Persistent platelet activation is related to very early cardiovascular events in patients with acute coronary syndromes

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Abstract

Introduction: Persistent platelet function while on antiplatelet therapy affects outcomes in patients with acute coronary syndromes (ACS).

Aim: To evaluate whether platelet reactivity measured by collagen-epinephrine (CEPI) or collagen-ADP (CADP) closure times (CT) with Platelet Function Analyzer 100® (PFA-100) is related to very early, in-hospital cardiovascular events in patients with ACS.

Methods: The study included 91 patients with ACS undergoing percutaneous coronary intervention (PCI) with stent implantation who were treated with aspirin and clopidogrel. Patients were stratified in accordance with both CEPI-CT (<190 s or >190 s), reflecting aspirin resistance, and our own cut-off point for CADP-CT measured at a mean of 6 days after admission. In-hospital events included re-infarction, cardiac arrest, recurrent angina, severe arrythmias, pulmonary oedema and cardiogenic shock.

Results: Patients were divided into 4 study groups: group 1 with CADP-CT <104 s (n=10, 11.0%), group 2 with CEPI-CT <190 s (n=10, 11.0%), group 3 with CADP-CT <104 s and CEPI-CT <190 s (n=9, 9.9%) and a control group with both CT values above the cut-off limits (n=62, 68.1%). The baseline clinical characteristics and received treatment of each subgroup were similar. A test for a trend between controls, group 1 or 2 and group 3 disclosed statistical significance (p <0.001). When analysed separately, only patients from group 3 had a higher incidence of negative outcomes compared to controls (p <0.005; relative risk RR – 9.0; 95% CI 2.4-33.9).

Conclusions: Enhanced platelet function after PCI when measured under high shear rates by both PFA-100® cartridges is independently associated with the most unfavourable in-hospital clinical outcome.

Key words: PFA-100®, acute coronary syndrome, antiplatelet agents, cardiovascular events

Kardiol Pol 2007; 65: 40-45

Introduction

Antiplatelet therapy is a cornerstone of cardiovascular medicine. It has an influence on percutaneous coronary intervention (PCI) success in patients with acute coronary syndromes (ACS) [1]. On the other hand, both aspirin and clopidogrel resistance are becoming an emerging clinical entity [2-6]. These phenomena may be related to polymorphism in platelet receptor or enzyme genes, genetic polymorphisms of enzymes taking part in drug metabolism, alternative pathways of platelet activation, patient non-compliance, pretreatment platelet activation, various degrees of drug absorption or drug interactions.

Antiplatelet treatment improves short- and long-term prognosis in patients with ACS. The short-term benefits of antiplatelet therapy were described in randomised clinical trials. The "Second International Study of Infarct Survival" (ISIS-2) trial demonstrated that aspirin use significantly reduced in-hospital mortality in acute

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Received: 24 April 2006. **Accepted:** 19 October 2006.
The study was sponsored by Medical University of Warsaw scientific grants 1WR/NM1/05 and 1WR/NM2/05.

ST-elevation myocardial infarction (MI) [7]. The "Clopidogrel in Unstable angina to prevent Recurrent Events" trial in patients treated with percutaneous coronary intervention (PCI-CURE) showed a 30% relative risk reduction in the primary endpoint which included MI, urgent revascularisation, or cardiovascular mortality at 30 days [8]. Therefore, persistent platelet function despite standard antiplatelet therapy may determine short- and long-term outcomes.

Several studies using the Platelet Function Analyzer (PFA-100®) disclosed enhanced platelet function in patients with acute MI [9, 10]. They also found that results of the PFA-100® test are independent predictors of MI size measured by markers of cardiac necrosis.

In the present study we wanted to check for the first time whether platelet function assessed by means of PFA-100® is related to very early, in-hospital cardiovascular events in patients with ACS treated with PCI.

Methods

The study included 91 consecutive patients who underwent PCI with stent implantation in the course of ACS [62 patients with ST-elevation myocardial infarction (STEMI) and 29 patients with non-ST-elevation acute coronary syndrome (NSTE-ACS)] and received aspirin and clopidogrel according to the current guidelines [1]. All patients received a loading dose of 300 mg of aspirin followed by daily regimen of 75 mg and a loading dose of either 300 or 600 mg of clopidogrel followed by 75 mg daily. Exclusion criteria consisted of concomitant IIb/IIIa inhibitor administration, active neoplasm or a history of neoplasm, severe renal insufficiency (plasma creatinine >4 mg/dl) or severe hepatic insufficiency, haemorrhagic diathesis, hematocrit <35% and >50%, total platelet count <150.000/μl or >500.000/μl and alcohol abuse. Informed consent was obtained from all participating patients. Permission for the study was obtained from the local ethics committee.

