Clinica Chimica Acta 495 (2019) 205–209

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Clinical performance of non-criteria antibodies to phospholipids in Chinese patients with antiphospholipid syndrome

Shulan Zhang[a], Ziyan Wu[a], Wen Zhang[a], Fengchun Zhang[a], Yongzhe Li[b,*], Yudong Liu[c,*]

[a] Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Beijing, China
[b] Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[c] Department of Clinical Laboratory, Peking University People's Hospital, Beijing, China

ARTICLE INFO

Keywords:
Antiphospholipid syndrome (APS)
Antiphospholipid antibodies (aPLs)
Non-criteria aPLs
Thrombosis
Seronegative APS

ABSTRACT

Background: Increasing evidence suggests the role of non-criteria aPLs as important supplements to the current criteria aPLs in APS. In this study, we evaluated the clinical performance of a panel of non-criteria antibodies to phospholipid antigens, including, phosphatidylserine (aPS), phosphatidylinositol (aPI), sphingomyelin (aSM), phosphatidylcholine (aPC) and phosphatidylethanolamine (aPE) in a well-defined Chinese APS cohort.

Methods: A total of 229 subjects were tested, including 86 patients with APS, 104 disease controls (DCs) and 39 healthy controls (HCs). Serum IgG/IgM aCL, IgG/IgM aβ2GP1, IgG/IgM aPS, IgG/IgM aPI, IgG/IgM aSM, IgG/IgM aPC, and IgG/IgM aPE were tested by ELISA.

Results: The presence of aPE, aPS, aPI, aPC, and aSM in patients with APS and Disease Controls were 8.1% (7/86) and 1.0% (1/104), 37.2% (32/86) and 9.6% (10/104), 50.0% (43/86) and 8.7% (9/104), 23.3% (20/86) and 1.0% (1/104), and 18.6% (16/86) and 1.9% (2/104), respectively. In criteria aPLs, aCL IgG demonstrated the highest positive likelihood ratio (LR+) of 35.75, followed by LA (LR+ of 13.51) and aCL IgM (LR+ of 11.64). In non-criteria aPLs, aPC IgG demonstrated the highest LR+ of 24.94 followed by aSM IgM (LR+ of 14.97). Importantly, the non-criteria aPLs were detected in 18.8% (3/16) of seronegative APS patients. The criteria aPLs, including LA, IgG aCL and IgG aβ2GPI, were significantly correlated with both arterial thrombosis and venous thrombosis, while the non-criteria aPLs, including IgG aPS, IgM aPS, IgG aPI and IgG aPC were significantly associated with arterial thrombosis but not venous thrombosis.

Conclusions: In summary, our findings indicate that those non-criteria aPLs may be particularly helpful for patients in whom APS is highly suspected, but conventional aPLs are repeatedly negative as well as for predicting APS patients with arterial thrombosis.

1. Introduction

Antiphospholipid syndrome (APS) is a thrombophilic autoimmune disorder characterized by the presence of a variety of autoantibodies against phospholipids (PL) and/or PL-binding proteins (antiphospholipid antibodies, aPLs) and a wide series of clinical manifestations, from recurrent arterial and/or venous thrombotic events to recurrent fetal loss [1–6]. Although the 2006 revised international diagnostic criteria for APS recommend lupus anticoagulant (LA), anti-cardiolipin (aCL) and anti-β2-glycoprotein 1 (aβ2GP1) antibodies for routine tests [7], increasing evidence has highlighted the role of non-criteria aPLs as important diagnostic supplements to the current criteria aPLs in the diagnosis of APS, especially in identifying patients with clinical evidence of APS, but seronegative for the criteria markers (seronegative APS), as well as those at high risk of thrombosis or recurrent fetal loss [4,8,9].

In addition to aCL, one of the diagnostic criteria aPLs, a number of autoantibodies targeting phospholipids, including phosphatidylserine (aPS), phosphatidylinositol (aPI), sphingomyelin (aSM), phosphatidylcholine (aPC) and phosphatidylethanolamine (aPE) have been identified in patients with APS [10,11]. Over the past two decades, a number of home-made or commercial ELISA kits have been developed for detecting those non-criteria aPLs. Despite tremendous efforts has been made to elucidate their clinical relevance in APS, the results were inconsistent, especially regarding whether those aPLs could predict patients with at high risk of thrombosis or recurrent fetal loss [12–19].

A possible explanation for those discrepancies mentioned above is the differences in genetic/environmental factors. To our knowledge,

* Corresponding authors.
E-mail addresses: LiYZ@pumch.cn (Y. Li), yudongliu1983@126.com (Y. Liu).

https://doi.org/10.1016/j.cca.2019.04.065
Received 5 December 2018; Received in revised form 10 March 2019; Accepted 15 April 2019
Available online 16 April 2019

Table 1
Demographic, clinical characteristic, and aPL profiles in patients with APS and controls.

