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Anti-β2GPI-domain I antibody is associated with extra-criteria manifestations in a large prospective antiphospholipid syndrome cohort in China

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ABSTRACT

Background Anti-β2GPI-domain I (β2GPI-DI) antibody is pathogenic in patients with antiphospholipid syndrome (APS), but its additional clinical associations and diagnostic value are controversial.

Methods A total of 378 patients were included, of which 119 patients diagnosed with primary APS, 50 with APS secondary to SLE (SAPS group), 209 with SLE without APS (SLE group). Serum anti-β2GPI-DI IgG was measured using chemiluminescent immunoassay. Extra-criteria manifestations were analysed, including thrombocytopenia, autoimmune haemolytic anaemia, valvular lesions, APS nephropathy and non-vascular neurological manifestations.

Results In 169 patients with APS, 55 (32.5%) were positive for anti-β2GPI-DI IgG, accounting for 77.5% of those with anti-β2GPI IgG positivity. It is shown that 96.4% of those with anti-β2GPI-DI IgG also showed triple positivity in classic antiphospholipid antibodies (aPLs). The positivity of anti-β2GPI-DI IgG was significantly associated with recurrent thrombosis before APS diagnosis (p=0.015), microvascular thrombosis (p=0.038), but not with pregnancy morbidity (PM). Notably, patients with extra-criteria manifestations showed significantly higher positivity (p=0.001) and titres (p<0.001) in anti-β2GPI-DI IgG, especially for thrombocytopenia and APS nephropathy. In multivariable analysis, anti-β2GPI-DI IgG positivity (OR 2.94, 95% CI 1.29 to 6.70), secondary APS, arterial hypertension and Coombs' test positivity independently predicted extra-criteria manifestations (C-index 0.83, 95% CI 0.77 to 0.90). After a median follow-up of 25 months, patients with anti-β2GPI-DI IgG also showed a tendency of more extra-criteria events, but not thrombotic events. Anti-β2GPI-DI was positive among 8.1% of the SLE controls, and showed high specificity (91.9%) in diagnosing SAPS among patients with SLE as compared with classic aPLs.

Conclusion Anti-β2GPI-DI IgG was associated with extra-criteria manifestations in patients with APS. Further studies are warranted to validate its predictive values and potential role in daily practice.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The potential role of anti-β2GPI-domain I (β2GPI-DI) in antiphospholipid syndrome (APS) diagnosis and events prediction has been indicated among general or high-risk population, but its clinical associations among patients with APS were still controversial.

WHAT THIS STUDY ADDS

⇒ Anti-β2GPI-DI positivity was highly correlated with triple positivity in classic antiphospholipid antibodies, and also associated with microvascular events and extra-criteria manifestations, instead of thrombotic events and pregnancy morbidities.

⇒ In patients with SLE, this antibody showed high specificity in diagnosing APS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Anti-β2GPI-DI stratifies patients with APS at higher risk of microvascular events or extra-criteria manifestations.

⇒ Further studies with larger sample sizes are still needed to confirm its predictive value for such events.

INTRODUCTION

Antiphospholipid syndrome (APS) is a systemic autoimmune disorder, which is characterised by occurrence of venous and/or arterial thrombosis, as well as obstetric complications. The 2006 Sydney APS classification criteria was proposed to assist clinical research and limit overdiagnosis.[1] In fact, antiphospholipid antibodies (aPLs)-related clinical spectrum other than thrombosis and PM were recognised, referred to as 'extra-criteria' manifestations. These clinical features affect many organs, including non-thrombotic neurological manifestations

(such as chorea, myelitis), haematological manifestations (thrombocytopenia and haemolytic anaemia), nephropathy, valvular heart disease and livedo reticularis.[2 3] Recognition and diagnosis of these complex extra-criteria events are crucial, as these might affect management decisions and patient outcomes.

In the current classification criteria, the functional assay for lupus anticoagulant (LAC) and solid-phase assays for anticardiolipin (aCL) antibodies (IgG and IgM) and anti-β2 glycoprotein I (anti-β2GPI) antibodies (IgG and IgM) are required.[1] LAC was reported to be a strong independent risk factor for thrombosis in APS.[4] Individuals with triple aPLs positivity also showed increased risk of thrombosis in asymptomatic aPL carriers,[5] as well as patients with APS.[6] Moreover, the global APS score and modified antiphospholipid score (aPL-S) were developed and demonstrated to improve risk stratification for thrombosis in primary APS (PAPS group).[7 8] The role of classic aPLs have been expanded from qualitative diagnostic markers to quantitative risk assessment markers. However, novel prediction markers are in need to further stratify APS in more precise ways, considering its complex clinical spectrum.

