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Vascular Thrombosis Associated with Antiphospholipide Syndrome

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A B S T R A C T

The aim of this study was to present our diagnostic and therapeutic experience with antiphospholipide syndrome (APS) and vascular thrombosis. Ninety-nine patients with positive antiphospholipide antibodies (aPL) and vascular thrombosis were included in the study: forty patients, according to clinical classification criteria, had primary antiphospholipide syndrome (PAPS), and fifty-nine patients had secondary antiphospholipide syndrome (SAPS). In PAPS group, 82.5% of the patients were LA-positive, 37.5% of the patients were IgG aCL-positive, 27.5% of the patients were IgM aCL-positive, and 15% of the patients were IgG antiβ2GPI-positive. In SAPS group, 61% of the patients were LA-positive, 50.8% of the patients were IgG aCL-positive, and 47.5% of the patients were IgM aCL-positive. Administered therapy was low molecular weight heparin (LMWH) throughout 7 days, followed by warfarin with prothrombin time maintained between 2.0 and 3.0 INR.

***Key words**: antiphospholipid syndrome, vascular thrombosis, antibodies*

Introduction

Antiphospholipide syndrome (APS) is characterized by venous and/or arterial thrombosis, recurrent pregnancy loss and presence of antiphospholipide antibodies[1]. Two antiphospholipide antibodies related to repeat thrombosis episodes are anticardiolipin antibodies (aCL) and lupus anticoagulant (LA)[3]. Anti-β2-glycoprotein I (anti-β2GPI) antibodies are found in sera of many, but not all patients with APS. Primary antiphospholipide syndrome (PAPS) occurs in patients without clinical evidence of another autoimmune disease, whereas secondary antiphospholipide syndrome (SAPS) occurs in association with autoimmune or other disease[2].

The most common clinical manifestation of antiphospholipide syndrome is thrombosis, which can affect vessels of any organ. Venous thrombosis, particularly of the lower limb, occurs in up to 55% of patients with this syndrome, half of which also have pulmonary embolism[3–5]. Arterial thrombosis affects the brain, causing transient ischemic attacks (TIA) or strokes. Other anatomical sites for arterial thrombosis are heart, eye, kidney and peripheral arteries[3–5]. Vascular occlusion may occur due to embolism from central sources such as vegetations on a mitral or aortic valve, which are reported in up to 4% of patients with antiphospholipide syndrome[5].

We present our diagnostic and therapeutic experience involving patients with APS and vascular thrombosis.

Subjects and Methods

Ninety-nine patients who fulfilled Sapporo classification criteria for APS, revised according to Sydney criteria, were included in the study[6,7]. Forty of those patients had PAPS, while 59 had SAPS. All patients had aPL anti-

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body level determined, and as a clinical manifestation they had vascular thrombosis affecting one of the organs. Lupus anticoagulant was determined with test of Dade Behring Marburg firm using LA 1 screening reagent and LA 2 confirming reagent, which is simplified reagent Dilute Russell's Viper Venom Test (DRVVT) for lupus anticoagulant verification. Anticardiolipin antibodies were determined with Autostat^{TM}II ACA test of Hycor firm, for every isoclass, based on enzyme-linked immunosorbent assay (ELISA), which is used for detection of specific antibodies against cardiolipin in human serum. Antiβ_2-glycoprotein-1 antibodies were identified with ETI-Beta-Glycoprotein I IgG kit for quantitative analysis of IgG antibodies against β_2-glycoprotein 1 in human serum. Preliminary tests, prothrombin time, activated partial thromboplastin time, fibrinogen and thrombin time (DADE Behring) were used for coagulogram screening, and platelet number was determined with Cell-Dyn-4000. Protein C (APC resistance) was determined with ProC Global test (DADE Behring). Factor V von Leiden mutation was determined according to Bertin[8], prothrombin mutation was determined according to Poort[9], and C667T mutation MTHFR was determined according to Frosst[10]. Data input and processing was done in a computer table calculator Microsoft Excel 2003, whereas software packages SPSS 13.0 for Windows and Statistica 6.0 were used for statistical analysis.

