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ORIGINAL ARTICLE

Anti-domain 1 of beta2-glycoprotein I aids risk stratification in lupus anticoagulant-positive patients

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Abstract

Lupus anticoagulant (LA) is considered a risk factor for thromboembolism (TE) and adverse pregnancy outcomes (APOs). However, quite a few patients diagnosed with LA positivity do not suffer these adverse events. Further testing of anticardiolipin (aCL), anti-beta2-glycoprotein I (anti-β2GPI) or anti-domain 1 of β2GPI (anti-D1) may help to assess the occurrence risk of TE and APOs. Therefore, we aimed to study how to stratify LA-positive patients. In our study, 167 LA-positive patients were consecutively enrolled from January 2015 to December 2016. Serum aCL and anti-β2GPI (IgG, IgM and IgA) and anti-D1 IgG were simultaneously measured. Among these patients, 114 (68.3%) were followed for an average of 36.5 months for TE and APOs. The outcomes showed that 105 patients experienced TE and/or APOs, and 62 patients were LA carriers. Anti-D1 had good consistency with triple positivity (LA+, aCL+, anti-β2GPI+) (kappa = 0.742). Elevated anti-D1 was related to increased risks for TE [odds ratio (OR) 29.87, 95% confidence interval (CI) 8.05–110.74] and APOs (OR 8.73, 95% CI 3.41–22.31). Area under curve showed that the diagnostic power of anti-D1 for TE and APOs was 0.856 (95% CI 0.743–0.970) and 0.682 (95% CI 0.599–0.765), respectively. Survival analysis revealed that patients with high anti-D1 titres had a high cumulative incidence of APOs (hazard ratio 4.66, 95% CI 1.46–14.87). In conclusion, anti-D1, based on good consistency with triple positivity in LA-positive patients, has a stronger association with TE and APOs and, to some degree, could predict pregnancy outcomes. Therefore, anti-D1 may aid risk stratification in LA-positive patients.

Keywords Lupus anticoagulant · Thromboembolism · Adverse pregnancy outcomes · Anti-domain 1 of beta2-glycoprotein I · Antiphospholipid antibodies

Introduction

Antiphospholipid antibodies (aPL) are a heterogeneous family of autoantibodies present in the circulation and are frequently associated with thromboembolism (TE) or adverse pregnancy outcomes (APOs), which are the clinical manifestations of antiphospholipid syndrome (APS) [1]. Furthermore, aPL, such as anticardiolipin (aCL), anti-beta2-glycoprotein I (anti-β2GPI) and lupus anticoagulant (LA), are not only detected for the diagnosis of APS but are also deemed to play a pathogenic role in the occurrence of TE or APOs [2, 3]. A large number of studies assessing the risk of adverse events in patients with aPL showed that lupus anticoagulant (LA) was associated strongly with thrombosis and pregnancy morbidity [4–7]. However, quite a few patients with LA positivity may not suffer any adverse events for a long time [8]. Therefore, a well-stratified method for use in LA-positive patients is necessary in clinical practice and may play a key role in the promotion of individualized treatment.

Currently, anti-β2GPI is measured by targeting β2GPI antigen directly; the LA assay is mainly mediated by antibodies targeting prothrombin and β2GPI, and aCL positivity is primarily determined by β2GPI-dependent aPL [9]. Obviously, β2GPI is the most significant antigen targeted by aPL. However, it is still debated whether anti-β2GPI is the most clinically relevant test because of its unsatisfactory specificity [10]. However, it has been shown that antibodies specifically targeting domain 1 of β2GPI (anti-D1) associate

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much stronger with a history of thrombosis and pregnancy morbidity than antibodies targeting other domains of β2GPI [11–14]. In addition, in mouse models, purified anti-D1 IgG from APS patients or monoclonal anti-D1 IgG have been shown to induce thrombosis and foetal loss [15, 16]. And domain 1 has been proved to protect mice from thrombosis caused by aPL, which is considered a proof-of-concept for the pathogenic role of anti-D1 [17].

Therefore, in this study, we aimed to explore whether anti-D1 could well stratify patients with LA positivity for the risk of onset of TE or APOs to thereby improve the intervention programme for these patients.

