No-reflow and platelet reactivity in diabetic patients with ST-segment elevation myocardial infarction: is there a link?

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Introduction

The no-reflow phenomenon in percutaneous coronary intervention (PCI) is defined classically as the absence of flow after restoration of arterial patency. Further research has shown that despite flow restoration in myocardial infarction (MI) there is still a considerable percentage of patients with a lack of perfusion at the level of the microvasculature caused by microvascular obstruction (MVO) [1]. This has a deleterious effect on outcomes and should be considered as a form of no-reflow [2]. It can be diagnosed with angiography or as the absence of ST-segment normalization in ECG after PCI, but the reference method for MVO diagnosis is contrast-enhanced cardiac magnetic resonance (CMR) [3]. Causes of MVO are thought to include peripheral embolism caused by debris originating in and flushed from the atherosclerotic plaque, ischemia/reperfusion injury, and individual predispositions such as diabetes [4]. Recently, increased platelet reactivity was proposed as one of the reasons for MVO occurrence [5–7]. Increased platelet reactivity is present in diabetes and together can increase MVO. Nevertheless, there is still a considerable lack of data regarding platelet reactivity and MVO assessed with the reference method of CMR in diabetic patients with ST-segment elevation MI (STEMI).

Aim

The aim of the study was to assess the link between platelet reactivity and the occurrence of MVO in diabetic patients with STEMI.

Material and methods

This is a sub-study of a larger series of patients included in the previously reported data [8]. In brief the previous study included patients with STEMI treated with PCI and diagnosed with diabetes before hospital admission. To have a closer look into MVO we included patients with Thrombolysis In Myocardial Infarction (TIMI) flow grade 3 after PCI and defined MVO when myocardial perfusion grade (MPG) assessed with angiography was 0–1 while normal microvascular flow was defined when MPG was 2 or 3 [1]. Study exclusion criteria were cardiogenic shock, platelet count below 100 000/m^3 or above 450 000/m^3, known allergy to acetylsalicylic acid or thienopyridine derivatives, and use of GP IIb/IIIa blockers during PCI. Patients included in the study were treated according to guidelines [9]. Antiplatelet treatment included a loading dose of 600 mg clopidogrel and a loading dose of 300 mg aspirin given by the paramedics before hospital admission. Blood for platelet reactivity was collected into hirudin anticoagulant probes (Sarstedt, Germany) before the procedure, immediately after it and then 24 h after PCI. Up to 2 h after blood collection platelet reactivity to arachidonic acid (response to aspirin), adenosine diphosphate (ADP) (response to clopidogrel), collagen and thrombin receptor-activating peptide (TRAP) was assessed using a Multiplate impedance aggregometer (Roche, Switzerland). Agonists for platelet aggregation were obtained from the Multiplate manufacturer. High on aspirin and clopidogrel treatment platelet reactivity (HPR) were assessed according to proposed cut-offs [10]; clopidogrel HPR was diagnosed when aggregation with ADP was above

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468 AUC · min and aspirin HPR was diagnosed when aggregation with arachidonic acid was above 300 AUC · min.

In this sub-study conducted in the year 2015 we screened 41 patients and finally included 30 patients, in whom we additionally performed contrast-enhanced CMR 7 days after PCI. Eleven drop-outs at that stage of the study were due to hospital death in 3 cases and withdrawal of informed consent in 8 patients. All CMR examinations were performed on a 1.5 T scanner (Magnetom Avanto, Siemens, Erlangen, Germany) with standard acquisition methods. Images were obtained during suspended respiration at end-expiration. Cine and delayed enhancement images were acquired in three standard left ventricular (LV) long-axis views and a set of multiple contiguous short-axis slices from the atrioventricular ring to the apex. Late gadolinium enhancement (LGE) imaging was performed using a segmental inversion recovery sequence 15 min after intravenous injection of 0.2 mmol/kg gadobenate dimeglumine (Gd-BOPTA) (Multihance, Bracco, Konstanz, Germany). Images were analyzed with

Table I. Clinical characteristics of the studied group. Microvascular obstruction diagnosed according to the cardiac magnetic resonance late enhancement results

