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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IgA anti-APL antibodies and clinical events in Swedish cases of SLE

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List of abbreviations: SLE = systemic lupus erythematosus; Ig = immunoglobulin; aCL = anti-cardiolipin antibody; anti-β_2GPI = anti-β_2-glycoprotein-I; LA = lupus anticoagulant; aPL = antiphospholipid antibody; pSS = primary Sjögren's syndrome; RA = rheumatoid arthritis; APS = antiphospholipid syndrome; ACR = American College of Rheumatology; SLICC = Systemic Lupus International Collaborating Clinics; SDI = SLICC/ACR damage index; HCQ = hydroxychloroquine; DMARD = disease modifying anti-rheumatic drug

SUMMARY

Immunoglobulin (Ig) G- and IgM-class anti-cardiolipin antibodies (aCL) and lupus anticoagulant (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-β_2-glycoprotein-I (anti-β_2GPI) were included in the 2012 Systemic Lupus International Collaborating Clinics criteria. The present study aimed to evaluate IgG-/IgA-/IgM-aCL and anti-β_2GPI occurrence in relation to disease phenotype, smoking habits, pharmacotherapy, 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-β_2GPI. 76 (14%) SLE cases fulfilled the Sydney APS-criteria, and ≥1 aCL/anti-β2GPI isotype (IgG/IgA/IgM) occurred in 138 SLE patients (26%). 45 (9%) of the SLE cases had IgA-aCL, of whom 20 (4%) lacked IgG-/IgM-aCL. 74 (14%) tested positive for IgA anti-β_2GPI, 34 (6%) being seronegative regarding IgG/IgM anti-β_2GPI. 6 (1%) had APS manifestations but were seropositive regarding IgA-aCL and/or IgA anti-β_2GPI 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-β_2GPI occurrence associated inversely with Caucasian ethnicity (*OR* 0.21, 95% *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.

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 subsequently lead to irreversible organ damage, a consequence which is intimately associated with decreased quality of life and increased mortality [2-4]. In SLE, accrual of organ damage and prognosis has consistently been linked 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 anticoagulant (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 IgG/IgM isotype and/or a positive LA test and/or a persistent false-positive serologic 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 fulfill 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-β_2 glycoprotein-I (anti-β_2GPI) 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-β_2GPI 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-β_2GPI 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-β_2GPI in people with no history of APS-related events constitute an important independent risk factor for the development of such events [19]. On the contrary, 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% vs. 83%) but slightly lower specificity (84% vs. 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-β_2GPI 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-β_2GPI domain 1 IgG in relation to APS in SLE has recently been evaluated [26].
Since the presence of IgA aPLs is of uncertain clinical significance [12], the overall goal of this study was to evaluate IgA aCL and anti-β_2GPI antibodies in serum samples of 526 well-characterised 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 previously been described in detail [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. 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 were collected [30]. 49% 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 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 ≥ 99th percentile of the levels in controls [13]. This was determined for each aPL isotype using 507 control sera (75% females). 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 anticoagulant assays

IgG, IgA and IgM aCL and anti-β_2GPI 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-β_2GPI 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 categorised 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-β_2GPI isotype *plus* a positive LA test). In order to evaluate the potential additive value of IgA aPL analysis, we also studied cases categorised 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 Mann-Whitney *U* test. Furthermore, 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 χ^2 or Fisher's exact test when numbers were ≤ 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 ≥ 7.5 mg, and LA positivity (univariate model). Thereafter, all variables

significant in the univariate model were combined and a stepwise procedure eliminating non-significant ($P \geq 0.05$) variables at each step was performed until a multiple model with only significant variables remained (the model with highest pseudo-R2 with only significant predictors). Two-tailed P values < 0.05 were considered significant. Statistical analyses were performed with SPSS Statistics 23.0 (IBM, Armonk, NY, USA) or GraphPad Prism, version 6.07 (GraphPad Software, La Jolla, CA, USA).

