ORIGINAL ARTICLE

Association between microRNA-21 concentration and lipid profile in patients with acute coronary syndrome without persistent ST-segment elevation

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KEY WORDS

acute coronary syndrome, atherosclerosis, cholesterol, lipids, microRNA-21

ABSTRACT

INTRODUCTION MicroRNA (miRNAs) are noncoding RNAs involved in the regulation of gene expression. Certain miRNAs, especially miRNA-21 (miR-21), may be involved in lipid metabolism.

OBJECTIVES The aim of the study was to evaluate the association between plasma free circulating miR-21 levels and lipid fractions: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, and triglycerides (TG), as well as their atherogenic profile expressed as the ratio of individual lipid fractions (TC:LDL-C, TC:non-HDL-C, TG:HDL-C, and HDL-C:LDL-C) in patients with acute coronary syndromes without persistent ST-segment elevation (NSTE ACS).

PATIENTS AND METHODS The study group consisted of 34 patients diagnosed with NSTE ACS on admission to the emergency department. Plasma miRNA levels were determined by real-time polymerase chain reaction and the $_{\Delta\Delta}$Ct method. Serum lipid fractions were assessed after a minimum of 12-hour fasting during the first day of hospitalization.

RESULTS MiR-21 levels showed a significant inverse correlation with TC ($r = –0.5$; $P = 0.002$), LDL-C ($r = –0.5$; $P = 0.001$), and non-HDL-C ($r = –0.6$; $P < 0.001$) levels. Moreover, they were inversely correlated with TC:HDL-C$_{ratio}$ ($r = –0.6$; $P < 0.001$), LDL-C:HDL-C$_{ratio}$ ($r = –0.6$; $P < 0.001$), TG:HDL-C$_{ratio}$ ($r = –0.4$; $P = 0.037$), and TC:non-HDL$_{ratio}$ ($r = 0.6$; $P < 0.001$). In a multivariate analysis, miR-21 levels ($\beta = –0.41$; $P = 0.018$) and the need for revascularization ($\beta = 0.35$; $P = 0.027$) were independent predictors of non-HDL-C levels.

CONCLUSIONS Free circulating miR-21 levels inversely correlate with TC, LDL-C, and non-HDL-C and are an independent predictor of non-HDL-C levels in patients with NSTE ACS. Thus, the overexpression of miR-21 is associated with a less atherogenic lipid profile.

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INTRODUCTION Cardiovascular disease (CVD), and particularly coronary artery disease (CAD), remains the leading cause of premature mortality in Europe.[1] The causes of CVD are multifactorial, including some nonmodifiable risk factors, such as age and sex, and also some modifiable factors, such as tobacco smoking, dietary habits, low physical activity, high blood pressure, type 2 diabetes, and dyslipidemia. Lipid abnormalities are widely recognized in expert documents[2] as the crucial correctable risk factor for the development of atherosclerosis, and novel therapeutic approaches are being investigated.

MicroRNAs (miRNAs) are small, noncoding, and highly conservative regulatory RNAs, involved in the posttranscriptional regulation of gene expression. It is thought that over 30% of all human messenger RNAs can be regulated by specific miRNAs,[3] free circulating and vesicle-incorporated miRNAs (eg, exosomal miRNA).[4] Recently, advances in miRNAs research have shed new light on their potential role in the control of

numerous metabolic pathways involved in lipid metabolism.[5] They seem to be the key regulator of molecular processes that govern different lipid metabolic phenotypes. A single miRNA usually targets numerous genes, and 1 gene may be targeted by a group of miRNAs, thereby providing a complex mechanism to regulate the entire networks of genes.[3] This complexity of miRNA's metabolic action reflects the complex disease etiology. Despite intense research efforts aimed at the understanding of molecular mechanisms regulating cholesterol metabolism, the contribution of miRNAs to lipid homeostasis or dysregulation remains poorly understood. Among many miRNA candidates, miRNA-21 (miR-21) is of special interest regarding lipid homeostasis because it is involved in numerous processes including atherosclerosis progression,[6] adipogenesis,[7] obesity,[8] cardiovascular disease,[9] as well as cancer, inflammation, and organ development.[9]

We sought to evaluate the association between plasma free circulating miR-21 concentrations and serum lipid fractions: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, and triglycerides (TG), as well as the serum atherogenic profile (TC:LDL-C_{ratio}, TG:HDL-$C_{ratio,}$ TC:non-HDL-C_{ratio}, and HDL-C:LDL-C_{ratio}) in patients presenting with acute coronary syndromes without persistent ST-segment elevation (NSTE ACS).

PATIENTS AND METHODS

Study population The study group consisted of 34 patients admitted to the emergency department with chest pain or its equivalent (less than 24 hours from the onset of symptoms), without persistent ST-segment elevation on electrocardiography or new-onset left bundle branch block.

Informed consent was obtained from all patients, and the study protocol was approved by the Ethics Committee of the Medical University of Lodz in Poland (permission No.: RNN/9/13/KE).

Plasma miRNAs: laboratory procedures

Plasma collection and storage Blood samples were collected from all patients after hospital admission within 3 hours from admission before patient's transfer to a catheter laboratory. In every patient, 5 ml of blood were collected in standard EDTA-coated tubes. Plasma was isolated by centrifugation at 1000 × *g* for 10 minutes at 4°C, after which it was transferred into RNase-free tubes and stored at –80°C until further processing.

