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Endothelial progenitor dysfunction associates with a type I interferon signature in primary antiphospholipid syndrome

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Abstract

Objectives—Patients with antiphospholipid syndrome (APS) are at risk for subclinical endothelial injury, as well as accelerated atherosclerosis. In the related disease systemic lupus erythematosus, there is a well-established defect in circulating endothelial progenitors, which leads to an accrual of endothelial damage over time. This defect has been at least partially attributed to exaggerated expression of type I interferons (IFNs). We sought to determine whether these pathways are important in APS.

Methods—We studied 68 patients with primary APS. Endothelial progenitors were assessed by flow cytometry and functional assay. Type I IFN activity was determined by a well-accepted bioassay, while peripheral blood mononuclear cells (PBMCs) were scored for expression of IFN-responsive genes.

Results—Endothelial progenitors from APS patients demonstrated a marked defect in the ability to differentiate into endothelial cells, a phenotype which could be mimicked by treating control progenitors with APS sera. Elevated type I IFN activity was detected in the circulation of APS patients (a finding that was then replicated in an independent cohort). While IgG depletion from APS sera did not rescue endothelial progenitor function, the dysfunction was successfully reversed by a type I IFN receptor-neutralizing antibody.

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AUTHORSHIP AND CONFLICT OF INTEREST DISCLOSURES

The authors have no competing interests or conflicts to disclose. R.C.G., S.Y., A.A.G., N.M.K., C.N.-A., and D.H.-R. conducted experiments and analyzed data. A.R.C., W.J.M., P.L.B., and J.S.K. analyzed data and designed the study. All authors participated in writing the manuscript, and gave approval before submission.

Conclusions—We describe, for the first time to our knowledge, an IFN signature in primary APS and show that this promotes impaired endothelial progenitor function. This work opens the door to novel approaches that may mitigate vascular damage in APS, such as anti-IFN drugs.

Keywords

antiphospholipid syndrome; endothelial cells; interferons; atherosclerosis

INTRODUCTION

Endothelial dysfunction and preclinical endothelial damage are well-recognized complications of diseases such as diabetes, rheumatoid arthritis, and systemic lupus erythematosus (SLE). Not coincidentally, these diseases are also strongly associated with accelerated atherosclerosis and significant cardiovascular morbidity. Indeed, endothelial dysfunction and damage serve as predictors, and perhaps precipitators, of atherosclerotic lesions.

These preclinical endothelial abnormalities are at least partially attributable to low numbers and function of so-called endothelial progenitors.[1–3] Endothelial progenitors are a heterogeneous collection of circulating cells with proangiogenic functions including the production of cytokines like vascular endothelial growth factor (VEGF),[4] and the ability to home to ischemic tissue where they support angiogenesis.[5] Some subtypes of progenitors also likely differentiate into true endothelial cells (ECs) *in vivo*, although this concept is somewhat controversial and continues to be explored.[6, 7]

The first endothelial progenitor to be described was the classic endothelial progenitor cell (EPC). Based on surface markers, EPCs are believed to originate from the hemangioblast, the pluripotent bone marrow progenitor of both hematopoietic cells and ECs. EPCs are typically defined and quantified in peripheral blood based on the surface profile CD34⁺CD133⁺VEGFR2⁺. [8, 9] Another strategy for characterizing progenitors does not rely on surface markers, but rather the ability of cells from the peripheral blood mononuclear cell (PBMC) fraction to take on features of mature ECs over days to weeks when cultured under well-defined proangiogenic conditions.[1, 10] At the end of this period, EC-like cells are identified by their ability to take up low-density lipoprotein and bind specified lectins. While some of these cells are derived from the aforementioned EPCs, many have the surface profile CD14⁺CD45⁺, and are presumed to originate from the myeloid compartment.[4] These myeloid-derived cells were originally named circulating angiogenic cells (CACs),[4] which is the nomenclature that will be used in this report. Other names have also been applied in the literature including myeloid EPCs, early EPCs, and proangiogenic cells (PACs).[6, 7] Importantly, CACs, similar to classic EPCs, have potent proangiogenic properties.[4, 11]