β2GPI is the main antigen targeted by aPLs, as a 50 kDa molecule consisting of five homologous domains (domain I through V).[9] The remarkable heterogeneity of anti-β2GPI antibodies has been recognised. Recent studies have focused on an epitope spanning amino acids 40 to 43 (G40-R43 epitope) on the domain I of β2GPI (anti-β2GPI-DI), regarded as the most relevant antigenic target in APS pathogenesis.[10] Early proof-of-concept studies showed that anti-β2GPI-DI IgG antibodies were able to cause thrombosis in mice models, indicating its pathogenic role.[11 12] Moreover, strategies targeting β2GPI and β2GPI-D1 showed therapeutic effects in animal models, further proving their pathogenic role in APS in a direct way.[13 14] Accumulating data have been reported to explore the clinical associations of anti-β2GPI-DI IgG antibodies in various populations. In LAC carriers and patients with SLE, the presence of anti-β2GPI-DI IgG antibody was associated with significantly increased risks of thrombosis and PM.[15 16] However, the addition of anti-β2GPI-DI IgG to classic aPLs failed to improve ORs for clinical symptoms among patients with APS.[17] Several studies have reported that anti-β2GPI-DI antibody titres were higher in thrombotic APS than in obstetric APS,[18 19] while this was failed to be confirmed in other reports.[20–23] Limited follow-up data indicated that anti-β2GPI-DI antibody positivity could predict thrombotic events or PM in aPLs carriers,[15 23 24] while their associations with APS prognosis were largely unknown.

An integrated analysis of the clinical associations of anti-β2GPI-DI IgG antibodies was lacking in patients with APS, especially in terms of extra-criteria manifestations. In this study, we aimed to evaluate the prevalence and the clinical associations of anti-β2GPI-DI antibodies in a large prospective cohort of Chinese patients with APS.[25 26]

MATERIALS AND METHODS

Study participants

This was a prospective, single-centre study conducted at Peking Union Medical College Hospital and the National Clinical Research Center for Dermatologic and Immunologic Diseases. At the Chinese Rheumatism Data Center at Peking Union Medical College Hospital, we started constructing a database of patients with rheumatic disease in March 2010, including those with APS and SLE. Diagnosis of APS was defined according to the 2006 Sydney revised classification criteria,[1] while diagnosis of SLE was made based on the 1997 American College of Rheumatology (ACR) criteria[27] and the 2019 EULAR/ACR criteria.[28]

For this study, we included a total of 378 consecutive patients, and 119 of them were diagnosed with PAPS, 50 with APS secondary to SLE (SAPS group). A total of 209 patients with SLE (SLE group) without thrombotic events or pregnancy morbidities according to the 2006 Sydney APS classification criteria were included. These patients with SLE without APS were disease controls, who were recruited consecutively at the same time as the patients with APS in our centre. All the patients were followed until November 2021. Clinical manifestations were recorded, including vascular thrombosis, PM and extra-criteria manifestations, such as thrombocytopenia, autoimmune haemolytic anaemia, heart valve disease, APS nephropathy and non-vascular neurological events. All cases of PM underwent aetiological analysis before inclusion, excluding those caused by embryonic chromosomal abnormalities, uterine structural issues, endocrine factors, etc. The attribution of these cases to aPLs is determined jointly by obstetricians and rheumatologists. Thrombocytopenia was defined as a platelet count $<100\times10^9$/L, following the diagnostic criterion of immune thrombocytopenia.[29] Non-vascular neurological manifestations mainly included chorea, epileptic seizures, cognitive impairment.[2] Serum for aPLs analysis and plasma for LAC test were collected at the time of recruitment.

Laboratory tests

For each study subject, we analysed both IgG and IgM isotypes of aCL and anti-β2GPI with QUANTA Flash CLIA kits (INOVA Diagnostics, San Diego, California, USA, Werfen Group as sales agent (W-CLIA)).[30] As recommended by the manufacturer, cut-off values were defined as 20 U/mL for these antibodies. LAC was detected and measured according to the International Society on Thrombosis and Haemostasis (ISTH) recommendations.[31] A three-step procedure for Dilute Russell viper venom time testing and activated partial thromboplastin time were performed to quantify LAC. Based on in-house validations based on evaluation of normal samples and positive controls, LAC was considered positive if the ratio of screen/confirm time was >1.20. Anti-β2GPI-DI IgG was measured using QUANTA Flash chemiluminescent immunoassay (INOVA Diagnostics). The cut-off value was 20 CU as defined by the manufacturer.[21] Positive results

of either one, two or three criteria aPL assays (including aCL IgG/M, anti-β2GPI IgG/M antibodies and LAC) were defined as single, double and triple positivity.

Statistical methods

All statistical analysis was carried out using SPSS V.26.0 or R (V.3.6.2). We applied χ^2 test or Fisher's exact test for comparison of categorical variables, and Mann-Whitney U test for continuous variables. Sensitivities, specificities and accuracies were compared in the McNemar test to diagnose SAPS among SLE. Positive and negative predictive values (PPV and NPV), Youden's index and OR with 95% CI were shown. Multivariate logistic analysis was performed to evaluate the association between the clinical variables and extra-criteria events. Time-to-first-event outcomes were analysed using Cox proportional hazards models and displayed with Kaplan-Meier curves for the two groups (with/without anti-DI antibodies). Patients were censored at the time of events, loss to follow-up or the end of the study period. Two-tailed p values <0.05 were considered statistically significant.

