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CLINICAL STUDY

# MicroRNAs in pathophysiology of acute myocardial infarction and cardiogenic shock

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**ABSTRACT**
AIM: Levels of circulating miRNA are considered to be potential biomarkers of acute myocardial infarction and disease progression.
METHODS: In this study, the expression levels of circulating miRNA-1, miRNA-133 and miRNA-124a were investigated in a group of patients with acute myocardial infarction (STEMI) and cardiogenic shock (CS) compared to controls.
RESULTS: During the hospitalization period, miRNA-133 showed a significant up-regulation in the serum of STEMI and CS patients compared to controls, while the expression of miRNA-1 was significantly different only in CS. The expression of miRNA-124 was significantly higher in STEMI and CS. Furthermore, miRNA-1 expression was related to the level of circulating glucose in patients with STEMI. We also found a negative correlation between miRNA-133 and MMP-9 levels. MiRNA-124 expression was significantly related to the level of soluble ST2; the marker correlated to cardiac damage.
CONCLUSION: All selected miRNAs are potential markers of cardiac injury in cardiogenic shock, whereas miRNA-124a and -133 are markers of injury in STEMI. MiRNA-1 expression is related to circulating glucose in STEMI. None of miRNAs could be correlated to the extent of injury, progress of the disease, or prognosis of patient outcome. Therefore, the levels of circulating miRNA have no potential for becoming a biomarker of myocardial damage and as such would bring no further benefit compared to current markers *(Tab. 4, Fig. 1, Ref. 47)*. Text in PDF *www.elis.sk.*
KEY WORDS**:** microRNA, STEMI, cardiogenic shock, prognosis.

## Introduction

MicroRNAs (miRNAs) are conserved, single stranded, small (about 22 nucleotides), non-coding RNAs involved in post-transcriptional regulation (1). The differences in miRNAs expression have been implicated in disease onset and/or progression with potential of diagnostic and/or prognostic biomarkers (2). More than 200 miRNAs were found in the heart with the biological role as cellular regulators involved in metabolic processes, proliferation, growth of the endothelial cells and myocytes, angiogenesis, contractility and cardiac rhythm (3). MiRNA expression varies with the progression of cardiovascular diseases including acute ischemia, myocardial infarction, cardiac hypertrophy, and cardiac failure (4, 5).

Among the most expressed miRNAs in cardiac muscle are miRNA-1, miRNA-133, which are widely discussed in relation to coronary heart disease, myocardial infarction and heart failure (6–9). The miRNA-1 and miRNA-133 profiles in circulation have been shown to reflect myocardial damage and injury (6, 9). On the other hand, miRNA-124a has been related to apoptosis, cell proliferation and inflammation with targets genes affecting cell chemotaxis and infiltration (11, 11). Furthermore, the studies suggest a potential role of miRNA-124a in remodeling the cardiac tissue following a myocardial ischemic insult (12, 13). The remodeling of the tissue is connected to the imbalance in synthesis and degradation of extracellular matrix (ECM), which could be induced by many pathways, leading to altered activation of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) (14, 15).

We hypothesized that the variation in miRNA-1, miRNA-133 or miRNA-124a expressions might serve as sensitive and specific biomarkers of cardiac damage after MI with subsequent progression into cardiogenic shock. The study further focuses on evaluation of cardiac biomarkers such as BNP, NT-proBNP and sST2 and markers of extracellular matrix (ECM) remodeling in relation to selected miRNAs expression.

