $p = 0.851$	9.65/6.17, $p = 0.315$	2.50/2.01, $p = 0.850$

Correlations between miRNA levels and age were assessed using Spearman's rank correlation. Comparisons of miRNA levels across binary risk factors were performed using the Mann–Whitney U test.

DM, diabetes mellitus; HTN, hypertension; IDCM, ischaemic dilated cardiomyopathy; miR, microRNA; NIDCM, non-ischaemic dilated cardiomyopathy.



status of IDCM patients. Together, these findings indicate that circulating miRNA patterns associated with clinical compensation differ according to the underlying disease etiology.

NYHA functional class remains one of the most widely used clinical indicators of compensation status in HF and is embedded in the guidelines, risk stratification and trial design^[1,2]. It captures integrated physiological information including functional reserve, neurohormonal activation, vascular tone and congestion, and correlates with hospitalisation risk and short-term stability. Numerous biomarker studies have relied on NYHA class to stratify compensated versus decompensated states, and its use consistently correlates with hospitalisation risk, haemodynamic congestion and adverse outcomes^[27]. The distribution of congestion-related symptoms and signs in this cohort further supported the clinical relevance of the NYHA classification. In our study, orthopnoea and peripheral oedema were the most frequent findings among patients with NYHA III–IV, while elevated JVP and pulmonary rales also occurred more commonly in the decompensated state. The very high concordance in this cohort between NYHA class and bedside congestion signs ($\kappa = 0.91$) supports its validity as an indicator of short-term haemodynamic compensation in our analyses.

In the present study, circulating miR-29 levels were higher in patients with IDCM than in those with non-ischaemic disease, indicating a baseline aetiological difference consistent with the ischaemic remodelling substrate. Importantly, within IDCM, compensated patients exhibited significantly higher miR-29 levels compared with decompensated patients, whereas no compensation-related difference was observed in the non-ischaemic group. This pattern suggests that the elevated miR-29 signal in ischaemic disease is not merely an aetiological characteristic but is closely aligned with clinical compensation in the ischaemic setting rather than representing a general feature of cardiomyopathy.

miR-29 is a well-established regulator of ECM homeostasis, with known roles in modulating collagen synthesis and fibrotic signalling pathways, as demonstrated in experimental and human studies^[15,28,29]. In IDCM, where ischaemic injury, scar formation and matrix remodelling critically influence ventricular stiffness and filling pressures, excessive profibrotic signalling contributes to reduced compliance and clinical instability, whereas more regulated matrix turnover supports preserved ventricular compliance and haemodynamic stability^[30,31]. Within this context, higher circulating miR-29 levels in clinically compensated IDCM patients may reflect a biological milieu characterised by restrained profibrotic activity and more controlled ECM remodelling, thereby facilitating maintenance of short-term clinical stability. This interpretation is consistent with prior reports linking miR-29 to adaptive matrix-regulatory signalling in post-ischaemic remodelling^[28,32].

By contrast, miR-320 has been linked to mitochondrial dysfunction, oxidative stress, lipotoxicity and impaired fatty-acid metabolism—pathways that are particularly relevant to non-ischaemic cardiomyopathies^[20,33–35]. Experimental studies demonstrate that miR-320 can promote transcriptional programmes associated with reduced mitochondrial efficiency and altered cellular energetics, and clinical investigations have reported elevated circulating miR-

320 in metabolic cardiomyopathy and diabetes-related myocardial injury^[19,20,35].

In the present study, circulating miR-320 levels were elevated in both IDCM and NIDCM compared with controls, likely associated with global metabolic stress in advanced HF. However, only in NIDCM did miR-320 distinguish decompensated from compensated patients, with higher levels observed in those with clinical decompensation, consistent with well-established evidence that non-ischaemic cardiomyopathies depend more heavily on metabolic and mitochondrial energetic reserve than ischaemic forms^[8,36,37], where decompensated NIDCM patients may therefore be indicative of worsening metabolic instability, whereas compensated NIDCM patients may appear to maintain more preserved energetic signalling.

Importantly, these findings are associative, where circulating miR-29 should be interpreted as a marker of underlying remodelling dynamics accompanying clinical compensation and circulating miR-320 as a marker of decompensation-related metabolic stress rather than mediators of clinical status.