RESULTS

Patients' demographics and antibody profiles

A total of 169 patients with APS and 209 patients with SLE without APS were included (table 1). Among 169 patients with APS, there were 117 (69.2%) females and the median

Table 1 Demographic characteristics of patients with APS grouped by anti-β2GPI-DI IgG positivity and control patients with SLE

	APS (n=169)	Anti-β2GPI-DI IgG-positive APS (n=55)	Anti-β2GPI-DI IgG-negative APS (n=114)	SLE without APS (n=209)
Secondary APS	50 (29.6)	16 (29.1)	34 (29.8)	
Demographic features				
Female (n (%))	117 (69.2)	40 (72.7)	77 (67.5)	192 (91.9)
Age at diagnosis (median, IQR)	34 (31, 41)	34 (31, 40)	35 (31, 42)	33 (28, 40)
BMI (median, IQR)	23.9 (21.5, 26.4)	24.4 (22.0, 26.0)	23.6 (21.4, 26.5)	22.0 (20.1, 24.5)
Conventional risk factor				
Diabetes mellitus (n (%))	6 (3.6)	2 (3.6)	4 (3.5)	1 (0.6)
Arterial hypertension (n (%))	32 (18.9)	16 (29.1)	16 (14.0)	12 (7.5)
Smoking (n (%))	32 (18.9)	5 (9.1)	27 (23.7)	12 (7.2)
Coronary artery disease (n (%))	6 (3.6)	0 (0.0)	6 (5.3)	0 (0.0)
Thrombosis (n (%))	131 (77.5)	45 (81.8)	85 (75.4)	–
Venous thrombosis (n (%))	81 (47.9)	26 (47.3)	55 (48.3)	–
Arterial thrombosis (n (%))	56 (33.1)	22 (40.0)	34 (29.8)	–
Venous and arterial thrombosis (n (%))	19 (11.2)	6 (10.9)	13 (11.4)	–
Microvascular thrombosis (n (%))	33 (19.5)	16 (29.1)	17 (14.9)	–
Recurrent thrombosis before diagnosis (n (%))	114 (67.5)	25 (45.5)	30 (26.3)	–
Adverse pregnancy history (n (%))	68 (70.1, 97)	23 (74.2, 31)	45 (68.2, 66)	–
Pre-eclampsia, eclampsia and placental dysfunction (n (%))	21 (21.6)	9 (29.0)	12 (18.2)	–
Early miscarriages ≥1 (n (%))	30 (30.9)	12 (38.7)	18 (27.3)	–
Fetal death (≥10 weeks) (n (%))	35 (36.1)	9 (29.0)	26 (39.4)	–
Extra-criteria manifestations (n (%))	86 (50.9)	38 (69.1)	48 (42.1)	–
Thrombocytopenia (n (%))	72 (42.6)	29 (52.7)	43 (37.7)	–
Autoimmune haemolytic anaemia (n (%))	19 (11.2)	6 (10.9)	13 (11.4)	–
Valvular lesions (n (%))	12 (7.1)	5 (9.1)	7 (6.1)	–
APS nephropathy (n (%))	11 (6.5)	7 (12.7)	4 (3.5)	–
Non-vascular neurological manifestations (n (%))	15 (8.9)	8 (14.6)	7 (6.1)	–

Only female patients with at least one conception were included in the analysis of adverse pregnancy history.
Anti-β2GPI-DI, anti-β2GPI-domain I; APS, antiphospholipid syndrome; BMI, body mass index.

