Rheumatology, 2022, **00**, 1–5
https://doi.org/10.1093/rheumatology/keac596
Advance access publication 13 October 2022
Concise report

Clinical science

Antiphospholipid antibody positivity in early systemic lupus erythematosus is associated with subsequent vascular events

Nicola Farina[1,2], Ruya Abdulsalam[1], Thomas McDonnell[1], Charis Pericleous[1,3], Amrita D'Souza[1], Vera M. Ripoll[1], Jemma Webster[1], David A. Isenberg [1], Ian Giles[1], Anisur Rahman [1,*]

[1]Centre for Rheumatology Research, Division of Medicine, University College London, London, UK
[2]Unit of Immunology, Rheumatology, Allergy and Rare Diseases, IRCCS San Raffaele, Milan, Italy
[3]National Heart and Lung Institute, Imperial College London, London, UK

*Correspondence to: Anisur Rahman, Centre for Rheumatology Research, Division of Medicine, Fourth Floor Rayne Institute, 5 University Street, London WC1E 6JF, UK. E-mail: anisur.rahman@ucl.ac.uk

Abstract

Objective: aPL are found in the blood of 20–30% of patients with SLE. Although aPL cause vascular thrombosis in the antiphospholipid syndrome, it is not clear whether positive aPL levels in early SLE increase risk of subsequent vascular events (VE). In a previous analysis of 276 patients with SLE, we found that early positivity for ≥2 of IgG anti-cardiolipin (anti-CL), IgG anti-β_2-glycoprotein I (anti-β2GPI) and anti-domain I of β_2-glycoprotein I (anti-DI) showed a possible association with VE. Here we have extended that analysis.

Methods: Serum samples taken from 501 patients with SLE early in their disease had been tested for IgG anti-CL, anti-β2GPI and anti-DI by ELISA. Complete VE history was available for 423 patients of whom 23 were excluded because VE occurred before the diagnosis of SLE. For the remaining 400 patients we carried out Kaplan–Meier survival analysis to define groups at higher risk of VE.

Results: Of 400 patients, 154 (38.5%) were positive for one or more aPL, 27 (6.8%) were double/triple-positive and 127 (31.8%) were single-positive. There were 91 VE in 77 patients, of whom 42 were aPL-positive in early disease. VE were significantly increased in aPL-positive *vs* aPL-negative patients ($P=0.041$) and in double/triple-positive *vs* single-positive *vs* aPL-negative patients ($P=0.0057$). Omission of the IgG anti-DI assay would have missed 14 double/triple-positive patients of whom six had VE.

Conclusion: Double/triple-positivity for IgG anti-CL, anti-β2GPI and anti-DI in early SLE identifies a population at higher risk of subsequent VE.

Keywords: SLE, APS, cardiovascular

Rheumatology key messages

- Of 400 patients with SLE, 38.5% were positive for antiphospholipid antibodies in early disease samples.
- Positivity for ≥2 of IgG anti-cardiolipin, anti-β_2-glycoprotein I and anti-domain I was associated with increased risk of vascular events.

Introduction

aPL are the key serological feature of the APS, which is characterized by vascular thrombosis and pregnancy morbidity [1]. aPL are found in 20–30% of patients with SLE [2] but not all these patients develop vascular events (VE). The common clinical assays for aPL include anti-cardiolipin (anti-CL) and anti-β_2-glycoprotein I (anti-β2GPI) ELISAs as well as the lupus anticoagulant test. In many lupus units, it is common for these aPL assays to be carried out routinely at the time of diagnosis in patients with SLE. This results in identification of patients who test positive without ever having suffered thrombosis or pregnancy morbidity. There is no consensus about how to manage this scenario as the long-term risk of VE for these patients is unclear.

The N-terminal domain (domain I or DI) of β2GPI is believed to contain the major epitope for pathological anti-β2GPI antibodies [3]. Thus, IgG anti-DI have been suggested to be a more specific marker of thrombosis, which would add predictive power to analysis of future risk of VE in aPL-positive patients [4–6]. However, anti-DI tests are not yet in routine clinical use.

It is not clear to what extent positivity for aPL in early SLE is predictive of increased risk of VE subsequently. In a previous publication in *Rheumatology* we described measurement of IgG anti-CL, IgG anti-β2GPI and IgG anti-DI in the earliest available serum samples from 501 patients with SLE [7]. There was a trend towards increased risk of VE in patients who were positive for ≥2 of these antibodies (double/triple-

Received: 26 June 2022. **Accepted:** 17 September 2022

positive), but this did not reach statistical significance [7]. In that paper we only had data on complete vascular history for 276 patients, which limited our analysis. After further thorough analysis of the medical records of an extra 197 patients, we have complete vascular history plus aPL measurements in early samples from 423 patients and an additional 7 years' follow-up. We have therefore repeated and extended the analysis of associations between early aPL-positivity and subsequent VE in the current paper.

