Evaluation of the Diagnostic Value of Extra-criteria Antibodies for Antiphospholipid Syndrome Patients in a Chinese Cohort

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Abstract

Objective: Although specific anti-phospholipids antibodies (aPLs) have been used in the diagnosis of the antiphospholipid syndrome (APS) for years, new biomarkers are required to increase its diagnostic as well as risk-predictive power. This study aimed to explore the value of several extra-criteria aPLs in a Chinese cohort. Methods: A total of 312 patients including 100 patients diagnosed with primary APS, 51 with APS secondary to SLE, 71 with SLE, and 90 health controls were recruited. Serum anticardiolipin (aCL) IgG/IgM/IgA, anti-β2-glycoprotein I (aβ2GPI) IgG/IgM/IgA, anti-phosphatidylserine/prothrombin antibodies (aPS/PT) IgG/IgM, anti-annexin A5 antibodies (aAnxV) IgG/IgM were tested using ELISA kits. Results: Totally 30.46% and 6.62% of patients with APS were positive for aCL or aβ2GPI IgA respectively, while 39.07% and 24.50% were positive for aAnxV or aPS/PT for at least one antibodies (IgG or IgM). The addition test of aCL IgA and aAnxV IgM assist in identifying seronegative APS patients, and IgG aANxV was linked with stroke. Conclusion: Detection of aCL IgA, aβ2GPI IgA, aAnxV IgG/M, and aPS/PT IgG/M as biomarker provide additive value in APS diagnosis, and would help in risk prediction for APS patients in medical practice.

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Abstract

Objective: Although specific anti-phospholipids antibodies (aPLs) have been used in the diagnosis of the antiphospholipid syndrome (APS) for years, new biomarkers are required to increase its diagnostic as well as risk-predictive power. This study aimed to explore the value of several extra-criteria aPLs in a Chinese cohort.

Methods: A total of 312 patients including 100 patients diagnosed with primary APS, 51 with APS secondary to SLE, 71 with SLE, and 90 health controls were recruited. Serum anticardiolipin (aCL) IgG/IgM/IgA, anti-β2-glycoprotein I (aβ2GPI) IgG/IgM/IgA, anti-phosphatidylserine/prothrombin antibodies (aPS/PT) IgG/IgM, anti-annexin A5 antibodies (aAnxV) IgG/IgM were tested using ELISA kits.

Results: Totally 30.46% and 6.62% of patients with APS were positive for aCL or aβ2GPI IgA respectively, while 39.07% and 24.50% were positive for aAnxV or aPS/PT for at least one antibodies (IgG or IgM). The addition test of aCL IgA and aAnxV IgM assist in identifying seronegative APS patients, and IgG aANxV was linked with stroke.

Conclusion: Detection of aCL IgA, aβ2GPI IgA, aAnxV IgG/M, and aPS/PT IgG/M as biomarker provide additive value in APS diagnosis, and would help in risk prediction for APS patients in medical practice.

Keywords antiphospholipid syndrome, antiphospholipid antibodies, immunoglobulin A, anti-phosphatidylserine/prothrombin, anti-annexin V

Introduction

The antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by thrombosis and/or pregnancy morbidity with the persistent presence of medium or high titer of antiphospholipid antibodies (aPLs). The golden standard for APS diagnosis is the 2006 APS classification criteria (Sydney criteria), where at least one of the clinical criteria, as well as one of laboratory criteria including lupus anticoagulant (LA), high level of anti-cardiolipin (aCL), anti-β2 glycoprotein-I (aβ2GPI) immunoglobulin isotype G (IgG) or M (IgM), should be present(1). Despite its wide use in clinical practice, patients could remain persistently negative for criteria aPLs yet show typical APS clinical manifestations (defined as seronegative APS, SNAPS(2)), and a broader range of diagnostic biomarkers are required (3). Apart from standard criteria, other extra-criteria clinical and laboratory features have been found associated with APS in numerous studies, which involves heart valve disease, thrombocytopenia, neurological manifestations, anti-CL or anti-β2GpI IgA, anti-phosphatidylserine–prothrombin (aPS/PT) complex, anti-annexin A5 antibodies (aAnxV), etc(4, 5). Besides APS diagnosis, evaluation of non-criteria aPLs could also contribute to prognosis and risk assessment for associated clinical manifestations(6).

More specifically, numerous studies had been conducted to investigate the diagnostic value of aCL/aβ2GpI IgA for APS, which received contradictory results(7). Nevertheless, testing of IgA had been recommended by guidelines when criterial aPLs remained negative(8). In addition, aAnxV and aPS/PT are receiving continuous attention in recent years. AnxV is a phospholipid-binding protein highly expressed in vascular endothelial cells. It could bind tightly to exposed anionic phospholipids and assemble into a shield, which may prevent phospholipid-dependent coagulation reaction(9, 10). In a systemic review, AnxV resistance has been observed and analyzed to have a higher prevalence in APS compared to disease controls(11) and has been reported to be linked with hypercoagulable state as well as obstetric complications in APS patients(12, 13). Furthermore, its anticoagulant activity was reduced by plasmas of patients with APS and thromboembolism(14), and loss of maternal aAnxV increased the chance of placental platelet thrombosis and fetal loss(15). However, other studies found no significant association between thrombotic event or adverse pregnancy manifestations(16, 17).

