Kardiologia Polska 2012; 70, 7: 677–684 ISSN 0022–9032

ORIGINAL ARTICLE

Platelet activation and microvascular injury in patients with ST-segment elevation myocardial infarction

Jarosław Zalewski[1, 3], Monika Durak[1, 2], Piotr Lech[1, 2], Grzegorz Gajos[3, 4], Anetta Undas[4], Jadwiga Nessler[3], Agnieszka Rosławiecka[5], Krzysztof Żmudka[1, 2]

[1]Centre for Interventional Treatment of Cardiovascular Diseases, The John Paul II Hospital, Krakow, Poland
[2]Department of Haemodynamics and Angiocardiography, Institute of Cardiology, Jagiellonian University, Medical College, Krakow, Poland
[3]Department of Coronary Disease, Institute of Cardiology, Jagiellonian University, Medical College, Krakow, Poland
[4]Department of Experimental Cardiology and Cardiac Surgery, Institute of Cardiology, Jagiellonian University, Medical College, Krakow, Poland
[5]Department of Cardiovascular Diseases, The John Paul II Hospital, Krakow, Poland

Abstract

Background: Dual antiplatelet therapy reduces the risk of thrombotic complications after primary percutaneous coronary intervention (PCI).

Aim: To assess whether inhibition of platelet function attenuates microvascular damage in patients with ST-segment elevation myocardial infarction (STEMI).

Methods: We studied 83 STEMI patients treated with primary PCI. Platelet aggregation was measured on admission (ADM) and 4 days later (D4) by light transmission aggregometry after stimulation with 0.5 mM of arachidonic acid and after stimulation with 5 and 20 μM of adenosine diphosphate (ADP) on treatment with dual antiplatelet therapy with aspirin and clopidogrel. Platelet-neutrophil aggregate (PNA) and platelet-monocyte aggregate (PMA) were analysed by flow cytometry. Contrast-enhanced magnetic resonance imaging was performed 2–4 days after STEMI to detect the area of perfusion defect at rest and to determine the size of microvascular obstruction. Microvascular obstruction was expressed as a percentage of infarct area.

Results: Perfusion defect at rest was found in 56 (67.5%) patients whereas microvascular obstruction in 63 (75.9%) patients. Patients with perfusion defect at rest had on admission a significantly higher level of both PMA (7.0 vs. 4.5%, $p = 0.004$) and PNA (4.1 vs. 2.2%, $p = 0.016$), however there were no significant differences at D4. Platelet aggregation after stimulation with 5 μM of ADP on ADM was correlated ($r = 0.37$, $p = 0.004$) with microvascular obstruction area. Moreover, the higher the concentration of PMA_{ADM} ($r = 0.31$, $p = 0.016$), PNA_{ADM} ($r = 0.34$, $p = 0.006$) and PMA_{D4} ($r = 0.35$, $p = 0.005$) the larger the size of microvascular obstruction. Infarct size ($\beta = 0.43$, 95% CI 0.19 to 0.67, $p < 0.0001$), TIMI < 3 after PCI ($\beta = -0.27$, 95% CI –1.90 to –0.11, $p = 0.015$) and PMA_{D4} ($\beta = 0.21$, 95% CI 0.13 to 1.86, $p = 0.032$) independently influenced the size of microvascular obstruction ($R2 = 0.60$, $p < 0.0001$).

Conclusions: Excessive platelet activation during reperfusion in STEMI patients despite dual antiplatelet therapy is associated with greater microvascular impairment.

Key words: STEMI, platelet activation, platelet-leukocyte aggregates, microvascular obstruction

Kardiol Pol 2012; 70, 7: 677–684

Address for correspondence:
Jarosław Zalewski, MD, John Paul II Hospital, ul. Prądnica 80, 31–202 Kraków, Poland, tel: +48 12 614 35 01, e-mail: jzalewski@szpitaljp2.krakow.pl
Received: 14.09.2011 **Accepted:** 16.11.2011

INTRODUCTION

Histopathology studies indicate that plaque rupture or plaque ulceration are direct causes of thrombus formation, resulting in impairment or cessation of the epicardial coronary blood flow [1]. Collagen and other plaque components, especially those of extracellular matrix, interact with blood platelets, and this can lead to platelet aggregation and adhesion to the injured vascular wall. Then, tissue factor release initiates the coagulation cascade pathway and thrombus formation as a final result [2, 3]. In clinical terms, this process manifests itself as an acute coronary syndrome (ACS) [4].

Physiologically, platelets do not interact with leukocytes. However, increased concentrations of leukocyte-platelet aggregates were found, among others, in circulating blood of ACS patients [5]. It has been proposed that measurement of platelet-monocyte aggregate (PMA), as well as platelet-neutrophil aggregate (PNA) concentrations, can be a sensitive indicator of platelet activation [6, 7].

One of the complications of an ACS is the no-reflow phenomenon, related to sustained tissue perfusion deficit despite successful recanalisation of an epicardial coronary artery in the acute phase [8]. In experimental studies with use of electron microscopy, features of progressive reperfusion injury were spotted at the microvascular level within the no-reflow zone, including swollen endothelial cells without pinocytotic vesicles and showing intraluminal protrusions with gaps in the basement membrane. Occasional intraluminal fibrin deposits, platelet aggregates as well as extravascular erythrocytes were noted [9, 10]. Histopathology studies shown that in the early phase of infarction, contrast defects seen within the late enhancement zone on magnetic resonance imaging (MRI), namely areas of microvascular obstruction (MVO), correspond to areas of microvascular injury and reduced perfusion [11].

