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Risk factors for arterial thrombosis in antiphospholipid syndrome

Aleksandra Matyja-Bednarczyk [a], Jakub Swadźba [a], Teresa Iwaniec [a], Marek Sanak [a], Sylwia Dziedzina [a], Adam Ćmiel [b], Jacek Musiał [a,*]

[a] Department of Medicine, Jagiellonian University Medical College, Krakow, Poland
[b] Faculty of Applied Mathematics, University of Science and Technology, Krakow, Poland

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ABSTRACT

Introduction: Antiphospholipid syndrome (APS) is associated with the risk of both arterial and venous thrombosis. However, it is not known which factors might determine the location of thrombosis.
Materials and Methods: To retrospectively characterize factors associated with the risk of arterial thrombosis in a cohort of APS patients. Analysis included laboratory and clinical criteria of APS, together with classical cardiovascular risk factors and the possible role of platelet integrin $\alpha_2\beta_1$ (807 C/T) and $\alpha_{IIb}\beta_3$ (PI A1/2) genetic polymorphisms. We enrolled 163 APS patients (123 women and 40 men aged 21-75; mean age 43 years); 78 suffered from arterial thrombosis.
Results: There were no significant differences in the frequency or titers of different antiphospholipid antibodies with the exception of slightly increased frequency of IgG anticardiolipin antibodies (ACL) in the arterial thrombosis group. Livedo reticularis was observed significantly more often in the arterial thrombosis group, particularly in stroke patients.
In univariate analysis arterial thrombosis was associated with male gender (OR-2,201; p = 0,033), arterial hypertension (OR-2,81; p = 0,002) and hypercholesterolemia (OR-3,69; p = 0,001). On multivariate analysis arterial hypertension (OR = 1,78; p = 0,008) and hypercholesterolemia (OR = 2,001; p = 0,002) remained as independent risk factors for arterial thrombosis. Platelet glycoprotein polymorphisms studied did not show any significant associations with arterial thrombosis in APS patients.
Conclusions: Among APS patients those with ACL IgG antibodies, having livedo reticularis, and suffering from hypertension an hypercholesterolemia are at the increased risk of arterial thrombosis.

Introduction

Antiphospholipid syndrome (APS) is the most common cause of acquired thrombophilia. It is characterized by a set of clinical symptoms (history of venous or arterial thrombosis or obstetric complications) and the presence of characteristic type of antiphospholipid antibodies (APA). APA are directed against protein cofactors bound to negatively charged phospholipids. It seems that the presence of APA alone is not sufficient for the progression to APS, since it was shown that thrombosis develops in only about 8 % of APA-positive individuals within 5 years (DVT) [1]. Consequently, a "double hit" hypothesis was proposed, which postulated that besides APA an additional factor seems necessary to induce APS clinical manifestations (DVT) [2].

Antiphospolipid syndrome is associated with the risk of both arterial and venous thrombosis. Collective data analysis suggests that in two-thirds of cases thrombosis involves venous, and in one-third, arterial vascular bed [3]. For reasons that remain largely unknown, arterial thrombosis predominantly affects cerebral circulation, leading either to asymptomatic multiple, small ischemic episodes or fully blown ischemic stroke [4]. They affect mainly young subjects, and seem to correlate with adverse prognosis. It is, therefore, of most importance to identify possible factors favouring development of arterial thrombosis in APS. Such studies are largely missing [5].

In a few studies common platelet glycoprotein polymorphisms (glycoprotein Ia/IIa = integrin $\alpha_2\beta_1$, 807 C/T, and glycoprotein IIb/IIIa = integrin $\alpha_{IIb}\beta_3$, PI A1/Pl A2) have been shown to be independently associated with an increased risk of premature myocardial infarction and ischemic stroke, both in a general population and in APS patients [6–9].

Our study aimed to identify such factors associated with the risk of arterial thrombosis among laboratory and clinical APS criteria, common platelet glycoprotein polymorphisms and classical cardiovascular risk factors in a cohort of APS patients. We hope that identification of those factors in APA-positive individuals will most certainly lead in

Abbreviations: APA, Antiphospholipid antibodies; APS, Antiphospholipid syndrome; LR, livedo reticularis; RP, Raynaud's phenomenon; aCL, anticardiolipin; AT, Arterial thrombosis; SAPS, Secondary Antiphospholipid syndrome, PAPS, Primary Antiphospholipid syndrome; CNS, Central Nervous System; SLE, Systemic Lupus Erythematosus, LA, lupus anticoagulant, DNA, Deoxyribonucleic acid; DVT, deep vein thrombosis.

* Corresponding author at: Department of Medicine, Jagiellonian University Medical College, Skawinska 8, 31-006 Kraków, Poland. Tel./fax: +48 12 430 5314.
E-mail address: mmmusia@cyf-kr.edu.pl (J. Musiał).

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the future to the development of effective primary and secondary preventive strategies.

Materials and Methods

Patients

163 patients (123 women and 40 men aged 21-75; mean age 43 years) with APS diagnosed according to the updated APS classification criteria [10] were enrolled to the study between 2008 and 2011 from the Outpatient Clinic for Autoimmune Diseases of the Jagiellonian University Medical College.

This study was approved by the Jagiellonian University Ethical Committee. Before entering the study all patients gave a written informed consent.

A detailed history (an uniform questionnaire) was collected from all the patients by a physician and their past medical records were carefully analyzed. Prerequisite to enter the study was objectivity of the data. All thrombotic episodes had to be confirmed by the imaging techniques (U/S, CT and/or NMR). TIA was considered for analysis only if cerebral imaging demonstrated signs typical for cerebral ischemia. Pregnancy associated morbidity was considered typical for APS according to recently updated classification criteria [10].

