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Antiphospholipid autoantibody detection is important in all patients with systemic autoimmune diseases

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Abstracts

Antiphospholipid (aPL) autoantibodies are uncommon in systemic autoimmune diseases (SADs). However, the European PRECISESADS study provides the opportunity to better characterize this rare association. The study was composed of 1818 patients with SADs including 453 with systemic lupus erythematosus (SLE), 359 with rheumatoid arthritis (RA), 385 with systemic sclerosis (SSc), 367 with Sjögren's syndrome (SjS), 94 with mixed connective tissue disease (MCTD), and 160 with undifferentiated connective tissue disease (UCTD). Assays used for aPL determination include the lupus anticoagulant (LAC) analysis using the dilute Russell's viper venom time (dRVVT) assay plus anti-cardiolipin (aCL) and anti- β 2GPI autoantibodies of IgG and IgM isotype. Information regarding clinical and biological characteristics of SAD patients was available. Among SAD patients, the prevalence of aPL differs significantly between two groups: SLE (57.6%) and non-SLE SADs (13.7%, $p < 10^{-4}$). Next, association between aPL plus thrombosis and miscarriage were observed in both SLE and non-SLE patients. Thrombosis was best predicted in SLE patients by dRVVT (OR=6.1; IC95:3.5-10.3) and miscarriage by aCL \pm β 2GPI IgG (OR=2.5; IC95:1.2-5.2); while in non-SLE SADs the best predictors were aCL \pm β 2GPI IgG for thrombosis (OR=6.6; IC95:2.4-18.4) and aCL \pm β 2GPI IgM for miscarriage (OR=2.9; IC95:1.2-6.8). In the case of multiple positivity of aPL, the risk for thrombosis and miscarriage is increased. Central nervous system involvement characterized the SLE patients, in contrast to pulmonary and skin fibrosis, valve lesions, hypertension, elevated creatinemia, C4 fraction reduction, platelet reduction and inflammation that characterized the non-SLE SAD patients. Anti-PL determination remains important in SADs patients and should not be restricted to only SLE patients.

Key words: systemic autoimmune diseases, antiphospholipid autoantibodies, dRVVT, anti-cardiolipin, anti- β 2GPI

1 Introduction

Two forms of antiphospholipid syndromes exist, primary antiphospholipid syndrome (APS-I) in half of cases, and secondary APS (APS-II) when associated with another systemic autoimmune disease (SAD) [1]. In both cases, the persistent detection (>12 weeks) of antiphospholipid (aPL) autoantibodies is predominantly associated with arterial/venous thrombosis and/or pregnancy morbidity. The aPL spectrum includes the presence of lupus anticoagulant (LAC) and/or anti-cardiolipin (aCL) autoantibodies in association with anti- β 2 glycoprotein-I (a β 2GPI) autoantibodies of IgG and IgM isotypes [2, 3]. The main pathogenic target for aPL has been characterized and is located within the N-terminal domain (Domain I) of β 2GPI, a PL binding glycoprotein [4].

With an aPL prevalence ranging between 1% and 5% in the general population, the risk of thrombosis and/or pregnancy morbidity (mainly miscarriage) due to aPL remains scarce with 40-50 cases of thrombosis per 100,000 persons corresponding to an incidence of 5 new cases per 100,000 persons per year. Such risk significantly increases in those patients with SADs as observed in aPL positive SLE patients with a risk of thrombosis estimated at 50-70% during 20 years of follow-up [5]. Accordingly, we took advantage of the PRECISESADS study to better characterize the risk factors associated with aPL positivity not only in SLE patients but also in non-SLE SAD patients.

2 Material and methods

2.1 Study design and subjects

A total of 1,818 individuals with SADs were included in the observational, European multicentric, PRECISESADS study between 2015 and 2018 (Table 1). The study encompassed 453 patients with systemic lupus erythematosus (SLE), 359 with rheumatoid arthritis (RA), 385 with systemic sclerosis (SSc), 367 with Sjögren's syndrome (SjS), 94 with mixed connective tissue disease (MCTD), and 160 with undifferentiated connective tissue disease (UCTD). Diagnostic criteria used for patient inclusion followed criteria for SLE [6], RA [7], SSc [8], SjS [9]; and MCTD [10]. Patients with clinical features of SADs but not fulfilling any specific SADs criteria for at least 2 years were referred to as UCTD, and patients were excluded from this group if they fulfilled 3 out of the minimal 4 SLE classification criteria or fulfilled early Systemic Sclerosis criteria [11]. Primary APS was an exclusion criterion for this

study while the 100 primary APS patients and the 592 healthy controls (HC) from the PRECISESADS study were used to characterize the aPL assays as reported in supplementary figure S1 [12]. The Ethical Review Boards of the 18 participating institutions approved the protocol of the study, which adhered to the standards set by the International Conference on Harmonization and Good Clinical Practice (ICH-GCP), and to the Declaration of Helsinki. The study is registered with number NCT02890121 in ClinicalTrials.gov.

Of the 41 clinical parameters recorded on the PRECISESADS project, 16 were selected based on their information in the “Euro-Phospholipid-Project” [13] such as peripheral arterial/venous thrombosis, miscarriage, neurological manifestations (stroke, cognitive dysfunction, seizure, chorea, and psychiatric disorders), pulmonary manifestations (worsening lung function, fibrosis), cardiac manifestations (pericarditis, valve lesions), renal manifestations (abnormal creatinine, proteinuria), cutaneous manifestations (photosensitivity, skin fibrosis, Raynaud’s phenomenon), hematological manifestations (reduced platelet count), arthritis and hypertension. In addition information regarding a history of cancer and chronic infection were recorded based on their association with aPL positivity [1].

2.2 LAC detection by dRVVT

The STA-Staclot dilute Russell viper venom time (dRVVT) Screen® and Confirm® (Stago, Asnières-sur-Seine, France) assays were used to test LAC according to the international guidelines [14]. The assay is based on the use of a protease extracted from the venom of the Russell’s viper that directly activates the coagulation factor X in the presence of calcium ions and phospholipids added at low (screen assay) or high (confirm assay) concentrations. Confirmatory tests were performed in those samples with a screen ratio against reference plasma above 1.20 and the final result was expressed as screen ratio/confirm ratio according to the manufacturer’s instructions. In those patients with a screen ratio <1.2 and for screen ratio/confirm ratio calculation, the confirmatory ratio was fixed at 1. ROC curves were used to fix the best pairing of sensitivity/specificity for LAC at ratio ≥ 1.4 (specificity 95%) for positivity and at ≥ 1.6 for medium/high positivity (specificity 98%) when comparing HC with APS-I and as described in Supplementary figure S1.

2.3 aPL assay : aCL (IgG and IgM) and aβ2GPI (IgG and IgM) detection by CLIA

The IDS-Isys (Boldon, UK) is a fully automated chemiluminescent immunoanalyzer (CLIA) using coated magnetic particles (solid phase) coupled with bovine aCL and/or purified human β2-GPI from Technogenetics (Milan, Italy), which was used for aCL IgG/IgM and aβ2GPI IgG/IgM isotype determination [15]. In the presence of sera, the chemiluminescent signal, proportional to the autoantibody (Ab) tested, is expressed in Relative Light Units (RLU) and is then converted to GPL or MPL U/ml (U=units) using the “Harris” reference standard for aCL IgG and IgM, respectively. For aβ2-GPI IgG and IgM, results are expressed in AU/ml (Arbitrary Unit). Results were considered positive when values exceeded 20 units/mL [15], and to study their significance, positive samples were further dichotomized as low and medium/high.

2.4 Other biological parameters

The presence of anti-dsDNA, anti-Sm, anti-U1-RNP, anti-SSA 52kDa, anti-SSA 60kDa, anti-SSB, anti-centromere, anti-Scl70, and anti-CCP2 autoantibodies were tested by CLIA (IDS-Isys) as previously described [16]. Turbidimetry (SpaPlus, Binding Site, UK) was used for rheumatoid factor (RF) and complement fractions C3c and C4 determination. The inflammation criteria were defined by the presence of an erythrocyte sedimentation rate (ESR) >30 mm/h and/or a C-reactive protein (CRP) above the laboratory norm in the absence of a concurrent infection. Hypergammaglobulinemia was defined by IgG levels above the laboratory norm and/or >20% gammaglobulin within the past 12 weeks that has not resolved.

2.5 Statistical analysis

Continuous data are reported as mean ± standard deviation and differences were analyzed by performing a T test. For categorical data, differences among groups were analyzed using the Fisher's exact test and odds ratio (OR) when necessary. Receiver operating curves (ROC) were generated to determine the area under the curve (AUC) and the optimal cut-off values were chosen. Two-tailed p-values were reported and p-values less than 0.05 were considered significant. Statistical analyses were performed using GraphPad Prism version 7.0 (La Jolla, CA).

3 Results

3.1 aCL, a β 2GPI and dRVVT prevalence in systemic autoimmune diseases

In order to estimate the prevalence of aPL in SADs, we took advantage of the PRECISESADS study composed of 592 HCs and 1,818 patients with SADs (SLE, RA, SSc, SjS, MCTD and UCTD). Gender and sex-based differences were reported between HC and SADs (Table 1). Regarding the presence of aPL Abs in SADs, a higher prevalence was reported in the SAD group for dRVVT ($p < 10^{-4}$), aCL IgG ($p < 10^{-4}$), a β 2GPI IgG ($p < 10^{-4}$), while this was not significant for aCL and a β 2GPI IgM. For statistical purposes, the detection of aCL and/or a β 2GPI, referred to as aCL \pm a β 2GPI, was considered after making sure that aCL and a β 2GPI for each isotype were highly correlated ($r \geq 0.93$, $p < 10^{-4}$) and with a very good concordance assessment rate (Cohen's Kappa ≥ 0.85) when using CLIA as previously described [15], data not shown.