Despite proven pivotal role of platelets and their activation in the pathogenesis of an ACS [12], the influence of platelets on the extent of microvascular injury was not studied to date — hence it is not known whether pharmacological platelet inhibition reduces microvascular injury in patients with ST-segment elevation myocardial infarction (STEMI).

METHODS

Study group. Eighty three STEMI patients treated with primary percutaneous coronary intervention (PCI) were included in the study. Inclusion criteria were as follows: resting chest pain of > 20 min and < 12 h duration with ST-segment elevation of at least 1 mm in at least 2 limb leads and of at least 2 mm in at least 2 precordial leads or new left bundle branch block.

Exclusion criteria were as follows: lack of patient informed consent to participate in the study, cardiogenic shock or pulmonary oedema on admission, history of MI of the same region, history of coronary artery bypass grafting, any other acute condition, history of malignancy or autoimmune disorder, history of venous thromboembolism, liver disease (alanine aminotransferase > 1.5 ULN), chronic kidney disease (serum creatinine > 177 μmol/L), chronic antithrombotic or steroid treatment or contraindications for MRI (e.g. claustrophobia, atrial fibrillation).

The study was approved by the Ethics Committee of the Jagiellonian University. All patients expressed their informed consent for participation in the study.

Angiography. Coronary angiography was reviewed by two experienced observers blinded to the results of platelet function tests. Infarct-related artery (IRA), the presence and extent of intravascular thrombus, distal embolisation, collateral flow to the IRA and presence of significant (> 50%) narrowing of the epicardial coronary arteries were determined. Epicardial coronary artery flow was assessed by TIMI scale (Thrombolysis in Myocardial Infarction, [13]) and by TIMI frame count [14] at baseline and post-PCI. The extent of intravascular thrombus was assessed with use of TIMI thrombus grade [15]. Distal embolisation was defined as contrast defect with sudden amputation of one or more epicardial branches distal to the culprit lesion.

Electrocardiogram. 12-lead ECG was obtained on admission, immediately after PCI, and 1 and 6 h post-procedurally. In each ECG, ST-segment depression and elevation were assessed 80 ms after the J point, and their absolute values were then summed up. The residual ST-segment elevation (STR) at each time point was calculated with the following formula: $STR = \Sigma ST_{Time\ point}/\Sigma ST_{ADM}$ [16].

Enzymatic injury. Plasma MB isoenzyme of creatine kinase (CK-MB, IU/L) and troponin I (TnI, ng/mL) were measured on admission, after 90 min and then after 8, 16, 24, 48 h post-PCI. Area under the curve of CK-MB release was calculated for the first 48 h after the procedure (AUC, IU/L × h). Maximal value of TnI was specified.

Cardiovascular magnetic resonance. MRI was performed between day 2 and day 4 of the infarct onset with a 1.5-T scanner (Sonata, Siemens, Erlangen, Germany), according to the protocols described earlier [17]. Myocardial perfusion at rest was imaged during the first pass of a gadolinium contrast agent, with the saturation recovery spoiled-gradient-echo technique. Immediately after, T1-weighted three-dimensional inversion-recovery gradient-echo sequence was used for infarct size imaging and MVO assessment. In the latter technique, the left ventricle (LV) was completely encompassed by contiguous 8-mm-thick slices. The total contrast dose was 0.15 mmol/kg and inversion time was individually adjusted. MVO presence was assessed on scans performed 2–5 min post-contrast administration, whereas infarct area was assessed on scans performed 10–25 min post-contrast. MVO was defined as non-enhanced areas within the late enhancement area, i.e. within infarct zone. Its extent was then expressed as percent of the infarct area. All the images were analysed off-line.

Platelet function. Platelet aggregation and platelet-leukocyte aggregate concentrations were measured at two distinct time points: on admission (ADM) and on day 4 from the infarct onset (D4). Blood samples were drawn atraumati-

cally from the antecubital vein. For platelet aggregation assessment, samples drawn into 3.2% citrate were subject to further analysis within 30–60 min from their collection.

Light transmission aggregometry. Blood samples drawn into 3.2% citrate were centrifugated at 120 g for 10 min for platelet-rich plasma and then at 850 g for 10 min for platelet-deficient plasma. Next, platelets were stimulated with 0.5 mM arachidonic acid (AA) and 5 or 20 μM adenosine diphosphate (ADP-5, -20). Platelet aggregation was measured in two-channel light aggregometer Chronolog (Chrono-Log 490; Chrono-Log Corp., Haverton, Pennsylvania). For platelet-deficient plasma without stimulation, light transmission was 100% and for platelet-rich plasma without stimulation light transmission was 0%. Aggregation curves were recorded for 6 min. Platelet aggregation was expressed as a difference between maximal platelet aggregation after stimulation and aggregation assessed with light transmission in platelet-deficient plasma [18]. Hight platelet reactivity during therapy (HoTPR) was defined as aggregation exceeding 46% after stimulation with 5 μM ADP. Definition of HoTPR was adopted from American College of Cardiology Working Group consensus [19].

Platelet-leukocyte aggregates. 100 μL of full blood drawn into EDTA were incubated for 30 min at room temperature in 1 mL Cell-fix and then centrifugated at 400 g for 5 min. After removing the supernatant, fixed cell fraction was put in 1 mL PBS. To label PMA and PNA, 100 μL of fixed cell suspension was incubated for 30 min in the dark at room temperature either with mouse monoclonal antibodies anti-CD61-FITC (BD Bioscience, Poland) or with staining with an isotopic IgG_1 solution. In the next stage, erythrocytes were disintegrated by incubation in lytic solution for 10 min and then samples were centrifugated at 400 g for 5 min. After removing the supernatant, cell fraction was put in 0.5 mL PBS. Samples thus prepared were then analysed in a flow cytometer (FACSCalibur System, BD Bioscience, Warsaw, Poland) [20].

