

EXTENDED REPORT

Value of IgA anticardiolipin and anti- β_2 -glycoprotein I antibody testing in patients with pregnancy morbidity

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Objective: To study the prevalence of IgA antiphospholipid antibodies, particularly anticardiolipin antibodies (aCL) and anti- β_2 -glycoprotein I ($\alpha\beta_2$ GPI), in a cohort of patients with pregnancy morbidity.**Patients and methods:** Serum samples from four groups of patients were studied by an in house enzyme linked immunosorbent assay (ELISA). Group I: 28 patients with primary antiphospholipid syndrome (PAPS) (median age 32.5 years, range 25–34). Twelve patients had a history of thrombosis. All were positive for IgG/M aCL or lupus anticoagulant (LA), or both. Group II: 28 patients with unexplained pregnancy morbidity (median age 35 years, range 23–48). Seven had history of thrombosis. Nine patients were positive for IgG/M aCL. None from this group fulfilled Sapporo criteria for APS. Group III: 28 patients with systemic lupus erythematosus (SLE) (median age 34 years, range 25–52). Eleven had a history of thrombosis. Twenty one patients had IgG/M aCL and/or LA, but only 19 fulfilled Sapporo criteria for APS.**Results:** IgA aCL were found in 12, 6, and 14 patients from the groups with PAPS, unexplained pregnancy morbidity, and SLE, respectively. Most patients had these antibodies together with IgG/IgM aCL. Three patients from the group with unexplained pregnancy morbidity and two with SLE had IgA aCL alone. IgA $\alpha\beta_2$ GPI was present in one patient from each group. All IgA $\alpha\beta_2$ GPI were present together with IgG and/or IgM $\alpha\beta_2$ GPI.**Conclusions:** The prevalence of IgA aCL is high in patients with pregnancy morbidity, although IgA aCL are usually present together with IgG and/or IgM aCL. IgA $\alpha\beta_2$ GPI are not useful in identifying additional women with APS and pregnancy morbidity.

The antiphospholipid antibody (aPL) family includes a heterogeneous population of autoantibodies whose specificity is directed against phospholipids and their complex with plasma proteins. It is recognised that the presence of IgG and IgM anticardiolipin antibodies (aCL) and lupus anticoagulant (LA) is associated with thrombosis and pregnancy loss, making the presence of these antibodies essential to classify a patient as antiphospholipid syndrome (APS).¹ It has also been demonstrated that these antibodies are directed to plasma proteins bound to anionic phospholipids. The phospholipids may induce some conformational changes in the protein structure, and thus many of the antibodies against phospholipid binding proteins can be detected basically in the presence of phospholipids.^{2–4} So far, β_2 -glycoprotein I (β_2 GPI) and prothrombin are the best known and characterised phospholipid binding proteins.⁵

β_2 GPI is a glycoprotein with a molecular weight of 50 kDa and high affinity towards negatively charged phospholipid surfaces. The binding of aPL to β_2 GPI is dependent on oxidised irradiated surfaces or membranes containing anionic phospholipids.²

The association between IgA aPL and clinical manifestations of the APS is controversial. Some studies showed a higher frequency of these antibodies in patients with systemic lupus erythematosus (SLE) or APS, or both, suggesting that their detection, particularly IgA $\alpha\beta_2$ GPI, may be of value as a method for assessing the risk in these patients.^{6–7} In contrast, some series showed that the IgA aCL isotype is uncommon and not helpful in diagnosing APS.^{8–9}

Recently, IgA anti- β_2 GPI ($\alpha\beta_2$ GPI) have been found in patients with unexplained recurrent pregnancy loss and no other aPL, suggesting that these antibodies may be related to pregnancy morbidity.^{10–12} We designed this study to investigate whether IgA $\alpha\beta_2$ GPI are raised in women with a history of

unexplained recurrent miscarriage, fetal death, or premature birth in an attempt to find out whether there might be a correlation between these antibodies and pregnancy morbidity.