Prothrombin is another phospholipid-binding protein which forms complex and is often co-detected of antibodies together with phosphatidylserine (aPS/PT). An international multi-centre study confirmed the contribution of aPS/PT IgG in APS diagnosis IgG(18). Concerning its relation with clinical features such as thrombotic events or obstetric complications, conflicting results had been shown and confirmation is still needed(19, 20). Nevertheless, numerous studies have indicated a strong correlation between aPS/PT and

LA(21, 22). In addition, a higher level of aPS/PT was observed to be associated with high-risk "triple positive" patients (LA+, aCL IgG and/or IgM +, and aβ2GPI IgG and/or IgM+)(23), and may also add value to identification of SNAPS(3).

Studies design, including detection method, patient stratification, population heterogeneity, etc., may lead to contradictory results in different studies. Regarding the Chinese population, a previous study indicated an increase of both IgG and IgM aAnxV in primary APS patients and APS associated with other diseases. Significant associations were also observed between IgG aANxV and thrombotic events(24). Additionally, assessment of the diagnostic performance of aPS/PT revealed a significant correlation between thrombotic events and pregnancy loss with IgG aPS/PT(25, 26), which was confirmed by a recent study(27). Concerning aCL/aβ2GpI IgA, a study recently conducted by us in a large Chinese population revealed little added diagnostic value(28). Few studies have explored all of the above extra-criteria autoantibodies in the same patient groups, and their relations with more detailed clinical manifestations still need investigation. This study focused on evaluating the additive diagnostic value of aCL/aβ2GpI IgA, IgG and IgM for aANxV or aPS/PT to standard aPLs in a Chinese cohort. Correlation with clinical features including thrombotic events, obstetric complications, as well as microangiopathy was also explored.

Patients and methods

Patients recruitment

This was a single-center, prospective cohort study conducted at Peking Union Medical College Hospital (PUMCH) from May 2017 to January 2020. A total of 312 consecutive patients were included in this study, of which 100 patients had been diagnosed with primary APS (PAPS group), 51 with APS secondary to SLE (SAPS group), 71 with SLE (SLE group), and 90 health controls (HC group). Diagnosis of APS was defined by clinicians according to the 2006 Sydney revised classification criteria. Sera samples were collected and immediately profiled of aPL antibodies at the Key Laboratory of Department of Rheumatology, Peking Union Medical College Hospital (PUMCH). Besides aPL serology, clinical manifestations were recorded for PAPS, SAPS, and SLE groups, including thrombosis (arterial or venous), pregnancy morbidity, microangiography (deep venous thrombosis, pulmonary embolism, etc.), and history of adverse pregnancy. For HC group, only aPL serology information was present. The study was approved by ethics committee at PUMCH and fulfilled the ethical guidelines of the declaration of Helsinki. All subjects gave written informed consent.

Laboratory tests

IgG, IgM, and IgA isotypes of aCL and aβ2GPI, IgG and IgM isotypes of aPS/PT and aAnxV were analyzed with AESKULISA® ELISA Test Kits provided by Aesku. Diagnostics GmbH & Co. KG (Wendelsheim, Germany). Cut-off value was defined as 18 U/mL as recommended by the manufacturer. Lupus anticoagulant was detected and evaluated at the Key Laboratory according to the ISTH recommendations measuring Dilute Russell viper venom time (dRVVT)/activated partial thromboplastin time (>1.20 as positive).

Statistical analysis

Statistical analysis was performed using SPSS 26.0 or R (version 3.6.2). The $\chi 2$ test or Fisher's exact test was used for comparison of categorical variables, and Wilcoxon test was used for continuous variables after normality was explored with the Shapiro-Wilk test. Sensitivities, specificities, and accuracies in APS diagnosis were compared in the McNemar test. Youden Index, positive and negative predictive values (PPV and NPV), and odds ratio (OR) with 95% confidence interval (95% CI) were also shown. Receiver operating characteristic (ROC) curves of individual aPL as well as logistic regression analysis of aPLs profile were used to calculate the area under the curve (AUC), with 95% CI shown. Associations between aPL isotype positivity and clinical manifestation in patients with APS were explored and displayed in 95% CI. Two-tailed values of P less than 0.05 were considered statistically significant.