	APS (n = 86)	DC (n = 104)	HC (n = 39)	p-Value***
Gender (female/male)	67/17	81/23	14/25	0.997
Median age at study (max, min)	34 (9, 86)	37 (12, 85)	39 (25, 65)	0.053
Arterial thrombosis, % (n)	33.3(28)	5.8 (6)	0.0 (0)	< 0.001
Venous thrombosis, % (n)	47.6 (40)	26.0 (27)	0.0 (0)	0.003
Obstetric complications*, % (n)	50.0 (28/56)	46.6 (34/73)	0.0 (0/12)	0.372
aCL, % (n) **	58.1 (50)	3.8 (4)	0.0 (0)	< 0.001
aβ2GPI, % (n)	66.3 (57)	9.6 (10)	2.6 (1)	< 0.001
LA, % (n)	75.6 (65)	7.7 (8)	0.0 (0)	< 0.001
aPE, % (n)	8.1 (7)	1.0 (1)	0.0 (0)	0.046
aPS, % (n)	37.2 (32)	9.6 (10)	0.0 (0)	< 0.001
aPI, % (n)	50.0 (43)	8.7 (9)	0.0 (0)	< 0.001
aPC, % (n)	23.3 (20)	1.0 (1)	0.0 (0)	< 0.001
aSM, % (n)	18.6 (16)	1.9 (2)	2.6 (1)	< 0.001

*Percentage among females with reproductive history; **Either IgG or IgM; ***p-Value was calculated between APS patients and diseases controls. APS, antiphospholipid syndrome; DC, disease control; HC, health controls, aCL, anticardiolipin antibodies, aβ2GP1, anti-β2-glycoprotein 1 antibodies, LA, lupus anticoagulants; aPE, antiphosphatidylethanolamine, aPS, anti-phosphatidylserine, aPI, antiphosphatidylinositol; aPC, antiphosphatidylcholine; aSM antisphingomyelin.

few, if any, studies have assessed the clinical relevance of those aPLs in Chinese patients with APS. Moreover, little is known regarding the clinical utility of aSM in the diagnosis of APS and in stratifying APS-related clinical manifestations. This is an important endeavor as this information will greatly enhance our appreciation on the clinical utility of those non-criteria aPLs, particularly in their prognostic value for thrombosis and pregnancy complications. In this study, we evaluated the clinical performance of a series of non-criteria autoantibodies targeting phospholipids, including aPS, aPI, aSM, aPC and aPE in a well-defined Chinese APS cohort.

2. Materials and methods

2.1. Subjects and specimen collections

Sera from a total of 229 subjects were collected and analyzed. The serum was collected during the first visit of a patient who was tested for aPLs. The serum was then aliquoted and stored in −80°C. The patient was followed up until the final diagnosis of APS was made. Detailed demographics and clinical characteristics from patients have been previously published [20,21]. Specifically, the subjects included 86 patients with APS, 104 patients as disease controls (including 30 patients with non-APS thrombosis, 32 patients with non-APS pregnancy-related morbidity (PRM), and 42 patients with systemic lupus erythematosus (SLE)), and 39 healthy controls (HC). Patients with non-APS thrombosis included 10 patients with deep vein thrombosis, 7 patients with cerebral ischemic stroke, 5 patients with Renal artery thrombosis, 4 patients with pulmonary embolism, 2 patients with portal vein thrombosis, 1 patient with splenic infarction and 1 patient with cerebral venous sinus thrombosis. Patients with non-APS PRM included 23 patients with unknown reason for recurrent miscarriage, 4 patients with uterine fibroids, 2 patients with endometriosis, 1 patient with polycystic ovary syndrome, 1 patient with severe pre-eclampsia and 1 patient with gestational hypertension and severe pre-eclampsia. APS was diagnosed according to 2006 Sydney revised Sapporo guidelines [7]. Specifically, a combination of one positive clinical criterion and one positive laboratory criterion (LAC, aCL or aβ 2G1 antibodies determined by ELISA) on two different occasions separated by 12 weeks were used for the diagnosis of APS. Among those APS patients, 16 were defined as seronegative APS, as suggested by other studies [22]. Briefly, those patients fulfilled the clinical criteria for APS, but were negative for LA as well as IgG/IgM aCL and IgG/IgM aβ2GPI antibodies as determined by ELISA (Aesku Diagnostics). HCs were defined as no signs of infection or inflammation or other significant illnesses. Study protocols were reviewed and approved by the Ethical Committee of Peking Union Medical College Hospital (PUMCH) and informed consents were obtained from all participants.