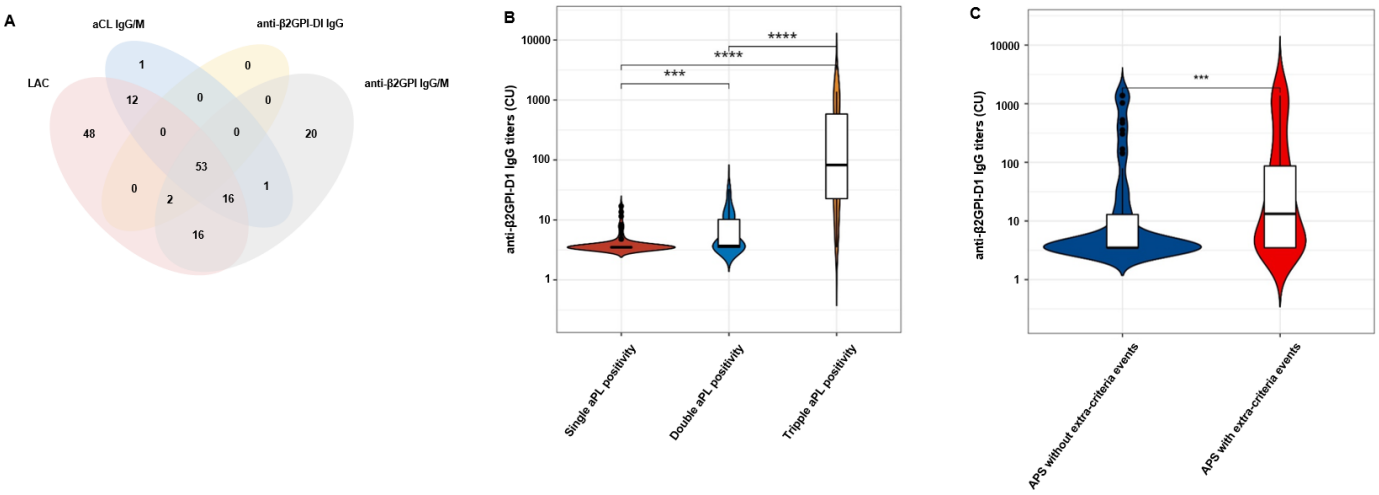

Figure 1 Distribution and association of anti-β2GPI-domain I (anti-β2GPI-DI) IgG with other antiphospholipid antibodies (aPLs) in antiphospholipid syndrome (APS). (A) Venn plot revealing the cross-positivity of aPLs in patients with APS. Numbers in the overlapping region represent the number of patients with multiple positive aPLs, while numbers in the non-overlapping region represent the number of patients with single positive aPL. (B) Serum titre of anti-β2GPI-DI IgG grouped according to the number of positive criteria antibodies. Single, double and triple refers to the positive numbers of the criteria aPLs, including anticardiolipin (aCL) IgG/M, anti-β2GPI IgG/M antibodies and lupus anticoagulant (LAC). (C) Serum titre of anti-β2GPI-DI IgG grouped according to the presence of extra-criteria events. The dark line within the box represents the median, and the length of the box represents the IQR. Mann-Whitney U test was performed. ***p<0.001; ****p<0.0001.

age was 34 years. Patients with APS were more commonly affected by arterial hypertension, smoking and coronary artery disease than patients with SLE. As for immunological profiles, the presence of aPLs was more common in the APS group, including anti-β2GPI-DI IgG (32.5% in APS, 8.1% in SLE, online supplemental table 1).

The proportion of females was higher in patients with SAPS (84.0%) than in PAPS (63.0%) ones (online supplemental table 2). More patients smoked in the PAPS group than the SAPS group. The positivity rates of LAC (96.0% vs 83.2%) and aCL-IgG/M (58.0% vs 41.2%) were higher in the SAPS group (online supplemental table 1). However, the proportions with positive anti-β2GPI IgG/M or anti-β2GPI-DI IgG were comparable between these two groups, which were 32.8% in PAPS and 32.0% in SAPS. Moreover, the titres of anti-β2GPI-DI IgG were also similar between patients with PAPS and SAPS (online supplemental figure 1A).

Antibody distribution and cross-positivity in APS

The associations between anti-β2GPI-DI IgG and other aPLs are shown in figure 1. All patients with anti-β2GPI-DI IgG positivity were also positive with anti-β2GPI IgG. Anti-β2GPI-DI IgG positivity was observed among 55 (77.5%) of 71 patients with APS with anti-β2GPI IgG antibody, 21.3% of those with anti-β2GPI IgM antibody and 37.4% of those with LAC. Meanwhile, all patients with anti-β2GPI-DI IgG were positive with LAC, and 96.4% of them also showed triple positivity in classic aPLs (table 2). The titres of anti-β2GPI-DI IgG antibodies were significantly higher in patients with triple aPLs positivity (82.50 CU, IQR 22.05–691.90) compared with those with single positivity (3.50, IQR 3.50–3.50) or double positivity (3.70, IQR 3.50–10.50) (p<0.001) (figure 1B).

Clinical association at recruitment

A total of 131 (77.5%) patients with APS experienced thrombosis and 68 (57.6%) had adverse pregnancy history, and 86 (50.9%) of them presented with extra-criteria manifestations (table 3). Notably, microvascular thrombosis (38.0% vs 11.8%, p<0.001), as well as extra-criteria manifestations (80.0% vs 38.7%, p<0.001), was significantly more common in patients with SAPS compared with patients with PAPS (online supplemental table 2).

In patients with APS, the positivity and titres of anti-β2GPI-DI IgG were similar between patients with thrombosis and PM (p>0.05, table 1, online supplemental figure 1B). Notably, they were significantly associated with recurrent thrombosis before APS diagnosis (p=0.015) and microvascular thrombosis (p=0.038) (table 1, online supplemental figure 1C), while it is comparable between patients with venous or arterial thrombosis. Extra-criteria manifestations were significantly more common in patients with anti-β2GPI-DI IgG than those without (69.1% vs 42.1%, p=0.001), and accompanied by higher anti-β2GPI-DI titres (p=0.002, figure 1C). In details, patients with thrombocytopenia or APS nephropathy presented with more anti-β2GPI-DI positivity and higher titres (table 1, online supplemental figure 1D,E). In multivariable analysis, anti-β2GPI-DI IgG positivity (OR 2.94, 95% CI 1.29 to 6.70), secondary APS (OR 6.19, 95% CI 2.41 to 15.87), arterial hypertension (OR 9.48, 95% CI 3.06 to 29.34) and Coombs' test positivity (3.53, 95% CI 1.37 to 9.07) independently predicted extra-criteria manifestations in patients with APS (C-index 0.83, 95% CI 0.77 to 0.90) (table 3) as well as in patients with PAPS (online supplemental table 3). Another model with similar C-index was

