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# Immunoglobulin A anti-phospholipid antibodies in Swedish cases of systemic lupus erythematosus: associations with disease phenotypes, vascular events and damage accrual

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## Summary

**Immunoglobulin (Ig) G- and IgM-class anti-cardiolipin antibodies (aCL) and lupus anti-coagulant (LA) are included in the 1997 update of the American College of Rheumatology (ACR-97) systemic lupus erythematosus (SLE) criteria. Despite limited evidence, IgA-aCL and IgA anti-$\beta_2$-glycoprotein-I (anti-$\beta_2$GPI) were included in the 2012 Systemic Lupus International Collaborating Clinics criteria. The present study aimed to evaluate IgG-/IgA-/IgM-aCL and anti-$\beta_2$GPI occurrence in relation to disease phenotype, smoking habits, pharmacotherapy, anti-phospholipid syndrome (APS) and organ damage among 526 Swedish SLE patients meeting ACR-97. Patients with rheumatoid arthritis (n = 100), primary Sjögren's syndrome (n = 50) and blood donors (n = 507) served as controls. Anti-phospholipid antibodies (aPL) were analysed by fluoroenzyme-immunoassays detecting aCL/anti-$\beta_2$GPI. Seventy-six (14%) SLE cases fulfilled the Sydney APS-criteria, and ≥ 1 aCL/anti-$\beta_2$GPI isotype (IgG/IgA/IgM) occurred in 138 SLE patients (26%). Forty-five (9%) of the SLE cases had IgA-aCL, 20 of whom (4%) lacked IgG-/IgM-aCL. Seventy-four (14%) tested positive for IgA anti-$\beta_2$GPI, 34 (6%) being seronegative regarding IgG/IgM anti-$\beta_2$GPI. Six (1%) had APS manifestations but were seropositive regarding IgA-aCL and/or IgA anti-$\beta_2$GPI in the absence of IgG/IgM-aPL and LA. Positive LA and IgG-aPL tests were associated with most APS-related events and organ damage. Exclusive IgA anti-$\beta_2$GPI occurrence associated inversely with Caucasian ethnicity [odds ratio (OR) = 0·21, 95% confidence interval (CI) = 0·06–0·72) and photosensitivity (OR = 0·19, 95% CI = 0·05–0·72). Nephritis, smoking, LA-positivity and statin/corticosteroid-medication associated strongly with organ damage, whereas hydroxychloroquine-medication was protective. In conclusion, IgA-aPL is not rare in SLE (16%) and IgA-aPL analysis may have additional value among SLE cases with suspected APS testing negative for other isotypes of aPL and LA.**

**Keywords**: anti-phospholipid antibodies, anti-phospholipid syndrome, autoantibodies, immunoglobulin A, systemic lupus erythematosus

## Introduction

Systemic lupus erythematosus (SLE) is a potentially severe autoimmune condition with an unpredictable disease course, often with fluctuations in disease activity over time [1]. Long-term inflammation and drug-related side effects may lead subsequently to irreversible organ damage, a consequence which is associated intimately with decreased quality of life and increased mortality [2–4]. In SLE, accrual of organ damage and prognosis has been linked consistently to the presence of anti-phospholipid antibodies (aPL), with or without clinical events related to the anti-phospholipid syndrome (APS) [5–7]. Presence of the lupus anti-coagulant (LA) has been identified as the laboratory finding with the highest predictive value regarding future organ damage in SLE [8].

The 1997 update of the American College of Rheumatology (ACR) classification criteria for SLE incorporated the presence of anti-cardiolipin antibodies (aCL) of immunoglobulin (Ig)G/IgM isotype and/or a positive LA test and/or a persistent false-positive serological test for syphilis [9]. Recent reviews conclude that 30–40% of all SLE cases display elevated levels of any aPL at some point during the disease course, yet only approximately half of these SLE cases will fulfil the APS classification criteria [10–12]. According to the Sydney classification criteria [13], APS is defined by vascular thrombosis and/or pregnancy morbidity and repeated raised defined levels of IgG and/or IgM isotype aCL and/or anti-$\beta_2$ glycoprotein-I (anti-$\beta_2$GPI) antibodies and/or a positive LA test.

Based on the results from some studies, it has been claimed that the assessment of IgA isotype aCL and/or anti-$\beta_2$GPI provides additional clinical value and identify IgG/IgM aPL and LA negative cases of APS in SLE [14–17]. Accordingly, the International Consensus Task Force on aPL antibodies recommends IgA isotype testing for both aCL and anti-$\beta_2$GPI when results of all other tests are negative and APS is still suspected [18]. Recently, it has been suggested that the presence of IgA anti-$\beta_2$GPI in people with no history of APS-related events constitute an important independent risk factor for the development of such events [19]. Conversely, other studies found that analysis of IgA aPL did not contribute to the recognition of APS in SLE patients [20–23]. Nevertheless, in addition to the IgG and IgM isotypes, IgA aPL was included in the most recent set of SLE classification criteria proposed by the Systemic Lupus International Collaborating Clinics (SLICC) group in 2012. In their validation set of the SLICC-12 criteria, a greater sensitivity (97 *versus* 83%) but a slightly lower specificity (84 *versus* 96%) compared with the 1997 ACR classification criteria was demonstrated [24]. However, it remains to be elucidated whether or not this update helps to identify SLE cases prone to develop APS-related events and future organ damage [23,25]. In Scandinavia, systematic assessment of IgA aCL and anti-$\beta_2$GPI in suspected or newly diagnosed cases of SLE is currently not a part of the general clinical routine. Furthermore, the importance of other aPLs, such as anti-phosphatidylserine/prothrombin complex IgG and anti-$\beta_2$GPI domain 1 IgG, in relation to APS in SLE has been evaluated recently [26].

As the presence of IgA aPLs is of uncertain clinical significance [12], the overall goal of this study was to evaluate IgA aCL and anti-$\beta_2$GPI antibodies in serum samples of 526 well-characterized Swedish SLE patients in relation to controls, other aPL isotypes, disease phenotypes, smoking habits, ongoing pharmacotherapy, APS-related events as well as the association with damage accrual in each domain of the SLICC/ACR damage index (SDI) [27].

