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Platelet aggregability and anticoagulant proteins activity during dobutamine stress echocardiography in asymptomatic patients four months after percutaneous coronary intervention

Agregabilnost trombocita i aktivnost antikoagulantnih proteina tokom stres ehokardiografije sa dobutaminom kod asimptomatskih bolesnika četiri meseca nakon perkutane koronarne intervencije

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Abstract

Background/Aim. Platelets aggregability (PA) and the activation of hemostasis during myocardial ischemia within physical or mental stress, can be one of many factors that influence the process of stent thrombosis after the percutaneous coronary intervention (PCI). The aim of the study is to investigate the relationship between the PA and activity of anticoagulant proteins with myocardial ischemia during the dobutamine stress echocardiography (DSE) in the asymptomatic patients 4 months after the PCI. **Methods.** The study population included 74 asymptomatic patients who had a successful PCI 4 months before a high-dose DSE. PA on epinephrine (EPI) and adenosine diphosphate (ADP) were determined by the Light Transmission Aggregometry (LTA), together with plasma activity of protein C and antithrombin before the DSE and at the peak stage of the stress test. The patients were divided into several groups on the basis of whether they have baseline or induced disturbance of segmental myocardial kinetics or not. All patients were on the clopidogrel and aspirin therapy at the time of DSE. **Results.** There were no statistically significant difference in the PA ADP (47.50% *vs* 50.20%; $p = 0.970$) as well as on EPI (59.30% *vs* 60.30%, $p = 0.600$) before and at the peak of DSE. A statistically significant difference was found in the anticoagulant activity of the antithrombin (84.85% *vs* 74.75%, $p = 0.001$) and protein C (77.75% *vs* 67.60%, $p < 0.001$). A significance of differences in antithrombin and the protein C, refered to the result before and at the peak levels of the test. There was no significant difference in the PA and plasma activity of anticoagulant proteins in the patients with or without induced myocardial ischemia at the peak of DSE. The patients who had an increased wall motion score index at the peak of DSE, had a higher EPI induced PA than the patients with normal myocardial contractility (68.60% *vs* 54.70%, respectively; $p = 0.017$). **Conclusion.** There are no changes in the PA before and after DSE, however, plasma activity of anticoagulant proteins decreased at the peak level of the test. The PA on EPI significantly increases at the peak of DSE in the patients with segmental myocardial hypocontractility.

Key words:
antithrombins; echocardiography, stress; percutaneous coronary intervention; platelet aggregation.

Apstrakt

Uvod/Cilj. Agregabilnost trombocita i proces aktivacije hemostaze tokom ishemije miokarda u sklopu fizičkog ili mentalnog stresa mogu biti jedan od brojnih faktora koji utiču na proces tromboze stenta nakon perkutane koronarne intervencije (PKI). Cilj rada bio je da se ispita povezanost agregabilnosti trombocita i aktivnosti antikoagulantnih proteina sa miokardnom ishemijom tokom dobutamin stres ehokardiografije (DSE) kod asimptomatskih bolesnika, četi-