Statistical analysis

All statistical analyses were performed with use of SPSS 12.01 statistical package. Continuous variables were presented as mean ± SD or median (interquartile range) and categorical variables as absolute values (percentage). Shapiro-Wilk statistics was used to check variable distribution. For normal distribution, Student t test was used and when the data did not follow normal distribution, Mann-Whitney U test was used. Categorical data were compared with χ^2 test. The Pearson or Spearman correlation coefficients were calculated to test variables with a normal or non-normal distribution, respectively. All the clinical, laboratory and angiographic variables that showed relationship with the size of MVO in the univariate model ($p \leq 0.2$), and did not show significant ($r > 0.3$) correlation with any other independent variable, were subsequently included into a multivariate model in order to identify variables independently determining the extent of MVO. All tests were 2-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Antiplatelet/antithrombotic therapy and platelet function. Clinical and angiographic characteristics of the study group are presented in Table 1. All patients were administered 300 mg aspirin at first medical contact, 53 (63.9%) of the patients received loading dose of clopidogrel and 45 (54.2%) of the patients received unfractionated heparin bolus of 5000 U. During PCI all patients received body weight-adjusted heparin bolus so that activated clotting time was 300–350 s in patients not receiving abciximab and 250–300 s in patients receiving abciximab.

Immediately before or after PCI, in additional 30 patients clopidogrel loading dose of 600 mg was administered. Median time from pain onset to PCI (t_I) was 219 (160; 334) min. On the other hand, time from aspirin loading dose to baseline blood sample drawing (t_{ASA}) was 50 (32; 82) min, time from clopidogrel loading dose to baseline blood sample drawing (t_{CLOP}) was 55 (32–77) min and to the beginning of PCI 80 (65; 113) min.

Table 1. Study group characteristics (n = 83)

Age [years]	59.0 ± 11.5
Male gender	62 (74.7%)
Weight [kg]	80.1 ± 13.3
Height [cm]	171 ± 8
Body mass index [kg/m^2]	27.4 ± 3.8
Cardiovascular risk factors:	
Hypertension	48 (57.8%)
Diabetes	15 (18.1%)
Dyslipidaemia	49 (59.0%)
Smoking	45 (54.2%)
Stroke	2 (2.4%)
Peripheral artery disease	1 (1.2%)
History of MI	5 (6.0%)
History of PCI	3 (3.6%)
Previous angina	14 (16.9%)
HF prior to MI (NYHA II–IV)	0
Chronic treatment with aspirin	8 (9.6%)
Anterior wall infarction	46 (55.4%)
Killip class on admission:	
I	80 (96.4%)
II	3 (3.6%)
Infarct related artery:	
LAD	48 (57.8%)
LCX	3 (3.6%)
RCA	32 (38.6%)
Aspiration thrombectomy	69 (83.1%)
Bare metal stent	81 (97.6%)
Stent diameter [mm]	3.44 ± 0.44
Total length of implanted stents [mm]	20.76 ± 9.04

MI — myocardial infarction; PCI — percutaneous coronary intervention; HF — heart failure; NYHA — heart failure classification according to New York Heart Association; LAD — left anterior descending; LCX — left circumflex; RCA — right coronary artery

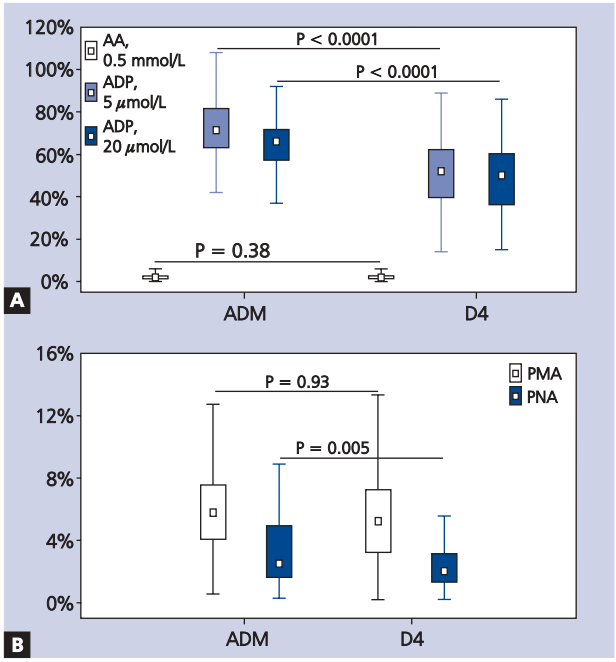


Figure 1. Platelet aggregation (**A**) and platelet-leukocyte aggregates (**B**). Box plot shows median and interquartile range (IQR) (Q3 to Q1), Q1 and Q3 are the first and the third quartile. Whiskers are drawn at Q3 + 1.5 × IQR, Q1 – 1.5 × IQR. Extreme values are omitted; PMA — platelet-monocyte aggregates; PNA — platelet-neutrophil aggregates; AA — arachidonic acid; ADP — adenosine diphosphate; ADM — admission; D4 — day 4 from infarct onset

