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Auto-antibodies to protein Z in patients undergoing off-pump coronary artery bypass surgery – preliminary results

Występowanie autoprzeciwciał przeciwko białku Z u pacjentów poddanych operacji pomostowania aortalno-wieńcowego bez użycia krążenia pozaustrojowego – obserwacje wstępne

Artur Słomka[1], Aleksandra Pieküś[2], Mariusz Kowalewski[3], Giuseppe Maria Raffa[4], Kamil Kozerawski[1], Wojciech Pawliszak[2], Lech Anisimowicz[2], Ewa Żekanowska[1]

[1]Department of Pathophysiology, Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland
[2]Department of Cardiac Surgery, Dr Antoni Jurasz Memorial University Hospital in Bydgoszcz, Poland
[3]Clinical Department of Cardiac Surgery, Central Clinical Hospital of the Ministry of Interior in Warsaw, Poland
[4]Cardiac Surgery and Heart Transplantation Unit, Department for the Treatment and Study of Cardiothoracic Diseases and Cardiothoracic Transplantation, IRCCS-ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad alta specializzazione), Palermo, Italy

Abstract

Background: Considerable attention has been focused over the past several years on the protein Z (PZ) system. However, little is known about the role of auto-antibodies to PZ (anti-PZ) in cardiac surgery patients.

Aim: In the present pilot study, we investigated plasma levels of anti-PZ in patients undergoing off-pump coronary artery bypass (OPCAB) surgery.

Material and methods: Thirty patients with coronary artery disease scheduled for OPCAB surgery were enrolled in this study. Plasma levels of anti-PZ IgM and anti-PZ IgG were measured before surgery and one week after surgery using enzyme-linked immunosorbent assay.

Results: None of the subjects were shown to have positive results for anti-PZ IgM and anti-PZ IgG either before and one week after OPCAB surgery.

Conclusions: These results suggest that anti-PZ autoantibodies are not an effective component of the PZ system in OPCAB patients.

Streszczenie

Wprowadzenie: W ostatnich latach wiele uwagi poświęca się ocenie układu białka Z (PZ). Niewiele wiadomo jednak na temat roli autoprzeciwciał przeciwko białku Z (anti-PZ) u pacjentów poddawanych zabiegom kardiochirurgicznym.

Cel badań: Celem tego pilotażowego badania była ocena osoczowego stężenia autoprzeciwciał anti-PZ u pacjentów zakwalifikowanych do operacji pomostowania aortalno-wieńcowego bez użycia krążenia pozaustrojowego (OPCAB).

Materiał i metody: Do badania włączono 30 pacjentów zakwalifikowanych do zabiegu OPCAB. Osoczowe stężenie autoprzeciwciał anti-PZ klasy IgM i IgG oceniono przed operacją i tydzień po niej przy użyciu testów immunoenzymatycznych.

Wyniki: Nie stwierdzono obecności autoprzeciwciał anti-PZ klasy IgM i IgG przed operacją OPCAB oraz tydzień po tym zabiegu.

Wnioski: Przeprowadzone badania pilotażowe wykazały, że autoprzeciwciała anti-PZ nie są istotnym składnikiem układu białka Z u pacjentów poddanych operacji pomostowania aortalno-wieńcowego bez użycia krążenia pozaustrojowego.

Keywords: auto-antibodies to protein Z, off-pump coronary artery bypass surgery
Słowa kluczowe: autoprzeciwciała przeciwko białku Z, pomostowanie aortalno-wieńcowe bez użycia krążenia pozaustrojowego

Introduction

The fundamental characteristic of hemostasis is a balance between pro– and anticoagulant systems. There is increasing evidence that an essential factor to achieving this balance is protein Z (PZ). PZ is a vitamin K-dependent glycoprotein which serves as a cofactor for the protein Z-dependent protease inhibitor (ZPI). Both proteins form a complex with each other that inhibits activated factor X (FXa) [1].

