Original Article

A Nomogram model for predicting the occurrence of no-reflow phenomenon after percutaneous coronary intervention using the lncRNA *TUG1*/miR-30e/*NPPB* biomarkers

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Background: Studies have shown that percutaneous coronary intervention (PCI) is considered as the essential therapeutic strategy for the patients with ST-segment elevation myocardial infarction (STEMI). However; no-reflow could still occur in a few patients after PCI. Studies have reported that biomarkers related to no-reflow pathogenetic components could play a prognostic role in the prediction phenomenon. Hence, this study explored the establishment of nomogram model for predicting the occurrence of no-reflow phenomenon after PCI using the lncRNA *TUG1*/miR-30e/*NPPB* biomarkers in patients with STEMI after PCI.

Methods: In this observational study, a total of 76 STEMI patients who underwent emergency PCI between January 2018 and December 2021were included. The patients after PCI, were divided into reflow (n=44) and no-reflow groups (n=32). The demographic, environmental and clinical risk factors were assessed and analysed between the groups. Quantitative RT-PCR was used to detect *TUG1*, miR-30e, and *NPPB* messenger RNA (mRNA) expression levels in the plasma of patients after PCI. Bioinformatic methods were used to predict the interaction of the plasma *TUG1*/miR-30e/*NPPB* axis. The risk factors in the no-reflow group were screened using a logistic-regression analysis, and a nomogram prediction model was constructed and validated. Subsequently, a gene set enrichment analysis revealed the function of lncRNA *TUG1*.

Results: Plasma lncRNA *TUG1* and *NPPB* were more highly expressed and miR-30e was more lowly expressed in the no-reflow group than the normal-reflow group (P<0.001). A negative correlation was observed between lncRNA *TUG1* and miR-30e, and between miR-30e and *NPPB*. However, a positive correlation was observed between lncRNA *TUG1* and *NPPB* mRNA. The bioinformatics analysis predicted multiple binding sites on the lncRNA *TUG1* and miR-30e. LncRNA *TUG1* [odds ratio (OR): 0.163, 95% confidence interval (CI): 0.021–0.944] and hs-CRP (OR: 2.151, 95% CI: 1.536–3.974) found to be as independent predictors. The C-index of this prediction model was 0.982 (95% CI: 0.956–1.000).

Conclusions: *TUG1* could function as an effective biomarker for no-reflow among patients with STEMI after PCT and the proposed nomogram may provide information for individualized treatment in patients with STEMI.

Keywords: No-reflow; ST-segment elevation myocardial infarction (STEMI); percutaneous coronary intervention (PCI)

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Introduction

Acute myocardial infarction is currently one of the most lethal diseases. The treatment strategy of choice for patients with ST-segment elevation myocardial infarction (STEMI) is direct percutaneous coronary intervention (PCI). However, despite the opening of the offending vessel, the distal myocardium remains ineffectively perfused in some patients. This is referred to as the no-reflow phenomenon, which can lead to swelling and even irreversible damage to the myocardial cells and can thus seriously affect patients' prognosis (1-3).

A study by Gupta, 2016 reported on the causes and mechanisms of the no-reflow phenomenon after PCI remain inconclusive (4). However, the microvascular embolism/spasm and endothelial injury mechanisms are much related to no-reflow phenomenon (5). Conventional and environmental risk factors such as age, gender, history, smoking, high blood pressure, diabetes, dyslipidaemia are the common risk factor for STEMI (6). The association of dyslipidaemia and diabetes with no-reflow could be due to the spasm and plugging in macrovascular tissues, intravascular thrombus, swelling in endothelial cells, and edema might worsen the intensity of microvascular obstruction leading to both elective and infarct-related PCI (7). C-reactive protein (CRP) is an independent risk predictor in patients with acute coronary disease, and it has been previously shown that PCI has been associated with an early rise in CRP (8).

Recent studies have reported numerous novel and reliable biomarkers have been used in clinical settings (9,10). The novel biomarkers are much needed to integrate within the clinical models to support the clinicians by identifying the patients at high risk for adverse clinical prognosis and provide accurate prognosis estimation with proper prevention program. Regarding to that, we have selected three biomarkers; brain natriuretic peptide precursor B (*NPPB*), taurine upregulated 1 (*TUG1*) and micro-ribonucleic acid-30e (miR-30e) that are related to STEMI (11-13).

The *NPPB* comprises 32 amino acids and is a neurohormone synthesised and secreted by the ventricular muscles as an indicator of ventricular function (14). Studies have shown that polymorphisms in the *NPPB* gene are strongly associated with diabetes, hypertension, myocardial infarction, and heart failure (11,12). The upstream regulatory pathway of *NPPB* is currently unclear and needs to be further explored. The overexpression of *TUG1* suggests a poor prognosis of heart attack (13). At the onset of STEMI, miR-30e, which is a microRNA (miRNA) associated with cardiomyocyte autophagy and apoptosis, was significantly downregulated. There may be a regulatory relationship between *TUG1*, miR-30e, and *NPPB*. Taking this into an account, this study sought to reveal the potential interrelationship between *TUG1*, miR-30e, and *NPPB* markers based on clinical relevance combined with bioinformatic methods to determine the mechanism of the no-reflow phenomenon in patients with STEMI after PCI and to improve the diagnosis and prognosis of the patients. However, the exact mechanism still remains unclear and the studies on risk factors affecting reflow are less in number and inconsistent. Hence, more models to determine the relevant risk factors acting no-reflow are much needed to formulate the preventive measures to treat the patients.

