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Value of Serum miR-23a, miR-30d, and miR-146a Biomarkers in ST-Elevation Myocardial Infarction

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The aim of this study was to analyze the relative expression level of miR-30d-5p, miR-23a-3p, and miR-146a-5p, and to comprehensively assess the diagnostic and predictive possibilities of these miRNAs. Their expression changes have not yet been sufficiently investigated during acute myocardial infarction. Therefore, it is important to comprehensively assess the diagnostic and predictive possibilities of these micro-ribonucleic acids (miRNAs).

Material/Methods: Random patients with ST-elevated myocardial infarction (STEMI) were enrolled into the study group. The control group was comprised of patients with no inflammation or ischemic heart disease who were hospitalized for minor elective surgery. The relative expression level for each miRNA was determined by reverse transcription quantitative polymerase chain reaction (RT-qPCR)-analysis.

Results: There were 88 participants enrolled into the study: 62 patients were diagnosed with STEMI and there were 26 healthy controls. Expressions of miR-30d-5p, miR-146a-5p, and miR-23a-3p were respectively 1.581-fold, 4.048-fold, and 4.857-fold lower in patients with STEMI compared to the control group patients (all *P* values were <0.001). Downregulation of miR-23a-3p was significantly negatively correlated with risk scores of GRACE (Global Registry of Acute Coronary Events) and APACHE II (Acute Physiology and Chronic Health Evaluation II). MiR-23a-3p was a fair predictor for STEMI: area under the curve (AUC)=0.806. Cox regression analysis revealed that expression levels of analyzed miRNAs were not significantly associated with negative endpoints at 1 month after the onset of STEMI.

Conclusions: All investigated miRNAs were differentially expressed when comparing patients with STEMI and control group individuals. The evaluation of miR-23a-3p expression levels in serum could be useful to assess the severity of STEMI and as a potential diagnostic biomarker of this condition. In addition, miR-23a-3p may provide limited short-term prognostic value for STEMI patients.

MeSH Keywords: Biological Markers • MicroRNAs • Myocardial Infarction

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Background

Micro-ribonucleic acids (miRNAs) are small non-coding transcripts. They play an important role in transcriptional activity of a cell, managing differentiation, metabolism, development, interaction and other processes. Some potential diagnostic, prognostic and predictive roles of miRNAs have already been described [1,2]. Nowadays, miRNAs are being widely explored as disease biomarkers.

MiRNAs have an important role in regulation of cardiovascular disease pathophysiology [3]. They are involved in the acute inflammatory process and systemic response to ischemic cardiac injury [4]. They are associated with myocardial ischemia-reperfusion injury and also associated with myocardial cell apoptosis [5]. Therefore, circulating miRNAs may have a potential as biomarkers of acute myocardial infarction (AMI) [6].

It has been established that miR-30d could be involved in systemic inflammatory response, and neovascularization processes after AMI [7]. A previous study reported miR-23a as a possible biomarker for coronary artery disease (CAD) [8]. It has been confirmed that miR-23a is related to myocardial hypertrophy [9]. Altered expression levels of miR-30d and miR-23a may be useful to identify non-infective systemic inflammatory response (SIR) processes, such as AMI [10]. The expression level change of stress related miR-146a was found to potentially attenuate cardiac dysfunction and apoptosis during AMI in mice [11].

As aforementioned, evidence has demonstrated that miR-30d-5p, miR-23a-3p, and miR-146a-5 could be involved in cardiovascular events. However, their expression level changes have not yet been sufficiently investigated during AMI. Most of the previous data on these miRNAs have been obtained from animal studies and there is little evidence of their value and clinical implication in humans. We decided to analyze expression levels of circulating serum miR-30d-5p, miR-23a-3p, and miR-146a-5 and to comprehensively assess the diagnostic and predictive possibilities of these miRNAs for patients with ST-elevated myocardial infarction (STEMI).

