Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

Platelet miR-587 may be Used as a Potential Biomarker for Diagnosis of Patients with Acute Coronary Syndrome

Hao Qiu [1], Yangyang Zhang [1], Qiang Zhao [1], Huaxin Jiang [2], Jingpeng Yan [1], Yanyan Liu [3]

[1] *Department of Cardiology, Affiliated Tengzhou Central People's Hospital of Jining Medical University, Jining Medical University, Tengzhou, China*
[2] *Department of Clinical Laboratory, Affiliated Tengzhou Central People's Hospital of Jining Medical University, Jining Medical University, Tengzhou, China*
[3] *Department of Pharmacology, Affiliated Tengzhou Central People's Hospital of Jining Medical University, Jining Medical University, Tengzhou,* China

SUMMARY

Background: **The current study aims to investigate the expression of miR-587 in platelets of patients with acute coronary syndrome (ACS) and its correlation with the severity of coronary artery disease.**
Methods: **One hundred thirty-nine patients with ACS who underwent coronary angiography (CAG) and hospitalized in the Department of Cardiology of Affiliated Tengzhou Central People's Hospital of Jining Medical University were selected. In addition, 42 subjects with normal CAG results were selected as the control group. The relative expression of platelet miR-587 was analyzed between ACS patients and control subjects.**
Results: **The relative expression of platelet miR-587 and Gensini score in acute myocardial infarction (AMI) patients were significantly higher than those in the unstable angina pectoris (UA) and control groups (median (IQR): 1.97 (0.47) vs. 1.49 (0.43) vs. 1.04 (0.35); 65.07 (21.6) vs. 44.58 (28.56) vs. 13.67 (11.8)). Furthermore, the relative level of miR-587 in ACS patients with three vessel lesions was significantly higher than those with double vessel lesion and single vessel lesion (2.68 (0.64) vs. 1.85 (0.53) vs. 1.22 (0.40)). Additionally, the relative expression of platelet miR-587 in patients with severe coronary artery disease was significantly higher than in those with moderate or mild coronary artery disease (2.44 (0.59) vs. 1.70 (0.49) vs. 0.96 (0.32)). Pearson's correlation analysis showed that the Gensini score and creatine kinase isoenzyme (CK-MB) of ACS patients were positively correlated with the expression of platelet miR-587 ($r = 0.785$, $p = 0.000$; $r = 0.806$, $p = 0.000$).**
Conclusions: **The expression of platelet miR-587 was closely related to the severity of coronary artery stenosis and could be used as a potential target for diagnosis or prognosis.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190703)

Correspondence:
Dr. Yanyan Liu
Department of Pharmacology
Affiliated Tengzhou Central People's Hospital
of Jining Medical University
Jining Medical University
277500 Tengzhou
China
Email: liuyanyanxinnei@163.com

Manuscript accepted August 28, 2019

KEY WORDS

acute coronary syndrome, coronary stenosis lesion, Gensini score, MiR-587

INTRODUCTION

In recent years, with the acceleration of the aging process in China, the incidence of coronary heart disease (CHD) has increased year by year [1]. Acute coronary syndrome (ACS) is a kind of acute and severe CHD, with a high fatality rate [2]. At present, the specific pathogenesis of ACS is still not clear [3]. According to the different degrees of myocardial ischemia and the

formation of collateral circulation, patients show different clinical symptoms [4]. Therefore, how to accurately judge the degree of vascular lesions and carry out reasonable stratified treatment is of great clinical value for improving the therapeutic effect and reducing the waste of medical resources.

At present, coronary angiography (CAG) is the gold standard for clinical diagnosis of CHD and objective evaluation of the degree of coronary stenosis [5]. However, it is expensive and invasive [6]. Meanwhile, the sensitivity of many biochemical markers is low [7]. Therefore, it is necessary to find new specific indicators to objectively evaluate the degree of coronary stenosis [8]. With the development of RNAomics, the potential of microRNAs (miRs) as a new diagnostic and therapeutic marker of cardiovascular diseases has been paid more and more attention in clinic [9,10].

A previous study has shown that miR-587 confers resistance to 5-fluorouracil (5-FU)-induced apoptosis in colorectal cancer cells [11]. In addition, miR-587 was also found to be abnormally expressed in glioblastoma multiforme (GBM) [12]. However, the expression pattern and diagnostic value of miR-587 in ACS patients have never been reported.