Results

Patient characteristics

Among 151 APS patients, there were 63(63.0%) females for PAPS, 45(88.2%) for SAPS, and the mean age for each were 36.3 and 32.9 years (Table 1). The mean age was 30.1 years in SLE group, of which 61(85.9%) were female, while HC group had 41(45.6%) female and a mean age of 43.4. Clinical manifestations were recorded for both APS and SLE patients and were selectively shown. Thrombosis was most commonly present, with 80(80.0%) for PAPS and 74.5% for SAPS, but not in SLE group. Patients were recorded for history of arterial or venous thrombotic events, pregnancy morbidity, microangiopathy, history of adverse pregnancy, and LA. Of all the clinical manifestations, prevalence of adverse pregnancy history was significant different between PAPS and SAPS group(χ^2=3.922,P =0.048).

Predictive power of aPLs in APS diagnosis

The diagnostic power of aPLs positivity (>18 U/ml) was evaluated for sensitivity, specificity, accuracy, Youden Index, PPV, NPV, and ORs in APS diagnosis from HC group in table 2. For IgA, the sensitivity and accuracy of the combination of aCL IgG, IgM, or IgA were significantly higher than that of aCL IgG or IgM (p<0.001), while specificity was lower (p=0.031). A similar result was observed for aCL or aB2GpI IgG or IgM or IgA compared to aCL or aB2GpI IgG or IgM. As for aAnxV, the sensitivity and accuracy of aAnxV IgG or IgM was significantly higher than that of aB2GpI IgG or IgM (P<0.001). In addition, a combination of aCL, aβ2GpI, or aAnxV IgG or IgM had significantly higher sensitivity (p=0.016) compared to that of aCL or aβ2GpI IgG or IgM.

As illustrated in figure 1, ROC curves were applied to evaluate the predictive value of aPLs or their combined positivity. Among individual aPLs, aβ2GP1 IgG (0.915), aCL IgA (0.853), aCL IgM (0.767), and aAnxV IgG (0.728) had the largest AUC values. Adding IgA, aAnxV or aPS/PT IgG or IgM to aCL or aβ2GpI IgG or IgM would both increase AUC (0.927, 0.951, and 0.936 compared to 0.925).

Cross-positivity analysis for four aPLs in APS patients

Among 151 APS patients, cross positivity of IgG, IgM, or IgA for aCL or aβ2GpI (a and b), as well as IgG or IgM for each of the four aPLs (c and d) were demonstrated with Venn diagram in figure 2. For patients positive for aCL, 16 were positive only for IgA. Concerning IgG isotype, aCL and aAnxV IgG were most often positive among APS patients. As for IgM isotype, there were 12 (7.9%) patients who were test positive only for aAnxV, and 4 (2.6%) were positive only for aPS/PT.

The number of patients positive only for each of the five non-criterial aPLs was also calculated among SPAPS and SNAPS patients (not shown). Totally 12 patients for aCL IgA, 9 for aAnxV IgG, 5 for aAnxV IgM, 3 for aPS/PT IgM, 1 for aPS/PT IgG, and 1 for aβ2GpI IgA were observed. On the other hand, among all 14 SNAPS patients, 7(50%) were positive for extra criteria aPLs. ACL IgA and aAnxV IgM each identified 3 of these patients individually, while 1 patient was positive for aPS/PT IgM and aβ2GpI IgA.

Distribution of antiphospholipid antibodies

The distribution of all criterial or non-criterial aPLs among different patient groups was shown in Figure 3. Levels of aPLs were calculated with (log(test value + 2)U/ml). The results of primary or secondary APS were compared to other groups. No significant difference was observed between primary and secondary APS, except for aCL IgM (p = 0.029) and aβ2GpI IgA (p = 0.043). Compared to HC, levels of IgG and IgA were significantly higher for four aPLs in both PAPS and SAPS group. However, IgM results varied for different aPLs.

Clinical manifestations of different aPLs in APS patients

Correlations between different aPLs and LA or clinical manifestations were shown with odds ratios in table 3. Presence of LA was significantly associated with IgG of aCL (ORs 9.0, 95% CI 2.6-31.0), aβ2GPI (ORs 14.1, 95% CI 1.9-107.2), aPS/PT (ORs 4.7, 95% CI 1.1-21.2), and aAnxV (ORs 21.5, 95% CI 2.8-163.0). Among all microangiopathy manifestations, stroke was significantly associated with aβ2GPI IgG (ORs 4.7, 95% CI 1.2–18.5) as well as aPS/PT IgG (ORs 6.5, 95% CI 1.6-25.9). Additionally, aPS/PT IgM was reversely associated with pregnancy loss in women (ORs 0.6, 95% CI 0.5-0.7).

Discussion

APS is an autoimmune disease featuring thrombosis and/or pregnancy morbidity which may lead to severe consequences. Detection of aCL and aβ2GPI as the golden standard in APS diagnosis is not satisfactory in the clinical scenario, and various potential aPLs have been extensively explored.

