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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).

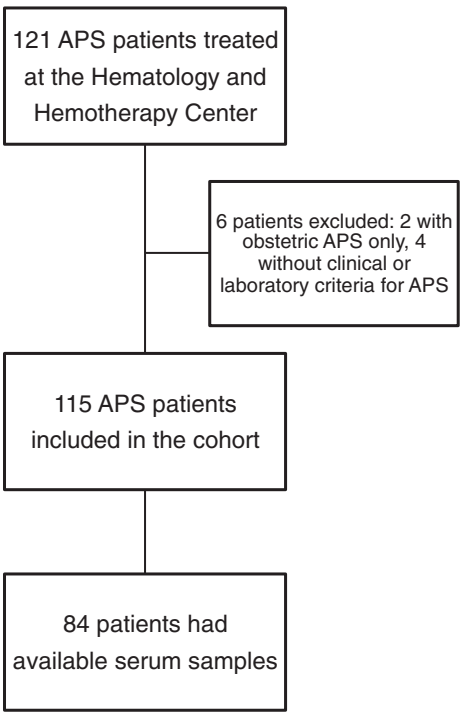

Fig. 1. Patient selection for the study. APS = antiphospholipid syndrome.

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

anticoagulant drug regimen or after a sufficient period of drug discontinuation.

For LAC, plasma samples were used and two assays based on different principles were applied: Dilute Russell's viper venom time (dRVVT) and Silica Clotting Time (SCT). Results of screening tests were potentially suggestive of LAC when their clotting times were longer than the local cut-off value (percentile 99th) and the results were confirmed for LAC at correction percentage of above the local cut-off value (99th percentile). The antiphospholipid antibodies with solid phase were tested in patient serum by "in house" ELISA immunological assays, with cardiolipin or β2GP1 as antigen (Sigma-Aldrich, USA), as previously described [16,17]. A calibration curve and commercial controls were used, positive patient samples were also used as positive controls, and samples were tested in duplicate. The local cut-off value for aβ2GP1 was determined by the 99th percentile.

The detection of aβ2GP1-Dm1 antibodies was performed in serum collected from patients during the follow-up and stored at −80 °C. The methodology used was chemiluminescent immunoassay (QUANTA Flash Domain 1 IgG; Inova Diagnostics), and the BIOFLASH equipment (Inova Diagnostics) was used. We used the cut-off value of 20 chemiluminescence units (CU) for IgG aB2GP1-Dm1 positivity. This value was established by Inova Diagnostics.

2.3. Statistical analysis

Descriptive statistics were used for categorical data and expressed as frequencies (percentage). Fisher exact test was used to compare categorical data. Continuous data were reported as median and interquartile and the difference in CU between groups was tested using Mann-Whitney or Kruskal-Wallis analysis, followed by the Dunn's multiple comparison test to identify the differences. ROC curve analysis was performed to evaluate whether aβ2GP1-Dm1 testing could discriminate distinct clinical features. In the graphs, CU values were represented as log10. Univariate logistic regression was used to evaluate the association of serological and clinical parameters with positive aβ2GP1-Dm1. A multivariate logistic regression analysis was performed with the parameters that were statistically associated with positive aβ2GP1-Dm1 by univariate analysis, with a P value of 0.1 or less, and adjusted for age and gender. The multivariate logistic regression models were run using block entry. Data were analyzed using SPSS for Windows version 15.0 (SPSS Inc., Chicago, IL, USA). Graphics were generated using GraphPad Prism, version 5 for Windows (GraphPad Software Inc., La Jolla, CA, USA). $P < 0.05$ was considered statistically significant.

3. Results

We included a total of 84 thrombotic APS patients (74% female) with a median age of 39 years (IQ 27–51 years). The median time from APS diagnosis was 6 years. Thirty-three patients (39%) had systemic autoimmune disease (31 of them had SLE) and the majority of patients presented with venous thrombosis (69%), followed by stroke, in 21% of patients. Thirty-six (43%) patients presented with recurrent thromboses and, among women who had ever been pregnant, 59% experienced additional APS related obstetric morbidities. Table 1 summarizes patient demographic features and clinical presentation. Antibody profile among patients was: 80% positive for LAC, 50% positive aCL, 54% positive aβ2GP1 and 26% triple positive.

