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Original article

Antiphospholipid patterns predict risk of thrombosis in systemic lupus erythematosus

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Abstract

Objective. We evaluated which aPL combinations increase the risk of future thrombosis in patients with SLE.
Methods. This prospective cohort study consisted of SLE patients who had been tested for all seven aPL (LA, aCL isotypes IgM, IgG and IgA, and anti-β_2-glycoprotein I isotypes IgM, IgG and IgA). Pooled logistic regression was used to assess the relationship between aPL and thrombosis.
Results. There were 821 SLE patients with a total of 75 048 person-months of follow-up. During the follow-up we observed 88 incident cases of thrombosis: 48 patients with arterial, 37 with venous and 3 with both arterial and venous thrombosis. In individual models, LA was the most predictive of any [age-adjusted rate ratio 3.56 (95% CI 2.01, 6.30), $P < 0.0001$], venous [4.89 (2.25, 10.64), $P < 0.0001$] and arterial [3.14 (1.41, 6.97), $P = 0.005$] thrombosis. Anti-β_2-glycoprotein I IgA positivity was a significant risk factor for any [2.00 (1.22, 3.3), $P = 0.0065$] and venous [2.8 (1.42, 5.51), $P = 0.0029$] thrombosis. Only anti-β_2-glycoprotein I IgA appeared to add significant risk to any [1.73 (1.04, 2.88), $P = 0.0362$] and venous [2.27 (1.13, 4.59), $P = 0.0218$] thrombosis among those with LA. We created an interaction model with four categories based on combinations of LA and other aPL to look at the relationships between combinations and the risk of thrombosis. In this model LA remained the best predictor of thrombosis.
Conclusion. Our study demonstrated that in SLE, LA remained the best predictor of thrombosis and adding additional aPL did not add to the risk, with the exception of anti-β_2-glycoprotein I IgA.

Key words: systemic lupus erythematosus, thrombosis, antiphospholipid antibodies, lupus anticoagulant, anticardiolipin, anti-β2-glycoprotein I, IgA isotype

CLINICAL SCIENCE

Rheumatology key messages

- In SLE, LA is still the best predictor of venous and arterial thrombosis.
- Adding other aPL with IgG and IgM isotypes did not add to the risk.
- The presence of anti-β2-glycoprotein I IgA is associated with thrombosis in SLE patients.

Introduction

APS has been classified as the development of venous and/or arterial thromboses, and/or pregnancy morbidity, in the presence of persistently raised levels of either the LA, aCL or anti-β_2-glycoprotein I [1]. The classification of APS can only be made if at least one clinical and one persistent laboratory criterion are met.

aPL were first described in patients with SLE [2, 3]. They are present in 11–40% of patients with SLE [4–6]. APS is a significant cause of morbidity and mortality in patients with SLE. In a 10-year prospective study, thrombosis was found as the cause in 26.7% of SLE patients who died, and was always associated with the presence of aPL [7].

It is well known that LA positivity is more strongly associated with both arterial and venous thrombosis than either aCL or anti-β_2-glycoprotein I antibodies [8, 9]. An unanswered question is which combinations of positive aPL add to the thrombosis risk.

Currently, the Sydney APS classification criteria include the IgG and IgM isotypes as a laboratory criterion

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[1]. However, there is controversy in the literature about the role of IgM isotypes. Large studies found that IgM aCL and IgM anti-β_2-glycoprotein I are not associated with thrombotic events [9–13]. Moreover, elevated titres of IgA isotypes have been shown to be associated with thrombosis [10, 14–16]. Recent publications have confirmed that anti-β_2-glycoprotein I IgA is a risk factor for the development of APS thrombosis [17–20].

