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Full Length Article

Clinical implications of the detection of antibodies directed against domain 1 of β2-glycoprotein 1 in thrombotic antiphospholipid syndrome☆

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ABSTRACT

Introduction: Antibodies directed against domain 1 of β2 glycoprotein 1 (aβ2GP1-Dm1) have been involved in the immunopathogenesis of antiphospholipid syndrome (APS). However, the clinical relevance of aβ2GP1-Dm1 in thrombotic APS has not yet been fully explored.
Objectives: To determine the frequency of aβ2GP1-Dm1 in a cohort of patients with thrombotic APS, and to evaluate whether testing for aβ2GP1-Dm1 could have a clinical impact upon the risk assessment of the disease.
Methods: Patients were tested for aβ2GP1-Dm1 antibodies by chemiluminescence (BioFlash/AcuStar®, ES). The presence of aβ2GP1-Dm1 was evaluated in different clinical presentations of the disease.
Results: Eight-four patients with a history of venous or arterial thrombosis were included. Forty-five (54%) patients had aβ2GP1 antibodies and 40% of them were positive for aβ2GP1-Dm1. Levels of aβ2GP1-Dm1 were higher in patients with systemic autoimmune disease (AUC = 0.665; 95% CI = 0.544–0.786; $P = 0.01$), positive antinuclear antibody (AUC = 0.654; 95% CI = 0.535–0.772; P = 0.01), triple antiphospholipid antibody (aPL) positivity (AUC = 0.680; 95% CI = 0.534–0.825; $P = 0.02$) and positive lupus anticoagulant (AUC = 0.639; 95% CI = 0.502–0.776; $P = 0.07$). In this cohort, aβ2GP1-Dm1 antibodies were not associated with the site of the first thrombosis (OR = 0,62, 95% CI = 0.20–1.94, $P = 0.42$), thrombosis recurrence (OR = 1.0, 95% CI = 0.37–2.71, $P = 1.0$) or pregnancy morbidity (OR = 1.5, 95% CI = 0.33–7.34, $P = 0.58$). In multivariate analysis, positivity for aβ2GP1-Dm1 antibodies was associated with the diagnosis of systemic autoimmune disease (OR = 4.01, 95% CI = 1.14–14.2; $P = 0.03$) and triple aPL positivity (OR = 3.59, 95% CI = 0.87–14.85; $P = 0.07$).
Conclusions: In the present cohort of thrombotic-APS patients, aβ2GP1-Dm1 antibodies were related to the diagnosis of systemic autoimmunity and complex serological profile of the disease, as triple aPL positivity and positive antinuclear antibody. Thus, our results suggest that testing for aβ2GP1-Dm1 antibodies may be useful for improving APS risk assessment.

1. Introduction

Antiphospholipid syndrome (APS) is an autoimmune thrombotic disease characterized by clinical manifestations of vascular thrombosis or pregnancy morbidity and persistent antiphospholipid antibodies (aPL) in serum, such as: lupus anticoagulant (LAC), IgM/IgG anticardiolipin (aCL) or IgG anti-β2 glycoprotein 1 (aβ2GP1) [1]. The risk of recurrent thrombosis and development of systemic autoimmunity is potentially high in patients with APS [2–4]. Therefore, the identification of critical

☆ List of abbreviations: aβ2GP1, Dm1, APS, aPL, LAC, aCL, ANA, PAPS, SAPS, SLE, UKNEQAS.

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markers that predict the prognosis of patients with thrombotic APS is crucial in order to improve the therapeutic approach to prevent further vascular events or complications.

The profile of aPL antibodies may identify APS patients with poor prognosis, since triple aPL positivity (LAC plus aCL plus aβ2GP1) and positive LAC are associated with a worse clinical course [5,6]. However, antibody profile is insufficient for risk stratification [7] and new risk markers are needed to identify high-risk patients with APS.

Different studies have demonstrated that pathologic autoantibodies in APS are mainly directed against the plasma β2-glycoprotein I (β2GP1) bound to phospholipids [8–10]. Particularly, antibodies directed against domain 1 of β2GP1 (aβ2GP1-Dm1) have been involved in the pathogenesis of thrombosis [11,12]. Moreover, evidence supports that aβ2GP1-Dm1 antibodies may be prevalent in patients with triple aPL positivity and, therefore, identify patients at risk [13].

Thus, we hypothesized that aβ2GP1-Dm1 could play a role as a risk marker of poor clinical course in thrombotic APS. In this context, the aim of this study was to evaluate the clinical implication of testing aβ2GP1-Dm1 for patients with thrombotic APS.

2. Material and methods

2.1. Study design and ethics

We evaluated the presence of aβ2GP1-Dm1 in a cohort of thrombotic APS patients treated at the Hematology and Hemotherapy Center at the University of Campinas, Brazil. Patients were enrolled between November 2013 and December 2014. Inclusion criteria comprised diagnosis of APS and history of at least one thrombotic episode. Patients who did not fulfill the diagnostic criteria for APS and patients without previous thrombosis were excluded. One hundred and twenty one patients diagnosed with APS were attended at the outpatient unit of the Hematology and Haemostasis Center at the University of Campinas during the enrollment period. Patients who were excluded had APS with obstetric complications only (2), positive aPL without APS (2) and lack of laboratory criteria for APS diagnosis (2). One hundred fifteen patients were included in the cohort and the serum samples of 84 patients were available for the present study (Fig. 1).