Twenty-one (25%) patients were positive for aβ2GP1-Dm1. Among the patients with positive antibodies against the whole β2GP1 protein ($n = 46$), 39% were positive for aβ2GP1-Dm1; in contrast, only 3% patients with negative aβ2GP1 were positive for aβ2GP1-Dm1 ($p < 0.001$). Anti-β2GP1-Dm1 also had a greater prevalence among patients with positive IgG aCL compared to those negative for IgG aCL (43 vs. 14%, $P = 0.01$).

The presence of aβ2GP1-Dm1 was not associated with age, gender, ethnicity, the site of thrombosis, recurrence or pregnancy morbidities (Table 1). However, considering APS etiology, a higher frequency of aβ2GP1-Dm1 was found in patients with secondary APS (SAPS) in comparison with PAPS (42 vs. 17.7%, $P = 0.04$). In addition, the serum levels of aβ2GP1-Dm1 were higher in patients with SAPS (CU = 5.8, IQ = 3.6–67.8) compared to PAPS (CU = 3.6, IQ = 3.6–5.5; $P = 0.004$), as illustrated in fig. 2.

Anti-β2GP1-Dm1 were detected more frequently in triple positive patients than in other aPL profile groups (46 vs. 17%, $P = 0.05$) and higher serum levels of aβ2GP1-Dm1 were found in triple positive and in double positive aβ2GP1 with LAC activity groups, as shown in fig. 3. Anti-β2GP1-Dm1 were more frequent in patients with positive ANA than in those with negative ANA (34 vs. 13%, P = 0.04); furthermore, the serum levels of aβ2GP1-Dm1 were higher in patients with positive ANA (CU = 9.6, IQ = 3.6–197.8) than in patients with negative ANA (CU = 3.6, IQ = 3.6–106.6, $P = 0.06$), as shown in fig. 4. Differences in aβ2GP1-Dm1 levels were observed in patients with SAPS versus PAPS (AUC = 0.665; 95% CI = 0.544–0.786; $P = 0.01$), positive antinuclear antibody (ANA) versus negative ANA (AUC = 0.654; 95% CI = 0.535–0.772; P = 0.01), triple aPL positivity versus other aPL profiles (AUC = 0.680; 95% CI = 0.534–0.825; $P = 0.02$) and positive LAC versus negative LAC (AUC = 0.639; 95% CI = 0.502–0.776; $P = 0.07$).

Table 1
Demographic and clinical features according to aβ2GP1-Dm1 positivity.

Demographic features	Total population	aβ2GP1-Dm1 positive	aβ2GP1-Dm1 negative	P value*
Patients, n (%)	84	21 (25%)	63 (75%)	–
Age at study inclusion, in years, median (IQ)	39 (27–51)	33 (27–46)	41 (26–51)	0.61
Age at the first thrombosis, in years, median (IQ)	28 (21–41)	25 (19–37)	27 (20–40)	0.53
Years from the first thrombotic event, median (IQ)	7 (3−13)	6 (2–14)	7 (3–14)	0.74
Years from APS diagnosis, median (IQ)	6 (2.5–10)	4 (2.5–13)	7.4 (2.5–12)	0.64
Caucasian: African descendents	72:12	18:3	54:9	1.00
Female: male	62:22	16:5	46:17	1.00

Clinical presentation	Total population	aβ2GP1-Dm1 positive	aβ2GP1-Dm1 negative	P value*
PAPS: secondary APS[a]	51:33	8:13	43:20	0.02
Venous: arterial thrombosis	58:26	17:5	41:21	0.58
VTE n (%)	46 (55%)	12 (26%)	34 (74%)	
Stroke n (%)	18 (21%)	4(22%)	14 (78%)	
Pregnancy morbidity[b] n (%)	24 (59%)	6 (25%)	18 (75%)	0.71
Recurrent thrombosis n (%)	36 (43%)	9 (25%)	27 (75%)	0.60

IQ: interquartile, APS: antiphospholipid syndrome, PAPS: primary APS, VTE: venous thromboembolism (deep vein thrombosis or pulmonary embolism).
* P value was calculated using Fisher exact test.
[a] 31 patients with SLE, 1 with Sjogren syndrome and 1 with mixed connective tissue disease
[b] Women who had ever been pregnant = 54.