The definition of persistent positivity in the Sapporo criteria (1999) [21] was 6 weeks, but was changed in the Sydney criteria (2006) [1] to 12 weeks. In our experience, aPL titres in SLE patients can fluctuate over time and contribute risk even at low and moderate titres [22]. Cross-sectional studies miss the true prevalence of aPL in SLE. In this prospective study, we evaluated which aPL combinations were associated with an increase in risk of future thrombosis in patients with SLE, using our longitudinal cohort in which patients were seen by protocol every 3 months.

Methods

Patient population

The Hopkins Lupus Cohort is a prospective longitudinal cohort of SLE patients ongoing since 1987. It was approved by the Johns Hopkins University School of Medicine Institutional Review Board on an annual basis. Informed written consent was obtained from all subjects. SLE patients were diagnosed according to revised ACR and SLICC criteria [23, 24]. At enrolment, a comprehensive medical history, including date of SLE diagnosis and information on prior thrombosis, was obtained from medical records and the patient. Visits were scheduled quarterly or more frequently, if medically necessary. At each clinic visit, laboratory tests were performed to complete SLE activity indices and for aPL (DRVVT and aCL at every visit; anti-β_2-glycoprotein, most recently available, or cohort entry).

Measurement of aPL

This analysis included SLE patients who had been tested for all seven aPL: LA, aCL isotypes IgM, IgG and IgA, and anti-β_2-glycoprotein I isotypes IgM, IgG and IgA. The DRVVT with confirmatory testing was performed as published [25]. Anti-β_2-glycoprotein I testing became available after 2003. This analysis was based on cohort experience from the first measurement of anti-β_2-glycoprotein I through October 2019. aCL and anti-β_2-glycoprotein I protein (ELISA IgG, IgM, IgA; Inova Diagnostics, San Diego, CA, USA) were defined as positive when the antibody titre exceeded 20 units (medium titre, following the Sydney classification criteria). The LA was determined by DRVVT with mixing studies and confirmatory studies if prolonged [25]. It was defined as positive if a patient had a DRVVT of 45 s or more, and a positive confirm ratio of more than 1.4. We excluded DRVVT measures if the patients were taking anticoagulants (warfarin/heparin). Among those visits with DRVVT of 45 s or more, 22% had missing confirm ratios. These values were imputed using multiple imputations.

Definition of the thromboses

The past history of thrombotic events was determined at cohort entry by review of all historical records and patient interviews, and was updated at subsequent visits. Any thrombosis was defined as any of the following: venous thrombosis [deep venous thrombosis (DVT), pulmonary embolus (PE) or other venous thromboses] or any arterial thrombosis [myocardial infarction (MI), cerebrovascular accident (CVA), digital ischaemia, or other arterial thromboses]. Venous thromboses were defined by ultrasound or venogram. Arterial thrombosis, in case of stroke, was identified by brain MRI or CT and, in case of MI, by appropriate electrocardiographic changes, creatine kinase or troponin change, or cardiac imaging. Other arterial thrombosis was identified as appropriate for the site involved. Patients who had a history of thrombosis before their first measurement of anti-β_2-glycoprotein I were excluded. Follow-up for each patient was either the first incident thrombosis or end of follow-up, which was the last recorded visit in the database.

Statistical analyses

For this prospective analysis, we constructed a dataset with one record for each month of follow-up for each patient. At each person-month, it contained a variable indicating whether the patient had experienced any thrombosis in that month. In addition, each record contained the aPL information of the patient supplied at the most recent prior clinic visit. In some instances, anti-β_2-glycoprotein I was not assessed at each visit, due to cost. Of those patients who had anti-β_2-glycoprotein I assessments, 62% had it measured only once. We used the most recent evaluation of this variable at a prior visit in our analysis.

Rates of thrombosis were calculated as the number of events, divided by the number of person-months at risk, and results were converted to rates per 1000 person-years. To assess the relationship between aPL and thrombosis, we used pooled logistic regression [26]. For analyses, including LA, we ran it for each imputed dataset. 'PROC MIANALYZE' in SAS 9.4 (SAS Institute, Cary, NC, USA) was then used to pool the results to obtain summary estimates. All rate ratios (RR) were adjusted for age at the person month.

