## Antibodies to domain I of β-2-glycoprotein I and IgA antiphospholipid antibodies in patients with 'seronegative' antiphospholipid syndrome

The standard serological tests included in the classification criteria[1] for antiphospholipid syndrome (APS) are those to detect immunoglobulin G (IgG) and IgM antibodies to cardiolipin (aCL) or β-2-glycoprotein I (anti-β2GPI) and the lupus anticoagulant. It is increasingly recognised, however, that some patients have typical thrombotic and non-thrombotic features of APS but test repeatedly negative in these routinely used assays. It has been suggested that these patients have the so-called seronegative APS (SN-APS).[2] In a retrospective study, there were no significant differences in clinical manifestations between 87 patients with seropositive APS and 67 with SN-APS.[3] Several authors have suggested that in these 'seronegative' patients, clinically relevant antibodies can be detected by looking for different isotypes, particularly IgA[2] and/or different antigen specificity[4] or by using different techniques[4 5] than those of the routine assays. In a recent paper, 79% of 24 patients with SN-APS had serum antibodies detectable by such strategies.[5] There is considerable evidence that IgA antiphospholipid antibody tests may be a useful diagnostic tool in APS.[6] Antibodies to domain I (DI) of β2GPI have attracted particular interest as they are strongly

**Table 1** Clinical features of patients in the study

| | APS group | SN-APS group |
|---|---|---|
| Number in study | 40 | 40 |
| Gender | | |
| Male | 4 | 0 |
| Female | 36 | 40 |
| Mean age at sample | 45.3 | 46.1 |
| Diagnosis | | |
| PAPS | 22 | N/A |
| SLE/APS | 18 | N/A |
| SN-PAPS | N/A | 35 |
| SN-APS/SLE | N/A | 5 |
| Thrombosis | 34 | 25 |
| Venous | 19 | 12 |
| Arterial | 15 | 13 |
| None | 6 | 15 |
| Pregnancies? | | |
| Yes | 33 | 39 |
| No | 3 | 1 |
| N/A | 4 | 0 |
| Pregnancy morbidity | 23 | 35 |
| Miscarriage | 12 | 29 |
| Stillbirth | 19 | 25 |
| Prematurity | 4 | 7 |
| Preeclampsia | 7 | 10 |
| None | 17 | 5 |
| LA positivity | | |
| Yes | 27 | 0 |
| No | 13 | 40 |

LA, lupus anticoagulant; PAPS, Primary Antiphospholipid Syndrome; SLE, systemic lupus erythematosus; SN-APS, seronegative antiphospholipid syndrome.

**Table 2** ELISA results

| | Seropositive APS (n=40) | SN-APS (n=40) |
|---|---|---|
| Standard ELISAs | | |
| No. (%) testing positive for IgG anti-CL | 18 (45%) | 0 |
| No. (%) testing positive for IgG anti-β2GPI | 6 (15%) | 0 |
| No. (%) testing positive for IgM anti-CL | 4 (10%) | 0 |
| No. (%) testing positive for IgM anti-β2GPI | 9 (22.5%) | 0 |
| Anti-DI ELISAs | | |
| No. (%) testing positive for IgG anti-DI | 11 (27.5%) | 3 (7.5%)* |
| No. (%) testing positive for IgM anti-DI | 9 (22.5%) | 0 |
| No. (%) testing positive for IgA anti-DI | 7 (17.5%) | 0 |
| Other IgA ELISAs | | |
| No. (%) testing positive for IgA anti-CL | 12 (30%) | 0 |
| No. (%) testing positive for IgA anti-β2GPI | 8 (20%) | 1 (2.5%)* |

The cut-off for positive in each assay was defined as 99th centile of the healthy control population.
*The titres of IgG anti-DI in the three positive patients were 16 absorbance units (AU), 15.3 AU and 22.2 AU respectively compared with the positive cut-off of 10 AU. The titre of IgA anti-β2GPI in the one positive patient was 16 AU compared with a positive cut-off of 9 AU.
anti-β2GPI, anti-β-2-glycoprotein; CL, cardiolipin; DI, domain I; Ig, immunoglobulin; SN-APS, seronegative antiphospholipid syndrome.

associated with thrombosis.[7–9] No formal analysis of anti-DI antibodies (of any isotype) or IgA antiphospholipid antibodies in patients with SN-APS has been reported.

Serum samples from 80 patients with APS (40 with seropositive APS fulfilling classification criteria[1] and 40 with SN-APS fulfilling clinical but not serological criteria) from St Thomas' Hospital (STH) and 200 healthy controls were tested at University College London (UCL) in nine ELISAs—IgG, IgM and IgA for each of aCL, anti-β2GPI and anti-DI. ELISAs were carried out blind to the clinical and serological information from STH using methods published previously[10] with appropriate modifications to detect IgA. We defined the cut-off for a positive result in each assay as the 99th centile of the healthy population.

Clinical features of the patients are shown in table 1 and results of the ELISAs in table 2. For ease of interpretation, table 2 groups the four criteria tests used in routine clinical practice (IgG aCL, IgM aCL, IgG anti-β2GPI and IgM anti-β2GPI) together at the top and the non-standard ELISAs (all anti-DI, IgA aCL and IgA anti-β2GPI) below. In the seropositive APS group, we found large numbers of samples that tested positive in the five non-criteria ELISAs. Thus 62.5% were positive in at least one of these assays. In the SN-APS group, we found no samples positive in the standard assays (thus 100% agreement with STH in tests at UCL done blind to STH results) but four (10%) were positive in one of the non-standard ELISAs.

In conclusion, this blinded serological analysis of seropositive and SN-APS cohorts confirms that anti-DI, IgA aCL or IgA anti-β2GPI antibodies, while present in a significant proportion of seropositive patients with APS, may also pick up a small proportion of patients with SN-APS. In this study, the IgG anti-DI assay had the highest pick-up rate (despite samples testing negative for anti-β2GPI), which is interesting given the accumulating evidence that IgG anti-DI antibodies are important in the pathogenesis of APS.[7–10]

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**Contributors** LC and CP carried out the laboratory experiments. MLB and MK recruited the patients and provided the samples and clinical information. AR, IG, CP and YI developed the anti-DI assays and designed the project. LC and AR wrote the final paper. All authors read and commented on the final manuscript.

**Funding** This work was funded by Arthritis Research UK Programme Grant 19423 and supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. YI is also supported by Arthritis Research UK Grant 20164. MLB is funded by the Louise Gergel Fellowship.

**Competing interests** None.

**Ethics approval** London Hampstead National Research Ethics Service Committee.

**Provenance and peer review** Not commissioned; externally peer reviewed.


**To cite** Cousins L, Pericleous C, Khamashta M, *et al*. *Ann Rheum Dis* 2015;**74**:317–319.

Received 15 August 2014
Revised 7 October 2014
Accepted 12 October 2014
Published Online First 30 October 2014

*Ann Rheum Dis* 2015;**74**:317–319. doi:10.1136/annrheumdis-2014-206483

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