Circulating miR-660-5p is associated with no-reflow phenomenon in patients with ST segment elevation myocardial infarction undergoing primary percutaneous coronary intervention

Jianwei Zhang, Lingjie He[1]

Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University; Beijing-*China*
[1]Department of Outpatient, Beijing Friendship Hospital, Capital Medical University; Beijing-*China*

Abstract

Objective: This study aims to investigate the association of circulating miR-660-5p with no-reflow phenomenon (NRP) in patients with ST segment elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PPCI).

Methods: Consecutive patients diagnosed with anterior STEMI within 12 h of pain onset were included; in these patients, coronary angiography confirmed that the left anterior descending artery was infarcted. Angiographic NRP was defined as a final thrombolysis in myocardial infarction (TIMI) flow 2 or 3 with a myocardial blush grade (MBG) <2. High miR-660-5p was defined as a value in the third tertile. The relationship of circulating miR-660-5p with NRP was assessed using Spearman correlation analysis and multiple logistic regression analysis.

Results: Fifty-two eligible patients were finally included in this study (mean age: 56±12.4 years, >65 years: 53.8%, male: 76.9%, and mean Body Mass Index: 26.3±3.5). The incidence of NRP was 38.5%. Circulating miR-660-5p was significantly related to the mean platelet volume (MPV). The patients were grouped into tertiles by miR-660-5p levels (Q1: <7.18, Q2: 7.18–11.31, Q3: >11.31). Those in the high microRNA-660-5p group had nearly a 6-fold higher risk of NRP than those in the low microRNA-660-5p group [odds ratio (OR) = 5.68, 95% confidence interval (CI) 1.40–23.07, p=0.015]. When analyzed by tertiles, relative odds of NRP were consistently increasing (OR1 for Q2 vs. Q1: 1.25, 95% CI: 0.27–5.73, p=0.770; OR2 for Q3 vs. Q1: 5.96, 95% CI: 1.33–26.66, p=0.02), despite multivariable adjustment. Receiver operating characteristic curve analysis demonstrated that the microRNA-660-5p level of 10.17 was the best cut-off level to predict the incidence of the NRP in patients undergoing PPCI with an area under the ROC curve (AUC) of 0.768 (95% CI: 0.636–0.890).

Conclusion: Circulating miR-660-5p was significantly associated with NRP, and it may be a useful biomarker to predict the incidence of NRP in patients with STEMI undergoing PPCI.

Key words: no-reflow phenomenon, primary percutaneous coronary intervention, ST segment elevation myocardial infarction, miR-660-5p

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Introduction

The China PEACE study has shown that the number of patients with acute myocardial infarction (AMI) undergoing primary percutaneous coronary intervention (PPCI) has increased significantly over the past 10 years in China (1). However, in-hospital mortality in patients with AMI was not significantly improved (1) due to the occurrence of no-reflow phenomenon (NRP) during PPCI, among other reasons. Nearly 40% of patients with ST segment elevation myocardial infarction (STEMI) did not obtain complete myocardial reperfusion by ST segment resolution or myocardial staining grading (MBG) analysis despite successful treatment of the culprit lesion (2, 3). NRP occurs when effective blood perfusion cannot be obtained in the ischemic myocardium after the occluded vessels opened in the presence of a mechanical obstruction such as a coronary dissection, embolism, thrombus, coronary spasm, or stenosis. NRP has been closely associated with in-hospital mortality, malignant

Address for Correspondence: Lingjie He, MD, Department of Outpatient, Beijing Friendship Hospital, Capital Medical University; Beijing-*China*
Phone: +13810390284 E-mail: hlj925@sohu.com
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HIGHLIGHTS

- Circulating miR-660-5p is significantly associated with no-reflow phenomenon (NRP) in patients with STEMI undergoing PPCI. MiR-660-5p is closely related to MPV. MiR-660-5p may be a useful biomarker to predict the incidence of NRP.

arrhythmias, and heart failure (4-6). Due to its extreme complexity, the pathogenesis of NRP has become an urgent scientific problem that requires effective intervention.