Antiphospholipid syndrome (APS) is a leading acquired cause of both thrombophilia and pregnancy loss. Approximately 50% of cases occur in patients with systemic lupus, while the remaining diagnoses comprise a standalone syndrome referred to as primary APS.[12] APS is characterized by the presence of so-called “antiphospholipid” antibodies. This historical name belies the fact that pathogenic antibodies in APS do *not* actually target

phospholipids, but rather clotting factors and other lipid-binding proteins. Indeed, the best-described antigenic target in APS is a protein that circulates at high levels in blood called β -2 glycoprotein I (β_2 GPI).[13, 14] Work over the past three decades has explored the ability of anti- β_2 GPI antibodies to stimulate the endothelium and platelets,[15] while more recent studies have also implicated activation of neutrophils and neutrophil extracellular traps (NETs) in APS pathogenesis.[16, 17]

In addition to its cardinal manifestations of thrombosis and pregnancy loss, APS is also characterized by seizures, cognitive decline, thrombocytopenia, and nephropathy, highlighting its status as a truly systemic autoimmune disease.[18] Further, accelerated atherosclerosis (which is not explained by traditional cardiovascular risk factors) is another recognized complication of APS.[19, 20] As presumed precursors to atherosclerosis, subclinical endothelial damage and dysfunction have been described in many,[21–25] but not all [23, 26] studies of primary APS. Indeed, a recent meta-analysis of APS case-control studies found evidence of both increased carotid intima-media thickness and decreased flow mediated dilation,[27] both of which are indicative of endothelial injury.

The status of endothelial progenitors has only been investigated to a limited extent in APS. Gresele and colleagues characterized 20 cases of primary APS, and in particular focused on patients with no traditional cardiovascular risk factors.[26] As compared with matched controls, they found no difference in the levels of EPCs ($CD34^+CD133^+VEGFR2^+$) in circulation.[26] Further, in a subgroup analysis of an SLE study, Denny and colleagues found no influence of anticardiolipin, anti- β_2 GPI, or lupus anticoagulant on circulating EPC numbers ($CD34^+CD133^+$).[1]

As mentioned above, there is a well-established reduction in the numbers and function of circulating endothelial progenitors in SLE, which potentially leads to an accrual of endothelial damage over time. This defect is at least partially attributable to elevated levels of type I interferons (IFNs).[28] Indeed, type I IFN expression correlates strongly with subclinical vascular disease,[29, 30] as well as abnormal numbers and function of endothelial progenitors.[1, 30] Endothelial progenitor defects in SLE have been observed both by flow cytometry of peripheral blood for $CD34^+CD133^+$ EPCs,[1] and by functional assays that simultaneously characterize both EPCs and CACs.[29, 30] Further, IFN-associated endothelial progenitor dysfunction has been reported in rheumatoid arthritis,[31] pediatric lupus,[32] and murine models of lupus.[33, 34] Mechanistically, IFN- α (the type I IFN most closely aligned with SLE) represses IL-1 signaling and thereby skews myeloid cells away from the CAC phenotype.[35] IFN- α has also been reported to function through the inflammasome to activate IL-18, a cytokine that turns out to be deleterious to CAC differentiation.[10] Of note, the experiments that mechanistically implicate type I IFNs in the endothelial progenitor dysfunction of SLE have primarily involved functional assays that simultaneously report on both EPCs and CACs. In this study, we set out to explore endothelial progenitor function in primary APS, with those findings leading us to also characterize type I IFN expression.

METHODS

See online supplementary methods for further details.

Human subjects

None of the 42 patients with primary APS met American College of Rheumatology criteria for SLE.[36]

In vitro differentiation into mature endothelial cells (ECs)

EC-enrichment culture media was prepared by supplementing MCDB 131 (Gibco) with endothelial growth medium (EGM) SingleQuot factors and 20% heat-inactivated fetal bovine serum. In some cases, the media was additionally supplemented with 30% patient or control sera. PBMCs were seeded into 48-well Human Fibronectin Cellware. After 12–14 days cells were incubated with markers of mature ECs, specifically fluorescein-conjugated Ulex europaeus agglutinin (UEA)-1 lectin and DiI-labeled acetylated low-density lipoprotein (LDL). In some experiments, a neutralizing monoclonal antibody against the type I IFN receptor was included in the media during the first 3 days of culture.

HeLa cell cultures

Cells were cultured in Eagle's minimal essential medium (EMEM) media (Lonza), which was supplemented with 50% patient or control sera.