Isolation of miRNAs Isolation of extracellular miRNAs from liquid samples was performed using Tri-Reagent LS (Sigma-Aldrich, St. Louis, Missouri, United States) together with the miRNeasy kit (Qiagen, Valencia, CA, United States). Briefly, 1 ml of Tri-Reagent LS was added to 0.2 ml of blood plasma, mixed by vigorous shaking for 10 seconds and then incubated for 10 minutes at room temperature to ensure complete dissociation of nucleoprotein complexes. Then 5 pg of synthetic miRNA-39 from Caenorhabditis elegans (cel-miRNA-39) were added as a spike-in control for purification efficiency. After supplying 200 µl of chloroform, the mixture was vigorously shaken for 15 seconds and allowed to stand for 5 minutes at room temperature. Following the centrifugation at 14 000 × *g* for 20 minutes at 40°C, total RNA was precipitated from the upper (aqueous) phase by adding 1.5 volumes of 100% ethanol. Purification of extracted total RNA was performed with miRNeasy columns (Qiagen) according to the manufacturer's instructions. From the Qiagen columns, RNA was eluted in 40 µl of RNase-free water.

miRNA quantification Reverse transcription was conducted using the TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, United States) according to the manufacturer's instructions. Quantification of miRNA was done using standard TaqMan® MicroRNA assays (Applied Biosystems): miR-21 and cel-miR-39 as control. The reactions were incubated in a 96-well plate at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. All reactions were run in duplicate.

Real time polymerase chain reaction analysis TaqMan polymerase chain reaction (PCR) assays were performed using the 7900HT Fast Real-Time PCR System (Applied Biosystems) and analyzed using the Sequence Detection System 2.3 software (Applied Biosystems). Fold induction values were calculated according to the equation $2^{-\Delta\Delta Ct}$, where $_{\Delta}Ct$ represents the differences in cycle threshold numbers between the target gene and spike-in control, and $_{\Delta\Delta}Ct$ represents the relative change in these differences between the study and control groups.

Lipid analyses Venous blood for each patient was drawn after 12-hour overnight fasting, within the first day of hospital stay. The blood was drawn from an antecubital vein and immediately centrifuged for 15 minutes at 3000 × *g* at 4°C. The serum samples were stored at –20°C until the biochemical analysis. The serum concentrations of TG, TC, LDL-C, and HDL-C were assessed by enzymatic methods using a fully automated analyzer, Cobas 6000 TM (Cobas c501 module; Roche Diagnostics International Ltd, Rotkreuz, Switzerland) with appropriate test kits.

Statistical analysis Data were presented as percentages for categorical variables and as mean with standard deviation or median with interquartile range depending on their distribution. The Shapiro–Wilk test was used for testing the normality of distribution. The *t* test for independent variables or the Mann–Whitney test was applied to test the intergroup differences depending on the variable distribution. The analysis of categorical variables was performed with the χ^2

TABLE 1 Characteristics of study patients

Variable	Study patients (n = 34)
male sex	24 (71)
age, y	66.5 ±10.9
body mass index, kg/m^2	27.9 ±4.0
cardiovascular risk factors	
diabetes mellitus	13 (38)
hyperlipidemia	31 (91)
hypertension	29 (85)
smoking	13 (38)
positive family history[a]	3 (9)
stroke	6 (18)
atrial fibrillation	5 (15)
prior myocardial infarction	10 (29)
prior percutaneous coronary intervention	12 (35)
prior coronary artery bypass grafting	2 (6)
left ventricular ejection fraction, %	46.9 ±10.7
laboratory values on admission	
creatinine, mg/dl	0.94 ±0.23
eGFR, ml/min/1.73 m^2	84.7 ±21.7
NT-proBNP, pg/ml	661 (200–1644)
C-reactive protein, mg/l	2.6 (1.1–12.2)
TC, mg/dl	185 (154–209)
LDL-C, mg/dl	103 (81–141)
HDL-C, mg/dl	50.6 ±15.3
non-HDL-C, mg/dl	139.3 ±53.4
TG, mg/dl	122 (94–160)
TC:HDL-C$_{ratio}$	4.0 ±1.4
TC:non-HDL-C$_{ratio}$	1.4 (1.3–1.5)
LDL-C:HDL-C$_{ratio}$	2.4 ±1.2
TG:HDL-C$_{ratio}$	2.8 (1.5–3.3)

Data are presented as mean ± standard deviation, median (interquartile range), or number (percentage) of patients.

a defined as evidence of coronary artery disease in a sibling or parent before 65 years of age in women or before 55 years of age in men

Conversion factors to SI units are as follows: for creatinine, 88.4; eGFR, 0.0167; NT-proBNP, 1; C-reactive protein, 9.524; TC, 0.0259; LDL-C, 0.0259; non-HDL-C, 0.0259; TG, 0.0114.

