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Paraoxonase-1 activity and oxidative stress in patients with anterior ST elevation myocardial infarction undergoing primary percutaneous coronary intervention with and without no-reflow

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ABSTRACT

Background: Reperfusion and ischemic injuries are pathogenetic mechanisms of no-reflow. Oxidative stress plays a critical role during ischemia as well as during the reperfusion phase following ST elevation myocardial infarction (STEMI). We sought to investigate the relationship between no-reflow with paraoxonase-1 (PON-1) activity and oxidative stress markers (total antioxidant capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), lipid hydro-peroxide (LOOH)) in patients with anterior STEMI undergoing primary percutaneous coronary intervention (PCI).

Methods: In this study, 319 consecutive anterior STEMI patients undergoing primary PCI were prospectively included (mean age 56.5 ± 12.5 years). The patients were divided into two groups as normal flow ($n = 231$) and no-reflow ($n = 88$) groups. Serum PON-1 activity was measured spectrophotometrically. TAC and TOS levels were determined by using an automated measurement method. LOOH levels were measured by ferrous oxidation with xylenol orange assay.

Results: PON-1 activity and TAC levels were significantly lower and TOS, OSI and LOOH levels were significantly higher in patients with no-reflow compared to normal flow group ($p < 0.05$, for all). On multivariate logistic regression analysis, PON-1 activity ($\beta = 0.976$, 95% CI = 0.962–0.990, $p = 0.001$) and OSI ($\beta = 1.094$, 95% CI = 1.042–1.148, $p < 0.001$) as well as diabetes, infarction time, thrombus score and initial SYNTAX score were independently associated with no-reflow.

Conclusion: In patients with no-reflow compared with normal flow, oxidants are increased, while serum PON-1 activity and antioxidants are decreased. This result shows that increased oxidative stress has a role in the pathogenesis of no-reflow.

1. Introduction

The no-reflow phenomenon is defined as the inability to reperfuse regions of the myocardium despite removal of a large epicardial coronary artery occlusion [1]. The patients with no-reflow exhibit a higher prevalence of; early postinfarction complications; left adverse ventricular remodeling; late repeat hospital stays for heart failure; and mortality [2]. Ischemic and reperfusion injuries are important pathogenetic mechanisms of no-reflow following ST elevation myocardial infarction (STEMI) [3]. Ischemia results in impaired antioxidant defense and subsequent reperfusion results in an increased concentration of reactive oxygen species (ROS) [3,4].

High-density lipoprotein (HDL) cholesterol exerts cardioprotective properties through its antioxidant activity and anti-inflammatory effects, which is largely maintained by paraoxonase-1 (PON-1) [5]. PON-1 protects lipoproteins against oxidative modification and to hydrolyze hydrogen peroxide, a major ROS produced under conditions of inflammation and atherosclerosis [5].

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Our hypothesis was that oxidative stress parameters and PON-1 activity will be associated with no-reflow phenomenon in patients with STEMI undergoing primary percutaneous coronary intervention (PCI). Therefore, we aimed to investigate PON-1 activity and oxidative stress markers in anterior STEMI patients undergoing primary PCI with and without no-reflow.

2. Methods

2.1. Study population

We prospectively included 319 consecutive patients with anterior STEMI who underwent primary PCI (240 male, 79 female; mean age 56.5 ± 12.5 years) between June 2012 and July 2013. On the basis of post-primary PCI infarct related artery flow, the patients were divided into two groups: no-reflow ($n = 88$) and normal flow groups ($n = 231$). STEMI was defined as resting chest pain lasting ≥30 min, together with new or presumed new ST segment elevation in ≥2 contiguous leads with the cutoff point ≥0.2 mV in anterior leads. The diagnosis was confirmed by coronary angiography in all patients.