RESULTS

While it is recognized that patients with primary APS are susceptible to endothelial dysfunction and damage,[27] the status of their endothelial progenitors has not been thoroughly investigated. In this study, we characterized the blood of 42 primary APS patients and 63 non-autoimmune controls as our primary cohort (Table 1). The patients and controls did not differ significantly for age or gender, and were always matched for these factors within individual experiments. The APS patients had antibody profiles and clinical manifestations as described in Table 1.

APS patients have a reduction in functional EPC/CACs

We first assessed classic EPCs in APS patients as compared with controls. Like others, we defined these cells as CD34⁺CD133⁺. [1, 32] Although there was a trend toward decreased EPC numbers in primary APS, this did not reach statistical significance (Figure 1A). We then turned our attention to a functional assay that characterizes a broader set of endothelial progenitors, including EPCs, but also CACs that are derived from the myeloid compartment. [1, 4] Because this assay characterizes the PBMC fraction for both EPCs and CACs, we will subsequently refer to progenitors scored by this assay as EPC/CACs. Indeed, PBMC fractions from primary APS patients displayed a striking reduction in the number of EPC/CACs that successfully differentiated into EC-like cells (Figure 1B–C). These results suggest that while there is no significant reduction in classic EPCs in primary APS, a broader look at endothelial progenitors does reveal a significant defect in patients as compared with controls.

APS sera impair the differentiation of control EPC/CACs into EC-like cells

We next tested whether the impairment of EPC/CACs observed in APS might be attributable to factors that PBMCs were exposed to in circulation. Specifically, we cultured control PBMCs with heterologous control or APS sera. We found that the APS sera (as compared with control sera) significantly reduced the number of EPC/CACs successfully differentiating into EC-like cells (Figure 2A–B). We asked whether APS IgG might directly impair the differentiation of EPC/CACs into EC-like cells, and found that depletion of total IgG from APS sera did not rescue EPC/CAC differentiation (Figure 2C–D). We also cultured control PBMCs in the presence of IgG purified from heterologous control or APS sera. Corroborating the depletion experiment, purified APS IgG did not interfere with EPC/CAC differentiation (Supplementary Figure S1). In summary, these data suggest that there are factors in APS sera (other than the antiphospholipid antibodies themselves) that impair successful differentiation of EPC/CACs into EC-like cells.

APS sera have elevated levels of type I IFNs

As discussed above, SLE is associated with a well-known defect in numbers and function of endothelial progenitors, a phenotype that is at least partially attributable to expression of type I IFNs. Further, the impact of type I IFNs has been especially demonstrable in functional experiments requiring EPC/CACs to differentiate into EC-like cells, such as the assay used here (Figure 2). We therefore explored whether sera from patients with APS might have evidence of exuberant type I IFN activity. To detect this activity, we used a well-accepted bioassay [29] and quantitation of three IFN-responsive genes judged to be relatively specific for type I IFNs as compared with type II IFNs such as IFN- γ . [37] Advantages of the bioassay include its avoidance of the ELISAs that have not always proven reliable or sensitive for IFN- α , [38] and also its ability to capture data on other biologically-active type I IFNs in addition to IFN- α . Interestingly, we found that APS sera strongly induce the expression of all three IFN-responsive genes in HeLa cells (as compared with heterologous control sera), consistent with the presence of higher type I IFN activity in APS (Figure 3). While HeLa cells have been used previously for the bioassay, [29] we also sought confirmation in cells that might have more relevance for EPC/CAC differentiation, namely human umbilical vein endothelial cells (HUVECs). Indeed, APS sera induced the expression of IFN-responsive genes in HUVECs (Supplementary Figure S2). We further went beyond the bioassays by testing for IFN- α directly in sera with a commercial ELISA that is designed to capture all relevant IFN- α subtypes. Again, we revealed upregulation of IFN- α in APS sera as compared with control (Supplementary Figure S3).

To our knowledge, this is the first report of increased IFN activity in primary APS. We therefore tested serum samples from an independent cohort of 26 primary APS patients, recruited from a different center (Supplementary Table S1). As compared with 26 non-autoimmune controls, the primary APS patients again showed robust IFN upregulation, as determined by the HeLa bioassay (Supplementary Figure S4). In summary, these data demonstrate evidence of elevated type I IFN activity in primary APS, spanning two independent patient cohorts.