Abbreviations: eGFR, estimated glomerular filtration rate (calculated using the Cockroft–Gault formula); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non NT-proBNP, N-terminal pro-B-type natriuretic peptide; TC, total cholesterol; TG, triglycerides

test, the χ^2 test with the Yates's correction, or the Fisher exact probability test. Correlations among continuous variables were assessed with the use of the Spearman rank correlation coefficient. A multivariate linear regression analysis was performed to determine independent factors affecting the main treatment goals in lipid parameters: LDL-C levels and non-HDL-C level. A P value of less than 0.05 was considered statistically significant. The statistical analysis was performed using the MedCalc software version 12.2.1.0 (MedCalc Software, Ostend, Belgium) and Statistica version 10.0 (StatSoft Poland, Kraków, Poland).

RESULTS

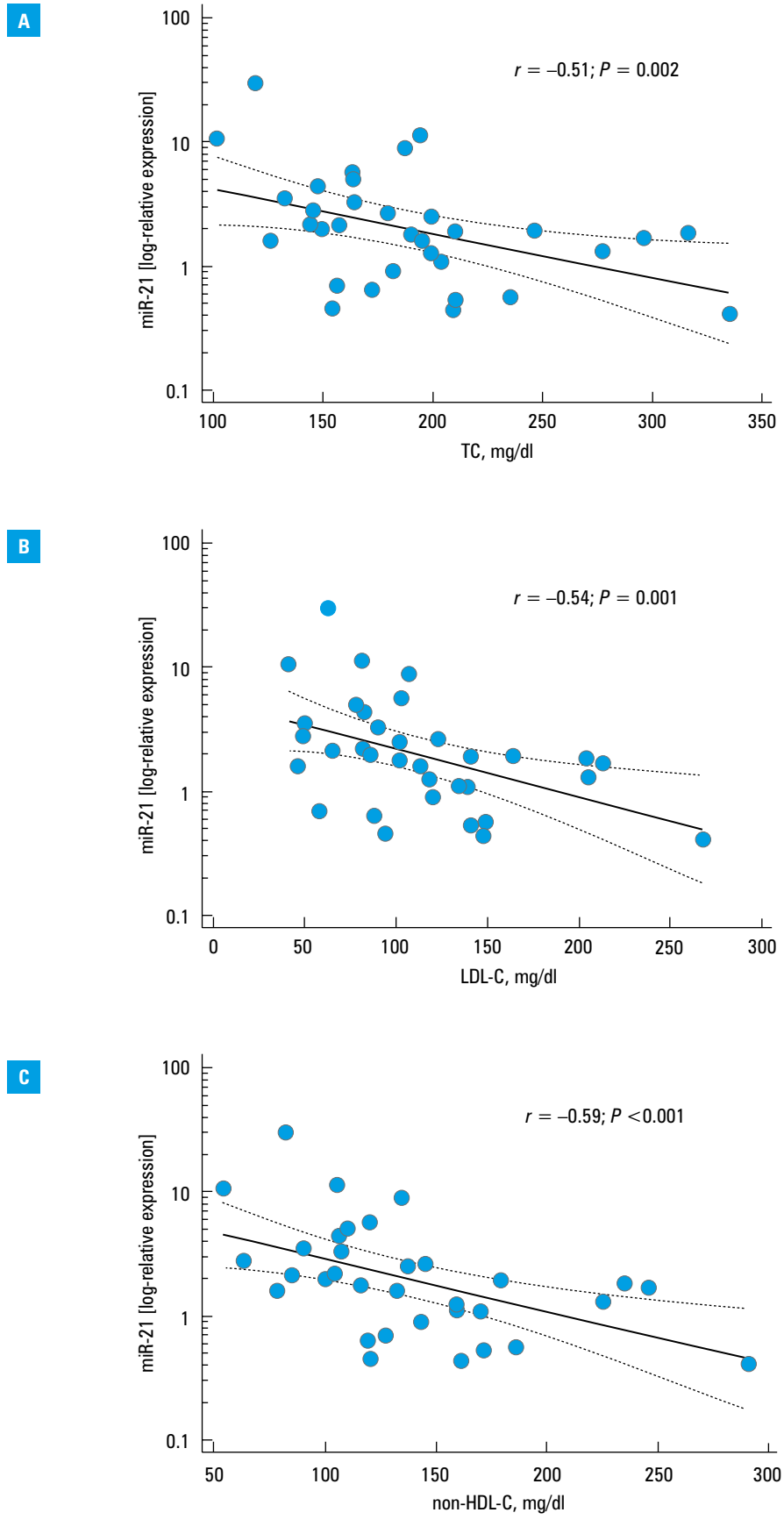
Characteristics of the study group The study patients were mostly male (71%); the mean age was 67 ±11 years. The majority of patients were diagnosed with hyperlipidemia (91%; on admission, all of them were treated with statins) and arterial hypertension (85%). More than every third patient (38%) was a cigarette smoker (active or former). In-hospital clinical presentation was consistent with the diagnosis of NSTE ASC in all patients. In 20 patients (59%), the final diagnosis was myocardial infarction without persistent ST-segment elevation (NSTEMI; positive cardiac troponins) and in 14 (41%)—unstable angina (negative cardiac troponins), according to the current guidelines.[10] The majority of the patients had coronary angiography during the hospital stay (n = 33, 97%). During the index hospitalization, percutaneous coronary intervention was performed in 26 patients (76%), predominantly in the NSTEMI group (95% vs 50%; P = 0.004). The main clinical characteristics of the study group are presented in TABLE 1.