MicroRNAs (miRNAs) are important posttranscriptional regulators of numerous biological processes such as cell growth, proliferation, differentiation, and apoptosis (7). Recently, many studies have reported that circulating microRNAs can be considered as biomarkers in cardiovascular disease (8). miR-660-5p has been positively associated with adverse cardiovascular outcomes in patients with STEMI (9). The overexpression of miR-660 increases the production of active platelets in vitro (10), indicating a potential role in NRP. However, there are no reports on the correlation between circulating miR-660-5p and NRP during PPCI. The purpose of this study is to investigate the association of circulating miR-660-5p levels with NRP in patients with STEMI undergoing PPCI.

Methods

We recruited patients diagnosed with anterior STEMI who were treated with PPCI within 12 h of pain onset from June 2017 to May 2018. STEMI was diagnosed based on prolonged chest pain (>30 min) and ST segment elevation >0.2 mv in two or more adjacent leads (11). Patients were finally included when coronary angiography confirmed that the infarction-related artery (IRA) was the left anterior descending artery. Patients who met the following criteria were excluded: age >85 years, history of myocardial infarction and PCI, long-term oral anticoagulant drug treatment, acute infections, malignancy, severe liver and kidney dysfunction, chronic inflammatory diseases, and overt heart failure (Killip III or IV). Upon admission, aspirin (300 mg) and clopidogrel (600 mg) were administered to all patients. The study was approved by the Beijing Anzhen Hospital Institutional Ethics Review Board, and written informed consent for the evaluation of their blood for scientific purposes were obtained.

The PPCI was performed and perioperative medication was administered in accordance with the relevant clinical guidelines (12, 13). In all procedures, 6- or 7-Fr guiding catheters through a transradial approach were used under a bolus of 70–100 IU/kg of heparin. Pre-dilatation was performed if necessary and second-generation drug eluting stents were directly implanted whenever possible. The type of stent was determined by the operator. Only the LAD artery was treated, and non-IRAs were treated after 3 months if necessary. A glycoprotein IIb/IIIa inhibitor and thrombus aspiration device were used at the discretion of the operator. Intracoronary nitrate was always administered after revascularization. We defined NRP as a final thrombolysis in myocardial infarction (TIMI) flow ≤2 or 3 with a myocardial blush grade (MBG) <2 (14). PPCI was considered successful when <30% residual stenosis was achieved after recanalization of the LAD artery. High miR-660-5p was defined as a value in the third tertile.

Venous peripheral blood was collected before PPCI, centrifuged at 3500 *g* for 5 min, and stored at –80°C in a centrifuge tube without RNA enzymes. The total RNA was extracted using an Ultrapure RNA extraction kit (Cat#CW0581, CWbio. Co. Ltd., China). A total of 5 μg of RNA was subjected to 1% agarose gel electrophoresis to determine the RNA integrity. A total of 1 μg of the original total RNA samples with a miRNA First Strand Synthesis Kit (Cat# CW2141, CWbio. Co. Ltd., China) was used for reverse transcription. The upstream primer of miR-660-5p was 5-TACCCATTGCATATCGGAGT-3, the downstream primer was 5-GCCAACCGAGAAGATGATG-3, the upstream primer of U6 was 5-GCTTCGGCAGCATCATACTAAATAAT-3, the downstream primer was 5-GCTTCACAATTGGCGTGCGTCATCATCATCAT-3, and the internal parameter was U6. An ABI7500 fluorescence quantitative polymerase chain reaction (PCR) instrument and miRNA Real-Time PCR Assay Kit (Cat# CW2142, CWbio. Co. Ltd., China) were used for real-time quantitative PCR. The qPCR procedure was performed at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. The operation was performed according to the manufacturer's protocol (15). The $2^{-\triangle\triangle CT}$ method was used to calculate the relative expression of RNA samples.

Statistical analysis

Continuous data are presented as mean ± standard deviation (SD) and were compared by one-way analysis of variance or the Kruskal–Wallis *H* test for those that were not normally distributed. The association between circulating miR-660-5p and other parameters was determined by Pearson's test or Spearman's test. Multiple logistic regression analysis was applied to verify the association of circulating miR-660-5p with NRP. Variables with an unadjusted p value <0.1 in the univariate analysis that have a significant effect on NRP were entered into the multivariate model. All statistical analyses were performed using SPSS 24.0 software (IBM Corp., Armonk, NY, USA). A p value <0.05 was required for statistical significance, and all tests were two-tailed.