Material and Methods

Ethics statement

The current monocentric, prospective study was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-2-19) and the study was carried out in accordance with the approved guidelines. All data were anonymized. Patients meeting the inclusion criteria were interviewed after hospitalization. The study purposes and benefits were explained. The informed consent form was approved by the Regional Ethics Committee, together with the description of the study, which was provided to the patient with sufficient time for potential participants to read the informed consent document, and ask questions prior to signing. Written informed consent was obtained from each individual.

Study population

Random patients with STEMI, who were admitted to a cardiac intensive care unit (CICU) and underwent emergency percutaneous coronary intervention (PCI) were enrolled into the study group. The control group was comprised of patients with no inflammation or ischemic heart disease who were hospitalized for minor elective surgery at Lithuanian University of Health Sciences between July 2018 and October 2018. Acute coronary syndrome (ACS), AMI, and STEMI were diagnosed according to international standards [12] by independent, experienced CICU physicians who were unaware of the miRNAs data. Patients were provided with treatment for STEMI, according to recent guidelines [13].

Data, describing demographics and severity of illness were gathered for each study participant. This included Killip class, Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, Global Registry of Acute Coronary Events (GRACE) scores, comorbidities and routine clinical blood test results. All participants underwent clinical evaluation, including physical examination and 12-lead electrocardiography.

Patients were excluded from the study if any of the following components presented: refusal to participate in the study at any stage, oncological disease, pregnancy, refusal to participate, convicts, patients under 18 years old, acute cerebrovascular disease, or coronary arteries free of significant lesions detected by PCI. Endpoints or events were determined by reviewing the medical records and by follow-up for up to 1 month. Primary outcome was the expression levels of circulating serum miR-30d-5p, miR-23a-3p, and miR-146a-5 in patients with STEMI and in healthy patients. Secondary outcomes were diagnostic and predictive possibilities of these miRNAs.

Blood sampling and data collection

Blood samples from patients with STEMI were collected simultaneously to other standard-care assessments to minimize/eliminate the need for additional punctures in the first 24 hours of CICU admission. Samples from the control group patients were drawn through venous puncture prior to surgical intervention. Collected blood specimens were stored in tubes containing clot activator and, were left at room temperature (15–25°C) from 10 minutes to 1 hour for complete clotting. Tubes were centrifuged at 3000 rpm for 10 minutes at 4°C, using a swinging bucket rotor. Afterwards, the resulting

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serum samples were transferred into new RNase/DNase-free Eppendorf tubes. Hemolysis in serum samples was measured using the QIAxpert spectrophotometer (Qiagen GmbH). We measured oxyhemoglobin absorbance at k = 414 nm [14]. The value of ≤0.2 absorption units (AU) was used as the threshold for samples inclusion [15]. Samples were stored at –80°C until further RNA purification, according to the protocol of manufacturer.

RNA extraction

The miRNeasy Serum/Plasma Kit (Cat No. 217184 Qiagen GmbH) was used to purify cell-free total RNA from human serum following the instructions of the manufacturer. In order to monitor miRNA purification and amplification, the corresponding *Caenorhabditis elegans* miR-39 (miRNeasy Serum/Plasma Spike-In Control, Cat No. 219610 Qiagen GmbH) was added after sample lysis. RNAs were eluted in 14 µL of RNase-free water.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The miScript PCR System (Qiagen GmbH) was used for the specific detection and relative quantification of microRNAs according to the manufacturer's protocol. Total RNA (1.5 µL) was reverse transcribed using the Reverse Transcription Kit (miScript II RT Kit Cat No. 218161 Qiagen GmbH). In the reverse transcription (RT) with miScript HiSpec Buffer, mature miRNAs were polyadenylated by poly(A) polymerase and reverse transcripted into complementary DNA (cDNA) with oligo-dT priming. cDNA samples were diluted in 200 µL of nuclease free water and were stored at –20°C until qPCR. Next, 2.0 µL of the diluted cDNA samples were used as a template for qPCR reactions using miScript Primer Assays (Qiagen GmbH) for miR-23a-3p, miR-30d-5p, and miR-146a-5p in combination with the miScript SYBR Green PCR Kit (Cat No. 218073 Qiagen GmbH), which contains the miScript Universal Primer (reverse primer). The combination of polyadenylation and the universal tag addition ensured that miScript Primer Assays do not detect genomic DNA. Rotor Gene Q real-time PCR cycler (Qiagen GmbH) was used for qPCR. Cel-miR-39-3p was used for normalization. The C_t value was defined as the number of PCR cycles required for the fluorescence signal to exceed the detection threshold value [16]. The relative expression level for each miRNA was calculated using the $2^{-\Delta\Delta Ct}$ method. C_t values below 36 were included.

