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Association of IgA Antiphospholipid Antibodies With Lupus Anticoagulant Phenotype and Characterization of T-Cell Immune Deviation

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ABSTRACT

Background: The serological diagnosis of antiphospholipid syndrome (APS) has long relied on IgG/IgM antiphospholipid antibodies (aPLs), whereas the clinical significance of IgA aPL remains controversial. Meanwhile, the role of T-cell immune imbalance in APS pathogenesis is increasingly recognized, but its association with specific antibody isotypes and the pathway by which it influences the lupus anticoagulant (LA) phenotype have not been systematically elucidated.

Objective: To evaluate the associations of different aPL isotypes with peripheral blood lymphocyte subsets and coagulation parameters, and to determine whether IgA aPL are independently associated with LA positivity within the measured antibody panel and how this relates to immune deviation.

Methods: A total of 212 patients who underwent aPL profiling (including IgA isotypes), lymphocyte subset analysis, and LA testing were retrospectively enrolled. Spearman correlation analysis was used to evaluate the correlations among antibody isotypes, immune cell subsets, and coagulation indices. Independent-samples *t*-test compared lymphocyte subsets between IgA-positive and IgA-negative groups. A bootstrap mediation model was constructed to examine whether IgA aPL mediate the relationship between $CD8^+$ T cells and the LA phenotype. Multivariable logistic regression, adjusted for inflammatory markers, age, sex, and immune cell subsets, was used to identify factors independently associated with composite laboratory LA positivity. Antibody concentrations were $\log_{10}$-transformed, and odds ratios (ORs) are expressed per 1-unit increase in $\log_{10}$ antibody concentration. Benjamini–Hochberg (BH) false discovery rate (FDR) correction was applied to exploratory correlation analyses.

Results: In unadjusted analyses, IgA aPL correlated positively with the percentage of $CD8^+$ T cells ($r = 0.170$, $p = 0.038$) and negatively with absolute $CD4^+$ T-cell count ($r = -0.173$, $p = 0.035$) and the CD4/CD8 ratio ($r = -0.182$, $p = 0.026$); however, after BH-FDR correction, most lymphocyte–aPL correlations were exploratory, and only the CD19+ B-cell/ACL-IgA correlation was borderline significant ($q \approx 0.050$). The absolute $CD4^+$ T-cell count in the IgA-positive group ($n = 14$) was significantly lower than that in the IgA-negative group ($n = 198$) (382.42 ± 232.17 vs. 637.72 ± 366.96, $p = 0.019$). Regarding coagulation, the correlation coefficients between IgA aPL and the LA surrogate marker ndRVVT-R ranged from 0.39 to 0.41 (both $p < 0.01$); ACL-IgA showed significant positive correlations with D-dimer ($r = 0.20$) and fibrin/fibrinogen (Fib) degradation product (FDP) ($r = 0.19$) (both $p < 0.01$), and these remained significant after FDR correction (D-dimer $q = 0.0013$; FDP $q = 0.0064$). Mediation analysis revealed no significant mediating effect of IgA aPL on the relationship between $CD8^+$ T cells and the LA phenotype (the 95% CI of the indirect effect included 0). In multivariable logistic regression adjusted for age, sex, NLR, and immune cell subsets, β_2-glycoprotein

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I (β2GPI)-IgA (OR = 1.101, 95% CI: 1.018–1.191, $p = 0.016$) and ACL-IgA (OR = 1.078, 95% CI: 1.023–1.137, $p = 0.005$) were each independently associated with LA positivity in separate models, whereas $CD4^+$, $CD8^+$, $CD19^+$ cells, and NLR were not statistically significant.

Conclusions: Within the measured antibody panel, IgA aPL were independently associated with LA positivity and showed a distinct immune association pattern characterized by reduced absolute $CD4^+$ T cells and a relative increase in CD8+ T-cell percentage. However, mediation analysis did not support IgA aPL as mediators (Ms) of the $CD8^+$–LA relationship. These findings are hypothesis-generating and require prospective validation with Treg phenotyping, apoptotic/PS exposure markers, and aPS/prothrombin time (PT) testing.

1 | Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by recurrent vascular thromboembolism, pathological pregnancy, and persistent positivity for antiphospholipid antibodies (aPLs). The current Sydney classification criteria identify lupus anticoagulant (LA), anti-cardiolipin (aCL) antibodies of the IgG/IgM isotypes, and anti-β_2-glycoprotein I (β2GPI) antibodies of the IgG/IgM isotypes as the core serological evidence for laboratory diagnosis [1, 2]. However, the limitations of this framework have been repeatedly demonstrated in clinical practice: a substantial proportion of patients with typical thrombotic or pregnancy loss histories remain persistently negative for the standard antibody profile and are consequently classified as "seronegative APS," a diagnostic gray zone lacking clear diagnostic criteria and therapeutic guidance [3]. The management dilemma of such patients stems fundamentally from the neglect of non-classical antibody isotypes—particularly IgA aPL—in the current criteria. Studies have indicated that the positivity rates of IgA aCL and IgA β2GPI in systemic lupus erythematosus-associated APS can be as high as 25.42% and 20.34%, respectively, and that these antibodies are associated with thrombotic events independently of the IgG/IgM isotypes [4, 5]. The exclusion of IgA aPL from the diagnostic framework may therefore result in the systematic underdiagnosis of a subset of APS patients with distinct immunopathological characteristics [6].

Although the epidemiological associations are becoming clearer, the core mechanism underlying the pathogenicity of IgA aPL remains far from being elucidated. Two critical knowledge gaps exist in the current literature. First, previous studies have largely focused on the direct associations of IgA aPL with hard clinical endpoints such as thrombosis and pregnancy morbidity, with little attention paid to their quantitative relationship with an intermediate phenotype—namely, LA. LA is considered the strongest predictor of thrombosis in APS [7], and its essence reflects the capacity of an antibody to interfere with phospholipid-dependent coagulation reactions in vitro [8]. Elucidating whether and to what extent IgA aPL can produce an LA effect constitutes the key functional evidence for establishing their pathogenic role. Second, the production of aPL is not an isolated humoral immune event but is deeply embedded in the immune network of T-B-cell collaborative dysregulation [9]. Clinical observations have documented that patients with primary APS exhibit total lymphopenia, decreased absolute $CD4^+$ T-cell counts, an inverted CD4/CD8 ratio, and an elevated proportion of activated $CD8^+$ T cells in the circulation [8–11]. In animal models, T cells are essential for the development and transfer of experimental APS, as T cell-depleted bone marrow cells failed to transfer the disease, whereas anti-CD4 treatment prevented aPL production and clinical manifestations [12, 13]. These findings strongly suggest that immune deviation within the T-cell compartment is an upstream immunological abnormality driving aPL production.

Nevertheless, studies integrating these two lines of evidence—the LA effect of IgA aPL and T-cell immune deviation—remain absent. A central question remains to be answered: is the abnormal distribution of peripheral blood lymphocyte subsets a pan-feature of APS, or is it specifically associated with a particular antibody isotype? If the latter, how should the causal chain among immune deviation, IgA aPL, and the LA phenotype be constructed? $CD4^+$ T-cell depletion may impair the regulation of autoreactive B cells and promote their class switching toward the production of IgA antibodies, which could then exert LA effects and procoagulant activity. In observational studies, however, univariate correlations between immune cells and coagulation indices are highly susceptible to confounding by covariation; therefore, multivariable models are required to dissect the independent contributions, and mediation analysis must be employed to test the proposed transmission pathways.

Against this background, the present study utilized a retrospective cohort of 212 patients who had undergone comprehensive aPL profiling (including IgA isotypes), peripheral blood lymphocyte subset analysis, and complete coagulation assessment. Through a systematic multistep analysis, we aimed to evaluate, rather than establish, the proposed pathway. The specific objectives were as follows: (1) to characterize the differential association patterns of different aPL isotypes (IgG, IgM, and IgA) with peripheral blood T-cell, B-cell, and NK-cell subsets; (2) to quantitatively compare the strengths of association between each antibody isotype and LA-sensitive assays as well as in vivo markers of fibrinolytic activation (D-dimer, FDP); and (3) to test whether IgA aPL statistically mediate the relationship between immune deviation and the LA phenotype through the combined application of mediation analysis and multivariable logistic regression, thereby clarifying whether IgA aPL are independently associated with LA positivity within the measured panel.