Results

Fifty-two eligible patients [mean age, 56 years (SD: 12.4)] were included in this study. Table 1 shows the baseline patient characteristics according to the tertiles of miR-660-5p levels. Approximately 53.8% of patients were older than 65 years, and 76.9% of them were male. The mean BMI was 26.3 kg/m^2 (SD: 3.5). The incidence of NRP was 38.5%. Patients were divided into tertiles by miR-660-5p levels (Q1: < 7.18, Q2: 7.18–11.31, Q3: > 11.31). A high miR-660-5p level was defined as a value in the third tertile (>11.31), while a low microRNA-660-5p level in the

Table 1. Baseline characteristics on admission according to microRNA-660-5p tertiles

	All (52)	Q1 (n=17)	Q2 (n=18)	Q3 (n=17)	*P* value
Age (years)	56±12.4	59±12.6	51.7±11.4	56.9±12.6	0.175
Age (>65 years), n (%)	28 (53.8)	11 (64.7)	8 (44.4)	9 (52.9)	0.539
Male, n (%)	40 (76.9)	13 (76.5)	14 (77.8)	13 (76.5)	NS
BMI, kg/m^2	26.3±3.5	25.6±2.5	27.2±3.5	26.1±4.3	0.424
Smokers, n (%)	39 (75)	13 (76.5)	14 (77.8)	12 (70.6)	0.924
Hypertension, n (%)	28 (53.8)	11 (64.7)	8 (44.4)	9 (52.9)	0.539
Systolic blood pressure (mm Hg)	120±16.9	119±14.9	119±16.3	121±20.0	0.949
Diastolic blood pressure (mm Hg)	77±12.7	75±11.0	76±12.6	79±14.7	0.626
Hypercholesterolemia, n (%)	24 (46.2)	9 (52.9)	8 (44.4)	7 (41.2)	0.832
Diabetes mellitus, n (%)	10 (19.2)	5 (29.4)	3 (16.7)	2 (11.8)	0.463
History of stroke/TIA, n (%)	9 (17.3)	5 (29.4)	2 (11.1)	2 (11.8)	0.422
Killip, n (%)					
I	19 (36.5)	4 (23.5)	8 (44.4)	7 (36.5)	0.448
>I	33 (63.5)	13 (76.5)	10 (55.6)	10 (63.5)	
Laboratory examination					
BNPmax	1322±1797.6	1781±2103.8	597±508.8	1632±2163.6	0.102
FBG	7.1±2.7	7.5±3.7	6.5±1.7	7.3±2.7	0.519
TG	1.6±1.1	1.8±1.4	1.5±0.8	1.6±0.9	0.512
LDL-C	2.7±0.8	2.5±0.8	2.7±0.8	2.9±0.8	0.311
HDL	1.0±0.2	0.9±0.1	1.0±0.2	1.0±0.2	0.155
TCHO	4.3±1.2	4.1±1.2	4.3±1.2	4.6±1.0	0.586
HGB	141.8±18.5	139.5±20.3	143.6±22.5	142±11.7	0.806
Creatine	59.4±14.9	61±17.6	57.9±12.9	58.6±14.3	0.744
D-dimer	0.2±0.2	0.1±0.1	0.2±0.2	0.1±0.1	0.529
WBC (109 L-1)	11.2±3.3	11.2±3.2	11.4±3.7	11.1±3.3	0.984
PLT (109 L-1)	237±57.7	249±57.8	236±65.1	225.5±49.7	0.502
MPV	12.0±2.3	11.1±2.3	11.3±1.7	13.7±2.1	0.001
TnT peak (ng/mL)	48.4±15.5	48.3±16.4	50.6±13.1	46±17.4	0.697
CK-MB peak (ng/mL)	247.5±209.9	261.4±181.46	202.7±131.4	281.1±292.3	0.524
CRP	8.6±13.9	13.0±21.2	4±6.2	8.9±8.7	0.157
LVEF (%)	55.2±7.3	53.8±8.07	55.2±6.9	56.5±7.1	0.568
Medication					
Statin, n (%)	52 (100)	17 (100)	18 (100)	17 (100)	NS
Beta-blocker, n (%)	49 (94.2)	16 (94.1)	16 (88.9)	17 (100)	0.765
ACEI/ARB, n (%)	42 (80.8)	11 (64.7)	15 (83.3)	16 (94.1)	0.102
Duration of hospitalization (days)	9±2.8	9±2.5	9±2.9	10±2.9	0.692
High MPV	37 (71.2)	10 (58.8)	11 (61.1)	16 (94.1)	0.037

