The relationship between myocardial fibrosis and myocardial microRNAs in dilated cardiomyopathy: A link between mir-133a and cardiovascular events

Abstract
It is unknown whether fibrosis-associated microRNAs: miR-21, miR-26, miR-29, miR-30 and miR-133a are linked to cardiovascular (CV) outcome. The study evaluated the levels of extracellular matrix (ECM) fibrosis and the prevalence of particular microRNAs in patients with dilated cardiomyopathy (DCM) to investigate any correlation with CV events. Methods: Seventy DCM patients (48 ± 12 years, EF 24.4 ± 7.4%) underwent right ventricular biopsy. The control group was comprised of 7 patients with CAD who underwent CABG and intraoperative biopsy. MicroRNAs were measured in blood and myocardial tissue via qPCR. The end-point was a combination of CV death and urgent HF hospitalization at the end of 12 months. There were differential levels of circulating and myocardial miR-26 and miR-29 as well as myocardial miR-133a when the DCM and CABG groups were compared. Corresponding circulating and myocardial microRNAs did not correlate with one another. There was no correlation between microRNA and ECM fibrosis. By the end of the 12-month period of the study, CV death had occurred in 6 patients, and a further 19 patients required urgent HF hospitalization. None of the circulating microRNAs was a predictor of the combined end-point; however, myocardial miR-133a was an independent predictor in unadjusted models (HR 1.53; 95% CI 1.14-2.05; P < .004) and adjusted models (HR 1.57; 95% CI 1.14-2.17; P < .005). The best cut-off value for the miR-133a level for the prediction of the combined end-point was 0.74 ΔCq, with an AUC of 0.67. The absence of a correlation between the corresponding circulating and myocardial microRNAs calls into question their cellular source. This study sheds new light on the role of microRNAs in ECM fibrosis in DCM, which warrants further exploration.

KEYWORDS
biopsy, dilated cardiomyopathy, fibrosis, microRNA, prognosis
1 | INTRODUCTION

Fibrosis of extracellular matrix (ECM) is a major pathology contributing to the progression from compensated dilated cardiomyopathy (DCM) to heart failure (HF).1 Increasing evidence indicates that microRNAs are important factors in cardiac remodelling, including ECM fibrosis. Thus far, five microRNAs, namely miR-21, miR-26, miR-29, miR-30 and miR-133a, have been found to be implicated in ECM fibrosis, mostly in animal studies.2-4 MicroRNAs function primarily inside cells and tissues, e.g. the myocardium; however, miRNAs have been recently detected in blood samples.5,6 We examined: (i) the relationship between circulating and myocardial microRNAs and ECM fibrosis, (ii) the relationship between myocardial microRNAs and cardiovascular (CV) outcome.

2 | MATERIALS AND METHODS

2.1 | Study population

Over a period of 14 months, 70 consecutive DCM patients were enrolled. DCM was diagnosed in line with currently accepted criteria.7,8 The study complies with the World Medical Association Declaration of Helsinki, and the study protocol was approved by the institutional review board and the ethics committee. All patients gave written informed consent. The control group was comprised of 7 patients with CAD who underwent CABG. During open-heart surgery, transmural needle biopsies of the anterior LV wall were performed.2

2.2 | Endomyocardial biopsy

Biopsy was performed in accordance with typical guidelines.9 Fibrosis was determined by a pathologist blinded to the clinical data. Collagen volume fraction (CVF) was assessed by quantitative morphometry.10

2.3 | Laboratory measurements

RNA was extracted from 100 µL of plasma performed with a mirVana kit (Life Technologies). Two microlitres of extracted RNA was used to perform reverse transcription with a TaqMan Advanced MicroRNA cDNA Synthesis Kit (Life Technologies). qPCR was conducted on 384-well plates with TaqMan Advanced Master Mix and TaqMan Advanced Assays targeting: hsa-miR-21-5p, hsa-miR-29b-3p, hsa-miR-30c-5p, hsa-miR-133a-3p, hsa-miR-26a-5p and hsa-miR-423-3p.11 Fifteen microlitre reactions were prepared with pipetting station Bravo (Agilent Technologies), and a real-time reaction was run and read on CFX384 Real-Time PCR Detection System (Bio-Rad).

2.4 | Statistical analysis

The combined end-point was composed of CV death and urgent HF hospitalization by the 12-month point. To examine any correlation between microRNAs and end-points unadjusted and adjusted for age, duration of disease, CVF, EF and NT-proBNP, Cox proportional hazard analyses were performed.

3 | RESULTS

3.1 | Baseline characteristics

At the 12-month follow-up, CV death had occurred in 6 (8.6%) patients and urgent HF hospitalization had been required for 19 (27.1%) patients. Thus, the combined end-point occurred in 25 patients (group 1), whereas the remaining 45 patients (group 2) were free from events. Patients with event-free survival had a significantly shorter duration of disease (17.8 ± 28.2 vs 36 ± 44.3 months, P < .05) and smaller LV end-diastolic diameters (33.8 ± 5.4 vs 38.8 ± 8.4 mm/m²; P < .004); however, LV volumes and EF were similar between the two groups. Patients from group 2 had less pulmonary hypertension [11 (24.4%) vs 16 (66.7%); P < .001], higher peak oxygen uptake (18.3 ± 6.1 vs 14.5 ± 5.5 mL/kg per min; P < .05) and lower NT-proBNP (2426.8 ± 4247 vs 5069.7 ± 6852 pg/mL; P < .05).