In this study, the diagnostic value of IgA for aCL or aβ2GPI, IgG/IgM for aANxV or aPS/PT was evaluated in APS patients. In brief, 45.70% and 6.62% of patients with APS were positive for aCL or aβ2GPI IgA respectively, while 30.46% and 24.50% were positive for aAnxV or aPS/PT for at least one antibodies (IgG or IgM). Adding IgA to criterial aPLs could increase the sensitivity in APS diagnosis. Detection of aANxV or aPS/PT, especially aAnxV IgG, could add value to diagnosis. IgG of aANxV or aPS/PT was significantly associated with LA, and IgG aANxV was linked with stroke.

Analysis of the predictive power indicates that although aCL IgA had relatively low specificity, adding IgA to aCL IgG or IgM/aCL or aβ2GpI IgG or IgM test could increase test sensitivity ($P < 0.001$). The sensitivity (39.07% compared to 29.14%, $P < 0.001$). and accuracy (60.58% compared to 55.19%, $P = 0.007$) of aAnxV IgG or IgM were both significantly higher than that of aβ2GPI IgG or IgM. Moreover, combination of aCL, aβ2GpI, or aAnxV IgG or IgM had significantly higher sensitivity (47.7% compared to 43.0%, $P = 0.016$) than that of aCL or aβ2GpI IgG or IgM. Statistic results suggested that adding aAnxV IgG or IgM to aCL or aβ2GpI IgG or IgM would both increase diagnostic value besides criterial antibodies. Meanwhile, there was no significant decrease in specificity (96.67%).

The result was further illustrated with ROC curves for each aPL and their combination. AUC of ACL IgA and aAnxV IgG ranked second and third (0.853 and 0.728) among individual aPLs. Addition of IgA, aAnxV or aPS/PT to aCL or aβ2GpI IgG or IgM would all increase their diagnostic power.

Venn diagram indicated the additive value of new aPLs from another perspective. Positive only for IgA isotype could point out an extra number of patients for both aCL (16, 10.6%) and aβ2GPI (4, 2.6%). Additionally, the number of patients positive for aAnxV IgG, aAnxV IgM, and aPS/PT IgM outperformed those of aβ2GPI, indicating their importance in APS clinical diagnosis. The result suggested that additional tests for extra criteria aPLs could provide unique value in the identification of SNAPS patients.

Besides predictive power, distribution, and comparison of aPLs among different patient groups were also examined. Between PAPS and SAPS, little significant difference was observed except for aCL IgM ($p = 0.029$) and aβ2GpI IgA ($p = 0.043$). Between PAPS and SLE, significantly higher titer of IgM aCL, IgA aCL, IgM aPS/PT, IgG AnxV, and IgM AnxV was observed ($p<0.001$). As for SAPS and SLE, only IgM aPS/PT showed a significant difference ($p = 0.015$). The results implied that both criterial and non-criterial aPLs had difficulty in distinguishing APS from SLE or APS secondary to SLE. Indeed, baseline information suggested little difference between PAPS and SAPS patients in age and most clinical manifestations (Table 1). It had been estimated in previous studies that around 40% of patients with SLE have aPL, and APS may develop in up to 50-70% of patients with both SLE and aPL(29). Nevertheless, levels of IgG for four aPLs were significantly higher in both PAPS and SAPS group compared to HC, which suggested their diagnostic value.

Finally, the relationship between aPLs and related clinical manifestations was calculated. In this study, no significant association was found between aPLs with any thrombotic events, which was contradictory with results from some previous studies conducted in the Chinese population (24-26). Concerning obstetric complication, aPS/PT IgM was reversely associated with pregnancy loss in women (ORs 0.6, 95% CI 0.5-0.7), which also showed conflicting results (25, 30, 31). For aAnxV, similar to a previous study, no significant relationship was observed(24). The different results might be due to the detection system. ELISA was chosen in this study, and the cut-off value provided by the manufacturer (18 U/ml for all the aPLs) may not reflect real aPL distribution in local population. Indeed, as illustrated in figure 2, 31 patients were negative for all IgG, while as many as 118 patients were negative for all IgM. It could be more suitable if 99th percentile strategy was adopted first to identify cut-off points for each aPLs.

Additionally, the relationship between aAnxV and aPS/PT IgG and LA was confirmed in our studies, and LA was found to be associated with IgG of all four aPLs. Regarding microangiopathy, a series of manifestations had been recorded for the patients (stroke, deep venous thrombosis, pulmonary embolism, etc.), and significant relationship with aPLs (aβ2GPI IgG and aPS/PT IgG) was present for stroke. Previous review has estimated an aPL positivity of 17% in patients with juvenile stroke (<50 years of age) (32). Although detection of aPS/PT alone may have less diagnostic value, it would still be valuable in risk prediction for and prevention of adverse clinical events.