Statistical analysis

Normal distribution data were expressed as mean ± standard deviation (SD). The normality of data was assessed with Kolmogorov-Smirnov or Shapiro Wilks tests. Groups were compared by independent samples *t*-test or one-way analysis of variance (ANOVA) for comparison between multiple groups. Non-normal distribution data were expressed as median (min, max) or median and interquartile range. For non-parametric statistics, Mann-Whitney U test or Kruskal-Wallis ANOVA were performed for comparison between groups. The difference in frequency distribution between groups was determined by chi-square test. Linear regression analysis was performed to determine correlations. Fold differences were calculated for the miRNA expression values in patients with STEMI compared with healthy controls. The 1-month survival was calculated by Kaplan-Meier curves with log-rank test. A *P* value <0.05 was considered statistically significant. The ability to distinguish STEMI group and control group was characterized by the receiver operating characteristic (ROC) curve. The sample size of at least 18 patients was determined based on previous studies during the analysis of all 3 miRNAs for assessing diagnostic value. The statistical analysis was performed using IBM SPSS Statistics software (v. 23.0 Chicago, IL, USA). Statistical tests were 2-sided with *P*<0.05 considered significant.

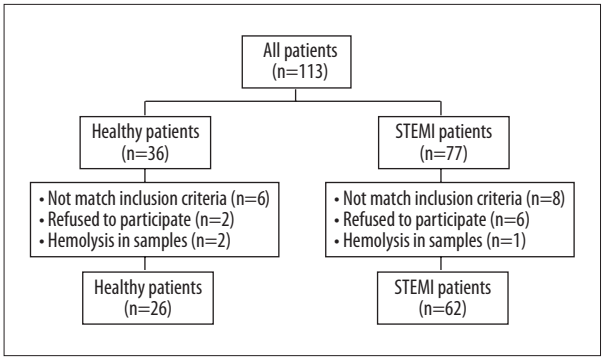

Figure 1. Study design. STEMI, ST-segment elevation myocardial infarction.

Results

Baseline characteristics

There were 113 potential candidates who were initially included in our study analysis, however 25 patients were excluded due to a mismatch of the inclusion criteria, refusal to participate, or hemolysis in samples (Figure 1). Finally, 88 participants were enrolled into the study: 62 patients were diagnosed with STEMI and there were 26 healthy controls. Table 1 presents a demographic flow chart and the laboratory data. The gender distribution, body mass index (BMI), blood pressure levels, heart rate (HR), mean hemoglobin (Hb), and hematocrit (HCT) did not differ between healthy participants and patients with STEMI. However, patients with STEMI were older, more frequent smokers, and suffered from arterial hypertension, diabetes, impaired kidney function,with higher levels of nitrogen containing compounds and elevated potassium and sodium

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Table 1. Clinical characteristics of groups.