Based on the affecting risk factors, a prediction model; Nomogram is a prediction model selected by multi-factor regression analysis could visualize the contribution of each affecting factor to the outcome events with more accuracy and reliability (15). In this study, a nomogram prediction model was established based on the risk factors affecting no-reflow after PCI in patients. We present the following article in accordance with the TRIPOD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-481/rc).

Methods

Patient information

A total of 76 patients with STEMI at The Second Affiliated Hospital of Nanchang University were enrolled in the study between 2018 and 2021. They were treated with PCI within 12 hours of the onset of the disease. The diagnostic criteria for STEMI were as follows: clinical symptoms of ischaemic chest pain (>30 minutes), creatine kinase-myocardial isoform, and/or cardiac troponin I values above the upper reference limit, and an electrocardiogram showing new ST-segment elevation or new significant LBBB. Patients were excluded from the study if they met the following exclusion criteria: had myocardial infarction due to non-atherosclerotic factors, such as aortic coarctation and acute non-embolism; had angina pectoris or coronary artery spasm with normal coronary flow; had STEMI to interrupt blood flow after invasive operations; had a severe infection and leukopeania or corticosteroid therapy; had hepatic or

renal dysfunction; had cardiogenic shock; had malignant tumours; and/or had other systemic critical diseases. Conventional and the clinical factors were taken from the medical record.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of The Second Affiliated Hospital of Nanchang University (No. 2018-069). Informed consent was obtained from all the patients.

Reflow diagnostics

Coronary flow was comprehensively evaluated before and after PCI according to the thrombolysis in myocardial infarction (TIMI) flow grade (16). The TIMI flow grade classification criteria were as follows: Grade 0, no reperfusion or occlusion, and no flow in distal vascular lesions; Grade 1, partial reperfusion, where the contrast medium penetrated the blockage but did not completely fill the distal coronary artery; Grade 2, partial reperfusion, where the contrast medium filled the entire coronary artery, but the rate of the contrast medium entry and clearance was slower than that of the normal coronary artery; and Grade 3, complete reperfusion, where the contrast medium filled and cleared rapidly in the coronary artery, and the flow rate was consistent with the normal vascular flow rate. Normal reflow was defined as a post-procedural TIMI flow grade of 3 with <20% residual stenosis of the vessels. No-reflow was defined as no significant mechanical obstruction, no significant residual stenosis or entrapment after emergency PCI, but an antegrade flow obstruction (TIMI flow grade ≤2) preventing the effective perfusion of the myocardium with components.

Sample collection

For all the patients, peripheral venous blood was collected within 1 hour of admission before the patient took aspirin, clopidogrel, or heparin, and was analyzed to obtain the complete blood counts and biochemical parameters. These blood samples were collected in tubes containing ethylenediaminetetraacetic acid (BD; Franklin Lakes, NJ, USA) and were centrifuged at 1,900 ×g for 10 minutes at 4 ℃ for 1 hour to remove blood cells. The separated plasma was then centrifuged at 16,000 ×g for 10 minutes at 4 ℃ to remove cellular debris with nucleic acids attached. The centrifuged plasma samples were then transferred to ribonuclease/deoxyribonuclease-free tubes and stored at –80 ℃ for further analysis.

Biochemical parameters

High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), high sensitive CRP, serum creatinine and Cystatin C were measured by Beckman Coulter AU5800 automatic biochemical analyzer.

Quantitative real-time PCR (qRT-PCR)

TRIZOL reagent (Invitrogen; Carlsbad, CA, USA) was used to extract total ribonucleic acids (RNAs). For the quantitative analysis of miR-30e, total RNA was isolated using the miRNeasy plasma kit (QIAGEN; Hilden, Germany). According to the manufacturer's instructions, the total RNA was extracted using stem-loop antisense primer mix and avian myeloblastosis virus transcriptase (TaKaRa; Dalian, China) reverse transcription complementary deoxyribonucleic acid. The SYBR QPCR kit (Toyobo, Osaka, Japan) was then used to perform qRT-PCR on an ABI 7500 real-time PCR system (Applied Biosystems; CA, USA) (17). The primer-pair sequences used were: *TUG1*, 5'-CTGAAGAAAGGCAACATC-3' (forward) and 5'-GTAGGCTACTACAGGATTTG-3' (reverse); miR-30e, 5'-ACACTCCAGCTGGGT GTAAACATCCTTGAC-3' (forward) and 5'-CTCAACTGGTGTCGTGGAGTCGG CAATTCAGTTGAGCTTCCA-3' (reverse); *NPPB*, 5'-CTTTCCTGGGAGGTCGT TCC-3' (forward) and 5'-GTTGCGCTGCTCCTGTAAC-3' (reverse); U6, 5'-CTCGCT TCGGCAGCACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse); and GAPDH, 5'-AAGAAGGTGGTGAAGCAGGC-3' (forward) and reverse, 5'-TCCACCACCCAGTTGCTGTA-3' (reverse). The quantification of messenger RNA (mRNA) and miRNA was performed using the $2^{-\Delta\Delta Ct}$ method and was normalized by glyceraldehyde-3-phosphate dehydrogenase or U6.