APS PBMCs display an IFN signature

Having demonstrated elevated type I IFNs in APS sera, we asked whether there was evidence that APS PBMCs had been exposed to higher-than-normal IFN activity *in vivo*. This is a relevant question as PBMCs from primary APS patients differentiated less efficiently into EC-like cells in the experiments of Figure 1. Indeed, primary APS PBMCs (similar to SLE PBMCs) showed upregulation of three IFN-responsive genes (Figure 4A–C and Supplementary Table S2). Again, these are three genes with relative specificity for type I IFNs as compared with IFN- γ . [37] When we segregated the patients according to whether they had positive testing for anti- β_2 GPI IgG by Sydney criteria, we found that PBMCs from the anti- β_2 GPI IgG-positive patients demonstrated higher levels of two of the three IFN-responsive genes, with a trend in the same direction for the third gene (Figure 4D). We additionally segregated patients by the other clinical manifestations and lab testing that are part of the Sydney criteria, but did not find other statistically-significant correlations (Supplementary Figures S5 and S6). In summary, these experiments suggest that in APS the fraction of cells containing EPC/CACs (i.e., PBMCs) has evidence of elevated IFN exposure *in vivo*.

Blockade of the type I IFN receptor rescues the differentiation of EPC/CACs into EC-like cells

Finally, we revisited the experiments of Figure 2, in which APS sera interfered with the differentiation of control EPC/CACs into EC-like cells. Specifically, we tested 10 APS sera (all patients were positive for anti- β_2 GPI IgG) in the presence or absence of a monoclonal antibody that neutralizes the type I IFN receptor. While the antibody had no effect in the context of control sera (Figure 5A), it rescued EPC/CAC differentiation when PBMCs were exposed to APS sera (Figure 5A–B). This result suggests that an important means by which APS sera reduce functional EPC/CAC differentiation is through type I IFN signaling.

DISCUSSION

Primary APS is associated with endothelial dysfunction and damage, and we now show that this may be at least partially attributable to a defect in endothelial progenitors. [27] Interestingly, the numbers of circulating classic EPCs were not appreciably different between primary APS and controls, which is in line with the work of another group. [26] However, when we looked more broadly at endothelial progenitors using a well-established functional assay, we found that primary APS patients have a clear reduction in numbers of functional EPC/CACs. After observing this phenotype in primary cells from APS patients, we also demonstrated that primary APS sera impair the ability of control EPC/CACs to take on features of EC-like cells in the functional assay. We considered the possibility that this might be attributable to the antiphospholipid antibodies themselves, but IgG depletion did not rescue EPC/CAC differentiation. Rather, we now favor the idea that circulating type I IFNs are a critical factor in preventing EPC/CAC maturation. Indeed, we found elevated IFNs in patient sera (which was replicated in an independent cohort), and an IFN signature in the PBMC fractions themselves. Importantly, we also showed that a neutralizing antibody against the type I IFN receptor leads to a restoration of normal EPC/CAC differentiation.

This work mirrors studies in SLE, where it has been suggested that type I IFNs tip myeloid-lineage cells away from the role of endothelial progenitors and towards antigen presentation (as dendritic cells, for example).[28] Although this is the first study that, to our knowledge, explicitly explores type I IFN expression in large primary APS cohorts, there are certainly other hints in the literature regarding IFN dysregulation in APS. For example, the expression of *MCPI* and *IFIT1* (two well-established IFN-regulated genes) correlates with anticardiolipin antibody levels in SLE patients with secondary APS.[39] Similarly, a pilot study of fluvastatin in antiphospholipid antibody-positive patients also suggested higher IFN- α levels by an ELISA assay.[40] Another very small study that included two patients with primary APS described a type I IFN signature in those patients.[41]

The source and driver of type I IFNs in primary APS remains to be elucidated. We considered the possibility that our cohort of primary APS patients may uniquely have more lupus features than most. This is argued against by the identification of elevated IFN activity in a completely independent cohort of primary APS patients. Further, of the 42 patients with primary APS from our original cohort, 20 had a positive anti-nuclear antibody (ANA) by HEp-2 immunofluorescence, most at a titer of 1:160 or less. This is in-line with other studies, which have found ANA prevalence on the order of 30–40% in primary APS.[42, 43] Eleven patients had detectable anti-dsDNA, but all at low titers; no patient had detectable anti-Sm. Further, no APS patient had a history of recurrent hypocomplementemia, nor was any patient hypocomplementemic at the time of the donation. Also, no patient designated as having primary APS was being treated by their hematologist or rheumatologist for SLE according to clinical documentation. As type I IFN activity has previously been shown to differentiate asymptomatic autoantibody-positive patients from those with at least some features of SLE, one wonders if that might also be true for our cohort.[44] We did not identify such features by retrospective chart review, but cannot rule out that they may develop going forward.

