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\(^4\) 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.\(^6\) 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
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 criteria1 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—lgG, IgM and lgA 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 previously10 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 (lgG 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, lgA aCL or lgA 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 lgG anti-DI assay had the highest pick-up rate (despite samples testing negative for anti-β2GPI), which is interesting given the accumulating evidence that lgG 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.

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