Materials and methods

Patients and samples

From January 2015 to December 2016, adult patients from outpatients and inpatients of Peking University Third Hospital who had tested positive for LA on two or more occasions at least 12 weeks apart were consecutively enrolled. We excluded patients who were older than 70 years, considering that elderly patients have a high probability of LA false positivity [18]. By reviewing the patients' medical records, the following clinical data were obtained: demographic and clinical characteristics, reasons for testing for LA, the history of TE and APOs events, and treatment. According to the classification criteria for definite Antiphospholipid Syndrome [1], TE was defined as one or more clinical episodes of arterial, venous, or small vessel thrombosis in any tissue or organ. In addition, APOs were defined here as follows: unexplained spontaneous abortion at < 10 weeks, with maternal or hormonal abnormalities and maternal and paternal chromosomal causes excluded; or unexplained death of a morphologically normal foetus at ≥ 10 weeks; or premature birth of a morphologically normal neonate at < 34 weeks because of placental insufficiency. Patients who persistently tested positive for LA but did not suffer TE and APOs were classified as "LA carrier". All included patients were simultaneously examined for aCL and anti-β2GP1 (IgG, IgM and IgA) and anti-D1 IgG antibodies, and were followed up for the occurrence of TE or APOs.

The study was performed in accordance with the Helsinki Declaration and was approved by the Peking University Third Hospital Medical Science Research Ethics Committee (ID number is IRB00006761-2016055). Informed consent was obtained from all patients.

Measurement of autoantibodies

For LA measurement, blood was collected using vacuum tubes (Becton Dickinson Medical Devices Co, Ltd, Franklin Lakes, New Jersey) containing 3.2% trisodium citrate (0.109 M). Platelet-poor plasma was obtained by double centrifugation within 2 h (15 min, 2000*g*). Blood samples for other aPL detection were collected in serum separation tubes and spun within 2 h (10 min, 2000*g*). Plasma and serum were stored at − 80 °C.

LA was determined according to the updated ISTH Lupus Anticoagulant Guidelines [18]. Screening and confirmatory test of Silica Clotting Time (SCT) and Diluted Russell's Viper Venom time (dRVVT) (Instrumentation Laboratory, Bedford, MA, USA) were performed on the automated coagulation analyser (ACL TOP®, Instrumentation Laboratory, Spain). The cut-off levels of the normalized ratio were 1.16 for SCT and 1.11 for dRVVT.

Serum aCL (IgG, IgM and IgA), anti-β2GPI (IgG, IgM and IgA) and anti-D1 IgG were detected by a chemiluminescence immunoassay (QUANTA Flash® assays, INOVA Diagnostics, Inc., San Diego, CA) on a BIO-FLASH Chemiluminescent Analyser (Biokit S. A., Barcelona, Spain) according to the manufacturer's instructions. The cut-off values for positivity of antibodies above were 20.0 CU according to the recommendations. The positivity of aCL/anti-β2GPI was defined as at least one subtype (IgG, IgM and IgA) positive.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 20 (SPSS, Inc., Chicago, IL) and GraphPad Prism 5.01 (GraphPad Software, San Diego, CA). The ratio of the LA difference was tested using one-way ANOVA, and a nonparametric Mann–Whitney *U* test was used to analyse the difference of antibody titres between groups. Cohen's kappa agreement was used to assess the consistency. The odds ratio (OR) was used to evaluate the association between aPL and clinical events. Receiver operating characteristic (ROC) curves were used to calculate the area under the curve (AUC) and defined the cut-off value. A Kaplan–Meier survival analysis and log-rank test were applied to estimate the cumulative incidence of APOs in the follow-up patients.

Results

Patient characteristics

A total of 167 LA-positive patients, aged 18–66 years, were included in the study. Among them, 16 patients experienced TE, 84 patients had a history of APOs, 5 patients had both TE and APOs, and 62 patients were LA carriers. Their clinical details are shown in Table 1. Compared with those in the LA carrier group, the dRVVT ratio (dRVVT-R) and SCT ratio (SCT-R) were significantly increased in patients with

Table 1 Demographic and clinical characteristics of LA-positive patients

Characteristics	Number
N (%)	167 (100)
Sex, *n* (%)	
Female	156 (93.4)
Male	11 (6.6)
Age, mean (SD), year	32.6 (8.1)
Reasons for testing, *n* (%)	
Antiphospholipid syndrome (APS)	64 (38.3)
Primary APS	40 (23.9)
APS associated with other diseases	24 (14.4)
Systemic lupus erythematosus	32 (19.1)
Thyroiditis	9 (5.4)
Undifferentiated connective tissue disease	8 (4.8)
Sclerosis	4 (2.4)
Thrombocytopenia	3 (1.8)
Ankylosing spondylitis	3 (1.8)
Behçet's disease	1 (0.6)
Infertility	29 (17.4)
Early miscarriage	14 (8.4)
Clinical manifestations, *n* (%)	
Thromboembolism	16 (9.6)
Venous thromboembolism	10 (6.0)
Arterial thromboembolism	5 (3.0)
Venous + arterial thromboembolism	1 (0.6)
Adverse pregnancy outcomes	84 (50.3)
Thromboembolism + adverse pregnancy outcomes	5 (3.0)
LA carrier	62 (37.1)