A related question is why previous studies focused on SLE patients have not seen a correlation between presence of antiphospholipid antibodies and IFN production.[45] We would point out that all of our patients meet criteria for APS, meaning their antibodies are durably positive, and also associated with thrombotic events or pregnancy morbidity. Further, within our population of APS patients, the best lab predictor of high IFN expression was anti- β_2 GPI, which was not considered in the most comprehensive prior work.[45]

It is possible that toll-like receptors (TLRs) may play a role in driving type I IFN production in APS (as has been well described in SLE). For example, an interesting study has demonstrated that antiphospholipid antibodies promote the translocation of TLR7/8 to the endosome in monocytes and plasmacytoid dendritic cells, where they may then initiate IFN signaling. Further, work from our group has shown that neutrophil extracellular traps (NETs) circulate at higher levels in primary APS, as compared with controls;[16] indeed, NETs have been shown to activate TLRs and type I IFN production in SLE.[46, 47] It is also worth noting that the best-described spontaneous mouse model of APS (NZW $\times$ BXSB F₁) overexpresses TLR7.[48] One might even speculate about a possible role of type I IFNs in promoting antiphospholipid antibody formation given numerous reports of iatrogenic APS in patients treated with IFN- α for hepatitis C and melanoma.[49–51]

A limitation of our study is that while our APS patient cohorts are well-characterized for standard clinical variables, we do not have data regarding endothelial function/dysfunction for the patients recruited into this study. Follow-up work will explore the EPC/CAC defect, as well as type I IFN expression, in a cohort where such data are available. It will also be of interest to explore the impact of type I IFNs on mature ECs in APS,[52, 53] and to consider anti-type I IFN therapeutics in both human and murine APS—a concept that has been met with mixed success to this point in SLE.[54, 55] Indeed, better and more targeted therapeutics for APS are clearly needed as both criteria (thrombosis, pregnancy loss) and non-criteria (accelerated atherosclerosis, cognitive decline) manifestations of APS frequently progress despite current standard-of-care treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. EPC/CACs show a diminished ability to differentiate into EC-like cells in primary APS. **A**, Classic EPCs (defined as CD34⁺CD133⁺) were quantified in peripheral blood of controls and primary APS patients by flow cytometry (units=EPCs/ml of blood). Each data point represents an individual control/patient. The mean for each group is represented by a horizontal line. **B**, PBMCs were isolated from either controls or primary APS patients, and then cultured under proangiogenic conditions for 12–14 days. EPC/CACs were scored for their ability to successfully differentiate into EC-like cells as determined by surface binding of both UEA-1 lectin and acetylated LDL. Again, means and individual data points are indicated. **C**, Representative photomicrographs from the experiments of panel B. UEA-1 lectin is stained green and acetylated LDL red. Scale bar=500 μ m.

Figure 2.

Primary APS sera interfere with the emergence of EC-like cells from control EPC/CACs. **A**, PBMCs were isolated from controls, and then cultured under proangiogenic conditions for 12–14 days. For the first 3 days of culture, media was supplemented with sera from either heterologous controls or primary APS patients. EPC/CACs were scored for their ability to successfully differentiate into EC-like cells as determined by surface binding of both UEA-1 lectin and acetylated LDL. Each data point represents an individual control/patient. The mean for each group is represented by a horizontal line. **B**, Representative photomicrographs from the experiments of panel A. UEA-1 lectin is stained green and acetylated LDL red.