## Materials and Methods

### SLE

SLE patients ($n$ = 526) diagnosed at the rheumatology clinics at the Linköping ($n$ = 231) and Karolinska (Stockholm) University hospitals ($n$ = 295) were included. All cases were classified as SLE according to the 1997 ACR criteria update [9], and both cohorts have been described in detail previously [28,29]. Altogether, 461 (88%) were prevalent cases and 65 (12%) had a newly diagnosed SLE (≤ 12 months' disease duration) at the time of sampling. Data on smoking habits (past/present/never) were recorded at the time-point of blood collection. A total of 476 of 526 (90%) cases were of Caucasian ethnicity, whereas the majority of non-Caucasian SLE patients had Asian or Hispanic origin. Detailed information regarding organ damage at the time of sampling in each separate domain of SDI was obtained by chart review for each patient [27]. SDI covers 12 organ systems and measures accumulated organ damage that has occurred since the disease onset, and is scored regardless of whether the damage can be attributed to SLE or to other causes [27]. In addition, data on APS classification including pregnancy morbidities and other APS-related events were collected [13]. SLE patient characteristics are detailed in Table 1.

### Disease controls and blood donors

Patients with primary Sjögren's syndrome (pSS) and patients with rheumatoid arthritis (RA) served as disease controls. None of these patients had a concomitant APS diagnosis. Sera from 50 patients with established pSS (94% women; mean age = 62 years) meeting the American–European consensus criteria was collected [30]. Forty-nine per cent of the pSS patients had a history of extra glandular disease; 90% were positive for anti-SSA antibodies (± anti-SSB). 51% received prednisolone, 53% were treated with hydroxychloroquine (HCQ) and 27% were prescribed other disease-modifying anti-rheumatic drugs (DMARDs), of which methotrexate was the most common (14%).

Sera from 100 patients with early RA included in TIRA-2 (Swedish acronym for 'timely interventions in RA') were collected. The patients were diagnosed with recent-onset RA by the ACR 1987 criteria (≤ 12 months since the first joint swelling) and included in Linköping's TIRA-2 cohort between 2006 and 2009 [31,32]. At sampling, 83% of the patients received DMARDs. The mean age was 55 years, 69% were women, 64% were anti-cyclic citrullinated peptide-2 antibody (anti-CCP2)-positive and 60% were

**Table 1.** Detailed characteristics of the 526 systemic lupus erythematosus (SLE) cases

| | |
|---|---|
| *Background variables* | |
| Females, *n* (%) | 475 (90·3) |
| Age at blood sampling, mean years (range, years) | 48·1 (18–88) |
| Caucasian ethnicity, *n* (%) | 476 (90·5) |
| Body mass index, mean (range) | 25·2 (14·2–59·1) |
| Ever smoker (former or current), *n* (%) | 263 (50·2) |
| Daily dose of prednisolone at blood sampling, mean (range, mg) | 5·4 (0–60) |
| *Disease variables* | |
| Age at diagnosis, mean years (range, years) | 35·1 (3–85) |
| Disease duration at blood sampling, mean years (range, years) | 15·0 (0–58) |
| Established disease at time for blood sampling, *n* (%) | 461 (87·6) |
| Meeting ACR-97 criteria, *n* (%) | 526 (100) |
| Number of fulfilled ACR-97 criteria, mean (range) | 5·8 (4–10) |
| SLEDAI-2K at blood sampling, mean (range) | 3·9 (0–28) |
| SLICC/ACR damage index, mean (range) | 1·7 (0–11) |
| *Clinical SLE phenotypes (ACR-97 defined), n (%)* | |
| (1) Malar rash | 260 (49·4) |
| (2) Discoid rash | 98 (18·6) |
| (3) Photosensitivity | 327 (62·2) |
| (4) Oral ulcers | 129 (24·5) |
| (5) Arthritis | 424 (80·6) |
| (6) Serositis | 210 (39·9) |
| Pleuritis | 189 (35·9) |
| Pericarditis | 84 (16·0) |
| (7) Renal disorder | 181 (34·4) |
| (8) Neurological disorder | 45 (8·6) |
| Seizures | 39 (7·4) |
| Psychosis | 11 (2·1) |
| (9) Haematological disorder | 354 (67·3) |
| Haemolytic anaemia | 26 (4·9) |
| Leucocytopenia | 228 (43·3) |
| Lymphopenia | 235 (44·7) |
| Thrombocytopenia | 90 (17·1) |
| Raynaud | 181 (34·4) |
| *Immunological features (ACR-97 defined), n (%)* | |
| (10) Immunological disorder | 338 (64·3) |
| Anti-dsDNA antibody (anti-dsDNA) | 310 (58·9) |
| Anti-Smith antibody (anti-Sm) | 89 (16·9) |
| (11) Anti-nuclear antibody (ANA)* | 519 (98·7) |
| Anti-Sjögren's syndrome A (Ro52) | 155 (29·5) |
| Anti-Sjögren's syndrome A (Ro60) | 213 (40·7) |
| Anti-Sjögren's syndrome B (La) | 131 (24·9) |
| Lupus anti-coagulant (LA) test positive† | 128 (25·7) |
| *Clinical APS phenotypes, n (%)* | |
| Anti-phospholipid syndrome (clinical diagnosis) | 98 (18·6) |
| Anti-phospholipid syndrome (defined by classification)‡ | 76 (14·4) |
| Any arterial event (MI, all cerebrovascular lesions) | 77 (14·6) |
| Myocardial infarction (MI) | 38 (7·2) |
| Angina pectoris | 25 (4·8) |
| Coronary bypass | 14 (2·7) |
| Valvular disease | 44 (8·4) |
| Valvular surgery | 8 (1·5) |
| Arterial embolism (MI, ischaemic stroke) | 73 (13·9) |
| Cerebrovascular lesions (ischaemic stroke, cerebral haemorrhage, TIA) | 61 (11·6) |
| Ischaemic stroke | 47 (8·9) |
| Cerebral haemorrhage | 9 (1·7) |
| Transient ischaemic attack (TIA) | 19 (3·6) |
| Venous thromboembolism (DVT and/or PE) | 72 (13·7) |
| Deep vein thrombosis (DVT) | 64 (12·2) |
| Pulmonary embolism (PE) | 23 (4·4) |
| Intermittent claudication | 7 (1·3) |
| Any miscarriage | 78 (16·4) |
| ≥ 3 miscarriages before the 10th week of gestation | 6 (1·3) |
| ≥ 1 miscarriage beyond the 10th week of gestation | 46 (9·7) |

*Positive by immunofluorescence microscopy (IF-ANA).