Results

There were 821 patients with a complete profile of 7 aPL with a total of 75 048 person-months of follow-up. Of the 821 patients, 94% were female, 51% were Caucasian, 40% were African-American and 50% were $<$30 years old at the time of SLE diagnosis. Forty-two percent of the patients entered the cohort within 1 year of their SLE diagnosis. The cumulative classification

criteria were 48% malar rash, 18% discoid rash, 50% photosensitivity, 53% oral ulcer, 72% arthritis, 47% serositis, 43% renal disorder, 8% neurological disorder, 70% hematological disorder, 87% immunological disorder and 98% ANA positivity, based on revised ACR classification criteria [24]. Additional SLICC classification criteria included 19% direct Coombs test, 58% low C3, 50% low C4 and 12% low CH50 [23].

Among these patients, there were 88 incident cases of thrombosis: 48 patients with arterial [CVA ($n = 20$), MI ($n = 12$), digital ischaemia ($n = 8$) or other arterial thromboses ($n = 8$)]; 37 patients with venous thrombosis [DVT/PE ($n = 33$) or other venous thromboses ($n = 4$)]; and 3 patients with both arterial and venous thrombosis (1 patient with DVT/PE and other arterial thrombosis, 1 patient with CVA and other venous thrombosis, and 1 patient with DVT and MI). The mean (S.D.) disease duration of SLE at the time of thrombosis for the 88 patients was 15 (9.3) years.

Tables 1–3 show the relationship between any, venous and arterial thrombotic events, and the most recent past assessment of each aPL. In individual models, LA was the most predictive of any [RR 3.56 (95% CI 2.01, 6.30), $P < 0.0001$], venous [4.89 (2.25, 10.64), $P < 0.0001$] and arterial [3.14 (1.41, 6.97), $P = 0.005$] thrombosis. Anti-β_2-glycoprotein I IgA positivity was a significant risk factor for any [2.00 (1.22, 3.30), $P = 0.0065$] and venous [2.8 (1.42, 5.51), $P = 0.0029$] thrombosis. There was no strong evidence that any aPL other than LA significantly associated with arterial thrombosis. aCL IgA showed a higher RR [3.08 (0.75, 12.65), $P = 0.1195$)] for any thrombosis, but it did not reach statistical significance, likely due to the small number of events and the small number with aCL IgA positivity.

Since the aPL are correlated, we examined whether an association between each specific aPL and thrombosis was seen after adjustment for the presence of LA. We found that the association between anti-β_2-glycoprotein I IgA any [1.73 (1.04, 2.88), $P = 0.0362$] and venous [2.27 (1.13, 4.59), $P = 0.0218$] thrombosis persisted after adjustment for LA. aCL IgG, IgM and IgA, and anti-β_2-glycoprotein I IgG and IgM positivity were not associated with increased risk of any, venous and arterial thrombosis after adjusting for LA. For aCL IgA, although the point estimate showed a positive association, the number of patients was relatively low, and this relationship did not reach statistical significance (Table 4).

Next, we fitted a model to look at the relationships between combinations of LA and other aPL and the risk of any, venous and arterial thrombosis. We created four categories based on combinations of LA and other aPL: both negative; both positive; LA positive, aPL (i) negative; LA negative, aPL (i) positive; where aPL (i) could be aCL IgG/M/A or anti-β_2-glycoprotein I IgG/M/A. In this interaction model, LA remained the best predictor of thrombosis. The risk of any thrombosis after adding anti-β_2-glycoprotein I IgA to LA was 2.3 times the risk for those who had LA without anti-β_2-glycoprotein I IgA. However, it did not reach statistical significance ($P = 0.1253$). Adding other aPL with different isotypes did not seem to increase the risk of any thrombosis (Table 5).