MATERIALS AND METHODS

Research subjects and grouping

One hundred thirty-nine ACS patients were selected from July 2016 to February 2018 in the Department of Cardiology, Affiliated Tengzhou Central People's Hospital of Jining Medical University. Inclusion criteria: 1) All patients were diagnosed as ACS by CAG, clinical symptoms, and auxiliary examinations. 2) Complete clinical data; and 3) informed consent signed by all patients. Exclusion criteria: 1) patients who did not meet the above inclusion criteria; 2) those who received thrombolytic, anticoagulant and antiplatelet drugs one month before the test; 3) those who received hormones, non-steroidal anti-inflammatory drugs and opioid receptor agonists one month before the test; 4) severe consciousness disorders, hypertension crisis and acute symptoms occurred before the treatment; 5) patients combined with heart failure, congenital heart disease, rheumatic myocarditis, viral myocarditis; 6) patients combined with severe immune system diseases, blood system diseases, acute and chronic severe infections, severe bleeding tendency; 7) patients with severe liver and kidney function damage and malignant tumors. Based on the Universal Definition of Myocardial Infarction (MI), the diagnosis of MI needs the rise and/or fall of cardiac biomarkers with clinical evidence of ischemia, defined by symptoms, electrocardiographic (ECG) changes, or new regional wall motion abnormalities [13]. Compared with MI due to an acute coronary syndrome (type 1 MI), type 2 MI is defined as a mismatch in myocardial oxygen supply and demand that is not due to unstable coronary artery disease (CAD) [13]. Hence, in the present study, we mainly focused on type 1 MI. According to the clinical manifestations and ECG changes of ACS patients, ACS patients were further divided into two subgroups, including acute myocardial infarction (AMI) (n = 68 cases) and unstable angina pectoris (UA) (n = 71 cases). In addition, 42 subjects with no CAD in our hospital at the same time were selected as the control group. For the controls, no abnormal results were found by CAG examination, and myocardial enzymes and troponin were negative. All peripheral venous blood samples were obtained from the AMI patients upon admission to hospital. This study has been approved by the Ethics Committee in Affiliated Tengzhou Central People's Hospital of Jining Medical University.

The degree of coronary artery lesion was evaluated by the Gensini scoring system. According to the Gensini score, 0 - 30 was defined as mild lesion, 31 - 60 was defined as moderate lesion, and > 60 was defined as severe lesion.

The number of stenosed coronary vessels was defined as follows: (1) Single-vessel lesion: any single vessel of the left anterior descending (LAD), left circumflex (LCX) and right coronary artery (RCA) with stenosis of more than 50%. (2) Two-vessel lesions: two single vessel stenosis of LAD, LCX, RCA > 50% or left main artery (LM) lesions. (3) Three-vessel lesions: LAD, LCX, and RCA were stenosis, or synchronous lesion of LM combined with RCA.

Extraction and purification of platelet

The blood samples were centrifuged at room temperature for 6 minutes at 160 g, and the middle supernatant was taken, which was platelet-rich plasma. Then, the same volume of HEPES solution was added to platelet-rich plasma and centrifuged at room temperature for 10 minutes at 300 g. After that, the supernatant was discarded. Three milliliters of HEPES solution was added and centrifuged at room temperature for 10 minutes at 300 g. The supernatant was discarded and platelets were collected. Platelet purity was measured by flow cytometry.

Platelet RNA Extraction

Total RNA from platelets was isolated according to the instructions of Ambion RNA extraction and separation kit (Takara Company, Japan). The extracted RNA solution was stored at -80°C.

RNA reverse transcription

RNA was reverse transcribed according to the instructions of PrimeScript™ RT reagent Kit (Takara Company, Japan). The cDNA was stored at -20°C for reserve.

