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Antiphosphatidylserine/prothrombin antibodies (aPS/PT) as potential diagnostic markers and risk predictors of venous thrombosis and obstetric complications in antiphospholipid syndrome

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Abstract

Background: The aim of the study was to determine the prevalence and clinical associations of antiphosphatidylserine/prothrombin antibodies (aPS/PT) with thrombosis and pregnancy loss in Chinese patients with antiphospholipid syndrome (APS) and seronegative APS (SNAPS).
Methods: One hundred and eighty six Chinese patients with APS (67 primary, 119 secondary), 48 with SNAPS, 176 disease controls (79 systemic lupus erythematosus [SLE], 29 Sjogren's syndrome [SS], 30 ankylosing spondylitis [AS], 38 rheumatoid arthritis [RA]) and 90 healthy donors were examined. IgG and IgM aPS/PT, IgG/IgM/IgA anticardiolipin (aCL) and IgG/IgM/IgA anti-β_2-glycoprotein I (anti-β2GPI) antibodies were tested by ELISA.
Results: One hundred and sixty (86.0%) of APS patients were positive for at least one aPS/PT isotype. One hundred and thirty five (72.6%) were positive for IgG aPS/PT, 124/186 (66.7%) positive for IgM aPS/PT and 99 (53.2%) positive for both. Approximately half of the SNAPS patients were positive for IgG and/or IgM aPS/PT. Highly significant associations between IgG aPS/PT and venous thrombotic events (odds ratio [OR] = 6.72) and IgG/IgM aPS/PT and pregnancy loss (OR = 9.44) were found. Levels of IgM aPS/PT were significantly different in APS patients with thrombotic manifestations and those with fetal loss ($p = 0.014$). The association between IgG/IgM aPS/PT and lupus anticoagulant (LAC) was highly significant ($p < 0.001$). When both were positive, the OR for APS was 101.6. Notably, 91.95% (80/87) of LAC-positive specimens were positive for IgG and/or IgM aPS/PT, suggesting aPS/PT is an effective option when LAC testing is not available.
Conclusions: Anti-PS/PT antibody assays demonstrated high diagnostic performance for Chinese patients with APS, detected some APS patients negative for criteria markers and may serve as potential risk predictors for venous thrombosis and obstetric complications.

Keywords: antiphosphatidylserine/prothrombin antibodies; antiphospholipid syndrome; lupus anticoagulant; seronegative APS.

Introduction

The antiphospholipid syndrome (APS) is a thrombophilic disorder characterized by clinical manifestations of vascular thrombosis and obstetric complications associated with the presence of specific antiphospholipid antibodies (aPLs). The conventional aPLs typically evaluated include anticardiolipin (aCL) antibody (IgG and IgM), anti-β_2-glycoprotein I (anti-β2GPI) antibodies (IgG and IgM) and lupus anticoagulant (LAC). The persistent presence of at least one of these three biomarkers at medium to high titers is recognized as the critical laboratory diagnostic criteria for the definite classification of patients with APS

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by international consensus detailed in the updated 2006 Sydney modification of the original 1999 "Sapporo criteria" [1, 2].

APLs are a heterogeneous group of immunoglobulins directed against phospholipids or specific phospholipid-binding plasma proteins such as β2GPI and prothrombin (PT) [3–5]. Antibodies to prothrombin have been investigated for years, but results have been variable and have not been included in the classification criteria. Atsumi et al. [6] showed that when prothrombin is complexed to phosphatidylserine however, the prothrombin-phosphatidylserine complex (PS/PT) has significantly improved performance over prothrombin alone. Numerous papers have since demonstrated the important role of aPS/PT in APS, lupus and other systemic inflammatory disorders [7–9] and have confirmed the enhanced performance of aPS/PT compared with antiprothrombin (aPT) antibodies [10, 11]. Anti-PS/PT antibodies are strongly correlated with risk of thrombosis and LAC, and levels are higher in "triple positive" (positive for aCL IgG and/or IgM, aβ2GPI IgG and/or IgM and LAC) patients [7, 10, 12–14]. Anti-PS/PT antibodies were recommended as helpful to assess the risk of thrombosis and diagnosis of APS by the task force on diagnostics at the 14th International Congress on APS [15].

The concept of seronegative APS (SNAPS) was first introduced in 2003 by Hughes and Khamashta to describe patients with clinical manifestations highly suggestive of APS, but persistently negative for LAC, IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies, the classical laboratory markers of APS [16]. Although the Sydney modification of the original APS classification criteria officially expanded the laboratory tests criteria by inclusion of anti-β2GPI antibodies and consequently was able to classify more patients, it is clear that some patients with clinical manifestations of APS remain seronegative for the conventional assays or have laboratory results of unclear clinical significance, which in both cases can lead to diagnostic uncertainty and possible delay in obtaining timely treatment. IgA aCL and IgA β2GPI antibodies, while not part of the official laboratory criteria markers, are suggested as useful supplemental assays. New biomarkers to accurately identify SNAPS patients are critically needed since failure to treat patients with APS can lead to serious consequences. As with classical APS, patients with SNAPS can have an accelerated progression resulting in multi-organ failure, a life-threatening medical condition known as catastrophic APS [17].

Although the assay for aPS/PT antibodies was first reported in 2000, the test can be difficult to produce, and until recently aPS/PT antibodies were rarely detected due to lack of standardized commercial kits available to clinical laboratories [18]. The present study was designed to evaluate the prevalence and significance of aPS/PT using a commercially available kit in a large cohort of very well-characterized patients with APS, SNAPS and other autoimmune diseases from the Shanghai area of China and to analyze the relationship of LAC, aPS/PT and other aPL antibodies with clinical manifestations.