Incident cases of thrombosis were reduced when we looked at venous and arterial thrombosis separately. In combinations with aCL, we did not find any cases with venous thrombosis. Adding other aPL (anti-β_2-glycoprotein I IgG/IgM) with different isotypes did not increase the risk of venous thrombosis. Anti-β_2-glycoprotein I IgA numerically increased the risk of venous thrombosis

Table 1 Relationship between any thrombosis and the most recent past assessment of each aPL

aPL		No. of any thrombotic events	Person-years	Rate per 1000 person-years	Age-adj. RR (95% CI)	*P*-value
LA	(−)	74	5936	12.5	1.00 (ref)	
	(+)	14	318	44.0	**3.56 (2.01, 6.30)**	**<.0001**
aCL-G	(−)	84	5986	14.0	1.00 (ref)	
	(+)	4	268	14.9	1.1 (0.4, 3)	0.859
aCL-M	(−)	85	6000	14.2	1.00 (ref)	
	(+)	3	254	11.8	0.83 (0.26, 2.61)	0.7446
aCL-A	(−)	86	6209	13.9	1.00 (ref)	
	(+)	2	45	44.4	3.08 (0.75, 12.65)	0.1195
aB_2GPI-G	(−)	84	6056	13.9	1.00 (ref)	
	(+)	4	198	20.2	1.48 (0.54, 4.05)	0.4442
aB_2GPI-M	(−)	79	5557	14.2	1.00 (ref)	
	(+)	9	697	12.9	0.91 (0.46, 1.81)	0.7797
aB_2GPI-A	(−)	68	5447	12.5	1.00 (ref)	
	(+)	20	807	24.8	**2.00 (1.22, 3.3)**	**0.0065**

aCL-G: aCL IgG; aCL-M: aCL IgM; aCL-A: aCL IgA; aB_2GPI-G: anti-β_2-glycoprotein I IgG; aB_2GPI-M: anti-β_2-glycoprotein I IgM; aB_2GPI-A: anti-β_2-glycoprotein I IgA; age-adj. RR: age-adjusted rate ratio. Bold values: LA was the most predictive of any [3.56 (2.01, 6.30), $P < 0.0001$] thrombosis. Anti-β_2-glycoprotein I IgA positivity was also a significant risk factor for any [2.00 (1.22, 3.3), $P = 0.0065$] thrombosis.

Table 2 Relationship between venous thrombosis and the most recent past assessment of each aPL

aPL		No. of venous thrombotic events	Person-years	Rate per 1000 person-years	Age-adj. RR (95% CI)	*P*-value
LA	(−)	32	5660	5.7	1.00 (ref)	
	(+)	8	284	28.2	**4.89 (2.25, 10.64)**	**<0.0001**
aCL-G	(−)	38	5688	6.7	1.00 (ref)	
	(+)	2	256	7.8	1.06 (0.26, 4.43)	0.9334
aCL-M	(−)	38	5704	6.7	1.00 (ref)	
	(+)	2	239	8.4	1.31 (0.32, 5.43)	0.7123
aCL-A	(−)	39	5900	6.6	1.00 (ref)	
	(+)	1	44	23.0	4.2 (0.57, 30.97)	0.1592
aB_2GPI-G	(−)	37	5745	6.4	1.00 (ref)	
	(+)	3	198	15.1	2.22 (0.68, 7.23)	0.1845
aB_2GPI-M	(−)	35	5267	6.6	1.00 (ref)	
	(+)	5	677	7.4	1.13 (0.44, 2.88)	0.803
aB_2GPI-A	(−)	28	5168	5.4	1.00 (ref)	
	(+)	12	776	15.5	**2.8 (1.42, 5.51)**	**0.0029**

aCL-G: aCL IgG; aCL-M: aCL IgM; aCL-A: aCL IgA; aB_2GPI-G: anti-β_2-glycoprotein I IgG; aB_2GPI-M: anti-β_2-glycoprotein I IgM; aB_2GPI-A: anti-β_2-glycoprotein I IgA; age-adj. RR: age-adjusted rate ratio. Bold values: LA was the most predictive of venous [4.89 (2.25, 10.64), $P < 0.0001$] thrombosis. Anti-β_2-glycoprotein I IgA positivity was also a significant risk factor for venous [2.8 (1.42, 5.51), $P = 0.0029$] thrombosis.