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ri meseca nakon PKI. **Metode.** Studijsku populaciju činila su 74 asimptomatska bolesnika koja su imala uspešnu PKI četiri meseca pre visokodozne DSE. Agregabilnost trombocita na epinefrin (EPI) i adenozin difosfat (ADP) određena je metodom optičke agregometrije – *light transmission aggregometry* (LTA) zajedno sa aktivnošću proteina C i antitrombina u plazmi pre i u piku opterećenja tokom stres testa. Bolesnici su bili podeljeni u nekoliko grupa shodno tome da li su imali poremećaje kontraktilnosti određenih segmenata miokarda u miru ili u naporu. Svi bolesnici su u vreme DSE bili na terapiji klopidogrelom i aspirinom. **Rezultati.** Nije bilo statistički značajne razlike u agregabilnosti trombocita na ADP (47,50 *vs* 50,20; $p = 0,970$) kao ni na EPI (59,30% *vs* 60,30%; $p = 0,600$) pre i u piku DSE. Statistički značajna razlika je utvrđena u aktivnosti antikoagulantnih proteina, antitrombina (84,85% *vs* 74,75%; $p = 0,001$) i proteina C (77,75% *vs* 67,60%; $p < 0,001$). Značajnost razlike u aktivnosti antitrombina i proteina C odnosi se na rezultat pre i u piku testa. Nije utvrđena značajna razlika u agregabilnosti trombocita i aktivnosti antikoagulantnih proteina u plazmi kod bolesnika sa ili bez indukovane ishemije u piku DSE. Bolesnici koji su imali povišen indeks pokretljivosti zida leve komore (*wall motion score index*) u piku DSE su imali veću agregabilnost trombocita na EPI nego bolesnici sa normalnom kontraktilnošću miokarda (68,60% *vs* 54,70% $p = 0,017$). **Zaključak.** Nema promena vrednosti u agregabilnosti trombocita pre i posle DSE, međutim, dolazi do smanjenja aktivnosti antikoagulantnih proteina u plazmi u piku testa. Agregabilnost trombocita na EPI značajno raste u piku DSE kod bolesnika sa segmentnom hipokontraktilnošću miokarda.

Ključne reči:
antitrombini; ehokardiografija, stres; perkutana koronarna inervencija; trombociti, agregacija.

Introduction

During the last decades, ischemic heart disease is the most common cause of morbidity and mortality in the developed world. Although the mortality rate from IHD declined over last four decades all over the world, it is still responsible for a third of all deaths in the patients older than 35 years [1, 2]. An important factor in the disease incidence and complications during the percutaneous coronary interventions (PCI) is a platelet aggregability, changes in these parameters and resistance to antiplatelet therapy [3–7]. The aggregation of platelets and activation process of hemostasis during myocardial ischemia within the physical or mental stress can be an important factor in stent thrombosis after the PCI [4].

Platelets are oval or round plates with a usual diameter of about 2 microns resulting from fragmentation of megakaryocytes in the bone marrow, liver, spleen and lungs, from where they are released into the bloodstream. The most important physiological functions of platelets are: active participating in all phases of hemostasis, both physical and chemical processes, as well as the release and activity of specific platelet factors. In addition, they have a role in the process and maintaining the integrity of the endothelium, phagocytosis, body detoxification and transport of goods [5–8]. Platelets play a key role in the pathophysiology of thrombosis after the plaque rupture. The plaque rupture occurs spontaneously in the patients with an acute coronary syndrome, or may be iatrogenic induced in the patients undergoing the PCI. Among the multiple mediators of platelet activation, adenosine diphosphate (ADP) plays a key role. Thienopyridines are irreversible inhibitors of the P2Y12 ADP receptor. Clopidogrel is a second generation thienopyridine that is, in combination with aspirin, proved to be superior to oral anticoagulants in the prevention of thrombotic complication after stenting of the coronary arteries [3, 8]. Protein C and antithrombin are natural plasma proteins that play an important role in the process of anticoagulation. Their deficiency leads to the development of procoagulant conditions, and one of the complications can be artery thrombosis [9–13]. The association of stress caused by ischemia, quantified by dobutamine stress echocardiography (DSE), and changes in platelet function and other parameters of hemostasis in patients after the PCI, have not been tested so far.

The aim of the study was to investigate the relationship between the platelets aggregability and activity of anticoagulant proteins with stress-induced myocardial ischemia during the dobutamine stress echocardiography in the patients after the PCI with stent implantation.

Methods

Subjects

The prospective study population included 74 patients with ischemic heart disease. At least 5 days before the PCI, the patients were on the dual antiplatelet therapy (aspirin 100 mg + clopidogrel 75 mg). All patients had completed the PCI 4 months before admission to the study and did not have angina. There was no difference in the dual antiplatelet therapy in the patients with the acute coronary syndrome and stable angina pectoris, both groups received the same dose of aspirin and clopidogrel. The study population was on average 58 years old and 35% of them were women. The blood samples had been taken from all patients before the test and at the maximum load of the SE [14, 15]. The platelets aggregability was determined by the method of Light Transmission Aggregometry (LTA) [3, 16] before and at the maximum load of the DSE. The LTA is used for the ADP and EPI tests. We also determined the concentration of protein C, and antithrombin in all plasma samples. The main characteristics of patients are shown in Table 1.