Platelet function analysis

To monitor the effectiveness of antiplatelet therapy we used a standardised point-of-care device – PFA-100®, Dade Behring, Germany [11-13]. The PFA-100® system mimics the in vitro bleeding time by creating an artificial vessel consisting of a sample reservoir, a capillary, and a biologically active membrane with a central aperture, coated with collagen plus ADP or collagen plus epinephrine. The application of a constant negative pressure aspirates the anticoagulated blood of the sample from the reservoir through the capillary (mimicking the resistance of a small artery) and the aperture (mimicking the injured part of the vessel wall).

A platelet plug gradually occludes the aperture; as a consequence, the blood flow through the aperture gradually decreases and eventually stops. The time needed for blood flow interruption (closure time or CT) is recorded. According to our own results we decided to use a cut-off limit of collagen/ADP cartridge (CADP-CT) <104 s and collagen/epinephrine cartridge (CEPI-CT) <190 s. All of the PFA-100® measurements were performed according to the manufacturer's instructions with all specimens collected in 3.2% (0.105 M) buffered sodium citrate. As shown in previous studies, a sustained antiplatelet effect of clopidogrel is present after 5 days of treatment [14]. Therefore, the platelet function analysis was performed after the acute phase of ACS (2-9 days after admission, median – 6 days).

Study protocol

On the basis of PFA-100® test results all patients were divided into 4 study groups: group 1 with CADP-CT <104 s (n=10, 11.0%), group 2 with CEPI-CT <190 s (n=10, 11.0%), group 3 with CADP-CT <104 s and CEPI-CT <190 s (n=9, 9.9%) and a control group with both CT values above the cut-off limits (n=62, 68.1%). Very early cardiovascular events were defined as in-hospital re-infarction, cardiac arrest, recurrent angina with changes in electrocardiogram characteristic for acute ischaemia, stroke, ventricular and supraventricular arrythmias requiring electrical cardioversion or intravenous infusion of antiarrythmic drugs, pulmonary oedema, cardiogenic shock or major bleeding (any intracranial bleeding, any bleeding requiring transfusion, any bleeding with haemoglobin drop of >5 g/dl or hematocrit drop of >15% compared to previous exam). All of the defined very early cardiovascular events were followed during the median in-hospital stay of 6 days (min. 3, max. 12) and excluded events which occurred during PCI.

Statistical analysis

All the results for continuous variables are expressed as means ± SD and skewed variables as the median with minimal and maximal values. To test the normal distribution, the Kolmogorov-Smirnov test was used. For the relation between clinical data we used χ^2 with Yates' correction for dichotomised comparisons or Fisher's exact test for categorical variables with a small number of expected frequencies. Student's t-test or Wilcoxon rank sum test was applied to compare any continuous variables with a normal or non-normal distribution.

Table I. Baseline characteristics of the studied population

Parameter	Group 1 n=10	Group 2 n=10	Group 3 n=9	Controls n=62	p group/control
Male [%]	7 (70)	7 (70)	7 (77.8)	41 (66.1)	NS
Median age (min., max.)	66 (38, 85)	59 (51, 82)	54.5 (48, 71)	58 (35, 86)	NS
Loading dose of clopidogrel [%]					
– 300 mg	1 (10)*	3 (30)	3 (33.3)	26 (41.9)*	0.08*
– 600 mg	9 (90)*	7 (70)	6 (66.7)	36 (58.1)*	0.08*
Previous MI [%]	3 (30)	3 (30)	1 (11.1)	9 (14.5)	NS
Diabetes mellitus [%]	1 (10)	2 (20)	1 (11.1)	10 (16.1)	NS
Dyslipidaemia [%]	4 (40)	5 (50)	2 (22.2)	20 (32.2)	NS
Hypertension [%]	4 (40)	6 (60)	4 (44.4)	32 (51.6)	NS
Cigarette smoking [%]	3 (30)	5 (50)	7 (77.8)	29 (46.8)	NS
NYHA class III/IV	1 (10)	1 (10)	0 (0)	1 (1.6)	0.05**
Number of coronary vessels with significant changes [%]					
1	6 (60)	6 (60)	3 (33.3)	33 (53.2)	NS
2	1 (10)	2 (20)	5 (55.5)	17 (27.4)	NS
3	3 (30)	2 (20)	1 (11.1)	12 (19.4)	NS
Type of ACS [%]					
NSTE-ACS	3 (30)	3 (30)	3 (33.3)	20 (22.6)	NS
STEMI	7 (70)	7 (70)	6 (66.7)	42 (77.4)	NS
In-hospital medical treatment [%]					
aspirin	10 (100)	10 (100)	9 (100)	61 (98.4)	NS
clopidogrel	10 (100)	10 (100)	9 (100)	62 (100)	NS
heparin (UFH/LMWH)	10 (100)	10 (100)	9 (100)	61 (98.4)	NS
β-blockers	10 (100)	10 (100)	9 (100)	60 (96.8)	NS
ACE-I	10 (100)	10 (100)	9 (100)	60 (96.8)	NS
statin	9 (90)	10 (100)	9 (100)	62 (100)	NS
Outcomes [%]					
re-infarction	0	1 (10)	1 (11.1)***	1 (1.6)	–
cardiac arrest	2 (20)	0	0	3 (4.8)	–
recurrent angina	0	1 (10)	1 (11.1)	1 (1.6)	–
arrhythmia	1 (10)	1 (10)	1 (11.1)	2 (3.2)	–
pulmonary oedema	0	0	1 (11.1)	1 (1.6)	–
cardiogenic shock	0	1 (10)	2 (22.2)	0	–
major bleeding	0	0	0	0	–