Table 3 Relationship between arterial thrombosis and the most recent past assessment of each aPL

Antiphospholipid antibody		No. of arterial thrombotic events	Person-years	Rate per 1000 person-years	Age-adj. RR (95% CI)	*P*-value
LA	(-)	44	5770	7.6	1.00 (ref)	
	(+)	7	299	23.4	**3.14 (1.41, 6.97)**	**0.005**
aCL-G	(−)	48	5811	8.3	1.00 (ref)	
	(+)	3	258	11.6	1.6 (0.5, 5.18)	0.4322
aCL-M	(−)	49	5821	8.4	1.00 (ref)	
	(+)	2	248	8.1	0.92 (0.22, 3.79)	0.9078
aCL-A	(−)	50	6029	8.3	1.00 (ref)	
	(+)	1	41	24.6	2.32 (0.31, 17.12)	0.4095
aB_2GPI-G	(−)	49	5878	8.3	1.00 (ref)	
	(+)	2	191	10.5	1.35 (0.33, 5.58)	0.6751
aB_2GPI-M	(−)	46	5383	8.5	1.00 (ref)	
	(+)	5	687	7.3	0.85 (0.34, 2.14)	0.727
aB_2GPI-A	(−)	42	5305	7.9	1.00 (ref)	
	(+)	9	764	11.8	1.53 (0.74, 3.14)	0.2499

aCL-G: aCL IgG; aCL-M: aCL IgM; aCL-A: aCL IgA; aB_2GPI-G: anti-β_2-glycoprotein I IgG; aB_2GPI-M: anti-β_2-glycoprotein I IgM; aB_2GPI-A: anti-β_2-glycoprotein I IgA; age-adj. RR: age-adjusted rate ratio. Bold value: LA was the most predictive of arterial [3.14 (1.41, 6.97), $P = 0.005$] thrombosis.

among those with LA, but statistically it was not significant ($P = 0.1081$). In arterial thrombosis, we did not find any additive risk of anti-β_2-glycoprotein I IgA to LA. In combinations with aCL IgA or anti-β_2-glycoprotein I IgG, we did not find any cases with arterial thrombosis.

Discussion

Based on the present prospective analysis of a large number of SLE patients, our study demonstrated that out of three aPL, LA remained the best predictor of risk of any, venous and arterial thrombosis. The presence of anti-β_2-glycoprotein I IgA, which is not a part of APS classification criteria, was also independently associated with thrombosis in SLE patients. Only anti-β_2-glycoprotein I IgA provides additional predictive value for thrombosis among SLE patients with LA positivity.

In most studies, it has been shown that in SLE, LA is the main predictor of thrombosis [9, 27–29]. In contrast, a few studies have suggested that the association of single LA positivity with thrombosis was weaker [30, 31]. In a population-based case–control study, LA with the concomitant positivity of anti-β_2-glycoprotein I or anti-PT