2 | Methods

2.1 | Study Design and Population

This was a retrospective cohort study that enrolled patients who visited Peking University Third Hospital between July 2025 and

July 2026 for suspected APS, systemic lupus erythematosus, unexplained thrombotic events, a history of pathological pregnancy, or other autoimmune diseases. All enrolled patients had concurrently undergone aPL profiling, peripheral blood lymphocyte subset analysis, and coagulation testing during the same clinical encounter. The study was approved by the Ethics Committee of Peking University Third Hospital (Approval Number S2024674).

2.1.1 | Inclusion Criteria

1. Patients for whom both "aPL profile (including IgA isotype)" and "lymphocyte subset" tests were clinically ordered within the study period.
2. Age ≥ 18 years.
3. Complete data: all test results were traceable, with no missing key variables.

2.1.2 | Exclusion Criteria

1. Critical missing data: incomplete results for the aPL profile (aCL IgG/IgM/IgA, β2GPI IgG/IgM/IgA) or lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16/CD56^+$).
2. Interference from anticoagulation: patients receiving therapeutic-dose anticoagulation (warfarin, direct oral anticoagulants) at the time of blood collection as this would compromise the accuracy of LA testing.
3. Severe hepatic or renal dysfunction: end-stage liver disease (Child-Pugh class C) or end-stage renal disease.
4. Active infection or malignancy: conditions that could significantly affect the distribution of peripheral blood lymphocyte subsets.

2.2 | Laboratory Measurements

2.2.1 | Lymphocyte Subset Analysis

Percentages and absolute counts of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, $CD16/CD56^+$ NK cells, and total $CD45^+$ lymphocytes were measured by flow cytometry. The CD4/CD8 ratio was calculated. Inflammatory markers, including SII, SIRI, NLR, dNLR, PLR, MLR, NPR, and PIV, were calculated from complete blood counts, as described in the Supporting Information.

2.2.2 | Coagulation and LA Testing

Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (Fib), and fibrin/Fib degradation products (FDPs) were measured on an ACL TOP 700 coagulation analyzer (Werfen). LA was assessed using two methods—silica clotting time (SCT) and dilute Russell's viper venom time (dRVVT)—performed on the same instrument. In accordance with the International Society on Thrombosis and Haemostasis (ISTH) guidelines, LA positivity was defined as a normalized SCT or dRVVT ratio >1.1 with confirmatory evidence of phospholipid-dependent inhibition. Individual assay ratios, including nSCT-R and ndRVVT-R, were reported separately as continuous laboratory measures. The D-dimer was measured by an immunoturbidimetric assay.

2.2.3 | aPL Profiling

Serum aCL IgG/IgM/IgA and anti-β2GPI IgG/IgM/IgA antibodies were detected using chemiluminescence immunoassays (CIA, QUANTA Flash assays, Inova Diagnostics) on a BIO-FLASH instrument (Biokit). All operations followed the manufacturer's standard operating protocols, and the manufacturer-recommended cut-off values were used to define positivity. Other routine blood parameters were obtained from the Department of Laboratory Medicine.

2.3 | Statistical Analysis

All data analyses were performed using SPSS 26.0 (IBM Corp.) and R software (version 4.2.1). The normality of continuous variables was assessed by the Shapiro–Wilk test. Normally distributed continuous variables are presented as mean $\pm$ standard deviation, while non-normally distributed ones are expressed as median (interquartile range). Categorical variables are presented as frequency (percentage). All tests were two-sided, and a p-value < 0.05 was considered statistically significant. For exploratory correlation analyses, Benjamini–Hochberg (BH) false discovery rate (FDR) adjustment was applied within each correlation matrix. Correlations with $q < 0.05$ after FDR correction were considered statistically significant; those with unadjusted $p < 0.05$ but $q \geq 0.05$ were described as exploratory. Exact p-values and q-values are provided in Tables S6–S9.

Correlation analysis: Spearman's rank correlation analysis was used to evaluate the correlations among lymphocyte subsets, aPL isotypes, and coagulation parameters, with correlation coefficients reported as r.

Between-group comparisons: patients were divided into two groups according to IgA aPL positivity. The correct IgA-positive subgroup is $n = 14$, and the IgA-negative group is $n = 198$ (total $n = 212$). Between-group differences in lymphocyte subsets and coagulation parameters were compared using independent-samples t-test (for normally distributed data) or Mann–Whitney U-test (for non-normally distributed data).

Mediation analysis: to examine whether IgA aPL mediate the effect of $CD8^+$ T cells on the LA phenotype, simple mediation models were constructed with $CD8^+$ T-cell percentage as the independent variable (X), β2GPI-IgA or ACL-IgA as the mediator (M), and normalized dRVVT ratio (ndRVVT-R) as the dependent variable (Y). The indirect effect ($a \times b$) and its 95% confidence interval (CI) were estimated using the bootstrap method with 5000 resamples; an indirect effect was considered statistically significant if the 95% CI did not include zero.

Multivariable logistic regression analysis: with composite laboratory LA positivity as the dependent variable, age, sex, NLR, absolute $CD4^+$ T-cell count, $CD8^+$ T-cell percentage, absolute $CD19^+$ B-cell count, and IgA aPL (represented separately by ACL-IgA and β2GPI-IgA) were entered as independent variables using the enter method. Results are expressed as odds ratios (ORs) with

95% CI. Antibody concentrations were $\log_{10}$-transformed, and ORs are expressed per 1-unit increase in $\log_{10}$ antibody concentration. The model with β2GPI-IgA served as the primary model, and the model with ACL-IgA was used as a sensitivity analysis. Model diagnostics, including VIF and Hosmer–Lemeshow tests, were assessed (Table S11). Because the IgA-positive subgroup was small, sensitivity analyses were performed, and multivariable estimates were interpreted cautiously.

3 | Results

3.1 | Baseline Characteristics of the Study Population

A total of 212 patients were enrolled in this study. The cohort was predominantly female, with 155 women (73.11%) and 57 men (26.89%). The mean age was 45.55 ± 16.46 years (mean ± SD) (Table 1 and Tables S1–S5).

The distribution of diagnostic categories was as follows: primary APS, 4 (1.9%); SLE-associated APS, 3 (1.4%); SLE without APS, 18 (8.5%); other autoimmune diseases, 9 (4.2%); haematological diseases, 8 (3.8%); neurological diseases, 48 (22.6%); respiratory diseases, 24 (11.3%); renal diseases, 11 (5.2%); reproductive disorders, 66 (31.1%); and other/unclassified, 21 (9.9%). Definite APS according to the Sydney criteria comprised seven patients (3.3%), including four with primary APS and three with SLE-associated APS. Documented APS clinical manifestations included arterial thrombosis in 7 (3.3%), venous thrombosis in 3 (1.4%), small-vessel thrombosis in 0, pregnancy morbidity in 36 (17.0%), and thrombocytopenia in 9 (4.2%).

Antibody-specific positivity was as follows: ACL-IgG, 13/212 (6.1%); ACL-IgM, 10/212 (4.7%); ACL-IgA, 12/212 (5.7%); β2GPI-IgG, 13/212 (6.1%); β2GPI-IgM, 6/212 (2.8%); β2GPI-IgA, 9/212 (4.2%); and composite IgA aPL, 14/212 (6.6%). Among the 14 patients positive for composite IgA aPL, seven were double-positive for both ACL-IgA and β2GPI-IgA; the remaining patients were positive for only one of the two IgA antibodies. Composite laboratory LA positivity was present in 39/212 patients (18.4%).

3.2 | Correlation Between Lymphocyte Subsets and aPLs

To delineate the association patterns between different aPL isotypes and peripheral immune cell distribution, Spearman's correlation analysis was employed to systematically evaluate the correlations of three isotypes (IgA, IgM, and IgG) of ACL and β2GPI antibodies with the percentages and absolute counts of peripheral blood T-cell, B-cell, and NK-cell subsets. The results revealed marked heterogeneity in the immune cell association patterns across the three isotypes (Figure 1), which could be categorized into three distinct profiles. After BH-FDR correction, most lymphocyte–aPL correlations were no longer statistically significant and are therefore described as exploratory; exact p-values and q-values are provided in Table S7.