Ethics approvals

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 6 (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 Figures 1A and 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-β_2GPI isotype between blood donors and population-based donors, but the population-based donors were slightly older ($P < 0.02$; the difference between medians was 2 years). IgG aCL was the only isotype which correlated significantly with age ($rho = -0.10$; $P < 0.05$). 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 Figure 1C. In total, 138 (26%) were positive for at least one antibody isotype. As demonstrated in Figure 2A, IgA aCL and/or anti-β_2GPI were found in 82 (16%) cases. Figure 2B illustrates the 45 (9%) IgA aCL positive cases, 20 (4%) of whom were positive in the absence of IgG/IgM isotypes; Figure 2C shows that 74 (14%) of the SLE cases were IgA anti-β_2GPI positive, 34 (6%) of whom were positive in the absence of IgG/IgM isotypes. Figure 2D demonstrates the overlap between exclusively IgA aPL positive SLE cases.

aPL levels vs. age, smoking habits and ethnicity in SLE

The levels of IgG- and IgA-class aPL antibodies were inversely correlated with age among SLE cases (IgG aCL *rho* = -0.09, IgA aCL *rho* = -0.09, IgG anti-β_2GPI *rho* = -0.10, IgA anti-β_2GPI *rho*

= -0.09; $P < 0.05$ for each comparison). A positive LA test and/or IgG anti-β_2GPI positivity were associated with being past or present tobacco smoker (Table 2). Regardless of seropositivity for other aPL isotypes, IgG anti-β_2GPI seemed to associate with Caucasian ethnicity (Table 2). On the contrary, non-Caucasian ethnicity associated significantly with exclusive positivity for IgA anti-β_2GPI (Table 3).

aPL isotypes vs. 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-β_2GPI 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 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 cyclosporine/sirolimus and salicylic acid.

APS-related events in exclusively IgA positive cases

As demonstrated in Figure 2D, we identified 8 cases (2%) who were exclusively IgA aCL positive, whereas 16 (3%) were exclusively IgA anti-β_2GPI positive. Of the 20 cases with

exclusively positive IgA aCL and/or anti-β_2GPI, 6 (1% of all SLE cases) had manifestations compatible with APS. Thus, given that IgA aPLs were included in the APS criteria, another 6 cases would have been classified as APS (provided testing above defined levels after ≥ 12 weeks) in addition to the 76 previously identified.

Factors associated with damage accrual

Table 4 illustrates factors and manifestations that were significantly associated with damage accrual. In the univariate model several factors were identified. However, in the multiple model disease duration (*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-R2 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-β_2GPI in well-characterised patients with SLE, whereof the majority 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-β_2GPI positivity without other isotypes was found to be associated with non-Caucasian ethnicity (representing less 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 cyclosporine/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-β_2GPI 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-β_2GPI 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-β_2GPI (Figure 2) was surprisingly limited. Yet, we feel confident with the results since 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-β_2GPI 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-β_2GPI antibodies associates with thromboembolic events [38], especially on the arterial side [39]. In addition, Tortosa *et al.* recently demonstrated an annual predictive value for APS events among isolated IgA anti-β_2GPI positive asymptomatic individuals of 3.1% over five years [19]. Similarly, studies of primary APS indicate larger clinical relevance of IgA anti-β_2GPI 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 significantly associated with APS events or organ damage. IgA anti-β_2GPI 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-β_2GPI in an Afro-American population with SLE as compared to other ethnicities [15]. However, in our hands, exclusive occurrence of IgA aCL did not contribute further with clinically useful information. Exclusive occurrence of IgA anti-β_2GPI significantly associated with photosensitivity and anti-SSA antibodies (Ro52 as well as Ro60), but these associations were based on only twelve 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 well in consistence

with earlier studies, including a review of primary APS [11, 42]. Cigarette smoking has previously been found 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-β_2GPI.

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 in part due to differences regarding disease severity and ethnicity, but most likely also to different methodological issues. Consensus guidelines and proposals for aPL testing have been published over the last two decades and resulted in improvements. Yet, methodological standardisation has not yet been reached. Developments regarding the definition of international units and reference materials for anti-β_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 the question regarding changes in aPL levels and aPL positivity over time unanswered. 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 long follow-up. RA and pSS may both clinically mimic SLE, 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, since pSS and RA are less commonly associated 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 & 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 rheumatologic 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-β_2GPI, 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 ≥ 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 obviously 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-β_2GPI, provided some additional clinical correlates, coinciding with non-Caucasian ethnicity and was inversely associated with photosensitivity. Yet, 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-β_2GPI, and LA [18, 54].