Table 4 Additive effect of other aPL adjusting for LA

Antibody combinations	ANY thrombosis		VENOUS thrombosis		ARTERIAL thrombosis	
	Age-adj. RR (95% CI)	*P*-value	Age-adj. RR (95% CI)	*P*-value	Age-adj. RR (95% CI)	*P*-value
Model 1: LA + aCL-G						
LA (+) *vs* (−)	3.9 (2.14, 7.09)	<0.0001	5.8 (2.56, 13.15)	<0.0001	3.11 (1.34, 7.23)	0.0084
aCL-G (+) *vs* (−)	0.63 (0.22, 1.81)	0.3910	0.47 (0.1, 2.13)	0.3289	1.04 (0.3, 3.62)	0.9449
Model 2: LA + aCL-M						
LA (+) *vs* (−)	3.88 (2.16, 6.98)	<0.0001	5.09 (2.28, 11.36)	<0.0001	3.41 (1.5, 7.78)	0.0035
aCL-M (+) *vs* (−)	0.53 (0.16, 1.73)	0.2938	0.76 (0.17, 3.31)	0.7157	0.61 (0.14, 2.63)	0.5083
Model 3: LA + aCL-A						
LA (+) *vs* (−)	3.42 (1.9, 6.13)	<0.0001	4.67 (2.11, 10.34)	0.0001	3.06 (1.36, 6.88)	0.007
aCL-A (+) *vs* (−)	1.86 (0.44, 7.89)	0.3981	2.14 (0.28, 16.56)	0.4659	1.57 (0.21, 11.97)	0.6623
Model 4: LA + aB_2GPI-G						
LA (+) *vs* (−)	3.68 (2.01, 6.71)	<0.0001	4.85 (2.09, 11.28)	0.0002	3.23 (1.41, 7.44)	0.0058
aB_2GPI-G (+) *vs* (−)	0.84 (0.29, 2.41)	0.7408	1.03 (0.29, 3.73)	0.9617	0.84 (0.19, 3.69)	0.8172
Model 5: LA + aB_2GPI-M						
LA (+) *vs* (−)	3.71 (2.08, 6.64)	<0.0001	4.99 (2.26, 11.02)	<0.0001	3.32 (1.47, 7.47)	0.0038
aB_2GPI-M (+) *vs* (−)	0.76 (0.38, 1.52)	0.4341	0.88 (0.34, 2.3)	0.8008	0.72 (0.28, 1.83)	0.4875
Model 6: LA + aB_2GPI-A						
LA (+) *vs* (−)	3.16 (1.76, 5.68)	0.0001	3.95 (1.77, 8.83)	0.0008	2.96 (1.31, 6.68)	0.0091
aB_2GPI-A (+) *vs* (−)	**1.73 (1.04, 2.88)**	**0.0362**	**2.27 (1.13, 4.59)**	**0.0218**	1.33 (0.64, 2.78)	0.4469

aCL-G: aCL IgG; aCL-M: aCL IgM; aCL-A: aCL IgA; aB_2GPI-G: anti-β_2-glycoprotein I IgG; aB_2GPI-M: anti-β_2-glycoprotein I IgM; aB_2GPI-A: anti-β_2-glycoprotein I IgA; age-adj. RR: age-adjusted rate ratio.

