Circulating miR-660-5p is associated with no-reflow phenomenon in patients undergoing primary percutaneous coronary intervention

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Research article

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Abstract

Background No-reflow phenomenon (NRP) is an important factor affecting the prognosis of patients with acute myocardial infarction undergoing primary percutaneous coronary intervention (PPCI). This study aims to investigate the association of circulating miR-660-5p with NRP in ST segment elevation myocardial infarction (STEMI) undergoing PPCI.

Methods 52 eligible consecutive patients diagnosed with anterior STEMI within 12h of pain onset, which coronary angiography confirms that infarct-related artery is left anterior descending artery (LAD), were included in this study. Angiographic NRP is define as the final TIMI flow of 2 or the final TIMI flow of 3 with myocardial blush grade (MBG) of <2.Effect of circulating miR-660-5p on NRP was assessed using Spearman correlation analysis and multiple linear regression analysis.

Results The incidence of NRP was 38.5%. Patients with higher miR-660-5p levels have significantly higher incidence of coronary NRP. At multivariable analysis, circulating miR-660-5p remained an independent predictor of NRP (odds ratio [OR]=1.34, 95%CI 1.10 to 1.63, p=0.004). Patients in higher microRNA-660-5p levels group have almost a 6-fold higher risk of NRP than that in lower microRNA-660-5p group (OR=5.68, 95%CI 1.40 to 23.07, p=0.015). When analyzed by tertiles, consistent trends of increasing relative odds of NRP were reported (OR1 for Q2 VS. Q1: 1.25,95%CI: 0.27-5.73, p=0.77;OR2 for Q3 VS. Q1: 5.96,95%CI: 1.33-26.66, p=0.02). Circulating miR-660-5p was related to MPV significantly.

Conclusion Circulating miR-660-5p is an independent predictor of NRP in STEMI patients undergoing PPCI.

Background

China PEACE study have showed that patients with acute myocardial infarction (AMI) undergoing primary percutaneous coronary intervention (PPCI) increased significantly over the past 10 years in China. However, there was no significant improvement in in-hospital mortality in patients with AMI [1]. One of the important reasons for poor prognosis was the occurrence of no-reflow phenomenon (NRP) during PPCI. Nearly 40% of ST segment elevation myocardial infarction (STEMI) patients could not get complete myocardial reperfusion by ST segment resolution or myocardial staining grading (MBG) analysis though successful treatment of the culprit lesion [2–3]. NRP is a phenomenon that effective blood perfusion can't be obtained in ischemic myocardium after the opening of the occluded vessels, in addition to the existence of mechanical obstruction such as coronary dissection, embolism, thrombus, coronary spasm or stenosis. Previous studies have confirmed that NRP is closely associated with in-hospital mortality, malignant arrhythmia and heart failure [4–6]. The causes of NRP are extremely complex and it is an urgent scientific problem to explore its pathogenesis and seek effective intervention.

MicroRNAs (miRNAs) are important posttranscriptional regulators of numerous biological processes including cell growth, proliferation, differentiation, and apoptosis [7]. Recently many studies reported that

circulating microRNAs can be considered as biomarkers in cardiovascular disease [8]. MiR-660-5p, a member of the microRNA family, has been shown to be positively associated with adverse cardiovascular outcome in STEMI patients[9]. For miR-660, overexpression of miR-660 increased production of active platelets in vitro[10], indicating a potential role for NRP. However, there is no report 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 no-reflow phenomenon in STEMI patients undergoing PPCI.

Methods

From June 2017 to May 2018, we recruited patients diagnosed with anterior STEMI treated with PPCI within 12h of pain onset. STEMI was diagnosed with prolonged chest pain (≥30 min) and ST-segment elevation≥0.2mv in two or more adjacent leads[11]. patients were finally included when coronary angiography confirms that the infarct-related artery (IRA) is left anterior descending artery. Patients who met the following criteria were excluded: age≥85years, previous myocardial infarction and PCI history, long-term oral anticoagulation drugs, acute infections, malignancy, severe liver and kidney dysfunction, chronic inflammatory diseases, and overt heart failure (Killip III or IV). All patients were treated with aspirin (300 mg) and clopidogrel (600 Mg) on admission. The study was approved by the Ethics Committee of the Catholic University, and all patients agreed to use part of their blood for scientific purposes.