Real-time quantitative polymerase chain reaction (qRT-PCR)

qRT-PCR was carried out using CFX-96-C1000 RT-PCR (Bio-Rad Company, USA). qPCR was detected by SYBR Green Supermix (Bio-Rad Laboratories, Inc.,

Hercules, CA, USA) using an ABI 7500 fluorescence quantitative PCR instrument (ABI). The total volume of the reaction system was 20 μL, including cDNA 2.0 μL, forward primer 1.0 μL; reverse primer 1.0 μL; 2 x mix 10 μL; ddH_2O 6.0 μL. The amplification conditions of qPCR were: pre-denaturation at 95°C for 5 minutes, then 40 cycles of denaturation at 95°C for 30 seconds and denaturation at 60°C for 30 seconds. U6 was used as an internal reference. Three repeated experiments were carried out for each sample. Relative mRNA expression was normalized to U6 using the $2^{-\Delta\Delta Cq}$ method [14].

Western blot

To determine the purity of platelets, western blot was carried out as previously determined [15]. In brief, platelet-rich plasma and platelets were treated with RIPA buffer (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China). Then, the protein was isolated by 12% SDS-PAGE. After that, the membrane was incubated with primary antibody against CD45 (ab10558, Abcam, Cambridge, UK) and GPIIb (ab134131, Abcam, Cambridge, UK). Following several washes with TBST, the membranes were incubated with HRP-conjugated goat anti-rabbit IgG for 2 hours at room temperature and then washed. The proteins were detected using enhanced chemiluminescence, according to the manufacturer's protocol (Merck KGaA, Darmstadt, Germany). GAPDH was used as an internal control.

Statistical analysis

All the data were analyzed by SPSS 17.0 statistical software. Data were provided as median with interquartile range (IQR). The two-tailed unpaired Student's *t*-tests were used for comparisons of two groups. The one-way ANOVA multiple comparison test followed by Tukey's post hoc test was used for comparisons of two more groups. The correlation analysis was performed by Pearson's correlation analysis. $p < 0.05$ was statistically significant.

RESULTS

Comparison of general data

Age, gender, body mass index (BMI), history of diabetes mellitus, history of hypertension, history of hyperlipidemia, and history of smoking were basically the same between the two groups, with no statistical difference ($p > 0.05$, Table 1).

Comparison of biochemical indexes between two groups

The results showed that the levels of blood platelets (PLT) and cardiac troponin I (cTnI) in ACS patients were higher than those in the control group. There was no significant difference in the levels of fasting blood glucose (FBG), total triglyceride (TG), total cholesterol (TC), high-density lipid-cholesterol (HDL-C) and low-density lipid-cholesterol (LDL-C) between the two groups (Table 2).

Comparison of platelet miR-587 expression and Gensini score in different groups

Then, we compared the Gensini score and platelet miR-587 expression in ACS patients and controls. As shown in Figure 1A, the Gensini score in the UA group (median (IQR): 44.58 (28.56)) and AMI group (65.07 (21.6)) were higher than those in the control group (13.67 (11.8)). Moreover, the Gensini score in patients with AMI were significantly higher than those in patients with UA (Figure 1A). Previous study has suggested that isolation of platelets is often contaminated by leukocytes [16]. Hence, we examined the expression of CD45, the leukocyte marker, and glycoprotein IIb (GPIIb), the platelet-specific marker in platelet-rich plasma (PRP) and purified platelets. Western blot assay showed that only GPIIb could be detected in the purified platelets (Figure 1B). Then, we examined the level of miR-587 in the platelets of the control, UA, and AMI groups. Our data showed that the relative expression of platelet miR-587 was highest in the AMI group (1.97 (0.47)) and higher in the UA group (1.49 (0.43)), compared with that of the control group (1.04 (0.35)) (Figure 1C).

Comparison of platelet miR-587 level in ACS patients with different lesion branches

The relative expression of platelet miR-587 in ACS patients with three-vessel lesions (2.68 (0.64)) was the highest, which showed a significant difference compared with two-vessel lesions (1.85 (0.53)) and single-vessel lesion (1.22 (0.40)) (Figure 2). The results showed that the higher the number of lesion vessels was, the higher the relative expression level of platelet miR-587 was.

Comparison of platelet miR-587 in patients with ACS with different degrees of coronary artery disease

The relative expression of platelet miR-587 was highest in the severe lesion group (2.44 (0.59)) and higher in the moderate lesion group (1.70 (0.49)), than that in the mild lesion group (0.96 (0.32)) (Figure 3). These data suggested that the expression level of platelet miR-587 gradually increased along with the severity of coronary artery disease.