## Material and methods

### *Patient population*

The study consists of 54 patients with ST-segment elevation myocardial infarction (STEMI), 14 patients with cardiogenic shock

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(CS) and 21 control subjects. Patients with cardiogenic shock and STEMI were hospitalized at the Coronary Care Unit of the Internal Cardiology Department, University Hospital Brno, Czech Republic. The STEMI diagnosis was based on symptoms consistent with myocardial infarction (MI) in conjunction with appropriate changes on electrocardiography (ECG), and elevation in the levels of myocardial necrosis markers (troponin I). The diagnosis of acute heart failure (AHF) was assessed according to clinical signs upon hospital admission and/or during hospitalization (Killip class I–IV). Cardiogenic shock in patients with previous MI was defined as hypotension with systolic blood pressure ≤ 90 mmHg lasting ≥ 30 min despite adequate left ventricular filling (pulmonary capillary wedge pressure (PCWP) or left ventricle end-diastolic pressure (LVEDP) > 15 mmHg) or if the patient required vasopressor therapy (dopamine ≥7 μg/kg/min or norepinephrine ≥ 0.15 μg/kg/min) longer than 30 min to keep systolic blood pressure ≥ 90 mmHg due to heart failure; additionally patients had signs of tissue hypoperfusion (oliguria < 20 ml/h, mottled and cold skin), signs of encephalopathy, and acidosis or blood lactate > 2 mmol/L. The reliability and reproducibility of echocardiographic parameters were evaluated by inter-observer variability using intra-class correlation coefficient (ICC). The intra-observer variability was up to 10 %. To evaluate the left ventricular (LV) hypertrophy and geometry remodeling, the LV weight index (indexed to BSA) with borders for male 125 g/m$^2$ and female 111 g/m$^2$, and relative wall thickness (RWT) with the cut off value > 0.42 was computed (16). Samples of venous blood were drawn for analysis before coronary angiography at hospitalization, in follow-up after 24 hours, and 6 months after MI onset.

As a control population, a total of 21 stable patients with LV ejection fraction > 60 %, without acute myocardial infarction, aortic or mitral valve disease were enrolled.

Written informed consent was obtained from individual participants of Caucasian origin before participation in the study. Experimental protocols of this study were approved by the local of the University Hospital Brno and by the Ethics Committee of the Masaryk University (Brno, Czech Republic). The study protocol complied with the Declaration of Helsinki.

Disease progression

The primary endpoint in the surviving STEMI patients was assessed as the presence of at least one of the insults in the follow-up: non-fatal myocardial infarction, revascularization (defined as any unplanned vessel coronary revascularization), stroke, hospitalization due to acute decompensation of heart failure, and death in the 5-year follow-up.

*Laboratory methods*

Serum collection and storage

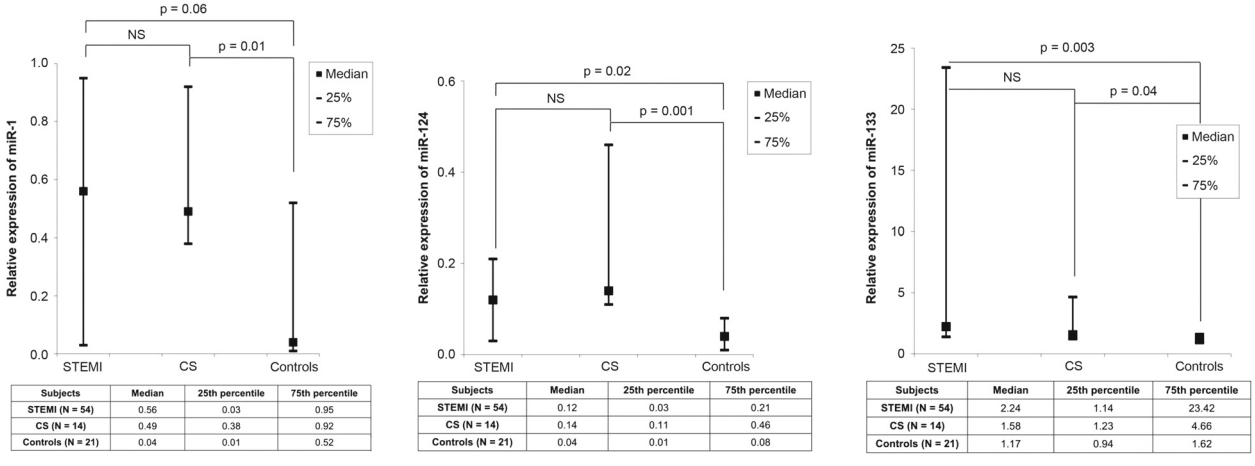
In brief, blood samples were obtained from all patients in the cardiac catheterization laboratory. Centrifugation was carried out for 15 min at 1000 g. Supernatant was aspirated, centrifuged, and stored at –80 °C until assayed.