Table 2 Antibody profiles of patients with APS grouped by anti-β2GPI-DI IgG positivity

Immunological profile	APS (n=169)	Anti-β2GPI-DI IgG positive (n=55)	Anti-β2GPI-DI IgG negative (n=114)	P value
ANA (n (%))	87 (51.8)	36 (65.5)	51 (45.1)	0.01
Low complement (n (%))	57 (34.1)	21 (38.2)	36 (32.1)	0.44
Coombs' test (n (%))	45 (28.3)	16 (30.8)	29 (27.1)	0.63
LAC (n (%))	147 (87.0)	55 (100.0)	92 (80.7)	<0.001
Anti-β2GPI IgG/M (n (%))	108 (63.9)	55 (100.0)	53 (46.5)	<0.001
Anti-β2GPI IgG (n (%))	71 (42.0)	55 (100.0)	18 (15.8)	<0.001
Anti-β2GPI IgM (n (%))	47 (27.8)	10 (18.2)	37 (32.5)	0.05
aCL IgG/M (n (%))	83 (49.1)	53 (96.4)	30 (26.3)	<0.001
aCL IgG (n (%))	83 (49.1)	53 (96.4)	30 (26.3)	<0.001
aCL IgM (n (%))	4 (2.4)	3 (5.5)	1 (0.9)	0.15
Single aPL positivity (n (%))	69 (40.8)	0 (0.0)	69 (60.5)	<0.001
Isolated LAC	48 (38.4)	0 (0.0)	48 (42.1)	<0.001
Isolated anti-β2GPI IgG/M	20 (11.8)	0 (0.0)	20 (17.5)	<0.001
Isolated aCL IgG/M	1 (0.6)	0 (0.0)	1 (0.9)	<0.001
Double aPL positivity (n (%))	31 (18.3)	2 (3.6)	29 (25.4)	<0.001
LAC–, anti-β2GPI IgG/M+, aCL IgG/M+	1 (0.6)	0 (0.0)	1 (0.9)	0.38
LAC+, anti-β2GPI IgG/M–, aCL IgG/M+	12 (7.1)	0 (0.0)	12 (10.5)	0.01
LAC+, anti-β2GPI IgG/M+, aCL IgG/M–	18 (10.7)	2 (3.6)	16 (14.0)	0.03
Triple aPL positivity (n (%))	69 (40.8)	53 (96.4)	16 (14.0)	<0.001
Triple positive IgG isotype	68 (40.2)	53 (96.4)	15 (13.2)	<0.001
Triple positive IgM isotype	4 (2.4)	3 (5.5)	1 (0.9)	0.08

χ^2 test or Fisher's exact test was used for comparison of categorical variables.
Single, double and triple refers to the positive numbers of the criteria aPLs, including aCL IgG/M, anti-β2GPI IgG/M antibodies and LAC.
aCL, anticardiolipin; Anti-β2GPI-DI, anti-β2GPI-domain I; aPL, antiphospholipid antibodies; LAC, lupus anticoagulant.

constructed with triple criteria aPLs positivity, secondary APS, arterial hypertension and Coombs' test positivity.

Events prediction in the follow-up

During a median follow-up of 25 months (IQR 21–34 months), 12 (7.1%) patients developed thrombotic events, including 8 venous thrombosis and 4 arterial thrombosis. Figure 2 showed event-free survival curve for patients with APS divided into two groups based on anti-β2GPI-DI IgG positivity. There was no statistically significant difference in terms of thrombotic events between these two groups (HR 1.23, 95% CI 0.39 to 3.93, p=0.73, figure 2A). Meanwhile, extra-criteria event occurred in four (2.4%) patients, including two with APS nephropathy and two with non-vascular neurological manifestations. While three of these patients were positive with anti-β2GPI-DI IgG, the increased risk failed to reach statistical significance (HR 6.53, 95% CI 0.68 to 62.79, p=0.10, figure 2B). Detailed antibody profiles of these patients are shown in online supplemental table 4.

Diagnostic performance of aPLs

The antibody profiles of the SLE controls without APS are shown in online supplemental table 1. In patients with SLE, anti-β2GPI-DI IgG antibody showed relatively high specificity (91.87%) and low sensitivity (32.00%) for the diagnosis of SAPS among the single aPL assays, while LAC presented the highest sensitivity (96.00%) and moderate specificity (79.38%) (table 4). The diagnostic performance of anti-β2GPI-DI IgG antibody was similar to triple criteria aPLs positivity.

Moreover, we evaluated the diagnostic performance of anti-β2GPI-DI IgG antibody for extra-criteria manifestations among patients with APS (online supplemental table 5). The presence of anti-β2GPI-DI IgG showed relatively high specificity (79.52%) and low sensitivity (44.19%) for predicting extra-criteria events, which was similar to triple criteria aPLs positivity as well.

DISCUSSION

Increasing evidence suggests the pathogenic role of anti-β2GPI-DI IgG in APS. In this study, we performed an integrated analysis of its clinical associations in a large Chinese APS cohort. The major findings were its significant associations with extra-criteria manifestations, especially thrombocytopenia and APS nephropathy.

