Detection of IgA Antiphospholipid Antibodies Does not Improve Thrombotic Antiphospholipid Syndrome Classification: A two-Center Study

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Abstract
Background: Thrombotic antiphospholipid syndrome (APS) is a systemic autoimmune disease; its diagnosis requires meeting both clinical and laboratory criteria. Prevalence rates of immunoglobulin (Ig) A anticardiolipin antibodies (aCL) and IgA anti-β2 glycoprotein I antibodies (aβ2GPI) remain unknown, and the clinical value of these antibodies to APS classification remains controversial. Therefore, we aimed to examine both items in the Chinese population.

Methods: Using chemiluminescence immunoassay, antiphospholipid antibodies (aPL) were quantified in 12,582 hospital-based general population, 278 thrombotic APS patients, and 233 healthy controls.

Results: In the general population, the positive rates of IgA aCL and IgA aβ2GPI antibodies were 2.87% and 1.99%, respectively. Furthermore, isolated IgA aPL-positivity rate was 0.72% in patients with APS, which was comparable to those in the general population (0.68%, p = 1) and in healthy controls (0.43%, p = 1). Among the IgA aPL-positive individuals in the general population, isolated IgA-positive individuals had lower serum levels of IgA antibodies (p = 0.007 for IgA aCL and p = 0.059 for IgA aβ2GPI). Regarding to APS classification, adding IgA aPL into conventional aPL assays may not improve and may even deteriorate the net reclassification index for APS; besides, no association between thrombosis and IgA aPL was observed.

Conclusions: this study assessed the prevalence of various aPL in Chinese population. IgA aPL may not enhance the classification ability of established laboratory criteria for thrombotic APS. Our data do not support the addition of IgA aPL to conventional aPL assays.

Keywords
thrombotic antiphospholipid syndrome, anti-β2 glycoprotein I antibody, anticardiolipin antibody, immunoglobulin A, classification

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Introduction
Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by recurrent thrombotic and/or pregnancy-related morbidity.1 Early diagnosis is essential to adopting appropriate antithrombotic strategies and preventing the onset of rapidly progressing and potentially life-threatening catastrophic APS. According to the 2006 updated Sapporo classification criteria, patients must meet both clinical and laboratory criteria to receive a diagnosis of APS.2 Lupus anticoagulant, immunoglobulin (Ig) G/IgM anticardiolipin antibodies (aCL), and IgG/IgM anti-β2 glycoprotein I antibodies (aβ2GPI) are classic antiphospholipid antibodies (aPL) used as laboratory diagnostic markers. However, in

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practice, some patients with clinical manifestations of APS repeatedly test negative for all these aPL. Accordingly, aPL that are not currently included in the classification criteria, such as IgA aCL and IgA αβ₂GPI, have been proposed as additional indicators to be considered in patients suspected of having seronegative APS.

The significance of IgA aPL in the development of thrombotic complications is of great interest. Previous studies involving a preclinical thrombosis mouse model have demonstrated that IgA aCL and αβ₂GPI were able to induce significantly larger thrombi and upregulate the expression of tissue factor. However, these data cannot prove the pathogenicity of IgA aPL because the influence of lupus anticoagulant or IgG aPL cannot be excluded. Some studies have suggested that there may be different subpopulations of αβ₂GPI, each recognizing different epitopes on β₂GPI and differing greatly in their pathologic effects. For example, IgA αβ₂GPI preferentially targeting the C-terminal portion of β₂GPI emerged as harmless in some studies and as pathogenic in others. Clinical findings on the association between IgA aCL and/or αβ₂GPI and APS-related thrombosis or obstetric complications vary substantially and are sometimes conflicting. The main factors that contribute to this heterogeneity are the differences in study populations and laboratory methods. Therefore, whether IgA aPL tests should be part of the routine diagnostic algorithm remains subject to debate. Studies with larger and well-characterized populations are required to clarify their clinical value.