IgA isotype antibodies are broadly associated with adaptive immune cells. IgA isotype antibodies were the only aPL isotype showing extensive correlations with T and B lymphocyte subsets, and the direction of these associations was highly consistent, all pointing to an immune deviation characterized by reduced $CD4^+$ T cells and B cells with a relative predominance of $CD8^+$ T cells. Specifically, ACL-IgA was significantly negatively correlated with absolute $CD19^+$ B-cell count ($r = -0.17$, $p = 0.004$; $q \approx 0.050$, borderline) and weakly negatively correlated with absolute $CD4^+$ T-cell count ($r = -0.13$, $p = 0.032$; exploratory after FDR) and absolute $CD8^+$ T-cell count ($r = -0.13$, $p = 0.028$; exploratory after FDR). β2GPI-IgA was positively correlated with $CD8^+$ T-cell percentage ($r = 0.17$, $p = 0.038$; $q = 0.1175$, exploratory) and negatively correlated with absolute $CD4^+$ T-cell count ($r = -0.17$, $p = 0.035$; $q = 0.1053$, exploratory) and the CD4/CD8 ratio ($r = -0.18$, $p = 0.026$; exploratory after FDR). Notably, β2GPI-IgA showed no significant correlation with absolute $CD8^+$ T-cell count ($r = 0.01$, $p = 0.895$), indicating that the elevated $CD8^+$ T-cell percentage was not attributable to an absolute expansion of this subset but rather to a relative shift caused by the reduction in $CD4^+$ T cells. This pattern was further corroborated by a between-group comparison: the IgA-positive group ($n = 14$) had significantly lower absolute $CD4^+$ T-cell counts than the IgA-negative group ($n = 198$) (Table 2), with the direction of the effect entirely consistent with the correlation analysis.

IgG isotype antibodies show essentially no association with lymphocyte subsets. In stark contrast to the IgA isotype, IgG isotype antibodies (ACL-IgG and β2GPI-IgG) exhibited no statistically significant correlations with any of the examined lymphocyte subset percentages or absolute counts, with all absolute correlation coefficients being less than 0.10. Only β2GPI-IgG showed a marginal negative correlation with absolute $CD16/CD56^+$ NK-cell count ($r = -0.15$, $p = 0.044$), which was no longer significant after correction for multiple comparisons. This negative finding suggests that the generation and maintenance of IgG aPL may be independent of fluctuations in circulating T- and B-cell numbers, fundamentally distinguishing them from IgA aPL.

IgM isotype antibodies: only ACL-IgM is selectively associated with NK cells. The immunological association profile of IgM isotype antibodies fell between those of IgA and IgG, exhibiting clear antibody specificity: ACL-IgM was significantly positively correlated with both $CD16/CD56^+$ NK-cell percentage ($r = 0.20$, $p = 0.002$; $q = 0.0195$) and absolute NK-cell count ($r = 0.16$, $p = 0.038$; exploratory after FDR), whereas β2GPI-IgM showed no significant correlations with any lymphocyte subset. This indicates that the elevated NK-cell count is a specific immunological feature associated with ACL-IgM, rather than a general property of the IgM isotype.

In summary, the peripheral immune association patterns of the three aPL isotypes displayed a distinct divergence: the IgA isotype broadly associated with adaptive immune deviation, the IgM isotype (specifically ACL-IgM) associated with innate NK cells, and the IgG isotype largely unrelated to the lymphocyte subset composition. This isotype-specific immunological association pattern strongly suggests that the production of different aPL isotypes may be driven by independent immunoregulatory mechanisms.

TABLE 1 | Baseline characteristics of the study population.

Parameter	Mean ± SD	Parameter	Mean ± SD
Blood routine		Composite inflammatory indices	
WBC ($\times10^9$/L)	6.87 ± 3.28	SII	700.01 ± 992.91
RBC ($\times10^{12}$/L)	4.14 ± 0.74	SIRI	1.56 ± 3.00
HGB (g/L)	123.42 ± 21.19	NLR	3.84 ± 8.64
HCT	0.38 ± 0.06	dNLR	0.88 ± 0.06
MCV (fL)	92.12 ± 6.65	PLR	150.74 ± 118.01
MCH (pg)	29.98 ± 2.75	MLR	0.29 ± 0.29
MCHC (g/L)	325.15 ± 14.60	NPR	0.03 ± 0.07
PLT ($\times10^9$/L)	213.96 ± 79.56	PIV	309.65 ± 498.82
RDW-CV (%)	13.48 ± 2.42	Routine coagulation	
RDW-SD (fL)	44.97 ± 7.46	PT(s)	11.11 ± 1.29
MPV (fL)	10.22 ± 0.99	INR	1.04 ± 0.12
PDW (fL)	11.30 ± 2.09	FIB (g/L)	2.92 ± 0.70
PCT (%)	0.22 ± 0.07	APTT(s)	32.14 ± 6.98
P-LCR (%)	26.19 ± 7.81	TT(s)	15.08 ± 1.68
LYMPH%	27.71 ± 11.36	FDP (μg/mL)	5.82 ± 11.93
LYMPH# ($\times10^9$/L)	1.76 ± 0.80	D-dimer (mg/L)	0.61 ± 1.81
Neut%	63.69 ± 12.43	Lupus anticoagulant	
Neut# ($\times10^9$/L)	4.53 ± 2.83	SCT-S(s)	44.71 ± 20.67
EO%	2.03 ± 2.84	SCT-C(s)	37.79 ± 6.31
EO# ($\times10^9$/L)	0.13 ± 0.18	nSCT-R	1.08 ± 0.41
BASO%	0.47 ± 0.31	dRVVT-S(s)	33.18 ± 10.71
BASO# ($\times10^9$/L)	0.03 ± 0.02	dRVVT-C(s)	26.97 ± 3.11
MONO%	6.10 ± 2.17	ndRVVT-R	1.04 ± 0.24
MONO# ($\times10^9$/L)	0.40 ± 0.20	—	—
CRP (mg/dL)	1.42 ± 3.23	—	—
Lymphocyte subsets		Antiphospholipid antibody profile	
$CD3^+$%	73.20 ± 10.46	ACL-IgG (U/mL)	14.52 ± 63.59
$CD4^+$%	39.60 ± 9.66	ACL-IgM (U/mL)	5.33 ± 8.89
$CD8^+$%	30.25 ± 12.06	β2GPI-IgG (U/mL)	52.36 ± 398.71
CD4/CD8 ratio	1.64 ± 1.25	β2GPI-IgM (U/mL)	3.21 ± 6.52
$CD19^+$%	13.15 ± 8.70	ACL-IgA (U/mL)	8.69 ± 19.32
$CD16/CD56^+$%	12.74 ± 7.66	β2GPI-IgA (U/mL)	8.07 ± 25.43
$CD45^+$ (cells/μL)	1599.90 ± 813.40	—	—
$CD3^+$ (cells/μL)	1155.90 ± 587.35	—	—
$CD4^+$ (cells/μL)	632.13 ± 354.61	—	—
$CD8^+$ (cells/μL)	459.66 ± 273.43	—	—
$CD19^+$ (cells/μL)	224.76 ± 204.76	—	—
$CD16/CD56^+$ (cells/μL)	206.57 ± 161.87	—	—

Abbreviations: ACL, anti-cardiolipin; CRP, C-reactive protein; dNLR, derived NLR; dRVVT, dilute Russell's viper venom time; FDPs, fibrin/fibrinogen degradation products; MLR, monocyte-to-lymphocyte ratio; ndRVVT-R, normalized dRVVT ratio; NLR, neutrophil-to-lymphocyte ratio; NPR, neutrophil-to-platelet ratio; nSCT-R, normalized SCT ratio; PIV, pan-immune-inflammation value; PLR, platelet-to-lymphocyte ratio; SCT, silica clotting time; SII, systemic immune-inflammation index; SIRI, systemic inflammation response index; β2GPI, β2-glycoprotein I.