Table 2. Reperfusion efficacy

Method and parameter	Value
Electrocardiography	
Residual ST-segment elevation [%]:	
Immediately post-PCI	36.5 ± 30.3
1 h post-PCI	19.4 ± 22.4
6 h post-PCI	15.4 ± 17.5
Enzymatic injury	
AUC for CK-MB [IU/L × h]	3918 ± 2319
TnI_{MAX} [ng/mL]	119 ± 85
Angiography	
TIMI prior to PCI:	
0/1	75 (90.4%)
2	6 (7.2%)
3	2 (2.4%)
TIMI post-PCI:	
0/1	1 (1.2%)
2	18 (21.7%)
3	65 (77.1%)
TFC post-PCI [frame]	34.9 ± 27.22
TTG post-PCI	0.18 ± 0.70
DE during PCI	11 (13.3%)
Magnetic resonance imaging	
PDR	56 (67.5%)
MVO	63 (75.9%)
MVO size (% IS)	17.5 ± 15.8

Data are shown as n (%) unless otherwise indicated. AUC — area under the curve of CK-MB release; TnI_{MAX} — maximal troponin I concentration; TIMI — Thrombolysis in Myocardial Infarction; TFC — TIMI frame count; TTG — TIMI thrombus grade; PCI — percutaneous coronary intervention; PDR — perfusion defect at rest; MVO — microvascular obstruction; IS — infarct zone; DE — distal embolisation

Median platelet count on admission was 211 (182; 246) K/μL and on day 4, 230 (168; 282) K/μL. At baseline, no correlations were found between t_{ASA} and aggregation after stimulation with AA ($r = 0.02$, $p = 0.88$). On the other hand, the longer the t_{CLOP}, the better ADP induced aggregation inhibition ($r = –0.3$ and $r = –0.32$ for ADP-5 and ADP-20, respectively; $p < 0.05$ for both). Platelet aggregation after stimulation with AA was 2.6% on admission and remained unchanged on day 4 (Fig. 1), whereas 5 and 20 μM ADP-induced aggregation changed significantly between admission and day 4 (by 29% and 23%, respectively; $p < 0.0001$ for both, Fig. 1). PMA concentration was not changed between admission and day 4 and PNA concentration significantly diminished (by 35%, $p = 0.005$, Fig. 1).

In diabetic patients PMA_{D4} was significantly higher that in non-diabetics (7.8 vs. 5.4%, $p = 0.044$). In the study group, diabetes and other coronary artery disease risk factors did not significantly affect the remaining platelet parameters.

Abciximab was administered during PCI in 14 (16.9%) patients. The administration of abciximab did not alter the platelet count (229 vs. 233 x K/μL in the abciximab and non-abciximab group, respectively, $p = 0.61$) and the platelet parameters on day 4 (AA-dependent aggregation [2.5 vs. 2.5%, $p = 0.94$], ADP-5 [46 vs. 52%, $p = 0.29$] ADP-20 [46 vs. 49%, $p = 0.65$], PMA aggregate concentration [7.3 vs. 6.1%, $p = 0.41$], PNA aggregate concentration [3.0 vs 2.4%, $p = 0.32$] and MVO [19.6 vs. 17.6%, $p = 0.68$].

Similarly, pantoprazole administration did not affect platelet parameters on day 4: ADP-5 (52 vs. 50% in the groups receiving and not receiving pantoprazol, respectively, $p = 0.70$) and ADP-20 (48 vs. 49%, $p = 0.81$), PMA aggregate concentration (6.0 vs. 7.3%, $p = 0.28$); PNA aggregate concentration (2.6 vs. 2.3%, $p = 0.54$).

At discharge 80 (96.4%) patients received beta-blocker, angiotensin converting enzyme and statins, 55 (66.3%) pantoprazol, 2 (2.4%) fibrates, calcium channel inhibitor and omeprazol, and 1 (1.2%) patient angiotensin receptor inhibitor.

Platelet function and reperfusion effectiveness. Parameters describing reperfusion effectiveness in the study group are presented in Table 2. STR immediately after PCI was moderately correlated with $ADP\text{-}5_{ADM}$ ($p = 0.034$, $r = 0.3$). Platelet-leukocyte aggregates on admission correlated with STR at 1 h post-PCI ($r = 0.35$, $p = 0.013$ for PMA and $r = 0.39$, $p = 0.005$ for PNA).

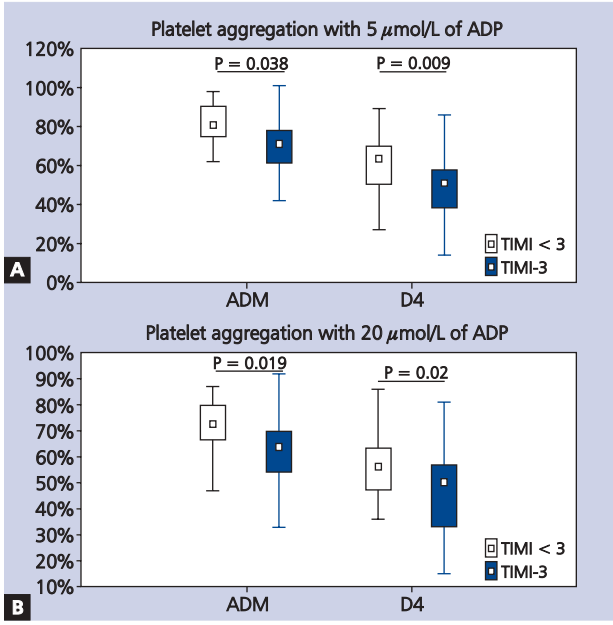


Figure 2. Platelet aggregation after stimulation with ADP of 5 μmol/L (**A**) and 20 μmol/L (**B**) in relation to epicardial blood flow restored after primary coronary angioplasty. Box plot shows median and interquartile range (IQR) (Q3 to Q1), Q1 and Q3 are the first and the third quartile. Whiskers are drawn at Q3 + 1.5 × IQR, Q1 – 1.5 × IQR. Extreme values are omitted; ADP — adenosine diphosphate; ADM — admission; D4 — day 4 from infarct onset; TIMI — Thrombolysis in Myocardial Infarction

Platelet aggregation as well as PMA at baseline were comparable in patient groups with spontaneously recanalised (TIMI-2/3) and occluded (TIMI-0/1) IRA prior to PCI. In patients with completely restored (TIMI-3) epicardial flow post-PCI, significantly higher platelet aggregation inhibition was noted on admission as well as on day 4 (Fig. 2).