a history of TE ($p < 0.0001$ and $p < 0.05$, respectively) and APOs ($p < 0.0001$ and $p < 0.05$, respectively), especially in patients who had both TE and APOs ($p < 0.0001$ and $p < 0.0001$, respectively). In addition, patients with TE had a higher dRVVT-R than patients with APOs only ($p < 0.05$) (Fig. 1).

Anti-D1 showed good consistency with triple positivity

Antiphospholipid antibodies profiles (summarized in Table 2) in LA-positive patients displayed that 75 patients expressed LA positivity only, 34 patients exhibited double positivity (LA+, aCL+, anti-β2GP1− or LA+, aCL−, anti-β2GP1+) and 58 patients showed triple positivity (LA+, aCL+, anti-β2GP1+). There were 60 patients showing the presence of anti-D1. Moreover, a higher anti-D1 value was observed in triple-positive patients (223.6, 42.8–737.2) than in double-positive (3.6, 3.6–56.5) and isolated LA-positive patients (3.6, 3.6–3.6) ($p < 0.0001$ and $p < 0.0001$, respectively). Anti-D1 titres of double-positive patients were also higher than those of isolated LA-positive patients ($p = 0.008$) (Fig. 2a).

Furthermore, the percentages of anti-D1-positive patients in the different groups were similar to those of triple-positive patients (Table 2). Therefore, a coherence analysis between anti-D1 and triple positivity was performed. The overall agreement between anti-D1 and triple positivity was 88.02%, with a Cohen kappa coefficient of 0.742 ($p < 0.0001$).

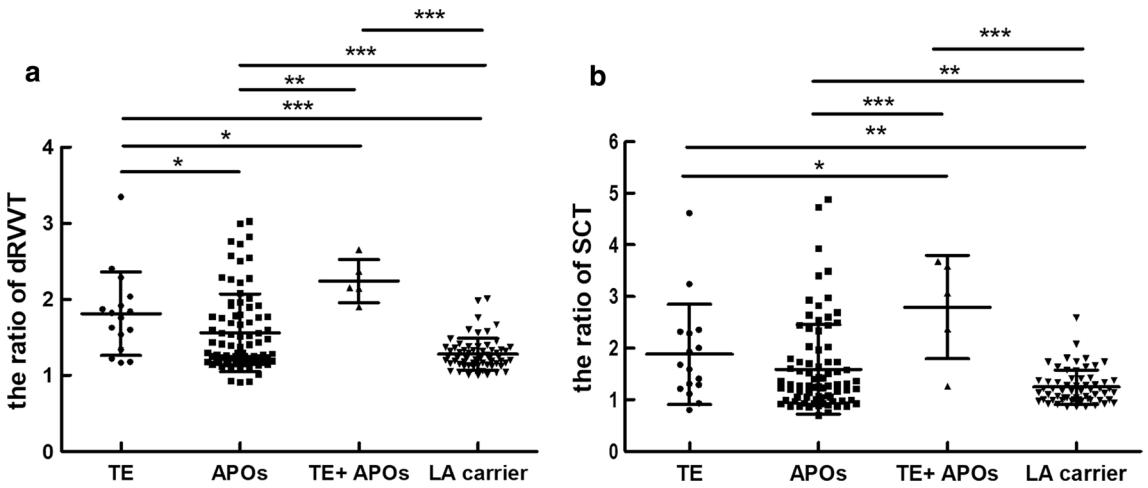

Fig. 1 The ratio of dRVVT (**a**) and SCT (**b**) in LA-positive patients with TE ($n = 16$), APOs ($n = 84$), TE + APOs ($n = 5$) and LA carriers ($n = 62$). *dRVVT* Diluted Russell's Viper Venom time, *SCT* Silica Clotting Time, *TE* thromboembolism, *APOs* adverse pregnancy outcomes. *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$