Table 3 Risk of extra-criteria manifestations in APS by univariable and multivariable logistic regression analysis

	Univariable regression analysis		Multivariable regression model—model 1*		Multivariable regression model—model 2†	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Secondary APS	6.35 (2.90 to 13.92)	<0.001	6.19 (2.41 to 15.87)	<0.001	5.60 (2.20 to 14.29)	<0.001
Demographic features						
Female	0.84 (0.44 to 1.62)	0.61				
Age at diagnosis	1.00 (0.97 to 1.03)	0.98				
BMI	0.93 (0.86 to 1.01)	0.10				
Conventional risk factor						
Diabetes mellitus	5.06 (0.58 to 44.28)	0.14				
Arterial hypertension	5.56 (2.15 to 14.38)	<0.001	9.48 (3.06 to 29.34)	<0.001	9.08 (2.94 to 28.03)	<0.001
Smoking	0.96 (0.44 to 2.07)	0.91				
Coronary artery disease	0.18 (0.02 to 1.61)	0.08				
Immunological profile						
ANA positivity	2.77 (1.48 to 5.18)	0.001				
Low complement	3.27 (1.66 to 6.48)	0.001				
Coombs' test positivity	4.99 (2.25 to 11.06)	<0.001	3.53 (1.37 to 9.07)	0.01	3.36 (1.30 to 8.66)	0.01
LAC positivity	5.68 (1.83 to 17.60)	0.003				
Anti-β2GPI IgG/M positivity	1.37 (0.73 to 2.57)	0.330				
aCL-IgG/M positivity	3.49 (1.85 to 6.56)	<0.001				
Single aPL positivity	0.36 (0.19 to 0.69)	0.002				
Double aPL positivity	0.64 (0.29 to 1.41)	0.27				
Triple aPL positivity	3.73 (1.94 to 7.16)	<0.001			3.00 (1.37 to 6.57)	0.01
Anti-β2GPI-DI IgG positivity	3.07 (1.55 to 6.08)	0.001	2.94 (1.29 to 6.70)	0.01		
C-index			0.83 (0.77 to 0.90)		0.83 (0.77 to 0.90)	

Single, double and triple refers to the positive numbers of the criteria aPLs, including aCL IgG/M, anti-β2GPI IgG/M antibodies and LAC.
*Model 1 was adjusted for secondary APS, arterial hypertension, Coombs' test positivity and anti-β2GPI-DI IgG positivity.
†Model 2 was adjusted for secondary APS, arterial hypertension, Coombs' test positivity and triple aPL positivity.
aCL, anticardiolipin; Anti-β2GPI-DI, anti-β2GPI-domain I; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; BMI, body mass index; LAC, lupus anticoagulant.

In our study, the prevalence and diagnostic value of anti-β2GPI-DI IgG in APS were largely similar to those reported in literature. A recent meta-analysis was performed including 767 patients with APS from 9 studies, and reported an overall median prevalence of 35.7%.[32] In our APS cohort, the general positivity rate was 32.5%, and comparable between PAPS (32.8%) and SAPS (32.0%). It has also been noted that the prevalence of anti-β2GPI-DI IgG varies significantly across different populations, which tends to be higher in aPLs carriers (41.9%)[24] or patients with persistent anti-β2GPI IgG (57.8%),[23] and lower in patients with SLE without APS (7%).[33] In this study, 50.9% of the patients with APS with anti-β2GPI IgG/M antibodies and 8.1% of the non-APS SLE controls were positive for anti-β2GPI-DI IgG. Considering this, it is critical to evaluate the clinical profiles of the control subjects and criteria antibody profiles of patients when calculating the diagnostic performance of anti-β2GPI-DI. Moreover, the methods of assays and choice of cut-off values may potentially affect the titres and positivity of results. Reports from literature, as well as our report, generally showed its high specificities (over 95%) and low sensitivities (ranging from 20% to 60%) for differentiating APS from various controls, including health subjects, patients with SLE or other autoimmune diseases, patients without APS suffering from thrombosis or obstetric events.[18 21 33–36] Compared with anti-β2GPI antibodies, the sensitivity of anti-β2GPI-DI was lower in diagnosing APS. This could be explained by the cross-positivity between these two antibodies, which showed that only 75.3% of those with anti-β2GPI-IgG positivity were positive with anti-β2GPI-DI (table 2). Moreover, this suggested the presence of antibodies against other epitopes of β2GPI in those with anti-β2GPI-IgG positivity, such as domain 4/5.[23] In anti-β2GPI antibodies carriers, the positivity rates and titres of anti-β2GPI-DI antibodies were significantly higher in those with classical triple aPLs positivity,[20 37] which was also confirmed by multiple studies