Additionally, we analyzed symptoms not included in the classification criteria of APS, but often associated with APA, namely: livedo reticularis (LR), Raynaud`s phenomenon (RP) and thrombocytopenia (<100,000/ μl).

Exclusion criteria included: recurrent or persistent atrial fibrillation, lack of consent to participate in the study and no adherence to a regular antiplatelet or anticoagulant regimen.

Methods

Blood was collected into plastic tubes containing 0.109 M sodium citrate and centrifuged twice (2000 g, 15 min in room temperature). Plasma was then immediately frozen in several aliquots (-70 °C). For further studies frozen plasma was thawed in a water bath at 37 °C immediately before testing. Lupus anticoagulant (LA) was determined as recommend by ISTH [11] using PTT LA (Diagnostica Stago, France) and DVV (American Diagnostica, USA) tests for screening, and Staclot LA (Diagnostica Stago, France) and DVV Confirm (American Diagnostica, USA) for confirmation on Behring Coagulation Timer (BCT) and Fibrintimer (Dade Behring, Germany) [12].

Serum levels of anticardiolipin (aCL) and anti-$\beta_2$glycoprotein I (aβ_2GPI) antibodies (of both IgG and IgM classes) were measured with a home-made ELISA kits, as described previously [13,14]. Monoclonal antibodies (HCAL and EY2C9) for IgG and IgM, respectively, were used as calibrators for the standard curve construction [15,16]. For all APA patients these values (determined at least twice at least 12 weeks apart) exceeded 99th percentile of a healthy population (100 healthy controls).

Genomic DNA was isolated from the peripheral blood samples collected on EDTA as anticoagulant. Cell rich plasma was prepared by addition of 1:10 volume of Dextrane 6% solution and leukocytes were collected by centrifugation. DNA was extracted using DNAzol Reagent (Invitrogen, USA), according to the manufacturer protocol. Genotoping for the platelet glycoprotein SNPs was performed using a commercial TaqMan assay and ABI 7900 HT real-time thermocycler (Applied Biosystems, USA). DNA samples were tested in the presence of all tree genotypes used as external controls. Laboratory performing the analysis is certified by Referenzinstitut fur Bioanalytik, Bonn, Germany. Samples were investigated by a technician blinded to the diagnosis.

Clinical and laboratory data, collected during the study were analyzed statistically, using parametric and non-parametric methods. All calculations were performed using Statistica v. 10 software package (Statsoft, USA).

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using χ^2 test. Correlation between variables was assessed using Pearson or Spearman's rank correlation coefficients, depending on the data distribution. All differences were considered statistically significant at p-values below 0.05. A multifactorial logistic regression was used to test for independent associations of parameters with multiple clinical variables. Generalized linear model for dichotomous response was used.

Genetic equilibrium of the single nucleotide variants was tested using χ^2 statistics of the counts observed and expected under Hardy-Weinberg equilibrium.

Results

Arterial thrombosis (AT) group consisted of 78 individuals who experienced at least one arterial thrombotic event; 36 subjects suffered from primary APS (PAPS) while in 42 APS was secondary (SAPS) to SLE. Thirty one patients experienced arterial episodes only, while in the remaining patients arterial thrombosis was accompanied by venous episodes and/or pregnancy complications (Table 1).

Ischemic stroke was the most common manifestation of arterial thrombosis, followed by transient ischemic attacks, myocardial infarction and arterial thrombosis located elsewhere (e.g. spleen, lower extremities, renal artery); overall, 123 episodes of arterial thrombosis were documented.

A comparative group consisted of remaining 85 APS patients (70 women and 15 men; 65 SAPS, 20 PAPS) who never experienced arterial thrombosis. Altogether, 94 episodes of venous thrombosis occurred in 67 patients, represented mainly by deep vein thrombosis (DVT), and less commonly by pulmonary embolism with only e few cases of upper extremity DVT and thrombosis of inferior vena cava. Nineteen patients experienced more than one episode of venous thrombosis. Thirty six women in this group had a history of obstetric complications; 18 with coexisting DVT.

Frequency of APA did not differentiate between AT and other APS patients, with the exception of IgG-class aCL antibodies. These were more frequently elevated in AT group ($\chi 2$ p = 0.036; OR = 2.032; 95% CI: 1.042 ÷ 3.956) (Table 2). APA titers (both aCL and aβ2GPI) did not differ between patients with arterial thrombosis and the remaining subjects (results not shown). We were also were unable to show any differences in the frequency of antiphospholipid antibody multiple positivity between the groups studied (Table 2).

Livedo reticularis was observed significantly more often in the arterial thrombosis group (21 patients, 29.6 %), than in the others (12 patients, 15.2 %) (p = 0.03; OR = 2.3; 95% CI: 1.055 ÷ 5.211). This association was particularly prominent for a group of stroke patients (51 patients/65 thrombotic events) (OR = 3,6; 95% CI: 1.2561 ÷ 4,61). There was no difference in the frequency of thrombocytopenia, observed in approximately one-fourth of all patients, both with and without of arterial thrombosis. The same was true for Raynaud's phenomenon.

Several well described and thoroughly verified classical cardiovascular risk factors have been defined in a general population. Their frequencies in the groups studied are presented in Table 3. APS patients with a history of arterial thrombosis were on average more than 4 years older than the others. AT was also more prevalent among men (32.1% - arterial thrombosis group vs. 17.6% - others). Arterial hypertension (systolic

Table 1
Characteristics of APS patients with arterial thrombosis.

	Number of patients
N (female/male)	78 (53/25)
arterial thrombosis only	31
arterial and venous thrombosis	27
arterial thrombosis and pregnancy complications	12
Arterial thrombosis, venous thrombosis and pregnancy complications	8