We have previously reported that IgA aPL may not provide added value in the diagnosis of APS in the Chinese population. However, that study was restricted to one center, and the 212 APS patients enrolled comprised 127 thrombotic APS patients, 62 obstetric APS patients, and 23 patients with both clinical signs. Emerging data have revealed some important differences between pure vascular and obstetric APS variants, which mainly involve a thrombophilic state and inflammation; thus, expanding subgroups of patients for further exploration is necessary. In addition, chemiluminescence immunoassay (CLIA) has become an alternative method to the classical enzyme-linked immunosorbent assay for aPL examination. CLIA has advantages in automation and standardization, which can provide a more stable and comparable measure of aPL.

In the present two-center study, 122 (43.88%) and 156 (56.12%) patients from northern and southwest China were enrolled, respectively. We focused on patients with clearly defined pure thrombotic APS and used CLIA for aPL detection to reduce the influence of the study population and testing method on the results. The present study aimed to obtain convincing evidence on the value of IgA aPL in thrombotic APS diagnosis.

Materials and Methods

Study Design and Participants

This was a retrospective, two-center study. We acquired all the routine IgG/IgM/IgA aPL detection results of participants from the West China Hospital (WCH, July 2019 to December 2020) and Peking Union Medical College Hospital (PUMCH, July 2019 to February 2020), using a laboratory information system. After reviewing their basic information, we recruited 12,582 non-duplicate individuals; these participants were considered as the hospital-based general population. In addition, we enrolled 278 eligible thrombotic APS patients who met the revised Sapporo classification criteria. A total of 233 apparently healthy individuals without infections, tumors, autoimmune diseases, or other inflammatory diseases were included as healthy controls. Baseline demographic and APS-relevant clinical characteristics were extracted from electronic medical records.

Comparisons of aPL levels and aPL-positivity rates were performed between the general population and APS patients, as well as healthy controls and APS patients, to estimate aPL prevalence differences. However, only healthy controls and patients with APS were included to evaluate the value of IgA aPL for APS classification.

Our research protocol conformed to the guidelines set forth by the Declaration of Helsinki. The study was approved by the ethical committees of the WCH and PUMCH. The requirement for informed consent was waived because of the retrospective nature of the study.

Measurement of serum Antiphospholipid Antibodies

The detection of aCL and αβ₂GPI antibodies was performed using a chemiluminescence immunoassay (iFlash 3000 Chemiluminescence Immunoassay Analyzer, YHLO Biotech Co. Ltd, Shenzhen, China). For aCL, the concentrations of IgA, IgG, or IgM antibodies were expressed in APL, GPL, or MPL U/mL, and values above 10 were considered positive according to the manufacturer’s recommendations. For αβ₂GPI, the concentrations of different antibody isotypes were all expressed in AU/mL, and 20 was regarded as the positive cutoff point. We defined the aPL as strongly positive when the obtained values were more than twice the positive threshold value, which meant aCL >20 and/or αβ₂GPI >40. Triple positivity was defined as simultaneous positivity for IgG aCL and/or αβ₂GPI, IgM aCL and/or αβ₂GPI, and IgA aCL and/or αβ₂GPI. Values below the lower limit of quantitation were imputed as half of the lower limit; for those above the upper limit, a value was arbitrarily assigned to the upper limit of +5.

Statistical Analyses

Statistical analysis was performed using SPSS for Windows (version 25.0; IBM Corp., Armonk, New York, USA). Descriptive statistics were summarized, using frequencies and percentages for categorical variables, and means and standard deviations or medians with interquartile ranges for quantitative variables, as suitable. Between-group comparisons were made using the Kruskal–Wallis approach and Student t-test for continuous variables, and the χ² test with Fisher’s exact test for nominal variables. The strength of the associations between aPL-positivity and APS-related clinical manifestations was
evaluated by calculating odds ratios (OR) with 95% confidence intervals (95% CI), using a binary logistic regression model. The net reclassification index (NRI) was calculated to evaluate any improvement in identifying APS patients when IgA aCL and αβ2GPI were added to the conventional aPL assays. A two-tailed p-value of <0.05 was considered statistically significant. The Bonferroni correction was applied to adjust for multiple comparisons.