2.2. Serum aPLs antibodies determination

Serum IgG/IgM aCL and IgG/IgM aβ2GP1 were determined by ELISA (Aesku Diagnostics, Wendelsheim, Germany) according to the manufacturer's instructions. The cutoff value for positivity of IgG aCL was set at 18 GPL/ml, and the cutoff value for positivity of IgM aCL was set at 18 MPL/ml based on the recommendations by the manufacturer. The cutoff values for positivity of IgG/IgM aβ2GP1 were set at 18 U/ml based on the recommendations by the manufacturer. Serum IgG/IgM aPS, IgG/IgM aPI, IgG/IgM aSM, IgG/IgM aPC, and IgG/IgM aPE were tested by ELISA (Aesku Diagnostics, Wendelsheim, Germany) according to the manufacturer's instructions. The cutoff values for positivity of those non-criteria aPLs were set at 18 U/ml based on the recommendations by the manufacturer. The detection ranges for IgG aCL and IgM aCL were 0–300 GPL/ml and 0–300 MPL/ml, respectively. The detection ranges for other aPLs were 0–300 U/ml. The intra- and inter-assay coefficients of variation (CV) were < 10% for all the tests (data not shown), which was in accordance with the Clinical and Laboratory Standards Institute (CLSI) protocol EP15-A2 [23].

2.3. Statistical analysis

One-way ANOVA was utilized to calculate the differences between groups. The $\chi 2$ test or Fisher exact test was utilized for comparison of categorical variables. Correlations between multiple aPLs and thrombosis or obstetrical complications were determined by logistic regression models. p values of < 0.05 were considered statistically significant. SPSS 20.0 statistical software package (SPSS Inc., Chicago, Illinois, USA) and Prism 5.02 (GraphPad Software, San Diego, California, USA) were utilized for all statistical tests.

3. Results

3.1. Non-criteria aPL profiles in patients with APS and controls

Overall, the presence of aPE, aPS, aPI, aPC and aSM in patients with APS were 8.1%, 37.2%, 50.0%, 23.3% and 18.6%, respectively. The presence of aPE, aPS, aPI, aPC and aSM in Disease Controls were 1.0%, 9.6%, 8.7%, 1.0% and 1.9%, respectively (Table 1). None of aPE, aPS, aPI, aPC were detected in healthy controls. aSM was detected in one healthy control (Table 1).

Table 2
The predictive power of multiple aPLs in the diagnosis of patients with APS vs. Disease Controls.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
LA	75.58	94.41	89.04	86.54	13.51	0.26
aCL IgG	50.00	98.60	95.56	76.63	35.75	0.51
aCL IgM	16.28	98.60	87.50	66.20	11.64	0.85
aβ2GPI IgG	65.12	93.71	86.15	81.71	10.35	0.37
aβ2GPI IgM	10.47	98.60	81.82	64.68	7.48	0.91
aPE IgG	5.81	99.30	83.33	63.68	8.31	0.95
aPE IgM	4.65	100.00	100.00	63.56	N/A	0.95
aPS IgG	29.07	93.01	71.43	68.56	4.16	0.76
aPS IgM	16.28	100.00	100.00	66.51	N/A	0.84
aPI IgG	47.67	93.71	82.00	74.86	7.57	0.56
aPI IgM	12.79	100.00	100.00	65.60	N/A	0.87
aPC IgG	17.44	99.30	93.75	66.67	24.94	0.83
aPC IgM	12.79	100.00	100.00	65.60	N/A	0.87
aSM IgG	12.79	98.60	84.62	65.28	9.15	0.88
aSM IgM	10.47	99.30	90.00	64.84	14.97	0.90

APS, antiphospholipid syndrome; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio; aCL, anticardiolipin antibodies; aβ2GP1, anti-β2-glycoprotein 1 antibodies; LA, lupus anticoagulants; aPE, antiphosphatidylethanolamine, aPS, anti-phosphatidylserine, aPI, antiphosphatidylinositol;

3.2. The predictive power of multiple aPLs in the diagnosis of patients with APS vs. Disease Controls

Assay performance parameters of each aPL were calculated to further assess the role of non-criteria aPLs in the diagnosis of patients with APS (Table 2). In criteria aPLs, aCL IgG demonstrated the highest positive likelihood ratio (LR+) of 35.75 with a sensitivity of 50.0% and a specificity of 98.6%, followed by LA (LR+ of 13.51, sensitivity of 75.58% and specificity of 94.41%) and aCL IgM (LR+ of 11.64, sensitivity of 16.28% and specificity of 98.60%) (Table 2). In non-criteria aPLs, aPC IgG demonstrated the highest LR+ of 24.94 with a sensitivity of 17.44% and a specificity of 99.30%, followed by aSM IgM (LR+ of 14.97, sensitivity of 10.47% and specificity of 99.30%) (Table 2). Of note, IgM aPS exhibited good diagnostic potential with a sensitivity of 16.28% and a specificity of 100%.