Patients with a recent history of myocardial infarction (MI), a previous PCI, a previous coronary artery bypass graft, late presentation (>12 h), unsuccessful primary PCI (residual stenosis >50% in the culprit lesion after procedure), pretreatment with thrombolytic or glycoprotein IIb/IIIa inhibitor therapy before primary PCI, infectious or inflammatory disease, severe liver or renal disease, neoplasm, or hematological disorders were excluded from the study. Patients taking antioxidant drugs such as statins, diuretics, angiotensin-converting enzyme inhibitors (captopril, zofenopril), beta-blocking agents (carvedilol, nebivolol), and vitamins (such as E and C) were excluded from the study. No dietary variation was present between the groups. Patients taking xenobiotics and alcohol were also excluded from the study. Exclusion criteria were applied to all the groups. The Local Ethics Committee approved the study protocol, and each participant provided written informed consent.

After assessment of detailed medical history and a complete physical examination, the baseline characteristics of patients including age, sex, hypertension, hyperlipidemia, diabetes mellitus (DM), current smoking status, family history of coronary artery disease (CAD), body mass index (BMI), and medications were recorded for all patients. Also, the time interval from the onset of symptoms to hospital admission was recorded in all patients. Left ventricle ejection fraction (EF) was measured using Simpson's method according to the suggestions of the American Society of Echocardiography [6].

2.2. Coronary angiography and percutaneous coronary intervention

Urgent diagnostic coronary angiography was performed according to the standard criteria in all patients. To achieve maximal dilatation, each coronary angiogram was preceded by an intracoronary injection of 100 mg nitroglycerine. Significant coronary artery disease was defined as at least 70% luminal diameter stenosis in at least one epicardial coronary artery. Primary PCI procedures were performed using the standard femoral approach with a 7-Fr guiding catheter. All patients were pretreated with loading doses of aspirin (300 mg) and clopidogrel (600 mg); they also received an intravenous bolus of heparin 50 IU/kg. After the guidewire insertion into the infarct related artery (IRA), thromboaspiration (Export™ 6F catheter, Medtronic, Santa Rosa, CA, USA) was performed whenever possible (when the anatomy of the coronary artery – curve and size – allowed it) in all patients with a TIMI Flow 0 and in all patients with a visible thrombus if TIMI Flow was 1 or more. Then, direct stenting was implanted whenever possible; in the remaining cases, balloon pre-dilatation was carried out. Bare-metal stents were mostly used. In each patient treated with tirofiban, it was administered after the primary PCI procedure in the coronary care unit. Baseline and post-primary PCI thrombolysis in myocardial infarction (TIMI) flow grade in IRA and post-PPCI TIMI myocardial perfusion grade (TMPG) were assessed by three independent interventional cardiologists. Intracoronary thrombus in IRA was identified angiographically after wiring of IRA and scored in five grades as described previously [7]. SYNTAX score was calculated as described previously [8].

2.3. The diagnosis of angiographic no-reflow

The diagnosis of no-reflow required the following criteria [2,3]: (a) angiographic evidence of reopening of the occluded coronary artery and successful stent placement with no evidence of flow-limiting residual stenosis (>50%), dissection, spasm, or apparent thrombus; (b) angiographic documentation of a TIMI flow grade of 2 or less, or a TIMI flow grade 3 with a TMPG 0 or 1, at least 10 min after the end of the PCI procedure. A TMPG 0 was defined when contrast failed to enter the vasculature and TMPG 1 was defined when contrast entered slowly, but failed to exit the vasculature [3].

2.4. Blood sampling

Venous blood samples were obtained before primary PCI at admission. Samples were taken from cubital vein into blood tubes and immediately stored on ice at 4 °C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples were stored at −80 °C until analysis of lipid parameters, PON-1 activity, lipid hydro-peroxide (LOOH), total oxidant status (TOS) and total antioxidant capacity (TAC).

Blood counts were measured by a Sysmex K-1000 (Block Scientific, Bohemia, New York) autoanalyzer within 5 min of sampling. Plasma triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose, uric acid, and creatinine concentrations were measured with an automated chemistry analyzer (Abbott Aeroset, Minnesota, USA) using commercial kits (Abbott). Activity of creatine kinase MB (CK-MB) was measured with an assay that uses 2 monoclonal antibodies (CK-MB STAT) on an Elecsys 2010 analyzer (Roche Diagnostics, Basel, Switzerland) by electrochemiluminescence immunoassay. Plasma NT-proBNP was measured by electrochemiluminescence (Roche Diagnostics, Basel, Switzerland). For the measurement of the platelet count and mean platelet volume (MPV), samples were analyzed within 20 min after collection using an automated hematology analyzer Sysmex XT 1800i (Roche Diagnostic, Shanghai, China).