Biochemical tests

Standard biochemical and hematological blood tests were performed immediately upon hospital admission before pPCI and 24 h after the onset of chest pain (including troponin I Abbott Laboratories, Abbott Park, IL, USA; BNP AxSYM BNP-Microparticle Enzyme Immunoassay, Abbott Laboratories; N-terminal prohormone of BNP (NTproBNP), Cobas E411 NTproBNP Imunoassay Kit, Roche Diagnostics, Indianapolis, IN, USA).

MiRNA

Total RNA extraction from serum was performed using the TRIzol reagent (Invitrogen, Carlsbad, CA). Reverse transcription into cDNA was performed using the TaqMan microRNA reverse transcription kit with specific hairpin primers (Applied Biosystems, Foster City, CA). The expression of miRNA-1, miRNA-133 and miRNA-124a was performed by real-time RT–PCR using an ABI 7700 system (Applied Biosystems). MiRNA-U6 was used as an endogenous control. For each individual sample,




| Subjects | Median | 25th percentile | 75th percentile |
|---|---|---|---|
| STEMI (N = 54) | 0.56 | 0.03 | 0.95 |
| CS (N = 14) | 0.49 | 0.38 | 0.92 |
| Controls (N = 21) | 0.04 | 0.01 | 0.52 |

| Subjects | Median | 25th percentile | 75th percentile |
|---|---|---|---|
| STEMI (N = 54) | 0.12 | 0.03 | 0.21 |
| CS (N = 14) | 0.14 | 0.11 | 0.46 |
| Controls (N = 21) | 0.04 | 0.01 | 0.08 |

| Subjects | Median | 25th percentile | 75th percentile |
|---|---|---|---|
| STEMI (N = 54) | 2.24 | 1.14 | 23.42 |
| CS (N = 14) | 1.58 | 1.23 | 4.66 |
| Controls (N = 21) | 1.17 | 0.94 | 1.62 |

**Fig. 1. Relative expression of miRNAs in acute myocardial infarction with ST elevations (STEMI) and cardiogenic shock (CS) patients in comparison to control subjects: A) miRNA-1; B) miRNA-124; C) miRNA-133**

**Tab. 1. Relationship of miRNAs expression to basic characteristics**

| | N (%) | miRNA-1 | p | miRNA-124 | p | miRNA-133 | p |
|---|---|---|---|---|---|---|---|
| Sex – male | 77.5 | 0.501 (0.142; 0.966) vs 0.525 (0.191; 0.895) | 0.852 | 0.078 (0.026; 0.202) vs 0.054 (0.035; 0.166) | 0.611 | 3.204 (1.292; 70.034) vs 2.479 (1.357; 14.420) | 0.629 |
| Diabetes mellitus | 28.6 | 0.164 (0.031; 0.624) vs 0.664 (0.217; 1.337) | **0.019** | 0.036 (0.020; 0.106) vs 0.087 (0.034; 0.204) | 0.067 | 22.572 (1.292; 51.268) vs 2.364 (1.385; 23.754) | 0.679 |
| Prior instability | 26.5 | 0.895 (0.423; 1.443) vs 0.399 (0.137; 0.841) | 0.061 | 0.091 (0.055; 0.202) vs 0.055 (0.021; 0.171) | 0.213 | 2.212 (1.272; 39.025) vs 4.110 (1.357; 49.866) | 0.872 |
| Hypertension | 48.9 | 0.399 (0.147; 0.895) vs 0.562 (0.192; 0.986) | 0.941 | 0.052 (0.023; 0.145) vs 0.087 (0.049; 0.201) | 0.287 | 14.420 (1.494; 51.268) vs 2.234 (1.172; 21.555) | 0.173 |
| HLP | 16.3 | 0.481 (0.142; 0.966) vs 0.586 (0.160; 0.864) | 0.918 | 0.095 (0.033; 0.180) vs 0.037 (0.023; 0.059) | 0.988 | 2.479 (1.414; 49.866) vs 8.876 (1.292; 51.268) | 0.150 |
| Lower extremity ID | 8.2 | 0.752 (0.332; 2.379) vs 0.438 (0.147; 0.946) | 0.755 | 0.231 (0.106; 0.469) vs 0.056 (0.026; 0.166) | 0.076 | 4.124 (1.439; 126.911) vs 2.841 (1.356; 49.866) | 0.895 |
| Smoking | 10.2 | 0.876 (0.864; 1.375) vs 0.434 (0.147; 0.895) | 0.271 | 0.031 (0.026; 0.042) vs 0.112 (0.031; 0.168) | 0.079 | 2.479 (1.464; 24.084) vs 4.856 (1.495; 49.866) | 0.997 |