Dobutamine stress echocardiography test

Four months after the PCI, all patients underwent the high-dose DSE. Dobutamine infusion was initiated with 10 μg/kg/min, then increased by 10 μg/kg/min every 3 minutes until a dose of 40 μg/kg/min was reached. Each stage was regularly monitored for the blood pressure and ECG. Also,

the side effects during the dobutamine test were followed in all patients. The regional wall abnormalities of the left ventricle were analyzed in accordance with the adopted the 16 divisions segmented model of the left ventricule by echocardiography recommended by American Cardiology Society. The regional wall-motion abnormality of each segment was evaluated by use of the 4-point scoring system: 1 = normal wall thickening; 2 = hypokinesia; 3 = akinesia; 4 = dyskinesia. Each segment of the left ventricle was individually assessed as viable if its regional contractility from the initial akinesia, hypokinesia or dyskinesia to hypokinesia or normokinesia were significantly improved at low doses of dobutamine while altering from the dyskinesia to akinesia was not considered as a marker of viability. If we had a worsening of motion from the baseline maintained motility after the dobutamine administration, these segments were evaluated as ischemic responses. Global mobility of the left ventricle in basal conditions and after the administration of low concentrations of dobutamine was determined based on the obtained values. The global mobility was presented like as Index of mobility – Wall Motion Score Index (WMSI), which is a measure of the average deviation from the ideal mobility segment that is preserved mobility of all 16 surveyed segments.

$$\frac{\text{WMSI} = \text{No. normokinesia} \times 1 + \text{No. hypokinesia} \times 2 + \text{No. akinesia} \times 3 + \text{No. diskinesia} \times 4}{16}$$

In physiological conditions, WMSI is 1, the WMSI value of 1.1 or higher, indicates a higher degree of the left ventricular dysfunction. WMSI was calculated in basal conditions and at the high dobutamine doses of 40 mg/kg. The findings of the DSE were considered positive when the regional wall motion of a normal or hyperkinetic segment was deteriorated. The interpretation of the test was done by an expert, echocardiographer.

Hemostatic parameters

At the same day when we performed the PCI, blood was sampled from the brachial veins in order to determine the platelets aggregability with the LOTA method. After the PCI, 4 months later, 2 samples of blood were taken from the brachial vein into the tubes containing sodium citrate, 3.8% below the minimum path, 30 min after standstill. The DSE was performed 2 to 3 hours after a blood cannula had been placed into the brachial vein. At the maximum load, blood was sampled from the venous cannula previously placed, into the two tubes containing 3.8% sodium citrate. Platelet rich plasma (PRP) was obtained by centrifugation at 150 x g for 10 min at room temperature. The platelet aggregation response to ADP (20 mmol/L) was recorded 5 min after addition of the agonists using the agregometar BCT-system (Dade-Behring, Germany). For determining the activity of protein C and antithrombin, platelet poor plasma (PPP) was taken, citrated and centrifuged at 2,000 x g for 15 min at room temperature and frozen at -80 °C to final weighing [4]. The activity of the protein C and antithrombin was determined by a colorimetric assay (Berichrom, Dade-Behring, Germany). All procedures were carried out according to the manufacturer's instructions.

Statistics

The Wilcoxon's test was used to compare two related samples as well those of the population without a normal distribution. The Mann-Whitney U test was used for comparison of two independent samples of the population who did not have a normal distribution. For the processing of the data, we used the SPSS (Statistical Package for the Social Sciences 20.0 for PC, SPSS Inc., Chicago, IL, USA). The values of $p < 0.05$ were considered significant, while those of $p < 0.01$ were considered to be statistically highly significant.

Results

The main characteristics of patients are shown in Table 1. There was no significant difference in the platelet aggregability both in the LTA tests on ADP ($p = 0.970$) and epinephrine (EPI) ($p = 0.600$), before and at the peak of the test (Table 2).