Materials and methods

Patient recruitment

The study included 186 consecutive patients with APS from the APS-SH database, which was established in 2000 by expert rheumatologists and statisticians at the Shanghai Jiaotong University School of Medicine (Shanghai, China). Patients included met the criteria for the classification of APS using the Sydney criteria [1, 2]. All patients had medical histories, laboratory tests, imaging studies and treatment documented and underwent a medical follow-up by expert rheumatologists. Serum samples were collected and frozen at −80 °C until testing. We also recruited 48 patients with SLE and definite thrombosis or miscarriage, but negative for criteria aPL laboratory markers. These patients were classified as patients with SNAPS. Patients with other autoimmune diseases, including 79 SLE without thrombosis and miscarriage, 29 SS, 30 AS and 38 RA patients classified using standard diagnostic criteria were also recruited [19–22]. Finally, samples from 90 age and sex-matched healthy donors with neither autoimmune nor infectious diseases were collected as healthy controls (HC).

The study was performed in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice and approved by the Institutional Review Broad of Ruijin Hospital (ID: 2016-61), Shanghai Jiaotong University School of Medicine, Shanghai, China.

Antibodies to phosphatidylserine-prothrombin (aPS/PT)

The presence of aPS/PT IgG and IgM antibodies was analyzed using semiquantitative QUANTA Lite® ELISA kits provided by Inova Diagnostics, Inc. (San Diego, CA, USA). The suggested cutoff values for IgG and IgM aPS/PT antibodies were defined as >30 units. All samples were tested blinded in duplicate in the laboratory of Ruijin Hospital. Note throughout this paper that the nomenclature "x"/"x" indicates "and/or."

Antibodies to cardiolipin (aCL) and β2-glycoprotein I (anti-β2GPI)

IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies were measured by in-house ELISA and commercial ELISA kits (Euroimmun, Luebeck, Germany) when patients were initially enrolled. The serum samples, which had been stored at −80 °C, were retested for IgG/IgM/IgA aCL

and IgG/IgM/IgA anti-β2GPI antibodies by commercial ELISA kits (QUANTA Lite®, Inova Diagnostics, Inc., San Diego, CA, USA). The suggested cutoff for IgG, IgM and IgA aCL is >20 GPL/MPL and for IgG, IgM and IgA anti-β2GPI is >20 units (calculated by the 99th percentile of healthy subjects). All samples were tested blindly and in duplicate.

Lupus anticoagulant (LAC)

Plasma samples were tested for LAC when enrolled into our database according to the recommended criteria from the ISTH Subcommittee on Lupus Anticoagulant-Phospholipid-dependent antibodies [23, 24], using the Automated Coagulation laboratory 300R (Instrumentation Laboratory, Milan, Italy). Samples were screened using the dilute Russell viper venom time (dRVVT) testing and activated partial thromboplastin time (Instrumentation Laboratory). Ratios higher than 1.10 that could not be corrected by the 50:50 mixture with normal plasma were considered as suggestive of LAC and subjected to dRVVT testing. The dRVVT coagulation test was performed in all samples using Diagen Russell's viper venom (Diagnostic Reagents Ltd, Oxon, UK) as described by Thiagarajan et al. [25]. Both screening and confirming steps were performed. The LAC was considered positive if the ratio of dRVVT screening time/dRVVT confirming time >1.20.

Statistical analysis

Statistical analyses were performed using SPSS 23.0 (IBM, Chicago, IL, USA) or Analyze-it ver 4.6 (Analyze-it Software, LTD). T-test, Mann-Whitney U-test, κ-test, Fisher's exact or χ^2-tests were applied. Comparisons between groups were expressed as odds ratio (OR) with 95% confidence interval (95% CI). Sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (LR+ and LR−), OR and receiver operating characteristic (ROC) analysis of the aPL assays tested were calculated. Multivariate analysis was performed in order to identify the independent factors that are positively associated with the dependent variable and to control for possible confounding parameters. Multivariate models are controlled for age and sex. The magnitude of each factor was expressed as an OR. p-Values <0.05 were considered significant.

Results

Clinical manifestations

A total of 186 patients with APS and 48 with SNAPS were examined, 164 (88.2%) female, 22 (11.8%) male, mean age 34.2 years. Primary APS (PAPS) was present in 67 patients (36.0%), whereas APS was secondary to other diseases in 119 patients (64.0%) (Table 1), including 94 patients (79.0%) with SLE and 25 patients (21.0%) with other autoimmune diseases.

The prevalence of major APS manifestations included 53 patients (28.5%) presenting with only arterial thrombosis (AT), 68 patients (36.5%) with only venous thrombosis (VT), 9 patients (4.8%) with both AT and VT and 56 patients (30.1%) with only fetal loss. A total number of 73 females in the pregnancy morbidity subset of APS group experienced 201 pregnancy episodes; of these, 145 had obstetric complications, including 44.3% (89/201) early fetal loss (<10 weeks), 21.4% (43/201) late fetal loss (≥10 weeks) and 18.8% (13/69) premature live births. Among the 48 SNAPS patients (female 41, male 7; mean age 38.9 years), 20 patients (41.7%) presented with only AT, 12 patients (25.0 %) with only VT, 8 patients (16.7%) with both AT and VT and 8 patients (16.7%) with only fetal loss (Table 1). In the subgroup of APS patients with thrombosis, stroke was the most frequent clinical manifestation

Table 1: Demographic and clinical variables of subjects.