Taken together, these findings suggest that clinical compensation in DCM represents a multidimensional phenotype influenced by factors such as neurohormonal balance, congestion, metabolic reserve, renal handling and ECM activity, rather than being determined by structural severity alone^[38,39]. Importantly, the present data suggest that the biological processes supporting clinical stability are not uniform across aetiologies. The observation that miR-29 tracked compensation specifically in IDCM, while miR-320 was associated with decompensation in NIDCM, is consistent with the notion that different dominant biological pathways constrain haemodynamic stability depending on the underlying substrate—predominantly ECM regulation in ischaemic cardiomyopathy and metabolic–energetic reserve in non-ischaemic cardiomyopathy. In this context, these miRNAs may function as integrative molecular signals that reflect which biological pathway is most relevant to maintaining or losing clinical compensation within a given aetiology. Although the observed associations were biologically plausible, they should be interpreted cautiously and considered exploratory and hypothesis-generating.

Conclusion

This study demonstrates that circulating miR-29 and miR-320 show distinct, aetiology-dependent clinical significance in DCM. miR-29 was associated with clinical compensation specifically in ischaemic disease, whereas miR-320 was associated with decompensation in non-ischaemic DCM, highlighting that clinical compensation is not a uniform biological condition but instead reflects distinct dominant biological constraints depending on the underlying aetiology. These findings suggest that clinical compensation in DCM may be influenced by distinct biological pathways according to the aetiology and may provide a framework for hypothesis generation in aetiology-specific phenotyping, warranting confirmation in larger and longitudinal studies investigating dynamic transitions between compensated and decompensated states.

Limitations

This study has several limitations. First, the sample size was modest, particularly after subgroup stratification, which may limit statistical power to detect smaller effect sizes. In addition, multiple statistical comparisons were performed without formal adjustment, increasing the risk of type I error. Therefore, the results should be interpreted with caution and considered exploratory and hypothesis-generating. Second, analyses were based on univariate comparisons without multivariable adjustment; therefore, residual confounding cannot be excluded. Multivariable modelling was not performed due to the limited sample size and risk of overfitting. Third, the cross-sectional design precludes assessment of temporal relationships or dynamic changes in clinical compensation. In addition, natriuretic peptide levels (brain natriuretic peptide [BNP] or N-terminal pro Brain natriuretic peptide [NT-proBNP]) were not available, which may have limited the robustness of compensation assessment based primarily on clinical evaluation. Finally, this was a single-centre study, which may limit generalisability, and validation in independent cohorts is warranted.

Conflicts of Interest

The authors have each completed the International Committee of Medical Journal Editors Form for uniform Disclosure of Potential Conflicts of Interest. No authors have any potential conflict of interest to disclose.

Funding

This work was supported by institutional research grant (Project No. 130601109). The authors have no other relevant affiliations or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilised in the production of this manuscript.

Ethics Approval

The study was approved by an institutional medical research ethics committee and conducted in accordance with the Declaration of Helsinki.

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Consent to Participate

All participants were provided an informed written consent to participate in the study in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments on humans.

Acknowledgment

The authors thank the staff of the participating cardiology and clinical pathology units for their assistance with patient recruitment and sample processing. The authors also acknowledge the contribution of all study participants. This work was supported by internal institutional resources, and no external funding was received. The authors declare that they have no conflicts of interest.

Abbreviations

AUC, area under the curve; BNP, brain natriuretic peptide; BP, blood pressure; bpm, beats per minute; cDNA, complementary deoxyribonucleic acid; CI, confidence interval; Ct, cycle threshold; DCM, dilated cardiomyopathy; DM, diabetes mellitus; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; EF, ejection fraction; HF, heart failure; HTN, hypertension; IDCM, ischaemic dilated cardiomyopathy; IQR, interquartile range; IVS, interventricular septum; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; MIQE, minimum information for publication of quantitative real-time PCR experiments; miRNA/miR, microRNA; NIDCM, non-ischaemic dilated cardiomyopathy; NT-proBNP–N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; ROC, receiver operating characteristic; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SD, standard deviation; VIF, variance inflation factor.

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