Analysis of the correlation between the expression of platelet miR-587 and Gensini score/CK-MB

Pearson's correlation analysis showed that the Gensini score of ACS patients was positively correlated with the expression of platelet miR-587 ($r = 0.785$, $p = 0.000$) (Figure 4A). Furthermore, we also analyzed the correlation between platelet miR-587 and CK-MB an important myocardial marker, which was mainly for the diagnosis of acute myocardial infarction and assessment of myocardial infarction area [17]. Pearson's correlation

Table 1. Analysis of general clinical data of patients.

Item	Control (n = 42)	ACS patients (n = 139)	p
Age (years)	60.43 (10.54)	63.15 (11.67)	0.371
Male (n, %)	25 (59.52)	74 (53.24)	0.468
BMI (kg/m^2)	24.12 (1.28)	25.67 (1.32)	0.237
Diabetes (n, %)	4 (9.52)	28 (20.14)	0.128
Hypertension	11 (26.19)	47 (33.81)	0.346
Hyperlipidemia	9 (21.43)	44 (31.65)	0.208
Smoking	9 (21.43)	37 (26.62)	0.475

Table 2. Comparison of examination results between the two groups at admission.

Item	Control (n = 42)	ACS patients (n = 139)	p
PLT (x 10^9//L)	168.53 (36.87)	235.62 (75.46)	0.000
cTnI (μg/L)	0.16 (0.35)	6.85 (12.45)	0.000
FBG (mM)	5.43 (2.15)	5.82 (1.56)	0.085
TG (mM)	1.89 (1.26)	2.14 (1.35)	0.562
TC (mM)	4.36 (1.62)	4.82 (1.86)	0.112
HDL-C (mM)	1.53 (0.36)	1.48 (0.28)	0.078
LDL-C (mM)	2.51 (0.32)	2.56 (0.38)	0.061

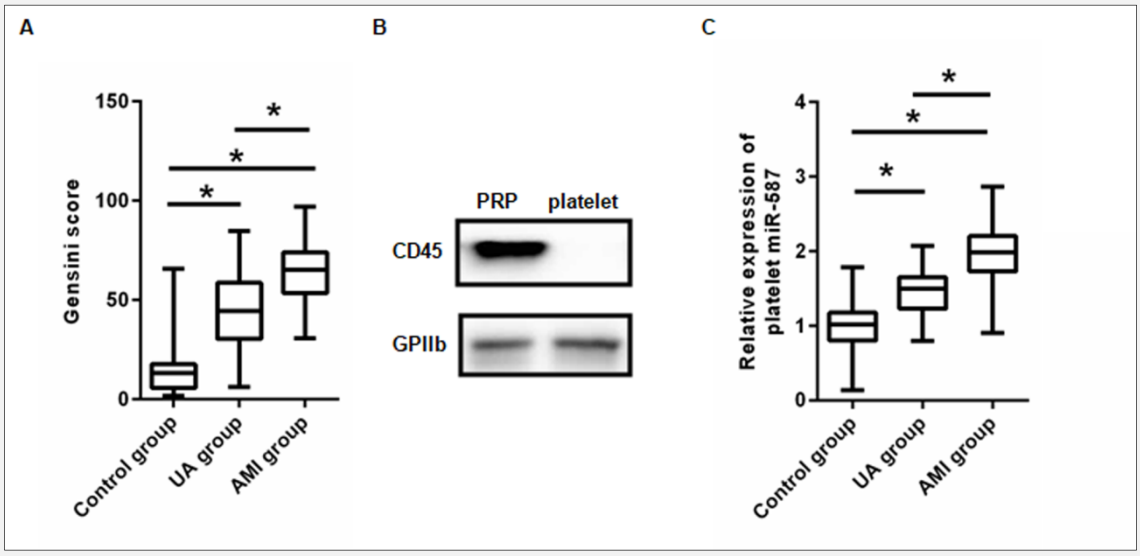

Figure 1. Platelet miR-587 expression and Gensini score were measured in different groups.

(A) The Gensini score gradually increased in the AMI group, UA group and control group. (B) Western blot assay showed that only GPIIb could be detected in the purified platelets and not in platelet-rich plasma (PRP). (C) Real time PCR was carried out to analyze the level of platelet miR-587. * - p < 0.05 vs. as indicated.