Based on the observation that aPL prevalence was significantly different between SLE (57.6%) and non-SLE patients (13.7%), as described in Figure 1, we have further differentiated SLE patients from non-SLE SAD patients. Differences were retrieved when considering individual assays: dRVVT functional assay (21.2% in SLE *versus* 7.0% in non-SLE SADs, $p < 10^{-4}$), aCL \pm a β 2GPI IgG Abs (10.8% in SLE *versus* 1.3% in non-SLE SADs, $p < 10^{-4}$) and aCL \pm a β 2GPI IgM Abs (7.1% in SLE *versus* 3.2% in non-SLE SADs, $p < 10^{-3}$); or double positivity between assays ($0.05 < p < 10^{-4}$). Based on the critical impact of aPL titers on clinical associations and as one may argue that aPL titers were reduced in non-SLE SADs, aPL were further ranked in low and medium/high titers showing a similar distribution between groups.

3.2 dRRVT in SLE and non-SLE SADs

The clinical picture of the dRVVT positivity spectrum (Figure 2) was characterized by venous and/or arterial thrombosis in both SLE ($p < 10^{-4}$) and non-SLE SADs ($p < 10^{-4}$) with specific associations that characterize medium/high LAC levels in SLE: miscarriage ($p = 0.05$) and involvement of the central nervous system ($p = 0.02$). In non-SLE-SADs, associations were retrieved between dRVVT positivity with pulmonary fibrosis ($p = 0.01$), worsening lung functions ($p = 0.002$), cardiac valve lesions ($p = 0.007$), hypertension ($p = 0.04$), and abnormal creatinemia ($p = 0.00002$). A low level of dRRVT in the non-SLE group was associated with a chronic infection report ($p < 10^{-4}$), pericarditis ($p = 0.03$), arthritis ($p = 0.01$) and Raynaud's phenomenon ($p = 0.00008$).

Regarding biological parameters tested, an association between dRVVT positivity with the C4 complement fraction ($p=0.03$), inflammation ($p=0.01$), hypergammaglobulinemia ($p=0.01$), and anti-SSA(60kDa)/SSB positivity was observed in the non-SLE SAD subgroup. In contrast, no association was retrieved for SLE.

3.3 aCL±aβ2GPI IgG autoantibodies in SLE and non-SLE SADs

Next, aCL±aβ2GPI IgG positivity at low and medium/high levels was explored revealing an association with venous and/or arterial thrombosis in both SLE ($p<10^{-4}$) and non-SLE SADs ($p<10^{-4}$) at medium/high levels (Figure 3). Regarding specific associations, miscarriage ($p=0.02$) was retrieved in SLE patients with aCL±aβ2GPI IgG Abs. Arthritis ($p=0.03$) was associated with aCL±aβ2GPI IgG Ab negativity and aCL±aβ2GPI IgG positivity at low level with a chronic infection ($p=0.008$) in non-SLE SADs. At the biological level, in the non-SLE SADs subgroup, associations concerned C3 and C4 complement fraction reduction ($p<0.0003$), hypergammaglobulinemia ($p=0.0007$), and SLE associated autoantibodies: anti-DNA ($p<10^{-4}$), anti-Sm ($p=0.05$), and anti-SSB Abs ($p=0.03$). Such effect on the biological parameters was not retrieved in the SLE subgroup.

3.4 aCL±aβ2GPI IgM autoantibodies in SLE and non-SLE SADs

In the SLE group (Figure 4), with regards to aCL±aβ2GPI IgM positivity at low and medium/high levels, an association was retrieved for medium/high levels with hypertension ($p=0.02$), fever ($p=0.02$), and a history of cancer ($p=0.04$). In contrast, the non-SLE SAD patients with aCL±aβ2GPI IgM Abs at low levels presented significantly more venous and/or arterial thrombosis ($p=0.02$), skin fibrosis ($p=0.005$), inflammation ($p=0.005$); and at medium/high levels miscarriages ($p=0.003$), and hypertension ($p=0.01$).

3.5 Odds ratio

Finally, the odds ratio (OR) for aPL association with thrombosis was explored at positive cut-off and for medium/high levels (Figure 5). In SLE patients, associations with thrombosis ranged for dRVVT from OR=6.1 (IC95: 3.5-10.3; $p<10^{-4}$) to OR=4.7 (IC95: 2.6-8.5; $p<10^{-4}$) at medium/high level; and for aCL±β2GPI IgG from OR=3.7 (IC95: 1.9-7.0; $p=0.0003$) to OR=6.3 (IC95: 3.1-12.8; $p<10^{-4}$) at medium/high level. Such association was retrieved in SLE for aCL±β2GPI IgM at medium/high levels (OR=3.8; IC95 1.5-9.6; $p=0.008$). In non-SLE SADs, aCL±β2GPI IgG (OR=6.6; IC95: 2.4-18.4; $p=0.001$) and dRVVT (OR=3.3; IC95: 1.9-5.6; $p<10^{-4}$) predicted thrombosis and this effect was increased when considering medium/high levels

OR=15.2 (IC95: 4.0-57.4; $p<10^{-4}$) for aCL±β2GPI IgG and OR=4.6 (IC95: 2.3-9.3; $p<10^{-4}$) for dRVVT. With regards to associations, dRVVT plus aCL±β2GPI IgG positivity (OR=9.4 at cut-off; IC95: 4.4-19.9; $p=10^{-4}$ and OR=9.1, IC95: 3.6-23.2 at medium/high levels, $p=10^{-4}$) and dRVVT plus aCL±β2GPI IgM positivity at medium/high levels (OR=11.0, IC95: 2.0-61.0, $p=0.007$) were highly predictive for thrombosis in the SLE group, while it was the association aCL±β2GPI IgG plus dRVVT (OR=15.5; IC95: 3.4-70.3; $p=0.001$) and, even more so, aCL±β2GPI IgG plus aCL±β2GPI IgM (OR=58.3; IC95: 5.3-436; $p=0.0002$) that was predictive of thrombosis in the non-SLE group.

For miscarriages (Figure 6), associations were retrieved when considering in SLE aCL±β2GPI IgG (OR=2.47 at cut-off; IC95: 1.16-5.22; $p=0.03$ and OR=2.9, IC95: 1.2-6.5; $p=0.02$ at medium/high levels), dRVVT at medium/high levels (OR=2.3, IC95: 1.2-4.7; $p=0.01$) and even more when aCL±β2GPI IgG were associated with aCL±β2GPI IgM (OR=5.1 at cut-off; IC95: 1.1-23.5; $p=0.02$). In non-SLE patients the best predictive factor was aCL±β2GPI IgM (OR=2.9; IC95: 1.23-6.80; $p=0.05$) and the risk was increased when aCL±β2GPI IgM were associated with dRVVT (OR=5.0; IC95: 1.3-19.8; $p=0.04$) or aCL±β2GPI IgG (OR=7.8; IC95: 1.3-47.0; $p=0.05$).

4 Discussion

In this study, performed with a European multi-center study of 1,818 patients with SADs, we observed: (i) that aPL detection characterizes patients with SAD; (ii) that aPL positivity is less frequent in non-SLE SADs such as SjS, RA, SSc, MCTD and UCTD than in SLE (13.7% versus 57.6%); (iii) that dRVVT and aCL±β2GPI IgG Abs perform similarly for association with thrombosis and at medium/high level with miscarriage in SAD, while aCL±β2GPI IgM Abs detection was associated with miscarriage in non-SLE SADs. In case of multiple positivity, the risk for thrombosis and miscarriage is increased as described in primary APS; (iv) the aPL risk for non-thrombotic events exists in SADs with differences observed between SLE and non-SLE SADs; and (v) the critical role of complement consumption, when associated with aPL positivity, is further supported.

Initially included in the classification criteria to discriminate SLE from other SADs[6], aPL detection is elevated in SLE with 11-30% of patients positive for the LAC test, 17-40% for

aCL±β2GPI, supporting a total prevalence of aPL ranging from 30% to 45%[17-19], which is in agreement with our report. In contrast, in non-SLE SAD patients, results from the literature, usually performed in limited cohorts, are more contrasted and range from 0 to 57% as described in SSc[20]. Similarly, conflicting results are reported in RA with an aPL prevalence of 28% according to a meta-analysis reported by Olech et al [21], but to only 10.4% in the report of Kim et al who found LAC in a cohort of 376 RA patients as the most prevalent test (6.6%) followed by aCL±β2GPI in 3.2% of the cases [22]. Regarding SjS, again important differences are observed with an aPL prevalence ranging from 2% to 37% [23, 24] From our knowledge, there is no data reporting aPL in UCTD and MCTD. This discrepancy in the literature for aPL detection in non-SLE patients, however, could be partially explained by differences regarding patient selection and recruitment, the diversity of diagnostic tests used for aPL detection, and the use of different cut off points. To circumvent these limitations, a large population of non-SLE SAD patients was selected (n=1365), primary APS patients were excluded from this study, and aPL were detected in a single center with robust techniques (Russel's clotting time and CLIA), the optimal cut-off for positivity was evaluated, and aPL were ranked in low and medium/high levels. Our study supports the concept that aPL are not restricted to SLE but can be detected in other SADs, at lower prevalence (9.75% in RA to 20.63 in UCTD), and that such detection is associated with clinical symptoms including thrombosis and miscarriage, which is an argument to test aPL Ab in non-SLE SAD patients.

The thrombotic risk associated with aCL±aβ2GPI of IgG and IgM isotypes and LAC has been studied in patients with SLE. There are consistent data demonstrating an association between aCL±aβ2GPI of IgG isotype at medium/high titers and an increased risk of venous and/or arterial thrombosis in SLE patients [25]. In contrast, when considering aCL±aβ2GPI IgM detection such an association was not observed as reported in a study conducted in 796 patients with SLE [26]. This statement was confirmed by other authors who failed to associate aCL±aβ2GPI IgM positivity in patients with primary and secondary APS manifestations with thrombosis [27, 28]. Horbach et al found, using a multivariate analysis approach, that LAC performed better than aCL±aβ2GPI of IgG in predicting venous thrombosis (OR 6.55; 95% CI 2.36-18.17) and arterial thrombosis (OR 9.77; 95% CI 1.74-31.15) in SLE [29]. According to the Hopkin's lupus cohort analysis, the presence of any aPL

(OR 1.84) increases the risk for arterial and/or venous thrombosis in patients with SLE and this risk was highest with LAC (OR 4.16) as 50% of LAC positive patients, in the following 20 years, have developed a venous thrombosis/pulmonary embolus [30]. This is in agreement with our study confirming the association between LAC in SLE (OR=6.1; IC95:3.5-10.3) and aCL/a β 2GPI IgG in non-SLE SADs (OR=6.6; IC95:2.4-18.4) with thrombosis, and the absence of association for thrombosis with regards to aCL \pm a β 2GPI IgM.