2.4.1. Measurement of serum paraoxonase-1 activity (PON-1)

Measurement of serum PON-1 activity was performed in the absence of NaCl (basal activity). The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbency at 412 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17,000 M^{-1} cm^{-1} [9]. PON-1 activity was expressed as U L^{-1} serum. Coefficient of variation (CV) for measurement of serum PON activity was 2%.

2.4.2. Measurement of lipid hydro-peroxide (LOOH), total oxidant status (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI)

Serum LOOH levels (as a crucial biomarker of oxidative stress) were measured with the ferrous ion oxidation–xylenol orange (FOX-2) assay as previously described [10]. The TAC and TOS levels

of serum were measured as described previously [11,12]. The oxidative stress index (OSI) was calculated as the ratio of TOS to TAC. The unit for total antioxidative capacity of plasma, was expressed as μmol Trolox equivalent/L, and the total oxidant status of plasma as the micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eq/L).

2.5. Statistical analysis

Statistical analysis was carried out using SPSS 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Data are expressed as mean value ± SD. Continuous variables were tested for normality using the Kolmogorov–Smirnov test. An independent-simple *t*-test was used in the analysis of continuous variables. Categorical variables were compared using the Chi-square test. Multivariate, stepwise backward conditional logistic regression analysis was used to determine the independent predictors of no-reflow. All significant parameters in the univariate analysis were selected in the multivariate model. A receiver–operator characteristic curve analysis was carried out to identify the optimal cutoff point of OSI and PON-1 in patients with STEMI and no-reflow. The value of the area under the curve was calculated as a measure of the accuracy of the test. A two-tailed *p*-value of less than 0.05 was considered as significant.

3. Results

In this study, no-reflow and normal flow groups included 88 (27.6%) and 231 (72.4%) patients, respectively. In the no-reflow group, number of patients with angiographic documentation of a TIMI flow grade of 2 or less was 73, and number of patients with TMPG 0 or 1 was 15.

3.1. Baseline characteristics

A comparison of the baseline characteristics is shown in Table 1. Patients with no-reflow were older, had higher rates of female sex, DM, hypertension, and higher values of peak CK-MB, MPV, neutrophil, neutrophil/lymphocyte, NT-proBNP, glucose, and compared with patients in the normal flow group ($p < 0.05$, for all). Also, patients with no-reflow had higher infarction time, and lower values of lymphocyte than patients with normal flow ($p < 0.05$, for all).

3.2. PON-1 activity and oxidative stress parameters of groups

A comparison of the PON-1 activity and oxidative stress parameters of groups were shown in Table 2. Patients with no-reflow group had lower PON-1 activity and TAC values and higher TOS, OSI and LOOH values than normal flow group ($p < 0.001$, for all).

3.3. ST segment resolution and PON-1 activity and oxidative stress parameters (Table 3)

Even with satisfactory angiographic results of primary PCI, 64 of 231 (27.7%) patients had failure of ST segment resolution (STR < 70%). Those patients had also lower PON-1 activity and TAC values and higher TOS, OSI and LOOH values than patients with angiographically normal flow and STR > 70% ($p < 0.001$, for all).

3.4. Angiographic and interventional characteristics (Table 4)

Initial TIMI flow grade, SYNTAX score and thrombus grade score after wiring were different between the groups ($p < 0.05$, for all).

Table 1
Comparison of baseline, laboratory and clinical characteristics of groups.