†Data available in 499 of 526 cases.

‡According to Miyakis *et al.* [13]

ACR = American College of Rheumatology; SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; SLICC = Systemic Lupus International Collaborating Clinics.

IgM rheumatoid factor (RF)-positive. During 8 years of follow-up, none of them developed SLE.

The Sydney criteria for APS require cut-off levels corresponding to the ≥ 99th percentile of the levels in controls [13]. This was determined for each aPL isotype using 507 control sera (75% female). Of these, 212 were healthy blood donors from Linköping University hospital (mean age = 44 years) and 295 were controls from the general population, Karolinska University hospital (mean age = 48 years) without any history of thrombosis or pregnancy morbidity, as defined in the APS criteria.

### aPL and anti-coagulant assays

IgG, IgA and IgM aCL and anti-$\beta_2$GPI were analysed in the accredited clinical immunology laboratories at Linköping, Uppsala and Karolinska University hospitals using fluoroenzyme-immunoassays (Phadia-250 instrument; Thermo-Fisher Scientific Phadia AB, Uppsala, Sweden). The defined cut-off level for each isotype was for aCL, IgG/IgA/IgM 26 GPL-U/ml, 17 APL-U/ml and 34 MPL-U/ml, and for anti-$\beta_2$GPI, IgG/IgA/IgM 31, 13 and 7·2 U/ml, respectively. LA was determined by the dilute Russell's viper venom time (dRVVT) method (Siemens Healthcare Diagnostics, Erlangen, Germany) in Linköping, and by a modified dRVVT (Biopool, Umeå, Sweden) using Bioclot LA at Karolinska.

### Definitions

aPL-positive cases were categorized as being 'independently positive' for an isotype (i.e. regardless of being positive

for other isotypes or LA) or 'exclusively positive' for an isotype (i.e. isolated positive for the specific antibody, meaning absence of other aPL isotypes and LA) or 'triple-positive' (i.e. at least one positive isotype of aCL combined with any anti-$\beta_2$GPI isotype plus a positive LA test). In order to evaluate the potential additive value of IgA aPL analysis, we also studied cases categorized as being positive for at least one IgA isotype in the absence of other isotypes and LA.

### Statistical analyses

Comparisons of aPL levels between groups were performed using the Mann–Whitney *U*-test. Furthermore, the Mann–Whitney *U*-test was used to establish potential differences in aPL levels within blood donors and SLE cases. Correlation analyses between aPL levels and age in SLE, disease controls and blood donors were calculated using Spearman's rho.

Associations between (a) aPL antibody positivity and (b) SLE phenotypes, APS-related events, pharmacotherapy and damage accrual were examined by $\chi^2$ or Fisher's exact test when numbers were $\leq 5$.

Poisson regression was used to establish the empirical relationship between damage accrual (global SDI score) and each of the isotypes, age, disease duration, smoking habits, hypertension, lupus nephritis, ongoing treatments with HCQ and statins, a daily dose of prednisolone of $\geq 7{\cdot}5$ mg and LA positivity (univariate model). Thereafter, all variables significant in the univariate model were combined and a stepwise procedure eliminating non-significant ($P \geq 0{\cdot}05$) variables at each step was performed until a multiple model with only significant variables remained (the model with highest pseudo-$R^2$ with only significant predictors). Two-tailed *P*-values < 0·05 were considered significant. Statistical analyses were performed with SPSS Statistics version 23.0 (IBM, Armonk, NY, USA) or GraphPad Prism, version 6.07 (GraphPad Software, La Jolla, CA, USA).

### Ethical approval

Oral and written informed consent was obtained from all participants. The study protocols were approved by the regional ethics review boards in Linköping regarding SLE (M75-08/2008) and early RA (M168-05), in Stockholm regarding SLE (03-556/031216), and in Uppsala regarding pSS (2006/217/2).

## Results

### aPL levels among disease controls and blood donors

Fourteen (14%) of the RA patients and six (12%) of the pSS cases (without APS diagnosis) tested positive for at least one aPL isotype. The distribution of aPL levels in RA and pSS controls are demonstrated in Fig. 1a,b. No differences were found in aPL levels with regard to age or sex among disease controls. There were no significant differences regarding the levels of any aCL/anti-$\beta_2$GPI isotype between blood donors and population-based donors, but the population-based donors were slightly