The two greatest risk factors for miscarriage are elevated levels of aPL and a history of previous fetal loss as these women have up to 80% risk of current pregnancy loss [31]. Both aCL \pm a β 2GPI IgG and IgM Ab detection are associated with an increased risk of miscarriage [30-35]. Such associations were retrieved in our study, in SLE medium/high titer aCL \pm a β 2GPI IgG and dRVVT were associated with miscarriages, while in the non-SLA SADs group it was the association between aCL \pm a β 2GPI IgM detection and miscarriage that was retrieved. However, when the whole population is considered instead of a selected SAD population, the association between aCL \pm a β 2GPI IgM detection and miscarriage is lost, which supports the idea of restricting aCL \pm a β 2GPI IgM Ab research to SAD patients [36]. Compared to single positivity, patients with double positivity have a higher risk for miscarriage and it's of particular note that triple positivity is exceptional (n=10/1818) in our study, which is in contrast to primary APS patients [37].

What about other clinical associations with aPL in SADs? Association with pulmonary fibrosis was retrieved in the non-SLE SAD patient group (LAC) as described in patients with SSc [38], while others did not **have such an association** [39, 40]. Regarding the risk of stroke and cerebral venous thrombosis, LAC when diagnosed with SLE was found to increase the risk by 2.5 (95% CI 1.08 to 5.94) [41]. Murray et al. demonstrated in SLE that the prevalence of cognitive impairment, measured by verbal memory and verbal fluency metrics, ranges from 11.9% (50/420) in aPL-negative SLE patients to 21% (57/274) in those with a positive aPL test, supporting a 2.1-fold increased risk for cognitive impairment for the later (95% CI 1.3–3.4)[42, 43]. A risk for LAC was retrieved for central nervous involvement in the SLE group of our study (p=0.02). From our knowledge, our large study further highlights other rare clinical associations with aPL not previously described in SADs such as hypertension, an abnormal creatinemia not associated with proteinuria, arthritis and skin fibrosis. As a consequence, the heterogeneity retrieved in the literature may be explained in part by the fact that most

of the reported studies tested a relatively small number of patients, which could be responsible for a lower statistical power, in contrast to our study. Another explanation is related to the existence of confounding factors that could present aPL positivity beside SADs, such as malignant tumors or infections. These confounding factors were limited in our study and, when present, associated with aCL at low levels.

Several studies have shown consumption of complement proteins through the classical and alternative pathways responsible for miscarriages, in association with inflammation, and vascular clots during primary APS [44]. The critical role played by the complement system in aPL pathophysiology is further supported by the observations that an increase in complement activation (C4d) on platelets coated with aPL Ab is observed in patients with SLE both *in vitro* and *in vivo* [45]; and that mice deficient in C3, C5 or C5a receptors are protected from fetal loss induced by the injection of aPL [46]. Then it is not surprising that we observed in SAD patients with aPL significantly lower levels of complement fractions and thrombopenia compared to SAD patient without aPL. With the exception of aCL±β2GPI IgG in the non-SLE group with hypergammaglobulinemia and with anti-SSB Abs, association between aPL and autoantibodies was not reported in our study and in line with previous observations.

Regarding strengths of the study, the current work is one of the first studies exploring clinical and biological associations with aPL in a large and multicentric study of patients with SADs. In order to minimize differences between centers and reagents, autoantibody detections, including aPL determination, were centralized in the PRECISESADS' study. This large study has further permitted us to better characterize the prevalence of aPL Abs in SAD as this information was contradictory in the non-SLE SAD patients. Assays for aPL determination were optimized with regards to the technology selected (dRRVT, CLIA), redefinition of the optimal cut-off for positivity, and the ranking of aPL in low and medium/high levels in order to take into account possible complicating factors such as a chronic infection or a cancer, factors that were limited in our study. However, the study has potential limitations such as information for each patient on the decision whether or not to take an anticoagulant treatment was missing as well as the facts that aPL determination was conducted at one time point and not repeated later as recommended by the APS criteria, and that all patients recruited were of European origin.

In conclusion, association between aPL profile and SAD from a large study has permitted better characterization of the prevalence of aPL in SADs. Furthermore, we found differences in the aPL profile, clinical and biological manifestations when SAD were dichotomized into SLE and non-SLE SADs. The main message from this work is that aPL determination remains important in SADs patients and should not be restricted to SLE patients.

Authorship contribution statement: AM, PLM, EB, and YR: Formal analysis and writing original draft; EB, GP, SN, HG: Investigation; MAR, JF: Project administration; and MAR: Funding acquisition.

Declaration of Competing Interest: All authors declare that they have no conflict of interest.

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Table 1: Antiphospholipid (aPL) autoantibody prevalence in healthy controls (HC) and in patients with systemic autoimmune diseases (SADs). As aPL prevalence was higher in the SLE sub-group, the SAD group was further subdivided into patients with systemic lupus erythematosus (SLE, n=453) and into non-SLE patients with rheumatoid arthritis (RA, n=359), systemic sclerosis (SSc, n=385), Sjögren's syndrome (SjS, n=367), mixed connective tissue disease (MCTD, n=94), and undifferentiated connective tissue disease (UCTD, n=160). The cut-off for positivity and the cut-off for medium/high levels are indicated (see material and methods and supplementary figure S1). The combination of aCL±aβ2GPI referred to the detection of aCL and/or aβ2GPI autoantibodies.

	HC (n=592)	All SADs (n=1818)	Statistics all SADs versus HC	SLE (n=453)	Statistics SLE versus HC	Non-SLE SADs (n=1365)	Statistics non-SLE versus HC	Statistics SLE versus non-SLE
Age (mean±SD)	47±13	54±14	p<10 ⁻⁴	46±14	p=0.55	57±13	p<10 ⁻⁴	p<10 ⁻⁴
Sex (F:H)	467 : 125	1603 : 215	p<10 ⁻⁴	422 : 31	p<10 ⁻⁴	1181 : 184	p<10 ⁻⁴	p=0.0002
Current treatment								
Antimalarial	-	660 (36.3%)	p<10 ⁻⁴	318 (70.2%)	p<10 ⁻⁴	342 (25.1%)	p<10 ⁻⁴	p<10 ⁻⁴
Steroids	4 (0.7%)	662 (36.4%)	p<10 ⁻⁴	233 (51.4%)	p<10 ⁻⁴	429 (31.4%)	p<10 ⁻⁴	p<10 ⁻⁴
Immunosupresant	-	657 (36.1%)	p<10 ⁻⁴	160 (35.3%)	p<10 ⁻⁴	497 (36.4%)	p<10 ⁻⁴	p=0.671
Biologics	-	171 (9.4%)	p<10 ⁻⁴	0	p<10 ⁻⁴	171 (12.5%)	p<10 ⁻⁴	p<10 ⁻⁴
Comorbidity								
Chronic infections	1 (0.2%)	25 (1.4%)	p=0.01	10 (2.2%)	p=0.004	15 (1.1%)	p=0.07	p=0.13
History of cancer	16 (2.7%)	137 (7.5%)	p<10 ⁻⁴	25 (5.5%)	p=0.03	112 (8.2%)	p<10 ⁻⁴	p=0.08
dRVVT (>1.4)	23 (3.9%)	192 (10.6%)	p<10 ⁻⁴	96 (21.2%)*	p<10 ⁻⁴	96 (7.0%)	p=0.01	p<10 ⁻⁴

Medium/high dRVVT (>1.6)	13 (2.2%)	105 (5.8%)	p=0.0007	60 (13.2%)	p<10 ⁻⁴	45 (3.3%)	p=0.24	p<10 ⁻⁴
aCL IgG (>20 U/mL)	0	62 (3.4%)	p<10 ⁻⁴	46 (10.2%)	p<10 ⁻⁴	16 (1.2%)	p=0.005	p<10 ⁻⁴
aβ2GPI IgG (>20 U/mL)	1 (0.2%)	58 (3.2%)	p<10 ⁻⁴	48 (10.6%)	p<10 ⁻⁴	10 (0.7%)	p=0.04	p<10 ⁻⁴
aCL±aβ2GPI IgG (>20 U/mL)	1 (0.2%)	66 (3.6%)	p<10 ⁻⁴	49 (10.8%)	p<10 ⁻⁴	17 (1.3%)	p=0.04	p<10 ⁻⁴
Medium/high aCL±aβ2GPI IgG (>60 U/mL)	1 (0.2%)	46 (2.5%)	p=0.0005	37 (8.2%)	p<10 ⁻⁴	9 (0.7%)	p=0.292	p<10 ⁻⁴
aCL IgM (>20 U/mL)	15 (2.5%)	61 (3.4%)	p=0.42	26 (5.7%)	p=0.01	35 (2.6%)	p=0.99	p=0.002
aβ2GPI IgM (>20 U/mL)	18 (3.0%)	70 (3.8%)	p=0.45	30 (6.6%)	p=0.007	40 (2.9%)	p=0.89	p=0.001
aCL±aβ2GPI IgM (>20 U/mL)	19 (3.2%)	76 (4.2%)	p=0.35	32 (7.1%)	p=0.007	44 (3.2%)	p=0.89	p=0.0007
Medium/high aCL±aβ2GPI IgM (>40 U/mL)	8 (1.4%)	37 (2.0%)	p=0.37	20 (4.4%)	p=0.004	17 (1.2%)	p=1.0	p<10 ⁻⁴
dRVVT + aCL±aβ2GPI IgG	1 (0.2%)	49 (2.7%)	p=0.0003	39 (8.7%)	p<10 ⁻⁴	7 (0.5%)	p=0.48	p<10 ⁻⁴
dRVVT + aCL±aβ2GPI IgM	3 (0.5%)	41 (2.3%)	p=0.01	23 (5.1%)	p<10 ⁻⁴	15 (1.1%)	p=0.31	p<10 ⁻⁴
aCL±aβ2GPI IgG + aCL±aβ2GPI IgM	1 (0.2%)	22 (1.2%)	p=0.04	14 (3.1%)	p=0.0002	5 (0.4%)	p=0.78	p<10 ⁻⁴
dRVVT + aCL±aβ2GPI IgG + aCL±aβ2GPI IgM	0	10 (0.6%)	p=0.15	7 (1.6%)	p=0.008	3 (0.2%)	p=0.61	p=0.003