3.3. Relationships among multiple non-criteria aPLs and criteria aPLs in patients with APS

The distributions and relationships among multiple non-criteria aPLs and criteria aPLs in patients with APS are illustrated by a Venn diagram in Fig. 1. Overall, 16 patients (18.6%, 16/86) were seronegative APS, who were negative for all of the three criteria aPLs (LA, aCL and aβ2GP1). Of note, the non-criteria aPLs were detected in 3 of those patients (18.8%, 3/16) (Fig. 1). In addition, among APS patients that were negative for IgM/IgG aCL and IgM/IgG aβ2GP1, non-criteria aPLs were detected in 6 patients (13.3%, 6/45) (Fig. 1).

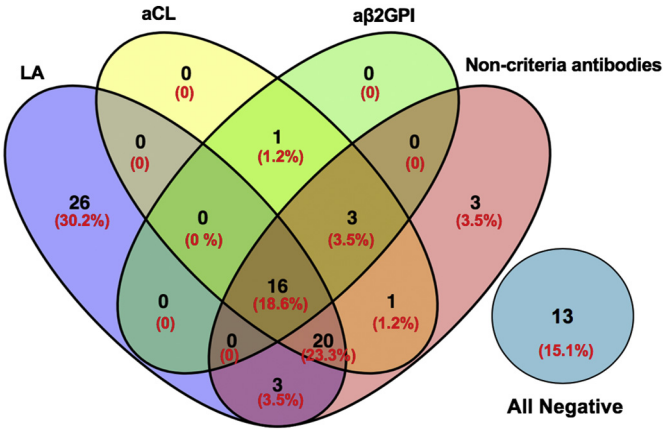

Fig. 1. Relationships among multiple non-criteria aPLs and criteria aPLs in patients with antiphospholipid syndrome (APS). aCL, anticardiolipin antibodies; aβ2GP1, anti-β2-glycoprotein 1 antibodies; LA, lupus anticoagulants; aPE, antiphosphatidylethanolamine, aPS, anti-phosphatidylserine, aPI, antiphosphatidylinositol; aPC, antiphosphatidylcholine; aSM antisphingomyelin.

3.4. Associations between multiple aPLs and thrombosis/obstetrical complications

The correlations between multiple aPLs and thrombosis/pregnancy morbidities were evaluated (Table 3). The criteria aPLs, including LA, IgG aCL and IgG aβ2GPI, were significantly correlated with both arterial thrombosis and venous thrombosis. While IgG aPS, IgM aPS, IgG aPI and IgG aPC were significantly associated with thrombosis, they were all correlated with arterial thrombosis but not venous thrombosis (Table 3). Among those non-criteria aPLs, IgG aPI displayed the highest OR of 4.209 in identifying APS patients at high risk of arterial thrombosis, followed by IgG aPC (OR, 3.964) and IgM aPS (OR, 3.563) (Table 3).

4. Discussion

In the present study, we evaluated the clinical performance of a series of non-criteria autoantibodies targeting phospholipids, including IgG/IgM aPS, IgG/IgM aPI, IgG/IgM aSM, IgG/IgM aPC and IgG/IgM aPE in the diagnosis of APS in a well-defined Chinese cohort. Although those non-criteria aPLs did not exhibit better diagnostic potentials than the current criteria aPLs in the diagnosis of APS, they were present in 18.8% (3/16) of seronegative APS. In addition, different from criteria aPLs which were significantly associated with both arterial thrombosis and venous thrombosis, some of those non-criteria aPLs, including IgG aPS, IgM aPS, IgG aPI and IgG aPC were significantly correlated with arterial thrombotic events alone. Collectively, our findings delineate the clinical relevance of those non-criteria aPLs, especially when it comes to distinguishing arterial thrombosis from venous thrombosis or diagnosing seronegative APS.

In this study, we found that aPE were less common than the rest non-criteria aPLs (IgG/IgM of 8.1% (7/86), IgG aPE of 5.81% (5/86) and IgM aPE of 4.65% (4/86), respectively). Although PE is one of the primary lipid components of the cell membrane, it is a neutral phospholipid [10]. As aPLs are mainly react with negatively charged phospholipids and cofactors, the neutral nature of PE may explain the low prevalence of aPE in our APS cohort. In addition, we failed to find a significant association between aPE and thrombosis events, which is consistent with a study by Bertolaccini et al., who also failed to demonstrate an association of aPE with thrombotic events in SLE [24]. A

Table 3
Correlations between multiple aPLs and thrombosis or obstetrical complications in clinically suspicious patients.