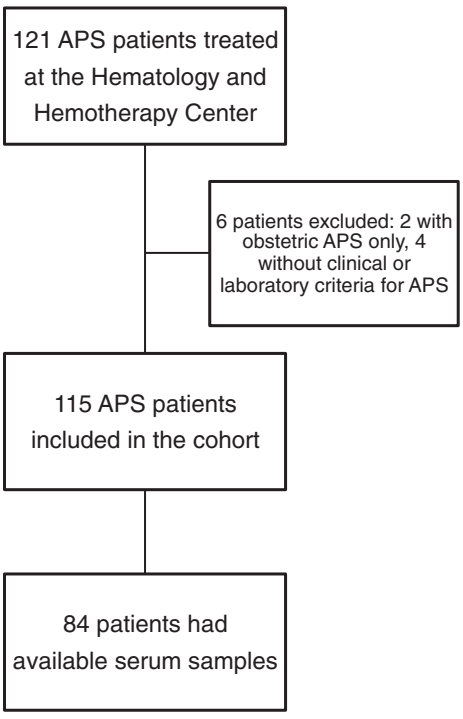

Fig. 1. Patient selection for the study. APS = antiphospholipid syndrome.

APS was diagnosed in patients with persistent positive aPL antibody plus a history of thrombosis or obstetric complications. Persistent positive aPL were defined as persistent positive LAC; persistent positive IgG or IgM aCL at moderate to high titles (>40 GPL or MPL) or persistent positive (> the 99th percentile) IgG/IgM anti-beta2 glycoprotein 1 (aβ2GP1), at two distinct times, with an interval of at least 12 weeks [1]. To ensure quality, the laboratory participated annually in an external quality control for antiphospholipid antibodies, provided by United Kingdom National External Quality Assessment Service for Blood Coagulation (UK NEQAS).

Thrombotic events were confirmed by imaging examinations, such as ultrasound (US), computerized tomography (CT), magnetic resonance (MR), ventilation/perfusion lung scan, or biopsies, according to the site of thrombosis. In cases of clinical suspicion of recurrent venous thrombosis, a new US was performed and the results were compared with those of the last available examination. Recurrent venous thrombosis was diagnosed in cases in which a previously fully compressible segment (contralateral or ipsilateral) was no longer compressible or when there was an increase in the residual thrombosis. New arterial thrombosis was diagnosed when there were symptoms of ischemia and new abnormalities on imaging examinations (CT or MR). Diagnosis of myocardial infarction depended on the alterations of electrocardiogram and cardiac enzymes.

The cohort had been followed for a median time of 6 years, since APS diagnosis. Patients were evaluated every month for oral anticoagulation control, clinical features were recorded every 6 months and routine laboratory tests including peripheral blood smear, blood glucose, lipids, renal function and for autoimmunity (as outlined below) were performed at least once a year. In order to prevent thrombosis recurrence, after the diagnosis of thrombotic APS was confirmed, patients received prolonged anticoagulant treatment with warfarin; patients with arterial thrombosis also received antiplatelet agents.

All patients were screened at diagnosis and annually, during the follow-up, for concomitant autoimmune disease (secondary APS) with the following tests: antinuclear antibodies (ANA), complement C3 and C4, anti-double-stranded DNA (dsDNA). In addition, in the presence of clinical signs and symptoms, such as proteinuria or hematological disorders, further investigations were performed as necessary. The diagnosis of SLE was confirmed according to established criteria [14,15].

We retrospectively reviewed the demographic and clinical features recorded at diagnosis and during follow-up. The demographic features evaluated were: age at study inclusion, age at the first thrombosis, number of years since the first thrombotic event, number of years since APS diagnosis, ethnicity and gender. The clinical parameters analyzed were: vascular bed of thrombosis, history of recurrent thrombosis, concomitant obstetrical and vascular APS and etiology of APS (primary or secondary to systemic autoimmune disease).

The profiles of aPL antibodies were evaluated as follows: single positive aβ2GP1 (aβ2GP1 +/aCL −/LAC −), double positive aβ2GP1 and aCL with no LAC activity (aβ2GP1 +/aCL +/LAC −), double positive aβ2GP1 with LAC activity (aβ2GP1 +/aCL −/LAC +) and triple positive (aβ2GP1 +/aCL +/LAC +).

The study was conducted in compliance with the Helsinki Declaration. The local Ethical Committee on Human Research approved this study and written informed consent was obtained from patients or their attending relatives.

2.2. Laboratory procedures

The detection of aPL was performed at APS diagnosis following the international guidelines from the International Society of Thrombosis and Haemostasis (ISTH) and Clinical and Laboratory Standard Institute (CLSI). Blood was collected in 0.109 M sodium citrate at a proportion of 9:1 and in serum separating tubes, prior to the initiation of any