This study has some limitations. Compared to similar studies, the sensitivity for autoantibodies is not very high, which may influence the results of sequence comparison. Since different detection methods and manufacturers vary greatly in antibody measurement, contradictory results could arise(33). In the future, quantitative/semi-quantitative detection methods such as chemiluminescence analysis (CLIA) could be applied to reduce systemic detection error. In addition, both patients and health individual involved in the study were relatively homogenous, and may not reflect real-life condition. A larger sample size and inclusion of patients with a wider range of associated diseases or clinical features could further complement the study.

Conclusion

In conclusion, detection of aCL IgA, aβ2GPI IgA, aAnxV IgG/M, and aPS/PT IgG/M as biomarker provide additive value in APS diagnosis, especially aCL IgA and aAnxV IgG. Detecting aCL IgA and aAnxV IgM assist in identifying seronegative APS patients. IgG of aANxV or aPS/PT was significantly associated with LA, and IgG aANxV was linked with stroke, which would assist in risk prediction for APS patients in medical practice.

Author Contributions

All authors were involved in the design of this study. CH, SL, ZX, HY, HJ, and JZ contributed to the collection of blood samples and other experimental procedures. YS and WQ were involved in data collection and pre-processing. CH and SL analyzed the data and wrote the manuscript. JZ, QW, XT, ML, and YZ contributed to the recruitment of patients and evaluation of clinical data.

Conflict of Interest

All authors declare no conflicts of interest.

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Table 1. Demographic and clinical variables of subjects (n=312).

	APS (151)	APS (151)	APS (151)	SLE (71)	SLE (71)
	Primary (100)	Primary (100)	Secondary (51)		
Gender (female/male)	63/37	45/6	45/6	61/10	41/49

	APS (151)	APS (151)	APS (151)	SLE (71)	SLE (71)
Mean age (years ± SD)	36.3±12.1	32.9±10.2	32.9±10.2	30.1±8.2	43.4±12.
Clinical manifestations					
Thrombosis, n (%)	80(80.0%)	38(74.5%)	38(74.5%)	0	NA
Pregnancy morbidity, n (%)	33(33.0%)	16(31.4%)	16(31.4%)	0	NA
Thrombosis + pregnancy morbidity, n (%)	13(13%)	3(5.9%)	3(5.9%)	0	NA
LA, n (%)	73(73.0%)	44(86.3%)	44(86.3%)	17(23.9%)	NA
History of arterial thrombosis, n (%)	43(43.0%)	21(41.2%)	21(41.2%)	0	NA
Stroke, n (%)	4(4.0%)	2(3.9%)	2(3.9%)	0	NA
Coronary heart disease, n (%)	9(9.0%)	2(3.8%)	2(3.8%)	0	NA
Eye involvement, n(%)	3(3.0%)	1(2.0%)	1(2.0%)		
Lower limb artery occlusion, n (%)	1(1.0%)	0	0	0	NA
History of venous thrombosis, n (%)	47(47.0%)	24(47.1%)	24(47.1%)	0	NA
Deep vein thrombosis, n (%)	19(19.0%)	7(13.7%)	7(13.7%)	0	NA
Pulmonary embolism, n (%)	19(19.0%)	2(3.9%)	2(3.9%)	0	NA
Upper limb vein thrombosis, n (%)	0	1(2.0%)	1(2.0%)	0	NA
Renal vein thrombosis, n (%)	1(1.0%)	0	0	0	NA
Portal vein thrombosis, n (%)	4(4.0%)	1(2.0%)	1(2.0%)	0	NA
Cerebral venous and sinus thrombosis, n (%)	3(3.0%)	1(2.0%)	1(2.0%)	0	NA
Central retinal venous occlusion, n (%)	1(1.0%)	0	0	0	NA
Microangiopathy, n (%)	57(57%)	24(47.1%)	24(47.1%)	0	NA
TP, n (%)	38(38%)	*28(54.9%)	*28(54.9%)	21(29.6%)	NA
Heat valve disease, n (%)	0	6(11.8%)	6(11.8%)	0	NA
Non-stroke CNS manifestations, n (%)	4(4.0%)	4(7.8%)	4(7.8%)	0	NA
Antiphospholipid syndrome nephropathy, n (%)	6(6.0%)	2(3.8%)	2(3.8%)	0	NA
Autoimmune hemolytic anemia, n (%)	1(1.0%)	5(9.8%)	5(9.8%)	0	NA
Thrombotic Microangiopathy, n (%)	0	1(2.0%)	1(2.0%)	0	NA
Hemolytic uremic syndrome, n (%)	1(1.0%)	0	0	0	NA
History of adverse pregnancy, n (%)	37(37%)	20(39.2%)	20(39.2%)	4(5.6%)	NA
Early fetal loss (<10 weeks), n (%)	12(12.0%)	8(15.7%)	8(15.7%)	4(5.6%)	NA
Late fetal loss (10-28 weeks), n (%)	19(19.0%)	12(23.5%)	12(23.5%)	0	NA
Placental insufficiency, n (%)	14(14.0%)	7(13.7%)	7(13.7%)	0	NA