In spite of the fact that the role of the PZ system in coagulation is well recognized, little is known about the non-genetic mech-

anism(s) regulating PZ levels in circulation. To date, a few clinical studies were focused on the detection of auto-antibodies to PZ (anti-PZ) [2-6]. Despite these findings, anti-PZ antibodies have not been investigated in the patients undergoing cardiac surgery. The purpose of the pilot analysis presented here was to evaluate the presence or absence of anti-PZ antibodies in patients undergoing off-pump coronary artery bypass (OPCAB) surgery. We have previously observed the significant effect of OPCAB surgery on PZ and ZPI levels [7]. Thus, in this initial report we have focused on the evaluation of the next component of the PZ system, anti-PZ antibodies. This study extends our previous research on the PZ system in OPCAB individuals [7].

Table I. Baseline characteristics of the study participants.

Parameter [unit]*		OPCAB patients (n=30)
Age [years]		63 (58-66)
BMI [kg/m^2]		29 (26-30)
SBP [mmHg]		131 (125-140)
DBP [mmHg]		82 (70-89)
LVEF [%]		60 (55-66)
Pre-operative laboratory data	sCr [mg/dL]	0.87 (0.83-0.95)
	eGFR [mL/min/1.73 m^2]	89 (80-96)
	HGB [g/L]	147 (140-152)
	RBCs [10^6/μL]	4.9 (4.6-5.2)
	WBCs [10^3/μL]	6.7 (5.9-7.8)
	PLTs [10^3/μL]	199 (160-220)
	CRP [mg/L]	2.23 (0.58-6.83)
Pre-operative hemostatic data	aPTT [s]	40.3 (36.7-42.1)
	INR	1.29 (1.21-1.36)
	Fibrinogen [g/L]	2.93 (2.64-3.53)
	AT [%]	95 (84-98)
Current smokers, n (%)		5 (17%)
Medical history, n (%)	Previous MI	17 (57%)
	Previous AIS	2 (7%)
	Hypertension	28 (93%)
	Diabetes mellitus	8 (27%)
	Dyslipidemia	19 (63%)
	AF	4 (13%)
Type of surgical procedures, n (%)	Touch	18 (60%)
	Non-touch	12 (40%)
Procedure duration [minutes]		218 (180-270)
Number of grafts		2 (2-3)
Post-procedural hospital stay [days]		7 (7-8)
Pre-operative medications, n (%)	ASA	30 (100%)
	Statin	27 (90%)
	β-blockers	29 (97%)
	ACEIs	7 (23%)
	Clopidogrel	4 (13%)
	LMWH	21 (70%)
	CCB	15 (50%)
Post-operative medications, n (%)	ASA	30 (100%)
	Statin	30 (100%)
	β-blockers	30 (100%)
	ACEIs	26 (80%)
	Clopidogrel	20 (67%)
	LMWH	30 (100%)
	CCB	5 (17%)
In hospital mortality, n		0

*values are median (interquartile range, IQR) or number (%) of patients as appropriate.
Abbreviations:
ACEIs: angiotensin-converting-enzyme inhibitors, AF: atrial fibrillation, AIS: acute ischemic stroke, aPTT: activated partial thromboplastin time, ASA: acetylsalicylic acid, AT: antithrombin, BMI: body mass index, CCB: calcium channel blockers, DBP: diastolic blood pressure, eGFR: estimated glomerular filtration rate, HGB: hemoglobin levels, CRP: C-reactive protein, INR: international normalized ratio, LMWH: low-molecular-weight heparin, LVEF: left ventricular ejection fraction, MI: myocardial infarction, n: number, PLTs: platelets, RBCs: red blood cells, SBP: systolic blood pressure, sCr: serum creatinine, WBC: white blood cells,

Material and Methods

In the current study, we used the same cohort of patients that were reported on previously [7]. The participants' enrolment, data collection, and the definitions of the clinical variables have been described in detail [7]. Briefly, we recruited male patients aged 45-80 years who underwent elective first-time isolated OPCAB surgery. Those patients undergoing emergency, redo, or concomitant valvular surgery were excluded. Additionally, subjects suffered from recent myocardial infarction (≤ 1 month), systemic malignancy, known bleeding disorders, renal and/or liver insufficiency, active hepatitis, pre-operative anemia (hemoglobin < 130 g/L), severe obesity (body mass index, BMI ≥ 40 kg/m^2) and mental disorders were excluded. All individuals gave written informed consent, and the study was approved by the local Bioethical Committee.