Abbreviations: ACE-I – angiotensin-converting enzyme inhibitor, ACS – acute coronary syndrome, LMWH – low molecular weight heparin, MI – myocardial infarction, NSTE-ACS – non-ST-elevation acute coronary syndrome, NYHA III/IV – New York Heart Association scale for chronic heart failure (class III and IV), STEMI – ST-elevation myocardial infarction, UFH – unfractionated heparin

** test for group 1 compared to controls,*

*** test for group 1 or 2 compared to controls,*

**** re-infarction due to subacute stent thrombosis 3 days after successful PCI with stent implantation*

Relative risk of cardiovascular events for any significant risk factors was additionally calculated. All tests were two-sided with the significance level of $p < 0.05$. All statistical analyses were performed with SAS software version 9e (SAS Institute Inc., Cary, NY).

Results

Baseline characteristics of the studied population are presented in Table I. Unless there were any major contraindications, all of the patients received standard treatment for ACS consisting (apart from aspirin and clopidogrel) of unfractionated heparin and/or low molecular weight heparin, β-blockers, angiotensin-converting enzyme inhibitor and statin (either atorvastatin or simvastatin). Negative outcomes during hospitalisation occurred in 11 (12.8%) patients and consisted of 21 pre-defined events, presented by study group in Table I. One of the re-infarctions occurred in the process of subacute stent thrombosis 3 days after successful stent implantation, an event which is a marker of antiplatelet resistance. No major bleeding or stroke

was registered during hospitalisation. Discrepancies in baseline characteristics included a loading dose of clopidogrel in group 1 compared to controls and frequency of advanced chronic heart failure (NYHA class III/IV) in groups 1 and 2, compared to controls. However, we did not find any differences in platelet function between patients who received 300 and 600 mg of clopidogrel.

The test for a trend between controls, group 1 or 2 and group 3 disclosed statistical significance (p <0.001). Ratios of patients experiencing early events by study group are presented in Figure 1. When analysed separately, only patients from group 3 had a higher incidence of negative outcomes compared to controls (p <0.005; relative risk [RR] – 9.0; 95% confidence interval [CI]: 2.4-33.9), while patients from groups 1 and 2 did not (p=0.17). Because of the small number of single event types we did not analyse them separately. Because NYHA class III/IV chronic heart failure could influence the study results we performed an additional analysis with exclusion of these patients, obtaining similar results (p for trend = 0.002).

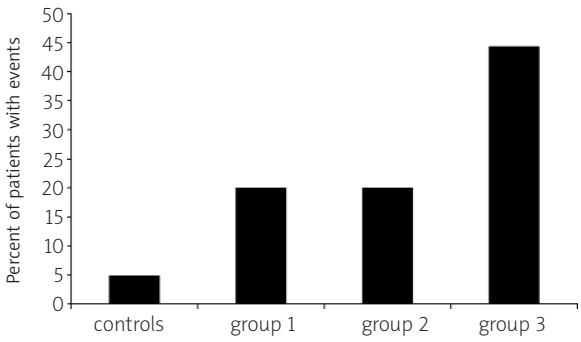

Figure 1. Proportion of patients who experienced early in-hospital cardiovascular events by study group (p for trend <0.001)

Discussion

In our study almost one third of patients showed persistent platelet function with one or two PFA-100® cartridges. A recent study by Gianetti et al. found a statistically significant relation between platelet function measured with PFA-100® and long-term cardiovascular events in patients with ACS [15]. We proved that platelet function measured with the same method was augmented in patients with ACS treated invasively and receiving standard antiplatelet treatment (aspirin and clopidogrel) who experienced negative in-hospital events. Persistent platelet activation may be at least partially explained by possible existence of antiplatelet resistance. On the other hand we cannot exclude other possible mechanisms of persistent platelet function such as pretreatment platelet activation and inherited or acquired platelet deficiencies.