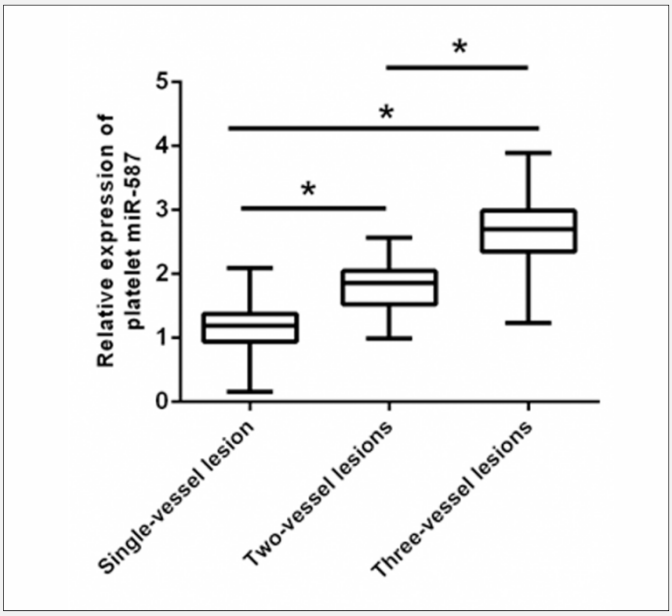

Figure 2. Platelet miR-587 was enhanced along with the increased number of lesion branches.

* **- p < 0.05 vs. as indicated.**

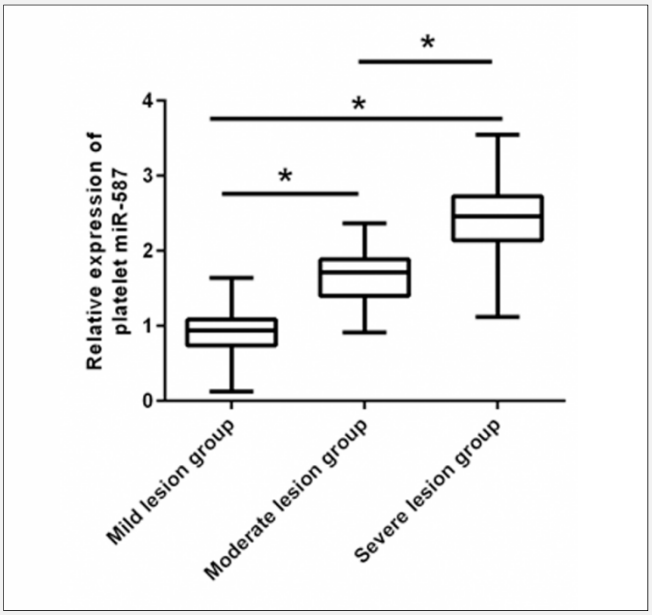

Figure 3. The expression level of platelet miR-587 gradually increased along with the severity of coronary artery disease.