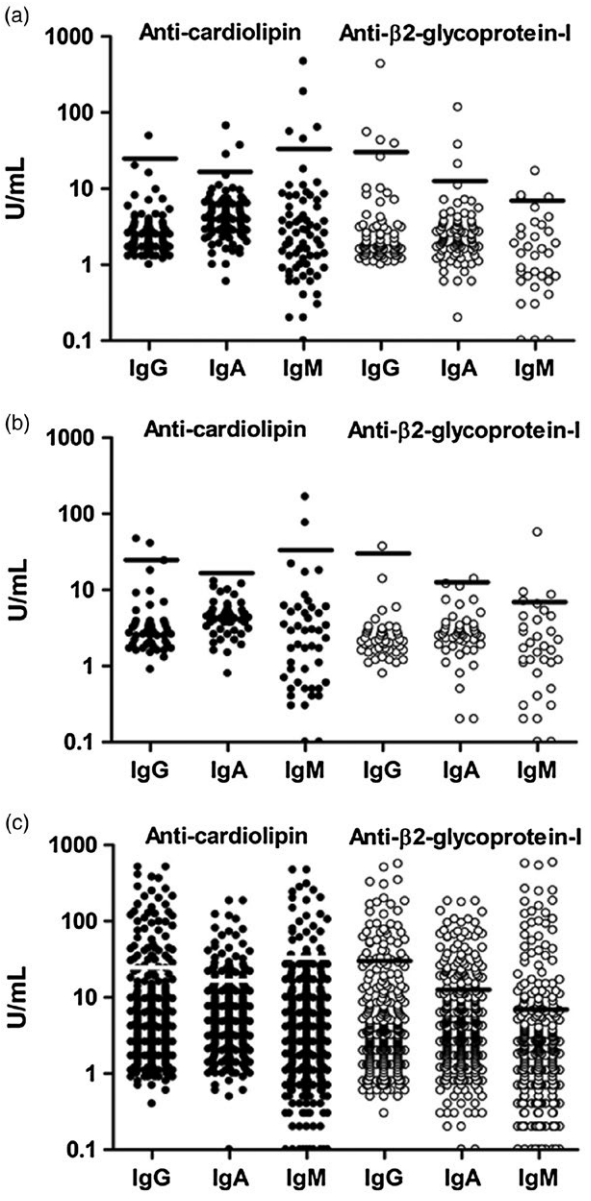


**Fig. 1.** (a–c) Serum levels of anti-phospholipid antibody (aPL) isotypes were determined by fluoroenzyme-immunoassays. Cut-off level corresponded to the 99th percentile of the levels of healthy controls. Closed circles represent anti-cardiolipin antibodies (aCL), while open circles represent anti-$\beta_2$-glycoprotein-I (anti-$\beta_2$GPI). (a) Serum levels of aCL and anti-$\beta_2$GPI isotypes in 100 rheumatoid arthritis (RA) controls. (b) Serum levels of aCL and anti-$\beta_2$GPI isotypes in 50 primary Sjögren's syndrome (pSS controls. (c) Serum levels of aCL and anti-$\beta_2$GPI isotypes in 526 systemic lupus erythematosus (SLE) cases.

older ($P < 0{\cdot}02$; the difference between medians was 2 years). IgG aCL was the only isotype which correlated significantly with age ($rho = -0{\cdot}10$; $P < 0{\cdot}05$). No differences were identified comparing aPL levels in women and men.

### aPL levels among cases with SLE

The levels of each separate aPL isotype among cases with SLE are shown in Fig. 1c. In total, 138 (26%) were positive for at least one antibody isotype. As demonstrated in Fig. 2, IgA aCL and/or anti-$\beta_2$GPI were found in 82 (16%) cases. Figure 2 illustrates the 45 (9%) IgA aCL-positive cases, 20 (4%) of whom were positive in the absence of IgG/IgM isotypes; Fig. 2 shows that 74 (14%) of the SLE cases were IgA anti-$\beta_2$GPI-positive, 34 (6%) of whom were positive in the absence of IgG/IgM isotypes. Figure 2 demonstrates the overlap between exclusively IgA aPL-positive SLE cases.

### aPL levels *versus* age, smoking habits and ethnicity in SLE

The levels of IgG- and IgA-class aPL antibodies were correlated inversely with age among SLE cases (IgG aCL rho = –0·09, IgA aCL rho = –0·09, IgG anti-$\beta_2$GPI rho = –0·10, IgA anti-$\beta_2$GPI rho = –0·09; $P < 0{\cdot}05$ for each comparison). A positive LA test and/or IgG anti-$\beta_2$GPI positivity were associated with being a past or present tobacco smoker (Table 2). Regardless of seropositivity for other aPL isotypes, IgG anti-$\beta_2$GPI seemed to associate with Caucasian ethnicity (Table 2). Conversely, non-Caucasian ethnicity was associated significantly with exclusive positivity for IgA anti-$\beta_2$GPI (Table 3).

### aPL isotypes *versus* APS-related events and pharmacotherapy

In total, 76 SLE patients (14%) fulfilled the APS classification criteria. Table 2 presents the significant associations between antibody specificities and SLE phenotypes, APS-related events, positivity for other autoantibodies, pharmacotherapy and damage accrual regardless of the number of positive aCL/anti-$\beta_2$GPI isotypes and/or LA. Triple-positive cases as well as cases with a positive LA test and/or IgG aPL were associated with most APS events and damage in several organ domains of the SDI.

Table 3 shows significant associations regarding exclusive occurrence of individual aPL isotypes and LA, as well as