PPCI operation and perioperative medication were performed in accordance with the relevant clinical guidelines [12-13]. All PPCI operations were performed by 6 or 7 Fr guiding catheters through transradial approach. A bolus of 70-100 IU/Kg of heparin was given. 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 LAD was treated, and non-IRA were treated after 3 months if necessary. Glycoprotein IIb/IIIa inhibitor and thrombus aspiration device were determined by the operator. Intracoronary nitrate was always given after revascularization.We define the angiographic no-reflow as the final TIMI flow of 2 or the final TIMI flow of 3 with myocardial blush grade (MBG) of ≤2[14]. The success of PPCI was defined as≤30% residual stenosis after recanalization of LAD. A high miR-660-5p group was defined as a value in the third tertile .

Venous peripheral blood was collected before PPCI and centrifuged with at 3500 g for 5min. After that, it was stored at -80 °C centrifuge tube without RNA enzyme. The total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and purified using mirVana PARIS kit (CWbio.Co.Ltd, Cat# CW0581) and cel-miR-39 was used as the external reference. NanoDrop ND-1000 (Thermo Scientific, Inc.) was used to evaluate the concentration and purity of RNA samples. The TaqMan MicroRNA Reverse Transcription Kit (CWbio.Co.Ltd,Cat#CW2141) was used for reverse transcription. The upstream primers of mir-660-5p were 5-TACCCATTGCATATCGGAGT-3, the downstream primers were 5-GCCAACCGAGAAGATGATG-3, the upstream primers of U6 were 5-GCTTCGGCAGCATCATACTAAATAAT3,

the downstream primers were 5-GCTTCACAATTGGCGTGCGTCATCATCATCAT3, and the internal parameters were U6. AB7500 fluorescence quantitative PCR instrument and UltraSYBR Mixture (With ROX) (CWbio.Co.Ltd, Cat# CW0956) were used for real-time quantitative PCR. The operation has followed the manufacturer's protocol [15]. 2-△△CT method was used to calculate the relative expression of RNA samples.

Statistical analysis

Continuous data were displayed by mean ± standard deviation (SD). Groups of continuous data were compared by one-way analysis of variance (ANOVA) or kruskal-wallis *H* test. The association between circulating miR-660-5p and other parameters was determined by Pearson test or Spearman test. Multivariable logistic regression analysis was applied to identify whether miR-660-5p is independently associated with NRP. Variables with unadjusted p-value <0.1 in univariate analysis and that known to have a significant effect on NRP were entered in the multivariate model. All statistical analyses were performed using SPPS 24.0 software (IBM Corp., Armonk, NY, USA). A P-value of 0.05 was required for statistical significance and all tests were two-tailed.

Results

52 eligible patients were included in this study. Table 1 shows the baseline characteristics of patients according to tertiles of miR-660-5p levels. The mean age was 56 years old, 53.8% of whom were over 65 years old, and 76.9% of them were males. Patients were divided into tertiles by miR-660-5p (Q1: ≤ 7.18, Q2: 7.18-11.31, Q3:11.31). A high miR-660-5p group was defined as a value in the third tertile (11.31), while a low microRNA-660-5p was defined as a value in the lower two tertiles (≤11.31).Compared with lower tertile group, patients in the higher tertile have significantly higher mean platelet volume (MPV). There was no significant difference between other variables.

Table 1

Baseline characteristics of patients according to tertiles of miR-660-5p levels

	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)	-
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 (mmHg)	120 ± 16.9	119 ± 14.9	119 ± 16.3	121 ± 20.0	0.949
Diastolic blood pressure (mmHg)	77 ± 12.7	75 ± 11.0	76 ± 12.6	79 ± 14.7	0.626
Hypercholesterolaemia, 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 class, n (%)					
Killp class Ⅰ	19(36.5)	4(23.5)	8(44.4)	7(36.5)	0.448
Killip class□□	33(63.5)	13(76.5)	10(55.6)	110(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

Data given as mean ± SD or n (%). Q1: ≤7.18, Q2: 7.18–11.31, Q3: ≥11.31.