Methods

All patients entered into the lupus cohort at University College London Hospital (UCLH) fulfil the American College of Rheumatology criteria for SLE [8] or previous criteria extant at the time of entering the cohort. The earliest available serum samples from 501 patients were retrieved from storage at −80°C. For over 90% of patients, this earliest sample was obtained within 2 years of diagnosis. IgG anti-CL, anti-β2GPI and anti-DI in these samples were measured by ELISA, as described in our previous papers [5, 7]. Cut-offs for positivity were defined as 20.9 IgG anti-DI units (GDIU), 18.9 IgG anti-β2GPI units (GBU), and 17.9 anti-CL units (GPLU), based on the 99th percentile for healthy controls measured in our laboratory.

Clinical information on VE was obtained from patient records and from interviews with patients with written informed consent and with ethics approval from the London Hampstead Research Ethics Committee (reference no. 12/LO/0373). VE were defined as any of the following: venous thrombosis, pulmonary embolism, coronary disease with proof by blood tests and/or imaging, and cerebrovascular attack with proof on imaging. Twenty-three patients were excluded from the analysis because they suffered VE before the diagnosis of SLE. Thus, we present results from 400 patients here.

Data were analysed using Stata (StataCorp, College Station, TX, USA) by using Kaplan–Meier (KM) survival estimates. The log-rank test was used to assess the significance of the differences between different groups of patients, stratified according to aPL profile found in the KM curves. Patients were censored at the time of death, loss to follow-up or the end of the study period.

Results

The ELISA results are depicted in the Venn diagram in Supplementary Fig. S1, available at *Rheumatology* online. Of 400 patients, 154 (38.5%) were positive for one or more aPL (aPL-positive) and 246 (61.5%) were aPL-negative, 27 (6.8%) were double/triple-positive and 127 (31.8%) were single-positive. The most common antibody was IgG-anti-DI, present in 108 (27%) patients. Supplementary Table S1 (available at *Rheumatology* online) shows demographic details of the 400 patients stratified in terms of early aPL-positivity.

There were 91 VE in 77 patients over a median follow-up period of 13 (IQR 9–18, max 40) years. There were 54 arterial VE and 37 venous VE. Supplementary Fig. S1B, available at *Rheumatology* online, shows the early aPL serology of these 77 patients, of whom over half were aPL-positive (42/77 = 55%). Of note, 18/77 (23%) were only positive for IgG anti-DI and would not have been identified as seropositive by testing only anti-CL and anti-β2GPI as included in the Sydney criteria [1].

We carried out Kaplan–Meier survival analysis using Stata, comparing VE outcomes in groups with different early sample serology. As shown in Fig. 1A, aPL-positive patients were significantly more likely to develop VE than aPL-negative patients ($P = 0.041$). Comparison of double/triple-positive *vs* single-positive *vs* negative patients also showed a statistically significant difference between groups ($P = 0.0057$) with the highest risk of VE in the double/triple-positive group (Fig. 1B).

Analysis of data for individual antibody-specificities showed significantly increased risk of VE in single anti-β2GPI-positive *vs* aPL-negative patients ($P = 0.0010$) but this was based on only three events in six patients (Fig. 2A). There were no significant differences between single anti-DI-positive *vs* aPL-negative patients or single anti-CL-positive *vs* aPL-negative patients (Fig. 2B and C).

Overall, 12 of 27 (44.4%) double/triple positive patients had VE compared with 30 of 127 (23.6%) single-positive patients and 35 of 246 (14.2%) aPL-negative patients.

The majority of the patients had low-titre aPL. The Sydney criteria for APS [1] require aCL level ≥40 GPLU for the diagnosis of APS. In our cohort 23/60 anti-CL-positive subjects had anti-CL ≥40 GPLU, 3/21 anti-β2GPI-positive subjects had anti-β2GPI ≥40 GBU, and 34/108 anti-DI-positive subjects had anti-DI ≥40 GPLU, The vast majority had either single IgG anti-DI positivity ($n = 31$) or single IgG anti-CL positivity ($n = 19$). There were only four patients who had more than one of these antibodies at high-titre. As shown in Supplementary Fig. S2, available at *Rheumatology* online, high-titre IgG aPL-positivity was not associated with increased risk of VE compared with low-titre positivity ($P = 0.29$).