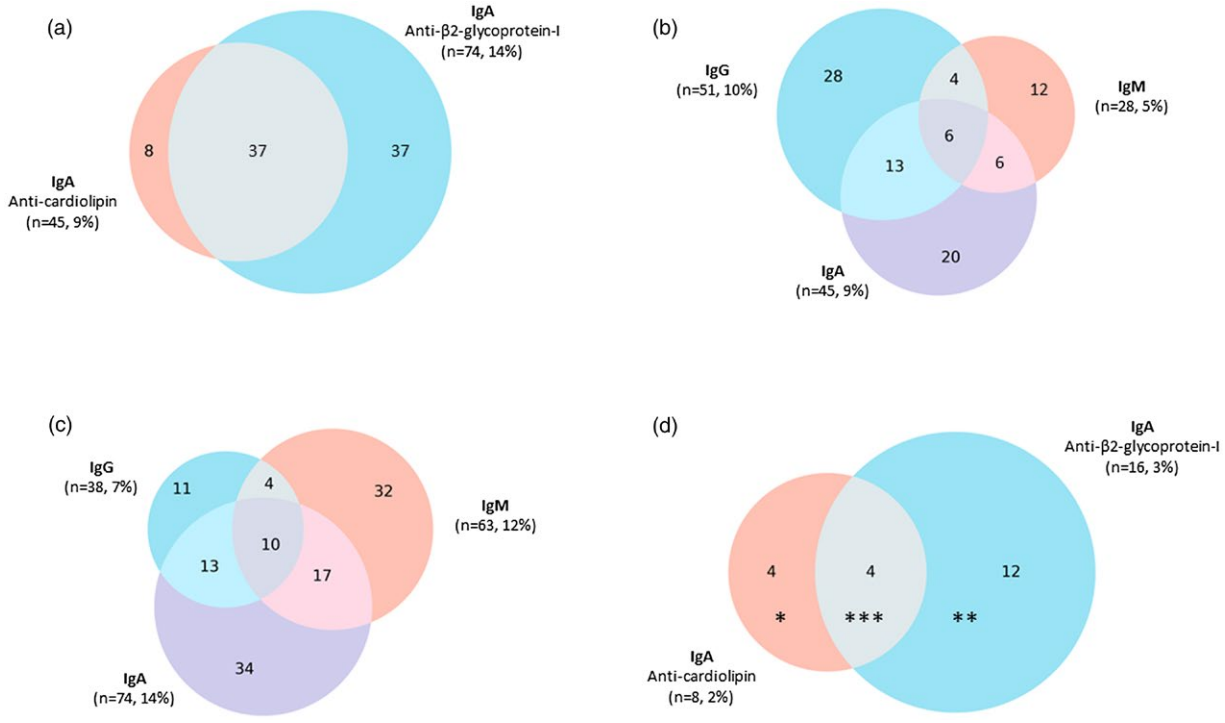


**Fig. 2.** (a) Distribution of immunoglobulin (Ig)A anti-cardiolipin antibody (aCL) and IgA anti-β2-glycoprotein-I (anti-$\beta_2$GPI)-positive cases in the full systemic lupus erythematosus (SLE) cohort; 82 (16%) of the SLE cases had IgA positivity, 45 (9%) of aCL and 74 (14%) of anti-$\beta_2$GPI type. (b) Distribution of IgG/A/M isotypes of aCL in the SLE cohort; 90 (17%) SLE cases were positive for at least one aCL isotype. (c) Distribution of IgG/A/M isotypes of anti-$\beta_2$GPI in the SLE cohort. 121 (23%) SLE cases were positive for at least one anti-$\beta_2$GPI isotype. (d) Distribution of exclusively IgA aCL and IgA anti-$\beta_2$GPI-positive cases in the SLE cohort; 20 (4%) of the SLE cases had IgA positivity, eight (2%) of aCL and 16 (3%) of anti-$\beta_2$GPI type. Each asterisk (*) indicates one patient with an anti-phospholipid syndrome (APS)-related event.

**Table 2.** Significant associations between disease phenotypes/serologies/damage/pharmacotherapy and each independent anti-cardiolipin antibodies (aCL)/anti-$\beta_2$-glycoprotein-I (anti-$\beta_2$GPI) isotype and lupus anti-coagulant (LA) (pos/neg) expressed by odds ratios (OR) with 95% confidence intervals

| | aCL | | | anti-$\beta_2$GPI | | | LA | Triple-positive[#] |
|---|---|---|---|---|---|---|---|---|
| | IgG (51/526) | IgA (45/526) | IgM (28/526) | IgG (38/526) | IgA (74/526) | IgM (63/526) | (128/499) | (49/499) |
| Caucasian ethnicity (*n* = 476) | | | | 8%[*†] | | | | |
| Ever smoker (*n* = 263) | | | | 2·39 (1·18–4·83) | | | 1·73 (1·15–2·60) | |
| Malar rash (*n* = 260) | 0·48 (0·26–0·88) | | 0·26 (0·10–0·66) | | | 0·49 (0·29–0·85) | 0·50 (0·33–0·76) | 0·46 (0·25–0·86) |
| Discoid rash (*n* = 98) | | | | | | 0·42 (0·17–0·99) | | |
| Photosensitivity (*n* = 327) | 0·35 (0·20–0·64) | 0·53 (0·29–0·96) | | 0·49 (0·26–0·95) | 0·46 (0·28–0·76) | 0·42 (0·25–0·72) | 0·62 (0·41–0·93) | 0·50 (0·28–0·91) |
| Thrombocytopenia (*n* = 90) | 1·99 (1·02–3·85) | | | | | | | |
| Raynaud (*n* = 181) | | | | | | 0·45 (0·24–0·84) | | |
| Anti-dsDNA antibody (*n* = 310) | | 2·23 (1·13–4·40) | | | 1·78 (1·04–3·03) | | | |
| Anti-SSA/Ro52 (*n* = 155) | | | | 0·25 (0·09–0·73) | | 0·26 (0·12–0·59) | 0·60 (0·37–0·96) | |
| Anti-SSA/Ro60 (*n* = 213) | 0·48 (0·25–0·93) | | | | | 0·33 (0·17–0·62) | 0·61 (0·40–0·93) | 0·51 (0·26–0·99) |
| Anti-SSB (*n* = 131) | 0·30 (0·12–0·77) | | | 0·15 (0·04–0·63) | | 0·33 (0·15–0·75) | 0·53 (0·32–0·89) | 0·39 (0·16–0·95) |
| APS, clinical (*n* = 98) | 4·82 (2·64–8·80) | 6·03 (3·25–11·20) | 3·59 (1·64–7·87) | 6·21 (3·16–12·20) | 4·46 (2·63–7·56) | 4·02 (2·30–7·01) | 12·55 (7·51–20·98) | 7·75 (4·17–14·41) |
| APS, classification (*n* = 76) | 5·35 (2·86–9·98) | 6·64 (3·52–12·53) | 4·31 (1·93–9·61)[*] | 7·17 (3·61–14·23) | 4·72 (2·70–8·26) | 3·96 (2·20–7·13) | 10·51 (6·02–18·35) | 9·65 (5·13–18·18) |
| Any arterial event (*n* = 77) | | | | 2·16 (1·01–4·64) | | | 3·01 (1·78–5·09) | 2·33 (1·17–4·63) |
| Valvular disease (*n* = 44) | 4·22 (2·01–8·85)[*] | | | | | | 2·48 (1·29–4·76) | 2·37 (1·03–5·43)[*] |
| Arterial embolism (myocardial infarction or ischaemic stroke) (*n* = 73) | 2·09 (1·04–4·21) | | | | | | 2·61 (1·52–4·48) | 2·21 (1·09–4·47) |
| Cerebrovascular lesion (*n* = 61) | 2·34 (1·13–4·84) | | | | | | 3·27 (1·85–5·81) | 2·14 (1·01–4·55) |
| Ischaemic stroke (*n* = 47) | 3·35 (1·59–7·09)[*] | | | 2·44 (1·01–5·89)[*] | 2·31 (1·14–4·68) | | 4·53 (2·36–8·66) | 3·05 (1·41–6·60)[*] |
| Transient ischaemic attack (*n* = 19) | | | | | | | | 3·76 (1·29–10·92)[*] |
| Venous thrombo-embolism (deep vein thrombosis or pulmonary embolism) (*n* = 72) | | 2·32 (1·14–4·71) | | 2·36 (1·10–5·09) | | 1·95 (1·01–3·75) | 2·88 (1·70–4·89) | 2·56 (1·28–5·10) |
| Deep vein thrombosis (*n* = 64) | | 2·73 (1·34–5·58) | | 2·27 (1·28–5·99)[*] | | 2·31 (1·19–4·47) | 3·00 (1·74–5·18) | 3·02 (1·50–6·06) |
| Pulmonary embolism (*n* = 23) | | | | | | 13%[*‡] | | |
| Intermittent claudication (*n* = 7) | 7·36 (1·60–33·86)[*] | | | | 8·55 (1·87–39·03)[*] | | 7·50 (1·44–39·15)[*] | 7·71 (1·67–35·52)[*] |
| Neuropsychiatric damage (*n* = 129) | 2·38 (1·31–4·32) | | | | | | 2·03 (1·30–3·17) | 1·91 (1·03–3·55) |
| Cardiovascular damage (*n* = 73) | 3·00 (1·55–5·81) | | | | | | 2·19 (1·26–3·81) | 2·21 (1·09–4·47) |
| Peripheral vascular damage (*n* = 49) | | 2·96 (1·37–6·40)[*] | 2·89 (1·11–7·50)[*] | 2·81 (1·21–6·51)[*] | 2·17 (1·07–4·39) | 2·65 (1·30–5·40) | 2·87 (1·56–5·30) | 3·34 (1·58–7·07)[*] |
| Any miscarriage (*n* = 78) | 2·51 (1·26–4·97) | | | 2·22 (1·01–4·85) | | | 1·71 (1·01–2·91) | 2·29 (1·14–4·61) |
| ≥1 miscarriage (beyond the 10th week of gestation) (*n* = 46) | 3·04 (1·39–6·64)[*] | | | 3·17 (1·34–7·50)[*] | | | 2·18 (1·15–4·12) | 2·66 (1·19–5·96)[*] |
| Warfarin (ongoing) (*n* = 103) | 2·76 (1·49–5·11) | 3·07 (1·65–5·74) | 4·60 (2·12–9·98) | 3·20 (1·62–6·31) | 2·84 (1·67–4·85) | 2·91 (1·66–5·10) | 6·83 (4·22–11·06) | 4·39 (2·38–8·08) |
| Salicylic acid (ongoing) (*n* = 114) | 2·15 (1·16–3·98) | 1·93 (1·02–3·67) | | | 2·08 (1·22–3·54) | | 1·84 (1·16–2·93) | |