* **- p < 0.05 vs. as indicated.**

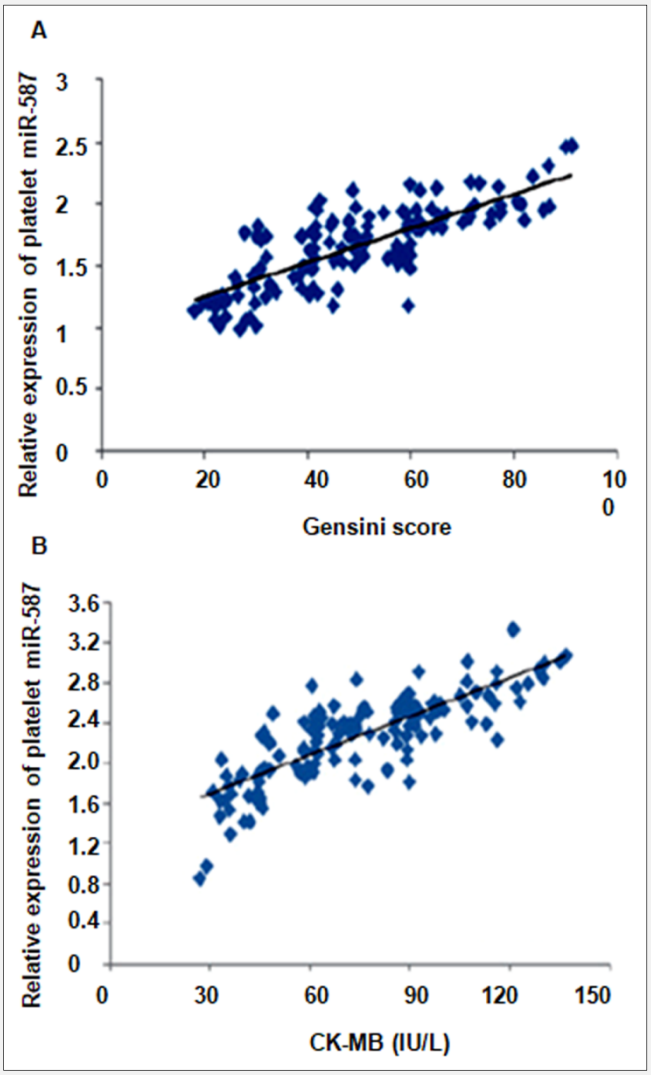

Figure 4. Analysis of the correlation between the expression of platelet miR-587 and Gensini score/CK-MB in ACS patients.

Pearson's correlation analysis showed that the Gensini score (A) and CK-MB (B) of ACS patients were positively correlated with the expression of platelet miR-587.

assay showed that platelet miR-587 positively correlated with CK-MB ($r = 0.806$, $p = 0.000$) (Figure 4B).

DISCUSSION

CHD is one of the most lethal cardiovascular and cerebrovascular diseases in the world, including China [1]. In recent years, the incidence of CHD has been increasing year by year, which has brought great economic burden to patients families and social medical care [18]. ACS belongs to the most serious and complex critical diseases of CHD [19]. Many studies have confirmed that the formation of unstable atherosclerotic plaques in the coronary artery is closely related to the occurrence and development of ACS [8,20,21]. Once the plaque ruptures, platelets aggregate rapidly to form a white thrombus [22]. If the plaque ruptures seriously, a large number of adherent fibrin will form a red thrombus, leading to complete occlusion of the coronary artery [23]. Therefore, platelets are a key factor affecting this series of pathological processes [23]. They not only release a large number of cytokines to participate in the occurrence and development of ACS, but also activate

or release inflammatory factors to participate in the formation of atherosclerosis [8,23].

At present, CAG is the most reliable and accurate diagnostic method for CHD [7]. However, because of the high cost of CAG examination, the need for patients to make an appointment in advance, and the high risk of invasive examination, many patients are unwilling to accept CAG examination [24]. Hence, the judgement of early coronary artery lesions in ACS patients and the choice of follow-up treatment options are affected [25]. In addition, some other biochemical indicators, including cTnI, BNP, and CRP, are still used as diagnostic tools [5]. However, they are only used as auxiliary means to detect coronary artery lesions, so the sensitivity and specificity of judging the degree of coronary artery lesions are low [6].

MiRNAs are a class of non-coding negative regulatory RNAs in eukaryotic cells, which can inhibit RNA transcription by binding to the target gene 3'-untranslated region [26]. At present, the research on the relationship between miRs and cardiovascular diseases has become a hotspot [27]. Through bioinformatics and gene chip technology, the differentially expressed microRNAs are extensively identified between ACS patients and healthy people, indicating that a large number of miRs are involved in the process of coronary atherosclerosis, platelet activation, vulnerable plaque rupture, and so on [26,28]. However, although gene chip technology is simple and easy to use, it has a large amount of screening information. Meanwhile, its sensitivity is low and the cost is expensive. qRT-PCR is the most commonly used and reliable detection method in clinical laboratory. Therefore, we hope to explore the use of qRT-PCR to detect platelet miR-587 as a feasible diagnostic marker in the course of CHD.

