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Full Length Article

Clinical significance of anti-domain 1 β2-glycoprotein I antibodies in antiphospholipid syndrome

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ABSTRACT

Background: Antiphospholipid syndrome (APS) is characterized by the presence of circulating antiphospholipid antibodies (aPL) in patients with thrombosis and/or pregnancy morbidity. In APS patients anti-domain 1 β2-glycoprotein I (anti-D1 β2GPI) IgG antibodies correlate strongly with thrombosis and to the lesser extent, with pregnancy complications.
The aim of this study was to assess clinical utility of the anti-D1 β2GPI antibodies in the diagnosis and risk stratification of antiphospholipid syndrome.
Patients/methods: In this retrospective study 202 autoimmune patients were studied (primary APS – 58, secondary - 45 SLE – 99). Anticardiolipin (aCL) and anti-β_2GPI (aβ_2GPI antibodies) (IgG and IgM class) together with anti-D1 IgG were tested with QUANTA Flash chemiluminescent immunoassay and lupus anticoagulant (LA) with coagulometric methods.
Results: The highest anti-D1 values were observed in triple positive patients as compared to patients with other antiphospholipid antibody profiles. A strong correlation was found between levels of anti-D1 IgG and a β2GPI IgG antibodies for all patients analyzed (Spearman's $\rho = 0.87$; $p < 0.0001$). Anti-D1 IgG antibodies increase specificity resulting from classic aPL positivity but at the expense of sensitivity. Anti-D1 test does not add accuracy in predicting APS thrombotic complications on the top of accuracy offered by classic aPL tests and their profiles.
Conclusions: Anti-D1 IgG antibodies did not add diagnostic power to the standard laboratory aPL tests as assessed by this retrospective study. A true clinical significance of anti-D1 antibodies in thrombotic risk stratification of aPL positive patients will require a properly designed clinical prospective trials.

1. Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized clinically by vascular thrombosis and/or pregnancy morbidity and serologically by the presence of antiphospholipid antibodies (aPL) in plasma. Antiphospholipid antibodies are currently detected by a coagulometric functional assay (lupus anticoagulant; LA) and solid-phase assays (antibodies against cardiolipin [aCL] and β2-glycoprotein I [aβ2GPI] of IgG and/or IgM isotype) [1]. Antiphospholipid antibodies are a heterogeneous group of autoantibodies directed against plasma protein complexes or single plasma proteins. The most important epitopes targeted by aPL are β2-glycoprotein I and prothrombin [2,3]. β2GPI (apolipoprotein H) is synthesized by endothelial cells, hepatocytes and trophoblast cells. It consists of 326 amino acids forming 5 homologous domains of approximately 60 amino acids each. Domain 5, located at the C terminus, contains hydrophobic core that binds to the plasma cell membrane via interactions with negatively charged phospholipids. This process induces a conformational change in β2GPI molecule, exposing hidden epitopes especially within domain 1, which enables domain-specific antibody generation and binding [4,5]. According to the recent findings IgG class anti-β2GPI antibodies (aβ2GPI) and (β2GPI-dependent) LA carry the strongest risk for thrombotic complications [6,7,8]. However, not all patients with positive aβ2GPI develop aPL-related clinical symptoms. This is, at least in part, because of the heterogeneity of aβ2GPI antibodies; a typical feature of all antiphospholipid antibodies. Few years ago an epitope on the β2GPI domain 1 has been identified and suggested as the most relevant antigenic target involved in pathogenic β2GPI/anti-β2GPI antibody binding [9]. This epitope spans aminoacids 40 to 43 (the G40-R43 epitope) on the domain 1 of β2GPI protein [4]. In APS patients antibodies of the IgG class directed against β2GPI domain 1 (anti-D1) seem to correlate strongly with the risk for thrombosis and to the lesser extent, with pregnancy complications [10,11]. Anti-D1 antibodies in high titers have also been found in patients with multiple aPL positivity; an accepted hallmark of thrombotic risk [12,13]. However, the precise diagnostic value

Abbreviations: APS, antiphospholipid syndrome; aCL, anticardiolipin; aβGPI, anti-beta2 glycoprotein I; anti-D1, anti-domain I antibodies; CIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; ROC, Receiver-operating characteristics; SLE, systemic lupus erythematosus.