Bioinformatics analysis

Bioinformatics methods were used to predict competitive endogenous RNA (ceRNA) interaction relationships. Interaction sites among long non-coding RNA (lncRNA) *TUG1*, miR-30e, and *NPPB* were predicted using RNAhybrid (18) and miRbase (19). The Gene Expression Omnibus (GEO) database was used to retrieve the data

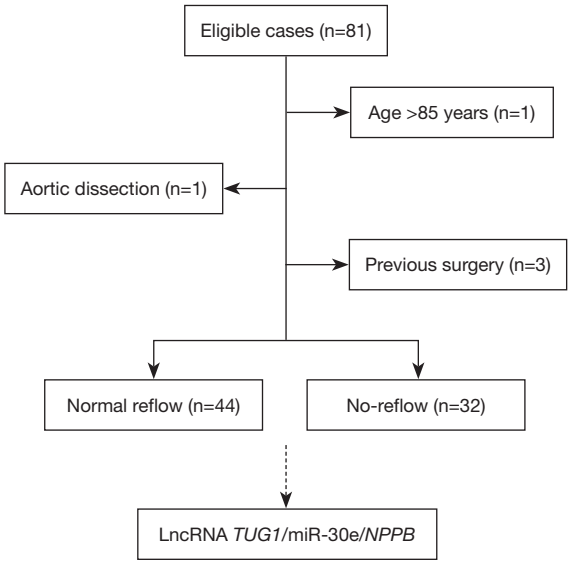

Figure 1 Patient screening flow chart. We screened 81 patients with STEMI treated with PCI. Patients of advanced age (age >85 years), with aortic coarctation, and history of previous surgery were excluded from this study. LncRNA, long non-coding RNA; *NPPB*, natriuretic peptide precursor B; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction; *TUG1*, taurine upregulated 1.

of patients with STEMI undergoing PCI. The retrieved GSE61144 transcriptome data were used to analyze the enrichment pathway of lncRNA *TUG1*. The gene set enrichment analysis (GSEA) of pathways was performed using the cluster Profiler package (20).

Statistical analyses

All the statistical analyses were performed using R software (version 3.6.0). The *t*-test was used to compare the continuous variables between the 2 groups. The chi-square test or Fisher's exact test was used to compare discrete variables. The correlation analysis was performed using the Pearson's test. The receiver operating characteristic (ROC) curves and areas under the curve (AUCs) were used to assess the predictive power of no-reflow occurrences (21). A P value <0.05 was considered statistically significant.

Results

Patient characteristics

Figure 1 illustrates the study flow chart. A total of 76 patients with STEMI were included. Of the patients, 32 had no-reflow and 44 had normal reflow. The basic characteristics of these 2 patient groups are shown in *Table 1*. Among the 44 patients in the normal-reflow group, there were 33 men and 11 women, with a mean age of 54.89±8.97 years. Among the 32 patients in the no-reflow group, there were 23 men and 9 women, with a mean age of 63.00±11.69 years. Compared to the patients in the normal-reflow group, patients in the no-reflow group were older and a significantly higher percentage had a Killip class >2 at admission, multi-vessel disease, a longer pain-to-balloon time, higher levels of blood glucose, and high-sensitivity C-reactive protein (hs-CRP) levels (P<0.05; *Table 1*). Conversely, HDL-C and left ventricular ejection fraction (LVEF) at admission were lower in the no-reflow group than the normal-reflow group (P<0.05; *Table 1*). There were also no significant differences between the 2 groups in terms of gender distribution, history of hypertension, history of diabetes, smoking status, dyslipidemia, body mass index, and other biochemical parameters (P>0.05).

Expression levels of lncRNA TUG1/miR-30e/NPPB between the 2 groups

Based on our findings, miR-30e and *NPPB* expression levels differed between the 2 groups. To investigate the potential role of lncRNA *TUG1*, miR-30e, and *NPPB* in no-reflow PCI, we extracted and isolated plasma from patients at the time of admission. We used qRT-PCR to detect *TUG1*, miR-30e, and *NPPB* mRNA expression levels in plasma after PCI in 44 and 32 patients with normal reflow and no reflow, respectively. As *Figure 2* shows, plasma lncRNA *TUG1* and *NPPB* were more highly expressed in the no-reflow group. In this study, miR-30e was also found to be less expressed in the no-reflow group than the normal-reflow group.

