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.

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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 Cervera R, Conti F, Doria A, *et al*. Does seronegative antiphospholipid syndrome really exist? *Autoimmun Rev* 2012;11:581–4.
3 Rodriguez-Garcia JL, Bertolaccini ML, Cuadrado MJ, *et al*. Clinical manifestations of antiphospholipid syndrome (APS) with and without antiphospholipid antibodies (the so-called 'seronegative APS'). *Ann Rheum Dis* 2012;71:242–4.
4 Ortona E, Capozzi A, Colasanti T, *et al*. Vimentin/cardiolipin complex as a new antigenic target of the antiphospholipid syndrome. *Blood* 2010;116:2960–7.
5 Conti F, Capozzi A, Truglia S, *et al*. The mosaic of "seronegative" antiphospholipid syndrome. *J Immunol Res* 2014;2014:389601.
6 Bertolaccini ML, Amengual O, Andreoli L, *et al*. 14th International Congress on Antiphospholipid Antibodies Task Force. Report on antiphospholipid syndrome laboratory diagnostics and trends. *Autoimmun Rev* 2014;13:917–30.
7 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.
8 Ioannou Y, Romay-Penabad Z, Pericleous C, *et al*. In vivo inhibition of antiphospholipid antibody-induced pathogenicity utilizing the antigenic target peptide domain I of beta2-glycoprotein I: proof of concept. *J Thromb Haemost* 2009;7:833–42.
9 Pericleous C, Ruiz-Limon P, Romay-Penabad Z, *et al*. Proof-of-concept study demonstrating the pathogenicity of affinity-purified IgG antibodies directed to domain I of beta2-glycoprotein I in a mouse model of anti-phospholipid antibody-induced thrombosis. *Rheumatology (Oxford)* 2014. doi:10.1093/rheumatology/keu360
10 Ioannou Y, Pericleous C, Giles I, *et al*. Binding of antiphospholipid antibodies to discontinuous epitopes on domain I of human beta(2)-glycoprotein I: mutation studies including residues R39 to R43. *Arthritis Rheum* 2007;56:280–90.