**Results**

**Demographic and Clinical Characteristics of the Study Groups**

In the hospital-based general population, 78.21% were female, while in the healthy and APS patients, the proportion of females was relatively close to that of males, and the female-to-male ratio was nearly 11:9 (Table 1). Age distribution was similar in all groups. The rates of venous and arterial thrombosis in patients with APS were 69.78% and 65.11%, respectively. The rates of potential thrombosis-related risk factors, such as smoking (22.66%), hypertension (21.58%), hyperlipidemia (12.95%), diabetes mellitus (7.91%), malignancy (6.12%), and autoimmune diseases (29.50%) were also evaluated in the APS group.

**Levels and Positive Rates of aPL in Different Groups**

Except for IgM aCL, the positive rates of aPL were highest in APS patients, followed by those in the general population and healthy controls. IgG was the most prevalent aPL isotype in patients with APS, whereas the reverse was true in healthy controls. The rate of aPL-positivity in APS patients was significantly different from those in the other groups (Table 2).

Among all study participants, APS patients had significantly greater aPL levels than did other participants, except for IgM aCL, which showed comparable levels in APS cases and

**Table 1.** Baseline characteristics of the study groups.

| Characteristics                  | Hospital-based general population (n = 12,582) | APS patients | Healthy controls (n = 233) | p1 | p2 |
|----------------------------------|-----------------------------------------------|--------------|----------------------------|----|----|
| Sex, n (%)                       |                                               |              |                            |    |    |
| Male                             | 2742 (21.79)                                  | 111 (41.80)  | 103 (44.21)                |    |    |
| Female                           | 9840 (78.21)                                  | 116 (41.80)  | 130 (55.79)                |    |    |
| Age, year, mean ± SD             | 40.7 ± 16.1                                   | 46.2 ± 15.3  | 44.1 ± 12.3                |    |    |
| Smoker, n (%)                    |                                               |              |                            |    |    |
| Hypertension, n (%)              |                                               |              |                            |    |    |
| Hyperlipidemia, n (%)            |                                               |              |                            |    |    |
| Diabetes mellitus, n (%)         |                                               |              |                            |    |    |
| Malignancy, n (%)                |                                               |              |                            |    |    |
| Autoimmune diseases, n (%)       |                                               |              |                            |    |    |
| Venous Thrombosis, n (%)         |                                               |              |                            |    |    |
| Arterial Thrombosis, n (%)       |                                               |              |                            |    |    |

**Table 2.** Positive rates of antiphospholipid antibodies in different groups.

| Antiphospholipid antibodies | Hospital-based general population (n = 12,582) | APS patients (n = 278) | Healthy controls (n = 233) | p1     | p2     |
|-----------------------------|-----------------------------------------------|------------------------|----------------------------|--------|--------|
| IgA aCL                     | 361 (2.87)                                    | 45 (16.19)             | 2 (0.86)                   | <0.001 | <0.001 |
| IgG aCL                     | 905 (7.19)                                    | 109 (39.21)            | 0 (0)                      | <0.001 | <0.001 |
| IgM aCL                     | 328 (2.61)                                    | 28 (10.07)             | 7 (3.00)                   | <0.001 | 0.003  |
| IgA αβ2GPI                  | 250 (1.99)                                    | 40 (14.39)             | 2 (0.86)                   | <0.001 | <0.001 |
| IgG αβ2GPI                  | 532 (4.23)                                    | 81 (29.14)             | 0 (0)                      | <0.001 | <0.001 |
| IgM αβ2GPI                  | 843 (6.70)                                    | 61 (21.94)             | 11 (4.72)                  | <0.001 | <0.001 |

Abbreviations: aCL, anticardiolipin antibody; αβ2GPI, anti-β2 glycoprotein I antibody; APS, antiphospholipid syndrome; p1, hospital-based general population versus total APS patients; p2, healthy controls versus total APS patients. Bonferroni correction was applied to adjust for multiple comparisons. Significant p-values are shown in bold.

The data are presented as frequencies (percentages). Bonferroni correction was applied to adjust for multiple comparisons. Significant p-values are shown in bold.
healthy controls. However, among the positive individuals, we found significant differences only in IgG aPL and IgM aβ2GPI distribution between the general population and APS groups (Figure 1).