Figures

Figure 1: **Prevalence of aPL (A) in SLE patients (n=453) and (B) in non-SLE patients (n=1365) from the European PRECISEADS study. C: aPL prevalence according to the assay (dilute Russell's viper venom time [dRVVT], anticardiolipin antibodies [aCL], anti- β 2 glycoprotein-I antibodies [a β 2GPI], immunoglobulin M [IgM], immunoglobulin G [IgG]) and the clinical group (systemic lupus erythematosus [SLE], mixed connective tissue disease [MCTD], Sjögren's syndrome [SjS], systemic sclerosis [SSc], rheumatoid arthritis [RA], undifferentiated connective tissue disease [UCTD]).**

Figure 2: **Comparison of clinical and biological characteristics in SLE and non-SLE-patients according to their dRVVT status at low (screen ratio/confirm ratio = 1.4-1.6) and medium/high levels (screen ratio/confirm ratio \geq 1.6). A : clinical and biological characteristics in SLE ; B : clinical and biological characteristics in non-SLE. Statistical values are indicated when significant ($p < 0.05$, Fisher's exact test). Abbreviations: SLE : systemic lupus erythematosus ; dRVVT : dilute Russell's viper venom time ; . CNS : Central nervous system ; PNS : peripheral nervous system; DNA: anti-DNA; Sm: anti-Sm; U1 RNP: anti-U1 ribonucleoprotein; SSA/SSB: anti-sicca syndrome A/B; Centro: anti-centromere; RF: rheumatoid factor; Scl70: anti-topoisomerase I; CCP: anti-cyclic citrullinated peptide.**

Figure 3: **Comparison of clinical and biological characteristics in SLE and non-SLE-patients according to their aCL \pm a β 2GPI IgG status at low (20-60 U/mL) and medium/high levels (\geq 60 U/mL). A : clinical and biological characteristics in SLE ; B : clinical and biological characteristics in non-SLE. The combination of aCL \pm a β 2GPI referred to the detection of aCL and/or a β 2GPI autoantibodies and statistical values are indicated when significant ($p < 0.05$). See figure 2 for abbreviations.**

Figure 4: **Comparison of clinical and biological characteristics in SLE and non-SLE-patients according to their aCL \pm a β 2GPI IgM status at low (20-40 U/mL) and medium/high levels (\geq 40 U/mL). A : clinical and biological characteristics in SLE ; B : clinical and biological characteristics in non-SLE. The combination of aCL \pm a β 2GPI referred to the detection of aCL and/or a β 2GPI autoantibodies and statistical values are indicated when significant ($p < 0.05$). See figure 2 for abbreviations.**

Figure 5: **Odds ratios (95% CI) for arterial/venous thrombosis in SLE and in non-SLE group (A/B and C/D, respectively) according to the antiphospholipid (aPL) profile at cut-off for positivity (upper panel) and at medium/high level (lower panel).** Statistical values are indicated when significant ($p < 0.05$). Abbreviations: dRVVT : dilute Russell's viper venom time ; aCL: anticardiolipin antibodies; anti- $\beta 2$ glycoprotein-I antibodies ; IgM : Immunoglobulin M : IgG : Immunoglobulin G.

Figure 6: Odds ratios (95% CI) for miscarriage in SLE and in non-SLE group (A/B and C/D, respectively) according to the antiphospholipid (aPL) profile at cut-off for positivity (upper panel) and at medium/high level (lower panel). Statistical values are indicated when significant ($p < 0.05$). See figure 5 for abbreviations.