MiRNA-21 expression We observed a considerable variation in the relative expression level of miR-21 between individuals (mean, 3.52 ±5.44; median, 1.89 [range, 0.42–30.02]). Due to its skewed (coefficient of skewness = 3.88, P <0.001) and non-normal distribution (Shapiro–Wilk test for normal distribution: P <0.001), a logarithmic transformation was used for the subsequent correlation analysis.

Correlations of miRNA-21 levels with lipid profile The levels of free circulating miR-21 proved to be significantly inversely correlated with TC (r = –0.51; P = 0.002), LDL-C (r = –0.54; P = 0.001), and non-HDL-C (r = –0.59; P <0.001) levels, as well as with secondary measures of atherogenicity: TC:HDL-C$_{ratio}$ (r = –0.60; P <0.001), LDL-C:HDL-C$_{ratio}$ (r = –0.59; P <0.001), TG:HDL-C$_{ratio}$ (r = –0.36; P = 0.037) and TC:non-HDL-C$_{ratio}$ (r = 0.60; P <0.001). There was no significant correlation between HDL-C or TG and miR-21 levels (r = 0.26, P = 0.144, and r = –0.24, P = 0.173, respectively). FIGURE 1 shows the correlations of miR-21 levels with individual lipid fractions and ratios.

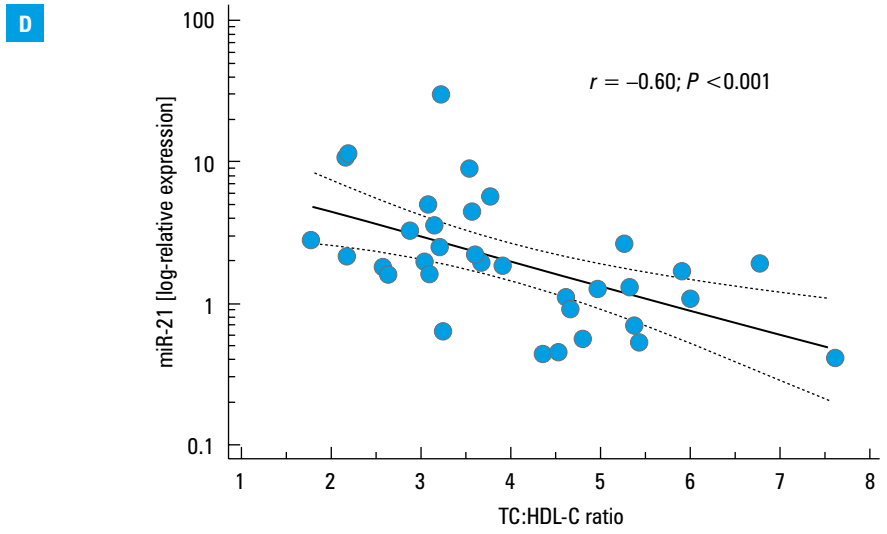
In a secondary analysis, we looked at the principal clinical data in both subgroups, based on the median value split (in the entire study group, the median value of relative miR-21 expression was 1.89). The patients with miR-21 levels higher than the median value had significantly lower levels of TC, LDL-C, non-HDL-C and less atherogenic serum lipid profile judged from a lower TC:HDL-C$_{ratio}$ and LDL-C:HDL-C$_{ratio}$ and higher TC:non-HDL-C$_{ratio}$ (TABLE 2) compared with patients with lower miR-21 levels. Except for a more frequent need for percutaneous coronary angioplasty in patients with miR-21 levels lower than the median value (94% vs 59%; P = 0.039), no other clinical or laboratory parameters were different between the subgroups (TABLE 2).

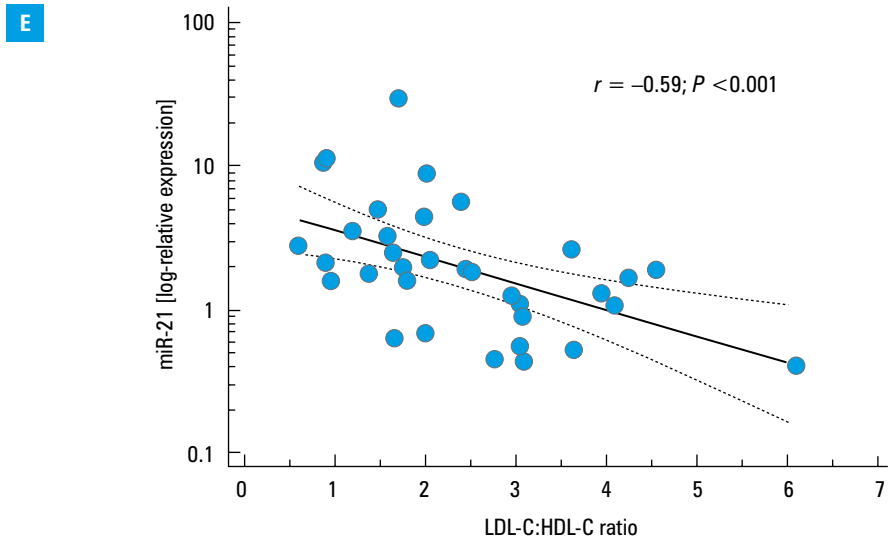
FIGURE 1
Correlations of miRNA-21 (miR-21) levels with individual lipid fractions and ratios; **A** – correlation of miR-21 with total cholesterol (TC); **B** – correlation of miR-21 with low-density lipoprotein cholesterol (LDL-C); **C** – correlation of miR-21 with non-high--density lipoprotein cholesterol (non-HDL-C); **D** – correlation of miR-21 with TC:HDL-C_{ratio}; **E** – correlation of miR-21 with LDL-C:HDL-C_{ratio}; **F** – correlation of miR-21 with TC:non-HDL-C_{ratio}