Table 1

Basic characteristics of patients included in the study

Basics parameters	Patients (n =74)
Age (years), mean ± SD	58 ± 9
Male, n (%)	48 (64.9)
Diabetics, n (%)	16 (21.6)
Smokers, n (%)	53 (71.6)
Treated hypertension, n (%)	71 (95.9)
Hypercholesterolemia, n (%)	32 (47.1)
BMI (kg/m^2), mean ± SD	27.05 ± 3.92
Body surface (m^2), mean ± SD	1.97 ± 0.22
STEMI patients, n (%)	11 (14.9)
NTEMI patients, n (%)	13 (17.6)
UAP patients, n (%)	18 (24.3)
SAP patients, n (%)	32 (43.2)

n – number of patients; SD – standard deviaton; BMI – body mass index; STEMI – ST-elevation myocardial infarction; NTEMI – non ST-elevation myocardial infarction; UAP – unstable angina pectoris; SAP – stable angina pectoris.

Table 2

Differences in the platelet aggregation and anticoagulation protein activity before and at the end of the stress echo test

Hemostatic parameters	Stress test, median (IQR)		*p* value
	before	at the end	
Platelet aggregability on ADP (%)	47.50 (31.65–74.40)	50.20 (31.90–71.30)	0.970
Platelet aggregability on EPI (%)	59.30 (44.60–74.90)	60.30 (44.50–74.50)	0.600
Antithrombin activity (IU/L)	84.85 (63.10–98.40)	74.75 (45.43–92.25)	0.001
Protein C activity (IU/L)	77.75 (39.88–105.00)	67.60 (32.30–97.25)	< 0.001

IQR – interquartile range; ADP – adenosine diphosphate; EPI – epinephrine.

A statistically significant difference was found in the activity of the anticoagulant proteins, antithrombin (p = 0.001) and protein C (p < 0.001) (Table 2). Significant ischemia was found in 16 of 74 patients. At the peak of the test, we registered very significant drop in the concentration of antithrombin and protein C levels (Table 2).

There was no significant difference in the platelet aggregability on ADP in the groups of patients with the positive and negative stress echo test (p = 0.829). The group with the positive stress echo test had the median of platelet aggregability on ADP of 38.10% with the inter-quartile range (IQR) from 30.05% to 72.80%, while that with the negative stress echo test on ADP had the median of platelet aggregability on ADP of 52.00% with IQR from 32.65% to 71.35% (Figure 1).

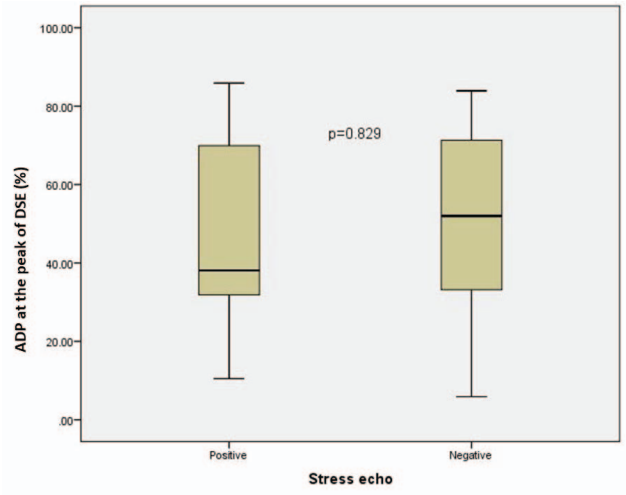

Fig. 1 – Platelet aggregability on adenosine diphosphate (ADP) in the groups of patients with positive and negative DSE test (stress echo). DSE – dobutamine stress echocardiography.

There was no significant difference in the platelet aggregability on EPI between groups (p = 0.465). The group with the positive stress echo test had median of platelet agregregability on EPI of 62.00% with IQR from 51.30% to 80.70%, while the group with negative stress echo test had the median of platelet aggregability on EPI of 59.00% with IQR from 43.83% to 73.60% (Figure 2). There was no statistically significant difference between groups regarding antithrombin activity (p = 0.081). The group with the positive stress echo test had the median of antithrombin activity of 86.30 IU/L with IQR from 58.00 IU/L to 101.25 IU/L, and the group with negative stress echo test had the median of antithrombin activity of 70.00 IU/L, with IQR from 36.35 IU/L to 87.50 IU/L (Figure 3).