Interaction of plasma TUG1/miR-30e/NPPB axis

Our bioinformatics analysis predicted that lncRNA *TUG1* and miR-30e have multiple binding sites. As *Figure 3A,3B* show, there are binding sites on the structure of lncRNA *TUG1*, miR-30e-3p, and miR-30e-5p. MiR-30e-3p and miR-30e-5p were also found to interact with *NPPB* mRNA. To further validate the interaction between *TUG1*, miR-30e, and *NPPB* mRNAs, we performed a Pearson correlation analysis to examine their expression. As *Figure 3C* shows, we found a negative correlation between lncRNA

Table 1 Basic characteristics of patients included in this study

Characteristics	No-reflow (n=32)	Normal reflow (n=44)	t/χ^2	P value
Age, years	63.00±11.69	54.89±8.97	3.285	0.002**
Male	23 (71.88)	33 (75.00)	0.093	0.760
BMI, kg/m^2	26.71±4.40	25.37±3.84	1.409	0.163
Smoking	13 (40.63)	16 (36.36)	0.143	0.706
Hypertension	14 (43.75)	14 (31.82)	1.134	0.287
Diabetes mellitus	17 (53.13)	14 (31.82)	3.482	0.062
Dyslipidemia	11 (34.38)	11 (25.00)	0.792	0.374
Killip class >2 at admission	6 (18.75)	3 (6.82)	2.526	0.112
Multi-vessel disease	23 (71.88)	19 (43.18)	6.169	0.013*
LVEF on admission	39.69±5.22	50.52±6.05	–8.153	<0.001**
Pain-to-balloon time, min	244.34±60.31	180.45±67.08	4.275	<0.001**
WBC, 10^9/L	6.33±1.64	6.01±1.79	0.792	0.431
Platelet, 10^9/L	252.01±85.52	257.02±87.33	–0.249	0.804
Blood glucose, mmol/L	11.71±1.25	10.83±0.84	3.467	0.001**
HDL-C, mmol/L	1.63±0.40	1.83±0.40	–2.216	0.030*
LDL-C, mmol/L	2.77±0.78	2.52±0.65	1.54	0.128
Serum creatinine, μmol/L	55.32±13.87	56.61±13.11	–0.415	0.679
Cystatin C, mg/L	0.81±0.26	0.90±0.26	–1.533	0.129
hs-CRP, mg/L	25.89±6.53	13.03±2.26	10.686	<0.001**

Data are presented as mean ± standard deviation or number (%). *, P<0.05; **, P<0.01. BMI, body mass index; LVEF, left ventricular ejection fraction; WBC, white blood cell; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein.

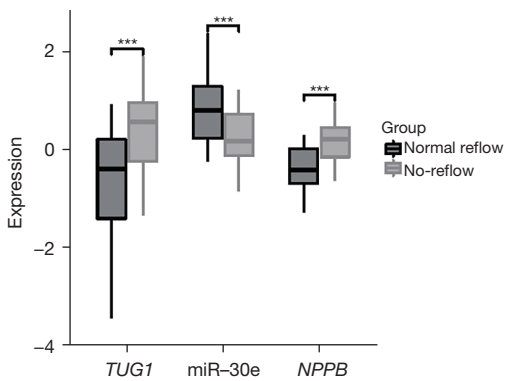

Figure 2 LncRNA *TUG1*, miR-30e, and *NPPB* expression between the no-reflow and normal-reflow patient groups. LncRNA *TUG1* and miR-30e were detected by qRT-PCR. LncRNA *TUG1* and *NPPB* expression were higher in the no-reflow group than the normal-reflow group. MiR-30e expression was lower in the no-reflow group than the normal-reflow group. ***, P<0.001. *NPPB*, natriuretic peptide precursor B; qRT-PCR, quantitative real-time PCR; *TUG1*, taurine upregulated 1; LncRNA, long non-coding RNA.

TUG1 and miR-30e expression in the plasma of patients from the normal-reflow and no-reflow groups. Similarly, there was a negative correlation between miR-30e and *NPPB* expression (*Figure 3D*). However, there was a positive correlation between lncRNA *TUG1* and *NPPB* mRNA expression (*Figure 3E*).

Logistics regression analysis of no-reflow risk factors

We tested different factors as independent variables in the no-flow logistic regression analysis of risk factor prediction. The univariate analysis identified age, LVEF on admission, pain-to-balloon time, multi-vessel disease, blood glucose, HDL-C, hs-CRP, lncRNA, *TUG1*, miR-30e, and *NPPB* as risks (*Table 2*). Among them, advanced age, low LVEF on admission, long pain-to-balloon time, multi-vessel disease, blood glucose, hs-CRP, lncRNA, *TUG1*, and *NPPB* were risk factors for no reflow. Additionally, HDL-C and miR-30e were identified as protective factors for no reflow. We