Enzymatic injury expressed as area under the curve of CK-MB release within initial 48 h of reperfusion significantly correlated with AA-dependent platelet aggregation ($r = 0.47$, $p < 0.0001$) and PNA on admission ($r = 0.34$, $p = 0.014$).

Patients with and without resting perfusion defect on MRI study did not differ significantly in terms of time that elapsed from loading doses of aspirin and clopidogrel to the blood sample drawing, AA-dependent platelet aggregation on admission (2.5 vs. 2.7%, $p = 0.80$) and on day 4 (2.4 vs. 2.0%, $p = 0.30$) and baseline ADP-5 (73 vs. 69%, $p = 0.37$) and ADP-20 (64 vs. 61%, $p = 0.49$) as well as on day 4 (49 vs. 54%, $p = 0.34$ for ADP-5, 48 vs. 52%, $p = 0.12$, for ADP-20). At baseline, patients with resting perfusion defect had significantly higher concentrations of both PMA (7.0 vs. 4.5%, $p = 0.004$) and PNA (4.1 vs. 2.2%, $p = 0.016$). However, no significant differences were noted on day 4 (7.1 vs. 4.6%, $p = 0.08$; 2.5 vs. 1.8%, $p = 0.14$, respectively).

Among 53 patients who were administered clopidogrel loading dose prior to admission, 48 (90.5%) met the HoTPR criterion and 49 patients (out of 83) met the criterion on day 4. Patients with high platelet reactivity on clopidogrel therapy at baseline, subsequently demonstrated significantly larger MVO (2.6 vs. 19.0%, $p = 0.016$). there were no significant differences of MVO extent in patients with or without HoTPR seen on day 4 (16.3 vs. 18.9%).

Platelet aggregation ADP-5_{ADM} correlated significantly with MVO area (Fig. 3). Moreover the greater the PMA_{ADM}, PNA_{ADM} and PMA_{D4} concentrations the larger MVO area. No correlations were found between MVO extent and ADP-dependent platelet aggregation on day 4 from infarct onset.

Independent determinants of MVO size. Results of linear multivariate regression for identification of factors independently determining MVO extent are presented in Table 3. Before inclusion to the multivariate model, independent variables such as infarct size, ADP-5_{ADM}, PMA_{ADM}, PMA_{D4}, PNA_{D4}, diabetes, time of ischaemia, symptoms of heart failure at the time of index infarction, chronic treatment with aspirin, were found related ($p < 0.2$ for each independent variable) to size of MVO in the univariate model.

Significant correlations were noted ($p < 0.05$ for each) between independent variables ($r = 0.5$ for PMA_{ADM} and PNA_{ADM}, $r = 0.43$ for PMA_{D4} and symptoms of heart failure, $r = 0.42$ for AA_{ADM} and diabetes, $r = 0.4$ for infarct size and AA_{ADM}, $r = 0.37$ for AA_{ADM} and symptoms of heart failure, $r = 0.35$ for PMA_{D4} and diabetes, $r = 0.34$ for infarct size and PNA_{ADM}, and $r = 0.32$ for infarct size and PMA_{ADM}).

