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Circulating miR-423_5p fails as a biomarker for systemic ventricular function in adults after atrial repair for transposition of the great arteries

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ABSTRACT

Background: Recently, the microRNA miR-423_5p was identified as a biomarker for left ventricular heart failure. Its role in patients with a systemic right ventricle and reduced ejection fraction after atrial repair for transposition of the great arteries has not been evaluated.
Methods: In 41 patients and 10 age- and sex-matched healthy controls circulating miR-423_5p concentration was measured and correlated to clinical parameters, cardiac functional parameters assessed by magnetic resonance imaging, and cardiopulmonary exercise testing.
Results: Levels of circulating miR-423_5p showed no difference between patients and controls. Further, there was no correlation between miR-423_5p and parameters of cardiopulmonary exercise testing or imaging findings.
Conclusions: In patients with a systemic right ventricle and reduced ejection fraction miR-423_5p levels are not elevated. Therefore, circulating miR-423_5p is not a useful biomarker for heart failure in this patient group.

1. Introduction

MicroRNAs are endogenous, highly conserved, noncoding RNAs that regulate the stability and subsequent translation of nascent mRNA transcripts [1]. There are estimated to be more than 1000 different miRNAs, many of which are expressed in a tissue and cell-specific manner [2]. It was discovered only recently that miRNAs are also abundantly present in blood [2]. Aberrant expression profiles of miRNAs have been identified in blood of subjects with certain forms of cancer and myocardial injury [2-5].

A recent study demonstrated a number of miRNAs as putative biomarkers for heart failure [2]. Tijsen and colleagues performed miRNA arrays on RNA isolated from patients with heart failure defined by the Framingham criteria and elevated NT-proBNP levels and compared these with healthy controls and patients with dyspnea attributable to other causes. One circulating miRNA in particular, miR-423_5p, was able to distinguish heart failure cases from the other cases with an area under the curve of 0.91 [2].

In patients after atrial repair for transposition of the great arteries (D-TGA), in whom the right ventricle supports the systemic circulation, dysfunction of the right ventricle is the most important contributor to morbidity and mortality [6]. Further progressive deterioration of systemic right ventricular function is common [6]. The role of microRNAs, in particular miR-423_5p, has not been evaluated in this patient group.

2. Materials and methods

2.1. Participants

This study is part of an exercise intervention trial, which has been registered at www.clinicaltrials.gov (No. NCT00837603) [7].

Patients with previous atrial redirection procedure (Mustard) for D-TGA who met all the inclusion criteria were eligible for the study. The inclusion criteria were: stable NYHA class I/II, unchanged medication (angiotensin converting enzyme inhibitors, beta-blockers) for the last 6 months, no physical training program at present, and the physical and mental ability to follow a controlled training program. Exclusion criteria were: heart failure NYHA III-IV, recent onset or change of heart failure medication <6 months, pregnancy, pacemaker or defibrillator implantation, history of ventricular arrhythmias, renal/liver insufficiency, claustrophobia, and mental retardation.

The control group consisted of 10 healthy age- and gender-matched volunteers.

All laboratory tests were performed at baseline before the start of the training program.

2.2. Measurement of circulating microRNA

Plasma concentration of miR-423_5p was determined by quantitative reverse transcription PCR [8]. Total RNA was isolated from plasma by the Master Pure RNA

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Table 1
Clinical characteristics of the study population.

		Patients	Controls	p
Total number		41	10	
Age in years		29.2 ± 3.6	30.3 ± 4.6	n.s.
Sex	Female	14 (34.1%)	3 (30%)	n.s.
	Male	27 (65.9%)	7 (70%)	
NYHA class	I	26 (63.4%)		
	II	15 (36.6%)		
NT-proBNP in pg/ml	Total	199 ± 152		
	NYHA I	196 ± 135		n.s. vs. NYHA II
	NYHA II	202 ± 183		
Peak VO_2 in ml/min/kg		25.2 ± 5.5		
EQO_2		30.9 ± 6.6		
$EQCO_2$		29.4 ± 5.5		
RVEF in %		47.6 ± 10.9		
RVEDV-index		92.2 ± 40.6		
RVESV-index		47.4 ± 24.7		
SV-index		44.8 ± 19.2		
RV mass-index		89.0 ± 34.6		

Peak VO_2 = peak oxygen uptake, EQO_2 = ventilatory equivalent for oxygen, $EQCO_2$ = ventilatory equivalent for carbon dioxide, RV = right ventricle, EDV = end-diastolic volume, ESV = end-systolic volume, EF = ejection fraction, SV = stroke volume. All MRI parameters were indexed to body surface area.

Purification Kit (Epicentre Biotechnologies). Briefly, 50 μL plasma was incubated with 1 μL Proteinase K (50 μg/μL) and 400 μL Tissue and Cell Lysis Solution at 65 °C for 15 min. 5 fmole *Caenorhabditis elegans* spike-in miRNA cel-miR-54 was added as a normalization control as previously described [8]. 250 μL of protein precipitation reagent were then added and the samples were centrifuged at 10,000 *g* for 10 min. RNA was recovered from the supernatant using 600 μL of isopropanol and resuspended in 25 μL DEPC-treated water. 2.5 μL of RNA was reverse transcribed using iScript Select cDNA Synthesis Kit (Bio-Rad) and miRNA-specific stem loop primers (Applied Biosystems). Quantitative PCR was performed using iQ supermix (Bio-Rad) and miRNA-specific TaqMan hybridization probes (Applied Biosystems). Five-fold serial dilutions were done for reverse transcribed cDNA and standard was employed on each PCR plate separately.