Variables	Normal flow group (*n*: 231)	No-reflow group (*n*: 88)	*p* value
Baseline characteristics			
Age, years	54.1 ± 11.4	62.6 ± 13.0	**<0.001**
Gender (male)	181 (78.4%)	59 (67.0%)	**0.027**
BMI, kg/m^2	27.1 ± 4.2	27.1 ± 3.9	0.965
Heart rate, beats/min	85.2 ± 15.6	89.7 ± 14.9	0.111
SBP, mm Hg	131.5 ± 26.2	126.9 ± 20.5	0.141
DBP, mm Hg	81.4 ± 15.6	80.0 ± 12.6	0.315
Hypertension, *n* (%)	78 (33.8%)	42 (47.7%)	**0.015**
Diabetes mellitus, *n* (%)	62 (26.8%)	50 (56.8%)	**<0.001**
Smoking, *n* (%)	140 (60.6%)	55 (62.5%)	0.430
Hyperlipidemia, *n* (%)	31 (13.4%)	15 (17.0%)	0.256
Family history, *n* (%)	103 (44.6%)	39 (44.3%)	0.534
Laboratory findings			
Glucose, mg/dl	159.1 ± 69.6	201.6 ± 89.3	**<0.001**
Total cholesterol, mg/dl	204.3 ± 41.0	214.2 ± 46.9	0.194
Triglyceride, mg/dl	160.2 ± 146.9	161.8 ± 146.9	0.954
HDL-C, mg/dl	40.2 ± 10.8	41.7 ± 9.9	0.440
LDL-C, mg/dl	132.1 ± 34.6	140.2 ± 38.6	0.209
Peak CK-MB, ng/ml	181.4 ± 105.2	203.8 ± 102.7	0.090
Creatinine, mg/dl	0.91 ± 0.6	0.89 ± 0.29	0.870
Uric acid, mg/dl	5.4 ± 1.5	5.6 ± 1.6	0.567
Hemoglobin, mg/dl	14.6 ± 1.5	14.7 ± 1.8	0.964
MPV, fL	10.3 ± 0.9	11.2 ± 1.1	**<0.001**
Platelet count, ×10^9/L	256.4 ± 72.8	253.8 ± 96.4	0.856
WBC, ×1000/μL	13.1 ± 4.4	13.6 ± 4.5	0.551
Neutrophil, ×1000/μL	9.7 ± 4.4	11.7 ± 3.6	**<0.001**
Lymphocyte, ×1000/μL	2.5 ± 1.3	1.9 ± 0.8	**<0.001**
Neutrophil/lymphocyte	5.3 ± 3.8	7.3 ± 3.8	**<0.001**
NT-proBNP, pg/ml	618.7 ± 1359.7	1475.7 ± 1835.1	**<0.001**
Clinical characteristics			
Pre-infarct angina, *n* (%)	82 (35.5)	30 (34.1)	0.461
Killip 2–4, *n* (%)	19 (8.2)	9 (10.2)	0.357
Initial IABP, *n* (%)	2 (0.9)	0 (0.0)	0.524
Cardiac arrest, *n* (%)	4 (1.7)	1 (1.1)	0.654
Infarction time, h	4.3 ± 3.1	6.1 ± 3.7	**<0.001**
Door-balloon time, min	26.4 ± 7.5	27.2 ± 7.9	0.437
Ejection fraction, %	43.9 ± 7.2	42.5 ± 5.7	0.293
Previous medications			
Statin use, *n* (%)	31 (13.4)	12 (13.6)	0.545
ACE use, *n* (%)	44 (19.0)	16 (18.2)	0.499
ARB use, *n* (%)	33 (14.3)	15 (17.0)	0.324
OAD use, *n* (%)	56 (24.0)	46 (56.3)	**<0.001**
ASA use, *n* (%)	31 (13.4)	14 (15.9)	0.342
Beta-blocker use, *n* (%)	24 (10.4)	12 (13.6)	0.263

Abbreviations: BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, HDL-C; high-density lipoprotein cholesterol, LDL-C; low-density lipoprotein cholesterol, MPV; mean platelet volume, WBC; white blood cell, NT-proBNP; N terminal pro-brain natriuretic peptide, IABP; intra-aortic balloon pump, ACE; angiotensin-converting enzyme, ARB; angiotensin receptor blocker, OAD; oral anti-diabetic, ASA; acetyl salicylic acid.
Significance *p* values ($p < 0.05$) were indicated in boldface.

3.5. Predictors of no-reflow in anterior STEMI

Multivariate logistic regression analysis showed that diabetes, MPV, ratio of neutrophil/lymphocyte, infarction time, thrombus

Table 2
Comparison of oxidative stress markers and PON-1 activities between the groups.

