SHORT COMMUNICATION

Increased serum microRNA-21 levels reflect cardiac necrosis rather than plaque vulnerability in patients with acute coronary syndrome: a pilot study

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Introduction Stable atherosclerotic plaque manifests clinically as stable coronary artery disease (SCAD), whereas unstable or vulnerable plaque—as acute coronary syndrome (ACS), which is further divided into unstable angina (UA) and myocardial infarction with and without ST-segment elevation (STEMI and NSTEMI, respectively). Rupture of a vulnerable plaque leads to thrombus generation, coronary artery occlusion, and subsequent cardiac muscle necrosis with dynamically increased serum troponin I levels, which is clinically defined as type 1 myocardial infarction.[1] The process of plaque destabilization and cardiac necrosis is associated with extracellular matrix alterations, in which metalloproteinases, such as matrix metalloproteinase 9 (MMP-9), play a pivotal role.[2] MMP-9 is upregulated epigenetically by microRNA-21 (miRNA-21), which downregulates the expression of MMP-9 inhibitors—reversion-inducing-cysteine-rich protein with Kazal motifs and tissue inhibitor of metalloproteinase 3.[3,4] We aimed to investigate whether miRNA-21 expression is enhanced in patients with ACS (with and without cardiac necrosis), as compared with patients with SCAD and healthy controls.

Methods **Study design and inclusion criteria** In this pilot study, participants were categorized into the following groups: STEMI, NSTEMI, UA, SCAD, and healthy controls, according to the corresponding European Society of Cardiology Guidelines (SCAD, 2013; UA/NSTEMI, 2015; STEMI, 2017). Moreover, in all groups (except for controls), the presence of coronary atherosclerotic plaque was confirmed by coronary angiography, and plaque burden was assessed by the Gensini score.[5] Patients from STEMI and NSTEMI groups presented with type 1 myocardial infarction.

Exclusion criteria Patients with at least one of the following criteria were excluded from the study: presence of malignant neoplastic, active autoimmune, or rheumatic disease; surgery or invasive intervention in the previous 6 months; estimated glomerular filtration rate (calculated using the Modification in Diet of Renal Disease formula) of less than 45 ml/min/1.73 m^2; diabetes mellitus; and active infection in the previous 3 months. Additional exclusion criteria for the SCAD group were previous ACS and percutaneous coronary intervention (PCI) with stent implantation.

Study population A total of 60 patients and volunteers were initially recruited to the study. All persons provided written informed consent for participation. Finally, 43 individuals were enrolled: 1) ACS group (n = 25), including 8 patients with STEMI, 9 patients with NSTEMI, and 8 patients with UA; 2) SCAD group (n = 8); and the control group (n = 8).

Blood samples Blood samples for miRNA analysis and blood investigations (hemoglobin, lipid panel, creatinine) were taken within the first 24 hours of admission before PCI. Blood samples in EDTA tubes were centrifuged at 1200 × *g* for 10 minutes to separate plasma from blood cells. Then, the plasma was aliquoted into microcentrifuge tubes, frozen on dry ice, and immediately stored at –80°C. Blood troponin I levels in patients with ACS were measured on admission and after at least 8 hours from admission, after PCI.

Detection of hsa-miR-21-5p The miRNA particles measured in the plasma samples were as follows: cel-miR-39-3p (a "spike-in" control), hsa-miR-93-3p, hsa-miR-191-5p (control miRNAs), and hsa-miR-21-5p (study miRNA). Isolation of miRNA and quantitative reverse transcription polymerase chain reaction were performed with the use of TaqMan Advanced miRNA Assays (Thermo Fisher Scientific, Waltham, Massachusetts, United States), according to the manufacturer's protocol. The obtained cycle-of-threshold (Ct) values for study hsa-mir-21-5p, as well as hsa-mir-191-5p and hsa-mir-93-3p as endogenous controls, were normalized by the Ct of cel--miR-39-3p and were used to calculate relative expression using the $2^{-\Delta\Delta Ct}$ method.

Ethical approval The study was approved by the local Research Ethics Committee of the Medical University of Warsaw, Warsaw, Poland (KB/55/2016 and KB/26/A/2017).

Statistical analysis All analyses were performed using STATISTICA 13 (StatSoft Inc., Tulsa, Oklahoma, United States). Results were presented as mean and SD, standard error of the mean (SEM), or median values and interquartile ranges (IQRs) according to the normality of distribution (Shapiro–Wilk test). The *t* test, Cochrane–Cox test (for nonequal variances), and the analysis of variance F test were used for normally distributed continuous variables. The Kruskal–Wallis test, Mann–Whitney test, and the Spearman rank correlation coefficient (r_s) were used for nonnormally distributed continuous variables. Categorical variables were compared using the χ^2 test. *P* values of less than 0.05 were considered significant.