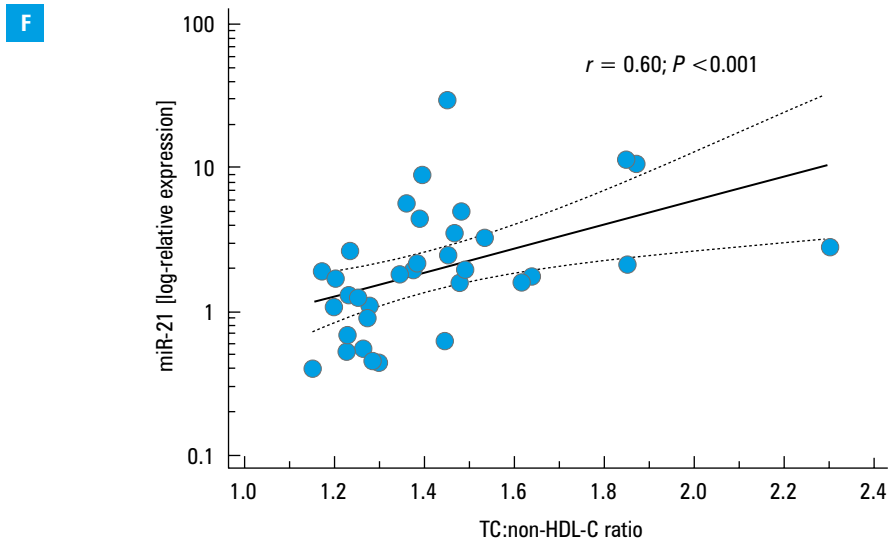


In the multivariate regression analysis, we sought to find the independent predictors affecting the main treatment goals of lipid parameters: LDL-C and non-HDL-C levels. MiR-21 levels significantly contributed only to non-HDL-C levels together with the need for revascularization, even after adjustment for age, time of ischemia, enzymatic peri-infarct injury (high-sensitivity cardiac troponin levels on admission), diabetes mellitus, hypertension, and left ventricular systolic function (TABLE 3).

The calculated post-hoc study power (with α = 0.05) for the observed difference in LDL-C levels (134 mg vs 82 mg/dl; P = 0.007) was 81.4%,







and for the mean difference in non-HDL-C levels (167 mg vs 112 mg/dl; $P = 0.001$), it was 93.6%.

Free circulating miRNA-21 levels are independent of myocardial necrosis, renal function, inflammatory parameters, body mass index, and diabetes There was no significant difference in the measured levels of free circulating miR-21 between patients with NSTEMI and those with unstable angina (median with the interquartile range of the relative expression: 1.6 [1.0–3.2] vs 2.0 [1.1–2.8]; $P = 0.653$). Moreover, we observed no significant correlation

TABLE 2 Characteristics of patients depending on the median miRNA-21 level

Variable	miRNA-21 <median (n = 17)	miRNA-21 ≥median (n = 17)	*P* value
male	12 (71)	12 (71)	1.000
age, y	62.9 ±12.6	68.19 ±8.3	0.228
body mass index, kg/m^2	28.19 ±3.2	27.89 ±4.8	0.959
cardiovascular risk factors			
diabetes mellitus	7 (41)	6 (35)	0.724
hyperlipidemia	16 (94)	15 (88)	1.000
hypertension	14 (82)	15 (88)	1.000
smoking	7 (41)	6 (35)	0.724
positive family history[a]	0 (0)	3 (18)	0.227
stroke	3 (18)	3 (18)	1.000
atrial fibrillation	1 (6)	4 (24)	0.335
prior myocardial infarction	4 (24)	6 (35)	0.452
prior percutaneous coronary intervention	6 (35)	6 (35)	1.000
prior coronary artery bypass grafting	1 (6)	1 (6)	1.000
left ventricular ejection fraction, %	45.8 ±10.5	47.9 ±11.1	0.513
NSTEMI	11 (65)	9 (53)	0.486
coronary angiographic characteristics			
3-vessel disease	2 (12)	3 (18)	1.000
percutaneous coronary intervention	16 (94)	10 (59)	0.039
laboratory values on admission			
creatinine, mg/dl	0.879 ±0.22	1.009 ±0.23	0.066
eGFR, ml/min/1.73 m^2	91.69 ±19.2	77.39 ±22.3	0.050
NT-proBNP, pg/ml	211 (171–3442)	720 (435–1510)	0.805
C-reactive protein, mg/l	3.0 (1.2–15.4)	2.5 (1.1–4.0)	0.476
TC, mg/dl	203 (182–235)	163 (145–187)	0.006
LDL-C, mg/dl	134 (102–149)	82 (65–103)	0.007
HDL-C, mg/dl	48.3 ±13.8	52.99 ±16.7	0.449
non-HDL-C, mg/dl	167.0 ±55.1	111.7 ±34.9	0.001
TG, mg/dl	150 (113–160)	108 (82–134)	0.121
TC:HDL-C$_{ratio}$	4.6 ±1.3	3.4 ±1.2	0.005
TC:non-HDL-C$_{ratio}$	1.3 (1.2–1.4)	1.5 (1.4–1.6)	0.005
LDL-C:HDL-C$_{ratio}$	3.0 ±1.2	1.9 ±1.0	0.007
TG:HDL-C$_{ratio}$	3.6 ±2.7	2.4 ±1.4	0.068