Variables	Normal flow group (*n*: 231)	No-reflow group (*n*: 88)	*p* value
TAC (μmol Trolox Eq/L)	0.98 ± 0.19	0.87 ± 0.16	**<0.001**
TOS (μmol H_2O_2 Eq/L)	17.1 ± 7.2	25.7 ± 9.3	**<0.001**
OSI (μmol H_2O_2 Eq/L)	18.3 ± 9.0	30.9 ± 12.9	**<0.001**
LOOH (μmol tBLOOH L^{-1})	8.9 ± 2.3	11.0 ± 2.3	**<0.001**
PON-1 (U L^{-1})	112.2 ± 60.4	64.2 ± 27.4	**<0.001**

Abbreviations: TAC; total antioxidant capacity, TOS; total oxidant status, OSI; oxidative stress index, LOOH; lipid hydro-peroxide, PON; paraoxonase.
Significance *p* values ($p < 0.05$) were indicated in boldface.

Table 3
Comparison of oxidative stress markers and PON-1 activities according to ST segment resolution.

Variables	Normal flow and STR > 70% (*n*: 167)	Normal flow and STR < 70% (*n*: 64)	*p* value
TAC (μmol Trolox Eq/L)	1.0 ± 0.2	0.9 ± 0.2	**<0.001**
TOS (μmol H_2O_2 Eq/L)	14.7 ± 4.7	23.4 ± 8.6	**<0.001**
OSI (μmol H_2O_2 Eq/L)	15.2 ± 6.1	26.5 ± 10.2	**<0.001**
LOOH (μmol tBLOOH L^{-1})	8.5 ± 1.7	10.0 ± 3.1	**<0.001**
PON-1 (U L^{-1})	128.9 ± 60.8	68.6 ± 29.9	**<0.001**

Abbreviations: STR; ST segment resolution, TAC; total antioxidant capacity, TOS; total oxidant status, OSI; oxidative stress index, LOOH; lipid hydro-peroxide, PON; paraoxonase.
Significance *p* values ($p < 0.05$) were indicated in boldface.

score, PON-1 activity, OSI and initial SYNTAX score were independent predictors for no-reflow in anterior STEMI ($p < 0.05$, for all). Predictors of no-reflow were shown in Table 5.

3.6. ROC curve analysis

The cutoff value of PON-1 activity obtained by the ROC curve analysis was 66.9 U L^{-1} for the prediction of no-reflow (sensitivity: 70%, specificity: 67%). The area under the curve (AUC) was 0.755 (95% CI: 0.700–0.809, $p < 0.001$).

The cutoff value of OSI obtained by the ROC curve analysis was 21.0 arbitrary unit for the prediction of no-reflow (sensitivity: 76%, specificity: 71%). The area under the curve (AUC) was 0.791 (95% CI: 0.735–0.848, $p < 0.001$).

The ROC curves of PON-1 and OSI for predicting the presence of no-reflow were shown in Fig. 1B and C.

4. Discussion

The main findings of this study are that the PON-1 activity and OSI are independent predictors of the no-reflow phenomenon in patients with anterior STEMI treated with primary PCI. Other independent predictors were initial SYNTAX, score diabetes, MPV, ratio of neutrophil/lymphocyte, infarction time and thrombus score. The cutoff values of PON-1 activity and OSI obtained by the ROC curve analysis for the prediction of no-reflow were 66.9 U L^{-1} (sensitivity: 70%, specificity: 67%) and 21.0 arbitrary unit (sensitivity: 76%, specificity: 71%), respectively.

In patients with STEMI treated with primary PCI, the incidence of the no-reflow phenomenon varies from 5% to 50% [3]. In a recent study, angiographic no-reflow was observed at 32.8% of patients with STEMI who underwent primary PCI [13]. In present study, 27.6% of patients with anterior STEMI treated with primary PCI showed evidence of no-reflow. No-reflow is thought to be caused by the variable combination of several pathogenetic mechanisms: distal atherothrombotic embolization, ischemic injury, reperfusion injury and susceptibility of coronary microcirculation to injury [3].