APS = anti-phospholipid syndrome.

[*]Fisher's exact test.

[#]At least one positive isotype of aCL combined with any anti-$\beta_2$GPI isotype plus a positive LA test.

[†]OR not possible to calculate as none of 50 (0%) non-Caucasian patients have anti-$\beta_2$GPI immunoglobulin (Ig)G; 38 of 476 (8%) Caucasian patients have anti-$\beta_2$GPI IgG.

[‡]OR not possible to calculate as none of 23 (0%) patients with pulmonary embolism do not have anti-$\beta_2$GPI IgM; 64 of 503 (13%) of patients with pulmonary embolism have anti-$\beta_2$GPI IgM.

 

**Table 3.** Significant associations between disease phenotypes/serologies/damage/pharmacotherapy and each exclusively positive anti-cardiolipin antibodies (aCL)/anti-$\beta_2$-glycoprotein-I (anti-$\beta_2$GPI) isotype or lupus anti-coagulant (LA), as well as for cases positive for ≥ 1 immunogobulin (Ig)A aPL in the absence of other isotypes or LA, expressed by odds ratios (OR) with 95% confidence intervals

| | aCL | | | anti-$\beta_2$GPI | | | LA | ≥1 IgA isotype (aCL/anti-$\beta_2$GPI) |
|---|---|---|---|---|---|---|---|---|
| | IgG (51/526) | IgA (45/526) | IgM (28/526) | IgG (38/526) | IgA (74/526) | IgM (63/526) | (128/499) | (82/526) |
| Male sex (*n* = 51) | | | | | | | 2·17 (1·02–4·64) | |
| Caucasian ethnicity (*n* = 476) | | | | | 0·21 (0·06–0·72)* | | | |
| Non-Caucasian ethnicity (*n* = 50) | | | | | 4·76 (1·39–16·67)* | | | |
| Photosensitivity (*n* = 327) | | | | | 0·19 (0·05–0·72)* | | | |
| Serositis (*n* = 210) | | | | | | | 1·88 (1·09–3·23) | |
| Anti-dsDNA antibody (*n* = 310) | | | | | | 4%*† | | |
| Anti-SSA/Ro52 (*n* = 155) | | | | | 3·51 (1·09–11·23)* | | | |
| Anti-SSA/Ro60 (*n* = 213) | | | | | 4·41 (1·18–16·49)* | | | 2·79 (1·16–6·72) |
| APS, clinical (*n* = 98) | | | | | | | 4·10 (2·32–7·26) | |
| APS, classification (*n* = 76) | | | | | | | 2·99 (1·61–5·56) | |
| Any arterial event (*n* = 77) | | | | | | | 2·65 (1·40–5·02) | |
| Valvular disease (*n* = 44) | | | | | | | 2·27 (1·03–5·04) | |
| Arterial embolism (myocardial infarction *OR* ischaemic stroke) (*n* = 73) | | | | | | | 2·10 (1·07–4·15) | |
| Cerebrovascular lesion (*n* = 61) | | | | | | | 2·95 (1·50–5·83) | |
| Ischaemic stroke (*n* = 47) | | | | | | | 2·95 (1·40–6·25) | |
| Ocular damage (*n* = 88) | | | 3%*‡ | | | | | |
| Pulmonary damage (*n* = 32) | | | | | | | 2·63 (1·07–6·48)* | 3·96 (1·25–12·57)* |
| Musculoskeletal damage (*n* = 95) | | | | | | | 1·98 (1·07–3·67) | |
| Ciclosporin/ sirolimus (ongoing) (*n* = 13) | | | 48·40 (2·82–829·82)* | | | | | 8·72 (2·15–35·37)* |
| Warfarin (ongoing) (*n* = 103) | | | | | | | 3·40 (1·91–6·04) | |
| Salicylic acid (ongoing) (*n* = 114) | | | | | | | 1·92 (1·06–3·48) | 3·12 (1·33–7·33)* |

APS = Anti-phospholipid syndrome.