There was no statistically significant difference between groups regrading activity of protein C (p = 0.240). The stress echo test positive group had the median of protein C activity of 64.50 IU/L with IQR from 11.20 IU/L to 94.43 IU/L, while the negative echotest group had the median of protein C activity of 67.70 IU/L with IQR from 35.10 IU/L to 99.00 IU/L (Figure 4).

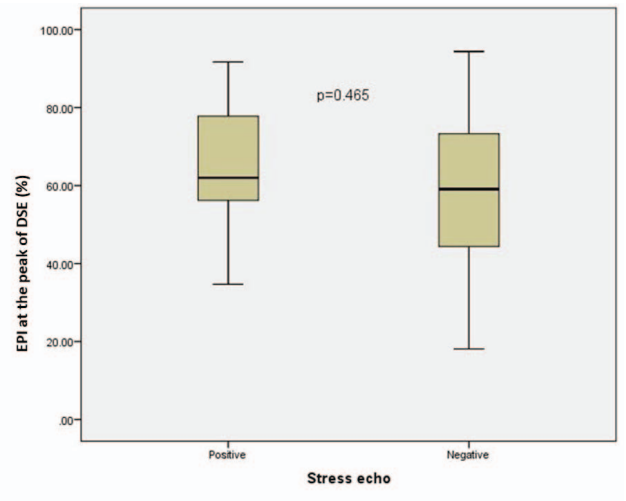

Fig. 2 – Platelet aggregability on epinephrine (EPI) in the groups of patient with positive and negative DSE test (stress echo). DSE – dobutamine stress echocardiography.

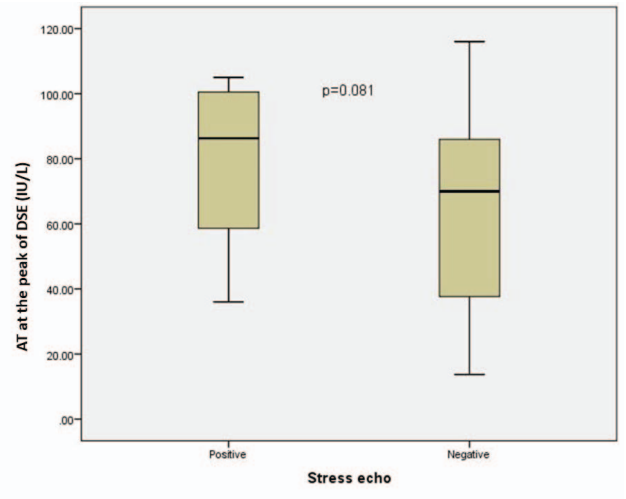

Fig. 3 – Antithrombin (AT) activity in the groups of patients with positive and negative DSE test (stress echo).

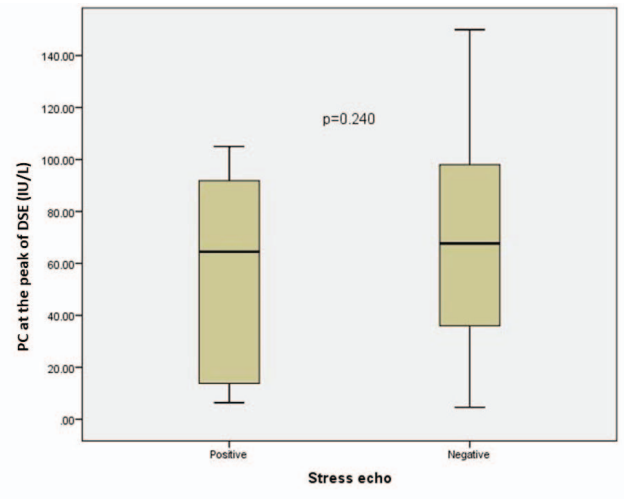

Fig. 4 – Protein C (PC) activity in groups of patients with positive and negative dobutamine stress echocardiography (DSE) test (stress echo).