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of anti-D1 antibodies in the APS thrombotic risk assessment is largely unknown. There are also doubts about the comparability of various methods of their detection. This is mainly because domain 1epitope in question is exposed only upon a conformational change of the β2GPI molecule. This exposure might differ across available commercial kits which in turn could be crucial for their diagnostic utility [14,15].

The aim of this study was to assess clinical utility of the anti-domain 1 β2GPI antibodies in the diagnosis and risk stratification of antiphospholipid syndrome.

1.1. Patients

The study included 202 consecutive autoimmune patients (Outpatient Clinic for Autoimmune Patients, Department of Internal Medicine, Jagiellonian University Medical College, Kraków, Poland) definitely diagnosed with SLE and/or APS. The total group of subjects screened included 395 subjects. Antiphospholipid syndrome was diagnosed according to the updated APS criteria [1]. Systemic lupus erythematosus was diagnosed whenever at least 4 ACR criteria were met [16]. Objective data on the presence or absence of venous thrombosis, arterial thrombosis, and obstetric complications were available for all patients. Additionally, a group of 120 sex- and age- matched healthy volunteers (84 women and 36 men; mean age 44.6 years, range 20–75 years) were used to calculate the value of the 99th percentile of a healthy population. This study was approved by local Ethics Committee, and informed consent was obtained from all patients according to the Declaration of Helsinki. All samples were tested for aCL and β2GPI antibodies in both IgG and IgM classes using chemiluminescent immunoassay (CIA). Autoantibodies to β_2GPI-Dm1 IgG were also measured by the CIA method. All samples were also examined for the presence of lupus anticoagulant (LAC).

1.2. Sample preparation

Blood samples for antiphospholipid antibody detection were collected in serum separation tubes and spun for 10 min at 3500 rpm at room temperature within 2 h from sampling. Serum samples were then stored at −80 °C for further study. For lupus anticoagulant measurement blood was drawn in 3.2% (0.109 mol/l) sodium citrate tubes (one part sodium citrate to nine parts venous blood). Platelet-poor plasma was prepared by double centrifugation within 2 h (10 min/3500 rpm, and 10 min/14,000 rpm) and stored at −80 °C for further analysis.

1.3. Measurement of autoantibodies

1.3.1. aCL and aβ2GPI antibodies

All samples were tested for aCL and β_2GPI antibodies of IgG and IgM isotypes with QUANTA Flash chemiluminescent immunoassay (CIA) according to the manufacturer's instructions.

QUANTA Flash® (Inova Diagnostics Inc., San Diego, CA, USA) aCL and aβ2GP1 are semi-quantitative immunoassays that are run on the fully automated BIO-FLASH® instrument (Biokit S.A., Barcelona, Spain). Results are expressed in (arbitrary) chemiluminescent units (CU).

For the purpose of this study and in accordance with ISTH SSC guidelines on aCL and aβ2GPI testing [20] values above the 99th percentile of 120 sex- and age-matched healthy subjects were defined as positive.

1.3.2. IgG antibodies to domain 1of β2GPI (anti-D1 IgG)

Anti-D1 IgG were measured by a chemiluminescent assay (QUANTA Flash Domain I IgG; Inova Diagnostics) according to the manufacturer's instructions. This method uses recombinant β_2GPI-D1 (comprising amino acid 1–64) coated onto paramagnetic beads and designed for the BIO-FLASH analyzer. The relative light units (RLUs) detected by the analyzer are proportional to the amount of isoluminol conjugate that is bound to the human IgG, which in turn is proportional to the amount of anti-D1 antibodies (in arbitrary units) bound to the antigen on the beads. The cut- off value for anti-D1 IgG was also calculated as 99th percentile of the results obtained in 120 healthy donors [17,18].