	Thrombosis	Arterial thrombosis	Venous thrombosis	Obstetrical complications
	p value, Odds ratio (95% CI)			
LA	< 0.001, 4.941 (2.723, 8.965)	< 0.001, 5.176 (2.391, 11.208)	< 0.001, 3.569 (1.956, 6.513)	0.228, 1.553 (0.757, 3.183)
aCL IgG	< 0.001, 5.194 (2.565, 10.518)	< 0.001, 7.000 (3.188,15.371)	0.004, 2.625 (1.338, 5.151)	0.052, 2.435 (0.975, 6.082)
aCL IgM	0.109, 2.271 (0.814, 6.339)	0.268, 2.033 (0.615, 6.721)	0.452, 1.495 (0.521, 4.293)	0.517, 0.556 (0.138, 2.247)
aβ2GPI IgG	< 0.001, 4.104 (2.242, 7.514)	< 0.001, 7.617 (3.438, 16.876)	0.004, 2.426 (1.320, 4.457)	0.183, 1.642 (0.790, 3.416)
aβ2GPI IgM	0.233, 2.070 (0.612, 7.000)	0.063, 3.581 (0.988, 12.976)	1.000, 0.902 (0.232, 3.511)	0.699, 1.368 (0.266, 7.029)
aPE IgG	1.000, 0.827 (0.148, 4.615)	0.595, 0.848 (0.802, 0.896)	1.000, 1.215 (0.217, 6.799)	1.000, 1.362 (0.186, 9.955)
aPE IgM	0.632, 1.679 (0.232, 12.139)	1.000, 0.849 (0.803, 0.897)	0.582, 2.462 (0.340, 17.847)	1.000, 1.356 (0.083, 22.123)
aPS IgG	0.026, 2.251 (1.087, 4.661)	0.003, 3.408 (1.477, 7.861)	0.477, 1.319 (0.614, 2.832)	0.803, 0.840 (0.305, 2.314)
aPS IgM	0.010, 4.572 (1.387, 15.071)	0.023, 3.563 (1.116, 11.379)	0.248, 1.893 (0.631, 5.684)	0.733, 0.658 (0.158, 2.744)
aPI IgG	0.001, 2.956 (1.552, 5.626)	< 0.001, 4.209 (1.952, 9.076)	0.124, 1.673 (0.865, 3.236)	0.643, 1.217 (0.530, 2.795)
aPI IgM	0.107, 3.079 (0.874, 10.846)	0.063, 3.581 (0.988, 12.976)	0.735, 1.406 (0.398, 4.971)	1.000, 0.664 (0.118, 3.749)
aPC IgG	0.033, 3.004 (1.051, 8.587)	0.008, 3.964 (1.336, 11.760)	0.059, 2.610 (0.937, 7.274)	0.699, 0.524 (0.098, 2.798)
aPC IgM	0.107, 3.079 (0.874, 10.846)	0.063, 3.581 (0.988, 12.976)	0.735, 1.406 (0.398, 4.971)	0.699, 1.368 (0.266, 7.029)
aSM IgG	0.066, 2.831 (0.895, 8.952)	0.109, 2.756 (0.798, 9516)	0.168, 2.178 (0.704, 6.742)	0.467, 0.431 (0.084, 2.214)
aSM IgM	1.000, 1.114 (0.305, 4.064)	0.365, 0.845 (0.798, 0.894)	0.484, 1.651 (0.451, 6.048)	1.000, 0.897 (0.145, 5.540)

APS, antiphospholipid syndrome; aCL, anticardiolipin antibodies; aβ2GP1, anti-β2-glycoprotein 1 antibodies; LA, lupus anticoagulants; aPE, antiphosphatidylethanolamine, aPS, anti-phosphatidylserine, aPI, antiphosphatidylinositol; aPC, antiphosphatidylcholine; aSM antisphingomyelin. Clinically suspicious patients, patients with recurrent arterial and/or venous thrombotic events, and/or recurrent fetal loss and tested for aPLs.

multicenter study from Europe showed that aPE were significantly associated with thrombosis when using patients with thrombosis and matched controls as study subjects [15]. The differences in study subjects may account for those discrepancies. Antibodies against another neutral phospholipid, aPC, have received less attention than aPE. In our study, we found that aPC displayed a higher prevalence in APS compared to aPE (IgG/IgM of 23.3% (20/86), IgG aPE of 17.44% (15/86) and IgM aPE of 12.79% (11/86), respectively), which was different from a study showing that the prevalence of aPC was 15.9%, while the prevalence of aPE was 18.2% [17]. Importantly, we found that IgG aPC displayed a LR+ of 24.94, which was higher than those from criteria aPLs, including LA (LR+ of 13.51), IgM aCL (LR+ of 11.64), IgG aβ2GP1 (LR+ of 10.35) and IgM aβ2GP1 (LR+ of 7.48). In addition, we showed that IgG aPC can predict patients at risk of arterial thrombosis. Taken together, our data indicate that aPC may have a better clinical performance than aPE in the diagnosis of APS in Chinese cohort.