Table 3

Differences in the platelet aggregability and anticoagulation protein activity compared with the Wall Motion Score Index (WMSI) at the peak of stress echo test

Hemostatic parameters	WMSI = 1 (n = 39) median (IQR)	WMSI > 1 (n = 35) median (IQR)	*p* value
Platelet aggregability on ADP (%)	49.70 (30.30–66.43)	65.20 (32.75–77.40)	0.275
Platelet aggregability on EPI (%)	54.70 (41.05–67.48)	68.60 (52.15–85.25)	0.017
Antithrombin activity (IU/L)	65.80 (33.50–82.15)	75.30 (56.15–98.65)	0.115
Protein C activity (IU/L)	63.00 (26.35–99.00)	84.00 (33.40–91.75)	0.921

IQR – interquartile range; n – number; ADP – adenosine diphosphate; EPI – epinephrine.

In the groups of patients with WMSI equal to 1.0 or greater than 1.0, it was found that there was a statistically significant difference between the groups in determining platelet aggregability on EPI, while concerning other parameters, platelet aggregability on ADP, antithrombin and protein C activities, there was no significant difference between the groups at the peak of the DSE test (Table 3).

Discussion

The most important finding in our investigation was that no one in the group of asymptomatic coronary patients, 4 months after the PCI, had an increase in the EPI and ADP dependent patelets aggregability at the peak of DSE; furthermore they had decreasing activity of both protein C and antithrombin. In the subgroup of patients with the detected regional motion abnormality at the peak of DSE, we measured the higher EPI induced platelets aggregability than in the subgroup of patients with a completely normal ventricular contractile function. These findings implicate a potential hypercoagulable state, especially during the inotropic and chronotropic myocardial stress in the coronary patients after the PCI, particularly in the presence of wall motion disturbances.

The treadmill test, which was used in most of the studies of hemostasis during physical exercise, had pretty or rather low sensitivity in the diagnosis of myocardial ischemia in comparison to the DSE [17–21] , which limits its capability to induce changes in hemostasis with appearance of ischemia. The DSE was used in our study, because of its better sensitivity to detect ischemia, so we could expect that the changes in hemostasis can be firmly associated with myocardial ischemia. The DSE lasts 12 min and it is not realistic to expect that in certain patients, due to the positive inotropic effects, dobutamine may cause adverse events. In our study, there were no such events on the effect of dobutamine during the test. Also, we found that the platelets aggregability on EPI was significantly higher in the patients with the regional left ventricle wall motion disturbances. They associate chronotropic and inotropic stress of myocardium and insufficient coronary flow with catecholamines induced enhanced platelets aggregability which can cause arterial thrombosis, especially at the spot of coronary stents.

In our previous study, which included 37 asymptomatic coronary patients, 4 to 8 months after the PCI, with even more sophisticated single photon emission computed tomography (SPECT) adenosine-exercise stress testing, we showed a significant decrease of antithrombin, but not the protein C activity without changes in the platelets aggregability at the peak of stress test [4]. In the adenosine-exercise stress SPECT test, redistribution of the blood flow and tachycardia induced ischemia, while in the DSE tachycardia and inotropic myocardial stimulation were the main factors causing ischemia. These differences, in the way of inducing ischemia, may be the reason for the EPI induced platelets aggregability did not change in the adenosine-exercise SPECT test and it was increased at the peak of DSE in the patients with the regional wall motion dysfunction.

The limitation of this study is a relatively small number of patients.

Conclusion

There are no changes in the platelets aggregability before and after the DSE, however, plasma activity of anticoagulant proteins decreased at the peak level of the test. The platelet aggregability on EPI significantly increases at the peak of DSE in the patients with a pronounced segmental myocardium hypocontractility (elevated WMSI). There is a need for more clinical trials, with a larger number of patients, to optimize the antiplatelet therapy in the patients with the coronary artery disease who had the PCI. Also, it is essential to determine additional hemostatic parameters which can interplay with the stress, myocardial ischemia and antiplatelet agents.

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