	STEMI (n=62)	HP (n=26)	P
Gender, male (n, %)	46 (74.2)	20 (76.9)	0.791
Age (years)*	64 (12)	42 (13)	<0.001
Smoking (n, %)	29 (46.8)	2 (7.7)	0.001
Body mass index (kg/m^2)*	29 (6)	28 (6)	0.331
Diabetes (n, %)	15 (24.2)	2 (7.7)	0.084
Hypertension (n, %)	43 (69.4)	2 (7.7)	<0.001
Arrhytmia (n, %)	14 (22.6)	0	–
Ischemic heart disease (n, %)	28 (45.2)	0	–
SBP (mmHg)*	134 (29)	140 (10)	0.383
DBP (mmHg)*	78 (19)	82 (14)	0.433
MBP (mmHg)*	97 (21)	101 (12)	0.371
Heart rate (t/min)n	75 (22)	78 (10)	0.562
Chronic kidney disease (n, %)	2 (3.2)	0	–
Acute kidney failure (n, %)	16 (25.8)	0	–
eGFR (mL/min/1.73 m^2)*	68 (28)	122 (13)	<0.001
Chronic obstructive pulmonary disease (n, %)	1 (1.6)	0	–
Medicines (n, %)	37 (59.7)	2 (7.7)	0.001
Allergy (n, %)	3 (4.8)	0	–
Glu (mmol/L)	7.7 (4.7; 36.5)	5.6 (5.2; 7.0)	<0.001
CREA (µmol/L)	93 (51; 721)	76 (48; 118)	0.01
UREA (mmol/L)	5.9 (3.8; 26.4)	4.9 (3.0; 6.6)	0.047
cTnI (ng/mL)	7.1 (0.01; 139)	–	–
CRP (mg/L)	4.0 (0.0; 175)	5.0 (2.0; 8.0)	0.984
Hb (g/dL)*	13.8 (16.2)	14 (15.2)	0.47
RDW (%)	13.8 (12.0; 19.0)	12.6 (12.0; 13.6)	0.002
HCT (%)*	41.5 (4.5)	41.8 (4.1)	0.775
PLT (×10^9/L)	205 (95; 482)	238 (177; 398)	0.034
WBC (×10^9/L)	10.8 (4.6; 21.1)	6.0 (3.5; 10.5)	0.000
K^+ (mmol/L)	4.1 (3.0; 5.8)	4.4 (4.0; 4.9)	0.000
Na^+ (mmol/L)	138 (124; 148)	141 (136; 143)	0.004
INR	1.1 (0.9; 1.7)	1 (0.9; 1.1)	0.06
APACHE II score	7.5 (1; 33)	2 (0; 4)	0.000
GRACE score*	127 (34)	–	–
KILLIP class	2 (1; 4)	–	–

Data are presented as the median (min, max) or proportion, as appropriate. * Data was normally distributed for these variables, they are presented mean ± standard deviation. STEMI – ST-elevated myocardial infarction; HP – healthy patients; SBP – systolic blood pressure; DBP – diastolic blood pressure; MBP – mean blood pressure; Glu – glucose; CREA – creatinine; eGFR – estimated glomerular filtration rate; cTnI – cardiac troponin I; CRP – C-reactive protein; Hb – hemoglobin; HCT – hematocrit; PLT – platelets; WBC – white blood cells; K^+ – potassium; Na^+ – sodium; INR – international normalized ratio; APACHE II score – Acute Physiology And Chronic Health Evaluation II; GRACE score – Global Registry of Acute Coronary Events.

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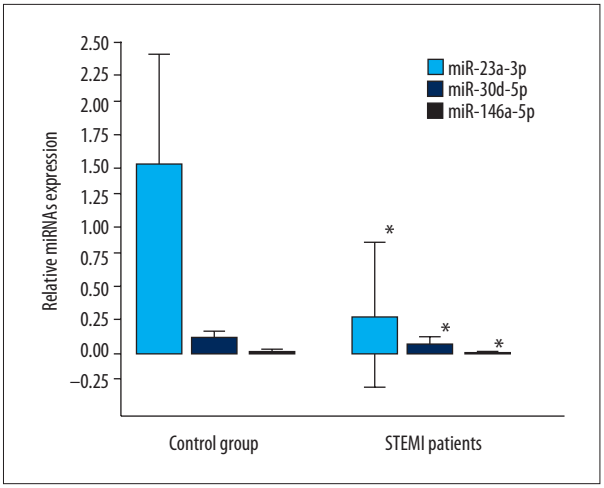

Figure 2. MiRNAs relative expression levels determined by RT-PCR-analysis. Values were normalized to cel-miR-39. Ratios calculated by $2^{-\Delta\Delta Ct}$ method. *P*<0.05 was considered statistically significant. Data presented as mean ± standard deviation. STEMI – ST-elevated myocardial infarction, miRNAs – microRNAs; RT-pPCR – reverse transcription quantitative polymerase chain reaction.