Discussion

This report underlines our previous finding in this cohort of patients that being positive for ≥2 of IgG anti-DI, IgG anti-CL and IgG anti-β2GPI in very early SLE identifies a group at high risk of developing VE in long-term follow-up [7]. If IgG anti-DI had not been measured, we would have identified only 13 anti-CL/anti-β2GPI double-positive patients including only six with VE (Supplementary Fig. S1B, available at *Rheumatology* online). Those 13 patients did not constitute a higher-risk group for VE on Kaplan–Meier analysis (Fig. 2D). Thus, we conclude that the main additive value of including the IgG anti-DI ELISA was not that anti-DI positivity by itself is a risk factor for VE, but that it increases the sensitivity for identifying patients positive for multiple aPL, who are a risk group for future VE. Almost half of the double/triple-positive group suffered VE. Of the 14 patients who would not have been identified in this double/triple-positive group without measuring IgG anti-DI, six suffered VE.

Positivity here refers to any value above the 99th percentile of the healthy population and not the higher values defined in the Sydney criteria for APS [1]. However, we carried out further analysis restricting our definition of aPL-positivity to patients with high levels of antibodies and found that high-titre positivity was not associated with increased risk of VE.

Our analysis is limited by the fact that we do not have data on lupus anticoagulant positivity because that cannot be