*P=0.048, significant different from primary APS

Table 2. The predictive value of different aPLs in APS diagnosis

	Sensitivity (%)	Specificity (%)	Accuracy (%)	Youden Index	PPV (%)	NPV (%)	OR (95% CI)
aCL IgG	37.09	100.00	60.58	0.371	100.00	48.65	[?]
aCL IgM	8.61	97.78	41.90	0.064	86.67	38.94	4.15(0.91-18.81)
aCL IgG or IgM	41.06	97.78	62.24	0.389	96.88	49.72	30.65(7.27-129.20)
aβ2GpI IgG	23.18	100.00	51.86	0.232	100.00	43.69	[?]
aβ2GpI IgM	7.95	98.89	41.91	0.068	92.31	39.04	7.68(0.98-60.12)

	Sensitivity (%)	Specificity (%)	Accuracy (%)	Youden Index	PPV (%)	NPV (%)	OR (95% CI)
aβ2GpI IgG or IgM	29.14	98.89	55.19	0.28	97.78	45.41	36.60(4.94-270.96)
aCL or aB2GpI IgG or IgM	43.05	97.78	63.48	0.408	97.01	50.57	33.26(7.89-140.10)
aCL IgA	30.46	92.22	53.53	1.2268	86.79	44.15	5.19(2.23-12.10)
aβ2GpI IgA	6.62	98.89	41.08	1.0551	90.91	38.70	6.31(0.79-50.16)
aCL IgG or IgM or IgA	51.66	91.11	66.39	1.4277	90.70	52.90	10.95(4.96-24.21)
aβ2GpI IgG or IgM or IgA	31.79	97.78	56.43	1.2957	96.00	46.07	20.51(4.85-86.79)
aCL or aB2GpI IgG or IgM or IgA	53.64	91.11	67.63		91.01	53.95	11.86(5.37-26.22)
P1	**<0.001**	**0.031**	0.052				
P2	0.125	1.000	0.375				
P3	**<0.001**	**0.031**	0.052				
aPS/PT IgG	18.54	96.67	47.72	0.152	90.32	41.43	6.60(1.95-22.40)
aPS/PT IgM	7.28	98.89	41.49	0.062	91.67	38.86	6.99(0.89-55.10)
aPS/PT IgG or IgM	24.50	95.56	51.03	0.201	90.24	43.00	6.99(2.40-20.32)
aCL, aB2GpI, or aPS/PT IgG or IgM	45.70	94.44	63.90	0.401	93.24	50.90	14.31(5.49-37.25)
P1'	**<0.001**	0.625	**<0.001**				
P2'	0.167	0.375	0.064				
P3'	0.125	0.250	1.000				
aAnxV IgG	30.46	100.00	56.43	0.305	100.00	46.15	[?]
aAnxV IgM	16.56	96.67	46.47	0.133	89.29	40.85	5.75(1.69-19.70)
aAnxV IgG or IgM	39.07	96.67	60.58	0.358	95.16	48.60	18.60(5.62-61.53)

	Sensitivity (%)	Specificity (%)	Accuracy (%)	Youden Index	PPV (%)	NPV (%)	OR (95% CI)
aCL, aβ2GpI, or aAnxV IgG or IgM	47.68	96.67	65.98	0.444	96.00	52.41	26.43(8.01-87.26)
P1"	0.648	1.000	0.503				
P2"	**<0.001**	0.500	**0.007**				
P3"	**0.016**	1.000	0.070				

PPV, positive predictive value; NPV, negative predictive value; OR, odds ratio; CI, confidence interval.P-values of sensitivity, specificity, and accuracy are calculated with McNemar test.

P_1: Comparison of result of aCl IgG or IgM or IgA to aCL IgG or IgM;P_2: Comparison of result of aβ2GpI IgG or IgM or IgA to aβ2GpI IgG or IgM;P_3: Comparison of result of aCL or aβ2GpI IgG or IgM or IgA to aCL or aβ2GpI IgG or IgM;$P_{1'}$: Comparison of result of aPS/PT IgG or IgM to aCL IgG or IgM;$P_{2'}$: Comparison of result of aPS/PT IgG or IgM to aβ2GpI IgG or IgM;$P_{3'}$: Comparison of result of aCL, aβ2GpI, or aPS/PT IgG or IgM to aCL or aβ2GpI IgG or IgM;P_1": Comparison of result of aAnxV IgG or IgM to aCL IgG or IgM; P_2": Comparison of result of aAnxV IgG or IgM to aβ2GpI IgG or IgM;P_3": Comparison of result of aCL, aB2GpI, or aAnxV IgG or IgM to aCL or aβ2GpI IgG or IgM;Odds ratios (ORs) with 95% confidence intervals (CIs) are shown.