DECLARATIONS

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Disclosure

There are no disclosures to report.

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. Dr. Frodlund had full access to all of 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: MF, AV, GG, JW, ÖD, ES, CS. Acquisition of data: MF, AV, GG, TS, KE, IG, AK, JR, ES, CS. Analysis and interpretation of data: MF, AV, GG, TS, KE, IG, AK, ÖD, JR, ES, CS.

FIGURE LEGENDS

Figure 1A-C: Serum levels of aPL isotypes were determined by fluoroenzyme-immunoassays. Cut-off level corresponded to the 99th percentile of the levels of healthy controls. Closed circles represent aCL, while open circles represent anti-β_2GPI.

1a) Serum levels of aCL and anti-β_2GPI isotypes in 100 RA controls.

1b) Serum levels of aCL and anti-β_2GPI isotypes in 50 pSS controls.

1c) Serum levels of aCL and anti-β_2GPI isotypes in 526 SLE cases.

Figure 2A: Distribution of IgA aCL and IgA anti-β_2GPI positive cases in the full SLE cohort. 82 (16%) of the SLE cases had IgA positivity, 45 (9%) of aCL and 74 (14%) of anti-β_2GPI type.

Figure 2B: Distribution of IgG/A/M isotypes of aCL in the SLE cohort. 90 (17%) SLE cases were positive for at least one aCL isotype.

Figure 2C: Distribution of IgG/A/M isotypes of anti-β_2GPI in the SLE cohort. 121 (23%) SLE cases were positive for at least one anti-β_2GPI isotype.

Figure 2D: Distribution of exclusively IgA aCL and IgA anti-β_2GPI positive cases in the SLE cohort. 20 (4%) of the SLE cases had IgA positivity, 8 (2%) of aCL and 16 (3%) of anti-β_2GPI type. Each asterisk (*) indicate one patient with an APS-related event.