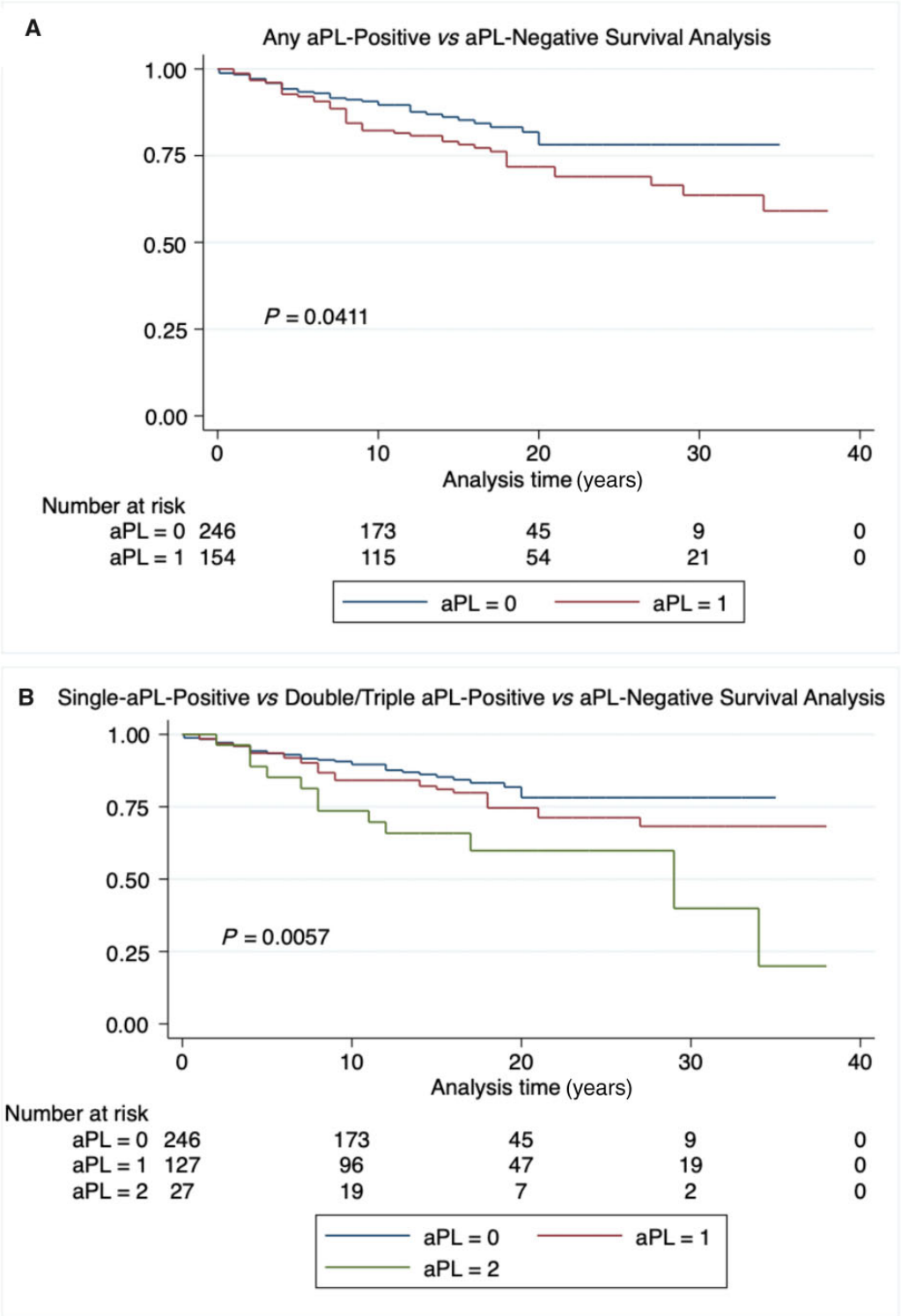

Figure 1. Kaplan–Meier survival curves comparing incidence of vascular events over time in groups stratified by serology in early disease samples. (**A**) Comparison of aPL-positive *vs* aPL-negative groups. (**B**) Comparison of single-positive *vs* double/triple positive *vs* negative groups. The overall *P*-value for difference between curves was 0.0057. Pairwise comparisons showed double/triple-positive *vs* aPL-negative $P = 0.0026$, single-positive *vs* aPL-negative $P = 0.19$ and double/triple positive *vs* single-positive $P = 0.025$

assessed in stored serum samples—though we previously showed that our double/triple-positive group were also at higher risk of developing lupus anticoagulant later in the disease [7]. Further limitations are that this is a single-centre cohort and ELISA results were from an in-house assay and that we did not measure IgM or IgA antibodies—which have also been shown to have predictive value for vascular thrombosis in patients with SLE [5].

This paper does not consider effects of traditional cardiovascular risk factors, medication or disease activity as these factors are the subjects of a separate larger manuscript currently under review. The current paper deals only with the