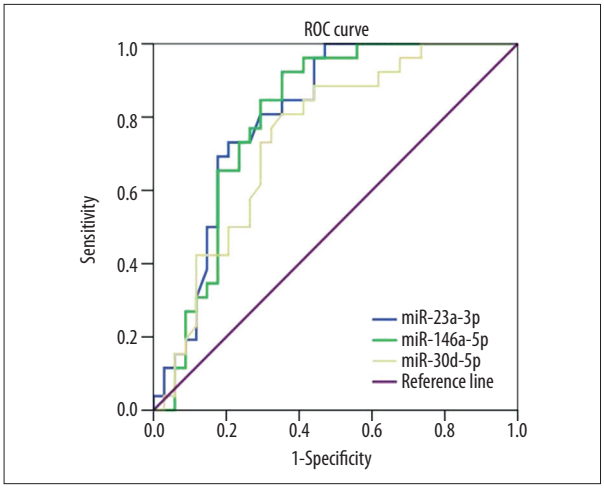

Figure 3. Diagnostic specificity and sensitivity of single miRNA testing in STEMI. MiR-23a-3p AUC 0.806 (95% CI 0.694–0.917); miR-146a-5p AUC 0.800 (95% CI 0.685–0.915); miR-30d-5p AUC 0.745 (95% CI 0.620–0.870). miRNAs – microRNAs; STEMI – ST-elevated myocardial infarction, AUC – area under the curve.

levels, in comparison to the control group individuals. Blood samples were taken 22±6 hours after the onset of symptoms.

Expression levels of miRNAs in the serum

All investigated miRNAs had differing expression levels between the 2 groups. As shown in Figure 2, the expression levels of miR-30d-5p, miR-146a-5p, and miR-23a-3p were respectively 1.581-fold, 4.048-fold, and 4.857-fold lower in patients with STEMI compared to control group patients (all *P* values were <0.001).

Diagnostic values of miR-30d-5p, miR-146a-5p, and miR-23a-3p

Receiver operating characteristic (ROC) analysis was performed on study and control group patients to assess the diagnostic value of miRNAs (Figure 3). The analysis showed that miR-23a-3p (area under the curve [AUC]=0.806) was a good predictor for STEMI. MiR-30d-5p and miR-146a-5p were fair predictors when AUC of 0.8 is a borderline between good and fair prediction. The high AUC of miR-23a-3p showed the potential diagnostic ability for patients with suspected ACS, especially during first 24 hours after hospital admission.

Correlation of expression values with mortality risk and disease severity

MiRNA expression values were correlated to mortality risk scores of GRACE and severity-of-disease scores of APACHE II. Only downregulation of miR-23a-3p was significantly negatively correlated with risk scores of GRACE and APACHE II (Figure 4). MiRNAs expression levels of the patients with high severity of disease and mortality risk were compared with expression levels of non-severe patients. Only expression levels of miR-23a-3p were 3.772-fold lower in patients with GRACE risk score >110 points (probability of in-hospital mortality >1.1%) and 3.274-fold lower in patients with APACHE II morbidity score >10 points (probability of in-hospital mortality >9%) comparing to non-severe STEMI patients; suggesting that a downregulation of circulating miR-23a-3p level may be associated with increased severity of STEMI and a higher risk of death.

Prognostic value of miR-30d-5, miR-146a-5p, and miR-23a-3p

Kaplan-Meier survival analysis was performed to investigate prognostic value. We divided patients into positive (>threshold value) and negative (<threshold value) groups. Threshold values were established from the ROC curves to be –1.829, –1.28, and –0.26 for miR-23a-3p, miR-30d-5p, and miR-146a-5p groups respectively. The sample size analysis showed that at least 12 individuals should be involved in miR-23-3p negative group and 40 patients in miR-23-3p positive group. The survival plot of the Kaplan-Meier curve predicted the miR-23a-3p-negative group (n=21) to have a lower cumulative survival rate than the positive group (n=41) at 1 month after STEMI (*P*=0.045, Figure 5), but there were no significant differences for miR-30d-5p and miR-146a-5p groups. Cox regression analysis revealed that expression levels of analyzed miRNAs were not significantly associated with negative endpoints at 1 month after the onset of STEMI.