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Figure 1

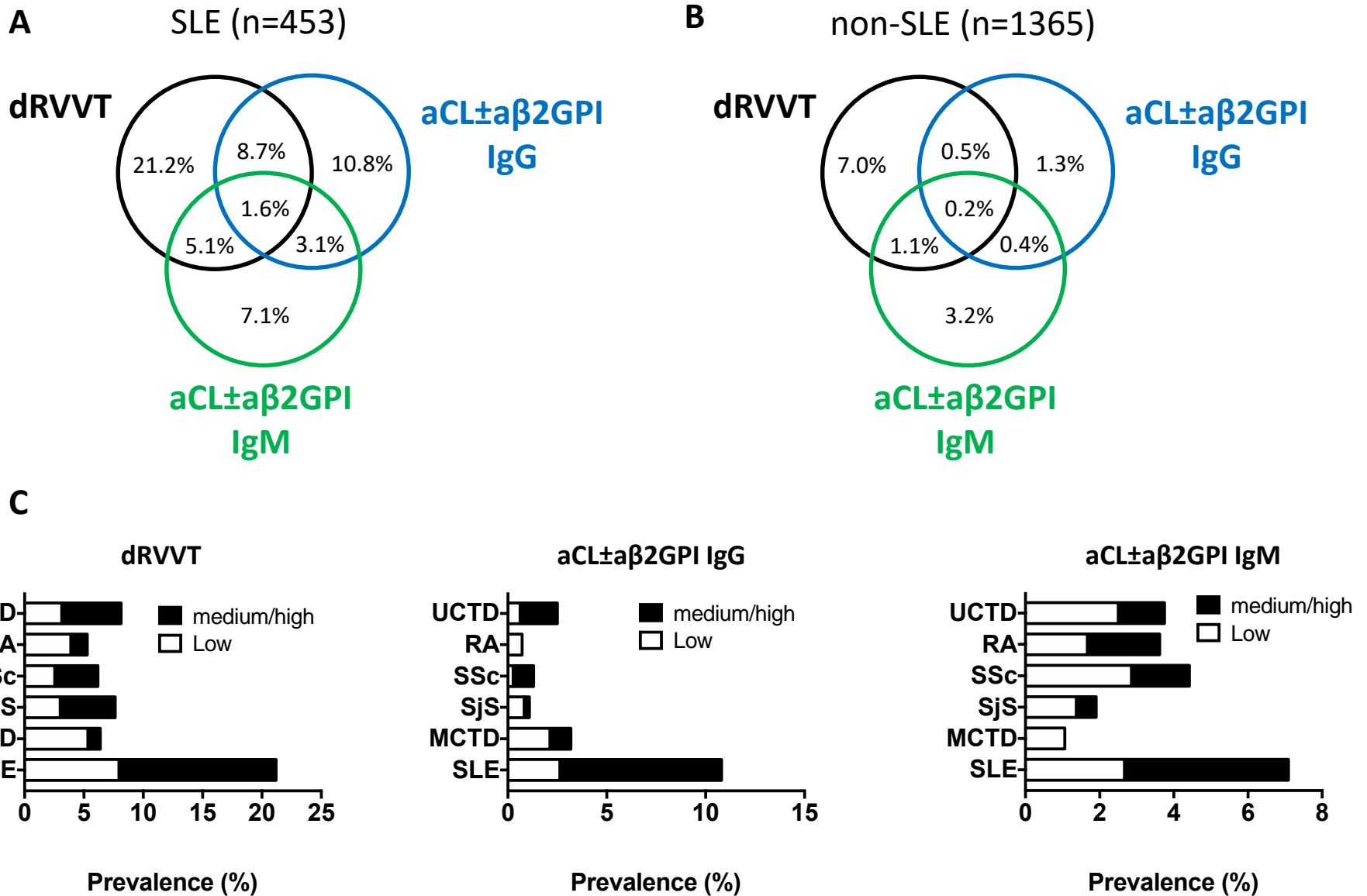


Figure 2

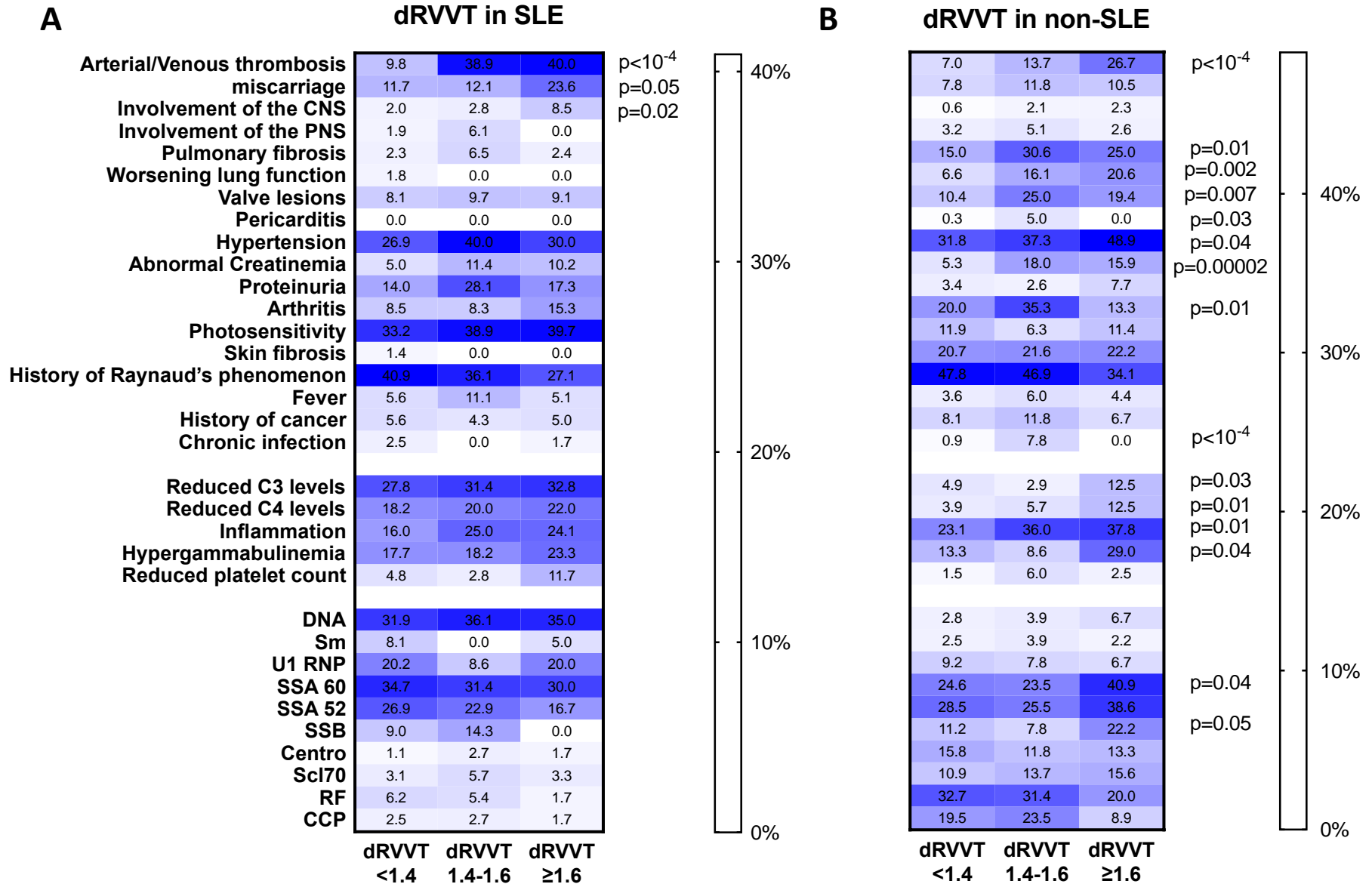


Figure 3

A

aCL±aβ2GPI IgG in SLE

Arterial/Venous thrombosis	13.6	8.3	46.0
miscarriage	11.9	16.7	28.1
Involvement of the CNS	2.8	0.0	5.4
Involvement of the PNS	2.0	0.0	3.0
Pulmonary fibrosis	2.0	0.0	11.5
Worsening lung function	1.0	0.0	5.0
Valve lesions	7.7	0.0	20.0
Pericarditis	0.0	0.0	0.0
Hypertension	27.4	41.7	35.1
Abnormal Creatinemia	6.0	0.0	10.8
Proteinuria	14.6	20.0	24.2
Arthritis	8.8	8.3	16.2
Photosensitivity	33.7	58.3	36.1
Skin fibrosis	1.2	0.0	0.0
History of Raynaud's phenomenon	40.1	45.5	27.0
Fever	5.5	16.7	8.3
History of cancer	6.2	0.0	0.0
Chronic infection	2.5	0.0	0.0
Reduced C3 levels	28.1	33.3	34.3
Reduced C4 levels	17.5	33.3	28.6
Inflammation	18.4	9.1	13.9
Hypergammabulinemia	18.5	30.0	12.0
Reduced platelet count	5.5	0.0	8.1
DNA	31.7	33.3	43.2
Sm	7.4	8.3	2.7
U1 RNP	18.8	16.7	24.3
SSA 60	34.9	16.7	27.0
SSA 52	26.0	25.0	16.2
SSB	8.9	0.0	2.7
Centro	1.2	0.0	2.7
Sci70	3.0	0.0	8.1
RF	6.2	0.0	0.0
CCP	2.7	0.0	0.0

p<10⁻⁴
p=0.02

B

aCL±aβ2GPI IgG in non-SLE

7.6	12.5	55.6
8.0	0.0	22.2
0.7	0.0	0.0
3.8	0.0	0.0
14.6	0.0	0.0
7.4	33.3	0.0
11.3	0.0	16.7
0.4	0.0	0.0
32.8	25.0	44.4
6.2	0.0	0.0
3.5	14.3	0.0
20.8	0.0	0.0
11.9	0.0	22.2
20.9	12.5	22.2
48.1	37.5	66.7
3.7	12.5	0.0
8.3	0.0	0.0
1.0	12.5	0.0
4.8	16.7	42.9
4.0	33.3	25.0
24.0	37.5	33.3
12.8	50.0	50.0
1.6	0.0	11.1
2.4	50.0	44.4
2.5	12.5	11.1
9.1	12.5	11.1
24.9	50.0	37.5
28.5	62.5	25.0
11.2	25.0	33.3
15.5	12.5	22.2
11.3	0.0	0.0
32.4	37.5	0.0
19.4	25.0	0.0