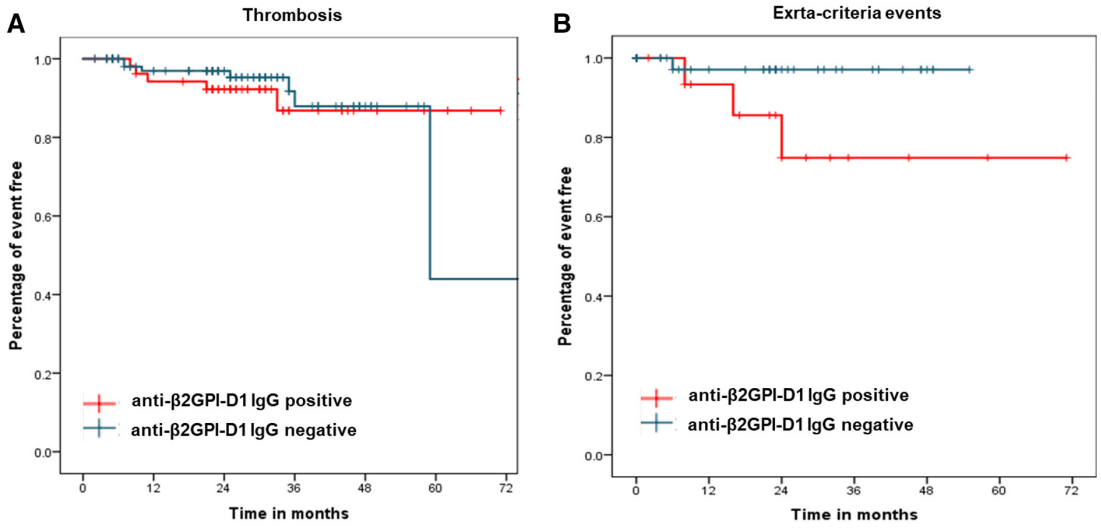

Figure 2 Thrombosis and extra-criteria events during the follow-up. Kaplan-Meier event-free survival curves of thrombosis (A) and extra-criteria events (B) in patients with APS grouped by anti-β2GPI-domain I (anti-β2GPI-DI) IgG positivity.

in patients with APS.[18 38 39] The presence of anti-β2GPI-DI correlated poorly with single aPL positivity (table 2). For example, a total of 48 (38.4%) patients had isolated LAC in our cohort, and none of them was positive with anti-β2GPI-DI. Addition of anti-β2GPI-DI antibodies to classic aPLs could hardly provide extra-diagnostic value of APS, this could be partially explained by its high level of agreement with triple positivity of classic aPLs (figure 1A).

The presence of anti-β2GPI-DI IgG was associated with increased risk of thrombosis and adverse pregnancy outcome in patients with LAC,[15] persistent anti-β2GPI-DI IgG[23] or SLE.[16] However, the interpretations of these findings are limited if these patients with events could have been diagnosed as APS by classic aPLs. Accordingly, the potential role of this antibody in routine practice depends on the its clinical associations within patients with APS. While some early studies demonstrated higher titres of this antibody in thrombotic APS than in obstetric APS,[18 19] more recent studies failed to show the same trend.[20 21] The observed discrepancies could be partially explained by their differences in study design, study populations and methodology of antibody measurements. Recently, it has been suggested that the titres and positivity of anti-β2GPI-DI IgG were associated with late PM, defined as late fetal loss (>10 weeks) or premature delivery.[21 23] We performed similar analysis and found no significant differences in either anti-β2GPI-DI IgG positivity (early PM 41.2% vs late PM 31.4%, p=0.46) or titres (online supplemental figure 1F). It is possible that the sample size of our study is insufficient and selection bias has led to inconsistent conclusions, and further studies with larger sample size are expected. More detailed analyses suggested the association of anti-β2GPI-DI IgG with recurrent thrombosis[20] or APS nephropathy,[40] which were also validated by data from our cohort. Prospective research is lacking concerning the predictive value of anti-β2GPI-DI for thrombosis or PM. In aPLs carriers, the presence of anti-β2GPI-DI was associated with thrombotic events during the follow-up.[24] A recent prospective study followed 44 patients with thrombotic APS for a median of 39 months, and found 4 new thromboses. All of these recurrent patients presented anti-β2GPI-DI positivity.[39] In our study, five out of the eight patients with thrombotic recurrence were positive for anti-β2GPI-DI, and further log-rank test failed to reveal significant predictive associations (figure 2A). Taken together, these results limit the use of anti-β2GPI-DI IgG in predicting thrombosis or PM among patients with APS.

In the recent decade, a large body of clinical studies emerged and suggested that extra-criteria clinical features were frequently associated with aPLs.[2] In the APS ACTION registry, 58% of the 642 patients with APS and 47% of the 162 aPL carriers reported at least one extra-criteria manifestations.[41] We have recently shown that thrombocytopenia could identify patients with PAPS at high risk of developing thrombotic events, PM and other severe extra-criteria events.[26] Notably, patients with triple aPLs positivity experienced the most thrombocytopenia, aPL nephropathy and cardiac valve disease.[41 42] It is also reported that aPL-positive patients with SLE demonstrated as higher frequency of thrombocytopenia, haemolytic anaemia and less IgG anti-β2GPI.[43] While several scoring systems for predicting APS thrombosis have been developed and validated,[8 44] reliable markers of extra-criteria features were lacking. In this study, we proposed that anti-β2GPI-DI IgG was a potential prediction marker of extra-criteria features in patients with APS. It was significantly associated with multiple extra-criteria features at the time of APS diagnosis (table 1). Meanwhile, extra-criteria manifestations were also more common in patients with SAPS compared with PAPS (online supplemental table 2). But the proportions of anti-β2GPI-DI IgG were the same between these two groups. Further multivariate regression analysis also proved that anti-β2GPI-DI