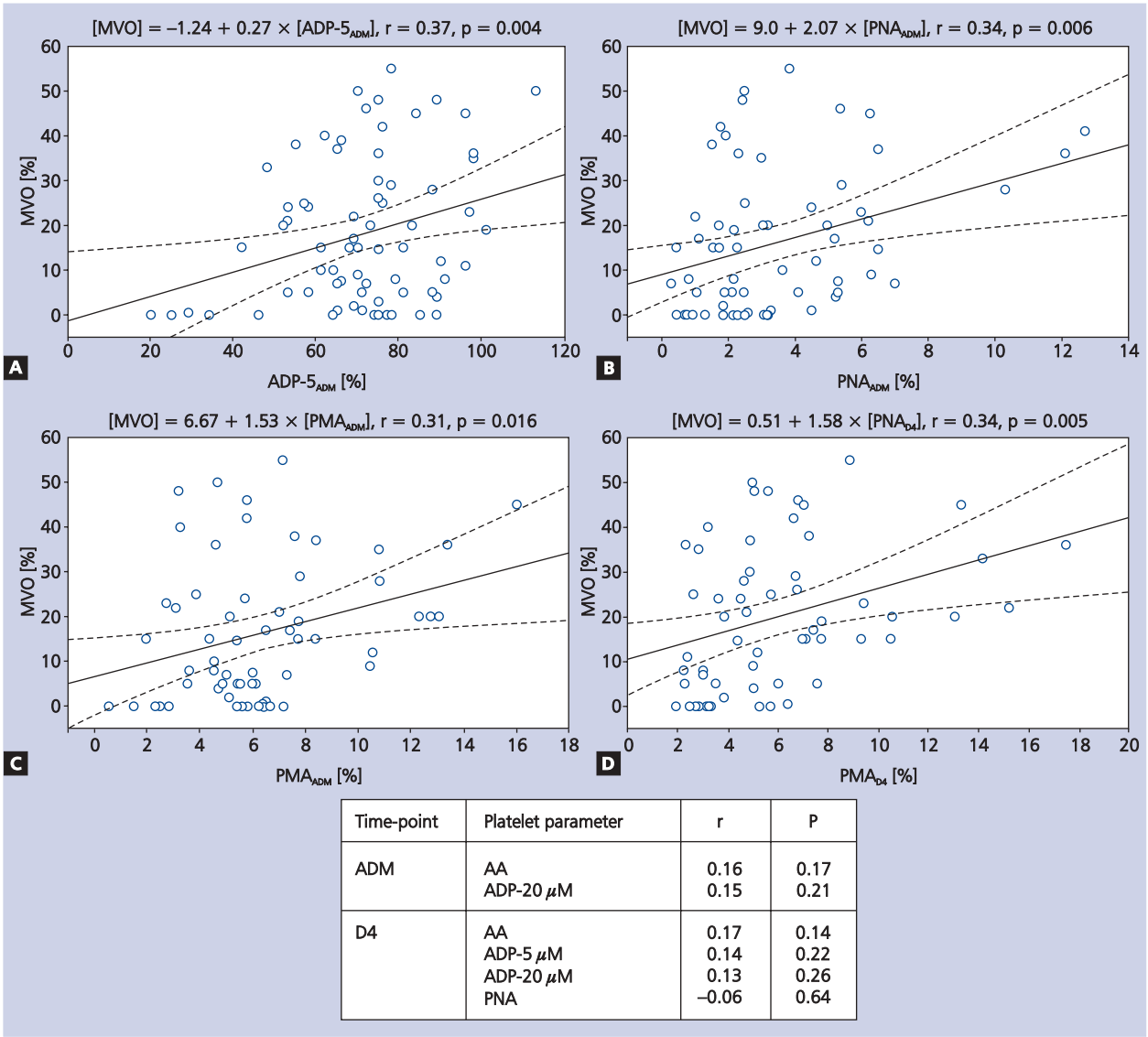
Finally, infarct size ($\beta = 0.43$, 95% CI 0.19–0.67, $p < < 0.0001$), TIMI < 3 post-PCI ($\beta = -0.27$, 95% CI –1.90 to –0.11, $p = 0.015$) and platelet-monocyte aggregates on day 4 from the infarct onset ($\beta = 0.21$, 95% CI 0.13–1.86, $p = 0.032$) were the factors that independently determined MVO size ($R2 = 0.60$, $p < 0.0001$).

DISCUSSION

Our study is the first to directly demonstrate that in STEMI patients treated with primary PCI the degree of platelet inhibition during dual antiplatelet therapy influences the extent of microvascular injury. Univariate analyses indicate, that the higher the baseline aggregation after 5 μM ADP stimulation, baseline PMA and PNA concentrations and day 4 PMA concentrations, the greater MVO. It should be underlined, that the correlations between all those variables remain moderate.

After inclusion of all the variables independently determining the extent of MVO zone, the only parameter that described platelet function on one hand and remained still independently related to MVO extent in STEMI on the other hand was PMA at day 4 after infarct onset.

The correlations that were identified between platelet parameters and MVO do not alter the fact, that in our study it was infarct size that was the strongest determinant of MVO extent. This observation remains in agreement with results published to date, in which MVO extent not only related to infarct size, but is in itself a clinically meaningful prognostic factor [21].



Time-point	Platelet parameter	r	P
ADM	AA	0.16	0.17
	ADP-20 μM	0.15	0.21
D4	AA	0.17	0.14
	ADP-5 μM	0.14	0.22
	ADP-20 μM	0.13	0.26
	PNA	–0.06	0.64

Figure 3A–D. Correlation between platelet function and the size of microvascular obstruction; PMA — platelet-monocyte aggregates; PNA — platelet-neutrophil aggregates; AA — arachidonic acid; ADP — adenosine diphosphate; ADM — admission; D4 — day 4 from infarct onset; MVO — microvascular obstruction; r — correlation coefficient

Table 3. Multivariate regression with microvascular obstruction as a dependent variable

Dependent variable	Independent variables [β per]	Variance, %	P	β	95% CI	
Microvascular obstruction	Final model	52.5	< 0.0001			
	Infarct zone [%]	20.9	< 0.0001	0.43	0.19	0.67
	TIMI < 3 post PCI [Y/N]	5.9	0.015	–0.27	–1.90	–0.11
	PMA_{D4} [%]	4.0	0.032	0.21	0.13	1.86
	$ADP\text{-}5_{ADM}$ [%]	3.9	0.053	0.20	–0.01	0.31
	Chronic treatment with aspirin [Y/N]	2.8	0.066	0.18	0.08	1.88
	Time of ischaemia [min]	0.1	0.56	0.01	–0.01	0.03

PMA_{D4} — platelet-monocyte aggregates at day 4 from infarct onset; $ADP\text{-}5_{ADM}$ — platelet aggregation after stimulation with 5 μM ADP on admission; β — correlation coefficient; CI — confidence interval for correlation coefficient; Y/N — yes/no

Studies published to date indicate that the presence of transmural late enhancement or MVO area is related to the lack of inotropic reserve, the lack of LV functional improvement over time as well as adverse LV remodelling [11, 22–24]. Wu et al. [21] demonstrated that large infarct zone (i.e. late enhancement > 30% of the LV mass) is related to primary end point rate of 71% (including death, heart failure, reinfarction or unstable angina) as compared to 30% in cases of smaller infarcts (< 18% of the LV mass) in 16-month follow-up.