Results

Baseline characteristics The groups were compared in terms of demographic parameters (age and sex), body mass index (BMI), history of hypertension, active smoking, and serum low-density lipoprotein cholesterol (LDL-C) levels, which constitute the major risk factors for atherosclerosis. In the first analysis (ACS vs SCAD vs controls), the SCAD group showed lower LDL-C levels than both the ACS and control groups as well as less frequent active smoking than the ACS group. The control group was younger and included mostly women, without active smokers. The second analysis (STEMI vs NSTEMI vs UA vs SCAD vs controls) revealed significant differences only between UA and STEMI groups in terms of active smoking. No differences in BMI and hemoglobin levels were shown in any of the analyses. The mean (SD) Gensini score was 59.1 (27.3) for STEMI, 86.8 (39.3) for NSTEMI, 66.3 (31.0) for UA, and 65.9 (54.5) for SCAD, without significant differences between groups. In the entire study population, 3 patients presented with carotid stenosis (2 from the NSTEMI and 1 from the UA group), and 1 patient from the UA group had peripheral artery disease. Among patients with myocardial infarction, 2 patients with STEMI (25%) and 6 patients with NSTEMI (66.7%) presented with the onset of chest pain more than 12 hours before admission.

Differences in relative hsa-miR-21-5p expression between 3 study groups The hsa-miR-21-5p expression in the ACS group showed nonnormal distribution. Patients from the ACS group had similar relative hsa-miR-21-5p expression as patients from the SCAD group and controls (median [IQR], 2.47 [1.46–4.68], 1.90 [1.02–3.25], and 1.81 [0.99–2.60], respectively; $P = 0.15$).

Differences in relative hsa-miR-21-5p expression between 5 study groups and correlation with troponin I The reciprocal analysis revealed that patients with STEMI had a higher relative hsa-miR-21-5p expression than patients with UA (mean [SEM], 5.96 [1.43] vs 2.31 [0.46], $P = 0.04$) and SCAD (mean [SEM], 5.96 [1.43] vs 2.32 [0.51], $P = 0.04$). The relative expression level did not differ between the UA and SCAD groups (mean [SEM], 2.31 [0.46] vs 2.32 [0.51], $P = 0.99$). The STEMI group, unlike the UA group, differed significantly in relative hsa-miR-21-5p expression levels from the control group. There was no difference between the SCAD and control group (mean [SEM], 2.32 [0.51] vs 1.79 [0.35], $P = 0.43$) (FIGURE 1). The hsa-miR-21-5p expression in the NSTEMI group showed nonnormal distribution (median [IQR], 1.49 [1.44–3.49]), and no significant differences were observed between NSTEMI and other groups. Moreover, patients with STEMI and NSTEMI showed a fair, although nonsignificant, correlation between serum troponin I levels after PCI and relative hsa--miR-21-5p expression ($r_s = 0.53$), with a negligible correlation between baseline troponin I levels and hsa-miR-21-5p expression.

Discussion

Darabi et al[6] showed that the relative expression level of hsa-miR-21-5p is elevated in patients with ACS, as compared with those with SCAD, and correlates positively with serum MMP-9 and high-sensitivity C-reactive protein levels.[6] However, our results demonstrated