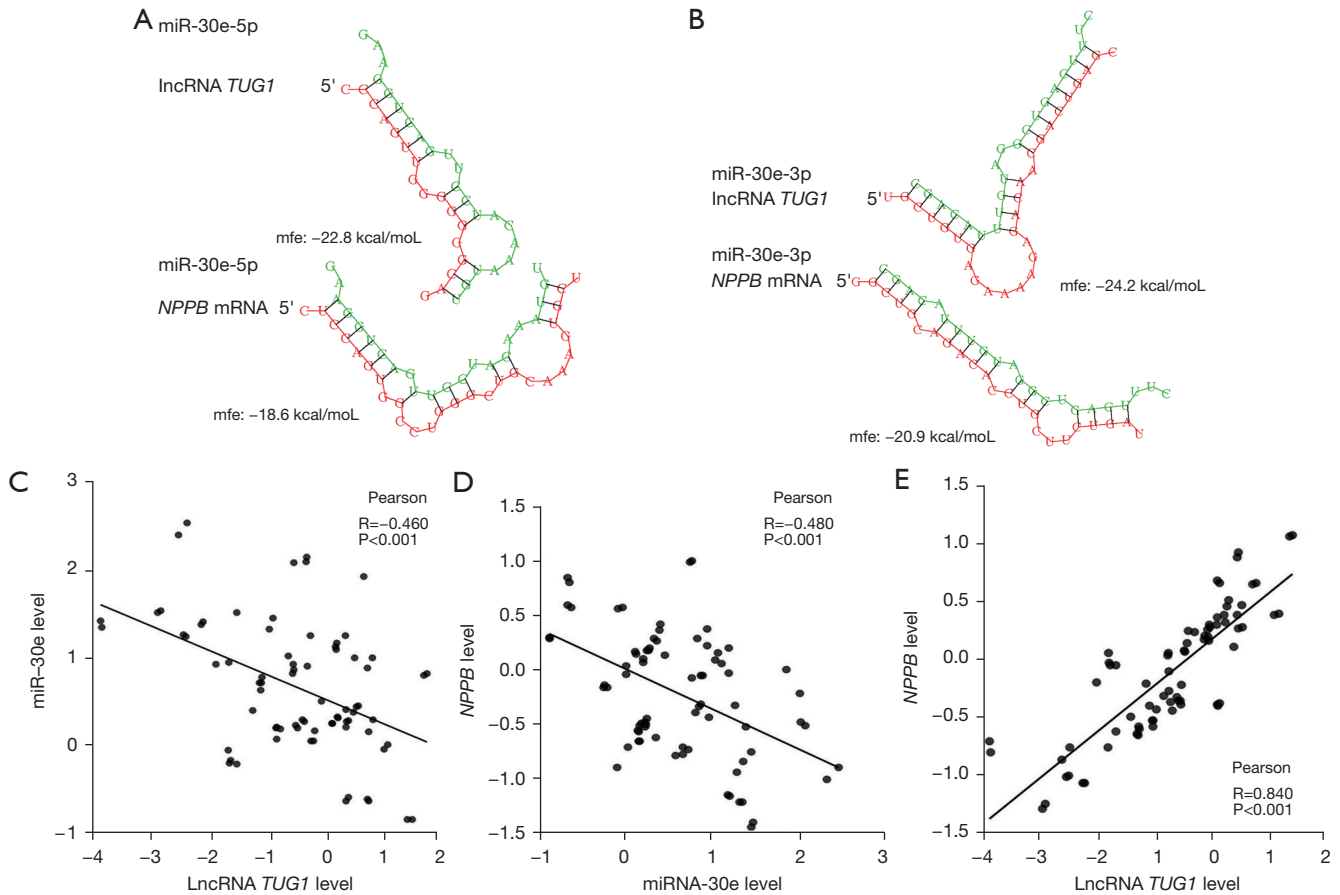

Figure 3 LncRNA *TUG1* may act as a molecular sponge for miR-30e to promote *NPPB* expression. The putative binding sites of miR-30e-5p (A) and miR-30e-3p (B) in lncRNA *TUG1* and *NPPB* mRNA were predicted using RNAhybrid. (C-E) A qRT-PCR analysis was conducted to investigate the correlation between lncRNA *TUG1*, miR-30e, and *NPPB* mRNA levels based on individual samples. Pearson correlation coefficients were used for the statistical descriptions. LncRNA, long non-coding RNA; *TUG1*, taurine upregulated 1; *NPPB*, natriuretic peptide precursor B; mfe, minimum free energy; qRT-PCR, quantitative real-time PCR.

further included hs-CRP, lncRNA, *TUG1*, miR-30e, and *NPPB* as independent variables in a multivariate logistic regression analysis, which identified lncRNA *TUG1* [odds ratio (OR): 0.163, 95% confidence interval (CI): 0.021–0.944] and hs-CRP (OR: 2.151, 95% CI: 1.536–3.974) as independent predictors (*Figure 4A*). A model was constructed based on multifactor logistic regression analysis, and the C-index of the model predicting no reflow was obtained as 0.982 (95% CI: 0.956–1.000). This model was validated by internal sampling, and the C-index obtained was 0.975.