HLP – hyperlipoproteinemia; ID – ischemic disease; results with $p < 0.05$ are in bold

the expression value was calculated based on reference miRNA (U6) according to a threshold cycle (Ct) value for each serum sample. To avoid the difficulties in detecting low expression values (>40 Ct), the preamplification was applied. The results are shown using relative quantification (ΔCt).

*Statistics*

Values are expressed as median with 25th and 75th percentile. The Kruskal–Wallis ANOVA and Mann Whitney U tests were used for the evaluation of miRNA expression levels and other continual variants. Correlation analyses were performed using two-tailed Spearman's rank correlation. Multivariate linear regression was applied for adjusting the influence of other variables. Parameters with $p < 0.10$ were taken into multivariate analysis, and if applicable, square root transformed. Statistica v. 10.0 (Statsoft Inc., Tulsa, OK) was used to analyze the data. The values with $p < 0.05$ were considered significant.

## Results

*Hospitalization phase*

The expression of miRNA-1, miRNA-124a and miRNA-133 in circulation was analyzed in 54 STEMI subjects, 14 subjects with cardiogenic shock (CS), and 21 controls. Different relative expression of miRNA-1 was detected between control subjects and group of patients with cardiogenic shock ($p = 0.01$) (Fig. 1). In the case of miRNA-124a (Fig. 1), significant differences were observed among control subjects, both STEMI patients ($p = 0.02$), and group of CS patients ($p = 0.001$). Similar results were found for miRNA-133 expression (Fig. 1); significant differences in relative expression levels were observed among the group of patients with STEMI and controls ($p = 0.003$), and between CS patients and control subjects ($p = 0.04$).

*Relation to clinical parameters*

Relations of studied miRNAs to selected clinical parameters are listed in Tables 1 and 2. Regarding the characteristics in anamnesis of patients, miRNA-1 expression was associated with diabetes mellitus ($p = 0.02$) (Tab. 1). MiRNA-1 expression was related to the level of circulating glucose in univariate analysis ($p < 0.0001$) and multivariate analysis ($p < 0.05$). Significant parameters in relation to miRNA-1 in univariate analysis (creatinine, ureic acid, LDL-cholesterol and glycated hemoglobin A1c (HbA1c)), were not significant in multivariate analysis. MiRNA-124a expression correlated to circulating levels of sST2 ($p = 0.0007$) in univariate analysis and multivariate analysis ($p < 0.05$). A relation of miRNA-133 to the levels of circulating MMP-9 in univariate analysis ($p = 0.005$) and multivariate analysis ($p = 0.05$) was observed. Parameters significant in univariate analysis, namely age and circulating levels of TNF-alpha, were not significant in multivariate analysis.

*Disease progression*

Subsequently, the STEMI patients were divided into subgroups: STEMI patients with endpoint ($n = 21$) and STEMI patients without endpoint ($n = 33$). The difference in miRNAs (miRNA-1, miRNA-124a, miRNA-133) expression was not statistically significant among STEMI patients with and without endpoint (Tab. 3), and even with LV hypertrophy evaluated in 33 patients with complete values (Tab. 4).