Results

Average age in PAPS group was 54.7±14.4, while in SAPS group it was 57.3±13.9. Ninety-nine patients, who fulfilled clinical classification criteria for APS were included in the study. Forty of them had PAPS, and 59 had SAPS. All patients had aPL antibody level determined, and as a clinical manifestation they had vascular thrombosis affecting one of the organs.

Table 1 shows distribution of patients according to gender. aPL antibody analysis showed that 82.5% of patients from PAPS group, and 61% of patients from SAPS group were LA-positive. In SAPS group, 50.8% of the patients were aCL-positive, while in PAPS group 37.5% of the patients were aCL-positive. Picture 1 shows share of specific aPL antibodies in patients with APS and vascular thrombosis. Distribution analysis results for patients with PAPS and SAPS, obtained by Kolmogorov-Smirnova test, lead to conclusion that analysed distributions are not normally distributed. Since the study included relatively small number of patients, distribution normality analysis needed to be done with Mann-Whitney test, which is a nonparametric test. On the significance level $p<0.05$, it is accepted as possible the hypothesis that between patients with primary antiphospholipide syndrome (PAPS) and patients with secondary antiphospholipide syndrome (SAPS) exists significant difference in β2-GPI IgG and IgA aCL values (Table 2). In order to examine connection that exists between analysed variables (LA, β2-GP1 IgG, IgG aCL, IgM aCL and IgA aCL), tables 3 and 4 show Spearman's correlation rang coefficients and belonging p-values obtained by testing significance of the hypothesis. Based on obtained results, it can be concluded that statistically significant correlation exists only between IgG aCL and IgM aCL variables, in both (PAPS and SAPS) groups of patients.

The most often clinical manifestation of APS was deep vein thrombosis (DVT) of lower leg, with 45% incidence in PAPS group and 32.2% incidence in SAPS group. Cerebral stroke had similar incidence, 27.5% in

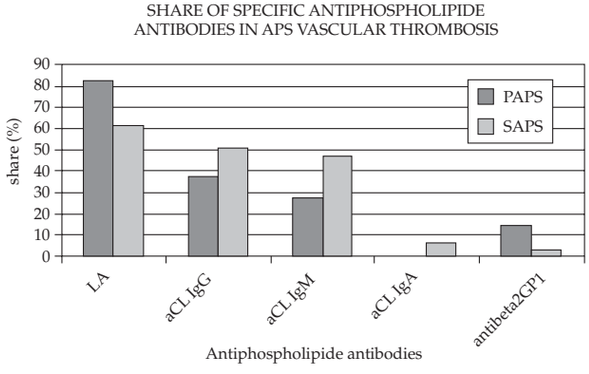
TABLE 1
DISTRIBUTION OF PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME (PAPS) AND PATIENTS WITH SECONDARY ANTIPHOSPHOLIPID SYNDROME (SAPS) ACCORDING TO GENDER

Gender	Antiphospholipide syndrome			
	PAPS		SAPS	
	Number of patients	Structure (%)	Number of patients	Structure (%)
Male	15	37.50	16	27.12
Female	25	62.50	43	72.88
Total	40	100.00	59	100.00

TABLE 2
RESULTS OF DIFFERENCE TEST FOR ANALYSED VARIABLE VALUES IN PATIENTS WITH PRIMARY ANTIPHOSPHOLIPIDE SYNDROME (PAPS) AND PATIENTS WITH SECONDARY ANTIPHOSPHOLIPIDE SYNDROME (SAPS) USING MANN-WHITNEY TEST

Variable	Z (Normal aproximation)	p-value
LA	–1.801	0.072
β2-GP1 IgG	–3.774	0.000
IgG aCL	–1.130	0.258
IgM aCL	–1.348	0.178
IgA aCL	–3.363	0.001

LA – lupus anticoagulant, aCL – anticardiolipin antibody, Ig – immunoglobuline antiβ2-GP1, IgG – antiβ2glycoprotein1



LA – lupus anticoagulant, aCL – anticardiolipin antibody, Ig – immunoglobuline antiβ2-GP1 IgG – antiβ2glycoprotein1