Data are presented as mean ± standard deviation, median (interquartile range), or number (percentage) of patients.

a defined as evidence of coronary artery disease in a sibling or parent before 65 years of age in women or before 55 years of age in men

For conversion factors and abbreviations, see TABLE 1.

between miR-21 levels and myocardial necrosis markers, such as high-sensitivity cardiac troponin T levels (r = –0.03; P = 0.859) and creatinine kinase-MB mass (r = –0.05; P = 0.783).

To determine whether renal function was related to miR-21 levels, we sought to evaluate its correlation with renal parameters; there were no correlations with estimated glomerular filtration rate (eGFR; r = –0.33; P = 0.060) or with serum creatinine levels (r = 0.24; P = 0.118). There was also no association of miR-21 expression with inflammatory indices such as C-reactive protein (r = 0.00; P = 0.998), white blood cell count (r = 0.15; P = 0.419), as well as no correlation with body mass index (r = –0.01; P = 0.963). The measured levels of miR-21 in diabetic subjects were similar to those in nondiabetic ones (median with the interquartile range of the relative expression: 1.9 [1.5–3.6] vs 2.0 [0.6–3.0]; P = 0.512).

DISCUSSION Our study showed that the levels of free circulating miR-21 in the early phase of NSTE ACS were significantly correlated with lipid profile, showing an inverse relationship with LDL-C, non-HDL-C, and TC levels and also with the atherogenic profile: TC:HDL-C$_{ratio}$, TC:non-HDL-C$_{ratio}$, LDL-C:HDL-C$_{ratio}$, and TG:HDL-C$_{ratio}$. Thus, our findings have shed new light on the

TABLE 3 Multivariable regression analysis of factors related to serum low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol levels

Variable	Non-HDL-C levels			
	univariate analysis		multivariate analysis[a]	
	β	*P* value	β	*P* value
age	–0.43	0.032	NS	NS
need for revascularization (PCI or CABG)	0.14	0.435	0.35	0.027
miR-21 levels	–0.34	0.047	–0.41	0.018
hs-cTn levels	–0.03	0.899	NS	NS
time from symptom onset	–0.03	0.857	NS	NS
DM	0.25	0.136	NS	NS
HA	0.23	0.216	NS	NS
LVEF	–0.17	0.383	NS	NS
model summary: R^2 (adjusted R^2)			0.27 (0.22)	
Variable	**LDL-C levels**			
	univariate analysis		multivariate analysis[a]	
	β	*P* value	β	*P* value
age	–0.38	0.173	NS	NS
need for revascularization (PCI or CABG)	0.16	0.57	NS	NS
miR-21 levels	–0.38	0.173	NS	NS
hs-cTn levels	–0.03	0.902	NS	NS
time from symptom onset	0.04	0.128	NS	NS
DM	0.21	0.4	NS	NS
HA	0.13	0.47	NS	NS
LVEF	–0.08	0.788	NS	NS
model summary: R^2 (adjusted R^2)			ND	

Data are presented as regression standardized coefficients (β) and *P* values.

a adjusted for all others covariates in table

Abbreviations: CABG, coronary artery bypass grafting (reference category: no need for revascularization); DM, diabetes mellitus (reference category: no evidence of DM); HA, arterial hypertension (reference category: no evidence of HA); hs-cTn, high-sensitivity cardiac troponin T; miR-21, microRNA-21; non-HDL-C, non-high-density lipoprotein cholesterol; ND, not determined; NS, not significant; PCI, percutaneous coronary intervention; others, see TABLE 1

potential biological effects of miR-21 observed in patients with acute coronary syndromes. Although several previous studies have investigated the in-vivo expression of different miRNAs in cell cultures and their relation with lipid metabolism, clinical studies of serum miRNAs in humans are rare. To our knowledge, this is probably the first clinical study reporting the relationship between free circulating miR-21 levels and the lipid profile in a clinically relevant scenario of acute NSTE ACS. Our study identified not only the correlation of miR-21 levels with particular lipid fractions, but also with the ratios of individual fractions, which are considered better predictors of coronary risk than the levels of individual lipid fractions alone.[11-13] This observation is strengthened by the finding of lower miR-21 levels in patients who required percutaneous coronary intervention, which may be related to miR-21-mediated protection from lipotoxicity.