*Fisher's exact test.

†OR not possible to calculate as none of 203 (0%) patients without anti-dsDNA have isolated anti-$\beta_2$GPI immunoglobulin (Ig)M. Eleven of 296 (4%) patients with anti-dsDNA have isolated anti-$\beta_2$GPI IgM.

‡OR not possible to calculate as none of 419 (0%) patients without ocular damage have isolated IgM aCL. Two of 80 (3%) patients with ocular damage have isolated IgM aCL.

 

**Table 4.** Poisson regression models to establish empirical relations with damage accrual (global SDI score)

| | Univariate model | | | Multiple model | |
|---|---|---|---|---|---|
| | OR* | 95% CI | Pseudo-$R^2$ | OR | 95% CI |
| Disease duration | 1·035 | 1·029–1·040 | 0·124 | 1·020 | 1·014–1·026 |
| Age | 1·036 | 1·031–1·040 | 0·230 | 1·034 | 1·029–1·040 |
| Ever smoker | 1·422 | 1·244–1·626 | 0·017 | 1·175 | 1·019–1·355 |
| Lupus nephritis | 1·232 | 1·076–1·410 | 0·003 | 1·498 | 1·289–1·742 |
| Daily prednisolone dose ≥ 7·5 mg (ongoing) | 1·325 | 1·151–1·526 | 0·008 | 1·727 | 1·485–2·008 |
| Statins (ongoing) | 1·822 | 1·488–2·230 | 0·023 | 1·249 | 1·013–1·539 |
| LA positivity | 1·521 | 1·315–1·760 | 0·171 | 1·268 | 1·092–1·471 |
| HCQ (ongoing) | 0·686 | 0·598–0·787 | 0·021 | 0·851 | 0·732–0·989 |
| Hypertension | 1·367 | 1·175–1·589 | 0·024 | | |
| Triple positivity | 1·298 | 1·058–1·593 | <0·001 | | |
| Total pseudo-$R^2$ (multiple model) | | | | | 0·471 |

Pseudo-$R^2$ is different from the $R^2$ used in ordinary least-squares regression models. However, it will give an approximation of how well the independent variables are related with the outcome [sum of global SLICC/ACR damage index (SDI)]. CI = confidence intervals.

*The odds ratios (OR) can be interpreted as follows: an increase of 1 year of disease duration is associated with 3·5% higher score (OR = 1·035) in the number of SDI points, and ongoing treatment with hydroxychloroquine (HCQ) is associated with 31·4% less score (OR = 0·686, 1–0·686 = 0·314) in the sum of global SDI score compared to those not having ongoing treatment with HCQ.

one column with ≥ 1 IgA isotype demonstrating the potential additive value of introducing analysis of IgA aPLs. LA showed significant associations with several types of damage and APS-related events. Cases exclusively positive for ≥ 1 IgA isotype associated with presence of anti-SSA/Ro60 antibodies, organ damage of the pulmonary domain, use of cyclosporin/sirolimus and salicylic acid.

### APS-related events in exclusively IgA-positive cases

As demonstrated in Fig. 2, we identified eight cases (2%) who were exclusively IgA aCL-positive, whereas 16 (3%) were exclusively IgA anti-$\beta_2$GPI-positive. Of the 20 cases with exclusively positive IgA aCL and/or anti-$\beta_2$GPI, six (1% of all SLE cases) had manifestations compatible with APS. Thus, given that IgA aPLs were included in the APS criteria, another six cases would have been classified as APS (provided testing above defined levels after ≥ 12 weeks), in addition to the 76 identified previously.

### Factors associated with damage accrual

Table 4 illustrates factors and manifestations that were associated significantly with damage accrual. In the univariate model several factors were identified. However, in the multiple model disease duration [odds ratio (OR) = 1·020], age (OR = 1·034), past/present) smoking (OR = 1·175), meeting the ACR-defined nephritis criterion (OR = 1·498), LA positivity (OR = 1·268), daily treatment with ≥ 7·5 mg prednisolone (OR = 1·727), ongoing use of statins (OR = 1·249) and ongoing treatment with HCQ (OR = 0·851) remained in the model. The overall pseudo-$R^2$ was 0·471, indicating that almost 50% of the total variation of global SDI scores could be explained by the significant factors included in the multiple model (Table 4).

## Discussion

The main objective of this study was to evaluate the frequencies of IgA aCL and anti-$\beta_2$GPI in well-characterized patients with SLE, the majority of whom had established disease, in relation to disease phenotypes, vascular events, smoking habits and accrual of organ damage. We identified a subgroup of patients with IgA aPL antibodies (16%), in some cases even in the absence of IgG and IgM isotypes (4%). The presence of IgA anti-$\beta_2$GPI positivity without other isotypes was found to be associated with non-Caucasian ethnicity (representing fewer than 10% of cases in the study). Apart from ethnicity, exclusive positivity of ≥ 1 IgA aPL antibody showed significant associations with anti-Ro60 positivity, pulmonary damage and ongoing use of cyclosporin/sirolimus or salicylic acid.

In previous studies of SLE, exclusive occurrence of IgA aCL has been demonstrated in 4–17%, but reports regarding its association with clinical APS are inconsistent [15,20,23]. In two studies, no associations were found between IgA aCL occurrence and clinical APS-related events [20,21], whereas other studies observed associations between IgA aCL and deep vein thrombosis and/or pregnancy loss [15,23]. In the study by Samarkos *et al.*, the occurrence of IgA aCL did not improve sensitivity, specificity or the positive predictive value for APS diagnosis [23]. In contrast, some reports have indicated that a positive IgA anti-$\beta_2$GPI test is associated with clinical manifestations of APS [15–17], whereas other studies have been

inconclusive [20,23,33]. Thus, the clinical relevance of IgA aPLs in APS-related events of SLE cases remains obscure. According to Meijide *et al.*, there is not yet enough evidence to recommend routine analysis of IgA aCL and/or IgA anti-$\beta_2$GPI in order to increase the diagnostic accuracy of APS [34]. However, comparisons of different studies may be hampered by differences in study populations and lack of diagnostic gold standards regarding methodology, including definition of cut-off levels for positive results [33,35–37]. In our study, the overlap between IgA aCL and IgA anti-$\beta_2$GPI (Fig. 2) was surprisingly limited. However, we feel confident with the results, as cut-off levels for both assays were based on samples from more than 500 blood donors.