Table 5 Relationship between aPL with LA and risk of ANY thrombosis

Antibody combinations		No. of events	Person-years	Rate (per 1000 person-years)	Age-adj. RR (95% CI)	*P*-value
(1) LA and aCL-G	LA (+), aCL-G (−)	11	232	47.4	1.00 (ref)	
	LA (+), aCL-G (+)	3	86	34.7	0.76 (0.21, 2.74)	0.6715
	LA (-), aCL-G (+)	1	181	5.5	0.12 (0.02, 0.93)	0.042
	LA (-), aCL-G (−)	73	5754	12.7	0.27 (0.14, 0.51)	<0.0001
(2) LA and aCL-M	LA (+), aCL-M (−)	12	254	47.2	1.00 (ref)	
	LA (+), aCL-M (+)	2	64	31.2	0.63 (0.14, 2.85)	0.5537
	LA (−), aCL-M (+)	1	190	5.3	0.11 (0.01, 0.84)	0.0334
	LA (−), aCL-M (−)	73	5746	12.7	0.26 (0.14, 0.49)	<0.0001
(3) LA and aCL-A	LA (+), aCL-A (−)	13	303	42.9	1.00 (ref)	
	LA (+), aCL-A (+)	1	16	64.1	1.42 (0.18, 11)	0.7352
	LA (−), aCL-A (+)	1	29	33.9	0.74 (0.1, 5.74)	0.7739
	LA (−), aCL-A (−)	73	5906	12.4	0.29 (0.16, 0.52)	<0.0001
(4) LA and aB_2GPI-G	LA (+), aB_2GPI-G (−)	11	246	44.7	1.00 (ref)	
	LA (+), aB_2GPI-G (+)	3	72	41.4	0.96 (0.27, 3.46)	0.9481
	LA (−), aB_2GPI-G (+)	1	126	7.9	0.18 (0.02, 1.39)	0.1000
	LA (−), aB_2GPI-G (−)	73	5810	12.6	0.28 (0.15, 0.53)	<0.0001
(5) LA and aB_2GPI-M	LA (+), aB_2GPI-M (−)	11	234	47.1	1.00 (ref)	
	LA (+), aB_2GPI-M (+)	3	85	35.4	0.73 (0.2, 2.64)	0.6333
	LA (−), aB_2GPI-M (+)	6	612	9.8	0.2 (0.08, 0.56)	0.0018
	LA (−), aB_2GPI-M (−)	68	5324	12.8	0.27 (0.14, 0.51)	<0.0001
(6) LA and aB_2GPI-A	LA (+), aB_2GPI-A (−)	7	220	31.8	1.00 (ref)	
	LA (+), aB_2GPI-A (+)	7	98	71.4	2.28 (0.8, 6.52)	0.1253
	LA (−), aB_2GPI-A (+)	13	709	18.3	0.58 (0.23, 1.45)	0.2438
	LA (−), aB_2GPI-A (−)	61	5227	11.7	0.37 (0.17, 0.8)	0.0119

aCL-G: aCL IgG; aCL-M: aCL IgM; aCL-A: aCL IgA; aB_2GPI-G: anti-β_2-glycoprotein I IgG; aB_2GPI-M: anti-β_2-glycoprotein I IgM; aB_2GPI-A: anti-β_2-glycoprotein I IgA; age-adj. RR: age-adjusted rate ratio.

showed the highest thrombosis risk. In contrast, single LA positivity without anti-β_2-glycoprotein I or anti-PT did not carry an increased risk for DVT [32].

In the present study having anti-β_2-glycoprotein I IgA appeared to add significant risk to any thrombosis risk, and venous thrombosis risk in patients who were also LA positive. However, in arterial thrombosis, there was no strong evidence that any other aPL had additive risk to LA. Other groups have suggested that different antibody profiles were better predictors of thrombosis risk status. The best known profile is called 'triple positivity', which requires simultaneous positivity of LA, aCL and anti-β_2-glycoprotein I [33]. Sciascia *et al*. [34] evaluated 23 possible combinations of aPL profiles in SLE patients, and found that combining LA, anti-β_2-glycoprotein I and aPS-PT had the best diagnostic accuracy for both thrombosis and pregnancy loss. A Japanese group devised an antiphospholipid score (aPL-S) by testing multiple aPL, including three tests for LA (aPTT, kaolin clotting time and DRVVT) and six antiphospholipid assays (IgG and IgM aCL, IgG and IgM anti-β_2-glycoprotein I, IgG and IgM aPS-PT) to diagnose APS and to predict the risk of thrombosis [35].