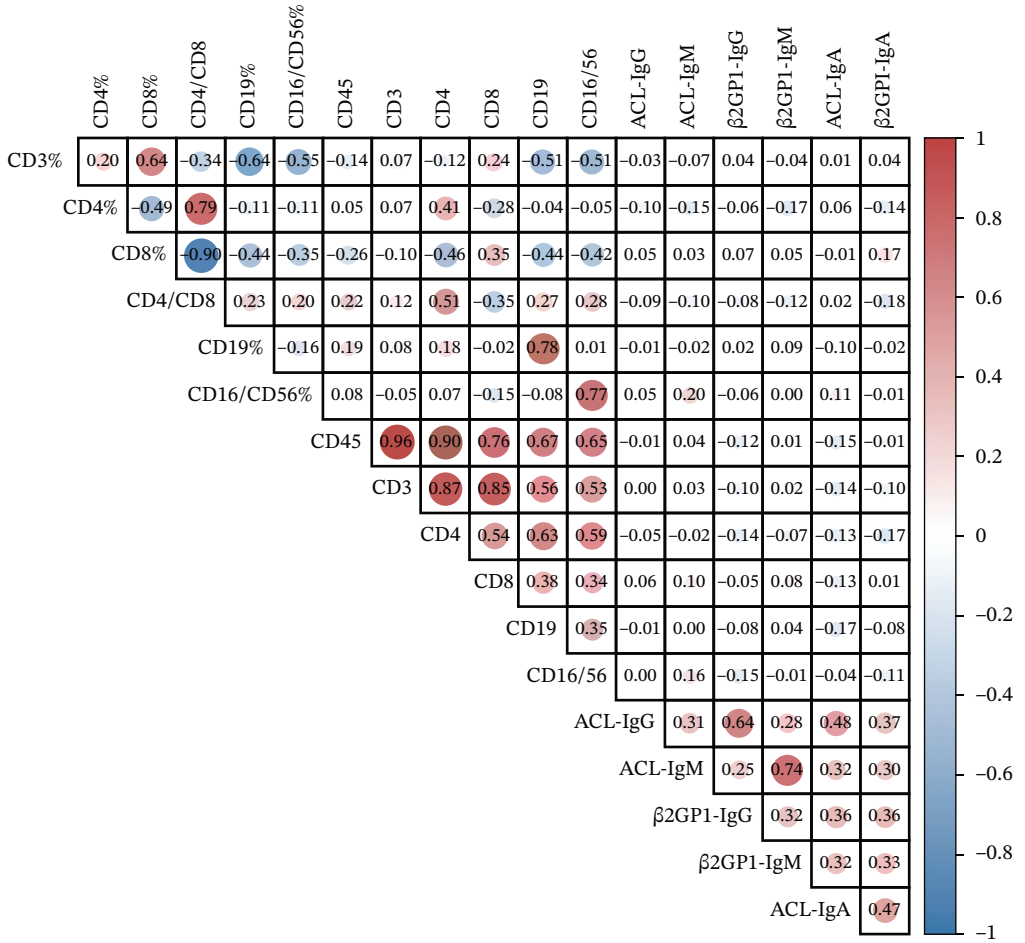


FIGURE 1 | Heatmap of correlations between different antiphospholipid antibody isotypes and peripheral blood lymphocyte subset. *Note:* the analysis included $n = 212$ patients. Spearman correlation coefficients are shown; asterisks indicate unadjusted p-values. Exact p-values, q-values, and 95% CIs are provided in Table S7.

TABLE 2 | Comparison of peripheral blood lymphocyte subsets between IgA aPL-positive and IgA aPL-negative groups (mean ± SD).

Parameter	IgA-negative ($n = 198$)	IgA-positive ($n = 14$)	t	p
CD3%	73.49 ± 10.64	74.23 ± 11.77	−0.229	0.819
CD4%	40.01 ± 9.63	36.23 ± 14.79	1.267	0.207
CD8%	30.33 ± 12.50	35.62 ± 11.30	−1.425	0.156
CD4/CD8	1.68 ± 1.32	1.25 ± 0.99	1.101	0.272
CD19%	13.11 ± 9.17	10.55 ± 7.09	0.948	0.345
CD16/CD56%	12.50 ± 7.67	14.37 ± 9.47	−0.807	0.421
CD45	1595.89 ± 845.63	1139.09 ± 601.25	1.838	0.068
CD3	1155.94 ± 606.94	835.23 ± 477.68	1.792	0.075
CD4	637.72 ± 366.96	382.42 ± 232.17	2.375	0.019*
CD8	456.49 ± 283.41	426.03 ± 277.01	0.361	0.719
CD19	223.63 ± 217.14	145.69 ± 119.02	1.229	0.221
CD16/56	204.06 ± 166.11	148.98 ± 116.38	1.129	0.260

*$p < 0.05$.

3.3 | Correlation Between aPL Isotypes and Coagulation Parameters

Spearman's correlation analysis was used to assess the associations between the three aPL isotypes (IgG, IgM, and IgA) and coagulation parameters, including routine coagulation tests (PT, APTT, Fib, FDP, and TT), LA-sensitive assays, and the fibrinolytic marker D-dimer. The results revealed a pronounced antibody isotype-dependent pattern (Figure 2). After BH-FDR correction, ACL-IgA remained significantly correlated with the D-dimer and FDP, whereas several other correlations were exploratory. Exact *p*- and *q*-values are provided in Table S8.

Among the three isotypes, IgA antibodies exhibited the most prominent and consistent correlations with LA-sensitive assays and fibrinolytic markers. ACL-IgA was significantly positively correlated with dRVVT-S ($r = 0.36$, $p < 0.01$), ndRVVT-R ($r = 0.39$, $p < 0.01$), and nSCT-R ($r = 0.26$, $p < 0.05$), with a positive trend for dRVVT-C ($r = 0.18$, $p > 0.05$) and a marginal positive correlation with SCT-S ($r = 0.17$, $p > 0.05$). β2GPI-IgA exhibited a highly consistent pattern, showing significant positive correlations with dRVVT-S ($r = 0.35$, $p < 0.01$), ndRVVT-R ($r = 0.41$, $p < 0.01$), nSCT-R ($r = 0.20$, $p < 0.05$), and dRVVT-C ($r = 0.21$, $p < 0.01$), with a positive trend for SCT-S ($r = 0.15$, $p > 0.05$). Notably, the correlation coefficients between IgA antibodies and ndRVVT-R reached 0.39–0.41, the highest among all antibody isotypes. In addition, ACL-IgA showed significant positive correlations with FDP ($r = 0.19$, $p < 0.01$; $q = 0.0064$), D-dimer ($r = 0.20$, $p < 0.01$; $q = 0.0013$), and Fib ($r = 0.19$, $p < 0.05$; $q = 0.2719$, exploratory after FDR). β2GPI-IgA showed a positive trend with FDP ($r = 0.15$, $p > 0.05$) and a weaker, directionally consistent correlation with D-dimer ($r = 0.15$, $p > 0.05$). These results indicate that IgA antibodies are not only associated with the in vitro LA phenotype but also with actual in vivo levels of fibrin formation and degradation (D-dimer and FDP).

The correlation between IgM isotype antibodies and coagulation parameters was comparatively weaker. β2GPI-IgM was significantly positively correlated with SCT-S ($r = 0.28$, $p < 0.01$), dRVVT-S ($r = 0.30$, $p < 0.05$), and ndRVVT-R ($r = 0.31$, $p < 0.05$), and also positively correlated with APTT ($r = 0.21$, $p < 0.05$). ACL-IgM exhibited a positive trend with SCT-S ($r = 0.18$, $p > 0.05$), but the overall effect sizes were lower than those of the IgA isotype. With respect to fibrinolytic markers, IgM antibodies showed only weak and nonsignificant correlations with FDP and the D-dimer.