p<10⁻⁴

p=0.03

p=0.008

p=0.00001
p=0.00003

p=0.0007
p=0.0001

p<10⁻⁴
p=0.05

p=0.03

80%

70%

60%

50%

40%

30%

20%

10%

0%

40%

20%

0%

IgG
<20 IgG
20-60 IgG
≥60

IgG
<20 IgG
20-60 IgG
≥60

Figure 4

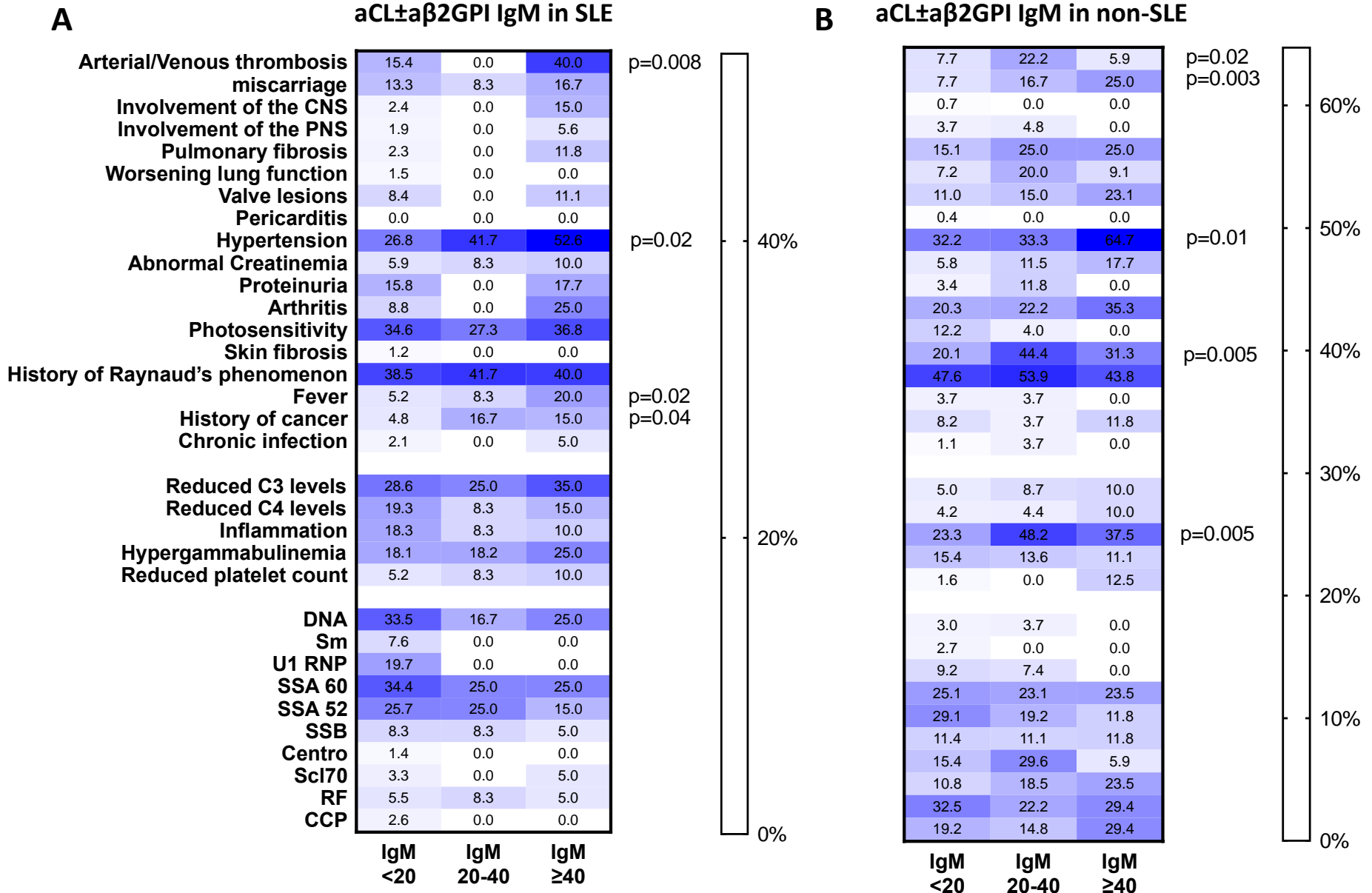


Figure 5

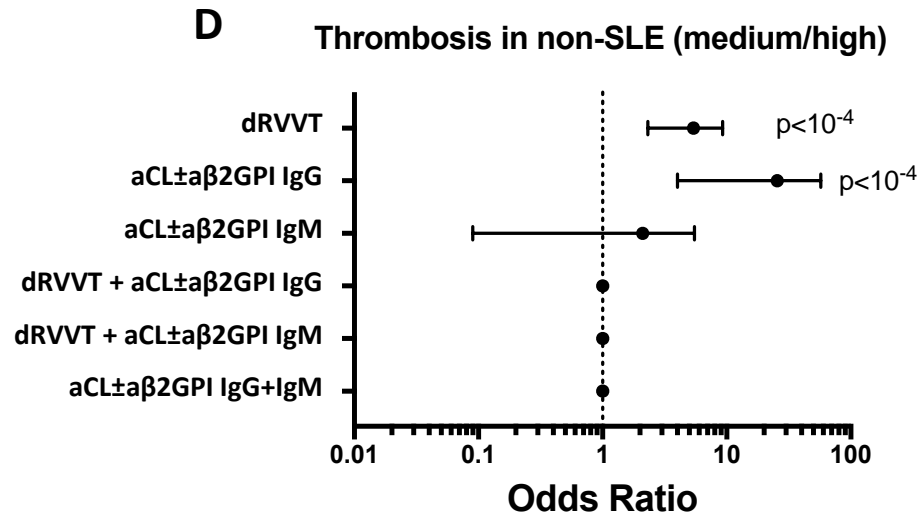
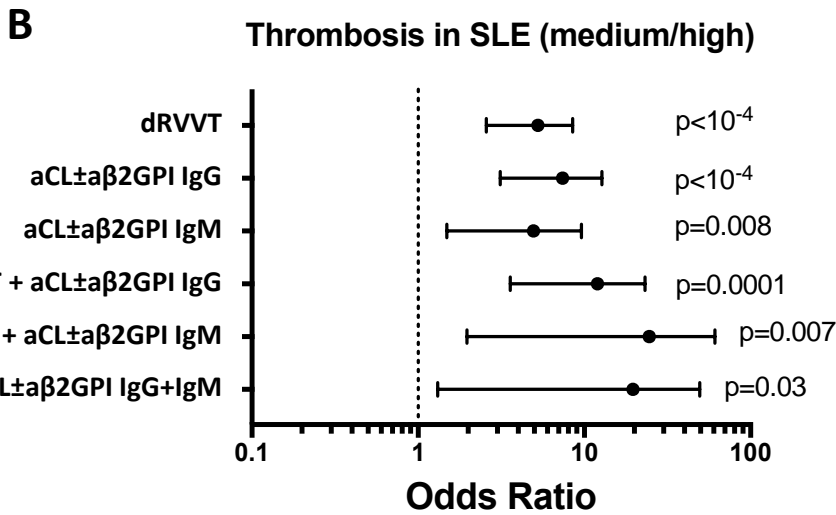
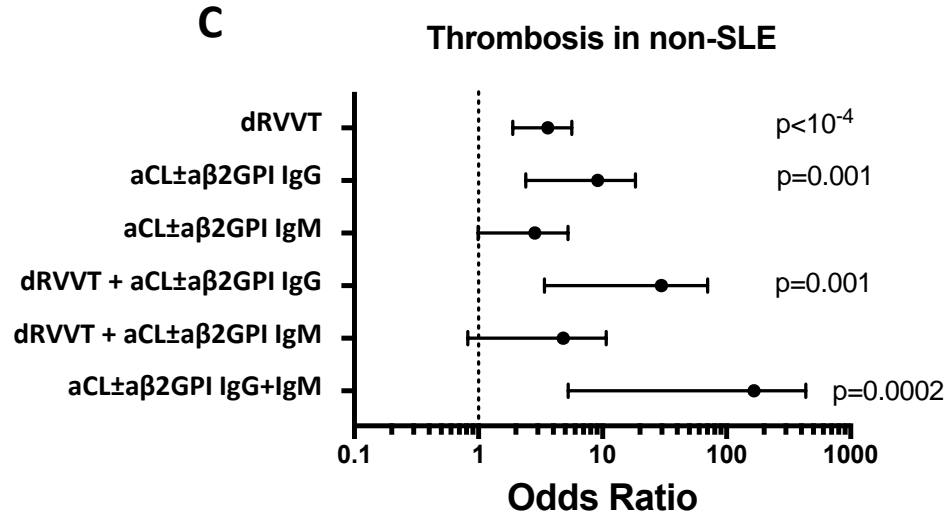
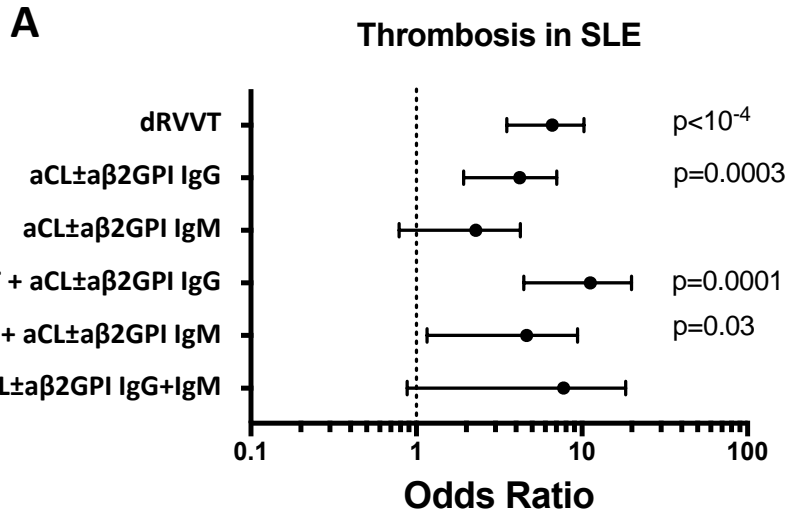


Figure 6

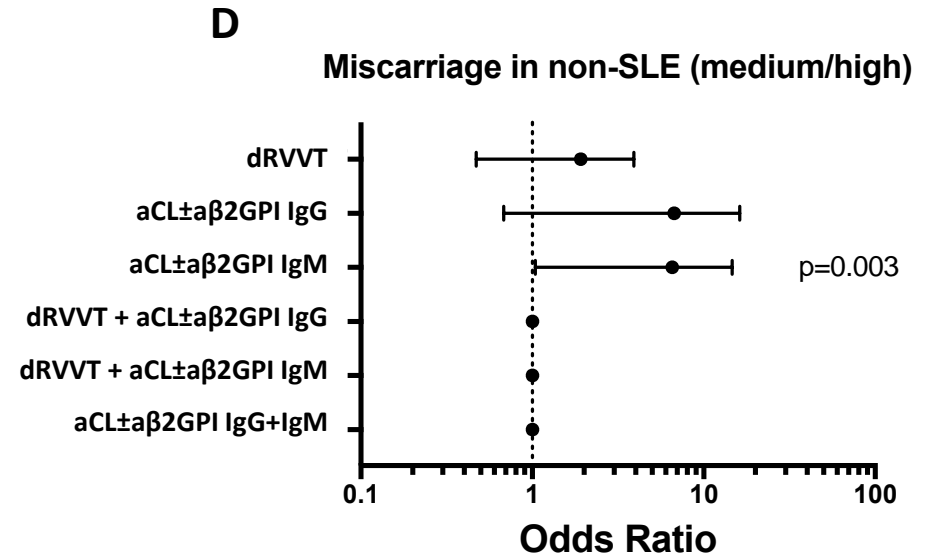
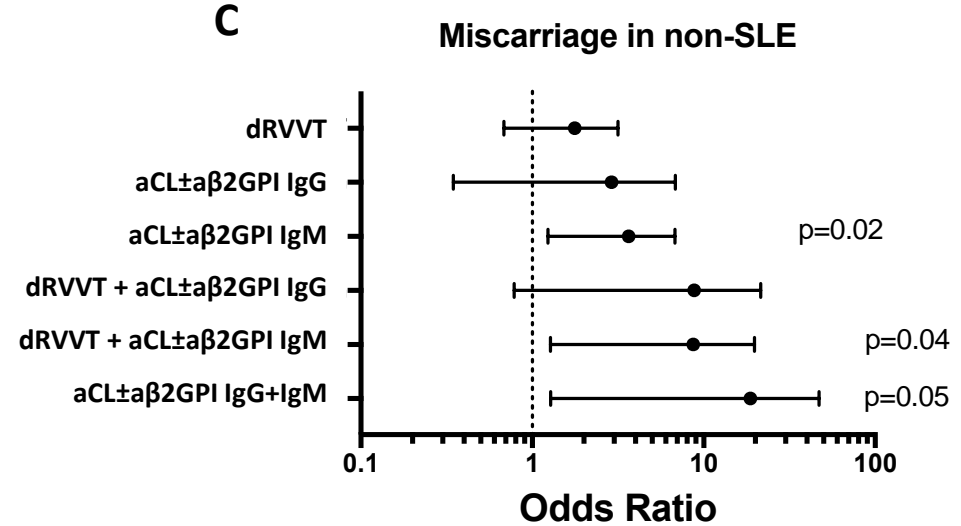
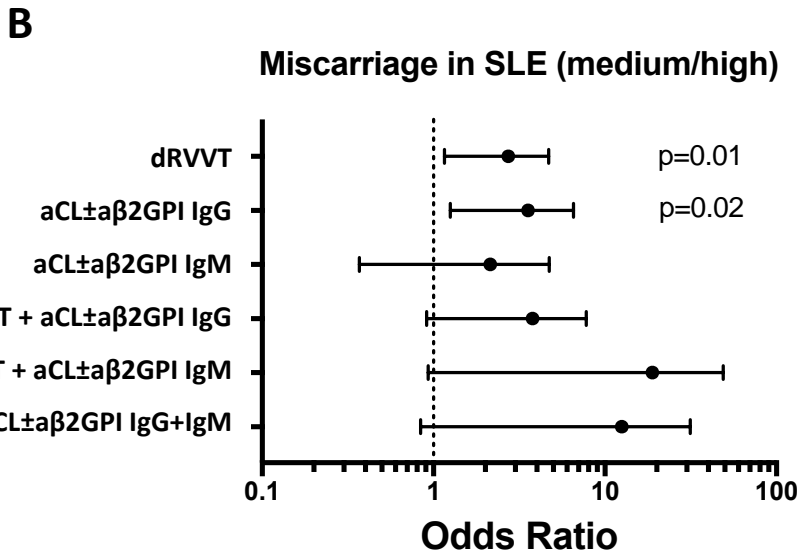
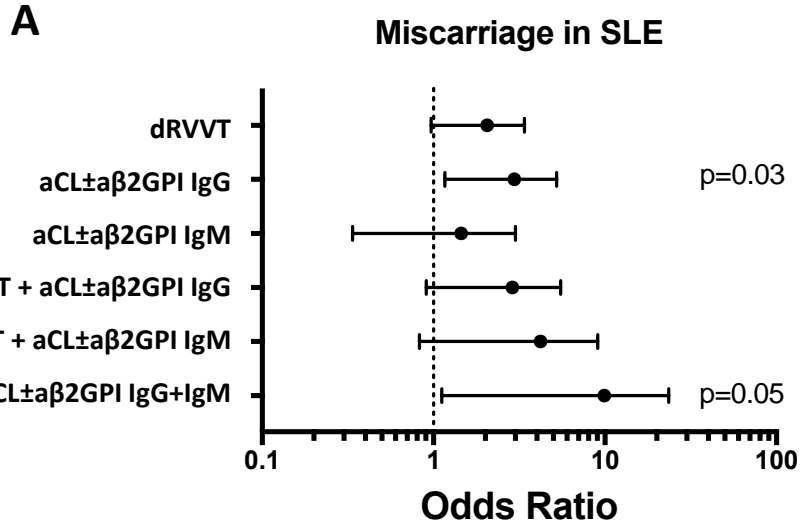