Other than LA, we found that only anti-β_2-glycoprotein I IgA was an independent significant risk factor for any and venous thrombosis. IgA isotypes of aPL were not included in the Sydney APS criteria, due to conflicting data on the association with APS manifestations [1]. Although acceptance of the IgA isotype in APS remains controversial, its diagnostic and clinical significance in SLE had been well demonstrated. Since 2012, the IgA isotype of the aPL has been accepted as a laboratory criterion for SLE in the SLICC SLE Classification Criteria [23]. Anti-β_2-glycoprotein I IgA antibodies are frequently seen in SLE patients, with a high association with thrombosis [36]. In a study of patients with isolated IgA anti-β_2-glycoprotein I positivity, the increased risk for thromboembolic events was observed only in patients with SLE [37]. A 5-year follow-up of 248 asymptomatic individuals with isolated IgA anti-β_2-glycoprotein I positivity showed that the presence of anti-β_2-glycoprotein I IgA was an independent risk factor for APS events [18]. The pathogenic role of anti-β_2-glycoprotein I IgA in APS patients has also been demonstrated in an experimental mouse model. Purified IgA anti-β_2-glycoprotein I isolated from APS patients were injected into mice, and femoral vein clotting was observed. IgA anti-β_2-glycoprotein I antibodies were thrombogenic and upregulated tissue factor in mice [38]. These data support that IgA isotypes play a role in the pathogenesis of both primary or secondary APS.

There is controversy over the clinical significance of IgM isotypes, which are part of the Sydney APS classification criteria [12, 39, 40]. In SLE, IgM isotypes were not found to be associated with thrombosis [11, 13]. Similarly, in a systematic review, aCL IgM was not predictive for thrombosis [9]. Moreover, it has been shown that IgM aCL is not associated with lifetime thrombosis risk in SLE [10, 11]. In addition, IgA anti-β_2-glycoprotein I was more strongly associated with DVT and with stroke than was IgM [19]. IgA anti-β_2-glycoprotein I antibodies in secondary APS patients showed better utility than that provided by the IgM isotype [20]. In the present study, neither IgG nor IgM isotypes provide additional predictive value for thrombosis among SLE patients who were LA positive.

aPL thrombosis in SLE patients is thought to be greater with persistent aPL positivity. The definition of 'persistent positivity' was 6 weeks in the Sapporo criteria (1999) [21] and revised to 12 weeks in the Sydney criteria (2006) [1]. However, in SLE, aPL often fluctuate and are often at low or moderate titres, which still confer risk [41–43]. In Hopkins Lupus Cohort, SLE patients were seen quarterly and were tested for aPL (LA and aCL) at every visit. This allowed us to observe this fluctuation during routine clinical practice. The clinical significance of this fluctuation is still under debate. Some studies reported that the increased risk of thrombosis in SLE was associated with the presence of LA and with a persistently positive aCL [41, 44]. Male *et al*. [45] found, in multivariate analyses, that LA was the strongest predictor of thrombosis, even if grouping patients with one or two positive tests. For transiently positive antibodies, the strength of the association with thrombosis decreased for aCL and aPT, but remained the same for LA and anti-β_2-glycoprotein I. Therefore, they suggested serial testing is required only for aCL and aPT. We have shown that LA also fluctuates in SLE 20 years after diagnosis [46].

The main advantages of our study are a large number of patients with SLE, and the prospective assessment of both aPL and the thrombotic events. Retrospectively designed and cross-sectional studies on aPL were unable to assess the temporal relationship with APS manifestations in SLE patients.

This study is not without limitations. Although patients in this cohort were tested for aCL and LAat each visit, 60% of patients were measured for anti-β_2-glycoprotein I only once, due to cost. Thus, we could not compare the risk of thrombosis between persistently and transiently anti-β_2-glycoprotein I-positive patients. A second limitation is that even given the large number of patients, the number of thrombotic events in same categories was small.

In conclusion, LA remained the strongest predictor of the risk of thrombosis, and in individual models, anti-β_2-glycoprotein I IgA showed a significant association with thrombosis. Moreover, anti-β_2-glycoprotein I IgA was the only aPL that increased the risk for future thrombosis among patients who were also LA positive. This is the first US SLE cohort study to examine categories of combinations of aPL and thrombosis risk. Particularly for SLE, the 'triple positive' category did not confer additional risk over LA alone; however, anti-β_2-glycoprotein I IgA (an isotype not considered in 'triple positivity' [33] did.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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