IgG isotype antibodies demonstrated the weakest associations with coagulation parameters. ACL-IgG showed absolute correlation coefficients <0.15 with all coagulation indices, none reaching

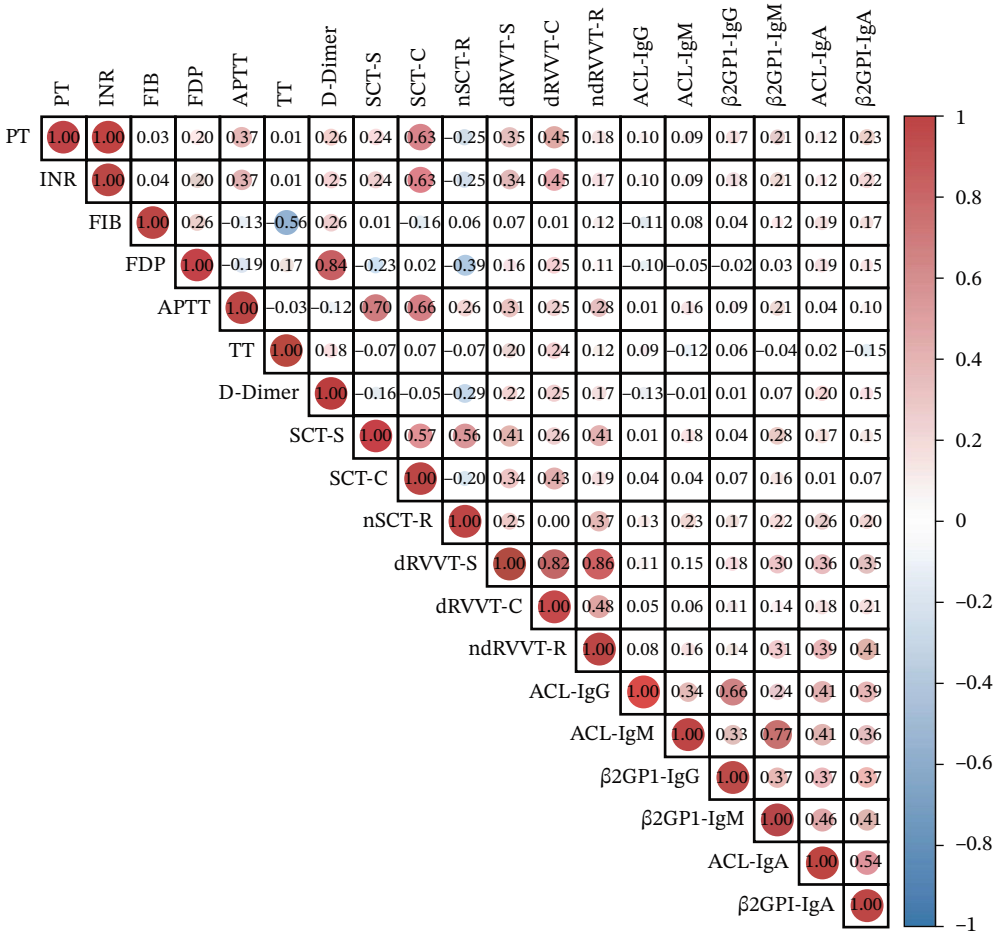


FIGURE 2 | Heatmap of correlations between antiphospholipid antibody isotypes and coagulation parameters. *Note:* the analysis included $n = 212$ patients. Spearman correlation coefficients are shown; asterisks indicate unadjusted *p*-values. Exact *p*-values, *q*-values, and 95% CIs are provided in Table S8.

statistical significance (all $p > 0.05$). β2GPI-IgG was weakly positively correlated with PT ($r = 0.17$, $p < 0.05$), INR ($r = 0.18$, $p < 0.05$; $q = 0.0404$), and dRVVT-S ($r = 0.18$, $p < 0.05$), but was not significantly associated with ndRVVT-R ($r = 0.11$, $p > 0.05$) or fibrinolytic markers.

Further comparison of the three isotypes revealed that only IgA antibodies displayed consistent, statistically significant correlations across all LA-sensitive assays and were also simultaneously associated with elevated Fib, FDP, and D-dimer levels. In contrast, IgG and IgM antibodies showed only sporadic, weak, and unstable correlation signals. This pattern clearly indicates that IgA aPLs—rather than the traditional IgG or IgM isotypes—may serve as a potential functional bridge linking the aforementioned immune deviation ($CD8^+$ T-cell predominance and $CD4^+/CD19^+$ cell reduction) to the LA phenotype and in vivo coagulation-fibrinolysis activation.

3.4 | Correlation Between Lymphocyte Subsets and Coagulation Parameters

To delineate the direct association between immune cell imbalance and coagulation abnormalities, we further analyzed the correlations between peripheral blood lymphocyte subsets and coagulation parameters (Figure 3), with the aim of exploring the differential impact of distinct immune cell subsets on the coagulation phenotype. Exact p-values and q-values are provided in Table S9.

The results showed that the percentage of $CD8^+$ T cells was consistently positively correlated with multiple LA-sensitive assays, including SCT-S ($r = 0.16$, $p = 0.017$), dRVVT-S ($r = 0.16$, $p = 0.041$), and dRVVT-C ($r = 0.14$, $p = 0.024$), and was also positively correlated with TT ($r = 0.20$, $p = 0.049$). However, the absolute $CD8^+$ T-cell count did not reach statistical significance with any of these parameters, indicating that an elevated relative proportion of $CD8^+$ T cells, rather than an absolute expansion of this subset, is closely associated with the LA-positive phenotype. In addition, the percentage of $CD3^+$ T cells was positively correlated with SCT-S ($r = 0.22$, $p = 0.032$) and dRVVT-C ($r = 0.18$, $p = 0.025$), further supporting an association between the overall proportional shift in the T-cell compartment and coagulation abnormalities. After FDR correction, these correlations were exploratory.

In contrast to $CD8^+$ T cells, $CD19^+$ B cells showed consistently negative correlations with LA-sensitive assays. The percentage of $CD19^+$ B cells was significantly negatively correlated with SCT-S ($r = -0.17$, $p = 0.043$) and dRVVT-C ($r = -0.23$, $p = 0.032$). The association with absolute $CD19^+$ B-cell count was similarly evident, reaching a correlation coefficient of −0.17 with dRVVT-C ($p = 0.004$) and exhibiting a marginally significant negative

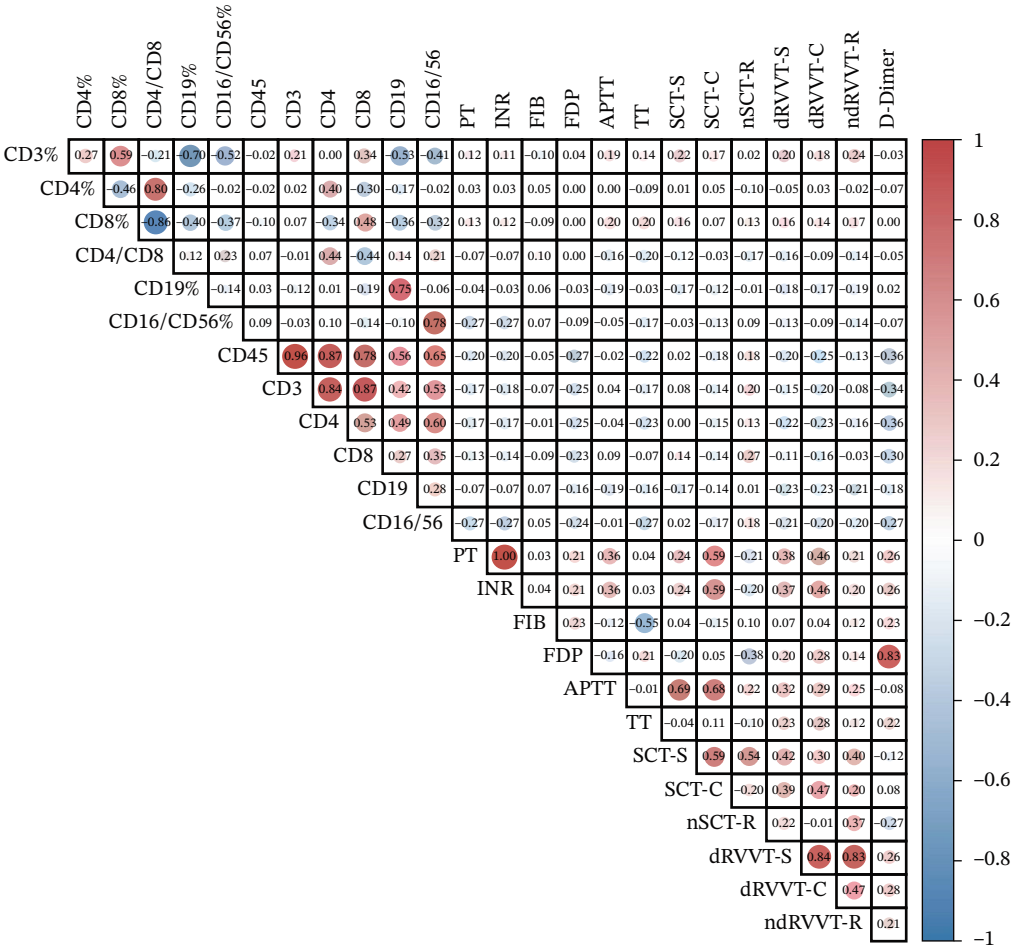


FIGURE 3 | Heatmap of correlations between lymphocyte subsets and coagulation parameters. *Note:* the analysis included $n = 212$ patients. Spearman correlation coefficients are shown; asterisks indicate unadjusted p-values. Exact p-values, q-values, and 95% CIs are provided in Table S9.

correlation with SCT-S ($r = -0.17$, $p = 0.053$). Furthermore, $CD19^+$ B-cell percentage showed a negative trend with APTT ($r = -0.19$, $p = 0.060$) and, although not statistically significant, was directionally consistent with dRVVT-S ($r = -0.18$, $p = 0.181$). These results indicate that higher peripheral blood B-cell levels are associated with shorter clotting times in LA-sensitive assays and a lower likelihood of LA positivity, suggesting a negative association between the $CD19^+$ B-cell count and the LA-positive phenotype, potentially conferring a protective effect.