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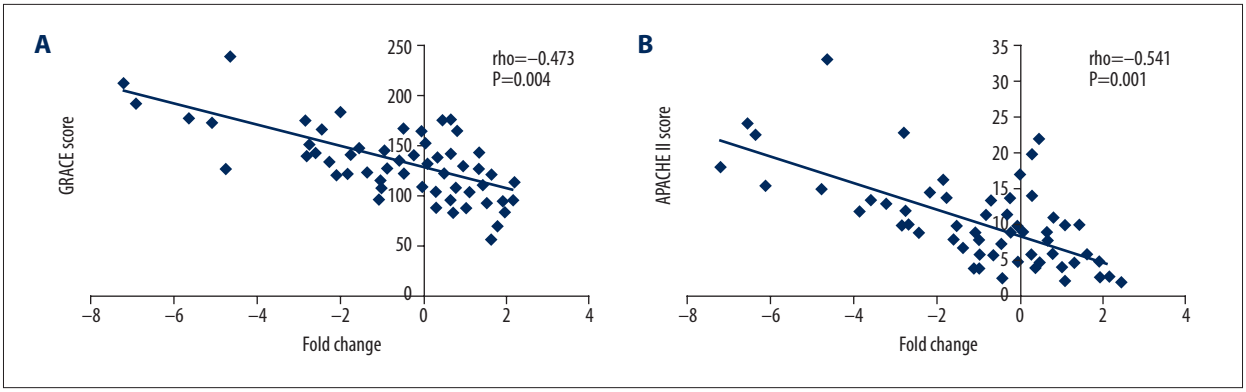

Figure 4. (**A, B**) Correlation between miR-23a-3p and risk scores of GRACE and APACHE II. Correlation trends are shown with the linear regression model including Spearman rho and the significances of the correlations. MiR-23a-3p in patients with STEMI negatively correlates with GRACE and APACHE II mortality risk scoring assessments. GRACE – Global Registry of Acute Coronary Events; APACHE II – Acute Physiology and Chronic Health Evaluation II; STEMI – ST-elevated myocardial infarction.

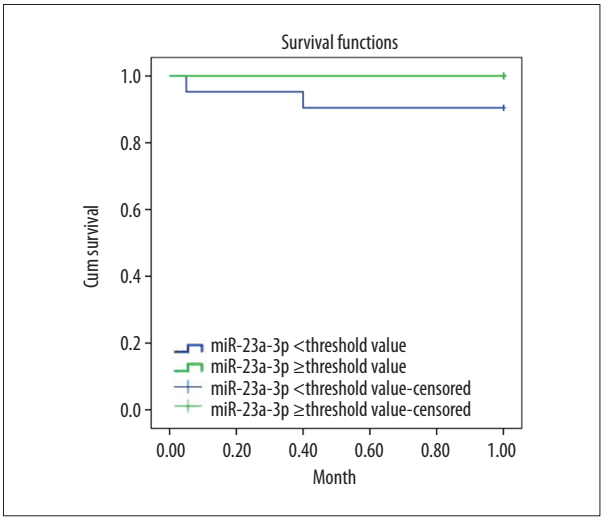

Figure 5. Kaplan-Meier survival curves of patients at 1 month (P=0.045) based on miR-23-3p levels (log-rank tests).

Expressions of miRNAs in STEMI patients with the acute kidney impairment

Estimated glomerular filtration rate (eGFR) was decreased (<60 mL/min/1.73 m^2) for 16 patients (25.8%) with STEMI. The role of kidney dysfunction in miRNAs expression levels was analyzed by comparing miRNAs between STEMI patients, with and without the acute kidney impairment, revealing no statistical significance of the miRNA expression (P>0.05).