BMI - body mass index; TIA - transient ischemic attacks; BNP - brain natriuretic peptide; FBG - fasting blood glucose; TG - triglyceride; LDL-C - low density lipoprotein C; HDL - high density lipoprotein; TCHO - total cholesterol; HGB - hemoglobin; WBC - white blood cell count; PLT - platelet count; MPV - mean platelet volume; TnT peak - troponin T peak; CK-MB - creatine kinase-MB; CRP - C-reactive protein; LVEF - left ventricular ejection fraction; ACEI - angiotensin-converting enzyme inhibitors; ARB - angiotensin receptor blocker; Q1: < 7.18, Q2: 7.18–11.31, Q3: > 11.31.

lower two tertiles (≤ 11.31). Compared with the lower tertile group, patients in the higher tertile group had a significantly higher mean platelet volume (MPV). There was no significant difference between the other variables. Table 2 displays the characteristics of coronary artery lesions and the procedural characteristics of PPCI. Ninety-four percent of patients with TIMI 0/1 grade on admission were admitted. Since 53.8% of the patients used tirofiban, only two patients underwent thrombus aspiration. Nearly half of the target lesions were at the proximal LAD artery. The incidence of angiographic NRP was 38.5%. Patients with higher microRNA-660-5p levels had a significantly higher incidence of NRP (Fig. 1).

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Table 2. Coronary artery lesions and procedural characteristics according to microRNA-660-5p tertiles

	All patients (52)	Q1 (n=17)	Q2 (n=18)	Q3 (n=17)	*P* value
Bifurcation, n (%)	6 (11.5)	1 (5.9)	2 (11.1)	3 (17.6)	0.561
Proximal LAD, n (%)	26 (50)	8 (47.1)	9 (50)	9 (52.9)	-
Syntax	16±4.5	18±5.3	14±3.7	15±3.7	0.020
Tirofiban infusion, n (%)	28 (53.8)	10 (58.8)	7 (38.9)	11 (64.7)	0.304
Pain to balloon time (min)	120.6±53.1	119±48.9	118±53.3	124±59.5	0.950
Thrombus-aspirating device usage, n (%)	2	1 (5.9)	1 (5.6)	0 (0)	-
Preoperative TIMI 0/1, n (%)	49 (94.2)	16 (94.1)	17 (94.4)	16 (94.1)	-
Preoperative MBG 0/1, n (%)	19 (36.5)	5 (29.4)	4 (22.2)	10 (36.5)	0.689
Mean diameter of stents, mm	3.0±0.37	3.4±0.45	3.2±0.49	3.3±0.47	0.748
Total length of stents, mm	22.7±6.83	20.1±5.32	20.5±5.54	23.6±7.67	0.235

TIMI - thrombolysis in myocardial infarction; LAD - left anterior descending coronary artery; MBG - myocardial blush grade; Q1: <7.18, Q2: 7.18–11.31, Q3: >11.31

Table 3. Relationship between microRNA-660-5p and coronary no-reflow phenomenon

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	Adjusted OR	95% CI	*P* value
PLT	0.99	0.98-1.00	0.125	0.99	0.98-1.04	0.212
LVEF	0.98	0.908-1.06	0.624	0.97	0.89-1.06	0.481
TnT peak	0.99	0.96-1.03	0.742	0.99	0.94-1.03	0.504
Tirofiban infusion, n (%)	1.5	0.48-4.65	0.483	1.15	0.27-4.84	0.852
Pain to balloon time (min)	0.99	0.98-1.00	0.27	0.99	0.98-1.01	0.213
MicroRNA-660-5p	1.3	1.10-1.56	0.003	1.34	1.10-1.63	0.004
High microRNA-660-5p	5.3	1.52-18.50	0.009	5.68	1.40-23.07	0.015
Q1	-	-	-	-	-	-
Q2	1.25	0.27-5.73	0.77	1.3	0.25-6.67	0.752
Q3	5.96	1.33-26.66	0.02	6.52	1.24-34.16	0.027