Independent of late enhancement area, the presence of MVO was related to significantly higher composite end-point rates (45 vs. 9%, p = 0.016), scar formation and adverse LV remodelling. It was recently reported, that the infarct zone as assessed 2 days, one week and 2 months post-infarction is the strongest determinant of LV remodelling. On the other hand, MVO is a significant determinant of infarct healing [25]. Detailed analyses of the studies including MRI point to the fact how important from the clinical point of view is the determination of independent factors influencing infarct size as well as MVO. Our study provides preliminary arguments that also platelet function parameters can influence infarct size as well as MVO in this setting.

Limitations of the study

Our study has several limitations. Firstly, the study group is rather small. However, platelet function was studied prospectively, with two methods and at two time points. Secondly, our study end-point was the size of MVO. Platelet function in relation to clinical end-points was not investigated, as in the context of small sample size it would not yield adequate statistical power. Lastly, our study focused on the in-hospital period. The effect of the parameters that we studied on the long-term clinical prognosis and the infarct scar healing was not an objective of our work.

CONCLUSIONS

Sustained excessive platelet activation despite double antiplatelet therapy in patients with acute MI is related to greater MVO extent. This provides additional rationale for adequate antiplatelet therapy in this patient group. Further larger prospective studies are needed, so as to define the goals of antiplatelet therapy in terms of aggregation level and platelet-leukocyte aggregate concentrations, in order to minimize microvascular injury, without increasing bleeding risk.

The work was funded by the Polish Ministry of Science and Higher Education grant [N402 187435 to KZ].

Conflict of interest: *none declared*

References

1. Virmani R, Burke AP, Farb A et al. Pathology of the vulnerable plaque. J Am Coll Cardiol 2006; 47: C13–18.
2. Meadows TA, Bhatt DL. Clinical aspects of platelet inhibitors and thrombus formation. Circ Res, 2007; 100: 1261–1275.
3. Furie B, Furie BC. Mechanism of thrombus formation. N Engl J Med, 2008; 359: 938–949.
4. Van de Werf F, Bax J, Betriu A et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. Eur Heart J, 2008; 29: 2909–2945.
5. Freedman JE, Loscalzo J. Platelet-monocyte agggregates: bridging thrombosis and inflammation. Circulation, 2002; 105: 2130–2132.
6. Michelson AD, Barnard MR, Krueger LA et al. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation, 2001; 104: 1533–1537.
7. Ott I, Neumann FJ, Gawaz M et al. Increased neutrophil-platelet adhesion in patients with unstable angina. Circulation, 1996; 94: 1239–1246.
8. Rezkalla SH, Kloner RA. No-reflow phenomenon. Circulation, 2002; 105: 656–662.
9. Kloner RA, Ganote CE, Jennings RB. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. J Clin Invest, 1974; 54: 1496–1508.
10. Kloner RA, Rude RE, Carlson N et al. Ultrastructural evidence of microvascular damage and myocardial cell injury after coronary artery occlusion: which comes first? Circulation, 1980; 62: 945–952
11. Hillenbrand HB, Kim RJ, Parker MA et al. Early assessment of myocardial salvage by contrast-enhanced magnetic resonance imaging. Circulation, 2000; 102: 1678–1683.
12. Szułdrzyński K, Zalewski J, Machnik A, Zmudka K. Elevated levels of 8-iso-prostaglandin F2alpha in acute coronary syndromes are associated with systemic and local platelet activation. Pol Arch Med Wewn, 2010; 120: 19–24.
13. The TIMI study group. The thrombolysis in myocardial infarction (TIMI) trial. N Engl J Med, 1993; 312: 932–936.
14. Gibson CM, Cannon CP, Daley WL et al. TIMI frame count: a quantitative method of assessing coronary artery flow. Circulation, 1996; 93: 879–888.
15. The TIMI IIIA Investigators. Early effects of tissue-type plasminogen activator added to conventional therapy on the culprit coronary lesion in patients presenting with ischemic cardiac pain at rest. Results of the Thrombolysis in Myocardial Ischemia (TIMI IIIA) trial. Circulation, 1993; 87: 38–52.
16. Schröder R, Dissmann R, Bruggemann T et al. Extent of early ST segment elevation resolution: a simple but strong predictor of outcome in patients with acute myocardial infarction. J Am Coll Cardiol, 1994; 24: 384–391.
17. Bogaert J, Dymarkowski S, Taylor AM. Clinical cardiac MRI: with interactive CD-ROM. Springer Verlag Inc. 2005.
18. Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol, 2007; 50: 1822–1834.
19. Bonello L, Tantry US, Marcucci R et al. Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. J Am Coll Cardiol, 2010; 56: 919–933.
20. May AE, Langer H, Seizer P et al. Platelet-leukocyte interactions in inflammation and atherothrombosis. Semin Thromb Hemost, 2007; 33: 123–127.
21. Wu KC, Zerhouni EA, Judd RM et al. Prognostic significance of microvascular obstruction by magnetic resonance imaging in patients with acute myocardial infarction. Circulation, 1998; 97: 765–772.
22. Kim RJ, Wu E, Rafael A et al. The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction. N Engl J Med, 2000; 343: 1445–1453.
23. Bogaert J, Kalantzi M, Rademakers F et al. Determinants and impact of microvascular obstruction in successfully reperfused ST-segment elevation myocardial infarction. Assessment by magnetic resonance imaging. Eur Radiol, 2007; 17: 2572–2580.
24. Gerber BL, Rochitte CE, Melin JA et al. Microvascular obstruction and left ventricular remodeling early after acute myocardial infarction. Circulation, 2000; 101: 2734–2741.
25. Ørn S, Manhenke C, Greve OJ et al. Microvascular obstruction is a major determinant of infarct healing and subsequent left ventricular remodelling following primary percutaneous coronary intervention. Eur Heart J, 2009; 30: 1978–1985.