Our results presenting the robust association between miR-21 and lipid levels are consistent with experimental results presented by Larsen et al,[14] who investigated the expression of miRNAs during the perinatal period in the rat model.It has been proved that the inhibition of miR-21 and miR-29a increases TC levels. The authors concluded that miR-21 is likely to functionally participate in the decrease of the cholesterol synthesis pathway.[14] They have also identified the sterol regulatory element-binding protein 1 (SREBP1) as the true target of miR-21 (downregulated by miR-21), showing that biochemical pathways related to sterol and lipid metabolism are affected by miRNAs. The SREBP family has been established as global physiological regulator of lipids and glucose metabolism.[15] Moreover, SREBP1 has been linked to the accumulation of lipids in various tissues and organs, often referred to as lipotoxicity.[16] That basic study provides potential molecular explanation for the findings from our study, where the overexpression of miR-21 also resulted in lower cholesterol levels and less atherogenic lipid indices. However, although most miRNAs have been highly conserved throughout evolution, this mechanism remains to be confirmed in the human model.

The potential role of miR-21 in protecting from the inflammation-mediated lipid accumulation process was investigated by Feng et al.[17] The authors demonstrated that miR-21 inhibit lipid--laden foam cell formation via the TLR4-NF-κB pathway in lipopolysaccharide-stimulated macrophages, implying a critical role of miR-21 in atherosclerosis progression.[17] These results are indirectly consistent with our findings showing that the overexpression of miR-21 resulted in a less atherogenic lipid profile. A study by Guo et al[18] demonstrated that miR-21 mediates some of the cellular anti-inflammatory effects of statins, and highlighted its role as the potential missing link between lipid metabolic imbalance and inflammation, both of which are the key factors in the development of atherosclerosis. Contrary to the findings for lipids, we observed no association between miR-21 levels and inflammation indices such as C-reactive protein and white blood cell count.

Endothelial cell (EC) function is essential for the development of CVD, by responding to shear stress, inflammation, hyperlipidemia, and regulating vessel wall homeostasis.[19] MiR-21 is also known to positively regulate endothelial NO synthase activity in ECs exposed to increased shear stress.[6,19] Riedel et al[20] investigated the HDL-C--mediated expression of miR-21 in ECs. Exposing the ECs to HDL-C isolated from the serum of patients with congestive heart failure resulted in reduced miR-21 expression, which could accelerate endothelial dysfunction and atherogenesis. The authors also concluded that differences in the HDL-C phenotype in patients with chronic heart failure may be explain an altered response to regulatory miRNA expression in ECs.[20] Moreover, HDL carries specific miRNAs,[21] and HDL-specific subfraction miRNAs have been suggested as novel biomarkers of CVD.[21,22] The ongoing DYS-HDL trial might provide additional information on the formation of dysfunctional HDL and its association with specific miRNAs.[21,22]

The discussed results are contradictory to those reported for cardiac tissue, where the overexpression of miR-21 was related to cardiac dysfunction and increased fibrosis,[23] possibly due to the different cellular environment and cell type. In our study, we observed no significant direct relation between the miR-21 expression and HDL--C fraction alone, as opposed to secondary indices: TC:HDL-C_{ratio}, LDL-C:HDL-C_{raio}, and TC:HDL-C_{ratio}. We can only speculate that the observed relations may be some molecular HDL-C-mediated interactions between miR-21 and ECs, which may contribute to the regulation of the lipid profile.

The liver is central to lipid metabolism.[24,25] Recent studies have suggested that posttranscriptional miRNAs-mediated gene regulation may be relevant to regulate hepatic lipid metabolism by modulation of numerous transcription factors present in liver tissue, including peroxisome proliferator activated receptors (PPARs).[26,27] Recently, it has been found that miR-21 and miR-27b negatively regulate PPARα and that the levels of PPARα protein are significantly decreased in human liver-derived cell lines by the overexpression of miR-21 and miR-27b.[27] Moreover, in the study of Ahn et al[28] on the mouse model, the miR-21 expression was decreased in subjects on high-fat diet. The authors identified fatty acid binding protein 7 as the novel target of miR-21, which was upregulated in high-fat diet and led to an increased fatty acid uptake and their transport to the liver, and finally, to hepatic steatosis. The authors also highlighted the association of miR-21 with PPARs and reversed lipid accumulation in tissues and cells by lycopene-induced normalization of the miR-21 expression, mediated by the regulation of PPARα and PPARγ. Also in the study of Li et al[29] on the ob/ob mouse model, which develops obesity and diabetes-like symptoms, the miR-21 expression in liver tissue was downregulated. Similar results were presented by Kim et al,[30] who demonstrated a significant decrease of the miR-21 expression in high-fat diet-induced obesity in the mouse model and has proved an increase in adipocyte proliferation associated with miR-21 downregulation. It seems that the association between the miR-21 and lipid levels might reflect a dietary status, as it has been shown in the described animal models, where a high-fat food intake led to the downregulation of miR-21. These pathways remain to be proved in humans.