Oxidative stress occurs if the quantity of free radicals exceeds the capacity of the endogenous antioxidant defense mechanisms. Oxidative stress supports pro-inflammatory, prothrombotic, proliferative and vasoconstrictor mechanisms related with atherogenic process and thereby alters normal endothelial function [14]. Present study showed that no-reflow group had increased oxidative stress assessed with TOS, OSI and LOOH levels. Moreover, in present study, antioxidants such as TAC and PON-1 activity were decreased in no-reflow group compared with normal flow group.

The role of oxidative stress in no-reflow pathogenesis is not yet fully clear. However, ROS and peroxides play a critical role during ischemia as well as during the reperfusion phase following acute MI [3,4,15]. The primary PCI procedure is associated with additional ROS-related damage either directly or indirectly through inflammatory pathways by generating a massive increase in oxygen at the injured site [16,17]. It has also been suggested that antioxidants, which physiologically protect against an excess of ROS, are consumed, thereby further increasing ROS-induced damage [16,17]. Obtaining proof of such antioxidant consumption could justify conditioning coronary blood flow during primary PCI either by repeated hypoxic periods [18] or with powerful antioxidants in order to limit ischemia reperfusion injury [19]. Eventually, ischemia results in impaired antioxidant defense system [15]. On the other hand, lethal reperfusion injury to both the endothelial cells and the cardiomyocytes is mainly related with the effects of oxidative stress [3]. At the time of reperfusion, a massive infiltration of coronary microcirculation by neutrophils and platelets occurs. Reintroduction of neutrophils in post-ischemic myocardium results in their activation, with subsequent adhesion to the endothelial surface and migration in the surrounding tissue. Activated neutrophils release oxygen free radicals, proteolytic enzymes, and pro-inflammatory mediators that can directly cause tissue and endothelial damage [3,20]. In present study, antioxidant defense system assessed with TAC was impaired, and oxidative stress assessed with TOS and LOOH levels was increased in no-reflow group compared to normal flow group. In present study, ischemic injury rather than reperfusion injury may be responsible for relationship between increased oxidative stress and no-reflow because blood samples were taken before primary PCI.

In present study, we assessed the no-reflow phenomenon by angiographically. No-reflow can also be assessed in the coronary care unit after primary PCI by assessing the STR. Failure of STR is considered as an established marker of no-reflow. Notably,

Table 4
Comparison of angiographic and interventional characteristics of groups.

Variables	Normal flow group (*n*: 231)	No-reflow group (*n*: 88)	*p* value
Angiographic characteristics			
Left main disease	9 (3.9%)	7 (8.0%)	0.118
Balloon pre-dilatation	67 (29.0%)	28 (31.8%)	0.359
Total stent length (mm)	20.5 ± 5.6	23.5 ± 6.2	**<0.001**
Stent diameter (mm)	3.3 ± 0.37	3.1 ± 0.35	**<0.001**
Mean stent count (*n*)	1.3 ± 0.6	1.6 ± 0.7	**<0.001**
Drug eluting stent, *n* (%)	67 (29.0%)	29 (33.0)	0.289
Bifurcation intervention, *n* (%)	24 (10.4%)	11 (12.5%)	0.428
Thrombectomy, *n* (%)	54 (23.4%)	23 (26.1%)	0.353
Glycoprotein IIb/IIIa inhibitors, *n* (%)	74 (32.0%)	34 (38.6)	0.163
After pre-dilation dissection, *n* (%)	17 (7.4%)	9 (10.2%)	0.266
Distal embolization, *n* (%)	16 (6.9%)	6 (6.8%)	0.596
Initial TIMI flow grade 0 or 1	157 (68.0%)	82 (93.2%)	**<0.001**
Thrombus grade score 2–5 after wiring	138 (59.7%)	75 (85.2%)	**<0.001**
Initial SYNTAX score	16.1 ± 6.1	19.6 ± 5.2	**<0.001**
Final SYNTAX score	11.4 ± 5.0	13.2 ± 4.7	**0.005**

Significance *p* values ($p < 0.05$) were indicated in boldface.

Table 5
Predictors of no-reflow in anterior ST elevation myocardial infarction.