Clinical variable	Whole group (*n* = 30) (1)	MVO present (*n* = 10) (2)	MVO absent (*n* = 20) (3)
Age [years] (mean ± SD)	59.1 ±9.4	58.1 ±10.6	59.5 ±9.2
Sex (men/women)	21/9	6/4	15/5
Culprit vessel (LAD/Cx/RCA)	10/5/15	4/2/4	6/4/10
Number of stents used, median (min.–max.)	1 (1–3)	1 (1–3)	1 (1–2)
Length of implanted stents, mean (median) [mm]	20 (18)	18 (18)	20 (18)
Diameter of implanted stents, mean (median) [mm]	3.0 (3.0)	3.2 (3.0)	3.0 (3.0)
Arterial hypertension, *n* (%)	25 (83)	9 (90)	16 (80)
Hypercholesterolemia, *n* (%)	24 (80)	8 (80)	16 (80)
Current cigarette smoking, *n* (%)	10 (33)	4 (40)	6 (30)
Positive family history, *n* (%)	4 (13)	1 (10)	3 (15)
Maximum troponin I level [ng/ml] (mean ± SD)	20.1 ±7.9	23.7 ±4.3*	19.1 ±3.8
HbA_{1c} on admission (mean ± SD) (%)	7.7 ±2.5	6.8 ±1.1	7.8 ±2.1
Left ventricular ejection fraction at discharge (mean ± SD) (%)	45.2 ±4.6	43.1 ±4.0*	47.2 ±3.4
Statins, *n* (%)	30 (100)	10 (100)	20 (100)
β-Blockers, *n* (%)	22 (73)	8 (80)	14 (46)
ACE-I, *n* (%)	30 (100)	10 (100)	20 (100)
Aspirin 1 × 75 mg, *n* (%)	30 (100)	10 (100)	20 (100)
Clopidogrel 1 × 75 mg, *n* (%)	30 (100)	10 (100)	20 (100)
Diabetes treatment at hospital admission, *n* (%):			
Insulin	7 (23)	2 (20)	5 (25)
Biguanides	10 (33)	5 (50)	5 (25)
Sulphonylurea derivatives	13 (43)	2 (20)	11 (55)
Diabetes treatment during acute STEMI phase, *n* (%):			
Insulin	15 (50)	5 (50)	10 (50)
Biguanides	14 (47)	5 (50)	9 (45)
Sulphonylurea derivatives	1 (3)	0 (0)	1 (5)

**p < 0.05 (2) vs. (3). MVO – microvascular obstruction, LAD – left anterior descending artery, Cx – circumflex artery, RCA – right coronary artery, ACE-I – angiotensin-converting-enzyme inhibitor, HbA_{1c} – glycated hemoglobin, SD – standard deviation.*

commercial software (Qmass MR, Medis Medical Imaging Systems, Leiden, the Netherlands). Left ventricular volumes and ejection fraction were calculated on the basis of end-diastolic and end-systolic manual endocardial tracings. Presence of MVO on LGE images was considered as the CMR criterion for no-reflow. Specifically, MVO was defined as a hypointense region surrounded by a hyperintense area (i.e. enhanced myocardium) on LGE images. Infarct size was quantified using computer-assisted planimetry and reported as percent of LV mass.

The study was approved by the Bioethics Committee of the Medical University of Silesia. All patients were required to sign an informed consent form for study participation.

Statistical analysis

Data are presented as mean ± SD for the normally distributed parameters. Depending on data distribution Student's *t*-test or Mann-Whitney *U* test was employed for comparison of 2 groups. For categorical or nominal variables the χ^2 test with adjustment for small numbers where applicable was used. Statistica 12 software (StatSoft, USA) was used for all calculations.

Results

Clinical characteristics of the study population are shown in Table I. Door-to-balloon time in the study was 74 ±19 min. Mean ± SD time from aspirin and clopidogrel loading dose and PCI was 57 ±17 min. The CMR defined MVO was present in 10 out of 30 patients. We found that platelet reactivity measured by four agonists was significantly higher in the group with MVO before and after the procedure, but not after 24 h (Table II). The HPR on aspirin was present in 10 patients before PCI and in 9 after PCI and it was more frequent in the MVO group in comparison with good myocardial flow on CMR (50% vs. 25%

Table II. Platelet reactivity and left ventricle performance according to microvascular obstruction diagnosed with cardiac magnetic resonance late enhancement