BMI: Body Mass Index; TIA: transient ischemic attacks; BNP: brain natriuretic peptide;FBG: fasting blood glucose; TG: Triglyceride; LDL-C: Triglyceride; HDL: High density lipoprotein; TCHO: Total cholesterol; HGB: Haemoglobin; 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;

	All (52)	Q1(n = 17)	Q2(n = 18)	Q3(n = 17)	P value
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 (10^9 L-1)	11.2 ± 3.3	11.2 ± 3.2	11.4 ± 3.7	11.1 ± 3.3	0.984
PLT (10^9 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)	0.158
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
Data given as mean ± SD or n (%). Q1: ≤7.18, Q2: 7.18–11.31, Q3: 11.31.					
BMI: Body Mass Index; TIA: transient ischemic attacks; BNP: brain natriuretic peptide;FBG: fasting blood glucose; TG: Triglyceride; LDL-C: Triglyceride; HDL: High density lipoprotein; TCHO: Total cholesterol; HGB: Haemoglobin; 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;					

Table 2 shows the characteristics of coronary artery lesions and the technical characteristics of PPCI. 94% patients with TIMI 0/1 grade on admission were admitted. Since 53.8% of the patients used tirofiban, only 2 patients underwent thrombus aspiration. Nearly half of the target lesions were at proximal of LAD.

The incidence of angiographic NRP was 38.5%. Patients in the higher microRNA-660-5p levels have significantly higher incidence of NRP (Fig. 1).

Table 2

Coronary artery lesions and procedural characteristics according to tertiles of miR-660-5p levels

	All (52)	Q1(n = 17)	Q2(n = 18)	Q3(n = 17)	P value
Syntax score	16 ± 4.5	18 ± 5.3	14 ± 3.7	15 ± 3.7	0.02
Proximal-LAD	26(50)	8(47.1)	9(50)	9(53)	NS
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	0.95
Thrombus-aspirating device usage, n (%)	2	1(5.9)	1(5.6)	0(0)	NS
TIMI 0/1, n (%)	49(94.2)	16(94.1)	17(94.4)	16(94.1)	NS
MBG0/1	19(36.5)	5(29.4)	4(22.2)	10(36.5)	0.689
Post-TIMI	9(17.3)	1(5.9)	1(5.6)	7(41.2)	0.01
Data given as mean ± SD or n (%). Q1: ≤ 7.18, Q2: 7.18–11.31, Q3:□11.31					
LAD: left anterior descending coronary artery; MBG: myocardial blush grade;					

As shown in Table 3, patients with higher microRNA-660-5p levels have a higher risk of NRP than that with lower microRNA-660-5p (odds ratio [OR] = 1.30, 95%CI 1.10 to 1.56, p = 0.003). At multivariable analysis, microRNA-660-5p remained an independent predictor of angiographic NR (odds ratio [OR] = 1.34, 95%CI 1.10 to 1.63, p = 0.004). Patients in the higher microRNA-660-5p levels group have almost a 6-fold higher risk of coronary no-reflow than that in lower microRNA-660-5p group (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 = 0.77;OR2 for Q3 VS. Q1: 5.96□95%CI: 1.33–26.66, p = 0.02).

Table 3
Relationship between miR-660-5p and 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	0.48
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
MiR-660-5p	1.3	1.10–1.56	0.003	1.34	1.10–1.63	0.004
High miR-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.

We also observed significant correlation between miRNA-660-5p and MPV ($P < 0.001$), while correlation did not find with other markers measured on admission (Table 4, Fig. 2).

Table 4
Correlation between circulating miR-660-5p and other parameters

	r	P Value
PLT	-0.85	0.549
MPV	0.567	0.0001
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.

Discussion

In this prospective study, we evaluated the association of circulating miR-660-5p with NRP in STEMI patients undergoing PPCI. To our knowledge, this is the first reported that miR-660-5p was an

independent predictor of NRP in STEMI patients 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. Since serum is easily measured, microRNAs may serve as disease biomarkers of AMI [16]. MiR-660-5p maybe has promise as a biomarker of NRP.