Variables	B	%95 CI (lower–upper)	*p*
Diabetes mellitus	0.385	0.159–0.936	**0.035**
MPV (fL)	4.293	2.533–7.275	**<0.001**
Neutrophil/lymphocyte	1.330	1.192–1.483	**<0.001**
PON-1 (U L^{-1})	0.976	0.962–0.990	**0.001**
OSI (μmol H_2O_2 Eq/L)	1.094	1.042–1.148	**<0.001**
Infarction time (h)	1.244	1.092–1.417	**0.001**
Thrombus grade score	0.156	0.044–0.554	**0.004**
Initial SYNTAX score	1.221	1.014–1.469	**0.035**

Abbreviations as Tables 1 and 2.
Significance *p* values ($p < 0.05$) were indicated in boldface.

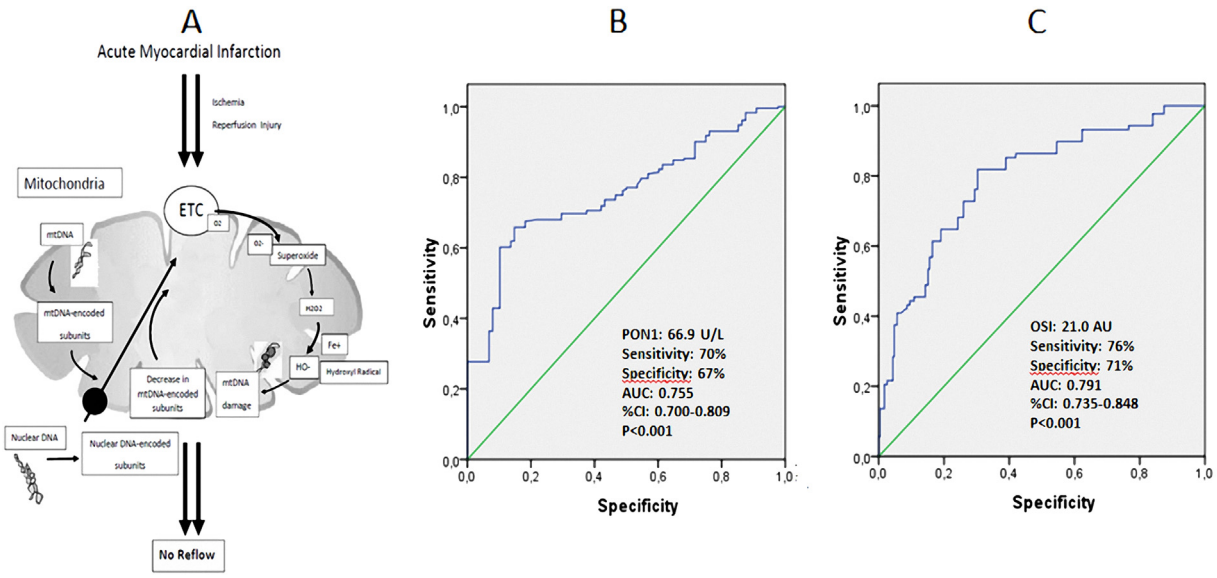

Fig. 1. A. The mechanism of mitochondrial oxidative stress in pathogenesis of acute myocardial infarction. B. The ROC curve analysis of PON-1 for predicting the presence of no-reflow. C. The ROC curve analysis of OSI for predicting the presence of no-reflow.

approximately one-third of patients with angiographically normal flow do not exhibit STR [3]. In present study, 64 (27.7%) patients had failure of STR even with satisfactory angiographic results of primary PCI. Those patients had also lower PON-1 activity and higher OSI values than patients with angiographically normal flow and STR > 70%.

There is direct evidence for a mechanistic link between activity of PON-1 with systemic oxidative stress and prospective cardiovascular risk, indicating a potential mechanism for the atheroprotective function of PON-1 [21]. Serum PON-1 activity is associated with both severity of CAD and incident of adverse cardiovascular events [21,22]. However relationship between no-reflow and PON-1 activity was not investigated in previous studies. Present study showed that the patients with no-reflow compared with normal flow had lower PON-1 activities. It is possible either that PON-1 declined as an acute reaction to the acute MI or that the decrease in its concentration and activity had occurred before the acute MI [21]. In present study, lower PON-1 activity could have been a consequence of an acute reaction to the STEMI as result of increased oxidative stress [17–19]. Kopetz et al. [23] reported that PON-1 level was increased in response to an oxidative stress environment during an acute coronary syndrome presentation in coronary slow flow phenomenon patients. It is also noteworthy to mention that PON-1 protein level was measured in that study, whereas we assessed PON-1 activity [24,25]. Thus there may be a compensatory increase in PON-1 protein level to account for the reduced PON-1 activity.