OR - odds ratio; CI - confidence interval; PLT - platelet count; LVEF - left ventricular ejection fraction; TnT peak - troponin T peak; Q1: <7.18, Q2: 7.18–11.31, Q3: >11.31

Table 4. Correlation between admission microRNA-660-5p and other parameters

	r	*P* value
PLT	-0.85	0.549
MPV	0.567	<0.001
WBC	0.068	0.633
CRP	0.045	0.749

PLT - platelet count; MPV - mean platelet volume; WBC - white blood cell count; CRP - C-reactive protein

As shown in Table 3, patients with higher microRNA-660-5p levels had a higher risk of NRP than those with lower microRNA-660-5p levels [odds ratio (OR)=1.30, 95% confidence interval (CI) 1.10–1.56, p=0.003]. In the multivariate analysis, microRNA-660-5p remained a strong predictor of angiographic NR (OR=1.34, 95% CI 1.10–1.63, p=0.004) after adjusting for age, sex, BMI, patients who are current smoker, those with hypertension, those with diabetes, and those who receive statin. Patients with higher microRNA-660-5p levels had a nearly 6-fold higher risk of NRP than those with lower microRNA-660-5p levels (OR=5.68, 95% CI 1.40 to 23.07, p=0.015). When analyzed by tertiles, consistent trends of increasing relative odds of angiographic NR were reported (OR1 for Q2 vs. Q1: 1.25, 95% CI: 0.27–5.73, p=0.770; OR2 for Q3 vs. Q1: 5.96, 95% CI: 1.33–26.66, p=0.020).

We also observed a significant correlation between miRNA-660-5p and MPV (p<0.001), while there was no correlation with other markers measured on admission (Table 4, Fig. 2).

As shown in Figure 3, the receiver operating characteristic (ROC) curve analysis demonstrated that microRNA-660-5p had moderate accuracy for predicting the incidence of the NRP in patients undergoing PPCI. The area under the ROC curve (AUC) was 0.768 (95% CI 0.636–0.890; p=0.001). The best microRNA-660-5p level for predicting the NRP in patients undergoing PPCI was 10.17, which exhibited the highest sensitivity (70%) and specificity (75%). The ROC curve analysis was also performed to determine the accuracy of MPV in predicting the incidence of the NRP and the AUC was 0.635 (95% CI 0.340–0.830; p=0.768).

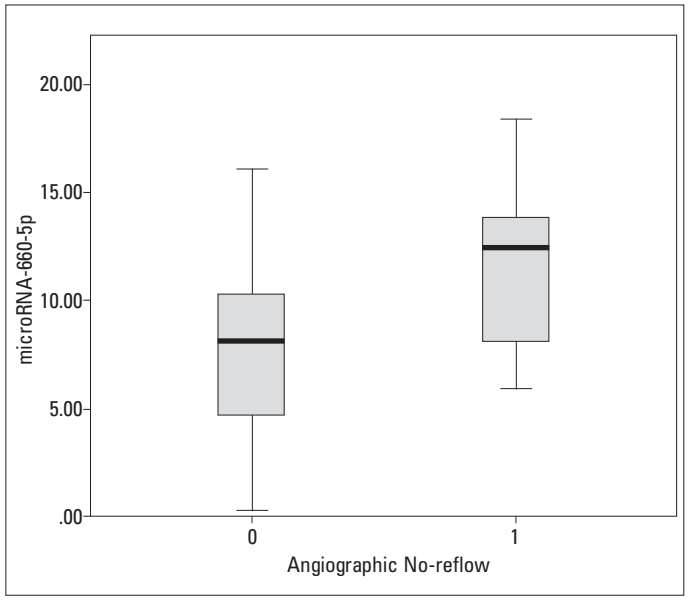

Figure 1. Comparison of miR-660-5p levels according to angiographic no-reflow phenomenon