*Follow-up*

In the group of patients with CS, the expressions of miRNA-1, miRNA-124a, and miRNA-133 were analyzed at hospitalization and after 24 hours. In the group of STEMI patients, the miRNAs expressions at hospitalization were compared with samples collected 6 months after MI onset. None of three selected miRNAs expressions was significantly different in the follow-up after 24 hours in CS patients (Tab. 3) or after 6 months in STEMI patients (Tab. 3).

## Discussion

Currently, increasing attention is paid to the miRNAs as potential biomarkers of various diseases including cardiovascular disorders.

*MiRNA-1 and miRNA-133*

In the context of the cardiovascular system, the most discussed are miRNA-1 and miRNA-133 (17). In the mammalian genome, miRNA-1 and miRNA-133 are encoded by two distinct gene clusters located on two different chromosomes with identical primary sequences of mature microRNA (8). In our study, miRNA-133 showed a significant up-regulation in the serum of STEMI patients and CS patients when compared with control subjects. The expression of miRNA-1 in CS patients compared to controls was significantly different. These results are in consensus with published studies describing increased circulating levels of miRNA-1 and

**Tab. 2. Correlation of miRNAs expression to selected clinical parameters.**

| STEMI (N = 47) | miRNA-1 | | miRNA-124 | | miRNA-133 | |
|---|---|---|---|---|---|---|
| Parameters | R | p | R | p | R | p |
| Age | 0.132 | 0.377 | 0.034 | 0.817 | 0.299 | **0.043** |
| Body Mass index | 0.154 | 0.300 | –0.265 | 0.065 | –0.122 | 0.413 |
| HR (/min) | 0.104 | 0.488 | –0.022 | 0.876 | 0.057 | 0.702 |
| Creatinine (umol/l) at entry | –0.352 | **0.015** | 0.003 | 0.978 | 0.024 | 0.870 |
| UA (umol/l) at entry | –0.343 | **0.019** | 0.175 | 0.232 | 0.081 | 0.593 |
| Glycemia (mmol/l) at entry | –0.523 | **0.0001** | –0.111 | 0.447 | –0.038 | 0.800 |
| LDL (mmol/l) at entry | 0.288 | **0.049** | 0.077 | 0.594 | 0.115 | 0.444 |
| Leukocytes | –0.057 | 0.699 | 0.097 | 0.505 | 0.132 | 0.378 |
| Troponin (ug/l) max | 0.224 | 0.133 | –0.010 | 0.946 | 0.023 | 0.879 |
| Hb (g/l) at entry | 0.118 | 0.426 | –0.090 | 0.535 | 0.028 | 0.850 |
| HbA1c (%) | –0.428 | **0.029** | –0.280 | 0.147 | 0.075 | 0.715 |
| BNP (pg/ml) at entry | 0.209 | 0.167 | 0.040 | 0.786 | 0.238 | 0.119 |
| NT-pro-BNP (pg/ml) at entry | 0.127 | 0.392 | 0.019 | 0.895 | 0.134 | 0.373 |
| TNFα (ng/ul) at entry | 0.089 | 0.699 | 0.020 | 0.928 | –0.442 | **0.045** |
| sST2 (ng/ul) at entry | 0.021 | 0.898 | 0.504 | **0.0007** | 0.017 | 0.919 |
| ACE (ng/ul) at entry | 0.066 | 0.664 | 0.105 | 0.480 | –0.121 | 0.430 |
| MMP-2(ng/ul) at entry | 0.043 | 0.772 | –0.057 | 0.699 | –0.144 | 0.343 |
| TIMP-1 (ng/ul) at entry | 0.090 | 0.551 | 0.160 | 0.276 | –0.229 | 0.121 |
| MMP-9 (ng/ul) at entry | –0.205 | 0.210 | 0.183 | 0.252 | 0.441 | **0.005** |
| MMP-8 (ng/ul) at entry | 0.286 | 0.106 | 0.116 | 0.504 | 0.161 | 0.378 |
| LVEDP max (mmHg) | 0.292 | 0.057 | –0.015 | 0.920 | 0.092 | 0.559 |
| EF LV 10–90% | 0.289 | 0.066 | 0.060 | 0.701 | –0.182 | 0.258 |
| dp/dt/P after RLVG | 0.122 | 0.446 | 0.170 | 0.274 | 0.151 | 0.350 |
| EF (%) at entry | 0.126 | 0.400 | 0.049 | 0.741 | –0.258 | 0.090 |
| EF (%) after 3 months | –0.006 | 0.973 | –0.204 | 0.305 | 0.059 | 0.779 |
| EF (%) after 6 months | 0.057 | 0.774 | –0.125 | 0.534 | 0.031 | 0.880 |
| EF (%) last know | 0.079 | 0.625 | 0.157 | 0.319 | –0.210 | 0.198 |