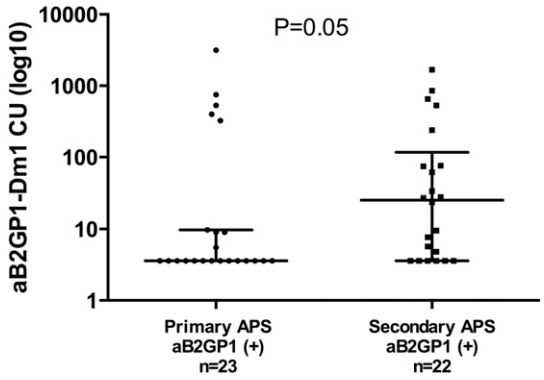

Fig. 2. Levels of antibodies against domain 1 of β2GP1 according to the diagnosis of systemic autoimmune disease in patients with positive aβ2GP1 antibody. *P* value was calculated using Mann-Whitney test. In the graph, CU values are represented as log10 and data range is illustrated as median and interquartile. APS = antiphospholipid syndrome. aβ2GP1 = anti-β2-glycoprotein 1.

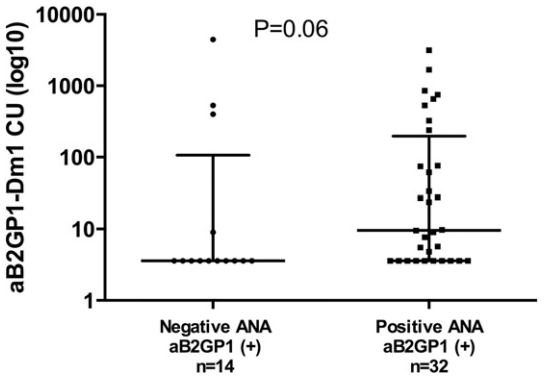

Fig. 4. Levels of antibodies against domain 1 of β2GP1 according to the ANA test results in patients with positive aβ2GP1 antibody. *P* value was calculated using Mann-Whitney test. In the graph, CU values are represented as log10 and data range is illustrated as median and interquartile. All patients had positive aβ2GP1 antibody. APS = antiphospholipid syndrome, aβ2GP1 = anti-β2-glycoprotein 1, ANA = anti-nuclear antibody.

The magnitude of the association between positive aβ2GP1-Dm1 with patient serological and clinical parameters was addressed by logistic regression analysis. The presence of aβ2GP1-Dm1 was 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 obstetrical morbidity (OR = 1.5, 95% CI = 0.33–7.34, $P = 0.58$). In multivariate analysis, patients with SAPS presented 3.5 times more chances of being positive for a β2GP1-Dm1 compared to PAPS. Similar rates were seen in patients with triple positivity for aPL antibodies (Table 2).

4. Discussion

In the present cohort of thrombotic APS patients, aβ2GP1-Dm1 antibodies were detected mainly in patients with SLE-APS or complex serological profiles, such as those with triple aPL positivity and positive ANA, suggesting that aβ2GP1-Dm1 antibodies may be more prevalent in patients with systemic autoimmunity. For patients with thrombosis and APS, the detection of those with SLE or higher risk for APS-related complications is important for treatment purposes.