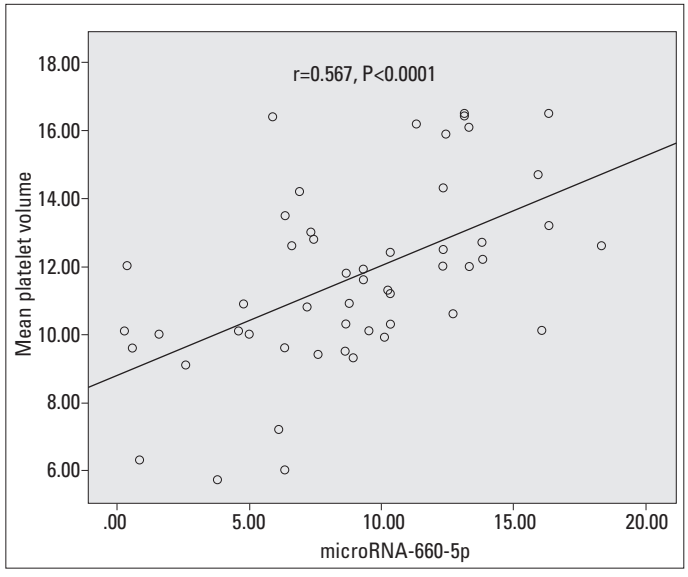

Figure 2. Correlation between miR-660-5p levels and mean platelet volume

Furthermore, when we combined mitral valve prolapse with miR-660-5p to analysis, the AUC was changed to 0.822 (95% CI 0.643–1.000; *p*=0.822), therefore suggesting the incremental prognostic value in measuring miR-660-5p on top of MPV.

Discussion

To the best of our knowledge, this is the first study to report that circulating miR-660-5p was significantly associated with NRP in patients with STEMI undergoing PPCI. Moreover, we also found that miR-660-5p was closely related to MPV. Many studies have shown that several microRNAs may be involved in coronary plaque rupture and local thrombus formation. Since serum is easily measured, microRNAs may serve as disease

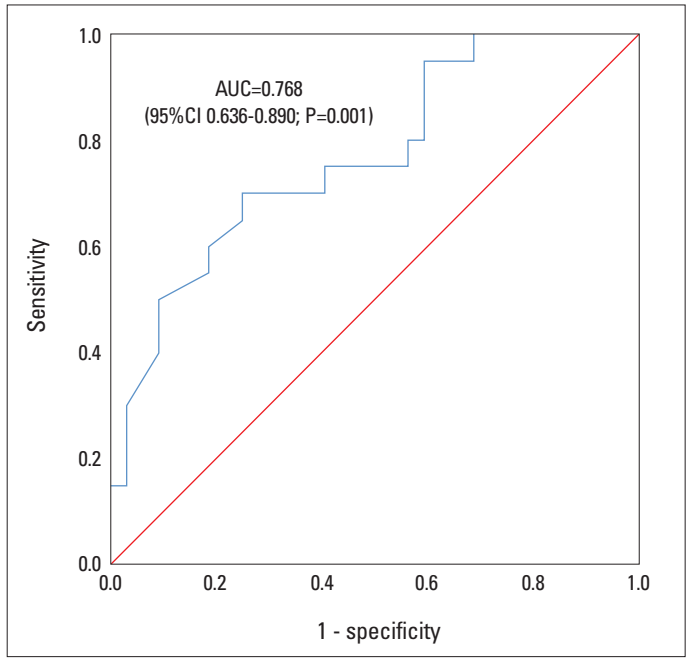

Figure 3. Receiver operating characteristic (ROC) curves of microRNA-660-5p as a marker to predict no-reflow phenomenon

biomarkers of AMI (16), with miR-660-5p as a promising biomarker of NRP.

Earlier studies have demonstrated that miR-660-5p differed significantly between patients who suffered from fatal AMI within 1–10 years and healthy controls, which was similar to our results (17). In addition, miR-660-5p has been significantly associated with adverse cardiovascular outcomes (cardiogenic death or recurrent myocardial infarction) in patients with STEMI, and it is an independent predictor of major adverse cardiovascular events (MACE) in patients with STEMI (9). However, the pathogenetic mechanisms, particularly NRP regulation, remain unclear.