TABLE 3
SPERMANS's CORRELATION RANG COEFFICIENTS FOR VARIABLES RELATED TO PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME (PAPS) AND CORRESPONDING p-VALUES OBTAINED BY TESTING HYPOTHESIS OF THEIR SIGNIFICANCE

	LA	β2-GP1 IgG	IgG aCL	IgM aCL	IgA aCL
LA	1.000	0.275 0.086	–0.224 0.165	–0.068 0.676	0.170 0.294
β2-GP1 IgG	0.275 0.086	1.000	–0.241 0.135	0.012 0.942	–0.060 0.712
IgG aCL	–0.224 0.165	–0.241 0.135	1.000	0.485 0.002	–0.041 0.803
IgM aCL	–0.068 0.676	0.012 0.942	0.485 0.002	1.000	0.212 0.188
IgA aCL	0.170 0.294	–0.060 0.712	–0.041 0.803	0.212 0.188	1.000

LA – lupus anticoagulant, aCL – anticardiolipin antibody, Ig – immunoglobuline, antiβ2-GP1 IgG – antiβ2glycoprotein1

TABLE 4
SPERMANS's CORRELATION RANG COEFFICIENTS FOR VARIABLES RELATED TO PATIENTS WITH SECONDARY ANTIPHOSPHOLIPID SYNDROME (SAPS) AND CORRESPONDING p-VALUES OBTAINED BY TESTING HYPOTHESIS OF THEIR SIGNIFICANCE

	LA	β2-GP1 IgG	IgG aCL	IgM aCL	IgA aCL
LA	1.000	0.130 0.327	–0.106 0.423	–0.022 0.866	0.025 0.848
β2-GP1 IgG	0.130 0.327	1.000	–0.017 0.899	0.001 0.994	0.188 0.153
IgG aCL	–0.106 0.423	–0.017 0.899	1.000	0.576 0.000	0.167 0.207
IgM aCL	–0.022 0.866	0.001 0.994	0.576 0.000	1.000	0.203 0.124
IgA aCL	0.025 0.848	0.188 0.153	0.167 0.207	0.203 0.124	1.000

LA – lupus anticoagulant, aCL – anticardiolipin antibody, Ig – immunoglobuline, antiβ2-GP1 IgG – antiβ2glycoprotein1

PAPS group, and 30.5% in SAPS group. Myocardial infarction (MI) had greater incidence in SAPS group with 22%, than in PAPS group with 12.4%. Peripheral arteries were affected almost the same in both groups of patients, 10% in PAPS group and 11.7% in SAPS group. Peripheral veins were least affected, with 5% incidence in PAPS group, and 3.4% incidence in SAPS group.

Discussion

From clinical point of view, it can be said that APS is a subject of many medical specialities. Given time, the syndrome has become described in detail. At first in case reviews, and then based on randomised controlled clinical trials. Treatment approach for this syndrome, interpretation of laboratory results, which are often contradictory, and connecting them with clinical manifestations present today's great challenge for clinical doctors.

One clinical and one laboratory criterion is necessary to make a diagnosis of APS [1]. The most often clinical manifestation of APS is vascular thrombosis, arterial or venous. Cerebral stroke and transient ischemic attack (TIA) are the most often presentations of arterial thrombosis, and recurrent cerebral strokes can cause ischemic dementia. Large blood vessels can also be affected in this syndrome, including aorta, causing aortal occlusion. Affection of peripheral blood vessels is variable and any artery can be affected, what causes ischemy of an end organ[11].

Prospective studies support a theory that antiphospholipid antibodies can be a risk factor for coronary artery disease and myocardial infarction, especially in young

and middle-aged people[12–14], and can present a risk for arteriosclerosis[15], and for occlusion of by-pass venous graft[16]. One study reported increased IgM aCL levels in men with coronary artery disease treated with percutaneous transluminal coronary angioplasty that restenosed[17]. Manifestation of valvular disease, the most common form of aPL-related cardiac involvement, may be seen in both primary and secondary APS patients. Specific findings range from valvular thickening to nodular excrescences (Liebman-Sacks), mitral and aortic regurgitation, and severe distortion and disfunction [18]. Deep vein thrombosis (DVT), often accompanied by pulmonary embolism (PE), is the most common manifestation of APS overall[11]. Venous distributions may include also ophthalmic veins, renal or splenic veins, hepatic veins, portal, mesenteric, adrenal veins[19].