The percentage of $CD16/CD56^+$ NK cells was significantly negatively correlated with SCT-C ($r = -0.17$, $p = 0.039$) and TT ($r = -0.27$, $p = 0.014$), but correlations with dRVVT-based parameters were weak and mostly nonsignificant, suggesting that the influence of NK cells on the coagulation system is primarily exerted through the intrinsic and common coagulation pathways rather than LA-specific pathways.

The correlations of lymphocyte subsets with routine coagulation parameters such as PT, INR, Fib, and FDP were generally weak and did not exhibit subset-specific consistent patterns. A consistent but very low-magnitude negative correlation with PT and positive correlation with INR were observed across multiple lymphocyte subsets (absolute counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD16/CD56^+$, and $CD19^+$ cells), suggesting that an overall reduction in lymphocyte counts may be associated with subtle alterations in the extrinsic coagulation pathway, though its clinical significance is limited.

Notably, D-dimer, an end product reflecting fibrin formation and degradation, was significantly negatively correlated with the absolute counts of several lymphocyte subsets, including $CD3^+$ T cells ($r = -0.34$, $p < 0.01$; $q = 7.1 \times 10^{-9}$), $CD4^+$ T cells ($r = -0.36$, $p < 0.01$; $q = 8.7 \times 10^{-9}$), $CD8^+$ T cells ($r = -0.30$, $p < 0.01$; $q = 2.9 \times 10^{-5}$), and $CD19^+$ B cells ($r = -0.18$, $p < 0.01$; $q = 7.5 \times 10^{-4}$). This pattern suggests that an overall reduction in lymphocyte numbers is closely associated with elevated levels of in vivo fibrin degradation products, further linking immune cell depletion to a prothrombotic state. Moreover, D-dimer was positively correlated with LA-sensitive assays (dRVVT-S: $r = 0.26$, $p < 0.01$; ndRVVT-R: $r = 0.21$, $p < 0.05$), indicating a synergistic relationship between the in vitro LA phenotype and in vivo coagulation activation.

In summary, at the level of LA-sensitive assays and fibrinolytic markers, immune cell subsets, and the coagulation/fibrinolytic system display a complex network of associations. An elevated $CD8^+$ T-cell percentage is positively correlated with the LA-positive phenotype, whereas an elevated $CD19^+$ B-cell count is negatively correlated; a global reduction in lymphocytes is closely associated with an elevated D-dimer. This pattern is highly consistent with the characteristics of IgA antibody-associated immune deviation described earlier, further supporting the involvement of cellular immune imbalance in the development and progression of the LA-positive phenotype and in vivo coagulation-fibrinolysis abnormalities.

3.5 | Lack of Mediating Effect of IgA Antibodies Between $CD8^+$ T Cells and the LA Phenotype

Based on the observed associations that $CD8^+$ T-cell percentage was positively correlated with the LA phenotype and that IgA antibodies were simultaneously associated with both immune deviation and the LA phenotype, we constructed simple mediation models to examine whether IgA aPL mediate the effect of $CD8^+$ T cells on the LA phenotype. Two independent simple mediation models were specified, with $CD8^+$ T-cell percentage as the independent variable (X), β2GPI-IgA or ACL-IgA as the M, and the normalized dRVVT ratio (ndRVVT-R) as the surrogate measure of the LA phenotype and the dependent variable (Y). The bootstrap method with 5000 resamples was used to test the indirect effect, and a 95% CI not containing zero was taken as the criterion for the statistical significance of the indirect effect. Exact results are provided in Table S10.

In the β2GPI-IgA mediation model, both the total effect ($c = 0.004$, $p = 0.031$) and the direct effect ($c' = 0.004$, $p = 0.035$) of $CD8^+$ T-cell percentage on ndRVVT-R were statistically significant. However, the a path from the independent variable to the M ($CD8^+$ T-cell percentage- β2GPI-IgA) was not significant (effect = 0.044, $p = 0.248$), and the bootstrap 95% CI for the indirect effect (ab) ranged from −0.001 to 0.003, including zero ($p = 0.732$), indicating a nonsignificant indirect effect. The results of the ACL-IgA mediation model were entirely consistent: the a path was not significant (effect = 0.057, $p = 0.387$), and the bootstrap 95% CI for the indirect effect was −0.001 to 0.003, also including zero ($p = 0.639$). In summary, neither β2GPI-IgA nor ACL-IgA exerted a significant mediating effect between the $CD8^+$ T-cell percentage and the LA phenotype. This null finding suggests that the effect of $CD8^+$ T cells on the LA-positive phenotype is primarily a direct one and does not depend on IgA aPL as an intermediate bridging molecule. This negative mediation analysis argues against a strong causal interpretation of the $CD8^+$–IgA aPL–LA pathway.

3.6 | Logistic Regression Analysis of Factors Associated With LA Positivity

The preceding correlation analyses demonstrated significant univariate associations of lymphocyte subset deviations, IgA aPL, and several systemic inflammatory indices with the LA phenotype (Sections 3.2–3.4). However, these analyses cannot answer a key multivariable question: after simultaneously adjusting for immune cell subsets, inflammatory burden, and antibody levels, which factors remain independently associated with LA positivity? To address this, we first selected an appropriate inflammatory marker as a covariate and then constructed multivariable logistic regression models.

Among the candidate inflammatory indices, several showed varying degrees of univariate correlation with the LA phenotype (represented by ndRVVT-R) (Figure 4). Spearman correlation analysis revealed that NLR was significantly positively correlated with ndRVVT-R ($r = 0.17$, $p = 0.021$), as was SIRI ($r = 0.17$, $p = 0.019$), and MLR ($r = 0.17$, $p = 0.048$); PLR showed a negative trend ($r = -0.15$, $p = 0.096$), whereas SII was not significantly correlated with ndRVVT-R ($r = -0.04$, $p = 0.952$). These results indicate that NLR and SIRI are the two inflammatory indices most closely associated with the LA phenotype. Given that NLR can be calculated simply from neutrophil and lymphocyte counts, boasts the highest clinical accessibility and reproducibility, and has been extensively validated in studies of autoimmune