Variables	APS (n = 186)	SNAPS (n = 48)	p-Value
Gender (female/male)	164/22	41/7	0.786
Mean age (years ± SD)	34.2 ± 13.9	38.9 ± 18.0	0.867
Onset type			
PAPS, n (%)	67 (36.0)	N/A	
SAPS, n (%)	119 (64.0)	N/A	
Clinical manifestations			
Thrombosis only, n (%)	113 (60.7)	36 (75.0)	0.096
AT only, n (%)	53 (28.5)	20 (41.7)	0.114
VT only, n (%)	68 (36.5)	12 (25.0)	0.182
AT + VT, n (%)[a]	9 (4.8)	8 (16.7)	0.012
Pregnancy morbidity only, n (%)	56 (30.1)	8 (16.7)	<0.01
Thrombosis + pregnancy morbidity, n (%)[b]	17 (9.1)	4 (8.3)	0.999

APS, antiphospholipid syndromes; PAPS, primary antiphospholipid syndrome; SAPS, secondary antiphospholipid syndrome; SNAPS, seronegative APS, patients with clinical manifestations highly suggestive of APS, but with persistently negative LAC, IgG/IgM aCL and IgG/IgM anti-β2-GPI antibodies; AT, arterial thrombosis; VT, venous thrombosis. [a]Including AT + VT. [b]Including thrombosis + pregnancy morbidity.

of AT, present in 46 patients (24.7%). For VT, the most frequent clinical manifestation was lower limb DVT, present in 74 patients (39.8%).

Prevalence of aPL and aPS/PT antibodies in study groups

All APS patients were tested by APS criteria assays, as well as IgA aCL and IgA β2GPI, and IgG/IgM aPS/PT. The results are shown graphically in Figure 1A for the total cohort of APS patients and Figure 1B for the subgroup of 48 SNAPS patients. In contrast to the strong reactivity of multiple antibodies in the APS group, only IgG and IgM aPS/PT, and to a lesser extent IgA β2GPI, were detected in the SNAPS subgroup. Using the 30-unit cutoff, 160 (86.0%) of APS patients were positive for at least one aPS/PT isotype. IgG aPS/PT antibodies were positive in 134/186 patients (72.0%), IgM aPS/PT antibodies were positive in 125/186 patients (67.2%) and both IgG and IgM aPS/PT antibodies were positive in 99/186 APS patients (53.2%) (Table 2). The highest prevalence of aPS/PT was in the APS group (72.1%), with a similar percentage seen in the PAPS (70.1%) and SAPS (73.1%) subgroups. Both IgG aPS/PT and IgM aPS/PT were more frequent in the APS group than in other disease and HC groups ($p < 0.001$). The performance of aPS/PT relative to the other aPLs assays in APS, SNAPS, SLE and HC groups is shown in Figure 2.

Thirty (38%) of the 79 patients with SLE and no current diagnosis of APS were positive for either IgG and/or IgM PS/PT antibodies. Of the 20 patients with only IgM aPS/PT antibodies, six patients were strongly positive with values >136 units. One SLE patient had only IgG PS/PT antibody >136 units, whereas three additional SLE patients were

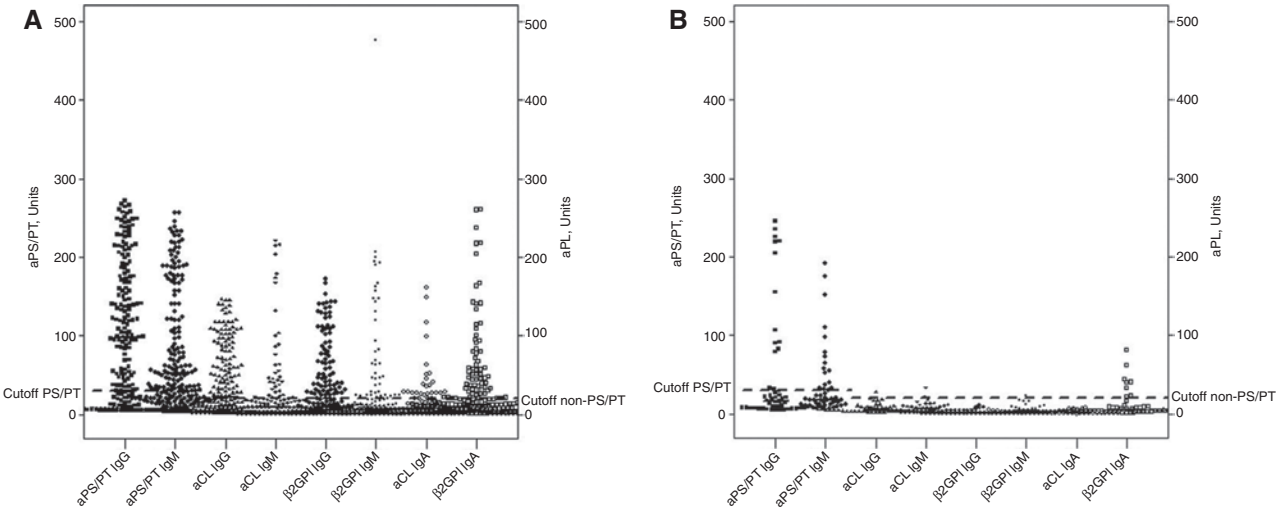

Figure 1: Distribution of aPLs levels (expressed in units) in study groups.
The cutoff for PS/PT is >30 units and cutoff for all other assays is >20 units. (A) Distribution of aPLs levels in APS group. (B) Distribution of aPLs levels in SNAPS group.

Table 2: Prevalence of aPS/PT in the study groups.