Profile Features of IgA aCL and aβ2GPI
Cross-positivity of the aPL isotype is summarized in Figure 2. Isolated positivity rates for IgA aCL were 0.68% (n = 86), 0.72% (n = 2), and 0.43% (n = 1) in the general population, APS patients, and healthy controls, respectively (p = 1 for both comparisons). In contrast, the differences in isolated IgG (both p < 0.001) or IgM (p = 0.030 for APS vs healthy controls; p < 0.001 for APS vs general population) aPL distributions were significant.

Within hospital-based general population, comparisons of IgA aPL levels in isolated and non-isolated IgA-positive individuals revealed higher serum IgA antibody levels in non-isolated individuals than in their counterparts (p = 0.007 for IgA aCL and p = 0.059 for IgA aβ2GPI; Figure 3). The median (interquartile range) levels of IgA aCL were 28.20 (15.60, 77.95) APL-U/mL and 19.45 (14.05, 38.20) APL-U/mL in the non-isolated and isolated IgA aCL-positive groups, respectively, while those of IgA aβ2GPI were 56.90 (32.05, 265.00) AU/mL and 38.10 (25.55, 132.50) AU/mL in the non-isolated and isolated IgA-positive aβ2GPI groups, respectively. Since the number of individuals with isolated IgA-positive status in the APS and control groups was small (2 patients and 1 patient, respectively), no follow-up concentration analysis was performed.

Adding IgA aCL and aβ2GPI did not Improve APS Classification Performance of Conventional aPL Assays
The ideal diagnostic test is one in which a large proportion of APS and a small proportion of non-APS individuals test positive. We assessed and compared the diagnostic performance of IgM and IgA aPL; both performed worse than did IgG aPL (Table 2). Therefore, we evaluated the predictive ability of IgA aPL for APS diagnosis over the conventional aPL assays by adding IgA aPL into the routine assays or by replacing IgM aPL with IgA aPL (Table 3). Combining IgA aPL with IgM/IgG aPL led to a small, statistically non-significant improvement in the classification of APS (NRI = 0.003, p = 0.663). However, the NRI showed a significant deterioration after IgM aPL were substituted by IgA aPL (NRI = -0.057, p = 0.027).

Correlations Between IgA aPL and APS-Related Clinical Manifestations
Table 4 presents the associations between major APS manifestations and IgA aPL with different positivity status. Among 278 thrombotic APS patients, no significant correlation was observed between IgA aPL and thrombosis (either venous or arterial thrombosis), stroke, autoimmune hemolytic anemia, cardiopulmonary and renal disorders. However, patients with IgA aPL-positivity (compared to IgA aPL-negativity), and IgG, IgM, and IgA triple aPL-positivity (compared to single or dual aPL-positivity) had increased odds of thrombocytopenia.
Discussion

This population-based study used data from the top two hospitals in China. Given the centers’ location, the participants recruited from the WCH and PUMCH are likely representative of the Chinese population. Although we found higher rates of IgA aCL and \( \alpha\beta_2\)GPI in APS patients than in other groups, IgA isotype antibodies tended to present with IgG and/or IgM aPL; consequently, the number of participants presenting with isolated IgA aPL was very limited. The fairly low prevalence of isolated IgA aPL was comparable between the APS and hospital-based general population, as well as healthy controls, resulting in no additional value of IgA aPL in APS classification observed in the present study.