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Figure 1A

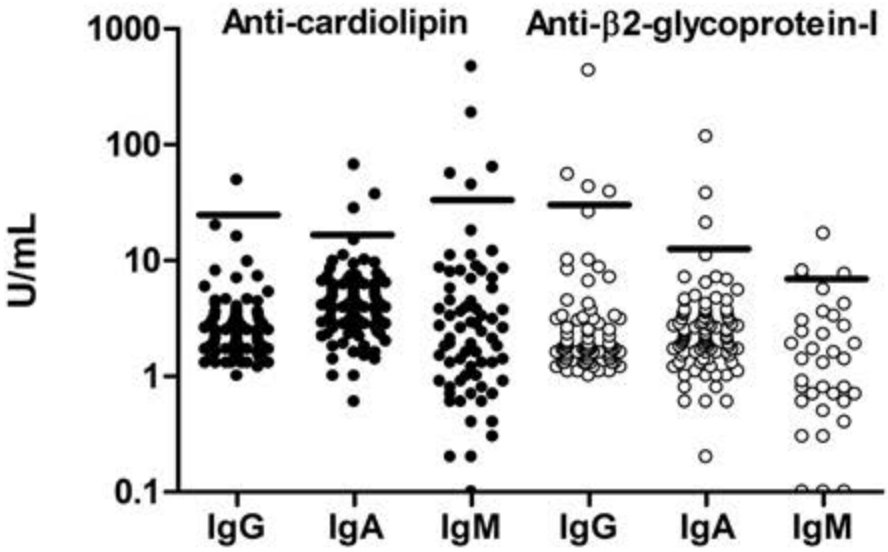


Figure 1B

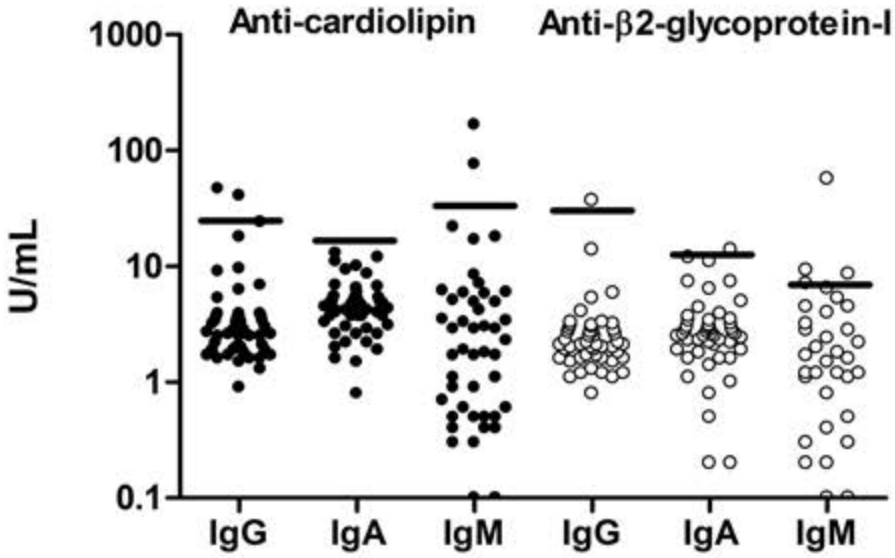


Figure 1C

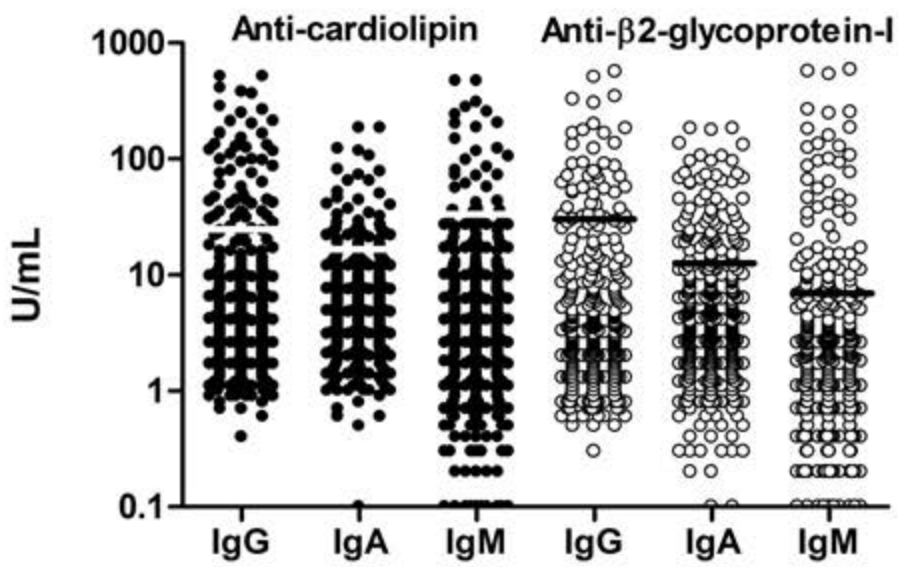
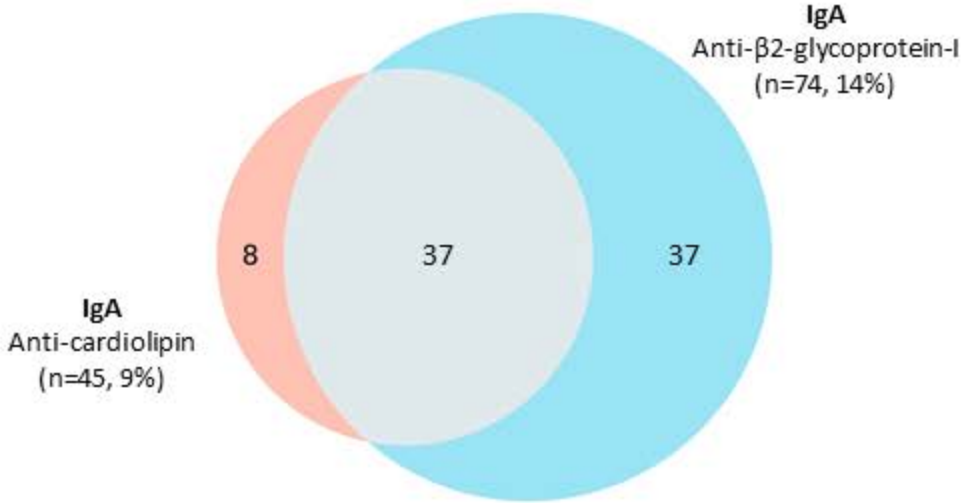