	No.	IgG or IgM aPS/PT, n (%)	IgG aPS/PT, n (%)	IgM aPS/PT, n (%)	Dual positivity, n (%)[a]
APS	186	160 (86.0)	134 (72.0)	125 (67.2)	99 (53.2)
PAPS	67	61 (91.0)	47 (70.1)	53 (79.1)	39 (58.2)
SAPS	119	99 (83.2)	87 (73.1)	72 (60.5)	60 (50.4)
SNAPS	48	25 (51.0)	14 (28.6)	17 (34.7)	6 (12.2)
SLE	79	30 (38.0)	10 (12.7)	26 (32.9)	6 (7.6)
SS	29	9 (31.0)	1 (3.4)	8 (27.6)	0 (0)
RA	38	0 (0)	0 (0)	0 (0)	0 (0)
AS	30	3 (10.0)	0 (0)	3 (10.0)	0 (0)
HC	90	7 (8.2)	0 (0)	7 (8.2)	0 (0)

aPS/PT, antiphosphatidylserine/prothrombin antibody; SLE, systemic lupus erythematosus; SS, Sjogren syndrome; AS, ankylosing spondylitis; RA, rheumatoid arthritis; HC, healthy controls. [a]IgG and IgM aPS/PT are both positive.

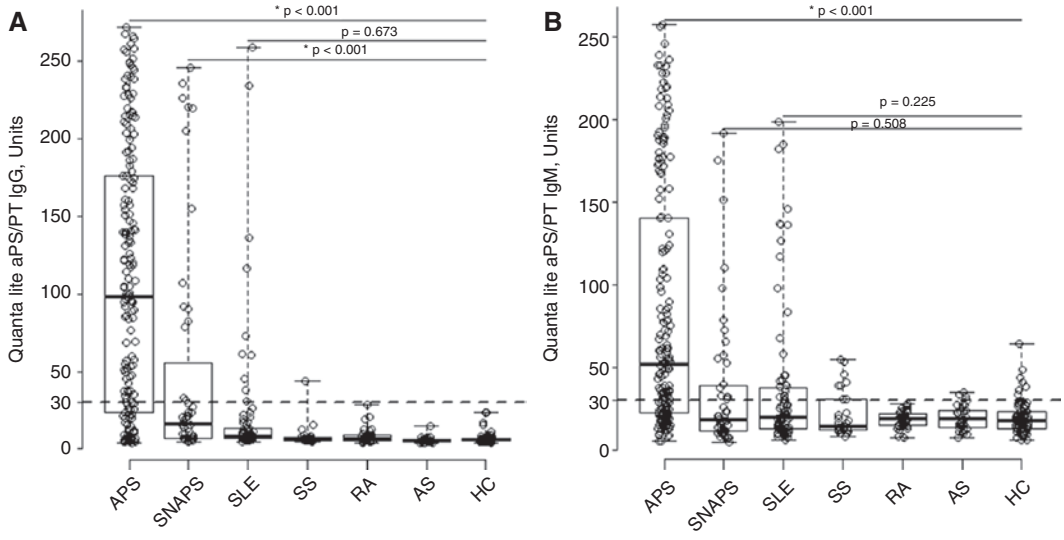

Figure 2: Distribution of IgG and IgM aPS/PT levels determined by ELISA in study groups.
The cutoff for IgG/M PS/PT is >30 units. (A) Distribution of IgG aPS/PT levels in APS, SNAPS, SLE, SS, RA, AS and HC groups. (B) Distribution of IgM aPS/PT levels in APS, SNAPS, SLE, SS, RA, AS and HC groups.

dual positive at levels >116 units for both IgG and IgM PS/PT antibodies.

Prevalence of aPS/PT antibodies in SNAPS

IgG and/or IgM PS/PT antibodies were detected in 25 (51%) of the SNAPS patients (Table 2 and Figure 2). IgG aPS/PT was positive in 14 (28.6%), IgM aPS/PT was positive in 17 (34.7%) and both were positive in 6 (12.2%) SNAPS patients. The prevalence of IgG aPS/PT in the SNAPS group was higher than the SLE group ($\chi^2 = 4.2868$, $p = 0.038$), other autoimmune diseases groups (RA, SS, AS, $p < 0.05$) or the HC group ($p < 0.001$). IgM aPS/PT positivity was significantly higher in the SNAPS patients compared with HC ($p < 0.001$), but not from the SLE ($\chi^2 = 0.0092$, $p = 0.924$) or SS ($\chi^2 = 0.2115$, $p = 0.646$) groups.

Clinical performance of aPL assays

IgG aPS/PT exhibited the highest sensitivity (72%) among the single aPL assays and showed high specificity (95.8%) (Table 3). IgM aPS/PT also gave a relatively good sensitivity of 67.2%, but the lowest specificity (83.1%) among the aPLs. ROC analysis for detection of APS showed anti- β2GPI IgG with an AUC of 0.874, followed closely by aPS/PT IgG and aCL IgG, both with AUCs of 0.849. LAC (AUC = 0.734) showed a significantly lower AUC than aPS/PT IgG ($p < 0.001$) (Figure 3). Notably, aβ2GPI IgA showed higher sensitivity than aCL IgM and aβ2GPI IgM. Among the aPL assays, the combination of anti-PS/PT IgG and IgM showed the highest sensitivity of 86.0% (160/186), with a specificity of 81.2%. In comparison, LAC had a sensitivity of 46.7% (87/186) with 100% specificity. Combining LAC and aPS/PT IgG/IgM assays resulted in sensitivity of 89.9% (167/186) and a specificity of 81.2% (Table 3).