Fasting venous blood was collected from patients twice: once on the day of surgery and once a week after surgery. The second time point was chosen based on previous papers in which different types of antibodies were measured at a similar time [8, 9]. Blood was drawn at 7:00 a.m into tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, USA) containing 3.2% sodium citrate with a 21-gauge butterfly needle (BD Vacutainer® Safety-Lok™, Becton Dickinson). Immediately following centrifugation at 2,500 x g for 20 min at +4°C, the plasma samples were frozen at – 80°C until analysis. All samples were thawed only once prior to use.

Plasma levels of anti-PZ IgM and anti-PZ IgG were determined with a ZYMUTEST Anti-PROTEIN Z IgM-Isotype kit and a ZYMUTEST Anti-PROTEIN Z IgG-Isotype kit, respectively (HYPHEN BioMed, SAS, Neuville-sur-Oise, France). The results were expressed as arbitrary units per milliliter (AU/mL), defined as per the upper limit of the normal range that corresponds to the mean plus 2 standard deviations value in a normal population, and were interpreted as negative if the AU was < 10, and as positive if it was ≥ 20. The results falling in between this range were regarded as grey zone results (≥ 10 AU/mL to < 20 AU/mL) [4].

Statistical analysis was conducted with STATISTICA® 13.1 (Dell Computer Corporation, Round Rock, Texas, USA). The data in Table I are shown using median (Me) and interquartile range (IQR). Auto-antibodies to PZ are presented as the results for each individual patient.

Results

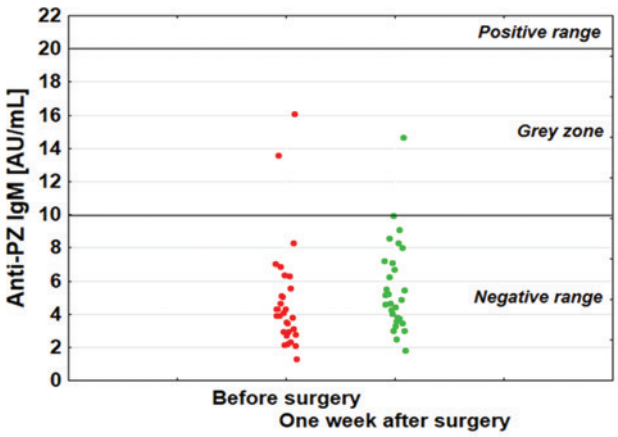
1. Demographic and clinical characteristics of the study population.

Table I summarizes the study population's characteristics. After applying exclusion criteria, a total of 30 patients were enrolled and all of them completed the study. The median age of the patients was 63 years and median BMI was 29 kg/m^2. Details of comorbidities were provided for all subjects. The most common among these comorbidities were hypertension (93%) and dyslipidemia (63%). The majority of patients (40%) underwent three bypass grafts. The touch technique was used in 60% patients. Pre– and postoperative drug use is presented in Table I. Of note, oral anticoagulants were not used after surgery in any OPCAB individuals.

2. Plasma levels of anti-PZ autoantibodies in patients before OPCAB surgery and one week after OPCAB surgery.

a) anti-PZ IgM isotype (Figure 1A).

Anti-PZ IgM autoantibodies before surgery were negative in 28 patients, and 2 patients had results within the grey zone. One week after OPCAB surgery the results were negative in 29 patients, and 1 patient had results within the grey zone. It is noteworthy that none of the subjects presented positive results at the two measurement time points.