Variable	CMR LE MVO present (*n* = 10)	CMR LE MVO absent (*n* = 20)	Statistical significance
Before PCI:			
AA induced aggregation (AUC, AU · min) (mean ± SD)	398 ±30	214 ±17	$p < 0.04$
ADP induced aggregation (AUC, AU · min) (mean ± SD)	1179 ±270	685 ±399	$p < 0.01$
TRAP induced aggregation (AUC, AU · min) (mean ± SD)	1426 ±443	1099 ±171	$p < 0.04$
COL induced aggregation (AUC, AU · min) (mean ± SD)	829 ±44	398 ±21	$p < 0.001$
AA induced aggregation (AUC, AU · min) (mean ± SD)	317 ±18	181 ±19	$p < 0.04$
After PCI:			
ADP induced aggregation (AUC, AU · min) (mean ± SD)	1015 ±379	592 ±356	$p < 0.01$
TRAP induced aggregation (AUC, AU · min) (mean ± SD)	1376 ±278	943 ±439	$p < 0.02$
COL induced aggregation (AUC, AU · min) (mean ± SD)	815 ±35	493 ±29	$p < 0.02$
24 h after PCI:			
AA induced aggregation (AUC, AU · min) (mean ± SD)	186 ±17	158 ±20	NS
ADP induced aggregation (AUC, AU · min) (mean ± SD)	309 ±33	301 ±24	NS
TRAP induced aggregation (AUC, AU · min) (mean ± SD)	811 ±79	682 ±110	NS
COL induced aggregation (AUC, AU · min) (mean ± SD)	301 ±82	314 ±98	NS
CMR determined LV parameters:			
LVEF (%) (mean ± SD)	38.1 ±9.5	53.1 ±8.9	$p < 0.001$
LVEDV [ml] (mean ± SD)	208.4 ±60.8	179.7 ±51.2	NS
LVESV [ml] (mean ± SD)	133.1 ±55.3	86.2 ±38.6	$p < 0.05$
Infarct size (% LV mass) (mean ± SD)	31.3 ±14.8	12.4 ±7.3	$p < 0.0001$

CMR LE – cardiac magnetic resonance late enhancement, MVO – microvascular obstruction, PCI – percutaneous coronary intervention, LV – left ventricle, LVEF – left ventricle ejection fraction, LVEDV – left ventricle end diastolic volume, LVESV – left ventricle end systolic volume, AA – arachidonic acid, ADP – adenosine diphosphate, TRAP – thrombin receptor activating peptide, COL – collagen, AUC – area under curve, AU · min – arbitrary units per minute, SD – standard deviation.

before PCI and 50% vs. 20% after PCI; $p < 0.05$ for both). HPR on clopidogrel was present in 24 patients before PCI and in 20 patients after PCI and it was more frequent in the MVO group in comparison with good myocardial flow on CMR (90% vs. 75% before PCI; $p < 0.05$). MVO was associated with significantly lower left ventricle ejection fraction, higher left ventricle end-systolic volume and larger infarct size. There was no significant difference between the two groups for time from antiplatelet treatment start to PCI (MVO present vs. MVO absent 58 ±11 vs. 55 ±14 min) nor door-to-balloon time (MVO present vs. MVO absent 77 ±22 vs. 73 ±19 min). There was a significant positive correlation between CMR defined infarct size (% of the left ventricle mass) and platelet reactivity before PCI to arachidonic acid ($r = 0.46$), ADP ($r = 0.55$) and collagen ($r = 0.46$; all $p < 0.05$).

Discussion

The impact of platelets on MVO presence was investigated previously by our group and others [7, 8, 11–13]. Interestingly, there are few studies comparing platelet reactivity in STEMI with MVO diagnosed with CMR, which seems to be a "gold standard" of MVO assessment [3]. We found that four different platelet activation pathways were highly reactive *in vitro* in MVO in comparison with good microvascular flow. This result adds to existing evidence; not only poor response to aspirin and clopidogrel was associated with more frequent MVO, but also increased platelet activation pathway through collagen and thrombin receptor. In other words, obtaining TIMI 3 flow in primary PCI does not guarantee good microvascular flow on CMR if platelets show higher reactivity in four different pathways. This association disappeared 24 h after PCI, which could only be attributed to the periprocedural phenomenon in patients with a good angiographic result according to TIMI flow. Zalewski *et al.* found that presence of MVO on CMR is associated with higher admission measured ADP induced reactivity of platelets (response to clopidogrel) but not arachidonic acid (response to aspirin). The difference for ADP reactivity was also present 4 days after PCI [7]. In their study 23% of patients had post-PCI TIMI less than 3 and different aggregometry than in our group, so although some results are concordant with ours they are difficult to compare. Others also showed that higher platelet reactivity assessed indirectly by means of platelet volume [11] or platelet microparticle levels [14] were all associated with presence of MVO on CMR, while lower reactivity to TRAP was associated with more frequent intramyocardial hemorrhage on CMR in patients with STEMI treated with primary PCI [15].

Main study limitation is the small number of patients included although we performed this subanalysis as hypothesis generating only. Strikingly, even with such small numbers the CMR defined MVO group showed a highly significant difference in periprocedural platelet reactivity, which also correlated with infarct size as assessed on CMR. As CMR is a "gold standard" for the diagnosis of MVO we did not investigate MVO based on ECG or contrast echocardiography.

Conclusions

Our study indicates that periprocedural higher platelet reactivity to arachidonic acid, ADP, TRAP and collagen may be associated with presence of MVO diagnosed with CMR in diabetic patients with STEMI treated with primary PCI.

Conflict of interest

The authors declare no conflict of interest.

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