Relationship of aPS/PT with LAC

The association between IgG or IgM aPS/PT antibodies and LAC was highly significant (OR = 14.32, 95% CI = 7.82–27, $p < 0.001$; OR = 7.84, 95% CI = 4.45–14.16, $p < 0.001$). Of the 135 patients positive for IgG aPS/PT, 69 (51.1%) were LAC positive, whereas 69/87 (80.2%) LAC-positive patients were IgG aPS/PT positive (Figure 4). Similarly, of the 125 patients with IgM aPS/PT positivity, 65/125 (52.0%) had LAC positivity, whereas of the 87 patients with positive LAC, 66/87 (75.9%) had positive IgM aPS/PT. Of the total 160 aPS/PT IgG/IgM-positive patients, 49.4% (79/160) were LAC positive. By contrast, of the 87 LAC positive sera, 90.8% (79/87) were PS/PT IgG and/or IgM positive. The combination of LAC and aPS/PT IgG yielded an OR of 101.6 (Table 3).

Clinical associations of aPS/PT

Finally, we investigated aPS/PT in subgroups of patients divided by disease manifestations: thrombosis, AT, VT and pregnancy loss. APS patients with thrombotic manifestations only displayed significantly lower IgM aPS/PT

Table 3: Diagnostic performance of specific aPL tests and combinations in APS group (sorted by highest OR).[a]

Parameters	Sens, %	Spec, %	PPV	NPV	LR+	LR–	OR
LAC	46.8	100.0	1.00	0.725	∞	0.532	∞
aPS/PT IgG/LAC	81.7	95.8	0.933	0.880	19.452	0.191	101.604
aPS/PT IgG/aCL IgG/LAC	84.9	93.9	0.908	0.897	13.918	0.161	86.406
aPS/PT IgG/LAC/aCL IgG/aβ2GPI IgG	84.9	93.1	0.898	0.897	12.304	0.162	76.179
aCL IgG/aβ2GPI IgG/LAC	70.4	96.9	0.942	0.821	22.710	0.305	75.325
aPS/PT IgG	72.0	95.8	0.924	0.828	17.143	0.292	58.566
aPS/PT IgG and IgM	53.2	97.7	0.943	0.746	23.130	0.479	48.362
aβ2GPI IgG	41.4	98.5	0.951	0.702	27.600	0.595	45.388
aCL IgG/M/A/aβ2GPI IgG/M/A/LAC	87.1	87.0	0.827	0.904	6.700	0.148	45.066
aCL IgG	51.1	97.7	0.941	0.737	22.217	0.501	44.368
aPS/PT IgG/M/aβ2GPI IgG/M/A/aCL G/M/A/LAC	93.0	74.3	0.721	0.937	3.619	0.094	38.533
aPS/PT IgG/M/LAC	89.8	81.2	0.773	0.918	4.777	0.126	38.028
aPS/PT IgG/M/LAC/aβ2GPI IgG/M	90.3	78.9	0.753	0.920	4.280	0.123	34.958
aPS/PT IgG/M/aβ2GPI IgGM/aCL GM/LAC	90.9	77.4	0.741	0.922	4.022	0.118	34.036
aPS/PT IgG/M/aCL IgG/M/A	88.7	79.3	0.753	0.908	4.285	0.142	30.119
aβ2GPI IgG/M	53.8	96.2	0.909	0.745	14.158	0.480	29.186
aPS/PT IgG/M/aβ2GPI IgG/M/A/aCL G/M/A	90.9	74.3	0.716	0.919	3.537	0.122	28.785
aPS/PT IgG/M/aβ2GPI IgG/M/aCL G/M	88.7	77.4	0.737	0.906	3.925	0.146	26.901
aPS/PT IgG/M	86.0	81.2	0.766	0.891	4.574	0.172	26.625
aPS/PT IgG/M/aβ2GPI IgG/M	87.6	78.9	0.748	0.900	4.152	0.157	26.544
aCL IgG/IgM	58.1	95.0	0.893	0.761	11.62	0.441	26.414
aCL IgG/IgM/IgA	58.1	94.6	0.885	0.760	10.759	0.443	24.429
aCL IgA	14.5	99.2	0.931	0.620	18.125	0.862	21.991
aCL IgG/M/A/aβ2GPI IgG/M/A	72.6	87.0	0.799	0.817	5.585	0.315	17.673
aβ2GPI IgG/M/A	64.0	90.0	0.821	0.778	6.400	0.400	16.053
aβ2GPI IgM	22.6	97.3	0.857	0.638	8.370	0.795	10.583
aPS/PT IgM	67.2	83.1	0.740	0.781	3.976	0.395	10.106
aCL IgM	20.4	96.9	0.826	0.631	6.581	0.821	8.120
aβ2GPI IgA	36.6	93.1	0.791	0.673	5.304	0.681	7.780

[a]SNAPS patients excluded from calculations, "/" = and/or.

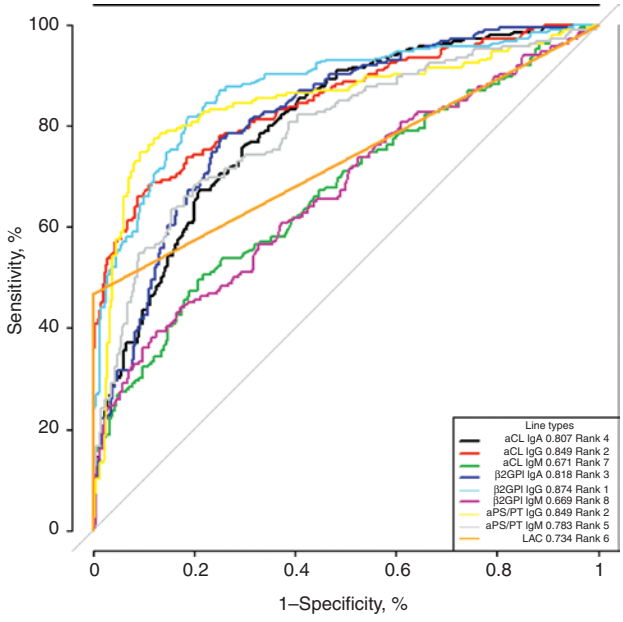

Figure 3: Comparison of receiver operating characteristic (ROC) curves and area under the curve (AUC) of aPL and LAC in APS group (n = 186).