Phosphatidylinositol (PI) and PS are negatively charged phospholipids. Antibodies to PI (aPI) and aPS were more commonly detected in our APS cohort than aPE and aPC (aPI, 50.0% (43/86) and aPS, 37.2% (32/86) vs. aPE 8.1% (7/86) and aPC 23.3% (20/86). Importantly, IgM aPS demonstrated good performance with a sensitivity of 16.28% and a specificity of 100%. A study from Saudi Arabia showed that aPS were present in 75% of confirmed APS cases, which was higher than those our study (37.2%, 32/86) [16]. Previous studies have indicated that aPS were associated with clinical features of APS [25–29]. We found that both IgG aPS and IgM aPS were significantly associated with arterial thrombosis but not venous thrombosis. Lopez et al. also found that aPS were strongly associated with arterial thrombosis ($p < .001$), but they also found that aPS were weekly but significantly correlated with venous thrombosis ($p = .01$) [27]. Of note, aPI demonstrated the highest prevalence among non-criteria aPLs assessed in this study (50.0%, 43/86), and aPI were detected in two seronegative APS patients. One patient, who had recurrent pregnancy failure, was only positive for IgG aPI, while the other patient with a history of stroke was positive for both IgM aPI and IgM aPS. Further, we showed that IgG aPI were significantly correlated with arterial thrombosis. Our findings were in agreement with previous reports showing that aPS and aPI may be implicated in stroke etiology [28,29]. A recent study by Castanon et al. failed to show any significant associations between IgG/IgM aPI and thrombosis [18]. The discrepancy may be due to the differences in sample size, ELISA kits for aPI or genetic/environmental factors. Taken together, our data show that both IgG/IgM aPS and IgG aPI may have promising diagnostic potentials in the diagnosis of APS, especially for seronegative APS.

Few, if any, studies have examined the clinical utility of aSM in the diagnosis of APS. In this study, we found that the prevalence of aSM was significantly higher in patients with APS than DC and HC. Importantly, IgM aSM displayed a LR+ of 14.97, which was also higher than those from LA (LR+ of 13.51), IgM aCL (LR+ of 11.64), IgG aβ2GP1 (LR+ of 10.35) and IgM aβ2GP1 (LR+ of 7.48). Further, although we failed to identify significant associations between IgG/IgM aSM and APS clinical manifestations, we did observe that IgG aSM was present in one seronegative APS patient. In fact, IgG aSM was the only aPL found in this APS patient. Thus, incorporating aSM into the aPLs panel may enhance the diagnostic sensitivity for APS.

Our study has a number of notable strengths. To the best of our knowledge, our study represents the first study in China investigating the clinical performance of those non-criteria aPLs in the diagnosis of APS, and the information obtained from our study will enhance our understanding of the clinical utility of those aPLs in Chinese APS patients. It should be noted, however, that our study has several limitations. First, the sample size was small, which may lead to potential analytical bias. Further studies with large cohorts are needed. Second, the subjects in our study were from a single institution, and these subjects were homogenous Han Chinese ethnic group. A multicenter study with various ethnic groups is needed to evaluate the generalizability of our results.

In summary, our study provides evidence regarding the clinical relevance of a number of non-criteria aPLs in the diagnosis and risk stratification of APS in Chinese patients. Our findings indicate that those non-criteria aPLs may be particularly helpful for patients in whom APS is highly suspected, but conventional aPLs are repeatedly negative as well as for predicting APS patients with arterial thrombosis. It is noteworthy that a multi-analyte aPLs detection system, which allows multiplex detection of aCL, aβ2GPI, anti-phosphatidic acid antibody, aPC, aPE, anti-phosphatidylglycerol antibody, aPI, aPS, anti-annexin V antibody and anti-prothrombin antibody, has been developed [30]. Thus, our findings may also shed insights on the strategies of developing appropriate aPLs panels for multi-analyte detection systems in the diagnosis, clinical and therapeutic decision-making process in Chinese patients with APS.

Acknowledgments

This work was supported in part by the National Natural Science

Foundation of China Grants No. 81771661 (to SZ) and 81671618 (to YL); Chinese Academy of Medical Sciences (CAMS) Initiative for Innovative Medicine (CAMS-I2M) No. 2017-I2M-3-001 (to SZ and YL), The National Key Research and Development Program of China No. 2016YFC0903900 (to YL).

Conflict of interest

The authors have no conflicting financial interest.