APL distribution is ethnicity-dependent. Geographic and ethnic studies reported a high variability in IgA aPL prevalence;\(^{10,18}\) some of these studies indicated a considerable prevalence of IgA aPL in populations of African origin.\(^{19,20}\) However, the incidence of IgA aPL-positivity in Chinese
patients is largely unknown, except for the evidence from our previous study. In addition, the samples of previous epidemiological studies were limited, and most focused on older adults, pregnant women, and participants with hypercoagulation conditions (including stroke, cardiovascular events, or deep vein thrombosis), lacking data on individuals without apparent abnormality. Herein, we conducted the largest population-based aPL prevalence study in China to date, showing that IgA aCL and IgA α2GPI were positive in 2.87% and 1.99% of the general population, respectively. Given China’s large population size, there may be many individuals with aberrantly elevated IgA aPL levels. Concomitantly, considering the significant difference of IgA aPL positivity between APS patients and healthy controls, routine testing for IgA aPL may help identify a substantial number of potential APS cases. However, aPL profile cross-validity evaluation revealed that isolated IgA aPL-positivity was rare in patients with APS (0.72%). The high rate of co-occurrence of IgA aPL with another IgG/IgM aPL indicated that most cases involving IgA aPL-positivity can be detected, using the current aPL panel. Chayoua et al. and Vlagea et al. reached conclusions similar to those of the present study, showing that adding IgA aCL and α2GPI tests to routine assessments may not improve the APS detection rate. Alongside their positive rates, we examined the levels of IgA antibodies. IgA aCL and α2GPI levels were significantly lower in IgA-isolated-positive individuals. The aPL titer is important in stratifying the risk of thrombosis. Low-level aPL occurs frequently with infections, and exposure to certain drugs or cancer cells. In contrast, high levels of aPL strongly predict future thrombosis with and without underlying autoimmune diseases. Consequently, we hypothesized that single IgA-positive individuals may be less likely to develop APS than dual- or triple-positive individuals. IgA-positive individuals can be classified into two categories: first, multiple

### Table 3. Net reclassification index for APS using conventional aPL assays and new models (adding IgA aPL or replacing IgM aPL with IgA aPL)/

|                         | IgG/IgM/IgA aPL | Reclassified | Improved | Worsened | Net | NRI |
|-------------------------|-----------------|--------------|----------|----------|-----|-----|
| **APS patients**        |                 |              |          |          |     |     |
| -                       | 130             | 2            | 132      |          |     |     |
| +                       | 0               | 146          | 146      |          |     |     |
| Total                   | 130             | 148          | 278      |          |     |     |
| **Healthy controls**    |                 |              |          |          |     |     |
| -                       | 219             | 1            | 220      |          |     |     |
| +                       | 0               | 13           | 13       |          |     |     |
| Total                   | 219             | 14           | 223      |          |     |     |

|                         | IgG/IgM aPL     | Reclassified | Improved | Worsened | Net   | NRI |
|-------------------------|-----------------|--------------|----------|----------|-------|-----|
| **APS patients**        |                 |              |          |          |       |     |
| -                       | 130             | 2            | 132      |          | 2 (0.72%) | 0   | 0.72% | 0.003 |
| +                       | 31              | 115          | 146      | 2        | 12 (5.15%) | 1   | 0.43% | 4.72% |
| Total                   | 161             | 117          | 278      |          |       |     |     |
| **Healthy controls**    |                 |              |          |          |       |     |
| -                       | 219             | 1            | 220      | 1        | 12 (5.15%) | 1   | 0.43% | 4.72% |
| +                       | 12              | 1            | 13       |          |       |     |     |
| Total                   | 231             | 2            | 233      |          |       |     |     |