titers (median titer = 45.63 units) compared with patients with fetal loss only (median titer = 91.60 units) (p = 0.014). By contrast, there was no significant difference in the median IgG aPS/PT titers among the three thrombosis groups. We then investigated the relationship between IgG and IgM aPS/PT and APS-related clinical manifestations (Table 4). Using multivariate analysis, the most significant associations were aPS/PT IgG (OR = 10.50, 95% CI = 6.54–17.23, p < 0.001) with total thrombosis events, aPS/PT IgG and/or IgM with arterial thrombosis events (OR = 6.30, 95% CI = 3.58–11.57, p < 0.001), aPS/PT IgG (OR = 6.42, 95% CI = 3.91–10.76, p < 0.001) with venous thrombotic events, and aPS/PT IgG and/or IgM with fetal loss (OR = 10.41, 95% CI = 5.47–21.63, p < 0.001).

The association of aPS/PT antibodies with stroke and transient ischemic attack (TIA) has been reported previously. In the present cohort, 62 patients (46 with APS, 15 with SNAPS and 1 with SLE) had a history of stroke, and 88.7% (55/62) of these patients were positive for aPS/PT IgG and/or IgM. Of the 46 patients with APS and stroke, 35 (76.1%) were positive for aPS/PT IgG, 31 (67.4%) for aPS/PT

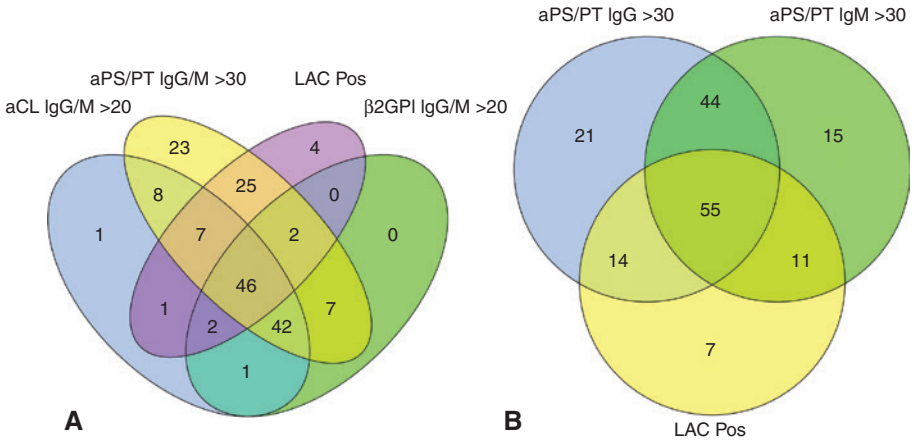

Figure 4: Venn diagram of the aPLs.
Venn diagram of serum reactivity of patients with APS. Serum was tested for aPS/PT and criteria aPLs by ELISA or by LAC. Numbers in individual fields indicate positive cases for a given reactivity or combinations of reactivities. (A) Venn diagram of aPS/PT IgG/M, aCL IgG/M, aβ2GPI and LAC. (B) Venn diagram of aPS/PT IgG, aPS/PT IgM and LAC.

IgM and 44 (95.6%) for aPS/PT IgG and/or IgM. In comparison, LAC was positive in 24/46 (52.2%) of the APS patients with a history of stroke. For the 15 SNAPS patients with a history of stroke, 10 (66.7%) were positive for aPS/PT IgG and/or IgM (4 IgG only, 4 IgM only and 2 both IgG and IgM). By definition, all SNAPS were LAC negative. Notably, all positive aPS/PT values for the SNAPS patients were >50 units.

Discussion

Delayed diagnosis of APS can have severe consequences for patients such as repeated pregnancy loss and/or thrombosis. New biomarkers are needed to improve diagnosis, prognosis and treatment. In addition to the conventional "criteria" markers (cardiolipin, β2GPI and LAC), numerous markers have been proposed and evaluated. The definition of "aPL positive", however, is still controversial. On the one hand, the lack of standardization among laboratories often makes it difficult for physicians to consistently identify "aPL positive" patients with APS. On the other hand, standard testing with current criteria aPLs may miss some APS patients with significant thrombosis and/or obstetric problems, but who are repeatedly negative for aCL, β2GPI and LAC of the SNAPS patients. While these patients are "seronegative" for the current criteria markers, they are seropositive for other aPL antibodies. Effective new markers could help decrease the number of seronegative patients and help more patients receive timely medical attention before devastating potential consequences.

Autoantibodies directed towards prothrombin and/or phosphatidylserine/prothrombin complexes have been extensively investigated for evaluation of patients with suspected APS [26]. Our study clearly demonstrated a higher prevalence of IgG/IgM aPS/PT in APS and SNAPS groups compared with other autoimmune disease groups. Furthermore, we demonstrated a strong association of aPS/PT with thrombosis and obstetric complications.