Table 4 Diagnostic performance of antiphospholipid antibodies for secondary APS in SLE

	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)	Accuracy (%)	Youden's index	OR (95% CI)
LAC	96.00	79.38	4.66	0.05	54.55	98.72	82.79	0.75	92.40 (21.53 to 396.54)
Anti-β2GPI-DI IgG	32.00	91.87	3.93	0.74	48.48	84.96	80.31	0.24	5.31 (2.45 to 11.52)
Anti-β2GPI IgG/M	56.00	78.71	2.63	0.56	39.44	87.85	74.21	0.35	4.71 (2.45 to 9.03)
aCL IgG/M	62.00	81.95	3.44	0.46	45.59	89.84	78.04	0.44	7.41 (3.78 to 14.52)
Single aPL positive	34.00	82.78	1.97	0.80	32.08	83.98	73.36	0.17	2.48 (1.25 to 4.92)
Isolated LAC	30.00	91.87	3.69	0.76	46.88	84.58	79.92	0.22	4.84 (2.21 to 10.58)
Isolated anti-β2GPI IgG/M	2.00	94.26	0.35	1.04	7.69	80.08	76.45	0.04	0.34 (0.04 to 2.64)
Isolated aCL IgG/M	2.00	96.65	0.60	1.01	12.50	80.48	78.38	0.01	0.59 (0.07 to 4.90)
Double aPL positivity (n (%))	18.00	93.78	2.89	0.87	40.91	82.70	79.15	0.12	3.31 (1.33 to 8.26)
LAC–, anti-β2GPI IgG/M+, aCL IgG/M+	0.00	95.69	0.00	1.04	0	80.00	77.22	NA	0.21 (0.01 to 3.65)
LAC+, anti-β2GPI IgG/M–, aCL IgG/M+	12.00	99.52	25.08	0.88	85.71	82.54	82.63	0.12	28.36 (3.33 to 241.53)
LAC+, anti-β2GPI IgG/M+, aCL IgG/M–	6.00	98.56	4.18	0.95	50.00	81.42	80.69	0.05	4.38 (0.86 to 22.40)
Triple aPL positive	48.00	90.91	5.28	0.57	55.81	87.96	82.63	0.39	9.23 (4.46 to 19.12)

aCL, anticardiolipin; Anti-β2GPI-DI, anti-β2GPI-domain I; aPL, antiphospholipid antibody; LAC, lupus anticoagulant; NA, not available; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.

IgG and SAPS were two independent factors associated with extra-criteria manifestations (table 3). In details, thrombocytopenia and APS nephropathy were more common in patients with anti-β2GPI-DI IgG, while those with SAPS experienced more thrombocytopenia, autoimmune haemolytic anaemia, valvular lesions and non-vascular neurological manifestations. This might implicate the different underlying pathogenesis among various extra-criteria manifestations. The two cases with APS nephropathy during the follow-up were positive with anti-β2GPI-DI IgG, but statistical analysis was limited by the sample size and follow-up duration.

Strengths of our study are its prospective design, relatively large sample size and integrated clinical profiling including extra-criteria features. There are several limitations to be noted. The study was based on data from a single centre, and the follow-up duration was relatively short. Future analysis based on the ongoing Chinese Anti-phosPholipid Syndrome cohorT cOllaborative NEtworks (NCT0523001) registry would be a complement to this, with participants from multiple centres and extended follow-up period. All the included subjects were Asians, and the conclusions should be validated in patients with other races/ethnicities before translating into clinical practice in different scenarios. All the subjects were diagnosed based on the positivity of aPLs measured by chemiluminescence technique (CLIA), and potential bias should be noted considering the discrepancies in detection aPLs between CLIA and ELISA.[45] Some studies have suggested that there are differences in the sensitivity and specificity of CLIA and ELISA methods, which are closely related to the choice of cut-off values.[46] We did not validate the manufacturer's cut-off value in local Chinese population. However, we have previously validated the diagnostic power of this CLIA system in Chinese patients with APS.[30] Limited data from a previous study also suggested a cut-off of 20 CU was appropriate in Chinese.[21] The time duration for the follow-up of patients with APS was relatively short, thus the event rate for extra-criteria manifestations was not enough for further statistical analysis. Also, treatment details were not included in the present study, which could limit our interpretations of the association between anti-β2GPI-DI IgG and thrombosis recurrence. Future studies with longer follow-up durations should be performed to validate the prediction value of anti-β2GPI-DI IgG for extra-criteria features and thrombosis recurrence, and to develop related risk prediction scores.

CONCLUSIONS

In this study, we found that anti-β2GPI-DI IgG was significantly associated with extra-criteria manifestations in patients with APS, especially thrombocytopenia and APS nephropathy. Further studies are warranted to validate its predictive values and potential role in daily practice.

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