The diagnosis of SLE in patients with antiphospholipid-associated thrombosis may be challenging since PAPS and SLE-APS may share clinical and serological manifestations, such as kidney disease, valvulopathy, positive ANA and hypocomplementemia. Furthermore, many patients do not fulfill the required diagnostic criteria for SLE and up to 10% of PAPS patients develop SLE in the course of the disease [18]. Indeed, PAPS and SLE-APS might represent distinct clinical manifestations of the same autoimmune disease, with different therapeutic approaches [19]. Therefore, additional biological markers would be useful tools to stratify patients at risk of developing SLE. In this context, our results are interesting as they demonstrated that the presence of aβ2GP1-Dm1 was more prevalent among SAPS patients, mainly SLE patients, with a specificity of 84%. Furthermore, the combination of positive aβ2GP1-Dm1 and ANA was present in only 6% of PAPS patients, resulting in 94% of specificity in distinguishing SAPS from PAPS. Therefore, our results demonstrated that aβ2GP1-Dm1 had higher specificity for the diagnosis of SAPS.

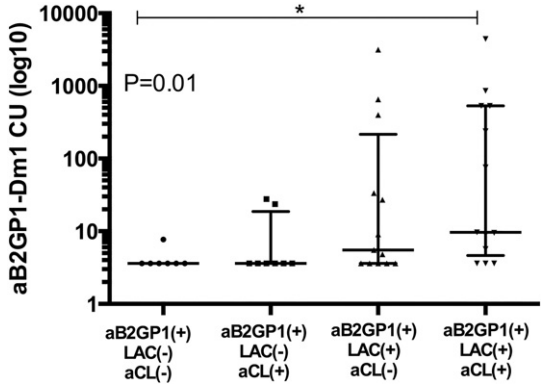

Fig. 3. Levels of antibodies against domain 1 of β2GP1 in aPL profiles: single positive aβ2GP1 without LAC activity (aβ2GP1+/aCL−/LAC−; $n = 7$), double positive aβ2GP1 without LAC activity (aβ2GP1+/aCL+/LAC−; $n = 8$), single positive aβ2GP1 with LAC activity (aβ2GP1+/aCL/LAC+; $n = 13$) and triple positive (aβ2GP1+/aCL+/LAC+; $n = 13$). *P* value was calculated using Kruskal-Wallis test; * indicates the place of differences (Dunn's test). In the graph, CU values are represented as log10 and data range is illustrated as median and interquartile. APS = antiphospholipid syndrome, aβ2GP1 = anti-β2-glycoprotein 1, LAC = lupus anticoagulant, aCL = anticardiolipin.

Indeed, autoantibodies are recognized as predictive markers for the development of autoimmune diseases and antiphospholipids are one of the earliest antibodies detected before the onset of SLE manifestations [20]. Conversely, the profile of the autoantibodies may represent a helpful marker in discriminating PAPS from SLE-APS. A recent clinical trial proposed that PAPS patients with positive ANA were twice as likely to evolve into SLE than those with negative ANA; and aDNA, anti-ribosomal P, anti-Ro/SS-A, anti-La/SS-B, and anti-U1RNP antibodies were not present in PAPS patients [21]. The prevalence of distinct aPL antibodies in SAPS has been previously demonstrated. Recently, Averina et al. suggested that the sensitivity of silica clotting time, a method to detect LAC, was higher in patients with systemic autoimmunity and triple aPL positivity [22]. In addition, Andreoli et al. suggested that the frequency of aβ2GP1-Dm1 was higher in patients with systemic autoimmune disease and the presence of this antibody was predictive of systemic autoimmunity [11]. Thus, similar to previous studies that suggested that the profile of autoantibodies would be a clue to SLE diagnosis, our findings further suggest that aβ2GP1-Dm1 may be a potential biological marker for discriminating PAPS from SAPS.

In addition to the diagnosis of SLE, the detection of APS patients with higher risk for APS-related complications is also a challenge in clinical practice, since antiphospholipid antibodies are associated with heterogeneous clinical presentations, ranging from asymptomatic to severe thrombotic disease [2]. Hyperlipidemia and arterial hypertension are clinical conditions associated with higher risk for APS-related thrombosis, as suggested by the Global Anti-Phospholipid Syndrome score (GAPSS) [7], however more specific predictive markers are needed [4, 23]. Considering APS-related complications, our results demonstrated that the presence of aβ2GP1-Dm1 had no impact upon the number of thrombotic events, the vascular bed of thrombosis or the occurrence of pregnancy morbidities. Therefore, in addition to the potential

Table 2
The association of positive aβ2GP1-Dm1 with laboratory and clinical parameters in univariate and multivariate analysis.