Our data showed that the expression level of platelet miR-587 in ACS patients was significantly higher than that in non-CHD subjects. Previous study has suggested that plaque rupture often occurs at sites of severe narrowing (mean 91%, range 67% to 99%) in fatal AMI [29]. Therefore, plaque rupture with thrombosis is unlikely to induce the fatal acute myocardial infarction in patients with mild to moderate coronary stenosis [29]. Hence, we evaluated miR-587 as an acute marker for MI (acute thrombosis) vs. severity of atherosclerosis/ CAD. Here, we found that the higher the degree of coronary lesion was, the higher the expression level of platelet miR-587 was found. Furthermore, we also found that the expression of miR-587 was up-regulated with the aggravation of ACS. Spearman's correlation analysis showed that the Gensini score of ACS patients was positively correlated with the expression of platelet miR-587, indicating that the expression level of platelet miR-587 directly affected the severity of CHD.

CONCLUSION

For the first time, we showed novel data that the expression of platelet miR-587 positively correlated with the occurrence of ACS and the severity of coronary artery lesions, which may serve as a potential molecular marker for the diagnosis and prediction of the severity of ACS.

Declaration of Interest:
We declare no conflicts of interest.

References:

1. Wang W, Ren D, Wang CS, Yao HC. Nardilysin: A potential biomarker for the early diagnosis of acute coronary syndrome. Int J Cardiol 2018;265:39 (PMID: 29885698).

2. Adam AM, Ali MA, Shah AA, et al. Efficacy of Hematological and Coagulation Parameters in the Diagnosis and Prognosis of Patients with Acute Coronary Syndrome. J Tehran Heart Cent 2018;13:115-25 (PMID: 30745924).

3. Adam AM, Nasir SAR, Merchant AZ, et al. Efficacy of serum blood urea nitrogen, creatinine and electrolytes in the diagnosis and mortality risk assessment of patients with acute coronary syndrome. Indian Heart J 2018;70:353-9 (PMID: 29961450).

4. Barrabes JA, Bardaji A, Jimenez-Candil J, et al. Characteristics and Outcomes of Patients Hospitalized With Suspected Acute Coronary Syndrome in Whom the Diagnosis is not Confirmed. Am J Cardiol 2018;122:1604-9 (PMID: 30213384).

5. Chacko S, Haseeb S, Glover BM, Wallbridge D, Harper A. The role of biomarkers in the diagnosis and risk stratification of acute coronary syndrome. Future Sci OA 2018;4:FSO251 (PMID: 29255623).

6. Charkhkar M, Vakili H, Kheirabadi M, Haddadnia J. Clustering-making model for diagnosis of acute coronary syndrome using PCA based on K-MICA algorithms. Am J Emerg Med 2018;36:160-1 (PMID: 28716591).

7. Cordero A, Rodriguez-Manero M, Garcia-Acuna JM, et al. Incidence and predictors of stroke in patients discharged with the diagnosis of acute coronary syndrome. Int J Cardiol 2019;276:20-5 (PMID: 30414748).

8. Graichen C, Tanner C. Diagnosis of Acute Coronary Syndrome. Am Fam Physician 2018;98:Online (PMID: 30215890).

9. Bai R, Yang Q, Xi R, Li L, Shi D, Chen K. miR-941 as a promising biomarker for acute coronary syndrome. BMC Cardiovasc Disord 2017;17:227 (PMID: 28830367).

10. Gacon J, Kablak-Ziembicka A, Stepien E, et al. Decision-making microRNAs (miR-124, -133a/b, -34a and -134) in patients with occluded target vessel in acute coronary syndrome. Kardiol Pol 2016;74:280-8 (PMID: 26365938).

11. Zhang Y, Talmon G, Wang J. MicroRNA-587 antagonizes 5-FU-induced apoptosis and confers drug resistance by regulating PPP 2R1B expression in colorectal cancer. Cell Death Dis 2016;7:e 2525 (PMID: 28005075).

12. Yerukala Sathipati S, Huang HL, Ho SY. Estimating survival time of patients with glioblastoma multiforme and characterization of the identified microRNA signatures. BMC Genomics 2016;17:1022 (PMID: 28155650).

13. Smilowitz NR, Subramanyam P, Gianos E, Reynolds HR, Shah B, Sedlis SP. Treatment and outcomes of type 2 myocardial infarction and myocardial injury compared with type 1 myocardial infarction. Coron Artery Dis 2018;29:46-52 (PMID: 28746145).

14. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods 2001;25:402-8 (PMID: 11846609).