Figure 2A



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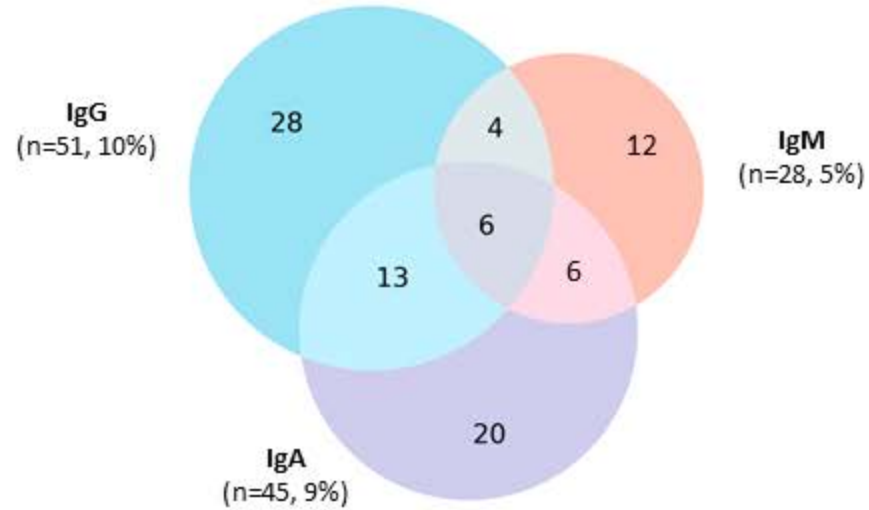

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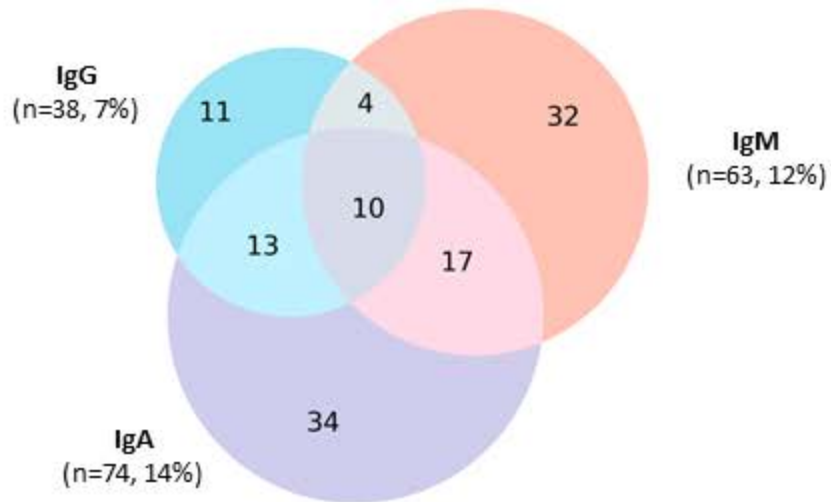