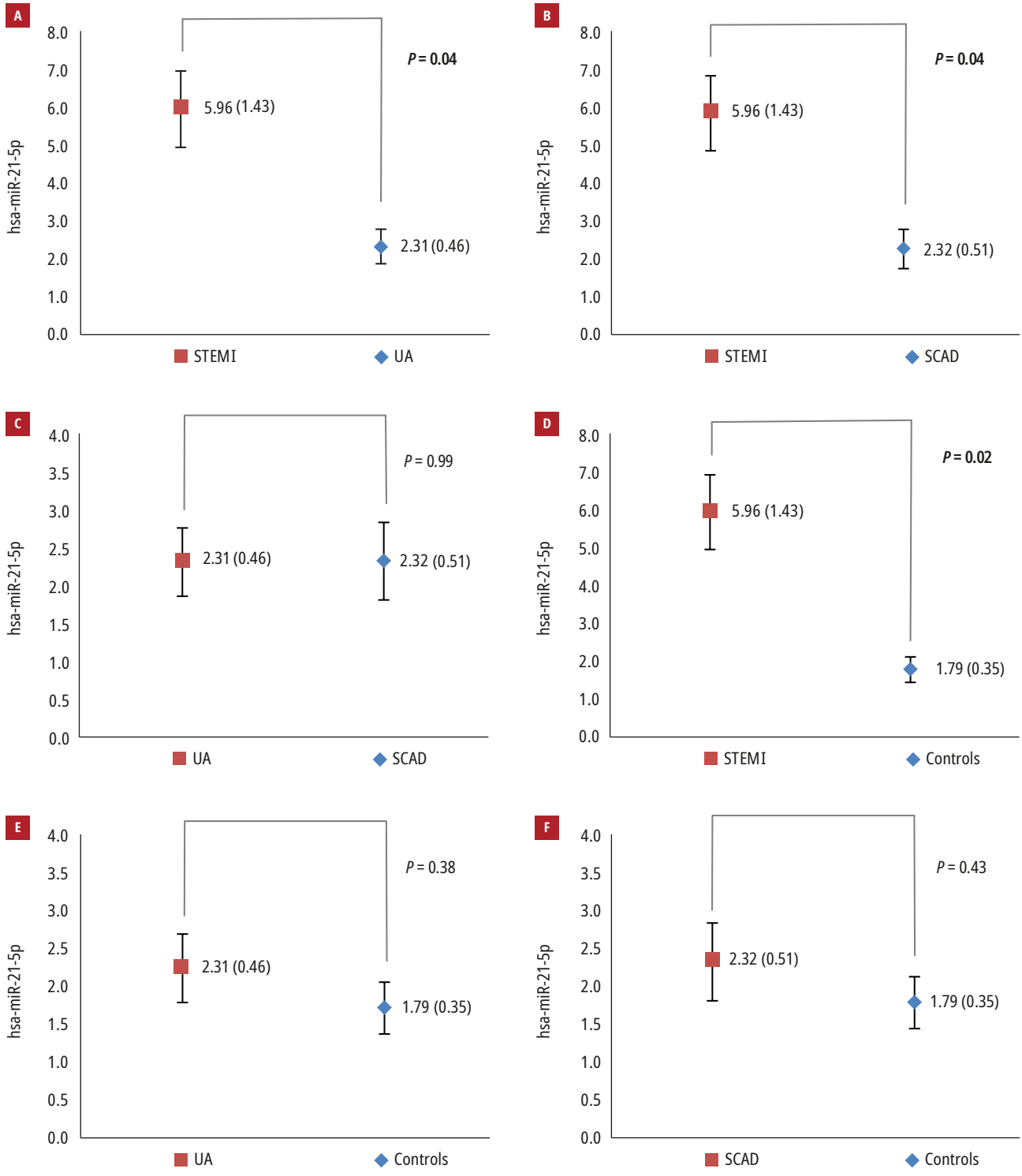

FIGURE 1 Correlations between study groups in terms of hsa-miR-21-5p relative expression level: **A** – patients with ST-segment elevation myocardial infarction (STEMI) and unstable angina (UA); **B** – STEMI and stable coronary artery disease (SCAD); **C** – UA and SCAD; **D** – STEMI and controls; **E** – UA and controls; and **F** – SCAD and controls. Data are presented as mean (standard error of the mean). A *P* value of less than 0.05 was considered significant. Significant values are marked in bold.

increased serum hsa-miR-21-5p levels in patients with STEMI, as compared with those with UA and SCAD, without differences between UA and SCAD groups.

There is some evidence that hsa-miR-21-5p is involved in extracellular matrix regulation during myocardial necrosis. A study on C57BL/6 mice confirmed increased murine miR-21 expression in the infarcted zone of mouse hearts.[7] In a human study, elevated serum hsa-miR-21-5p expression, measured 5 days after STEMI in 198 patients, was significantly correlated with the probability of left ventricular remodeling.[8] However, another study demonstrated a negative correlation between hsa-miR-21-5p and both LDL-C and total cholesterol levels in patients with

non–ST-segment elevation ACS, which suggests that this miRNA might participate in the regulation of lipid homeostasis in this population.[9]

In conclusion, diverse physiologic and pathophysiologic processes within the heart (eg, differentiation of cardiomyoblasts to cardiomyocytes, fibrosis, hypertrophy) depend on miRNA particles, which might function as "molecular switches" promoting the pathways that are either beneficial or detrimental.[10] The overexpression of certain miRNA particles might be associated with particular clinical situations. For instance, a study in 43 patients revealed that serum hsa-miR-124 expression levels distinguished patients with an occluded infarct-related coronary artery from those with patent arteries (area under the receiver operating characteristic curve, 0.787).[11]

Considering the above data, the analysis of miRNA particles might be a promising diagnostic and therapeutic method. Therefore, these particles should be investigated as integral elements of complex molecular pathways in cardiac pathobiology.

ARTICLE INFORMATION

CONFLICT OF INTEREST None declared.

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