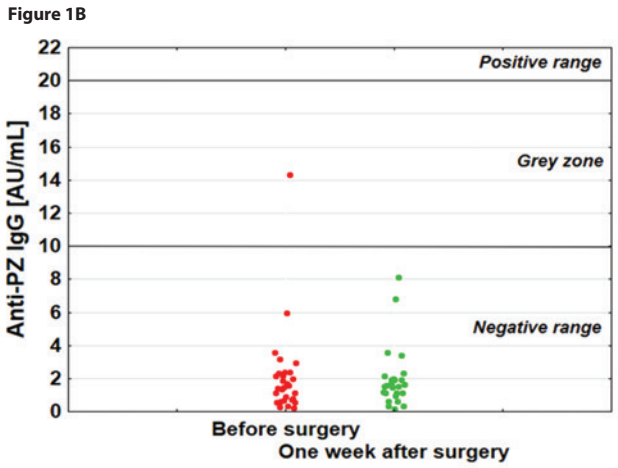


Figure 1. Plasma levels of auto-antibodies to protein Z of IgM (Figure 1A) and IgG (Figure 1B) isotypes among OPCAB patients before surgery and one week after surgery.

b) anti-PZ IgG isotype (Figure 1B).

Negative results for anti-PZ IgG autoantibodies were found in 29 patients before OPCAB surgery and in all patients (n=30) one week after surgery. Similarly to anti-PZ IgM, none of the patients had positive results for anti-PZ IgG either before and after OPCAB surgery.

Discussion

In the present study, we asked two major questions. Firstly, whether autoantibodies to PZ are present in circulation of patients scheduled for OPCAB surgery. Secondly, we aimed to investigate the present of auto-antibodies to PZ one week after surgery. Our study highlights the absence of auto-antibodies to PZ in the blood of the individuals undergoing OPCAB grafting.

The mechanism of blood anti-PZ antibody action is poorly understood. Several authors investigated the clinical importance of anti-PZ antibodies in a variety of pathological conditions associated with thrombosis [5, 6]. The study of Pardos-Gea et al. [5] revealed that the majority of subjects with arterial and venous thrombosis presented negative anti-PZ antibodies. Most importantly, the lack of anti-PZ antibodies was associated with significantly lower levels of PZ in individuals with thrombosis when compared to healthy controls. Consequently, the argument could be made that the process of thrombosis is not associated with anti-PZ IgG and IgM autoantibodies and that antibodies to PZ are not involved in the regulation of plasma PZ levels. In addition to this, anti-PZ autoantibodies, even when their presence was detected in circulation, did not significantly increase risk for thrombosis [6]. In our earlier study [7], we provide data supporting the clinical relevance of the PZ system: an increase in PZ levels and a decrease in ZPI levels have been reported one week after OPCAB surgery. Therefore, novel information on the effect of anti-PZ autoantibodies in cardiac surgery would be clinically significant. To our knowledge, the current study is the first analyzing anti-PZ autoantibodies in OPCAB subjects. This data provides evidence that anti-PZ antibodies are not an effective component of the PZ system in these individuals. Although our study raises several potential issues for the PZ system in cardiac surgery, there are some limitations. The main limitation of our monocentric study is the relatively low number of patients. Furthermore, our data only relates to elective, not emergency surgery. Nonetheless, we consider these initial results to be useful for other researchers who are interested in hemostasis of OPCAB patients.

Summing up, our study has shown that OPCAB patients did not display antibodies to PZ and that surgery had no influence on anti-PZ autoantibodies generation.

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Corresponding author:
dr Artur Słomka
Department of Pathophysiology
Nicolaus Copernicus University in Toruń
Ludwik Rydygier Collegium Medicum in Bydgoszcz
Skłodowskiej-Curie Str. 9, 85-094 Bydgoszcz
Poland
Tel. + 48 52 5853594
E-mail: artur.slomka@cm.umk.pl

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