References

[1] M.G. Tektonidou, Antiphospholipid syndrome nephropathy: from pathogenesis to treatment, Front. Immunol. 9 (2018) 1181.
[2] A. Turrent-Carriles, J.P. Herrera-Felix, M.C. Amigo, Renal involvement in Antiphospholipid syndrome, Front. Immunol. 9 (2018) 1008.
[3] R.A. Levy, F.C. Dos Santos, G.R. de Jesus, N.R. de Jesus, Antiphospholipid antibodies and Antiphospholipid syndrome during pregnancy: diagnostic concepts, Front. Immunol. 6 (2015) 205.
[4] S. Zhang, Z. Wu, W. Zhang, J. Zhao, G.L. Norman, X. Zeng, F. Zhang, Y. Li, Antibodies to phosphatidylserine/prothrombin (aPS/PT) enhanced the diagnostic performance in Chinese patients with antiphospholipid syndrome, Clin. Chem. Lab. Med. 56 (6) (2018) 939–946.
[5] A. Antovic, M. Sennstrom, K. Bremme, E. Svenungsson, Obstetric antiphospholipid syndrome, Lupus Sci. Med. 5 (1) (2018) e000197.
[6] J.S. Knight, H. Meng, P. Coit, S. Yalavarthi, G. Sule, A.A. Gandhi, R.C. Grenn, L.F. Mazza, R.A. Ali, P. Renauer, J.D. Wren, P.L. Bockenstedt, H. Wang, D.T. Eitzman, A.H. Sawalha, Activated signature of antiphospholipid syndrome neutrophils reveals potential therapeutic target, JCI Insight 2 (18) (2017).
[7] S. Miyakis, M.D. Lockshin, T. Atsumi, D.W. Branch, R.L. Brey, R. Cervera, R.H. Derksen, P.G. De Groot, T. Koike, P.L. Meroni, G. Reber, Y. Shoenfeld, A. Tincani, P.G. Vlachoyiannopoulos, S.A. Krilis, International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS), J. Thromb. Haemost. 4 (2) (2006) 295–306.
[8] N. Zohoury, M.L. Bertolaccini, J.L. Rodriguez-Garcia, Z. Shums, O. Ateka-Barrutia, M. Sorice, G.L. Norman, M. Khamashta, Closing the serological gap in the antiphospholipid syndrome: the value of "non-criteria" Antiphospholipid antibodies, J. Rheumatol. 44 (11) (2017) 1597–1602.
[9] P. Zigon, A. Podovsovnik, A. Ambrozic, M. Tomsic, A. Hocevar, N. Gaspersic, Z. Rotar, S. Praprotnik, S.S. Semrl, S. Cucnik, Added value of non-criteria antiphospholipid antibodies for antiphospholipid syndrome: lessons learned from year-long routine measurements, Clin. Rheumatol. 38 (2) (2019) 371–378.
[10] J.A. McIntyre, D.R. Wagenknecht, W.P. Faulk, Antiphospholipid antibodies: discovery, definitions, detection and disease, Prog. Lipid Res. 42 (3) (2003) 176–237.
[11] K.M.J. Devreese, T.L. Ortel, V. Pengo, B. de Laat, A. Subcommittee on Lupus Anticoagulant/Antiphospholipid, Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH, J. Thromb. Haemost. 16 (4) (2018) 809–813.
[12] H.L. Staub, M.L. Bertolaccini, M.A. Khamashta, Anti-phosphatidylethanolamine antibody, thromboembolic events and the antiphospholipid syndrome, Autoimmun. Rev. 12 (2) (2012) 230–234.
[13] M. Sanmarco, N. Bardin, The contribution of antiphosphatidylethanolamine antibodies in the diagnosis of the antiphospholipid syndrome, Lupus 21 (7) (2012) 727–728.
[14] M. Sanmarco, Is testing for antiphosphatidylethanolamine antibodies clinically useful? Curr. Rheumatol. Rep. 13 (1) (2011) 81–85.
[15] M. Sanmarco, S. Gayet, M.C. Alessi, M. Audrain, E. de Maistre, J.C. Gris, P.G. de Groot, E. Hachulla, J.R. Harle, P. Sie, M.C. Boffa, Antiphosphatidylethanolamine antibodies are associated with an increased odds ratio for thrombosis. A multicenter study with the participation of the European Forum on antiphospholipid antibodies, Thromb. Haemost. 97 (6) (2007) 949–954.
[16] H. Khogeer, A. Alfattani, M. Al Kaff, T. Al Shehri, O. Khojah, T. Owaidah, Antiphosphatidylserine antibodies as diagnostic indicators of antiphospholipid syndrome, Lupus 24 (2) (2015) 186–190.