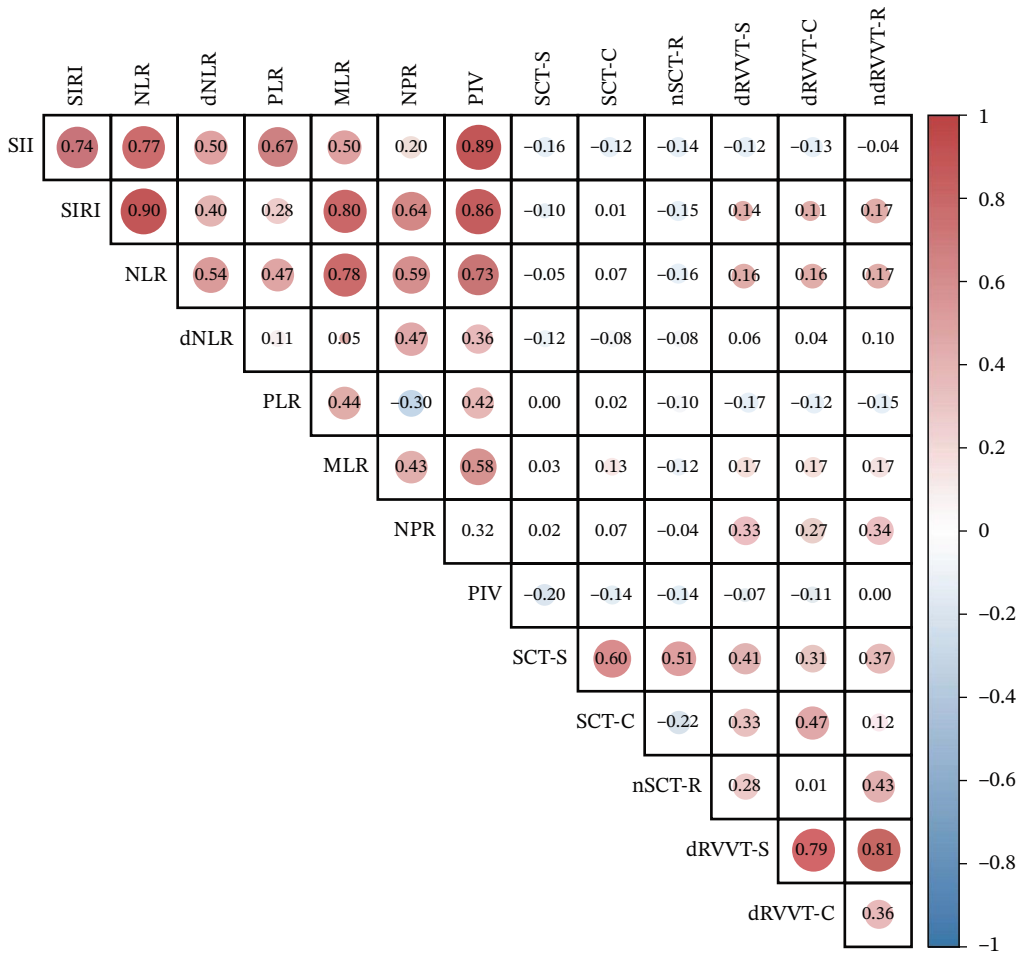


FIGURE 4 | Heatmap of correlations between composite inflammatory indices and lupus anticoagulant parameters. *Note:* the analysis included $n = 212$ patients. Spearman correlation coefficients are shown; asterisks indicate unadjusted *p*-values. Exact *p*-values and *q*-values are provided in Supporting Tables.

coagulation disorders, NLR was selected as the representative covariate for the inflammatory burden in the subsequent multivariable regression.

With composite laboratory LA positivity as the dependent variable, age, sex, NLR, absolute $CD4^+$ T-cell count, $CD8^+$ T-cell percentage, absolute $CD19^+$ B-cell count, and ACL-IgA were entered as independent variables in a multivariable logistic regression model (Table 3). Antibody concentrations were $\log_{10}$-transformed, and ORs are expressed per 1-unit increase in the $\log_{10}$ antibody concentration.

The results showed that after simultaneously adjusting for immune cell subsets, inflammatory markers, and demographic

TABLE 3 | Multivariable logistic regression analysis of factors associated with lupus anticoagulant positivity.

	Model 1 (β2GPI-IgA)		Model 2 (ACL-IgA)	
Variable	**OR (95% CI)**	***p*-Value**	**OR (95% CI)**	***p*-Value**
$CD4^+$T-cell count	0.999 (0.998–1.001)	0.598	1.000 (0.998–1.002)	0.724
$CD8^+$T-cell percentage	1.010 (0.966–1.057)	0.656	1.014 (0.969–1.060)	0.552
$CD19^+$B-cell count	0.999 (0.994–1.003)	0.553	0.999 (0.994–1.003)	0.526
NLR	1.045 (0.929–1.176)	0.462	1.052 (0.934–1.186)	0.403
β2GPI-IgA	1.101 (1.018–1.191)	0.016	—	—
ACL-IgA	—	—	1.078 (1.023–1.137)	0.005

Note: Models were adjusted for age and sex. LA positivity was defined according to ISTH guidelines as a normalized SCT or dRVVT ratio >1.1 with confirmatory evidence of phospholipid-dependent inhibition. Antibody concentrations were $\log_{10}$-transformed. The model with β2GPI-IgA served as the primary model, and the model with ACL-IgA was used as a sensitivity analysis.
Abbreviations: ACL, anti-cardiolipin; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; β2GPI, β2-glycoprotein I.

factors, in Model 1, β2GPI-IgA was independently associated with LA positivity (OR = 1.101, 95% CI: 1.018–1.191, $p = 0.016$), meaning that for every one logarithmic unit increase in β2GPI-IgA titer, the risk of LA positivity increased by approximately 10.1%. In contrast, $CD4^+$ T-cell count ($p = 0.598$), $CD8^+$ T-cell percentage ($p = 0.656$), $CD19^+$ B-cell count ($p = 0.553$), and NLR ($p = 0.462$) all failed to reach statistical significance after adjustment for antibodies.

To verify the robustness of these results, the logistic regression was repeated with ACL-IgA in place of β2GPI-IgA (Table 3, Model 2). The results demonstrated that ACL-IgA was also independently associated with LA positivity (OR = 1.078, 95% CI: 1.023–1.137, $p = 0.005$), while the immune cell variables ($CD4^+$: $p = 0.724$; $CD8^+$: $p = 0.552$; $CD19^+$: $p = 0.526$) and NLR ($p = 0.403$) remained nonsignificant. Model diagnostics showed acceptable calibration (Hosmer–Lemeshow $p = 0.709$ for Model 1 and $p = 0.821$ for Model 2), no significant multicollinearity (maximum VIF 1.510 and 1.672, respectively), and overall prediction accuracy of 87.02% and 87.12%. The EPV was 4.6 in both models, indicating that the number of events was small relative to the number of predictors; therefore, multivariable estimates should be interpreted cautiously. Exact diagnostics are provided in Table S11.

4 | Discussion

Through a multilayered network analysis spanning lymphocyte subsets, antibody isotypes, and coagulation phenotypes in 212 patients, this study has revealed three progressive and internally consistent core findings. First, different aPL isotypes display distinct immune cell association patterns [14]: IgA antibodies are broadly associated with adaptive immune deviation characterized by $CD4^+$ T-cell reduction and a relative increase in $CD8^+$ T cells; IgM antibodies show selective association with NK cells only for ACL-IgM, while IgG antibodies exhibit no correlation with any examined lymphocyte subset. Second, at the coagulation level, the strength of association between IgA antibodies and the LA phenotype far exceeds that of the traditional IgG/IgM isotypes, with correlation coefficients with ndRVVT-R reaching 0.39–0.41 and ACL-IgA demonstrating significant positive correlations with D-dimer ($r = 0.20$) and FDP ($r = 0.19$), thereby extending the interference effect from in vitro coagulation tests to in vivo fibrinolytic activation. Third, multivariable models rigorously adjusted for the inflammatory burden and immune cell subsets confirm that IgA antibodies (ACL-IgA and β2GPI-IgA) are independently associated with LA positivity within the measured antibody panel, whereas the numerical deviations of $CD4^+$, $CD8^+$, and $CD19^+$ lymphocytes all lose statistical significance. Collectively, these results point to a core conclusion: IgA aPL is associated with the LA-positive phenotype and may be a potentially important contributor linking upstream immune dysregulation to downstream coagulation abnormalities; however, this study cannot establish causality.

By taking LA, a standardized intermediate phenotype, as the point of departure, this study provides evidence with greater mechanistic relevance. The essence of LA is the antibody's ability to interfere with phospholipid-dependent coagulation reactions in vitro [15], directly reflecting its pathogenic potential [16]. In this study, the correlation of IgA antibodies with the dRVVT system was not only significantly stronger than that of the IgG/IgM isotypes but also independent of inflammatory levels and lymphocyte counts, suggesting that IgA antibodies may possess unique spatial conformations or charge characteristics, enabling more efficient crosslinking of β2GPI–phospholipid complexes and competitive inhibition of coagulation factor binding, thereby producing a stronger LA effect. More notably, the positive correlations of ACL-IgA with D-dimer and FDP extend this effect from the test tube to the actual in vivo processes of fibrin formation and degradation, providing a direct biochemical evidence chain for the procoagulant activity of IgA antibodies and compensating for the limitation of previous studies that relied solely on clinical events as endpoints [6, 17].