Table 3 Correlations between different aPLs and clinical manifestations among APS patients (n=151)

	Thrombosis	Arterial thrombosis	Venous thrombosis	Venous thrombosis	Pregnancy morbidity	Preg
aCL IgG	1.3(0.6-3.1)	1.6(0.8-3.2)	0.8(0.4-1.6)	1.2(0.6-2.7)	1.2(0.6-2.7)	1.0(
aCL IgM	0.6(0.2-1.9)	0.8(0.3-2.7)	0.7(0.2-2.3)	1.0(0.3-3.5)	1.0(0.3-3.5)	0.5(
aCL IgA	0.6(0.2-1.2)	0.8(0.4-1.5)	0.8(0.4-1.5)	0.9(0.4-1.9)	0.9(0.4-1.9)	1.5(
aβ2GPI IgG	1.7(0.6-5.0)	1.6(0.8-3.4)	1.0(0.5-2.1)	1.1(0.5-2.9)	1.1(0.5-2.9)	0.9(
aβ2GPI IgM	1.3(0.3-6.5)	0.4(0.1-1.6)	2.5(0.7-8.6)	0.9(0.2-4.2)	0.9(0.2-4.2)	0.6(
aβ2GPI IgA	1.1(0.2-5.2)	0.9(0.2-3.3)	1.8(0.5-6.7)	1.0(1.0-1.1)	1.0(1.0-1.1)	0.7(
aPS/PT IgG	1.3(0.4-3.6)	1.7(0.8-4.0)	0.7(0.3-1.6)	1.3(0.5-3.4)	1.3(0.5-3.4)	0.9(
aPS/PT IgM	1.2(0.2-5.8)	0.8(0.2-2.7)	2.1(0.6-7.6)	0.4(0.1-2.0)	0.4(0.1-2.0)	**0.6***
aAnxV IgG	1.7(0.7-4.3)	1.8(0.9-3.6)	0.8(0.4-1.7)	1.0(0.4-2.3)	1.0(0.4-2.3)	0.7(
aAnxV IgM	1.1(0.4-3.1)	1.3(0.6-3.1)	0.7(0.3-1.8)	1.0(0.3-2.6)	1.0(0.3-2.6)	0.9(

Odds ratios (ORs) with 95% confidence intervals (CIs) are shown. *P <0.05)

Figure 1. Comparison of receiver operating characteristic (ROC) curves and area under the curve (AUC). ORs with 95% CIs are shown.