Anti-D1 correlated more strongly with TE or APOs in LA-positive patients

LA-positive patients were regrouped into three parts, including 21 patients with TE, 89 with APOs (5 patients had both TE and APOs), and 62 LA carriers. The median and interquartile range of anti-D1 titres in the TE, APOs and LA carrier groups were 121.1 (31.8–407.5), 6.5 (3.6–259.6) and 3.6 (3.6–3.8), respectively. The levels of anti-D1 were significantly increased in TE and APOs group compared with those in the LA carrier group ($p < 0.001$ and $p < 0.001$, respectively) (Fig. 2b). The OR values for TE and APOs are shown in Table 3. Compared with the other aPL, anti-D1 had the highest OR for TE (29.87, 95% CI 8.05–110.74) and for APOs (8.73, 95% CI 3.41–22.31). Next, the ROC curve analysis for aPL was performed. The AUC of anti-D1 was 0.856 (0.734–0.970) for TE and 0.682 (0.599–0.765) for APOs. The optimal cut-off value of anti-D1 to help predict TE and APOs was 24.6 CU (the sensitivity and specificity were 81% and 90.3%) and 32.4 CU (the sensitivity and specificity were 57.2% and 91.9%), respectively (Table 3).

Table 2 Antiphospholipid antibodies profiles in LA-positive patients

	TE ($n=16$)	APOs ($n=84$)	TE + APOs ($n=5$)	LA carrier ($n=62$)
LA only (single positive)	2 (14.5%)	33 (39.3%)	0	40 (64.5%)
LA+ aCL+ anti-β2GP1− (double positive)	0	2 (2.4%)	0	3 (4.8%)
LA+ anti-β2GP1+ aCL− (double positive)	3 (18.8%)	14 (16.7%)	1 (20.0%)	11 (17.7%)
LA+ aCL+ anti-β2GP1+ (triple positive)	11 (68.8%)	35 (39.3%)	4 (80.0%)	8 (12.9%)
LA+ anti-D1+	11 (68.8%)	38 (42.9%)	5 (100.0%)	6 (9.7%)

TE thromboembolism, *APOs* adverse pregnancy outcomes

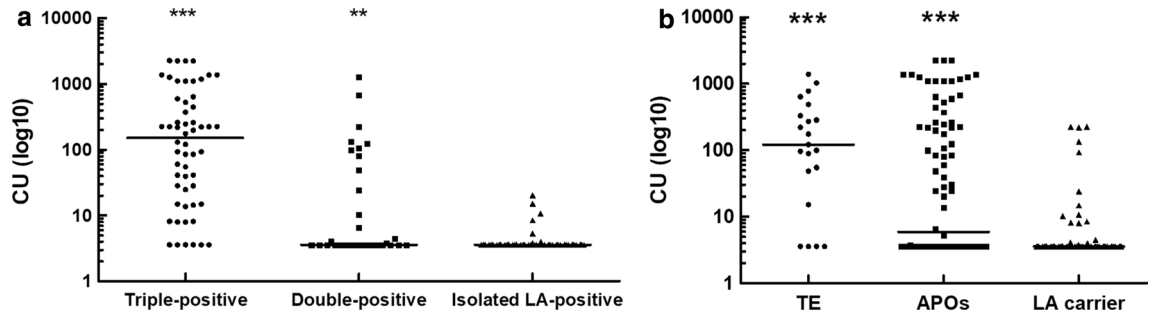

Fig. 2 Titres of anti-D1 in different antiphospholipid antibodies profiles (**a**) and clinical manifestations (**b**). Triple-positive (LA+, aCL+ and anti-β2GP1+, $n=75$), double-positive (LA+, aCL+, and anti-β2GP1− or LA+, aCL−, and anti-β2GP1+, $n=34$), and isolated LA positivity ($n=58$). *TE* thromboembolism ($n=21$), *APOs* adverse pregnancy outcomes ($n=89$), LA carrier ($n=62$). *CU* chemiluminescent units. $^{**}p<0.01$; $^{***}p<0.0001$