1.3.2.1. Lupus anticoagulant detection. Lupus anticoagulant (LA) was determined in a three-step procedure according to the guidelines of the ISTH [19,20]. Diluted Russell's viper venom time (dRVVT; LA1-screen; Siemens, Germany) and a sensitive activated partial thromboplastin time (PTT LA; Diagnostica Stago, France) were used for screening purposes, whereas LA2-confirm (Siemens, Germany) and Staclot LA (Diagnostica Stago, France) were run as confirmatory tests. Reference values for each test were established using 99th percentile of the healthy population.

1.4. Statistical analyses

Data were statistically evaluated using GraphPad Prism, version 6.05 (GraphPad Software, San Diego, USA) and Statistica 12 (StatSoft, Cracow, Poland). Due to non-Gaussian distribution, data of anti-Dm1 IgG antibodies (CU)a were transformed into log10. The difference between groups was tested using Mann-Whitney test.

Spearman's correlation and Cohen's *kappa* agreement tests were performed to assess the correlation between anti-Dm1 IgG and other antiphospholipid antibodies. p values < 0.05 were considered significant. Odds ratio (OR) was calculated to assess the association between aPL antibodies and thrombotic risk. ROC curves were used to assess clinical utility of aD1 IgG antibodies.

2. Results

2.1. Clinical characteristics of patients

All subjects studied were Caucasian. Among 202 patients APS was diagnosed in 103–58 with primary APS (PAPS), and 45 with secondary APS (SAPS). SLE was diagnosed in 99 patients. In the SAPS group, 44 patients were diagnosed with SLE and 1 with MCTD (Table 1). Additionally APS patients were grouped according to their classic aPL profile into triple positive (LA+, IgG/IgM aCL+, IgG/IgM aβ2GPI+, n = 79), triple positive, but only for the IgG class antibodies (LA+, IgG aCL+, IgG aβ2GPI+, n = 75), double positive (LA+ and/or IgG/IgM aCL+ and/or IgG/IgM aβ2GPI+, n = 10) and single positive (LA+ or IgG/IgM aCL + or IgG/IgM aβ2GPI, n = 14).

Ninety nine patients suffered from SLE only, with no clinical manifestations attributable to APS. SLE patients were also grouped according to their aPL profile (as above); triple positive (n = 7), triple positive for

Table 1
Demographic and clinical characteristics of the patients studied.

	APS (n = 103)	PAPS (n = 58)	SAPS (n = 45)	SLE (n = 99)
Sex				
Male (%)	22 (21.4)	17 (29.3)	5 (11.1)	12 (12.1)
Female (%)	81 (78.6)	41 (70.7)	40 (88.9)	87 (87.9)
Mean age – years (range)	45 (19–80)	45 (19–80)	46 (22–80)	44 (22–71)
APS - clinical criteria				
Thrombosis, n (%)	96 (90.6)	55 (94.8)	41 (91.1)	
- Venous thrombosis, n (%)	73 (70.9)	40 (69.9)	33 (73.3)	
- Arterial thrombosis, n (%)	33 (32.0)	19 (32.7)	14 (31.1)	
- Venous + arterial thrombosis, n (%)	11 (10.7)	5 (8.6)	6 (13.3)	
Obstetrical complications	24 (23.3)	15 (25.9)	9 (20.0)	
- Thrombosis + obstetrical complications, n (%)	17 (16.5)	12 (20.7)	5 (11.1)	
- Obstetrical complications only	7 (6.8)	3 (5.2)	4 (8.9)	

APS – antiphospholipid syndrome, PAPS – primary APS, SAPS – secondary APS, SLE – systemic lupus erythematosus.