This study is the first to precisely anchor the $CD4^+$ T-cell-depletion-dominant immune deviation to the IgA isotype at the population level rather than treating it as a universal feature of APS. The absolute $CD4^+$ T-cell count in the IgA-positive group decreased by approximately 40% compared with the IgA-negative group, and the nature of this deviation is a relative increase in $CD8^+$ percentage caused by $CD4^+$ T-cell reduction rather than an absolute expansion of $CD8^+$ cells—a judgment powerfully supported by the near-zero correlation coefficient between β2GPI-IgA and the absolute $CD8^+$ count ($r = 0.01$). This characteristic immune phenotype points to a unique pathological mechanism: $CD4^+$ T-cell loss may weaken the helper selection and immunoregulation of autoreactive B cells [9] and in a compensatory microenvironment enriched with mucosal-associated cytokines such as TGF-β, aberrantly drive β2GPI-specific B cells toward IgA class switching [18–20]. The negative correlation between the absolute $CD19^+$ B-cell count and ACL-IgA further supports this hypothesis and may reflect the abnormal retention or excessive consumption of autoreactive B cells in lymphoid tissues. In striking contrast, IgG antibodies show no association with circulating lymphocyte subsets, suggesting that plasma cells producing IgG aPL may mainly reside in the bone marrow, with their antibody secretion maintenance independent of peripheral blood T-cell number fluctuations. The distinct immune association patterns of the three isotypes confirm for the first time at the cellular level that different aPL isotypes are driven by independent immunoregulatory networks, providing a new dimension for the immunological heterogeneity of APS.

The nonsignificant mediation effect indicates that the elevation in $CD8^+$ T-cell percentage does not indirectly influence LA by upregulating IgA antibodies. Conversely, in the regression analysis, when IgA antibodies entered the model, the effects of all lymphocyte indicators disappeared. This phenomenon of effect disappearance inversely reveals that the univariate associations between immune cells and LA are essentially confounded by their covariation with IgA antibodies. A more plausible working model is that $CD4^+$ T-cell depletion and the resulting immune microenvironmental disturbance, as upstream events, drive the production of IgA autoantibodies; the IgA antibodies, as potentially important contributors, then act on phospholipid–protein targets, leading to the LA phenotype and in vivo fibrinolytic activation. Therefore, IgA antibodies may be an important node in this pathway—without IgA antibodies, T-cell deviation alone may be insufficient to trigger coagulation abnormalities.

This model is explicitly labeled as a hypothesis and requires future experimental testing.

An additional mechanism linking T-cell abnormalities to the antiphospholipid response may involve regulatory T-cell depletion and increased exposure of phosphatidylserine during abnormal cell death. Lai et al. [21] reported depletion of FoxP3$^+$ cells within the CD4$^+$CD25$^+$ regulatory T-cell compartment in SLE patients with APS, accompanied by increased lymphocyte mitochondrial oxidative stress. Because the present study measured total CD4$^+$ cells without defining the Treg compartment, the observed CD4+ reduction may encompass a clinically relevant loss of immune-regulatory cells. In parallel, phosphatidylserine becomes externalized on apoptotic cells, including dying T cells, generating an anionic membrane surface capable of binding β2GPI and prothrombin. Increased cell death and/or defective clearance of apoptotic material in SLE could therefore increase immune exposure to phosphatidylserine-associated protein complexes. In the setting of impaired Treg-mediated tolerance, this process could facilitate the development of antibodies to these complexes. Future studies should evaluate Tregs, cellular apoptosis/phosphatidylserine exposure, and anti-phosphatidylserine (anti-PS)/prothrombin antibodies together with conventional aPL isotypes to define more directly the relationship between T-cell dysregulation and the LA/APS phenotype [22].

We also emphasize that anti-PS antibodies and anti-PS/prothrombin complex (aPS/PT) antibodies should not be treated as interchangeable. PS externalization is the biological substrate created by dying cells, whereas aPS/PT is a defined serological specificity against a PS-associated protein complex [15]. Detection of aPS/PT should not be interpreted as proof of antibody binding to PS alone. In this retrospective cohort, aPS/PT testing was not performed in the original clinical workup, and stored samples were not available; this is an important limitation for a study focused on the LA phenotype. We therefore no longer claim that IgA aPL are independent or superior determinants of LA beyond the measured antibody panel. The independence of IgA aCL/anti-β2GPI was demonstrated only within the measured panel, and residual confounding by aPS/PT cannot be excluded.

Our findings have clear translational implications that remain hypothesis-generating. First, in patients who are LA-positive but negative for standard IgG/IgM ELISA, testing for IgA aPL should be prospectively evaluated to identify APS cases missed by the current criteria, thus potentially reducing the underdiagnosis of seronegative APS. Second, a reduction in the absolute CD4$^+$ T-cell count may serve as a simple immunological clue for predicting the presence of IgA antibodies, suitable for initial risk stratification. However, the present study evaluated laboratory associations, particularly LA-related assays, and did not evaluate clinical APS outcomes. Claims about diagnostic utility or risk stratification require prospective validation.

This study has several limitations. The single-center retrospective design cannot establish causality, and the predictive value of IgA antibodies for thrombotic events needs to be validated in prospective cohorts. Treg markers were not part of the original clinical flow cytometry panel, and stored samples were not available for supplementary Treg phenotyping. We therefore explicitly acknowledge the absence of CD4$^+$CD25highCD127low/−FoxP3$^+$ Treg quantification as a major limitation. Future studies should measure Treg frequency/function and relate it to IgA aPL, aPS/PT, LA status, and clinical APS manifestations. The sample size of the IgA-positive group is relatively small ($n = 14$), which may limit the statistical power to detect some differences; multivariable estimates may be unstable, and the results should be interpreted cautiously. Residual confounding by disease activity, glucocorticoids, and other immunosuppressive therapies cannot be excluded. The absence of thrombotic event records means that the conclusions remain at the laboratory phenotype level. Moreover, the molecular mechanisms by which IgA aPL directly inhibit phospholipid-dependent coagulation reactions await elucidation through in vitro experiments. Finally, because the original clinical workup did not include aPS/PT, Annexin V/PS exposure, apoptotic T-cell markers, or oxidative stress markers, these mechanistic questions remain unanswered and should be prioritized in future studies.

In summary, from an integrative immunocoagulation perspective, this study supports the hypothesis that IgA aPL is associated with the LA-positive phenotype and delineates an upstream CD4$^+$ T-cell depletion immune signature. These findings should be considered hypothesis-generating and require prospective validation with Treg phenotyping, apoptotic/PS exposure markers, and aPS/PT testing. This finding elevates the serological heterogeneity of APS from the level of antibody class differences to the level of immunoregulatory pathway divergence, opening new avenues for the molecular subtyping and targeted therapy of APS.

Author Contributions

Zhongjun Shen and Shuo Yang conceived the study and established its structure. Yuying Nie and Jingjin Tao drafted the manuscript and designed the figures. Liyan Cui participated in the critical revision of the manuscript. Liyan Cui supervised the entire study, oversaw the manuscript preparation and revision process, and is responsible for the overall content as the guarantor.

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Disclosure

No patients or members of the public were involved in the design, conduct, reporting, or dissemination of this research. All authors read and approved the final manuscript.

Ethics Statement

This study was reviewed and approved by the Ethics Committee of Peking University Third Hospital (Approval Number S2024674). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Information.** The Supporting Information accompanies this manuscript and provides additional methodological details and supporting results. It includes definitions for inflammatory markers, lymphocyte subsets, coagulation parameters, and antiphospholipid antibody measurements; the statistical framework, including Benjamini–Hochberg FDR correction and bootstrap mediation settings; and Tables S1–S11: diagnostic categories and APS manifestations (S1), antibody positivity definitions and thresholds (S2), prevalence of aPL and LA positivity (S3), antibody concentrations (S4), measured and unavailable immune/apoptosis-related variables (S5), correlation matrices for inflammatory markers, lymphocyte subsets, aPL isotypes, and coagulation/LA parameters (S6–S9), bootstrap mediation results (S10), and multivariable logistic regression model diagnostics (S11). Exact *p*-values, *q*-values, and 95% CIs are provided in the relevant supporting tables. The supporting data should be read in conjunction with the main text.