Evaluation of APS and SNAPS demographics suggested that the SNAPS group may have experienced fewer obstetric complications than the APS group ($p < 0.01$). The prevalence and level of IgG aPS/PT in SNAPS group was significantly higher than in the SLE group in our study, suggesting IgG aPS/PT may help the diagnosis of APS lacking positive "criteria" aPL. A follow-up study of IgG aPS/PT positive patients in our SLE group will evaluate patient outcome, specifically assessing the utility of aPS/PT antibodies for risk assessment of future thrombotic events. In addition to diagnostic value, studies have shown that aPS/PT antibodies may be strongly related to ischemic/ thrombotic cerebrovascular events. In a prospective study of 167 patients with TIA, Mullen et al. [27] showed by multivariate analysis adjusted by $ABCD^2$ risk score, that aPS/PT IgG antibodies were independently associated with stroke or death in patients with TIA (OR = 15.7, 95% CI = 2.0–125.6, $p = 0.009$). Akhter et al. [28] demonstrated that aPS/PT (IgG and IgG/IgM) were significantly associated with venous thrombosis (OR = 2.1, 95% CI = 1.2–3.8, $p = 0.015$) in a cohort of 326 SLE patients. The current study provides additional support of these observations. Among the 62 patients with a history of stroke (46 APS, 15 SNAPS, 1 SLE), IgG and/or IgM aPS/PT antibodies were positive in 88.7% (55/62) of the APS, SNAPS and

Table 4: Relationship of aPL with thrombosis and/or obstetric manifestations.

aPL assay	Thrombosis		Arterial thrombosis		Venous thrombosis		Pregnancy loss	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value
aCL IgG/M (≥40 PL)	7.72 (4.42–14.05)	<0.001	3.76 (2.13–6.66)	<0.001	3.78 (2.23–6.44)	<0.001	3.74 (2.10–6.67)	<0.001
aCL IgG (≥40 GPL)	9.49 (5.15–18.61)	<0.001	3.98 (2.22–7.15)	<0.001	4.18 (2.41–7.26)	<0.001	3.23 (1.76–5.91)	<0.001
aCL IgM (≥40 MPL)	3.93 (1.61–10.55)	0.004	1.38(0.44–3.72)	0..546	3.27 (1.32–7.92)	<0.001	4.26 (1.70–10.75)	<0.001
aβ2GPI IgG/M	6.21 (3.80–10.37)	<0.001	3.16 (1.85–5.39)	<0.001	3.96 (2.40–6.54)	<0.001	4.36 (2.54–7.49)	<0.001
aβ2GPI IgG	7.50 (4.24 13.84)	<0.001	3.95 (2.21–7.06)	<0.001	3.61 (2.09–6.20)	<0.001	3.37 (1.86–6.06)	<0.001
aβ2GPI IgM	3.22 (1.72– 6.24)	<0.001	1.62 (0.76–3.27)	0.192	2.68 (1.38–5.11)	<0.001	4.87 (2.48–9.60)	<0.001
LAC	7.85 (4.50–14.23)	<0.001	2.72 (1.53–4.78)	<0.001	4.56 (2.70–7.74)	<0.001	5.13 (2.92–9.07)	<0.001
aPS/PT IgG/M	9.10 (5.73–14.81)	<0.001	6.30 (3.58–11.57)	<0.001	5.58 (3.27–9.94)	<0.001	10.41 (5.47–21.63)	<0.001
aPS/PT IgG	10.50 (6.54–17.23)	<0.001	4.62 (2.76–7.88)	<0.001	6.42 (3.91–10.76)	<0.001	5.66 (3.35–9.75)	<0.001
aPS/PT IgM	4.24 (2.78–6.56)	<0.001	3.12 (1.89–5.23)	<0.001	3.24 (2.02–5.29)	<0.001	4.71 (2.79–8.12)	<0.001
aCL IgG + aβ2GPI IgG	9.36 (4.81–19.79)	<0.001	4.07 (2.18–7.58)	<0.001	4.09 (2.27–7.36)	<0.001	3.33(1.73–6.37)	<0.001
aCL IgG + aβ2GPI IgG + LAC	6.33 (2.79–16.27)	<0.001	3.99 (1.83–8.58)	<0.001	2.33 (1.07–4.88)	0.011	4.06 (1.77–9.35)	<0.001
aCL IgG + aβ2GPI IgG + aPS/PT IgG	8.90 (4.44–19.49)	<0.001	3.77 (1.97–7.19)	<0.001	4.27 (2.32–7.89)	<0.001	3.31 (1.67–6.51)	<0.001
Double (aCL + aβ2GPI)	6.93 (3.87–12.98)	<0.001	3.55 (1.96–6.40)	<0.001	3.68 (2.12–6.39)	<0.001	4.31 (2.36–7.88)	<0.001
Double (aCL + LAC)	6.38 (3.14–14.11)	<0.001	3.96 (1.99–7.87)	<0.001	2.36 (1.20–4.56)	0.002	3.97 (1.94–8.14)	<0.001
Double (aCL + PS/PT)	7.05 (4.03–12.85)	<0.001	4.03 (2.27–7.16)	<0.001	3.28 (1.91–5.60)	<0.001	3.79 (2.11–6.81)	<0.001
Double (aβ2GPI + LAC)	7.11 (3.52–15.67)	<0.001	3.12 (1.57–6.10)	<0.001	3.21 (1.68–6.06)	<0.001	4.47 (2.23–9.03)	<0.001
Double (aβ2GPI + PS/PT)	7.05 (4.21–12.15)	<0.001	3.72 (2.16–6.45)	<0.001	3.84 (2.31–6.40)	<0.001	4.95 (2.86–8.63)	<0.001
Double (PS/PT + LAC)	7.07 (4.02–12.94)	<0.001	3.22 (1.80–5.76)	<0.001	3.79 (2.21–6.49)	<0.001	4.90 (2.75–8.76)	<0.001
Triple (aCL + aβ2GPI + LAC) “Triple Pos”	5.32 (2.58–11.85)	<0.001	3.63 (1.77–7.36)	<0.001	2.21 (1.08–4.38)	0.025	5.29 (2.50–11.35)	<0.001
Triple (aPS/PT + aβ2GPI + LAC)	6.54 (3.22–14.43)	<0.001	3.32 (1.67–6.57)	<0.001	2.82 (1.45–5.38)	<0.001	4.48 (2.20–9.20)	<0.001
Triple (aCL + aβ2GPI + aPS/PT)	6.30 (3.51–11.81)	<0.001	3.83 (2.11–6.97)	<0.001	3.16 (1.79–5.53)	<0.001	4.42 (2.39–8.18)	<0.001
Quadruple(aCL + aβ2GPI + aPS/PT + LAC)	4.83 (2.33–10.80)	<0.001	3.93 (1.90–8.09)	<0.001	1.86 (0.88–3.78)	0.092	5.39 (2.50–11.84)	<0.001