3.2 | Comparison of microRNAs between DCM and CABG groups

DCM patients were younger (48.04 ± 12.1 vs 72.5 ± 3.4 years; P < .05) and had lower EF (24.4 ± 7.4 vs 55.6 ± 3.6%; P < .001). Both circulating miR-26 (~0.47 ± 0.79 vs 0.71 ± 0.75 ΔCq; P < .05) and tissue miR-26 (~1.4 ± 0.77 vs 1.9 ± 0.12 ΔCq; P < .05) were down-regulated in DCM, whereas both circulating miR-29 (7.71 ± 2.78 vs 3.8 ± 1.2 ΔCq; P < .01) and myocardial miR-29 (1.2 ± 0.9 vs 0.02 ± 0.18 ΔCq; P < .01) were up-regulated in DCM. Circulating miR-133a was similar in both groups, but myocardial miR-133a (0.91 ± 0.97 vs 1.93 ± 0.11 ΔCq; P < .05) was down-regulated in DCM.

3.3 | The relationship between the corresponding circulating and myocardial microRNAs

Only miR-21 was more highly expressed in the myocardium (1.08 ± 0.97 ΔCq) compared to blood levels (0.14 ± 0.6 ΔCq). The remaining microRNAs were at much higher concentrations in the bloodstream compared to cardiac tissue: miR-26 (blood: ~0.02 ± 0.79 vs myocardium: ~1.41 ± 0.77 ΔCq; P < .001), miR-29 (blood: 2.78 ± 0.78 vs myocardium: 1.22 ± 0.9 ΔCq; P < .001), miR-30 (blood: 4.39 ± 1.21 vs myocardium: ~0.42 ± 1.43 ΔCq; P < .001) and miR-133a (blood: 7.58 ± 2.28 vs myocardium: 0.91 ± 0.97 ΔCq; P < .001). None of the corresponding microRNAs correlated with one another in terms of their blood vs myocardium measurements.

3.4 | MicroRNAs, fibrosis and CV outcome

No correlation was observed between myocardial microRNAs and fibrosis. There was no difference in any of the circulating microRNAs...
Data are presented as mean ± SD.

### TABLE 1 Comparison of circulating and myocardial microRNAs between patients with and without a combined endpoint

| Parameter         | Group 1 (n = 45) | Group 2 (n = 25) | P-value |
|-------------------|------------------|-----------------|---------|
| miR-21 [ΔCq]      | 0.08 ± 0.61      | 0.23 ± 0.57     | .33     |
| miR-26 [ΔCq]      | −0.17 ± 0.8      | 0.18 ± 0.75     | .08     |
| miR-29 [ΔCq]      | 2.77 ± 0.53      | 2.8 ± 1.09      | .89     |
| miR-30 [ΔCq]      | 4.16 ± 1.03      | 4.57 ± 1.4      | .17     |
| miR-133a [ΔCq]    | 7.6 ± 2.1        | 7.4 ± 2.7       | .69     |
| tissue miR-21 [ΔCq] | 1.13 ± 0.96    | 1.01 ± 0.99     | .58     |
| tissue miR-26 [ΔCq] | −1.44 ± 0.82   | −1.34 ± 0.67    | .61     |
| tissue miR-29 [ΔCq] | 1.31 ± 0.92     | 1.06 ± 0.85     | .29     |
| tissue miR-30 [ΔCq] | −0.36 ± 1.7     | −0.51 ± 0.66    | .33     |
| tissue miR-133a [ΔCq] | 0.65 ± 0.75    | 1.36 ± 1.15     | .01     |

between patients with or without an event. However, tissue miR-133a was more highly expressed in patients with an event (Table 1). None of the circulating microRNAs was a predictor of the combined end-point. Among myocardial microRNAs, myocardial miR-133a was the only predictor of the end-point in unadjusted (HR 1.53; 95% CI 1.14-2.05; \( P < .004 \)) and adjusted (HR 1.57; 95% CI 1.14-2.17; \( P < .005 \)) models. The best cut-off value for miR-133a level for the prediction of end-point was 0.73 ΔCq, with a sensitivity of 58.3% and specificity of 71.1% (AUC 0.666). Patients with myocardial miR-29, miR-30 and miR-133a were down-regulated in our DCM cohort in comparison with CABG patients, whereas myocardial miR-26 and miR-133a were up-regulated in our DCM cohort in comparison with CABG patients, whereas myocardial miR-26 and miR-133a were down-regulated in DCM. Thus, the aetiology of HF seems to be crucial with respect to molecular cardiac pathology, including the distribution of microRNAs. We have previously reported relatively strong correlations between circulating miR-26 and miR-30 with CVF \( (r = .48, P < .01 \) and \( r = .72, P < .001 \), respectively). Surprisingly, the corresponding myocardial microRNAs did not have any relationship with ECM fibrosis. In a predominantly inflammatory DCM cohort, Besler et al reported that myocardial miR-133a expression was related to CV outcome in inflammatory DCM. Our findings extend this observation to the non-inflammatory DCM population.

### 4.1 Study limitations

The number of patients in the control cohort is small, which was as a result of a slow recruitment rate. Only selected microRNAs can be measured with qPCR. Although next-generation sequencing enables the analysis of virtually all microRNAs, we chose to use qPCR as our aim was to verify the role of the most “promising” microRNAs in relation to fibrosis.

### 5 CONCLUSIONS

The lack of correlation between corresponding circulating and myocardial microRNAs calls into question their cellular source. Based on the expression of a selection of circulating microRNAs (miR-21, miR-26, miR-29, miR-30 and miR-133a), no prediction can be made on the expression of their myocardial counterparts. No associations...
were found between myocardial microRNAs and fibrosis. Only myocardial miR-133a was found to be an independent predictor of prognosis. Although preliminary, this study sheds new light on the role of microRNAs in DCM pathology and warrants further exploration.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

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