In the review by Andreoli *et al.*, it was concluded that IgA anti-$\beta_2$GPI is of clinical importance regarding APS in patients with SLE, whereas the importance of IgA aCL is less clear [25]. This conclusion is supported by other studies showing that exclusive occurrence of IgA anti-$\beta_2$GPI antibodies associates with thromboembolic events [38], especially on the arterial side [39]. In addition, Tortosa *et al.* demonstrated recently an annual predictive value for APS events among isolated IgA anti-$\beta_2$GPI-positive asymptomatic individuals of 3.1% over 5 years [19]. Similarly, studies of primary APS indicate larger clinical relevance of IgA anti-$\beta_2$GPI compared to IgA aCL [40,41]. However, in the present study we found that the overall occurrence of at least one aCL isotype (including IgA) is indeed associated with APS-related events and vascular damage. Being exclusively IgA aPL-positive, however, was not associated significantly with APS events or organ damage. IgA anti-$\beta_2$GPI was more frequent than IgA aCL, and associated with non-Caucasian ethnicity. The latter is partly in line with the study by Cucurull *et al.*, who reported higher prevalence rates of IgA aCL and anti-$\beta_2$GPI in an African American population with SLE compared to other ethnicities [15]. However, in our hands, the exclusive occurrence of IgA aCL did not contribute further with clinically useful information. Exclusive occurrence of IgA anti-$\beta_2$GPI associated significantly with photosensitivity and anti-SSA antibodies (Ro52 as well as Ro60), but these associations were based on only 12 cases.

Our observation that IgG aPLs, LA, as well as triple-positive patients, had the largest number of significant associations with APS-related events and damage accrual is extremely consistent with earlier studies, including a review of primary APS [11,42]. Cigarette smoking has been found previously to associate with aPLs [29]. Herein, we identified significant associations between past or present tobacco smoking and positive LA test as well as with IgG anti-$\beta_2$GPI.

Development of organ damage, defined according to SDI, is highly predictive of prognosis and mortality in SLE [27,43,44]. The presence of aPLs, as well as manifest APS, is associated with increased morbidity and mortality, as well as a lower quality of life [5–7,45]. Hence, it is of major importance to analyse these antibody specificities and identify new SLE cases with significant risks of future pregnancy morbidity, other APS-related events as well as damage accrual.

The prevalence rates of aPLs in SLE studies deviate considerably, possibly due in part to differences regarding disease severity and ethnicity, but most probably also to different methodological issues. Consensus guidelines and proposals for aPL testing have been published during the last two decades and resulted in improvements. However, methodological standardization has not yet been reached. Developments regarding the definition of international units and reference materials for anti-$\beta_2$-GPI testing are ongoing, and may lessen the discordance in prevalence [18,35,46].

A limitation of the study is the cross-sectional design which leaves unanswered the question regarding changes in aPL levels and aPL positivity over time. In addition, the blood donors were healthy at the time of blood sampling, and were not followed over time. Nevertheless, an obvious strength of the present study is the use of disease control groups with a long follow-up. RA and pSS may both mimic SLE clinically, particularly in early disease. None of the disease controls met the APS classification criteria, although almost 20% had either aPL of at least one isotype and/or a positive LA test. The proportions of positive laboratory tests in the control groups were higher than we expected, as pSS and RA are associated less commonly with APS compared to SLE [47,48]. However, similar frequencies of aPL in pSS have been demonstrated for the IgG/IgM isotypes [47], and in a review by Olech and Merrill a mean prevalence of 28% was reported regarding aPL in RA [48]. Cardio- and cerebrovascular events are expected to be found, as it is well known that several rheumatological diseases have an increased risk for such events [49–52]. Two of the RA patients (one of whom was positive for both IgA aCL and IgA anti-$\beta_2$GPI, and the other positive with regard to IgM aCL only) and one patient with pSS (IgG aCL and LA-positive) had suffered from cardiovascular or cerebrovascular events.

Disease duration, age, tobacco smoking (past/present), lupus nephritis, use of statins and $\geq$ 7·5 mg prednisolone daily and a positive LA test were associated with damage accrual in the multiple model, whereas ongoing treatment with HCQ showed a protective effect. Our findings are well in line with the observations by the SLICC group

which reported that hypertension, LA positivity and HCQ constitute factors which associate with damage accrual over time [44], and the results are also compatible with data from the Hopkins Lupus Cohort and others [5,7,8,53]. In this context, accumulated corticosteroid dose would clearly have been valuable in the regression models, but this was unfortunately not available.

To conclude, the addition of IgA-class autoantibody analyses, especially IgA anti-$\beta_2$GPI, provided some additional clinical correlates, coinciding with non-Caucasian ethnicity, and was associated inversely with photosensitivity. However, further evaluations of the importance of IgA-class aPL antibody analyses are required before it is introduced in general clinical routine. Based on the results presented here, we agree with recent consensus documents, suggesting that serum IgA-class antibody analyses should be restricted to SLE patients with clinically suspected APS, who test negative for IgG and IgM aCL/anti-$\beta_2$GPI, and LA [18,54].

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The authors declare that they have no disclosures related to this manuscript.

## Author contributions

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. M. F. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: M. F., A. V., G. G., J. W., Ö. D., E. S. and C. S. Acquisition of data: M. F., A. V., G. G., T. S., K. E., I. G., A. K., J. R., E. S. and C. S. Analysis and interpretation of data: M. F., A. V., G. G., T. S., K. E., I. G., A. K., Ö. D., J. R., E. S. and C. S.

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