Scale bar=500 μm . **C**, Total IgG was depleted from 10 APS serum samples. These patients were all positive for anti- $\beta_2\text{GPI}$ IgG at baseline. For each group, the median, 25th/75th percentiles, and minimum/maximum are represented by box-and-whisker. Anti- $\beta_2\text{GPI}$ IgG was undetectable in the depleted samples. **D**, As in panel A, PBMCs were isolated from controls, and then cultured under proangiogenic conditions for 12–14 days. For the first 3 days of culture, media was supplemented with APS sera that were either depleted of IgG, or not. EPC/CACs were scored for their ability to successfully differentiate into EC-like cells as determined by surface binding of both UEA-1 lectin and acetylated LDL. Each pair of data points represents an individual patient.

Figure 3.

Primary APS sera have elevated levels of type I IFNs based on an established bioassay. As described in Methods, HeLa cells were treated with sera from either controls or primary APS patients for 6 hours, and then harvested for RNA preparation. Expression of the type I IFN-regulated genes *IFIT1* (panel **A**), *IFI44* (panel **B**), and *PRKR* (panel **C**) was scored by quantitative PCR. Each data point represents an individual control/patient. The median for each group is represented by a horizontal line.

Figure 4.

The PBMC compartment has evidence of a type I IFN signature in primary APS patients, similar to SLE patients. RNA was prepared from total PBMCs. Expression of the type I IFN-regulated genes *IFIT1* (panel A), *IFI44* (panel B), and *PRKR* (panel C) was scored by quantitative PCR. Each data point represents an individual control/patient. The median for each group is represented by a horizontal line. D, Data for primary APS patients are segregated based on whether the patients were negative or positive for anti- β_2 GPI IgG by the Sydney criteria. Again, medians and individual data points are indicated.

Figure 5.

A neutralizing antibody against the type I IFN receptor restores normal EPC/CAC differentiation in primary APS patients. **A**, PBMCs were isolated from controls, and then cultured under proangiogenic conditions for 12–14 days. For the first 3 days of culture, media was supplemented with sera from either heterologous controls or primary APS patients. For these same days, media was additionally supplemented with the aforementioned IFN receptor-neutralizing antibody, or isotype control. EPC/CACs were scored for their ability to successfully differentiate into EC-like cells as determined by surface binding of both UEA-1 lectin and acetylated LDL. Each pair of data points represents an individual control/patient. **B**, Representative photomicrographs from the experiments of panel A. UEA-1 lectin is stained green and acetylated LDL red. Scale bar=500 μ m.

Table 1

APS patients and controls

	Primary APS	Controls
N	42	63
Female	27 (64%)	42 (67%)
Age (y) *	45 ± 5 (21–76)	42 ± 3 (21–67)
Disease duration (y) *	9 ± 2 (1–27)	
White/Caucasian	40 (95%)	59 (94%)
Anti-β ₂ GPI IgG	18/42 (43%)	
Anti-β ₂ GPI IgM	8/42 (19%)	
Anti-β ₂ GPI IgA	13/42 (31%)	
Anticardiolipin IgG	30/42 (71%)	
Anticardiolipin IgM	11/42 (26%)	
Lupus anticoagulant	33/40 (83%)	
Triple positive	20/40 (50%)	
ANA	20/41 (49%)	
Anti-dsDNA ‡	11/40 (28%)	
Anti-SSA	1/37 (3%)	
Anti-SSB	1/37 (3%)	
Anti-Sm	0/37 (0%)	
Anti-RNP	4/37 (11%)	
Venous thrombosis	19/42 (45%)	
Arterial thrombosis	18/42 (44%)	
Small vessel thrombosis	2/42 (5%)	
Thrombocytopenia	10/42 (24%)	
Pregnancy morbidity	9/42 (21%)	
Livedo	8/42 (19%)	
Only thrombocytopenia	1/42 (2%)	
Only APS nephropathy	1/42 (2%)	
Warfarin	17/42 (40%)	
Aspirin	23/42 (55%)	
LMWH	3/42 (7%)	
Factor Xa inhibitor	4/42 (10%)	
Hydroxychloroquine	12/42 (29%)	
Immunosuppressants	2/42 (5%)	

* mean ± 95% confidence interval

‡ For the 11 positives, median=15.9 and maximum=63 (nl <7)

ANA, anti-nuclear antibody; APS, antiphospholipid syndrome; GPI, glycoprotein I; LMWH, low molecular weight heparin; RNP, ribonucleoprotein; SSA, Sjogren's syndrome A; SSB, Sjogren's syndrome B.