Table 3 Odds ratio and clinical utility of aPL for thromboembolism or adverse pregnancy outcomes in patients with LA positivity

aPL	OR (95% CI)		AUC (95% CI)	
	TE	APOs	TE	APOs
aCL IgG	15.71 (4.73–52.21)	3.61 (1.46–8.91)	0.847 (0.730–0.942)	0.657 (0.571–0.742)
aCL IgM	4.63 (0.94–22.72)	2.77 (0.74–10.39)	0.696 (0.550–0.842)	0.649 (0.553–0.726)
aCL IgA	8.55 (2.43–30.08)	4.80 (1.80–11.98)	0.778 (0.655–0.901)	0.654 (0.567–0.740)
Anti-β2GPI IgG	19.64 (5.74–67.26)	5.59 (2.29–13.65)	0.865 (0.760–0.976)	0.680 (0.594–0.758)
Anti-β2GPI IgM	2.16 (0.93–7.31)	1.12 (0.54–2.34)	0.626 (0.483–0.769)	0.604 (0.511–0.697)
Anti-β2GPI IgA	3.56 (0.92–13.85)	2.79 (0.79–6.73)	0.720 (0.582–0.857)	0.612 (0.522–0.702)
Triple positive	18.67 (5.07–56.21)	5.62 (2.54–12.85)	0.922 (0.846–0.999)	0.719 (0.640–0.799)
Anti-D1	29.87 (8.05–110.74)	8.73 (3.41–22.31)	0.856 (0.743–0.970)	0.682 (0.599–0.765)

OR odds ratio, *CI* confidence interval, *AUC* area under curve, *TE* thromboembolism, *APOs* adverse pregnancy outcomes

Titres of anti-D1 predicted pregnancy outcomes for LA-positive patients

Among all included patients, 114 patients (68.3%) were followed for an average of 36.5 months for the occurrence of TE or APOs. During the follow-up, none of patients suffered TE events; we observed that 37 patients were pregnant, whose details are shown in Table 4. There were 15 patients suffering APOs. The pregnancy outcomes were 1 early miscarriage (< 10 weeks), 7 foetal deaths (≥ 10 weeks), and 7 preterm births due to placenta insufficiency (< 34 weeks). The levels of anti-D1 were significantly increased compared with those in the non-APOs patients. Then, based on the anti-D1 cut-off value (32.4 CU), 37 pregnant LA-positive patients were divided into two groups: a high-value group ($n = 16$) and a low-value group ($n = 21$). The Kaplan–Meier survival analysis showed that high anti-D1 titres were associated with a significantly higher cumulative incidence of APOs compared with those in the low-value group ($p = 0.009$), as shown in Fig. 3, and the hazard ratio (HR) was 4.66 (95% CI 1.46–14.87).

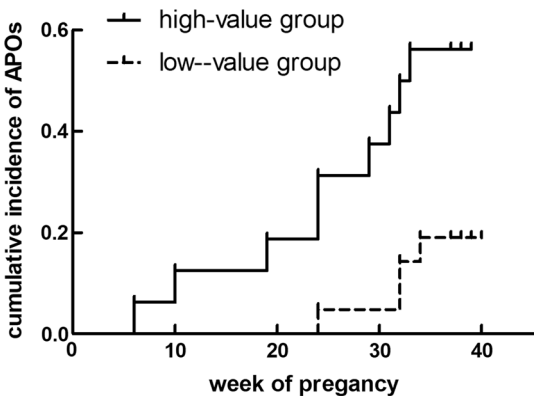

Fig. 3 Cumulative incidence of APOs in the high- and low-value groups of anti-D1 in the pregnant LA-positive patients during follow-up. high-value group ($n = 16$): anti-D1 ≥ 32.4 CU; low-value group ($n = 21$): anti-D1 < 32.4 CU. *APOs* adverse pregnancy outcomes

Discussion

The present study was aimed to find better indicators to assess the occurrence risk of TE or APOs in patients with LA positivity by simultaneously analysing aCL, anti-β2GP1 (IgG, IgM and IgA) and anti-D1 IgG. We found that anti-D1 showed good consistency with triple positivity (88.02%, kappa = 0.742) and presented a stronger association with TE (OR 29.87, 95% CI 8.05–110.74) and APOs (OR 8.73, 95% CI 3.41–22.31) than did aCL, anti-β2GP1 or triple positivity. Furthermore, in the follow-up patients, we found that high anti-D1 titres had a significantly higher cumulative incidence of APOs (HR 4.66, 95% CI 1.46–14.87). To some degree, anti-D1 could help predict pregnancy outcomes for LA-positive patients.