Characteristics	Univariate		Multivariate	
	Odds Ratio	95% CI	Odds Ratio	95% CI
Arterial vs. venous thrombosis	0.62	0.20–1.94 ($P = 0.42$)	–	–
Recurrent thrombosis	1.01	0.37–2.71 (P = 1.0)	–	–
Pregnancy morbidity	1.52	0.33–7.34 (P = 0.58)	–	–
SAPS	3.49	1.25–9.76 ($P = 0.01$)	4.01	1.14–14.22(P = 0.03)
Triple positive	4.04	1.05–15.61($P = 0.04$)	3.59	0.87–14.85 (P = 0.07)
ANA test	3.30	1.08–10.1 ($P = 0.03$)	1.21	0.23–6.19 (P = 0.82)

SAPS: secondary antiphospholipid syndrome; ANA: *anti*-nuclear antibody; CI: confidence interval. *Odds Ratio, 95% CI and P values were calculated using logistic regression.

association with systemic autoimmunity, we found no association between aβ2GP1-Dm1 and other APS-clinical presentations.

However, the profile of aPL antibodies has also been associated with the development of APS-related complications [24]. Positive LAC is suggested to be a strong risk factor for thrombosis, increasing the risk of thrombosis 5 to 16-fold [5]. Moreover, Pengo et al. proposed that patients with triple positivity for aPLs would present higher risk for thrombosis than those with negative LAC (double or single positive for aCL or aβ2GP1) [25]. Our results demonstrated that aβ2GP1-Dm1 antibodies were associated with triple aPL positivity and positive LAC. Therefore, we could hypothesize that positive aβ2GP1-Dm1 may be an additional marker for APS risk assessment, which is in agreement with previous studies [13,26].

The present study has the strength of having evaluated a homogeneous cohort of thrombotic APS patients, with a well-documented clinical presentation. Despite the increasing interest in aβ2GP1-Dm1, the clinical significance of this antibody as a biomarker of high risk APS has not yet been not established [24]. Clinical studies have reported the association of aβ2GP1-Dm1 and thrombosis in heterogeneous populations of patients presenting aPL (mainly asymptomatic positive aPL) [11,17,27]. Recent studies further confirmed that positive aβ2GP1-Dm1 is more specific of APS-related thrombotic than obstetric complications [28,29]. However, previous studies have not evaluated the clinical impact of aβ2GP1-Dm1 antibodies in the course of APS.

Nevertheless, there are some limitations that must be highlighted. Firstly, this is a retrospective study and therefore no conclusions can be drawn regarding the risk of developing SLE among PAPS patients with positive aβ2GP1-Dm1. A prospective study would be necessary to address this issue. Furthermore, clinical information may have been missed during the follow-up, as the registration of the data was not prospectively controlled. Another limitation is that aB2GP1-Dm1 was determined only once and a second test, with an interval of at least 12 weeks, would be necessary to confirm the persistence of the antibody. However, there is clinical evidence suggesting that aβ2GP1-Dm1 is not a transient antibody. Pengo et al. have reported that 93% of patients remained aB2GP1-Dm1 positive after 3 months of follow-up [13].

In conclusion, our results suggest that aβ2GP1-Dm1 antibodies may contribute to APS risk assessment, due to their association with the diagnosis of systemic autoimmunity and with more aggressive aPL profiles, such as triple positivity and lupus anticoagulant activity.

Conflict of interest

The authors have no conflicts of interest to declare.

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Addendum

SA Montalvão designed the study, performed the assays, collected and analyzed the data, and wrote the manuscript; PE Soares performed the assays; B Mazetto collected patients' samples and clinical data. S Saraiva collected patients' clinical data and revised the manuscript. S Appenzeller and J Annichino-Bizzacchi helped in the study design, analyzed the data and revised the manuscript; MP Colella and EV de Paula interpreted data and revised the manuscript. FA Orsi designed the study, collected and analyzed the data and wrote the manuscript.

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