Figure 2D

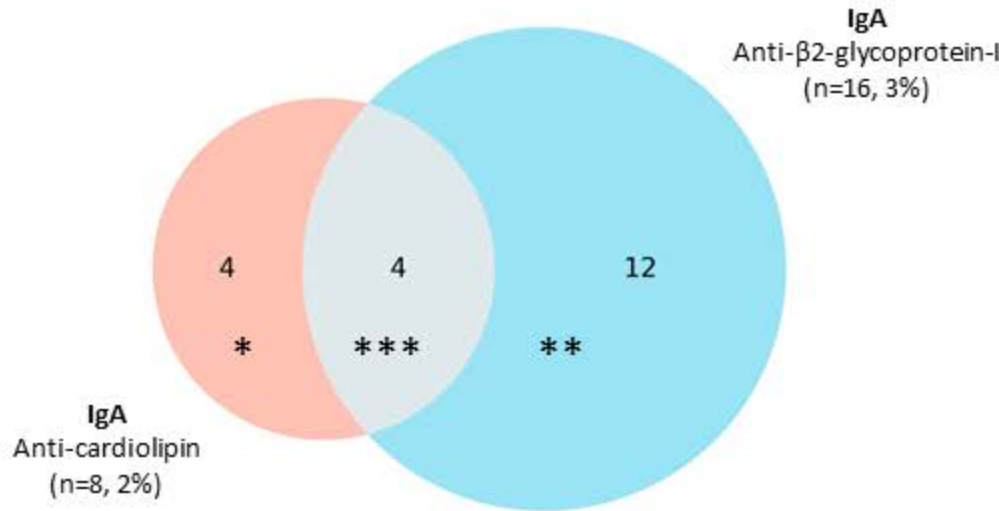

Table 1. Detailed characteristics of the 526 SLE cases.

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) Neurologic disorder	45 (8.6)
Seizures	39 (7.4)
Psychosis	11 (2.1)
9) Hematologic disorder	354 (67.3)
Hemolytic anemia	26 (4.9)
Leukocytopenia	228 (43.3)
Lymphopenia	235 (44.7)
Thrombocytopenia	90 (17.1)
Raynaud	181 (34.4)
Immunological features (ACR-97 defined), *n* (%)	
10) Immunologic disorder	338 (64.3)
Anti-dsDNA antibody (anti-dsDNA)	310 (58.9)
Anti-Smith antibody (anti-Sm)	89 (16.9)
11) Antinuclear 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 anticoagulant (LA) test positive[#]	128 (25.7)

Clinical APS phenotypes, n (%)	
Antiphospholipid syndrome (clinical diagnosis)	98 (18.6)
Antiphospholipid 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 by-pass	14 (2.7)
Valvular disease	44 (8.4)
Valvular surgery	8 (1.5)
Arterial embolism (MI, ischemic stroke)	73 (13.9)
Cerebrovascular lesions (ischemic stroke, cerebral hemorrhage, TIA)	61 (11.6)
Ischemic stroke	47 (8.9)
Cerebral hemorrhage	9 (1.7)
Transient ischemic 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]

Table 2. Significant associations between disease phenotypes/serologies/damage/pharmacotherapy and each independent aCL/anti-β_2GPI isotype and LA (pos/neg) expressed by *odds ratios (OR)* with 95% confidence intervals.

	aCL			**anti-β2GPI**			**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%*[a]				
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* ischemic 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)
Ischemic 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 ischemic 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%*[b]		
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)	

* Fisher's exact test

At least one positive isotype of aCL *combined with* any anti-β2GPI isotype *plus* a positive LA test.

[a] *OR* not possible to calculate since 0 of 50 (0%) of none-Caucasian patients have anti-β2GPI IgG. 38 of 476 (8%) Caucasian patients have anti-β2GPI IgG.

[b] *OR* not possible to calculate since 0 of 23 (0%) of patients with pulmonary embolism do not have anti-β2GPI IgM. 64 of 503 (13%) of patients with pulmonary embolism have anti-β2GPI IgM.

Table 3. Significant associations between disease phenotypes/serologies/damage/pharmacotherapy and each exclusively positive aCL/anti-β_2GPI isotype or LA, as well as for cases positive for ≥1 IgA aPL in the absence of other isotypes or LA, expressed by *odds ratios (OR)* with 95% confidence intervals.

	aCL			**anti-β_2GPI**			**LA**	**≥1 IgA isotype** (aCL/anti-β_2GPI)
	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%*[a]		
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* ischemic stroke) (*n*=73)							**2.10** (1.07-4.15)	
Cerebrovascular lesion (*n*=61)							**2.95** (1.50-5.83)	
Ischemic stroke (*n*=47)							**2.95** (1.40-6.25)	
Ocular damage (*n*=88)			3%*[b]					
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)	
Ciclosporine/ 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)*

* Fisher's exact test

[a] *OR* not possible to calculate since 0 of 203 (0%) of patients without anti-dsDNA have isolated anti-β2GPI IgM. 11 of 296 (4%) of patients with anti-dsDNA have isolated anti-β2GPI IgM.

[b] *OR* not possible to calculate since 0 of 419 (0%) of patients without ocular damage have isolated IgM aCL. 2 of 80 (3%) of 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

Note: 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 SDI). *CI* = confidence intervals.

* The odds ratios (*OR*) can be interpreted as follows: an increase of one 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.