Spearman rank order coefficients are shown in the corresponding cells, results with $p < 0.05$ are in bold; results with $p < 0.05$ with Bonferroni correction are in bold and underlined. ACE – Angiotensin converting enzyme, BNP – B-type natriuretic protein, EF – Ejection fraction after MI, Hb – hemoglobin, HbA1c – glycated hemoglobine A1c, HR– Heart rate, LDL – Low density lipids, LV – Left ventricle, LVEDP– Left ventricular end diastolic pressure, MMP – Matrix metalloproteinase, NTproBNP – N-terminal prohormone of BNP, RLVG –Retrograde left ventriculography ,sST2 –solubile suppressor of tumorigeneicity 2, TIMP – Tissue inhibitor of MMP, TNF-α – Tumor Necrosis Factor alpha, UA – Ureic Acid

miRNA-133 in patients with MI (18–20). The source of elevated levels of miRNA-1 and miRNA-133 is likely in the damaged cardiac tissue (18, 21). The injury leads to deregulation of miRNA-1 and miRNA-133 and release from the damaged cardiomyocyte (22). In the model of chronic failing hearts, Kumarswamy et al have shown negative regulation of cardiomyocyte miRNA-1 expression and decreased miRNA-1 expression in vivo (23). Furthermore, it has been shown that inhibition of endogenous miRNA-133 results in cell hypertrophy, increased fetal gene expression, and expression of atrial natriuretic factor (ANF) (24). Zhang et al showed the effect of miRNA-133 and miRNA-1 on cell cycle progression (25). Although this fact suggests an association of increasing levels with progressive damage to the heart, the study failed to confirm the increase in levels of miRNA-1 and miRNA-133 in cardiogenic shock compared to myocardial infarction.

Regarding the prognostic value of miRNA-1 and miRNA-133, no significant changes in miRNAs expression were found 24 hours after the onset of CS or 6 months after MI onset. Even no significant differences were observed in STEMI patients with or without combined endpoint in the follow-up of 5 years. On the other hand, the prognostic value of miRNAs was evaluated in patients with acute coronary syndromes when the levels of miRNA-133 were significantly associated with the risk of death, with loss of additional prognostic information after adjusting for high-sensitive troponins (26). Similar results were published by Eitel (2012) in STEMI patients (23).

Concerning the clinical parameters, we found relation of miRNA-1 expression to the level of circulating glucose in patients with STEMI. Interestingly, higher miRNA-1 expression was found in non-diabetic patients, which could suggest the influence of "stress" glucose on miRNA-1 release. In consensus with our findings of high glucose and increased miRNA-1 expression, in previous reports, the increased expression of miRNA-1 was found in the ventricular samples from diabetic patients (27). The diabetic complications were related to high glucose-induced apoptosis of cardiomyocytes (28). Hyperglycemia induces oxidative stress and alters calcium homeostasis causing cell and mitochondrial dysfunctions (29, 30). On the other hand, Feng et al associated glucose-induced down-regulation of miRNA-1 with up-regulation of enothelin-1 and fibronectin in the endothelial cells (EC) (31). MiRNA-1 influences also EC dysfunction, leading to increased permeability and extracellular matrix production. Furthermore, miRNA-1 affects the apoptotic process, vasoactive mediators, and could be one of the mediators of cardiac hypertrophy (32). In consensus to mentioned data, a trend in increase of miRNA-1 expression in LV hypertrophy was found.