Multivariate models are controlled for age and sex.

SLE patients with a history of stroke. By contrast, LAC was positive in only 38.7% (24/62) of these patients.

Our study demonstrated the significant value aPS/PT IgG testing for evaluation of patients suspected of APS. Notably, 28.6% of the SNAPS group was found to have IgG aPS/PT antibodies. Whether these cases possibly belong to a different subset of APS patients merits further study. We found that 12.7% of SLE patients without thrombosis were positive for IgG aPS/PT. Of particular interest was the finding that 10 of the SLE patients had very high levels of IgG and/or IgM aPS/PT antibodies (116–199 units). Since patients with SLE can develop APS (and vice versa), the presence of the high aPS/PT antibody levels may indicate the future development of APS or may indicate unrecognized APS in these patients. Close follow-up of these patients will continue to determine if the high PS/PT value portended the development of clinical APS.

Our results showed a higher prevalence of aPS/PT compared with similar studies previously reported [29–31], perhaps reflecting the specific make-up of our very well-characterized clinical cohort. Overall, the IgG isotype showed higher specificity and prevalence than IgM for both criteria aPL antibodies and aPS/PT antibodies. We found low positivity (8.2%) for aPS/PT IgM in HC group (6 of 7 positives <50 units). Interestingly, the HC group showed more reactivity than the RA and AS disease groups. The same lot of kits were used for all testing. The HC group had no known morbidities, and we do not currently have an explanation for this observation. Our study showed aβ2GPI IgA displayed better performance than aCL IgM and aβ2GPI IgM in APS, SNAPS and SLE groups, consistent with other studies, indicating that IgA aβ2GPI might be more useful than measuring aCL IgM and aβ2GPI IgM [14, 32, 33].

The association of aPS/PT (IgG or IgM) antibodies with clinical manifestations of APS was statistically significant. In the multivariate analysis, AT was associated with the presence of aPS/PT, IgG aCL, IgG aβ2GPI and their combinations. Venous thrombosis and pregnancy loss were associated with the presence of IgG/IgM aPS/PT, IgG aPS/PT and LAC. Previous studies reported similar data. For example, Vlagea et al. [12] reported that the presence of IgG/IgM aPS/PT, specifically the IgG isotype, had a strong association with VT, and their study confirmed aPS/PT as an independent risk factor for such an event. Moreover, in our study, LAC demonstrated lower diagnostic performance in comparison with aPS/PT antibodies in APS group by ROC analysis. IgG aPS/PT and LAC were significantly associated with both vascular thrombosis and pregnancy morbidity. Compared with LAC, IgG aPS/PT was shown to be an increased risk factor for thrombosis and pregnant morbidity alone or when combined with "criteria" aPLs such as anticardiolipin and/or β2GPI antibody.

The strong correlation between aPS/PT and LAC activity has been reported previously [13, 34]. Despite efforts to improve standardization of LAC assays, accurate detection and intralaboratory reproducibility is still not fully achieved. Because the aPS/PT assay is not affected by anticoagulant treatment, it represents an important alternative to LAC, especially for patients who have already been anticoagulated when the serum is drawn or in those with unknown or uncertain anticoagulation history. During the preparation of our manuscript, the results of a study conducted in Beijing was published with similar observations and which agreed with our conclusion that aPS/PT antibodies could play an important role in the diagnosis of Chinese APS patients [35].

One limitation of our study is that the cohort of patients in our study were very well-characterized APS patients who had been identified and followed by clinical experts in APS. The cohort is therefore more homogeneous than would typically be encountered in a "real-life" patient population, and the high performance achieved by the assays in this study may not be so striking in a more heterogenous general population. Nevertheless, we believe that the strong performance achieved by the aPL assays tested, in particularly aPS/PT, provides support for the value of these assays to identify patients with APS, both those meeting and not meeting the current laboratory criteria for classification of APS. Additional prospective studies will help clarify and validate the utility of the antibody profiles examined in our study.

In summary, the present study supports aPS/PT as a promising biomarker for APS in the Chinese population. The aPS/PT assays showed high diagnostic efficiency for APS, detected patients negative for conventional "criteria markers" (SNAPS patients) who might not receive timely treatment with conventional testing schemes and supported previous studies, indicating that measurement of aPS/PT antibodies could serve as additional serological markers for thrombosis and obstetric complications. Although additional studies are needed, our results add strong support to recommendations for inclusion of aPS/PT antibodies as a new laboratory criteria biomarker for the formal classification of APS [26].

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