Figure 1

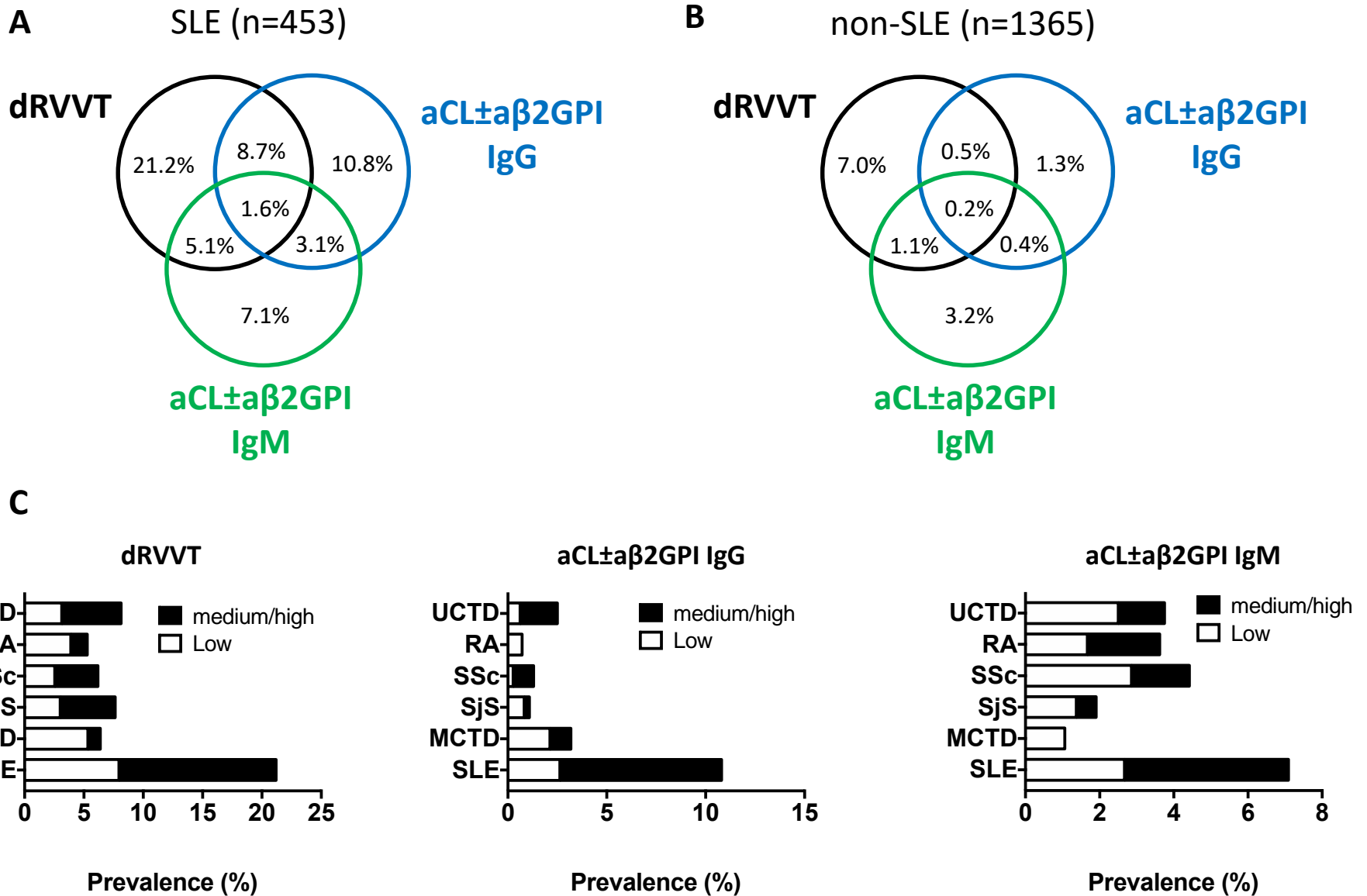


Figure 2

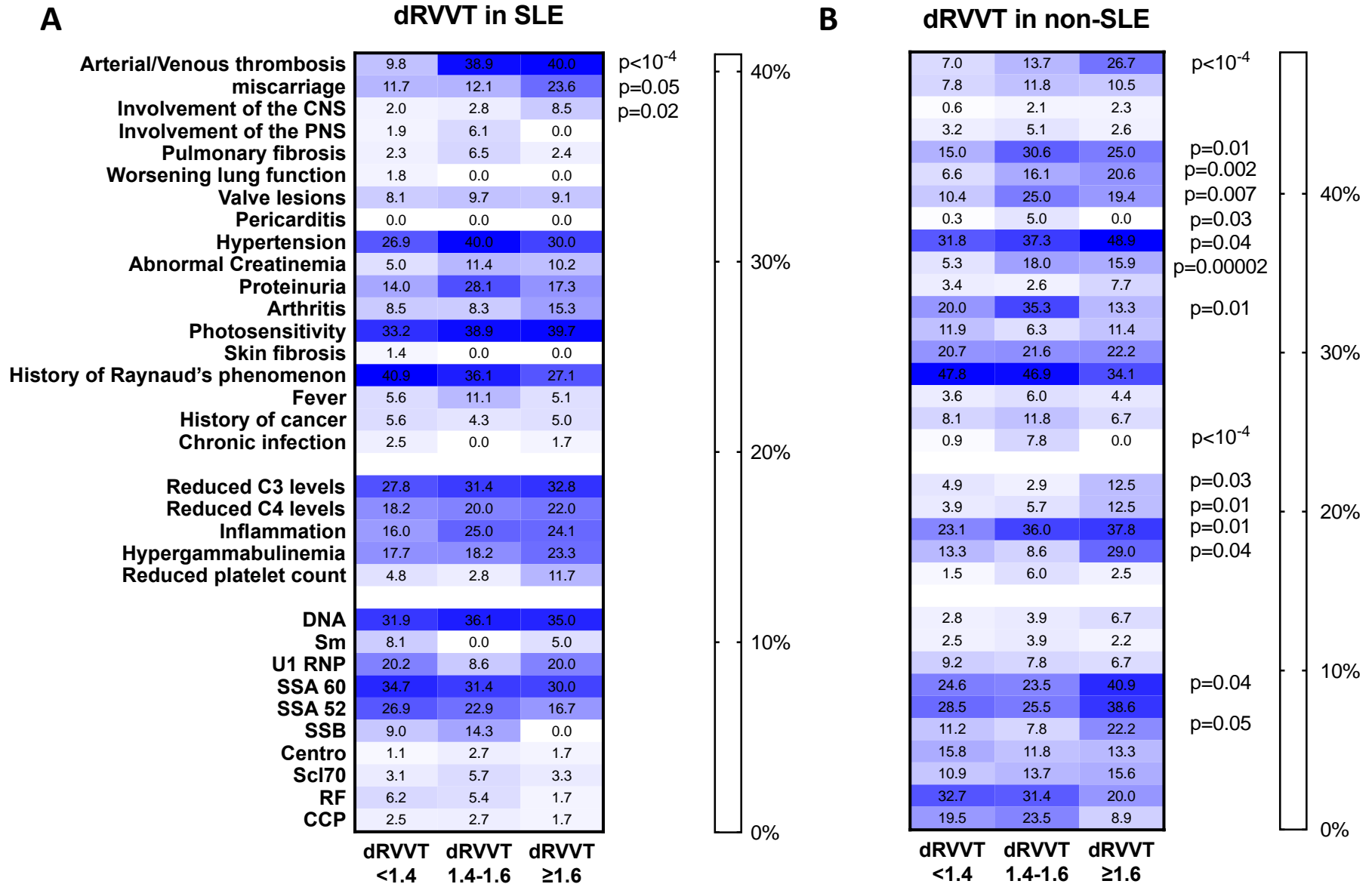


Figure 3

A

aCL±aβ2GPI IgG in SLE

Arterial/Venous thrombosis	13.6	8.3	46.0
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Involvement of the CNS	2.8	0.0	5.4
Involvement of the PNS	2.0	0.0	3.0
Pulmonary fibrosis	2.0	0.0	11.5
Worsening lung function	1.0	0.0	5.0
Valve lesions	7.7	0.0	20.0
Pericarditis	0.0	0.0	0.0
Hypertension	27.4	41.7	35.1
Abnormal Creatinemia	6.0	0.0	10.8
Proteinuria	14.6	20.0	24.2
Arthritis	8.8	8.3	16.2
Photosensitivity	33.7	58.3	36.1
Skin fibrosis	1.2	0.0	0.0
History of Raynaud's phenomenon	40.1	45.5	27.0
Fever	5.5	16.7	8.3
History of cancer	6.2	0.0	0.0
Chronic infection	2.5	0.0	0.0
Reduced C3 levels	28.1	33.3	34.3
Reduced C4 levels	17.5	33.3	28.6
Inflammation	18.4	9.1	13.9
Hypergammabulinemia	18.5	30.0	12.0
Reduced platelet count	5.5	0.0	8.1
DNA	31.7	33.3	43.2
Sm	7.4	8.3	2.7
U1 RNP	18.8	16.7	24.3
SSA 60	34.9	16.7	27.0
SSA 52	26.0	25.0	16.2
SSB	8.9	0.0	2.7
Centro	1.2	0.0	2.7
Sci70	3.0	0.0	8.1
RF	6.2	0.0	0.0
CCP	2.7	0.0	0.0
	IgG	IgG	IgG
	<20	20-60	≥60

