

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 criteria¹ for antiphospholipid syndrome (APS) are those to detect immunoglobulin G (IgG) and IgM antibodies to cardiolipin (aCL) or β -2-glycoprotein I (anti- β 2GPI) 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 IgA² and/or different antigen specificity⁴ 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 β 2GPI 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.

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—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 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 (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

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.

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}

Laura Cousins,¹ Charis Pericleous,¹ Munther Khamashta,² Maria Laura Bertolaccini,² Yiannis Ioannou,^{1,3} Ian Giles,¹ Anisur Rahman¹

¹Centre for Rheumatology Research, University College London, London, UK

²Lupus Research Unit, The Rayne Institute, King's College London School of Medicine, London UK

³Arthritis Research UK Centre for Adolescent Rheumatology, University College London, London, UK

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

LC and CP are joint first authors, who contributed equally to the work.

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.

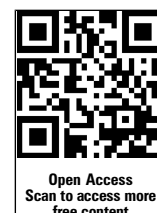
Competing interests None.

Ethics approval London Hampstead National Research Ethics Service Committee.

Provenance and peer review Not commissioned; externally peer reviewed.



OPEN ACCESS



Open Access
Scan to access more
free content

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/3.0/>



CrossMark

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

REFERENCES

- 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.
- Cervera R, Conti F, Doria A, *et al.* Does seronegative antiphospholipid syndrome really exist? *Autoimmun Rev* 2012;**11**:581–4.
- 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.
- Ortona E, Capozzi A, Colasanti T, *et al.* Vimentin/cardioliipin complex as a new antigenic target of the antiphospholipid syndrome. *Blood* 2010;**116**:2960–7.
- Conti F, Capozzi A, Truglia S, *et al.* The mosaic of "seronegative" antiphospholipid syndrome. *J Immunol Res* 2014;**2014**:389601.
- 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.
- 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.
- 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.
- 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
- 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.