Predictive value of plasma TUG1/miR-30e/NPPB for no reflow

To investigate whether lncRNA *TUG1*/miR-30e/*NPPB* can distinguish between normal reflow and no reflow, we applied logistic regression for the ROC analysis. As *Figure 4B* shows, the AUCs of *TUG1*, miRNA-30e, and *NPPB* in predicting no reflow under a single factor were 0.785, 0.752, and 0.804, respectively. LncRNA *TUG1*, miRNA-30e, and *NPPB* were further co-incorporated into the multifactorial analysis. As *Figure 4C* shows, the predicted AUC of the multifactorial logistic regression model was 0.827 (95% CI: 0.736–0.919). These results suggest that lncRNA *TUG1*, miRNA-30e, and *NPPB* can differentiate between normal reflow and no reflow in STEMI after PCI.

GSEA analysis of lncRNA TUG1

We conducted a GSEA to explore the possible role of the

Table 2 Logistics regression analysis of risk factors associated with no-reflow

Characteristics	OR	95% CI	P value
Male	0.852	0.304–2.426	0.760
Age, years	1.080	1.030–1.139	0.002**
BMI, kg/m^2	1.085	0.969–1.223	0.163
Hypertension	1.667	0.649–4.328	0.289
Diabetes mellitus	2.429	0.957–6.337	0.064
Dyslipidemia	1.571	0.576–4.314	0.375
Smoking	1.197	0.467–3.062	0.706
Killip class >2 at admission	3.154	0.763–15.986	0.126
LVEF on admission	0.647	0.505–0.768	<0.001**
Pain-to-balloon time, min	1.015	1.007–1.024	<0.001**
Multi-vessel disease	3.363	1.300–9.250	0.015*
WBC, 10^9/L	1.116	0.854–1.477	0.426
Platelet, 10^9/L	0.999	0.994–1.005	0.801
Blood glucose, mmol/L	2.343	1.433–4.204	0.002**
HDL-C, mmol/L	0.273	0.077–0.872	0.034*
LDL-C, mmol/L	1.683	0.873–3.414	0.130
Serum creatinine, μmol/L	0.993	0.958–1.027	0.674
Cystatin C, mg/L	0.242	0.036–1.460	0.131
hs-CRP, mg/L	1.939	1.502–2.917	<0.001**
TUG1	3.158	1.796–6.340	<0.001**
miR-30e	0.192	0.070–0.447	<0.001**
NPPB	15.638	4.593–69.602	<0.001**

*, P<0.05; **, P<0.01. OR, odds ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricular ejection fraction; WBC, white blood cell; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; *TUG1*, taurine upregulated 1; *NPPB*, natriuretic peptide precursor B.

independent predictor lncRNA *TUG1*. According to the GSEA, the gene ontology pathways associated with *TUG1* were the regulation of the dopamine receptor signalling pathway, the regulation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, the regulation of oxidoreductase activity, acting on NAD(P)H, oxygen as an acceptor, the positive regulation of vascular endothelial growth factor production, and the negative regulation of cellular response to oxidative stress (*Figure 5A*). The *TUG1*-associated Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways were leukocyte trans-endothelial migration, platelet activation, lipid, and atherosclerosis regulation, regulation of the chemokine signalling pathway, and regulation of actin cytoskeleton (*Figure 5B*). These results suggest that *TUG1* is associated with energy metabolism, vascular endothelial growth, oxidative stress, cellular chemotaxis, and atherosclerotic functions. In summary, *TUG1* plays an important role in no-reflow PCI among patients with STEMI. We also identified a potential lncRNA *TUG1*/miR-30e/*NPPB* pathway that upregulated *NPPB* in no reflow that may play an important role in the phenomenon.

Discussion

This study aimed to investigate the potential regulatory relationship among *TUG1*, miR-30e, and *NPPB*, to screen for no-reflow risk factors through clinical data combined via a bioinformatics analysis, and to develop a nomogram prediction model. The results showed that lncRNA *TUG1*, miR-30e, *NPPB*, and hs-CRP were risk factors for patients with STEMI after PCI no reflow. The clinical prediction model based on lncRNA *TUG1*, miR-30e, *NPPB*, and hs-CRP also had good predictive power. LncRNA *TUG1* and miR-30e expression and miR-30e and *NPPB* expression were both negatively correlated. Conversely, lncRNA *TUG1* and *NPPB* mRNA expression were positively correlated. We also found that the lncRNA *TUG1*/miR-30e/*NPPB* pathway was upregulated during STEMI. Thus, lncRNA *TUG1* may be a molecular sponge for miR-30e to promote *NPPB* expression, ultimately leading to cardiomyocyte injury and the onset of the no-reflow phenomenon after PCI.