**Abbreviations:** aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; NRI, net reclassification index.
| Table 4. IgA aPL and APS-related clinical manifestations. |
|-----------------------------------------------------------|
| Clinical manifestations | Positive IgA aPL | | Strongly positive IgA aPL | | Positive IgG&IgM&IgA aPL (Triple positivity) | |
|                          | Present (n=46) | Absent (n=232) | p | OR (95% CI) | Present (n=35) | Absent (n=243) | p | OR (95% CI) | Present (n=10) | Absent (n=268) | p | OR (95% CI) |
|--------------------------|----------------|----------------|---|-------------|----------------|----------------|---|-------------|----------------|----------------|---|-------------|
| Venous thrombosis       | 30 (65.22)     | 164 (70.69)    | 0.461 | 0.777 (0.398-1.518) | 24 (68.57)     | 170 (69.96)    | 0.867 | 0.937       | 7 (70.00)       | 187 (69.78)    | 0.988 | 1.011 (0.255-4.007) |
| Arterial thrombosis     | 31 (67.39)     | 150 (64.66)    | 0.722 | 1.130 (0.577-2.214) | 23 (65.71)     | 158 (65.02)    | 0.936 | 1.031       | 8 (80.00)       | 173 (64.55)    | 0.326 | 2.197 (0.457-10.553) |
| Stroke                   | 19 (41.30)     | 67 (28.88)     | 0.098 | 1.733 (0.903-3.326) | 14 (40.00)     | 72 (29.63)     | 0.217 | 1.583       | 5 (50.00)       | 81 (30.22)     | 0.195 | 2.309 (0.650-8.194) |
| Cardiopulmonary involvement | 13 (28.26)   | 97 (41.81)     | 0.089 | 0.548 (0.274-1.096) | 11 (31.43)     | 99 (40.74)     | 0.295 | 0.667       | 4 (40.00)       | 106 (39.55)    | 0.977 | 1.019 (0.281-3.696) |
| Renal involvement        | 7 (15.22)      | 36 (15.52)     | 0.959 | 0.977 (0.406-2.355) | 7 (20.00)      | 36 (14.81)     | 0.430 | 1.437       | 2 (20.00)       | 41 (15.30)     | 0.688 | 1.384 (0.284-6.752) |
| Autoimmune hemolytic anemia | 7 (15.22)  | 24 (10.34)     | 0.341 | 1.556 (0.627-3.860) | 5 (14.29)      | 26 (10.70)     | 0.530 | 1.391       | 1 (10.00)       | 30 (11.19)     | 0.906 | 0.881 (0.108-7.203) |
| Thrombocytopenia         | 24 (47.83)     | 75 (32.33)     | **0.046** | **1.919** (1.011-3.641) | 17 (48.57)     | 80 (32.92)     | 0.073 | 1.924       | 7 (70.00)       | 90 (33.58)     | **0.029** | **4.615** (1.166-18.271) |

Abbreviations: aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome.
Data are presented as frequencies (percentages). Significant p-values are shown in bold.
aPL-positive group, whereby IgA coexists with IgM/IgG antibodies and can be identified with the conventional aPL assays; second, isolated IgA-positive group with a low risk of APS due to the low levels of antibodies. This observation corroborates our conclusion that the detection of IgA antibodies is not associated with diagnostic advantages. The distribution of different aPL antibody levels was significantly different between individuals with and without APS (Figure 1). However, within the IgA aPL-positive individuals, we found no difference in IgA antibody levels between groups; conversely, the levels of IgG aCL, IgG aβ2GPI, and IgM aβ2GPI of the positive individuals were higher in the APS than in the other group, suggesting that APS patients may produce high levels of IgM/IgG aPL but not of IgA antibodies.

Finally, in the present study, thrombocytopenia was more common in IgA aPL-positive or IgG, IgM, and IgA isotype triple-positive APS individuals than in other participants. The pathogenesis of aPL-related thrombocytopenia remains unclear. Based on currently available data, secondary immune thrombocytopenia may be one of the pathogenetic mechanisms. Expression of platelet membrane glycoproteins increases after aPL stimulation, and the binding of aβ2GPI-β2GPI complex to receptors on the platelet membrane induces the activation and aggregation of platelets. In summary, aPLs (including IgA antibodies) may promote platelet aggregation; thrombocytopenia may be a consequence of platelet consumption. Similar to Vlagea et al., Bertolaccini et al., and others, we failed to find any association between thrombosis and IgA aPL. However, other previous studies have yielded contradictory results likely due to sample heterogeneity, including eligibility criteria, and differences in laboratory methods; further studies are required.

The present study has some limitations, which should be considered when interpreting its findings. First, we only used one platform for aPL detection, considering the poor agreement in detection of aPL between platforms; simultaneous application of multiple testing systems may enhance confidence in results. Second, we did not consider the impact of treatment on test findings. Third, we only selected the first aPL test results of patients with APS for statistical analysis.

In conclusion, in this two-center, large population-based study, we reported the prevalence of aCL and aβ2GPI in the Chinese population. IgA aPL usually coexists with IgG/IgM aPL, and isolated IgA aPL is present at a low frequency in the general population and in APS patients. The presence of IgA aPL is not associated with APS-related thrombosis. Therefore, the present findings suggest that including IgA aPL in APS classification may bring no additional value. Our results do not support the addition of IgA aCL and IgA aβ2GPI to conventional aPL for thrombotic APS diagnosis.