p<10⁻⁴
p=0.02

B

aCL±aβ2GPI IgG in non-SLE

7.6	12.5	55.6
8.0	0.0	22.2
0.7	0.0	0.0
3.8	0.0	0.0
14.6	0.0	0.0
7.4	33.3	0.0
11.3	0.0	16.7
0.4	0.0	0.0
32.8	25.0	44.4
6.2	0.0	0.0
3.5	14.3	0.0
20.8	0.0	0.0
11.9	0.0	22.2
20.9	12.5	22.2
48.1	37.5	66.7
3.7	12.5	0.0
8.3	0.0	0.0
1.0	12.5	0.0
4.8	16.7	42.9
4.0	33.3	25.0
24.0	37.5	33.3
12.8	50.0	50.0
1.6	0.0	11.1
2.4	50.0	44.4
2.5	12.5	11.1
9.1	12.5	11.1
24.9	50.0	37.5
28.5	62.5	25.0
11.2	25.0	33.3
15.5	12.5	22.2
11.3	0.0	0.0
32.4	37.5	0.0
19.4	25.0	0.0
IgG <20	IgG 20-60	IgG ≥60

p<10⁻⁴

p=0.03

p=0.008

p=0.00001
p=0.00003

p=0.0007
p=0.0001

p<10⁻⁴
p=0.05

p=0.03

80%

70%

60%

50%

40%

30%

20%

10%

0%

Figure 4

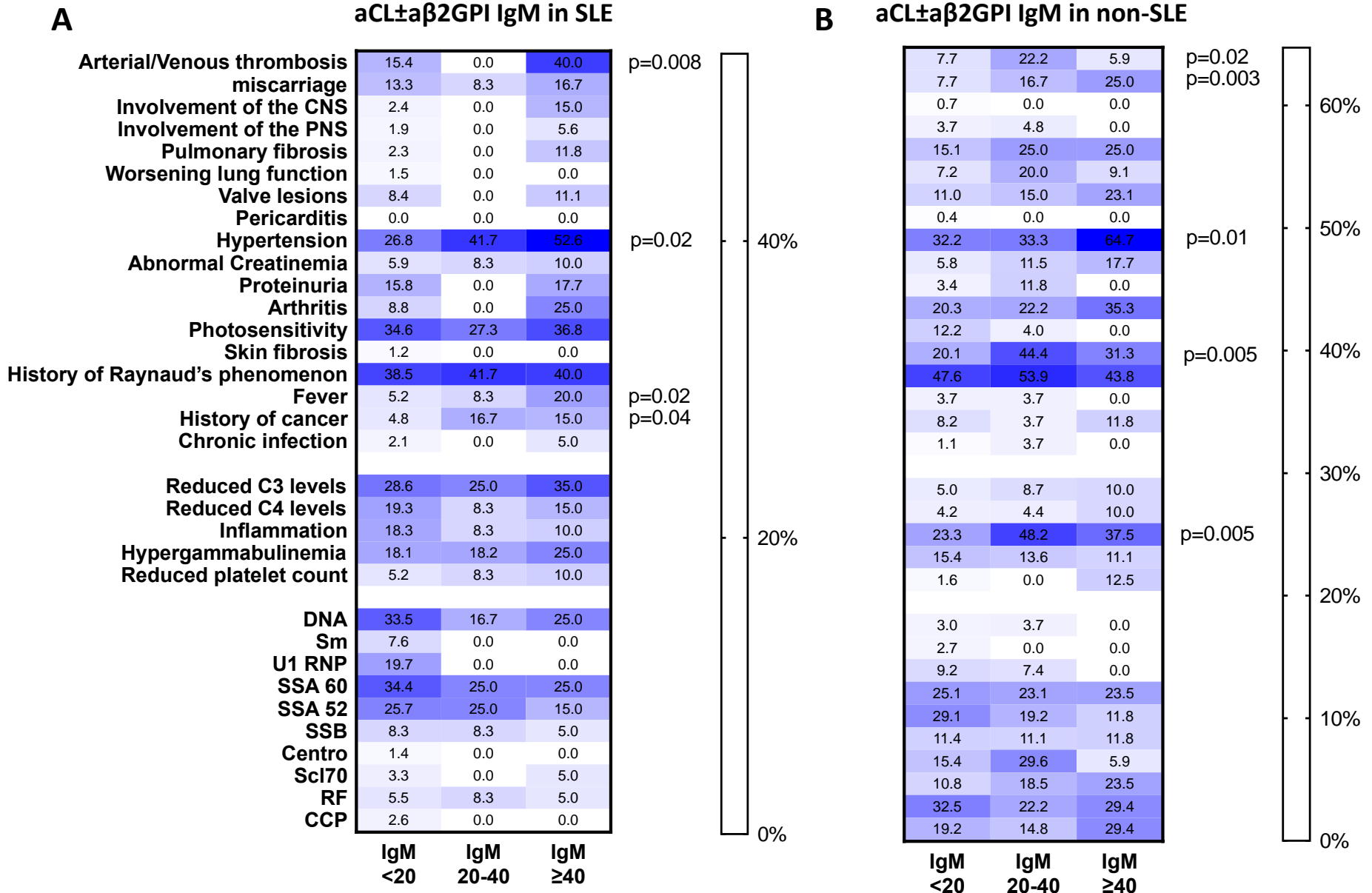


Figure 5

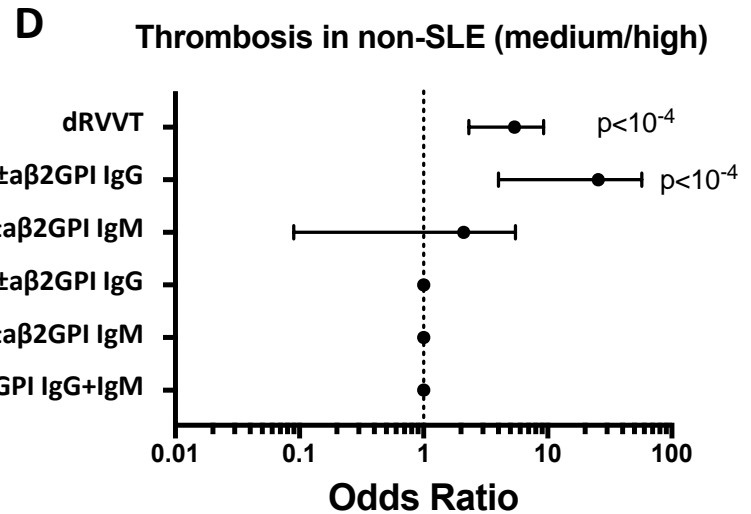
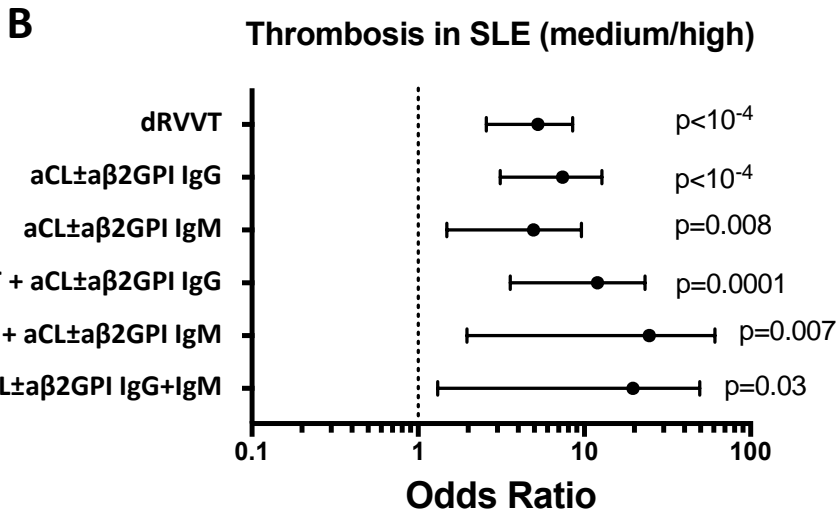
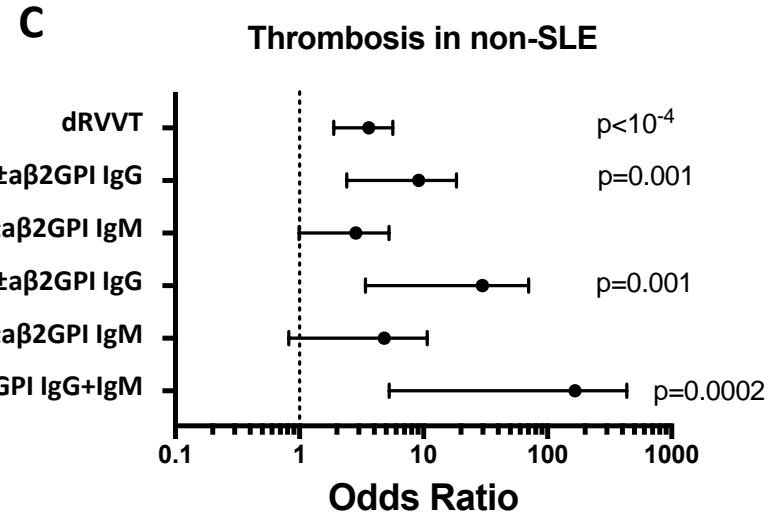
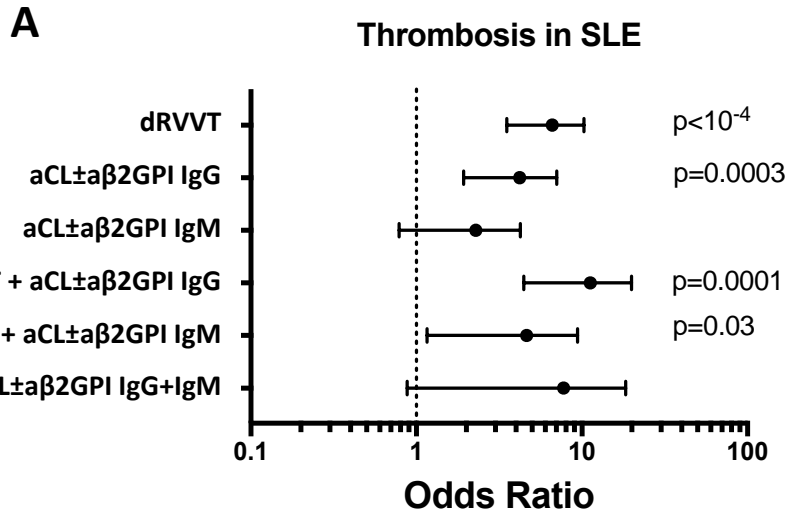


Figure 6