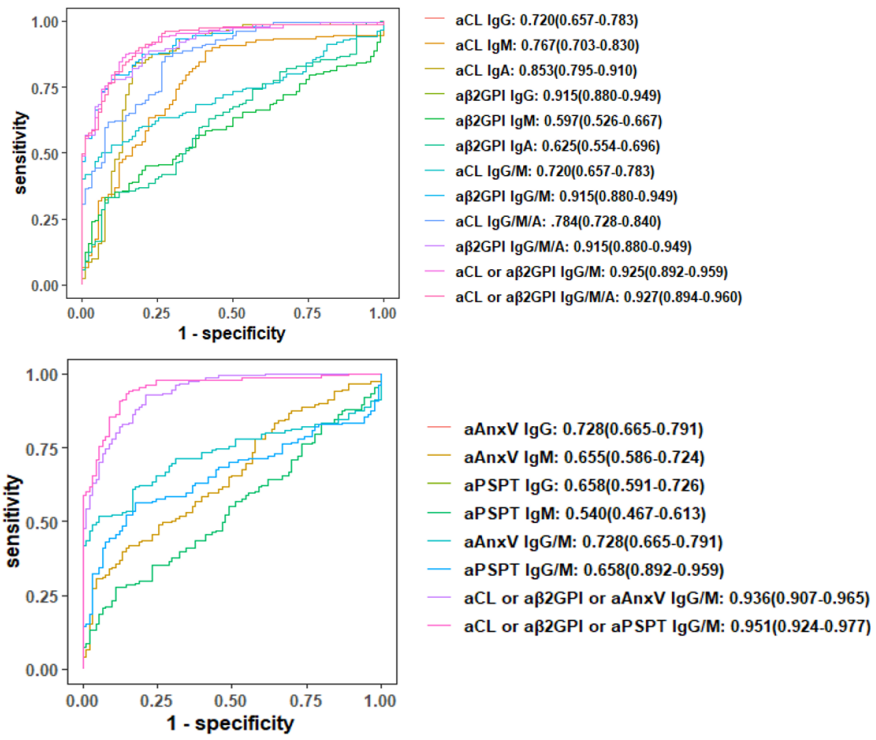
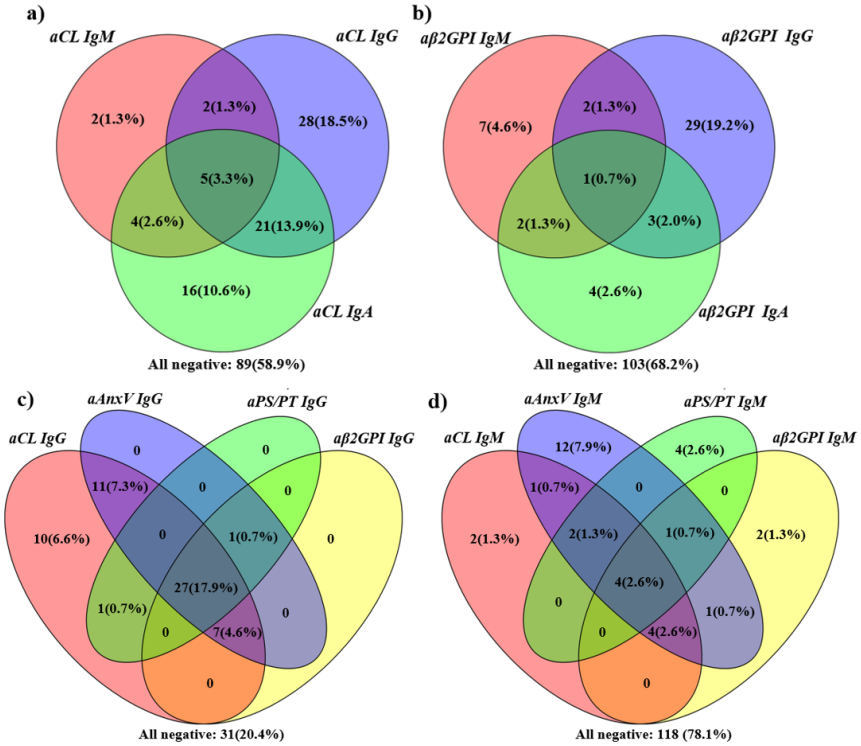
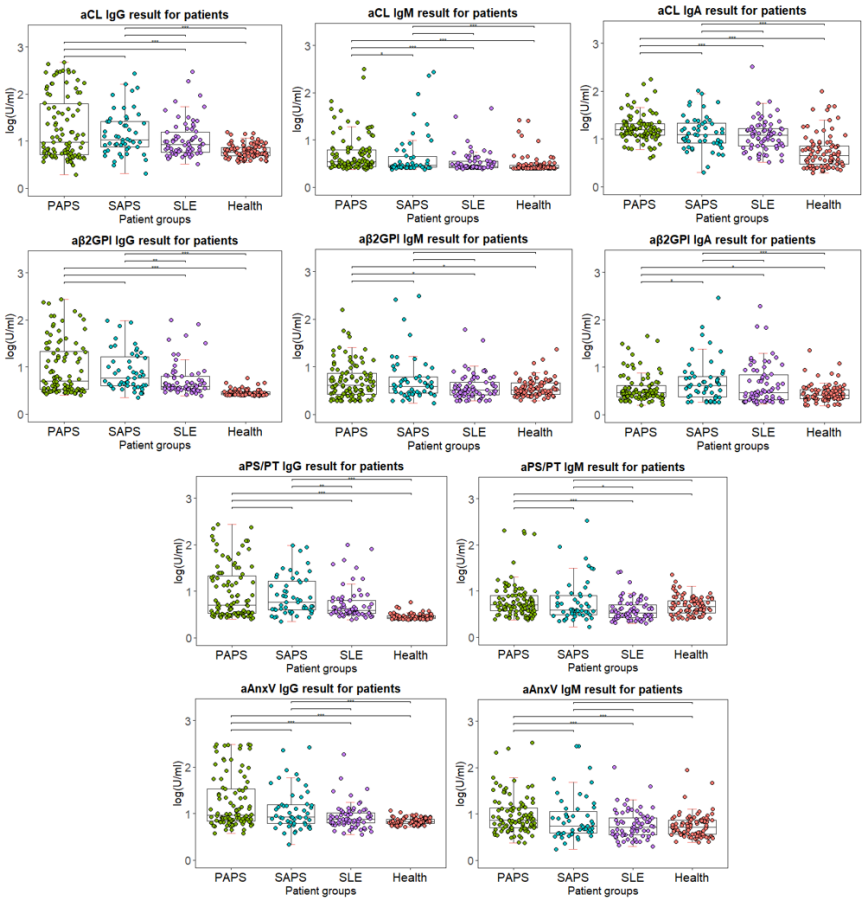


Figure2. Venn diagram of aPLs cross positivity analysis in APS group (n=151)



a) cross positivity for aCL; b) cross positivity for aβ2GpI; c) cross positivity for IgG; d) cross positivity for IgM

Figure 3. Distribution of IgG and IgM for four antibodies among different patient groups



Test results are calculated using lg(test value + 2), in order for the value to be shown in positive number. Wilcox's test is conducted comparing primary or secondary APS results to other patient groups. *p < 0.05. **p < 0.01, ***p < 0.001, NS: not significant.