MiR-660-5p has been shown to be associated with adverse cardiovascular outcomes (cardiogenic death or recurrent myocardial infarction) significantly in STEMI patients and it was an independent predictor of major adverse cardiovascular events (MACE) events in STEMI patients [9]. However, the pathogenetic mechanisms remains unclear, especially, how to participate in regulating no-reflow phenomenon.

Although the pathophysiology of NRP is not completely understood, several theories including reperfusion injury, distal thromboembolism with PCI, microvascular arterial spasm, endothelial dysfunction have been proposed [17]. It is well recognized that platelets play a key role in the occurrence of NRP. As platelet turnover is increased in STEMI, newly formed platelets with transient expression of inducible COX-2 enzyme may be released into the circulation, leading to thromboxane A2 production in amounts sufficient to initiate platelet aggregation [18]. Several studies have shown that there is a significant correlation between platelet aggregation and coronary microvascular blood flow reduction [19].The increased platelet volume has been linked to impaired reperfusion, more frequent NRP in patients treated with PPCI [20]. Oxidative stress has also been linked to development of microvascular obstruction. Specifically, sustained levels of NOX2, the catalytic subunit of NADPH oxidase that is released by platelet activation, results in a vicious cycle of platelet aggregate stabilization and thrombus growth that contributes to CNR [21]. Huczek et al found that patients with higher platelet reactivity assessed by PFA-100 have a statistically significant higher percentage of angiography NRP [22]. Aurigemma C et al. reported that platelet activation in patients with coronary artery microvascular occlusion (MVO) was significantly higher than that with complete myocardial reperfusion [23]. Thromboxane A2 (TXA2) is not only an important medium for platelet activation and aggregation, but also an important medium for platelet-induced coronary artery contraction. Giampaolo Niccoli et al. thought that plasma TXA2 level was an independent predictor of coronary angiography NR and lack of ST-segment resolution after PPCI [24].MiR-660-5p have been reported increased production of active platelets in experimental studies [10]. Moreover, miR-660-5p has a positive effect on megakaryopoiesis and the output of activated platelets [25]. MiR-660-5p has been speculated to regulate 2644 high confidence target [26], among which genes of "enzyme-linked receptor signal pathway", "HDAC class I mediated signal event" and " glycosphingolipid biosynthesis" were significantly enriched. 19 genes including GATA1, TAL1, TESC and IL-11, which are well-known regulators of megakaryocyte differentiation, are considered to be regulatory genes of megakaryopoiesis. Overexpression of miR-660-5p can make the colony forming unit (CFU)-MKs increase by 3.2 times and the percentage of hyperploid cells increase 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. So microRNA-660-5p maybe play a role in NFP with increased production of active 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 high MPV (≥ 10.3 fl) than patients with low MPV (< 10.3 fl)[27] .And MPV could be as an independent predictor of coronary no-reflow and mortality. Larger platelets are more active in metabolism and enzymes than smaller platelets. Several studies have shown that larger platelets produce more prothrombotic factors and more dense granules, aggregate preferentially and more rapidly[28].

In summary, these findings suggest that circulating miR-660-5p is of value to NRP. MiR-660-5p maybe play a role in NRP with increased production of active platelets and MPV. It may be considered for early evaluation of NRP in PPCI. Moreover, these findings indicate that incorporating circulating miR-660-5P 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 the possible other mechanisms are necessarily required.

There were some limitations in this study. Firstly, the sample size is small, so a larger sample size, multicenter, prospective studies are needed to confirm our results. Secondly, the lack of collection and analysis of miR-660-5p in normal population may make our study less rigorous. Thirdly, the potential mechanism of the correlation between miR-660-5p and NRP has not been elucidated, which needs to be fully clarified through further basic research.

Conclusion

In conclusion, circulating miR-660-5p is an independent predictor of NRP in STEMI patients undergoing PPCI.