LncRNAs regulate genes epigenetically, transcriptionally, post-transcriptionally, transliterally, and post-translationally. *TUG1* is a member of the lncRNA family, which regulates gene expression at multiple levels. *TUG1* reportedly participates in and mediates various biological processes. A study has shown that lncRNA *TUG1* is involved in the progression of multiple cardiovascular-related diseases (22). In the cardiovascular context, *TUG1* promotes hypoxia-induced injury and induces cardiomyocyte death (23). In the present study, we found that *TUG1* was associated with energy metabolism, vascular endothelial growth, oxidative stress, cellular chemotaxis, and atherosclerosis. LncRNA *TUG1* expression was also negatively correlated with miR-30e and was positively correlated with that of *NPPB* mRNA.

MiRNAs are important regulators of cardiac homeostasis and stress responses. By disrupting mRNAs that encode

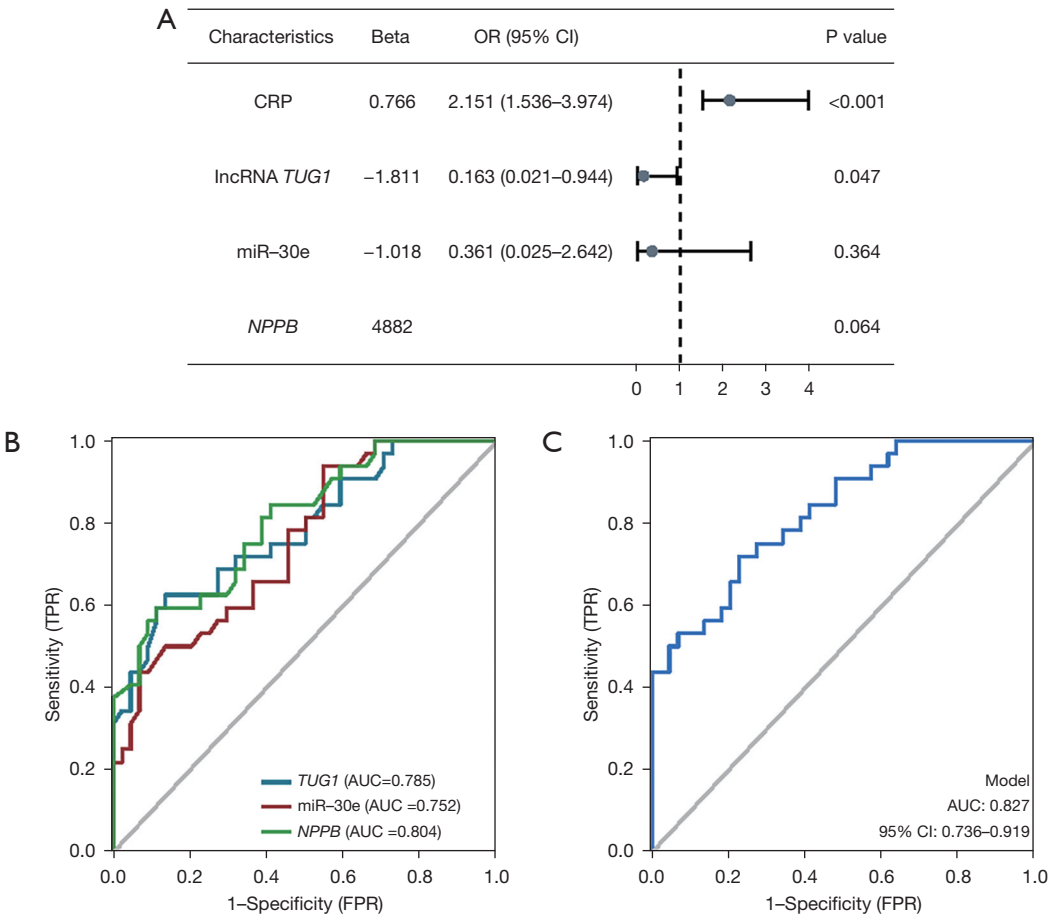

Figure 4 Predictive ability of lncRNA *TUG1*, miR-30e, *NPPB*, and hs-CRP for no-reflow in patients with STEMI after PCI. (A) Multifactorial analysis of lncRNA *TUG1*, miR-30e, *NPPB*, and hs-CRP in predicting no-reflow. (B) Single-factor analysis of lncRNA *TUG1*, miR-30e, and *NPPB* predicts no-reflow. (C) Multifactorial analysis of lncRNA *TUG1*, miR-30e, and *NPPB* predicts no-reflow. OR, odd ratio; CI, confidence interval; CRP, C-reactive protein; lncRNA, long non-coding RNA; *TUG1*, taurine upregulated 1; *NPPB*, natriuretic peptide precursor B; TPR, true positive rate; AUC, area under the curve; FPR, false positive rate; hs-CRP, high-sensitivity C-reactive protein; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention.

proteins in cellular metabolism, growth, calcium signalling, and programmed death pathways, microRNAs exert control over key biological processes, and are important regulators of the stable internal environment of cardiomyocytes (24,25). It has been shown that miR-30e is associated with cardiomyocyte protection after myocardial ischaemia/reperfusion (26). Members of the miR-30 family are abundantly expressed in cardiac tissue and are extensively involved in the pathophysiology of a variety of heart diseases (27-29). The miR-30 family also plays a protective role in diseases, such as myocardial hypertrophy, myocardial infarction, heart failure, and ischaemia-reperfusion injury (30-33). Further, a previous study showed that miR-30e-3p expression was significantly reduced after coronary microembolization (CME) (34). Autophagy is a mechanism that maintains homeostasis in cardiomyocytes (35). MiR-30e-3p has also been found to be involved in CME-induced cardiac dysfunction by possibly regulating myocardial autophagy (34). In the present study, miR-30e expression was found to be lower in the no-reflow group than the normal-reflow group. The bioinformatics analysis also predicted multiple binding sites for lncRNA *TUG1* and miR-30e and found that miR-30e-3p and miR-30e-5p interact with *NPPB* mRNA.