In the present study platelet function assessment was performed after a few days of hospitalisation when some of the cardiovascular events had already happened. It cannot be excluded that analysed events caused platelet activation on their own. Therefore, we cannot clearly determine the pathophysiological chain of dependence. A similar study should be performed with PFA-100® testing on admission or at least before cardiovascular events.

In the light of a recent review, the use of PFA-100® for therapeutic monitoring should be restricted to research studies and prospective clinical trials [3, 11]. We present further evidence of its clinical relevance. Although light transmittance aggregometry is the current gold standard for determining platelet function, this technique is labour intensive and requires the assay to be performed in a laboratory, not at bedside [11, 16, 17]. We are also aware of the fact that single assessment of platelet function and application of only one method to test platelet function may not be sufficient to fully diagnose the response to antiplatelet therapy. Because PFA-100® is highly dependent on von Willebrand factor (vWF), prolonged or shortened CT can to some extent reflect variability in plasma concentrations of vWF. In the future, multiple testing will be required to better determine antiplatelet resistance and platelet function.

New PCI guidelines published last year recommend a 600 mg loading dose of clopidogrel over lower doses [1]. Before that time some of our patients were receiving a loading dose of 300 mg. However, we did not discover any difference in platelet function between the groups. It is known that loading dose of clopidogrel is associated with platelet function, but prominent drug-induced platelet inhibition is seen only during the first 24 hours. Therefore, it is unlikely that loading dose had an influence on platelet function measured a few days later [18].

Conclusions

We showed that platelet function is enhanced in patients with ACS who suffer from negative in-hospital events. Correct identification of patients with persistent platelet activation may enable implementation of alternative treatment strategies. We believe that the presented findings merit attention and should be confirmed by future studies.

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Przetrwała aktywacja płytek u pacjentów z ostrymi zespołami wieńcowymi jest związana z bardzo wczesnymi zdarzeniami sercowo-naczyniowymi

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Streszczenie

Wstęp: Przetrwała aktywacja płytek w trakcie leczenia przeciwpłytkowego u pacjentów z ostrymi zespołami wieńcowymi (OZW) wpływa na częstość negatywnych zdarzeń.

Cel: Ocena, czy aktywność płytek mierzona w testach z kolagenem i epinefryną (CEPI-CT) oraz kolagenem i dwufosforanem adenozyny (CADP-CT) przy użyciu Platelet Function Analyzer 100® (PFA-100®) jest związana z bardzo wczesnymi, wewnątrzszpitalnymi zdarzeniami sercowo-naczyniowymi u pacjentów z OZW.

Metody: Do badania włączono 91 pacjentów z OZW leczonych przezskórną interwencją wieńcową (PCI) z implantacją stentu, którzy otrzymali kwas acetylosalicylowy (ASA) i klopidogrel. Pacjentów podzielono na grupy w zależności od wyniku CEPI-CT (<190 s lub >190 s) odzwierciedlającego oporność na ASA oraz na podstawie wyniku CADP-CT, wykonywanych po średnio 6 dniach od przyjęcia. Zdarzenia wewnątrzszpitalne obejmowały: dorzut zawału, nagłe zatrzymanie krążenia, nawrót dławicy, złośliwe arytmie, obrzęk płuc i wstrząs kardiogenny.

Wyniki: Pacjentów zaklasyfikowano do 4 grup: grupy 1. z CADP-CT <104 s (n=10; 11,0%), grupy 2. z CEPI-CT <190 s (n=10; 11,0%), grupy 3. z CADP-CT <104 s i CEPI-CT <190 s (n=9; 9,9%) oraz grupy kontrolnej z wynikami obu testów powyżej punktów odcięcia (n=62; 68,1%). Charakterystyka podstawowa oraz zastosowana terapia nie różniły się między grupami. Badanie trendu między grupą kontrolną, grupą 1. lub 2. oraz grupą 3. wykazało istotność statystyczną (p <0,001). Analiza zależności między poszczególnymi grupami wykazała, że jedynie pacjenci z grupy 3. narażeni są na istotnie większą częstość zdarzeń w porównaniu z grupą kontrolną (p <0,005; ryzyko względne RR – 9,0; 95% CI 2,4–33,9).

Wnioski: Przetrwała aktywacja płytek u pacjentów po PCI mierzona w warunkach symulujących warunki naturalne jest niezależnie związana z negatywnymi zdarzeniami wewnątrzszpitalnymi.

Słowa kluczowe: PFA-100®, ostre zespoły wieńcowe, leki przeciwpłytkowe, zdarzenia sercowo-naczyniowe

Kardiol Pol 2007; 65: 40-45

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Praca wpłynęła: 22.04.2006. **Zaakceptowana do druku:** 19.10.2006.

Praca finansowana z grantów Akademii Medycznej w Warszawie: 1WR/NM1/05 i 1WR/NM2/05.