There is a general consensus that the higher the titres of LA, the greater is the risk for thrombosis and pregnancy morbidity. In LA-positive patients we studied, 62.87% (105/167) of them had a history of TE and APOs, and the rest were LA carriers. In contrast, with LA carriers, dRVVT-R and SCT-R were significantly increased in patients with TE and APOs, especially in patients who had both TE and APOs. Moreover, we found that the proportion of isolated LA was small (53.8%) in patients with adverse events. However, in LA carriers, the proportion of isolated LA was larger (64.5%), and the values of dRVVT-R and SCT-R were lower. Therefore, these findings may suggest that non-TE and non-APOs LA are more frequently weakly positive and isolated. Nevertheless, no data indicate that weakly positive LA or isolated LA is not clinically relevant and what level should be defined as weakly positive LA [5]. Therefore, it is

Table 4 Clinical characteristics and pregnancy outcomes of LA-positive pregnant patients during follow-up

	APOs ($n = 15$)	non-APOs ($n = 22$)	p
Age mean (SD), year	29.5 (3.50)	30.2 (3.46)	0.555
Anti-D1/CU	372.2 (3.6–1108.9)	3.6 (3.6–20.1)	0.004*
Risk factors, *n* (%)			
Antiphospholipid syndrome	13 (86.7)	12 (54.5)	0.073
Systemic lupus erythematosus	2 (13.3)	2 (9.1)	> 0.999
Undifferentiated connective tissue disease	0	3 (13.6)	0.257
Hypertension	2 (13.3)	7 (31.8)	0.262
History of APOs	14 (93.3)	18 (81.8)	0.629
Treatment, *n* (%)			
Low molecular weight heparin	9 (60.0)	16 (72.7)	0.488
Low dose aspirin	10 (100.0)	18 (81.8)	0.283

APOs adverse pregnant outcomes

*Statistical significance ($p < 0.05$)

recommended that these people should be monitored continuously and treated properly. Then, in patients with TE or APOs, we found that there were 35.2% (37/105) patients with the absence of both aCL and anti-β2GPI (IgG/IgM/IgA). This phenomenon may be due to the existence of other antibodies, such as anti-annexin A5 and anti-prothrombin/anti-phosphatidylserine antibodies, which have been proven to display LA activity [19–22].

The multiple positivity of aPL profiles is increasingly considered an important parameter for evaluating the risk of thrombosis and obstetrical complications [23, 24]. We observed that 80% of patients had triple positivity in the group with both TE and APOs events, 68.8% patients were triple positivity in the TE group, and 39.3% of patients were triple positivity in the APOs group, but these percentages were still higher than that in the LA carrier group (12.9%). Interestingly, we discovered that the percentages of anti-D1-positive patients were quite similar to those of triple positivity in the different groups. In addition, anti-D1 had good consistency with triple positivity (kappa = 0.742). Moreover, the levels of anti-D1 were significantly higher in patients with triple positivity compared to those in patients with double or single positivity, which agreed with previous studies [13, 14, 25, 26]. Thus, these findings suggest that anti-D1 might be a marker of high-risk aPL profiles. Furthermore, compared with aCL and anti-β2GP1, anti-D1, in line with previous data, showed the highest OR for TE (29.87, 95% CI 8.05–110.74) [13, 21]. In our study, anti-D1 also had the highest OR for APOs (8.73, 95% CI 3.41–22.31), which is different from the former observations that anti-D1 had a weaker association with pregnancy morbidity [13]. However, a recent study, which included patients who had anti-β2GP1 IgG positivity, at least one previous pregnancy and no associated systemic autoimmune diseases, showed that anti-D1 had the highest OR (7.3, 95% CI 2.1–25.5) for late pregnancy morbidity [27]. These results should not be surprising, as the levels of anti-D1 were much higher in patients with APOs events than in patients without adverse events. The mechanism by which anti-D1 causes vessel occlusion and pregnancy failure is not elusive at present. The pathogenetic explanation might be that the anti-D1 recognized domain 1 of β2GP1 can disrupt the annexin A5 anticoagulant shield of trophoblasts and endothelial cells and expose the phosphatidylserine (PS) to thereby activate coagulation system, increase tissue factor (TF) activity of macrophages [15] and promote vascular cell adhesion molecule (VCAM) expression on endothelial cells and tumour necrosis factor α (TNF-α) released by monocytes [28], and activate the complement cascade [16]. Above all, anti-D1 seems to have potential for identifying LA-positive patients at high risk for TE and APOs.