A number of studies have also suggested a significant direct contribution of adipose tissue to miRNAs-mediated lipid metabolism. The expression of miR-21 in subcutaneous adipose tissue from healthy individuals showed a positive relationship with obesity.[7] Kim et al[8] reported the role of miR-21 in the differentiation of human adipose tissue-derived mesenchymal stem cells and explained the mechanism by the involvement of tumor growth factor-β action, suggesting miR-21 as potential therapeutic target in the management of metabolic diseases and prevention of obesity. However, in our study, we did not observe any association of free circulating miR-21 levels with body mass index or with diabetes, which may confirm the different roles of miRNAs depending on the cell and tissue type. In the murine model, Seeger et al[31] showed that long-term treatment with anti-miR-21 led to a significant weight loss, adipocyte size reduction, and a decrease in TG levels, without significant changes in serum TC levels. Importantly, our study was conducted in a distinct clinical setting of patients with NSTE ACS, which may explain some of the discrepancies with previously published data.

Study limitations Our study has numerous limitations. First, the studied population was relatively small, but nonetheless, the results seem to be robust and consistent. We studied patients with NSTE ACS, and thus our findings may not be valid in the population of stable patients. Although we could not detect correlations between

miR-21 levels and myocardial necrosis factors, it is uncertain whether myocardial damage had any effect on the miRNA expression.

Further studies should be performed on a larger group of patients representing the general population of cardiac patients to elucidate the observed association on the molecular level in humans.

Conclusions In cardiac patients, the overexpression of miR-21 negatively correlates with TC and LDL-C levels and is associated with a less atherogenic lipid profile defined as TC:LDL-C_{ratio}, TG:HDL-C_{ratio}, TC:non-HDL-C_{ratio}, and LDL--C:HDL--C_{ratio}. It seems that miR-21 may play an important role in lipid metabolism. To establish whether it can be used as a therapeutic target or disease marker and to study the molecular mechanisms of the presented relationships, further research is needed.

Contribution statement DM contributed to study design, concept, statistical analysis, data collection, data interpretation, preparation of the manuscript, and coordination of funding for the project; PL contributed to data interpretation and final revision of the manuscript; KW-D, KK, BM, KW-O, and PW-M contributed to data collection, data interpretation, and preparation of the manuscript; JD-K contributed to study design, concept, data interpretation, final revision of the manuscript; and coordination of funding for the project.

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Związek między poziomem wolnego krążącego mikroRNA-21 a profilem lipidowym pacjentów z ostrym zespołem wieńcowym bez przetrwałego uniesienia odcinka ST

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STRESZCZENIE

WPROWADZENIE MikroRNA (miRNAs) są niekodującymi cząsteczkami RNA zaangażowanymi w regulację ekspresji genów. Niektóre miRNAs, w szczególności miRNA-21 (miR-21), mogą być zaangażowane w metabolizm lipidów.

CELE Celem badania była ocena związku między poziomem wolnego krążącego miR-21 w osoczu a profilem lipidowym: cholesterolem całkowitym (TC), cholesterolem LDL (LDL-C), cholesterolem HDL (HDL-C), cholesterolem non-HDL (non-HDL-C) i triglicerydami (TG) oraz ich aterogennym profilem określanym jako stosunek stężeń poszczególnych frakcji lipidów (TC:LDL-C, TC:non-HDL-C, TG:HDL-C i HDL-C:LDL-C) w grupie pacjentów z ostrym zespołem wieńcowym bez przetrwałego uniesienia odcinka ST (*acute coronary syndromes without persistent ST-segment elevation* – NSTE ACS).

PACJENCI I METODY Do badania włączono 34 pacjentów, u których po przyjęciu na izbę przyjęć zdiagnozowano NSTE ACS. Poziom miRNA w osoczu oznaczano za pomocą techniki PCR w czasie rzeczywistym oraz metody $_{\Delta\Delta}$Ct. Stężenia poszczególnych frakcji lipidów w surowicy oznaczono po minimalnym okresie 12-godzinnego przebywania na czczo w trakcie pierwszej doby hospitalizacji pacjentów.

WYNIKI Stężenia miR-21 wykazały istotną ujemną korelację z TC ($r = -0,5$; $p = 0,002$), LDL-C ($r = -0,5$; $p = 0,001$) oraz non-HDL-C ($r = -0,6$; $p < 0,001$). Ponadto stężenia te były ujemnie skorelowane z TC:HDL-C ($r = -0,6$; $p < 0,001$), LDL-C:HDL-C ($r = -0,6$; $p < 0,001$), TG:HDL-C ($r = -0,4$; $p = 0,037$) oraz TC:non-HDL-C ($r = 0,6$; $p < 0,001$). Poziom miRNA-21 ($\beta = -0,41$; $p = 0,018$) oraz konieczność rewaskularyzacji ($\beta = 0,35$; $p = 0,027$) okazały się niezależnymi predyktorami poziomu non-HDL-C w analizie wieloczynnikowej.

WNIOSKI Poziom wolnego krążącego miR-21 koreluje negatywnie ze stężeniami TC, LDL-C i non-HDL-C oraz niezależnie wpływa na poziom non-HDL-C u pacjentów z NSTE ACS. Tym samym nadekspresja miRNA-21 wiąże się z mniej aterogennym profilem lipidowym.

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