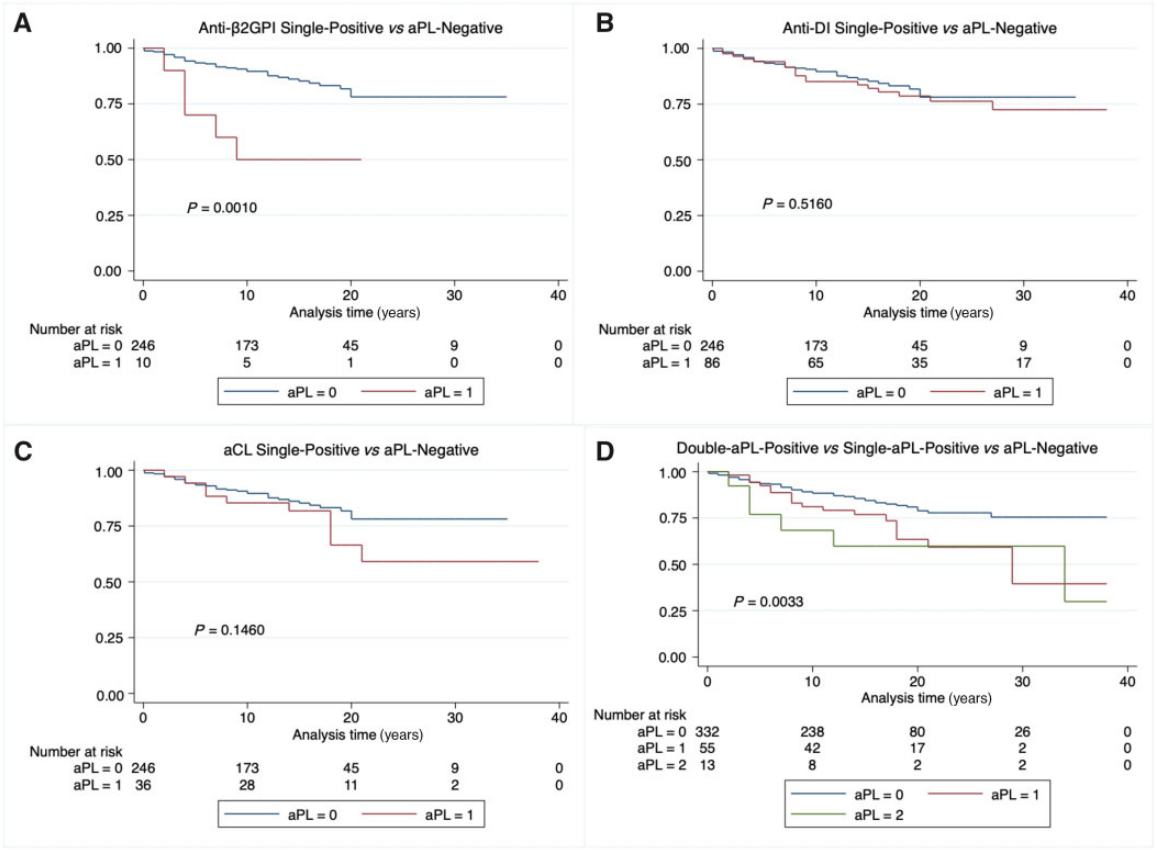

Figure 2. Further Kaplan–Meier survival curves comparing incidence of vascular events over time in groups stratified by serology in early disease samples. (**A**) Comparison of single anti-β2GPI positive *vs* aPL-negative groups. (**B**) Comparison of single anti-DI positive *vs* aPL-negative groups. (**C**) Comparison of single anti-CL positive *vs* aPL-negative groups. (**D**) An analysis carried out to simulate what would have happened if only the criteria assays—IgG anti-β2GPI and anti-CL—had been carried out. There would have been 332 aPL-negative, 55 single-positive and 13 double-positive patients. The increased risk in positive *vs* negative patients is still present but the survival curves for double-positive and single-positive patients do not diverge. The overall *P*-value for difference between curves was 0.0033. Pairwise comparisons showed double-positive *vs* aPL-negative $P = 0.0077$, single-positive *vs* aPL-negative $P = 0.015$ and double positive *vs* single-positive $P = 0.38$

issue of early aPL-positivity as a follow-up to our previous paper [7].

There is currently no consensus on how often to check aPL in patients with SLE who have not suffered a VE. Use of aspirin for primary prophylaxis of VE is recommended only in those with high-risk profiles [9]. Our results suggest that patients with SLE who test positive for ≥ 2 of IgG anti-DI, anti-CL and anti-β2GPI, even at low levels, at the time of diagnosis, may warrant more frequent aPL testing and consideration of aspirin.

Supplementary data

Supplementary data are available at *Rheumatology* online.

Data availability statement

Data are available on request to the authors.

Funding

This work was carried out at a centre supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre and supported by Arthritis Research UK programme grant 19423. V.M.R. was supported by LUPUS UK and the Louise Gergel Fellowship. T.M. is supported by a Medical Research Foundation Fellowship. C.P. is supported by Versus Arthritis Career Development Fellowship 21223.

Disclosure statement: C.P., I.G., T.M. and A.R. are co-inventors on a patent to develop an agent based on DI as a potential therapeutic agent for APS.

Acknowledgements

N.F., J.W., A.dS., D.A.I., I.G. and A.R. collected clinical data. C.P., V.M.R. and T.M. carried out laboratory assays, N.F. and R.A. analysed the data. A.R. wrote the final manuscript. All authors contributed to critical analysis of the manuscript and approved the final version.

References

1. Miyakis S, Lockshin MD, Atsumi T *et al.* International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4:295–306.

2. Petri M. Epidemiology of antiphospholipid syndrome. In: Khamashta M, ed. Hughes syndrome – antiphospholipid syndrome. 2nd edn. London: Springer, 2006: 23–4.
3. Pericleous C, Rahman A. Domain I: the hidden face of antiphospholipid syndrome. Lupus 2014;23:1320–3.
4. de Laat B, Pengo V, Pabinger I *et al.* The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. J Thromb Haemost 2009;7:1767–73.
5. Pericleous C, Ferreira I, Borghi O *et al.* Measuring IgA anti-β2-glycoprotein I and IgG/IgA anti-domain I antibodies adds value to current serological assays for the antiphospholipid syndrome. PLoS One 2016;11:e0156407.
6. Meneghel L, Ruffatti A, Gavasso S *et al.* Detection of IgG anti-Domain I beta2 Glycoprotein I antibodies by chemiluminescence immunoassay in primary antiphospholipid syndrome. Clin Chim Acta 2015;446:201–5.
7. Pericleous C, D'Souza A, McDonnell T *et al.* Antiphospholipid antibody levels in early systemic lupus erythematosus: are they associated with subsequent mortality and vascular events? Rheumatology (Oxford) 2020;59:146–52.
8. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
9. Tektonidou MG, Andreoli L, Limper M *et al.* EULAR recommendations for the management of antiphospholipid syndrome in adults. Ann Rheum Dis 2019;78:1296–304.