Reason for such widespread thrombosis occurrence that can affect any body organ lies in antiphospholipid antibody (aPL) patofisiology. Autoimmune aPL present nothing more than autoantibodies against phospholipide-binding proteins. B2GPI is the most important among numerous potentially relevant phospholipide-binding proteins, including prothrombin, protein C, S and Annexin V. Binding with phospholipide-binding proteins, they interfere with coagulation cascade, activating endothelial cells, causing growth factor expression on monocytes, and in that way causing thrombosis[20–22].

LA and aCL antiphospholipide antibodies are the most common in results of other authors, as well as ours. Risk for thrombosis in aPL-positive patients is best investigated in group of patients with SLE, where 12–30% of patients were aCL-positive[23,24], and 15–34% of them were LA-positive[24,25], while 38% of patients were positive to both aPL antibodies[26]. Around 50% of patients with SLE have a history of arterial or venous thrombosis[27,28]. Recurrent episodes tend to mimic the original vascular event, with venous thrombosis following venous occlusion and arterial thrombosis following arterial occlusion, although there are exceptions when patients occasionally develop both venous and arterial disease[26]. But occurrence of aPL in patients that do not have SLE or other autoimmune disease or malignancy, also carries a risk of vascular thrombosis. Besides traditional risks for thrombosis, such as pregnancy, surgery, long term immobilisation, LA occurrence presents up to 10 times higher risk for thrombosis, IgG class of aCL presents 3 times higher risk, and both antibodies together carry cumulative risk[29]. When such patients develop vascular thrombosis, they should be monitored for the rest of their life. Standard treatment presents heparin or LMWH, followed by warfarin, with prothrombin time maintained between 2.0 and 3.0 INR for venous thrombosis, while for recurrent and arterial thrombosis it is advisable for INR to range between 3.0 and 4.0, with addition of low dose aspirin (LDA)[30]. Warfarin therapy does not have to last several years for all patients. Given time, aPL level decreases or disappears entirely, suggesting that warfarin therapy should be cancelled. But before that, other prothrombotic factors, such as smoking, oral contraceptives and congenital risk factors, should be excluded[31]. Asymptomatic, persistently aPL-positive individuals do not benefit from low-dose aspirin for primary thrombosis prophylaxis, have a low overall annual incidence rate of acute thrombosis, and develop vascular events when additional thrombosis risk factors are present[32].

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ANTIFOSFOLIPIDNI SINDROM KAO UZROK VASKULARNIH TROMBOZA

S A Ž E T A K

Cilj nam je bio pokazati naša iskustva u dijagnosticiranju i liječenju vaskularnih tromboza u sklopu antifosfolipidnog sindroma (APS). 99 pacijenata s pozitivnim antifosofilipidnim antitijelima (aPL) i vaskularnim trombozama bilo je uključeno u studiju: 40 pacijenata, sukladno klasifikacijskim kliničkim kriterijima imalo je primarni antifosfolipidni sindrom (PAPS), te 59 sekundarni antifosfolipidni sindrom (SAPS). Svim pacijentima je određen profil protutijela. U grupi s PAPS 82,5% pacijenata bilo je LA pozitivno, 37,5% IgG aCL, 27,5% IgM aCL, te 15% na IgG antiβ2GPI. U grupi sa SAPS 61% bilo je pozitivno na LA, 50,8% na IgG aCL, 47,5% bilo je IgM aCL pozitivno. Na IgA aCL klasu antitijela bilo je pozitivno 6,7% pacijenata, dok na antiβ2GPI u grupi sa SAPS 3,4% pacijenata. Nije nađen niti jedan pacijent pozitivan isključivo na IgA aCL, kao ni na antiβ2GPI, te oni nemaju klinički značaj. Kao terapija korišten je niskomolekularni heparin (LMWH) kroz 7 dana, potom nastavljeno s varfarinom uz održavanje protrombinskog vremena između 2,0–3,0 INR.