Aktywacja płytek krwi a uszkodzenie mikrokrążenia u pacjentów z zawałem serca z uniesieniem odcinka ST

Jarosław Zalewski[1, 3], Monika Durak[1, 2], Piotr Lech[1, 2], Grzegorz Gajos[3, 4], Anetta Undas[4], Jadwiga Nessler[3], Agnieszka Rosławiecka[5], Krzysztof Żmudka[1, 2]

[1]Centrum Interwencyjnego Leczenia Chorób Serca i Naczyń, Krakowski Szpital Specjalistyczny im. Jana Pawła II, Kraków.
[2]Zakład Hemodynamiki i Angiokardiografii, Instytut Kardiologii, Uniwersytet Jagielloński, *Collegium Medicum*, Kraków
[3]Klinika Choroby Wieńcowej, Instytut Kardiologii, Uniwersytet Jagielloński, *Collegium Medicum*, Kraków
[4]Zakład Kardiologii i Kardiochirurgii Doświadczalnej, Instytut Kardiologii, Uniwersytet Jagielloński, *Collegium Medicum*, Kraków.
[5]Klinika Chorób Serca i Naczyń, Krakowski Szpital Specjalistyczny im. Jana Pawła II, Kraków

Streszczenie

Wstęp: Podwójna terapia przeciwpłytkowa zmniejsza ryzyko powikłań zakrzepowych po pierwotnej angioplastyce wieńcowej (PCI).

Cel: Celem pracy było zbadanie związku między stopniem zahamowania funkcji płytek krwi a wielkością obszaru uszkodzenia mikrokrążenia u pacjentów z zawałem serca z uniesieniem odcinka ST (STEMI).

Metody: Badaniem objęto 83 pacjentów ze STEMI leczonych pierwotną PCI. Agregację płytek krwi metodą agregometrii optycznej po stymulacji kwasem arachidonowym (0,5 mM) i dwufosforanem adenozyny (ADP, 5 i 20 μM) oraz stężenie agregatów płytkowo-monocytarnych (PMA) i płytkowo-neutrofilowych (PNA) metodą cytometrii przepływowej oznaczono 2-krotnie: w chwili przyjęcia (ADM) i w 4. dobie od początku MI (D4) w czasie stosowania podwójnej terapii przeciwpłytkowej kwasem acetylosalicylowym i klopidogrelem. Rezonans magnetyczny z podaniem kontrastu wykonano między 2. a 4. dobą od początku MI w celu stwierdzenia obecności strefy spoczynkowego ubytku perfuzji i określenia wielkości obszaru obstrukcji mikrokrążenia wyrażonego jako odsetek strefy MI.

Wyniki: Spoczynkowy ubytek perfuzji stwierdzono u 56 (67,5%) pacjentów, podczas gdy obstrukcję mikrokrążenia u 63 (75,9%) osób. Chorzy ze spoczynkowym ubytkiem perfuzji w chwili przyjęcia charakteryzowali się znamiennie wyższym stężeniem zarówno PMA (7,0 *v.* 4,5%; p = 0,004), jak i PNA (4,1 *v.* 2,2%; p = 0,016), niemniej jednak w 4. dobie hospitalizacji różnice te były nieistotne. Agreagacja płytek krwi po stymulacji 5 μM ADP przy przyjęciu umiarkowanie korelowała z wielkością obstrukcji mikrokrążenia (r = 0,37; p = 0,004). Stwierdzono, że im większe jest stężenie agregatów płytkowo-leukocytarnych (PMA_{ADM} r = 0,31; p = 0,016; PNA_{ADM} r = 0,34; p = 0,006 i PMA_{D4} r = 0,35; p = 0,005), tym większy obszar obstrukcji. Strefa zawału (β = 0,43, 95% CI 0,19–0,67, p < 0,0001), TIMI < 3 po PCI (β = –0,27; 95% CI od –1,90 do –0,11; p = 0,015) i PMA_{D4} (β = 0,21; 95% CI 0,13–1,86; p = 0,032) w sposób niezależny determinowały wielkość strefy obstrukcji mikrokrążenia (R2 = 0,60, p < 0,0001).

Wnioski: Nadmierna aktywacja płytek krwi podczas reperfuzji u pacjentów ze STEMI mimo stosowania podwójnej terapii przeciwpłytkowej wiąże się z obecnością większej strefy uszkodzenia mikrokrążenia.

Słowa kluczowe: zawał serca z uniesieniem odcinka ST, aktywacja płytek krwi, agregaty płytkowo-leukocytarne, obstrukcja mikrokrążenia

Kardiol Pol 2012; 70, 7: 677–684

Adres do korespondencji:
lek. Jarosław Zalewski, Krakowski Szpital Specjalistyczny im. Jana Pawła II, ul. Prądnica 80, 31–202 Kraków, tel: +48 12 614 35 01, e-mail: jzalewski@szpitaljp2.krakow.pl
Praca wpłynęła: 14.09.2011 r. **Zaakceptowana do druku:** 16.11.2011 r.