Abbreviations

AMI
acute myocardial infarction
PPCI
primary percutaneous coronary intervention
NRP
no-reflow phenomenon
STEMI
ST segment elevation myocardial infarction
MBG
myocardial staining grading
miRNAs
MicroRNAs
IRA
infarct-related artery

MPV
mean platelet volume
MACE
major adverse cardiovascular events

Declarations

Ethics approval and consent to participate

This study was approved by anzhen Hospital institutional medical ethical committee. All patients provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from Institutional Review Board of the Beijing Anzhen hospital of Capital Medical University but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Institutional Review Board of the Beijing Anzhen hospital of Capital Medical University.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Jianwei Zhang and Lingjie He designed the experiments; Jianwei Zhang and chengping hu performed the experiments; Jianwei Zhang and Yingxin Zhao analyzed the experimental results and wrote the manuscript. Both authors read and approved the final manuscript.

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Figures

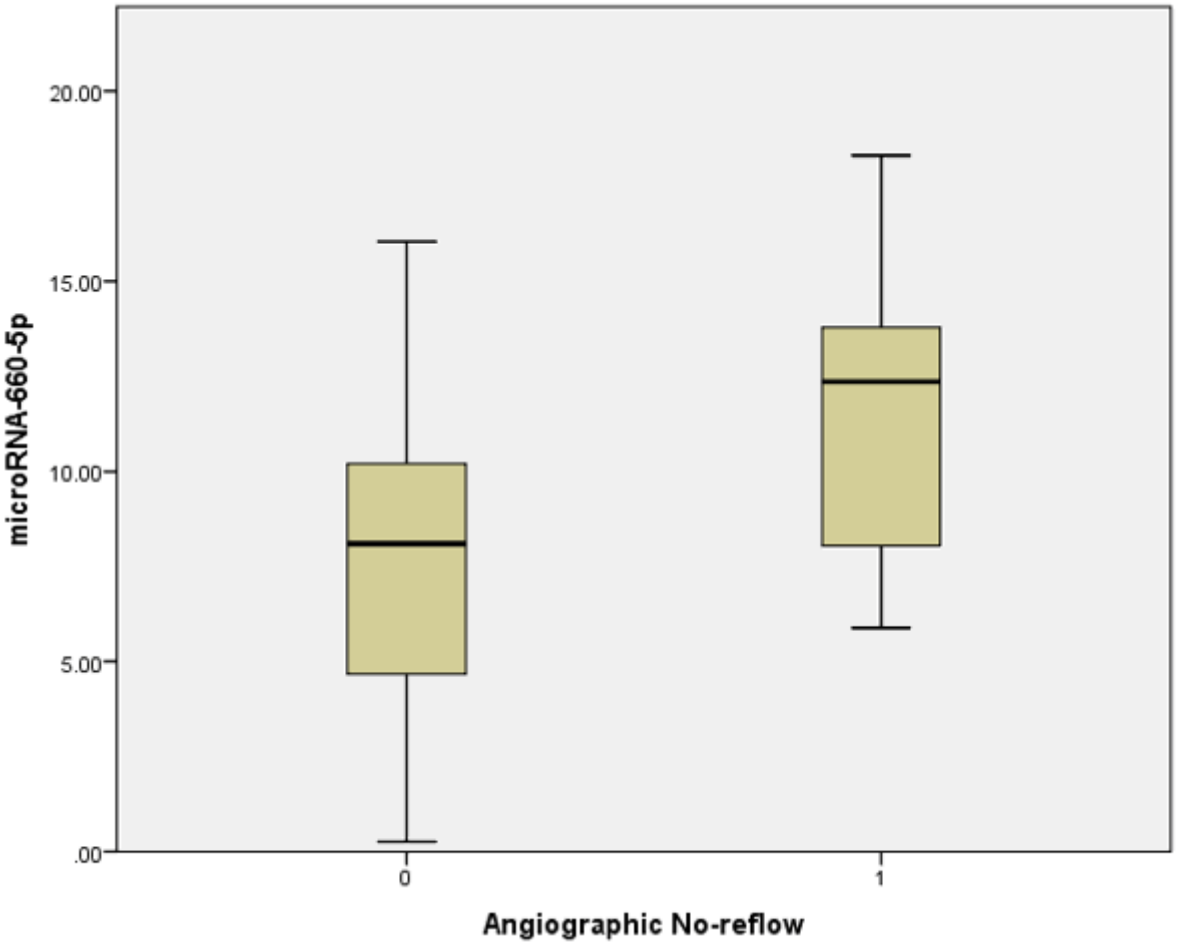

Figure 1

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

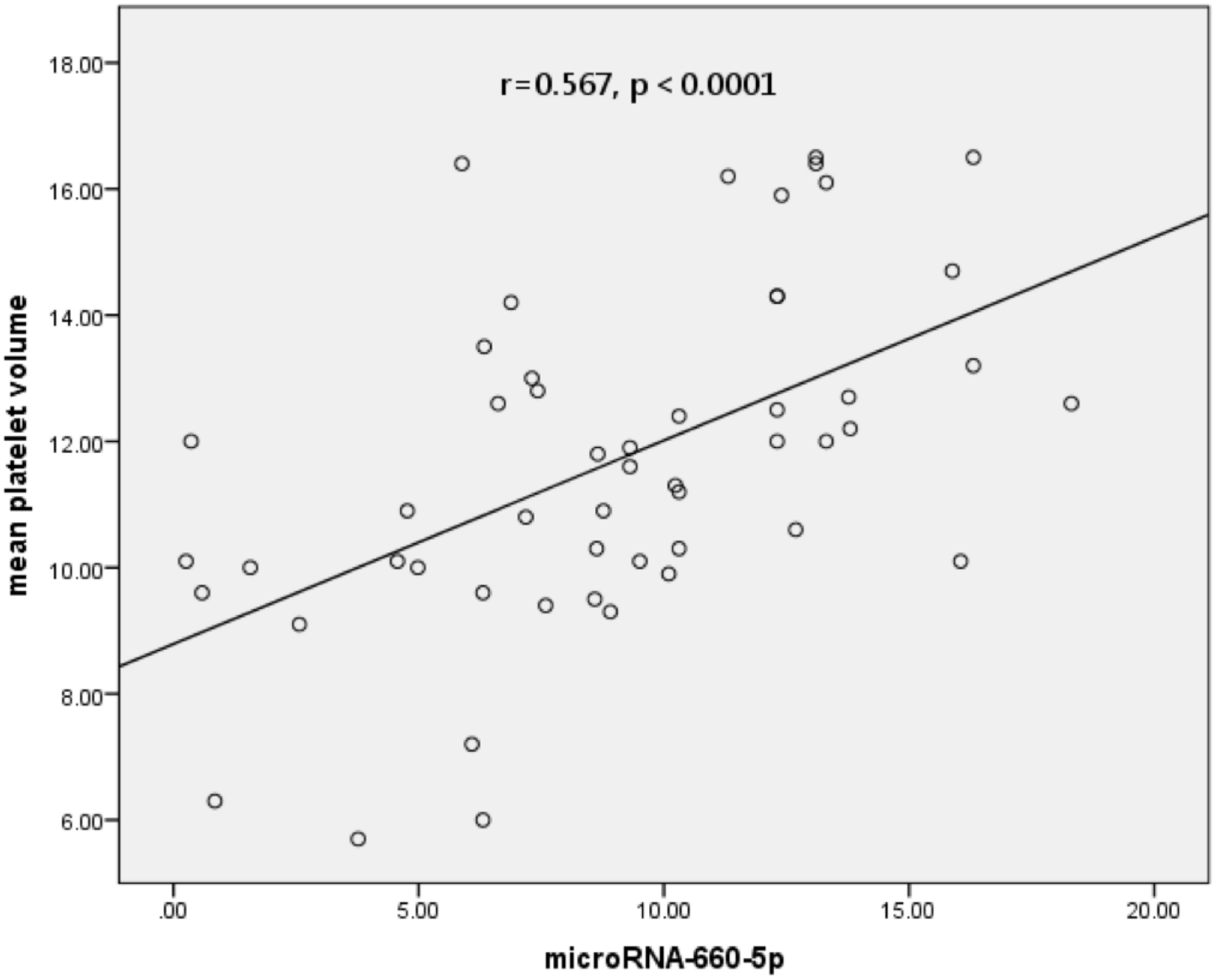

Figure 2

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