Although the pathophysiology of NRP is not completely understood, several theories including reperfusion injury, distal thromboembolism with PCI, microvascular arterial spasm, and endothelial dysfunction have been proposed (18). Platelets play a key role in the occurrence of NRP. As platelet turnover is increased in STEMI, newly formed platelets with transient expression of an inducible COX-2 enzyme may be released into the circulation, leading to thromboxane A2 production in amounts sufficient to initiate platelet aggregation (19). Platelet aggregation has been significantly correlated to coronary microvascular blood flow reduction (20) and to impaired reperfusion and more frequent NRP in patients treated with PPCI (21). Oxidative stress has also been associated with the development of microvascular obstruction. Specifically, sustained levels of NOX2, the catalytic subunit of nicotinamide adenine dinucleotide phosphate oxidase that is released by platelet activation, result in a vicious cycle of platelet aggregate stabilization and thrombus growth that contributes to CNR (22). Huczek et al. (23) found that patients with higher platelet reactivity assessed by PFA-100 had a significantly higher percentage of angiographic

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NRP that those with lower platelet reactivity. Aurigemma et al. (24) reported that platelet activation in patients with coronary artery microvascular occlusion was significantly higher than that in patients with complete myocardial reperfusion. Thromboxane A2 (TXA2) is an important medium not only for platelet activation and aggregation but also for platelet-induced coronary artery contraction. Niccoli et al. (25) concluded that the plasma TXA2 level was an independent predictor of coronary angiography NR and lack of ST segment resolution after PPCI. In experimental studies, miR-660-5p can increase the production of active platelets (10) and has a positive effect on megakaryopoiesis and the output of activated platelets (26). miR-660-5p has been speculated to regulate 2644 high confidence targets (27), among which genes of the "enzyme-linked receptor signal pathway," "HDAC class I mediated signal event," and "glycosphingolipid biosynthesis" were significantly enriched. Nineteen genes including GATA1, TAL1, TESC, and IL-11, which are well-known regulators of megakaryocyte differentiation, are considered regulatory genes of megakaryopoiesis. The overexpression of miR-660-5p enables the colony forming unit (CFU)-MKs to increase by 3.2 times and the percentage of hyperploid cells from 4% in the control group to 11% in the overexpression group (10). Although miR-660-5p could not increase the total number of cells, it increased the proportion of polyploid cells and the number of activated platelets.

Moreover, we also found that miR-660-5p was closely related to MPV. The incidence of NRP after PPCI was significantly higher in patients with a high MPV (≥10.3 fl) than in patients with a low MPV (<10.3 fl) (21). Furthermore, MPV was significantly associated with coronary NRP and mortality. Larger platelets are more active in metabolism and enzymes than smaller platelets. Larger platelets have been shown to produce more prothrombotic factors and more dense granules and aggregate preferentially and more rapidly (28).

In summary, these findings suggest that circulating miR-660-5p is significant in NRP as it results in increased production of active platelets and MPV. It may be considered for early evaluation of NRP in PPCI. Moreover, these indicate that incorporating circulating miR-660-5P levels into clinical decision making has the potential to guide treatment more accurately. Therefore, further studies to identify the optimal cut-off values and to clarify possible other mechanisms are necessary.

Study limitations

This study had some limitations. First, the sample size was small; thus, larger sample size, multicenter, prospective studies are needed to confirm our results. Second, the lack of collection and analysis of miR-660-5p levels in a normal population rendered our study less rigorous. Third, the potential mechanism of the correlation between miR-660-5p and NRP has not been elucidated and needs to be fully clarified through further basic research. Finally, because our study was an observational cohort study, there might be some confounding factors that have not been collected and adjusted, such as intraoperative medication.

Conclusion

In conclusion, circulating miR-660-5p was significantly associated with NRP in patients with STEMI undergoing PPCI, and it may be a useful biomarker to predict the incidence of NRP in patients with STEMI undergoing PPCI.

Ethics approval and consent to participate: This study was approved by the Institutional Medical Ethical Committee of Beijing Anzhen Hospital. All patients provided written informed consent.

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