[17] A.E. Tebo, T.D. Jaskowski, A.R. Phansalkar, C.M. Litwin, D.W. Branch, H.R. Hill, Diagnostic performance of phospholipid-specific assays for the evaluation of antiphospholipid syndrome, Am. J. Clin. Pathol. 129 (6) (2008) 870–875.
[18] A. Castanon, G. Pierre, R. Willis, E.N. Harris, E. Papalardo, Z. Romay-Penabad, A. Schleh, P. Jajoria, M. Smikle, K. DeCeulaer, A. Tebo, T. Jaskowski, M.M. Guerra, D.W. Branch, J.E. Salmon, M. Petri, E.B. Gonzalez, Performance evaluation and clinical associations of immunoassays that detect antibodies to negatively charged phospholipids other than cardiolipin, Am. J. Clin. Pathol. 149 (5) (2018) 401–411.
[19] V. Rodriguez-Garcia, Y. Ioannou, A. Fernandez-Nebro, D.A. Isenberg, I.P. Giles, Examining the prevalence of non-criteria anti-phospholipid antibodies in patients with anti-phospholipid syndrome: a systematic review, Rheumatology (Oxford) 54 (11) (2015) 2042–2050.
[20] S. Zhang, Z. Wu, J. Li, P. Li, S. Chen, X. Wen, L. Li, W. Zhang, J. Zhao, F. Zhang, Y. Li, Clinical performance of antibodies to prothrombin and thrombin in Chinese patients with antiphospholipid syndrome: potential interest in discriminating patients with thrombotic events and non-thrombotic events, Rheumatol. Int. 37 (4) (2017) 579–584.
[21] S. Zhang, Z. Wu, S. Chen, J. Li, X. Wen, L. Li, W. Zhang, J. Zhao, F. Zhang, Y. Li, Evaluation of the diagnostic potential of antibodies to beta2-glycoprotein 1 domain 1 in Chinese patients with antiphospholipid syndrome, Sci. Rep. 6 (2016) 23839.
[22] A. Hoxha, A. Ruffatti, E. Mattia, L. Meneghel, M. Tonello, E. Salvan, V. Pengo, L. Punzi, Relationship between antiphosphatidylserine/prothrombin and conventional antiphospholipid antibodies in primary antiphospholipid syndrome, Clin. Chem. Lab. Med. 53 (8) (2015) 1265–1270.
[23] Clinical and Laboratory Standards Institute (CLSI), User Verification of Performance for Precision and Trueness; Approved Guideline, CLSI Document EP15-A2, CLSI, Wayne, PA, USA, (2005).
[24] M.L. Bertolaccini, T. Atsumi, M.A. Khamashta, O. Amengual, G.R. Hughes, Autoantibodies to human prothrombin and clinical manifestations in 207 patients with systemic lupus erythematosus, J. Rheumatol. 25 (6) (1998) 1104–1108.
[25] A.E. Tebo, T.D. Jaskowski, H.R. Hill, D.W. Branch, Clinical relevance of multiple antibody specificity testing in anti-phospholipid syndrome and recurrent pregnancy loss, Clin. Exp. Immunol. 154 (3) (2008) 332–338.
[26] M.S. Sater, R.R. Finan, F.M. Abu-Hijleh, T.M. Abu-Hijleh, W.Y. Almawi, Anti-phosphatidylserine, anti-cardiolipin, anti-beta2 glycoprotein I and anti-prothrombin antibodies in recurrent miscarriage at 8-12 gestational weeks, Eur. J. Obstet. Gynecol. Reprod. Biol. 163 (2) (2012) 170–174.
[27] L.R. Lopez, K.J. Dier, D. Lopez, J.T. Merrill, C.A. Fink, Anti-beta 2-glycoprotein I and antiphosphatidylserine antibodies are predictors of arterial thrombosis in patients with antiphospholipid syndrome, Am. J. Clin. Pathol. 121 (1) (2004) 142–149.
[28] T. Kahles, M. Humpich, H. Steinmetz, M. Sitzer, E. Lindhoff-Last, Phosphatidylserine IgG and beta-2-glycoprotein I IgA antibodies may be a risk factor for ischaemic stroke, Rheumatology (Oxford) 44 (9) (2005) 1161–1165.
[29] H. Okuma, Y. Kitagawa, S. Takagi, Investigation of antiphosphatidyl-serine antibody and antiphosphatidyl-inositol antibody in ischemic stroke patients, Clin. Dev. Immunol. 2010 (2010) 439230.
[30] C. Nalli, V. Somma, L. Andreoli, T. Buttner, P. Schierack, M. Mahler, D. Roggenbuck, A. Tincani, Anti-phospholipid IgG antibodies detected by line immunoassay differentiate patients with anti-phospholipid syndrome and other autoimmune diseases, Auto Immun Highlights 9 (1) (2018) 6.