Moreover, by analysing the 37 pregnant LA-positive patients followed, we found that the high-value group had a much higher cumulative incidence of APOs. Therefore, this result suggests that high expression of anti-D1 confers a high risk for pregnancy complications in LA-positive patients. Among 15 patients suffering APOs, 1 patient was a LA carrier without a history of TE and APOs. Therefore, we recommend that women showing the presence of LA be advised to undergo testing for anti-D1 regardless of whether they have a history of adverse events. If a woman has a higher level of anti-D1, she should be advised to receive treatment that will possibly prevent adverse clinical events. Therefore, LA-positive patients can be stratified through the titres of anti-D1, which is an easy way to facilitate clinical determination. However, we do not mean that anti-D1 can replace the classic aPL panel, considering the unsatisfactory sensitivity of anti-D1. However, anti-D1 might contribute to a better, easier and more complete risk stratification for LA-positive patients.

Nevertheless, our study has some limitations. First, there is a bias in our studied population, as our hospital has a good reputation in obstetrics, gynaecology and reproductive science, which might reduce the value of anti-D1 to evaluate the risk for thrombosis. Second, we used samples from a single centre. A multicentre study is necessary to clarify the anti-D1 role.

Conclusions

In LA-positive patients, anti-D1, based on a good agreement with triple-positive aPL, showed a stronger association with the occurrence of TE or APOs and predicted the pregnancy outcomes to some degree. Therefore, anti-D1 may be a useful tool to aid risk stratification for LA-positive patients.

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Author contributions HG designed the research, interpreted data, performed statistical analysis and wrote the manuscript; YZ designed the research, interpreted data, AL and CW collected samples; SY and YZ analysed samples; JZ reviewed the manuscript; and RQ designed the research, identified sample characteristics and reviewed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies were got approval from the Ethics Committee of Peking University Third Hospital according to the 1964 Helsinki Declaration and its latter amendments or comparable ethical standards.

Informed consent Informed consent was signed from all participants included in the study.