Correlation of expression values with pro-inflammatory mediators

Afterwards, the interdependence of miRNAs to plasma levels of pro-inflammatory mediators (CRP, LEU) was analyzed, but no correlation was found.

Discussion

Serum levels of all investigated miRNAs were lower in STEMI patients compared to control group individuals. The difference in the expression level of miR-30d-5p was the lowest among the groups. Moreover, the diagnostic value of this miRNA was poorer compared to other miRNAs we investigated. MiR-146a-5p showed great STEMI diagnostic value, but did not correlate with mortality risk or severity-of-disease. This study found an impressive decreased level of miR-23a-3p in patients with STEMI compared with healthy patients during the first 24 hours after occurrence of myocardial injury. This finding is supported by previous investigations demonstrating miRNAs involvement in ACS processes [17–20]. Severe disease patients had significantly lower expression levels of miR-23a-3p compared to non-severe disease patients. This negatively correlated with the mortality risk scoring systems. MiR-23a-3p had a fair diagnostic value for STEMI.

Other studies have shown reduced levels of miR-23a in cases of heart injury [17,20]. A significant downregulation of miR-23a and miR-30a was reported by Han et al. in mice models with artificially induced atherosclerotic artery damage and in human patients with CAD in comparison to healthy controls, respectively [20]. Expression of miRNAs could be dependent on time following the onset of STEMI. The report by Wang et al. stated that plasma miR-23a expression level peaks at first 4 hours after the onset of acute MI and gradually returns to control levels over the next 3 days, exhibiting the same trend as cardiac troponin I (cTnI) [8]. Kegang et al. found that expression levels of miR-125-5p and miR-30d-5p were higher in patients with ACS at the very early stage of AMI and started to decrease after 9 hours from the onset [7]. A constant 24-hour long decline of miR-23a expression was detected after the onset of ischemia-reperfusion injury in a rat model [21]. In our study, blood samples were drawn 22±6 hours after the onset of STEMI symptoms. Keeping in mind that the response

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of miRNAs expression to treatment is rapid; this could be the reason why our results differed from some other studies where blood samples were collected at earlier phases of AMI. It should be mentioned that expression levels of downregulated miRNAs during early period of ACS gradually increase towards their baseline values after 3 months [22]. Moreover, circulating miRNAs could be differentially expressed in the serum and plasma of the same individual. Therefore, caution must be taken when comparing miRNAs data of various studies, where different obtainment sources are used to generate patterns of miRNAs expression levels in patients with AMI [21].

MI could be characterized as a local decrease of blood flow of the heart following a development of acute ischemia that leads to myocardial injury or necrosis [23]. Heart damage caused by MI depends on duration of ischemia and intensity of subsequent alterations due to reperfusion. Changes of miRNAs expression through different MI phases remain quite unexamined. In addition to necrosis, apoptosis also plays a role in tissue damage after MI, therefore, has pathological and therapeutic implications [12]. Studies show that apoptosis occurs after constant ischemia [24,25]. Therefore, it has been suggested that apoptosis is a major determinant of the infarct size [25]. Findings indicate that the role of miR-23a in cardiomyocyte apoptosis is controversial. For example, one study, using a rat model, revealed that the knockdown of miR-23a by tail injection of chemically modified antisense oligonucleotides (antagomiR) attenuated the ischemic/reperfusion injury and cardiomyocyte apoptosis, indicating that inhibition of miR-23a may have a protective effect [17]. Moreover, Long et al. showed that inhibition of miR-23a attenuated the downregulation of the antioxidant enzyme manganese superoxide dismutase (MnSOD) and cardiomyocyte apoptosis [26]. It could be hypothesized that the downregulation of miR-23a expression during the early phase of MI may be as a natural protection mechanism. On the other hand, another rat model study showed that the apoptosis rate of myocardial cell increased, while miR-23a expression was downregulated, indicating the protective role of miR-23a in myocardial cell apoptosis [21]. What is more, miR-23a could have an influence on the activity of NF-κB, as well as on NF-κB target genes that encode pro-inflammatory mediators, for example IL-6 and TNF-α [27].