2.3. NT-proBNP measurements

For analysis of NT-proBNP, venous blood was collected on the day before echocardiography and exercise testing. NT-proBNP was measured using an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics, Mannheim, Germany).

2.4. Echocardiography

A Philips iE 33 ultrasound system with a 2–4 MHz transducer and second harmonic imaging was used. Echocardiographic examinations were performed according to the recommendations for the assessment of systolic function/diameter and valvular heart disease issued by the American Society of Echocardiography [9, 10].

2.5. Magnetic resonance imaging (MRI)

CMR scans were acquired with a 1.5-Tesla Siemens Avanto scanner (Siemens, Erlangen, Germany). All subjects were examined in supine position. Routine assessment of anatomy was performed. Then a contiguous short axis stack of 7-mm steady state free precision cine images (3-mm gap) aligned orthogonal to the left ventricular axis from the apex to the atrioventricular ring was obtained. All images were acquired using breath-hold, ECG-triggered cine CMR.

Quantification of the short axis stack was done by planimetry by Simpson's method with dedicated software (Mass analysis; Medis, The Netherlands) for the RV. The endocardial border was traced manually at end-diastole and at end-systole to calculate the cross-sectional area of the ventricle at each level. End-systole and end-diastole were defined as the phases with the lowest and highest volume, respectively. RV end-diastolic volume, end-systolic volume, RV mass and RV stroke-volume were obtained. The RV free wall under the pulmonary valve was included in the assessment of RV volume. Ridges and bands from the RV wall of the interventricular septum were accounted to the RV volume. All scans of the patients were evaluated anonymously. Investigators performing the CMR and echocardiographic study were blinded to all clinical parameters.

2.6. Exercise testing

Cardiopulmonary exercise testing was performed on a bicycle with increasing workload of 25 W every 2 min. All patients exercised to the end of their tolerance. Ventilation was measured by a breath-by-breath method. Subjects breathed through a fitted mask and a hot-wire anemometer (Oxycon Delta; Jäger, Hoechberg, Germany) continuously measuring inspiration and expiration flow. Continuous measurements allowed the determination of minute ventilation (VE; l/min), maximum oxygen uptake ($peakVO_2$, ml/kg/min), O_2 pulse (oxygen uptake/beat, ml), carbon dioxide production (ml/min) and the oxygen (EQO_2) and carbon dioxide ($EQCO_2$) ventilatory equivalents.

2.7. Ethics

The trial was approved by the ethics committee at Hannover Medical School. The study complies with the Declaration of Helsinki, written informed consent was obtained from the patients and controls.

2.8. Statistical methods

We used SPSS 15.0 for statistical analysis. Continuous data are presented as mean ± standard deviation. Categorical data are presented as counts and proportions. Patient demographic and clinical characteristics were summarized as means ± standard deviation. Between group comparisons were examined using unpaired Student's *t* test for continuous and Fisher's exact test for categorical variables. One-way ANOVA was used if more than two groups were compared. For correlation Pearson's correlation coefficient was calculated. The significance level was set at $p<0.05$ and was two-sided.

3. Results

Forty-one patients and 10 age- and sex-matched healthy controls were enrolled in this study. Table 1 shows the clinical characteristics of the study population. Cardiopulmonary exercise testing, echocardiography and MRI were only performed in the patients.

3.1. MiR-423_5p in comparison between patients and controls

MiR-423_5p showed no difference between patients and healthy controls (0.000113 ± 0.000095 vs. 0.000129 ± 0.000034; p = 0.387; Fig. 1).

3.2. MiR-423_5p and systemic ventricular function

Patients were divided into three groups according to their systemic ventricular ejection fraction (RVEF) measured by MRI: group I (RVEF<40%, n = 8), group II (RVEF<50%, n = 13) and group III (RVEF≥50%, n = 20). MiR-423_5p levels were not different in comparison between group I and group II (0.000069 ± 0.000049 vs. 0.000150 ± 0.000118; p = 0.177) and group III (0.000107 ± 0.000087; p = 1.0), and also not significant between the later two (p = 0.588; Fig. 2). In contrast, NT-proBNP was significantly elevated in group I compared to group II (364 ± 177 pg/ml vs. 180 ± 159 pg/ml; p = 0.01) and to group III (145 ± 80 pg/ml; p = 0.001; Fig. 3).

3.3. MiR-423_5p in correlation with other clinical parameters

There was no difference in patients with NYHA class II compared to patients with NYHA I (0.000113 ± 0.000079 vs. 0.000129 ± 0.000101; p = 0.364) or controls (0.000129 ± 0.000034; p = 0.651). There was

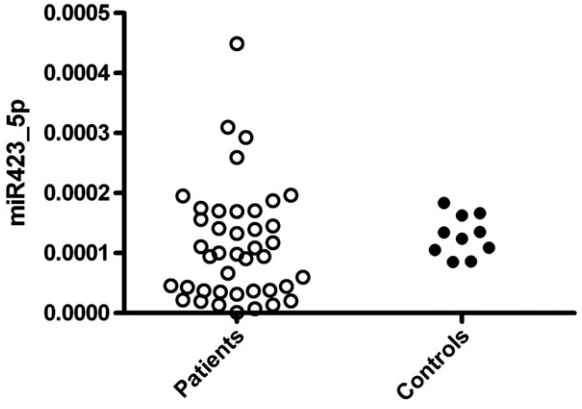

Fig. 1. MiR-423_5p levels in patients and healthy controls.