15. Rong F, Gao X, Liu K, Wu J. Methotrexate remediates spinal cord injury *in vivo* and *in vitro* via suppression of endoplasmic reticulum stress-induced apoptosis. Exp Ther Med 2018;15:4191-8 (PMID: 29731818).

16. Landry P, Plante I, Ouellet DL, Perron MP, Rousseau G, Provost P. Existence of a microRNA pathway in anucleate platelets. Nat Struct Mol Biol 2009;16:961-6 (PMID: 19668211).

17. Poyhonen P, Kylmala M, Vesterinen P, et al. Peak CK-MB has a strong association with chronic scar size and wall motion abnormalities after revascularized non-transmural myocardial infarction - a prospective CMR study. BMC Cardiovasc Disord 2018; 18:27 (PMID: 29422025).

18. da Silva Costa L, de Lima Lopes J, Takao Lopes C, Batista Santos V, Lucia Bottura Leite de Barros A. Prevalence and Associations Between Related Factors and Defining Characteristics of the Nursing Diagnosis Sedentary Lifestyle in Patients with Acute Coronary Syndrome. Int J Nurs Knowl 2018 (PMID: 30576086).

19. Demir MT, Baydin A, Amanvermez R, Erenler AK, Güzel M, Yücel O. Comparison of pentraxin-3 and ischemia-modified albumin with troponin in early diagnosis of acute coronary syndrome. Bratisl Lek Listy 2018;119:509-12 (PMID: 30160160).

20. Montero-Perez FJ, Quero-Espinosa FB, Clemente-Millan MJ, Castro-Giménez JA, de Burgos-Marín J, Romero-Moreno MÁ. Proposed clinical scale for the diagnosis of acute coronary syndrome in patients with an inconclusive electrocardiogram and myocardial injury biomarkers. Rev Clin Esp 2018;218:49-57 (PMID: 29317021).

21. Ossola P, Gerra ML, De Panfilis C, Tonna M, Marchesi C. Anxiety, depression, and cardiac outcomes after a first diagnosis of acute coronary syndrome. Health Psychol 2018;37:1115-22 (PMID: 30307271).

22. Parizadeh SM, Ferns GA, Ghandehari M, et al. The diagnostic and prognostic value of circulating microRNAs in coronary artery disease: A novel approach to disease diagnosis of stable CAD and acute coronary syndrome. J Cell Physiol 2018;233:6418-24 (PMID: 29215707).

23. Tipoo Sultan FA, Adnan G. Myocarditis mimicking acute coronary syndrome - the role of cardiac magnetic resonance imaging in the diagnosis. J Pak Med Assoc 2018;68:477-9 (PMID: 29540 892).

24. Katus H, Ziegler A, Ekinci O, et al. Early diagnosis of acute coronary syndrome. Eur Heart J 2017;38:3049-55 (PMID: 29029109).

25. Quadri G, D'Ascenzo F, Moretti C, et al. Complete or incomplete coronary revascularisation in patients with myocardial infarction and multivessel disease: a propensity score analysis from the "real-life" BleeMACS (Bleeding complications in a Multicenter registry of patients discharged with diagnosis of Acute Coronary Syndrome) registry. EuroIntervention 2017;13:407-14 (PMID: 28169217).

26. Li Z, Wu J, Wei W, et al. Association of Serum miR-186-5p With the Prognosis of Acute Coronary Syndrome Patients After Percutaneous Coronary Intervention. Front Physiol 2019;10:686 (PMID: 31231239).

27. Bagavad Gita J, George AV, Pavithra N, Chandrasekaran SC, Latchumanadhas K, Gnanamani A. Dysregulation of miR-146a by periodontal pathogens: A risk for acute coronary syndrome. J Periodontol 2019 (PMID: 30618100).

28. Li X, Yang Y, Wang L, et al. Plasma miR-122 and miR-3149 Potentially Novel Biomarkers for Acute Coronary Syndrome. PLoS One 2015;10:e0125430 (PMID: 25933289).

29. Qiao JH, Walts AE, Fishbein MC. The severity of atherosclerosis at sites of plaque rupture with occlusive thrombosis in saphenous vein coronary artery bypass grafts. Am Heart J 1991;122:955-8 (PMID: 1927881).