Using a tumor necrosis factor (TNF)-α-induced bone marrow mesenchymal stem cells (BM)-MSC injury model *in vitro* and a rat MI model *in vivo*, Mao et al. showed that miR-23a was involved in TNF-α-induced BM-MSC apoptosis through regulating caspase-7 and that the injection of BM-MSCs over-expressing miR-23a could improve left ventricular (LV) function and reduce infarct size in the rat MI model [28].

In our study, ROC analysis presented the high sensitivity and specificity of analyzed miRNAs, especially miR-23-3p (AUC 0.806). This may provide diagnostic information for patients with STEMI in the first 24 hours after the onset of symptoms. Surprisingly, the diagnostic value of miR-23a-3p and miR-146-5p were shown in one study to be only slightly lower than the high sensitive of cTnI (AUC 0.88; 95% CI 0.86–0.94) [29], which is currently the preferred biomarker for the diagnosis of AMI. So far, little data is known about prognostic value of miR-23a-3p, miR-30d-5p, and miR-146-5p in patients with STEMI. Jia et al. revealed no correlation of plasma miR-30d-5p expression and adverse cardiac outcomes at 1, 6, and 12 months after the onset of AMI [7], Junjie et al. found that reduced serum miR-30d expression in patients with acute heart failure along with lower hemoglobin and serum sodium levels, may predict an increased morbidity risk over a 1-year period [30]. We revealed that despite the diagnostic value, analyzed miRNAs were not good predictors of cardiovascular events and endpoints during a 1-month follow-up period for STEMI patients.

Alterations of specific miRNA expressions have been linked to severity of cardiovascular diseases in previous reports. Little information exists about the impact of altered miR-23a-3p, miR-30d-5p, and miR-146a-5p expression levels on the severity of STEMI. We found a significant correlation between reduced serum level of miR-23a-3p and increased severity of STEMI along with a higher risk of morbidity. Similar tendencies could be seen in other studies: a downregulation of plasma miR-155 and miR-126-5p had a negative correlation with SYNTAX severity score and reduced miR-126-5p level was significantly associated with multi-vessel disease in CAD patients [31,32]. Liu et al. found that upregulation of plasma miR-134, miR-3135b, and miR-2861 may be associated with the level of coronary artery calcification and expression levels of these miRNA were also significantly altered in patients with obstructive coronary artery disease, verified via angiography [33].

We found that eGFR was decreased for 25.8% of patients with STEMI. Circulating miRNA may be eliminated through kidneys, as Gidlof et al. showed that cardiospecific miR-1 and miR133a can be identified in urine 12 hours after the onset of STEMI [34]. Significantly lower expression levels of miR-23-3p were detected in sepsis-induced acute kidney injury patients [35]. This urged us to speculate whether the miRNAs investigated in our study were affected by renal function: however, the levels of miR-30d-5p, miR-23a-3p, and miR-146-5p did not significantly differ compared to patients with and without a decrease of eGFR. Finally, little information exists about renal clearance of miRNAs and further studies are needed in this field.

Conclusions

Our results indicated that miR-30d-5p, miR-23a-3p, and miR-146a-5 were downregulated in patients with STEMI compared

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to control group individuals. The high AUC of miR-23a-3p represented the potential diagnostic ability for patients with suspected STEMI. The evaluation of miR-23a-3p expression levels in serum could be useful to assess the severity of STEMI and as a potential diagnostic biomarker of this condition. In addition, miR-23a-3p may provide limited short-term prognostic value for STEMI patients.

Study limitations

There were some limitations to our study. This was a single-institution study and tour study had a relatively small sample size, therefore, results need to be interpreted with caution and confirmed by larger multicenter studies. No dynamics of miRNA expression during the early stages of ACS could be evaluated. The results may have been affected by renal insufficiency and the use of medications prior to administration at the CICU and during the hospitalization period before the collection of blood samples.

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