In our study, miRNA-133 was found to be negatively correlated with circulating levels of MMP-9, factor of ECM remodeling.

**Tab. 3. Relative expression of miRNAs in acute myocardial infarction with ST elevations (STEMI) and cardiogenic shock (CS) patients.**

A) Relative expression of miRNAs in STEMI patients divided regarding progression into combined endpoint

| | miRNA-1 | | | |
|---|---|---|---|---|
| STEMI – endpoint (with N=21; without N=33) | Median | 25th percentile | 75th percentile | p |
| With endpoint | 0.23 | 0.03 | 0.59 | 0.15 |
| Without endpoint | 0.56 | 0.04 | 0.95 | |
| | miRNA-124 | | | |
| | Median | 25th percentile | 75th percentile | p |
| With endpoint | 0.05 | 0.03 | 0.11 | 0.07 |
| Without endpoint | 0.12 | 0.03 | 0.21 | |
| | miRNA-133 | | | |
| | Median | 25th percentile | 75th percentile | p |
| With endpoint | 20.55 | 1.32 | 71.55 | 0.29 |
| Without endpoint | 2.24 | 1.41 | 23.43 | |

B) Relative expression of miRNAs in acute myocardial infarction with ST elevations (STEMI) without progression into combined endpoint in follow-up of 6 months

| | miRNA-1 | | | |
|---|---|---|---|---|
| STEMI follow-up (without endpoint N=33) | Median | 25th percentile | 75th percentile | p |
| At hospitalization | 0.56 | 0.03 | 0.95 | 0.16 |
| After 6 months | 0.38 | 0.23 | 0.77 | |
| | miRNA-124 | | | |
| | Median | 25th percentile | 75th percentile | p |
| At hospitalization | 0.11 | 0.04 | 0.21 | 0.06 |
| After 6 months | 0.20 | 0.10 | 0.50 | |
| | miRNA-133 | | | |
| | Median | 25th percentile | 75th percentile | p |
| At hospitalization | 2.24 | 1.41 | 23.42 | 0.17 |
| After 6 months | 1.91 | 1.02 | 8.17 | |

C) Relative expression of miRNAs in cardiogenic shock (CS) patients in follow-up of 24 hours

| | miRNA-1 | | | |
|---|---|---|---|---|
| CS (N=14) | Median | 25th percentile | 75th percentile | p |
| At hospitalization | 0.49 | 0.38 | 0.92 | 0.94 |
| After 24 hours | 0.68 | 0.40 | 1.32 | |
| | miRNA-124 | | | |
| | Median | 25th percentile | 75th percentile | p |
| At hospitalization | 0.14 | 0.11 | 0.46 | 0.35 |
| After 24 hours | 0.15 | 0.06 | 0.43 | |
| | miRNA-133 | | | |
| | Median | 25th percentile | 75th percentile | p |
| At hospitalization | 1.58 | 1.23 | 4.66 | 0.57 |
| After 24 hours | 1.27 | 0.97 | 1.80 | |

**Tab. 4. Relationship of miRNAs expression to left ventricular hypertrophy (LVH).**

| | LVH* (n=14) | Without LVH (n=19) | p |
|---|---|---|---|
| miR-1 at entry | 0.70 (0.217; 1.337) | 0.43 (0.031; 0.895) | 0.11 |
| miR-1 after 6 months | 0.58 (0.154; 1.009) | 0.15 (0.003; 0.454) | 0.08 |
| miR-124 at entry | 0.10 (0.008; 0.180) | 0.07 (0.033; 0.337) | 0.20 |
| miR-124 after 6 months | 0.11 (0.068; 0.166) | 0.32 (0.065; 0.448) | 0.51 |
| miR-133 at entry | 8.88 (1.464; 23.588) | 3.55 (1.292; 23.425) | 0.48 |
| miR-133 after 6 months | 1.12 (1.000; 2.462) | 1.78 (0.643; 14.787) | 0.25 |