In our study, initial TIMI flow in IRA was an angiographic predictor of no-reflow phenomenon. Several mechanisms may explain the protective role of initial residual blood flow in the IRA with regard to development of no-reflow. First, residual blood flow in the IRA is associated with reduced infarct size. Second, IRA with residual blood flow may have less thrombotic burden, meaning less distal embolization, which is considered a crucial factor for the development of no-reflow after PCI. Third, early restoration of blood flow may alleviate tissue ischemia and prevent or attenuate the full-blown microvascular damage [26].

Coronary distal emboli of different particle sizes can originate from epicardial coronary thrombus and from fissured atherosclerotic plaques, in particular during primary PCI [27]. The relevance of high thrombus burden at the site of the culprit artery in predicting distal embolization has been shown by Limbruno et al. [24]. In our study, thrombus grade in IRA was also an angiographic predictor of no-reflow phenomenon.

In present study, diabetes, MPV, N/L and initial SYNTAX score as well as OSI and PON-1 were independent predictors for no-reflow phenomenon following primary PCI. The relationships between no-reflow phenomenon with diabetes, MPV, N/L and initial SYNTAX score were reported in previous studies [3,13,28].

5. Clinical implications

Previous studies reported that antioxidant therapy before primary PCI may prevent no-reflow phenomenon following primary PCI [25,29]. Antioxidant treatment with Trolox and ascorbate, and particularly with both, can alleviate these processes and restore normal microvascular function [25]. Matsumoto et al. suggested that natural antioxidant levels might protect from no-reflow [29]. In that study, authors demonstrated that levels of vitamin C, vitamin E, and glutathione peroxidase obtained from coronary sinus before primary PCI were significantly lower in patients exhibiting no-reflow than in patients exhibiting normal myocardial reperfusion. The present study showed that that reduced PON-1 activity and increased OSI were independent predictors of no-reflow phenomenon. However, lower sensitivity and specificity of both parameters for prediction of no-reflow limit the possible clinical application of these findings in humans.

6. Study limitations

In present study, current smoking was not excluded from the study. The smoking may be affective on oxidative stress. However, smoking frequencies were not differ between the groups in present study. In present study, only anterior MI patients were included to the study because anterior MI is known as a risk factor for no-reflow [3]. However, in future studies, relationship between no-reflow with oxidative stress and PON-1 should be investigated in all MI patients. In addition blood samples were taken from cubital vein in present study. Coronary sinus blood samples could be more

useful for investigating of oxidative stress. However, taking coronary sinus blood sample was thought to be not cost effective.

The effects of vitamin E and other antioxidant vitamins on various cardiovascular diseases have been investigated by several clinical and epidemiological trials [25,29]. The Health Professional Follow-up Study [30] found a decrease in the risk of coronary artery disease in men and women with vitamin E supplementation. The Cambridge Heart Antioxidant Study (CHAOS) [31] reported about a 77% decrease in the incidence of non-fatal MI in patients receiving vitamin E versus placebo. However, these studies have important limitations. Uncontrolled confounding from unknown or unmeasured confounders can be similar in magnitude to the observed benefit effects, and antioxidant consumption may be merely a marker for different cardioprotective factor (such as exercise and diet) that are responsible for the observed health benefits. In addition, it is not clear whether the beneficial effect of vitamin E is due to its antioxidant properties or non-antioxidant properties. Our study has a cross-sectional design and do not provide an insight into the exact mechanisms that are responsible for the observed associations. We clearly showed that increased oxidative stress was associated with no-reflow phenomenon. But, further large scale, randomized studies are needed to assess the exact role of vitamin E or other antioxidant agents on no-reflow phenomenon in patients with acute STEMI.

In conclusion, OSI and PON-1 activity are independent predictors for no-reflow phenomenon following primary PCI. This result shows that increased oxidative stress has a role in the pathogenesis of no-reflow.

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