References

1. 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;4:295–306. https://doi.org/10.1111/j.1538-7836.2006.01753.x.
2. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: understanding the antibodies. Nat Rev Rheumatol. 2011;7:330–9.
3. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. N Engl J Med. 2013;368:1033–44.
4. Lockshin MD, Kim M, Laskin CA, et al. Prediction of adverse pregnancy outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies. Arthritis Rheum. 2012;64:2311–8. https://doi.org/10.1002/art.34402.
5. Yelnik CM, Laskin CA, Porter TF, et al. Lupus anticoagulant is the main predictor of adverse pregnancy outcomes in aPL-positive patients: validation of PROMISSE study results. Lupus Sci Med. 2016;3:e000131. https://doi.org/10.1136/lupus-2015-000131.
6. Gebhart J, Posch F, Koder S, et al. Increased mortality in patients with the lupus anticoagulant: the Vienna Lupus Anticoagulant and Thrombosis Study (LATS). Blood. 2015;125:3477–83. https://doi.org/10.1182/blood-2014-11-611129.
7. Urbanus RT, Siegerink B, Roest M, Rosendaal FR, de Groot PG, Algra A. Antiphospholipid antibodies and risk of myocardial infarction and ischaemic stroke in young women in the RATIO study: a case-control study. Lancet Neurol. 2009;8:998–1005. https://doi.org/10.1016/S1474-4422(09)70239-X.
8. Pengo V, Biasiolo A, Gresele P, et al. A comparison of lupus anticoagulant-positive patients with clinical picture of antiphospholipid syndrome and those without. Arterioscler Thromb Vasc Biol. 2007;27:e309–10. https://doi.org/10.1161/ATVBAHA.107.153536.
9. Bertolaccini ML, Amengual O, Andreoli L, et al. 14th international congress on antiphospholipid antibodies task force. Report on antiphospholipid syndrome laboratory diagnostics and trends. Autoimmun Rev. 2014;13:917–30. https://doi.org/10.1016/j.autrev.2014.05.001.
10. de Groot PG, Urbanus RT. The significance of autoantibodies against beta2-glycoprotein I. Blood. 2012;120:266–74. https://doi.org/10.1182/blood-2012-03-378646.
11. de Laat B, de Groot PG. Autoantibodies directed against domain I of beta2-glycoprotein I. Curr Rheumatol Rep. 2011;13:70–6.
12. de Laat B, Derksen RH, Urbanus RT, de Groot PG. IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. Blood. 2005;105:1540–5. https://doi.org/10.1182/blood-2004-09-3387.
13. de Laat B, Pengo V, Pabinger I, et al. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. J Thromb Haemost. 2009;7:1767–73. https://doi.org/10.1111/j.1538-7836.2009.03588.x.
14. Pengo V, Ruffatti A, Tonello M, et al. Antiphospholipid syndrome: antibodies to domain 1 of beta2-glycoprotein 1 correctly classify patients at risk. J Thromb Haemost. 2015;13:782–7. https://doi.org/10.1111/jth.12865.
15. Pericleous C, Ruiz-Limon P, Romay-Penabad Z, et al. Proof-of-concept study demonstrating the pathogenicity of affinity-purified IgG antibodies directed to domain I of beta2-glycoprotein I in a mouse model of anti-phospholipid antibody-induced thrombosis. Rheumatology. 2015;54:722–7. https://doi.org/10.1093/rheumatology/keu360.
16. Agostinis C, Durigutto P, Sblattero D, et al. A non–complement-fixing antibody to β2 glycoprotein I as a novel therapy for antiphospholipid syndrome. Blood. 2014;123:3478–87. https://doi.org/10.1182/blood-2013-11-537704.
17. Ioannou Y, Romay-Penabad Z, Pericleous C, et al. In vivo inhibition of antiphospholipid antibody-induced pathogenicity utilizing the antigenic target peptide domain I of beta2-glycoprotein I: proof of concept. J Thromb Haemost. 2009;7:833–42. https://doi.org/10.1111/j.1538-7836.2009.03316.x.
18. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on lupus anticoagulant/antiphospholipid antibody of the scientific and standardisation committee of the international society on thrombosis and haemostasis. J Thromb Haemost. 2009;7:1737–40. https://doi.org/10.1111/j.1538-7836.2009.03555.x.
19. de Laat B, Wu XX, van Lummel M, Derksen RH, de Groot PG, Rand JH. Correlation between antiphospholipid antibodies that recognize domain I of beta2-glycoprotein I and a reduction in the anticoagulant activity of annexin A5. Blood. 2007;109:1490–4. https://doi.org/10.1182/blood-2006-07-030148.
20. Amengual O, Forastiero R, Sugiura-Ogasawara M, et al. Evaluation of phosphatidylserine-dependent antiprothrombin antibody testing for the diagnosis of antiphospholipid syndrome: results of an international multicentre study. Lupus. 2017;26:266–76. https://doi.org/10.1177/0961203316660203.
21. Lee JS, Gu J, Park HS, Yoo HJ, Kim HK. Coexistence of anti-beta2-glycoprotein I domain I and anti-phosphatidylserine/prothrombin antibodies suggests strong thrombotic risk. Clin Chem Lab Med. 2017;55:882–9. https://doi.org/10.1515/cclm-2016-0676.
22. Bertolaccini ML, Sciascia S, Murru V, Garcia-Fernandez C, Sanna G, Khamashta MA. Prevalence of antibodies to prothrombin in solid phase (aPT) and to phosphatidylserine-prothrombin complex (aPS/PT) in patients with and without lupus anticoagulant. Thromb Haemost. 2013;109:207–13.
23. Otomo K, Atsumi T, Amengual O, et al. Efficacy of the antiphospholipid score for the diagnosis of antiphospholipid syndrome and its predictive value for thrombotic events. Arthritis Rheum. 2012;64(2):504–12. https://doi.org/10.1002/art.33340.
24. Oku K, Amengual O, Atsumi T. Antiphospholipid scoring: significance in diagnosis and prognosis. Lupus. 2014;23:1269–72. https://doi.org/10.1177/0961203314537361.
25. De Craemer AS, Musial J, Devreese KM. Role of anti-domain 1-beta2 glycoprotein I antibodies in the diagnosis and risk stratification of antiphospholipid syndrome. J Thromb Haemost. 2016;14:1779–87. https://doi.org/10.1111/jth.13389.
26. Iwaniec T, Kaczor MP, Celinska-Lowenhoff M, Polanski S, Musial J. Clinical significance of anti-domain 1 beta2-glycoprotein I antibodies in antiphospholipid syndrome. Thromb Res. 2017;153:90–4. https://doi.org/10.1016/j.thromres.2017.02.019.
27. Chighizola CB, Pregnolato F, Andreoli L, et al. Beyond thrombosis: anti-beta2GPI domain 1 antibodies identify late pregnancy morbidity in anti-phospholipid syndrome. J Autoimmun. 2018;90:76–83. https://doi.org/10.1016/j.jaut.2018.02.002.
28. Chighizola CB, Pregnolato F, Andreoli L, et al. Autoantibodies specific to a peptide of beta2-glycoprotein I cross-react with TLR4, inducing a proinflammatory phenotype in endothelial cells and monocytes. Blood. 2012;120:3360–70. https://doi.org/10.1182/blood-2011-09-378851.