Hs-CRP, a cytokine closely associated with inflammatory responses, is reportedly strongly associated with the

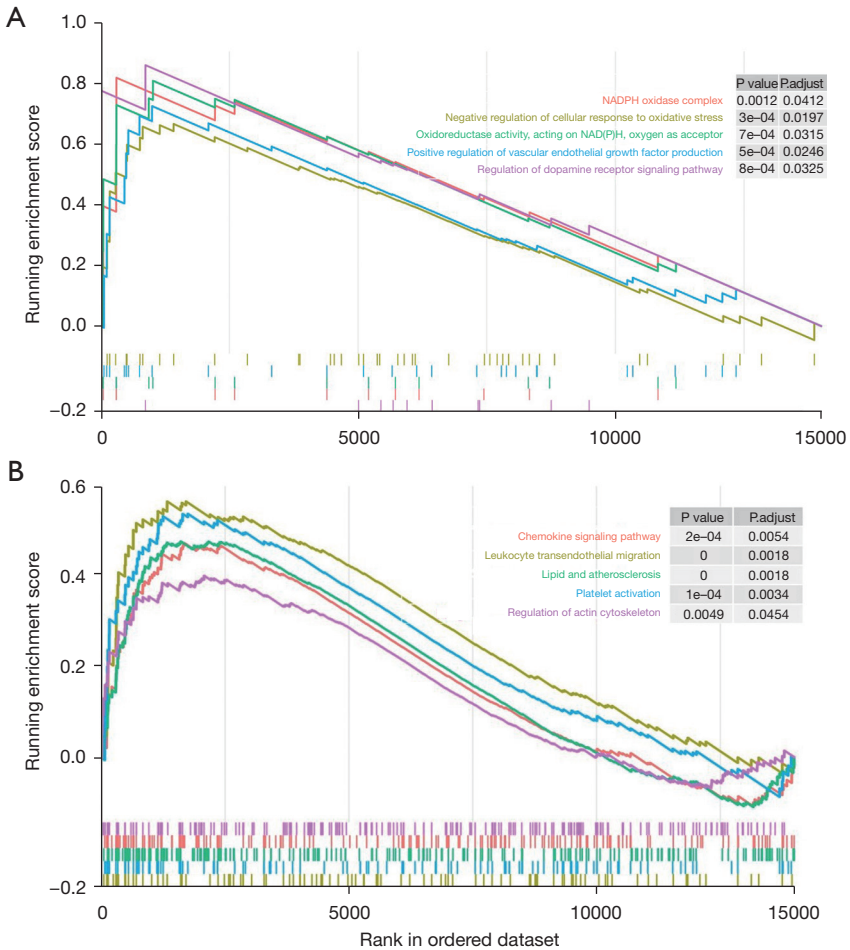

Figure 5 GSEA of *TUG1* in STEMI before and after PCI. In the presence of elevated *TUG1* expression, the enriched GO pathway (A) and enriched KEGG pathway (B) are shown. Vertical lines (middle) mark the positions of pathway genes in the ranked list. GSEA, gene set enrichment analysis; *TUG1*, taurine upregulated 1; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

development of cardiovascular disease and is recognised as a strong predictor of cardiovascular risk events (36,37). In the univariate logistic regression analysis of the present study, hs-CRP, lncRNA, *TUG1*, miRNA-30e, and *NPPB* were identified as risk variables. Further, lncRNA *TUG1* (OR: 0.163; 95% CI: 0.021–0.944) and hs-CRP (OR: 2.151; 95% CI: 1.536–3.974) were identified as independent predictors. However, the present study had a limited number of clinical samples, future prospective studies are needed with large samples, multi-regional, multicentre and multigene studies to verify the reliability of the nomogram's predictive ability.

Conclusions

This study was the first to reveal the potential regulatory relationship among *TUG1*, miR-30e, and *NPPB*. Clinical prediction models based on lncRNA *TUG1*, miR-30e, *NPPB*, and hs-CRP have good predictive power based on clinical data combined via a bioinformatic analysis. The potential mechanism of the no-reflow phenomenon in patients with STEMI after PCI was described, which provides new approaches and new therapeutic targets for the clinical management of STEMI.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-481/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-481/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-22-481/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethics Committee of The Second Affiliated Hospital of Nanchang University (No. 2018-069). Informed consent was obtained from all the patients.

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