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Declaration of Conflicting Interests
The authors declare that there is no conflict of interest.

Author Contributions
Bin Yang and Chaojun Hu designed the study; Zhenzhen Su, Zhuochun Huang, Jiuliang Zhao, Xiaofeng Zeng, and Mengtao Li recruited participants; Zhenzhen Su, Jing Hu, and Jiuliang Zhao performed experiments and collected data; Zhenzhen Su, Bin Yang and Chaojun Hu performed statistical analyses and interpreted results; Zhenzhen Su and Bin Yang drafted the manuscript; all authors reviewed the manuscript.

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References
1. Garcia D, Erkan D. Diagnosis and management of the antiphospholipid syndrome. N Engl J Med. 2018;378(21):2010-2021.
2. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4(2):295-306.
3. Nayef R, Uthman I, Aoun J, et al. Seronegative antiphospholipid syndrome. Rheumatology (Oxford). 2013;52(8):1358-1367.
4. Lakos G, Favaloro EJ, Harris EN, et al. International consensus guidelines on anticardiolipin and anti-β2-glycoprotein I testing: report from the 13th international congress on antiphospholipid antibodies. Arthritis Rheum. 2012;64(1):1-10.
5. Pierangeli SS, Liu XW, Barker JH, et al. Induction of thrombosis in a mouse model by IgG, IgM and IgA immunoglobulins from patients with the antiphospholipid syndrome. Thromb Haemost. 1995;74(5):1361-1367.
6. Murthy V, Willis R, Romay-Penabad Z, et al. Value of isolated IgA anti-β2-glycoprotein i positivity in the diagnosis of the antiphospholipid syndrome. Blood. 2012;120(12):3186-3193.
7. Chayoua W, Yin D, Kelchtermans H, et al. Is there an additional value in detecting anticardiolipin and anti-β2 glycoprotein I IgA antibodies in the antiphospholipid syndrome? Thromb Haemost. 2020;120(11):1557-1568.
8. de Groot PG, Urbanus RT. The significance of autoantibodies against β2-glycoprotein I. Blood. 2012;120(2):266-274.
9. Forastiero R, Martinuzzo M, de Larrañaga G, et al. Anti-β2glycoprotein I antibodies from leprosy patients do not show thrombogenic effects in an in vivo animal model. J Thromb Haemost. 2011;9(4):859-861.
10. Andreoli L, Fredi M, Nalli C, et al. Clinical significance of IgA anti-cardiolipin and IgA anti-β2Glycoprotein I antibodies. *Curr Rheumatol Rep*. 2013;15(7):343.

11. Vlagea A, Pascual-Salcedo D, Álvarez Doñoro R, et al. Iga anti-β2 glycoprotein I antibodies: experience from a large center. *Thromb Res*. 2018;162:38-43.

12. Žigon P, Podovšanik A, Ambrožič A, et al. Added value of non-criteria antiphospholipid antibodies for antiphospholipid syndrome: lessons learned from year-long routine measurements. *Clin Rheumatol*. 2019;38(2):371-378.

13. Danowski A, Kickler TS, Petri M. Anti-beta2-glycoprotein I: prevalence, clinical correlations, and importance of persistent positivity in patients with antiphospholipid syndrome and systemic lupus erythematosus. *J Rheumatol*. 2006;33(9):1775-1779.

14. Liu T, Gu J, Wan L, et al. “Non-criteria” antiphospholipid antibodies add value to antiphospholipid syndrome diagnoses in a large Chinese cohort. *Arthritis Res Ther*. 2020;22(1):33.

15. Hu C, Li X, Zhao J, et al. Immunoglobulin A isotype of antiphospholipid antibodies does not provide added value for the diagnosis of antiphospholipid syndrome in a Chinese population. *Front Immunol*. 2020;11:568503.

16. Meroni PL, Borghi MO, Grossi C, et al. Obstetric and vascular antiphospholipid syndrome: same antibodies but different diseases? *Nat Rev Rheumatol*. 2018;14(7):433-440.