*LV hypretrophy was assessed as RWT > 0.42 and/or LV weight index above125 g/m$^2$ in male and 111 g/m$^2$ in female

A similar pattern was reported on cardiomyocytes during hyperhomocysteinemia, when the expression of MMP-9 increased with an increase in H3K9 acetylation, while miRNA-133a decreased (33). Recently, the low miRNA-133 expression was associated with cardiac fibrosis, dilatative cardiomyopathy, sudden cardiac death (34), cardiac hypertrophy and heart failure (23, 35). On the other hand, higher miRNA-133 expression has been associated with low-grade heart failure (36) and antiapoptotic effects (37). The role of alterations of miRNA-133 has been demonstrated in remodeling processes leading to cardiomyocyte hypertrophy in diabetes (38, 39). Interestingly, miRNA-133 is likely essential for regeneration of the injured heart (40). In consensus with this hypothesis, miRNA-133 emerges as a key element of the reverse pressure overload hypertrophy remodeling process in valve replacement in aortic stenosis (41). The mentioned facts suggest a possibly great potential of miRNA-133 in the prevention of heart remodeling processes.

*MiRNA-124a*

MicroRNA-124a is concerned as a potential regulator of proliferation, migration, and inflammatory phenotype of certain types of cells. Furthermore, miRNA-124a plays role in chemokine production and immune cells infiltration (10, 11). Despite the small number of studies dealing with the relationship between miRNA-124a and cardiovascular system, the increase in miRNA-124a was associated with ischemia in ischemic stroke (42). In our study, expression of miRNA-124a was higher both in STEMI patients and those with CS compared to control subjects. The values were not different after 24 hours from the development of cardiogenic shock, even in the follow-up after 6 months. MiRNA-124a is able to regulate the differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) into cardiomyocytes (13, 43). Bone marrow-derived mesenchymal stem cells (BMSCs) may trans-differentiate into cardiomyocytes, replace apoptotic myocardium and improve myocardial functions after the damage. Expression levels of miRNA-124a in the heart was shown to be similar to those in BMSCs, thus suggesting a potential role of miRNA-124a in remodeling of the cardiac tissue (12). In our study, MiRNA-124a was related to the levels of soluble ST2, a component of IL-33/ST2 system. The sST2 is considered to be a potential biomarker of cardiomyocyte stretch and prognostic biomarker in patients with myocardial infarction and heart failure (44–46). Also, sST2s stimulates the synthesis of extracellular matrix (ECM) by increasing collagen and fibronectin levels, and likewise is able to increase matrix metalloproteinase (MMP) activities (47).

Our study has several limitations. Firstly, these results are from an observational study and the subgroups analyses were limited by a small number of patients, especially in case of patients with cardiogenic shock. Secondly, the echocardiographic assessment

of left ventricular hypertrophy in STEMI and CS patients was not analyzed. The measurement of wall thickness and LV weight on a larger group would provide additional information in the evaluation of LV hypertrophy.

## Conclusion

In conclusion, miRNA-1, -124a, and -133 are potential markers of cardiac injury in cardiogenic shock patients, whereas miRNA-124a and -133 are markers of injury in the STEMI patients. From all miRNAs tested, none could be correlated to the extent of injury, progress of the disease, or prognosi of patient outcome. Therefore, the use of any of these miRNAs as a biomarker of myocardial damage would bring no further benefit compared to current markers, such as troponin I. Nonetheless, the fact that the miRNA-1 expression correlated with circulating glucose in patients after myocardial infarction is.an interesting finding. Although our findings would need further confirmation in other, preferably larger studies, a better understanding of the role of microRNA-dependent regulation of the processes of disease development may lead to new targeted therapies and new diagnostics for cardiovascular impairment in humans.

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