17. Wang C, Guan X. Laboratory diagnosis of antiphospholipid syndrome. *Front Lab Med*. 2018;2(3):97-101.

18. Uthman I, Khamashta M. Ethnic and geographical variation in antiphospholipid (hughes) syndrome. *Ann Rheum Dis*. 2005;64(12):1671-1676.

19. Molina JF, Gutierrez-Ureña S, Molina J, et al. Variability of anticardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. *J Rheumatol*. 1997;24(2):291-296.

20. Cucurull E, Garhari AE, Diri E, et al. Iga anticardiolipin and anti-beta2-glycoprotein I are the most prevalent isotypes in African American patients with systemic lupus erythematosus. *Am J Med Sci*. 1999;318(1):55-60.

21. Petri M. Epidemiology of the antiphospholipid antibody syndrome. *J Autoimmun*. 2000;15(2):145-151.

22. Manoussakis MN, Tzioufas AG, Silis MP, et al. High prevalence of anti-cardiolipin and other autoantibodies in a healthy elderly population. *Clin Exp Immunol*. 1987;69(3):557-565.

23. Andreoli L, Chighizola CB, Banzato A, et al. Estimated frequency of antiphospholipid antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. *Arthritis Care Res (Hoboken)*. 2013;65(11):1869-1873.

24. Sciascia S, Sanna G, Khamashta MA, et al. The estimated frequency of antiphospholipid antibodies in young adults with cerebrovascular events: a systematic review. *Ann Rheum Dis*. 2015;74(11):2028-2033.

25. Bradacova P, Slavik L, Uhlová I, et al. Current promising biomarkers and methods in the diagnostics of antiphospholipid syndrome: a review. *Biomedicines*. 2021;9(2):166.

26. van den Hoogen LL, van Roon JAG, Radstake TRDJ, et al. Delineating the deranged immune system in the antiphospholipid syndrome. *Auto Immun Rev*. 2016;15(1):50-60.

27. Kelchtermans H, Pelkmans L, de Laat B, et al. IgG/IgM antiphospholipid antibodies present in the classification criteria for the antiphospholipid syndrome: a critical review of their association with thrombosis. *J Thromb Haemost*. 2016;14(8):1530-1548.

28. Devreese K.M.J. How to interpret antiphospholipid laboratory tests. *Curr Rheumatol Rep*. 2020;22(8):38.

29. Martirosyan A, Aminov R, Manukyan G. Environmental triggers of autoreactive responses: induction of antiphospholipid antibody formation. *Front Immunol*. 2019;10:1609.

30. Bazzan M, Montaruli B, Vaccarino A, et al. Presence of low titre of antiphospholipid antibodies in cancer patients: a prospective study. *Intern Emerg Med*. 2009;4(6):491-495.

31. Somers E, Magder LS, Petri M. Antiphospholipid antibodies and incidence of venous thrombosis in a cohort of patients with systemic lupus erythematosus. *J Rheumatol*. 2002;29(12):2531-2536.

32. Calcaterra I, Ambrosino P, Vitelli N, et al. Risk assessment and antithrombotic strategies in antiphospholipid antibody carriers. *Biomedicines*. 2021;9(2):122.

33. Tomasello R, Giordano G, Romano F, et al. Immune thrombocytopenia in antiphospholipid syndrome: is it primary or secondary? *Biomedicines*. 2021;9(9):1170.

34. Bertolaccini ML, Atsumi T, Escudero Contreras A, et al. The value of IgA antiphospholipid testing for diagnosis of antiphospholipid (hughes) syndrome in systemic lupus erythematosus. *J Rheumatol*. 2001;28(12):2637-2643.

35. Pignatelli P, Ettorre E, Menichelli D, et al. Seronegative antiphospholipid syndrome: refining the value of “non-criteria” antibodies for diagnosis and clinical management. *Haematologica*. 2020;105(3):562-572.

36. Tortosa C, Cabrera-Marante O, Serrano M, et al. Incidence of thromboembolic events in asymptomatic carriers of IgA anti β2 glycoprotein-I antibodies. *PLoS One*. 2017;12(7):e0178889.