PATIENTS AND METHODS

Patients

This study comprised 84 patients with history of pregnancy morbidity, defined by ≥ 3 miscarriages before the 10th week of gestation, ≥ 1 fetal death beyond the 10th week of gestation, and/or ≥ 1 premature birth before the 34th week of gestation due to severe pre-eclampsia or eclampsia, or severe placental insufficiency, as established by Wilson *et al*.¹³ 100 apparently healthy controls were also studied. Patients were included in three groups: 28 women with well defined primary APS (PAPS) according to the international consensus criteria¹ (median age 32.5 years, range 25–34); 28 women with unexplained pregnancy morbidity, (median age 35 years, range 23–48); 28 women fulfilling at least four of the American College of Rheumatology criteria for SLE¹³ (median age 34 years, range 25–52).

Of the patients with PAPS, 12 had a history of thrombosis (five arterial, six venous, and one patient both arterial and venous events). All patients from this group were positive for IgG/M aCL and/or LA, according to the 1999 Sapporo criteria,¹ and 13 for IgG/M $\alpha\beta_2$ GPI. In the group with unexplained pregnancy morbidity, seven patients had a history of

Abbreviations: $\alpha\beta_2$ GPI, anti- β_2 -glycoprotein I; aCL, anticardiolipin antibodies; aPL, antiphospholipid antibodies; BSA, bovine serum albumin; ELISA, enzyme linked immunosorbent assay; LA, lupus anticoagulant; PAPS, primary antiphospholipid syndrome; PBS, phosphate buffered saline; SLE, systemic lupus erythematosus

Table 1 Demographic data and thrombotic history in patients with PAPS, patients with unexplained pregnancy morbidity, and patients with SLE

Group	Mean age (range) Years	Thrombotic history No (%)	Arterial events No (%)	Venous events No (%)
PAPS (n=28)	32.5 (25–34)	12 (43)	6 (21)	7 (25)
Unexplained pregnancy morbidity (n=28)	35 (23–48)	7 (25)	1 (4)	6 (21)
SLE (n=28)	34 (25–52)	11 (39)	4 (14)	7 (25)

All patients were female. PAPS, primary antiphospholipid syndrome; SLE, systemic lupus erythematosus.

Table 2 Autoantibody profile in patients with PAPS, patients with unexplained pregnancy morbidity, and patients with SLE

	aPL positive No (%)	aCL No (%)	IgG aCL No (%)	IgM aCL No (%)	LA No (%)	a β_2 GPI No (%)	IgG a β_2 GPI No (%)	IgM a β_2 GPI No (%)
PAPS (n=28)	28 (100)	26 (93)	14 (50)	25 (89)	15 (54)	13 (46)	12 (43)	4 (14)
Unexplained pregnancy morbidity (n=28)	9 (32)	9 (32)	2 (7)	8 (29)	0 (0)	8 (29)	7 (25)	1 (4)
SLE (n=28)	21 (75)	21 (75)	20 (71)	20 (71)	5 (18)	11 (39)	10 (36)	4 (14)

PAPS, primary antiphospholipid syndrome; SLE, systemic lupus erythematosus; aPL, antiphospholipid antibodies; aCL, anticardiolipin antibodies; LA, lupus anticoagulant.

None from the group with unexplained pregnancy morbidity and 19 patients from the group with SLE fulfilled the Sapporo criteria for the APS.

thrombosis (one arterial and six venous). Only nine patients were positive for IgG/M aCL and eight for IgG/M a β_2 GPI at low titres. Therefore, none of these patients fulfilled Sapporo criteria for the classification of APS.¹ Thrombosis occurred in 11 patients with SLE (four arterial and seven venous). IgG/M aCL and/or LA antibodies were present in 21 patients, but only 19 fulfilled Sapporo criteria for APS. Tables 1 and 2 summarise these data.

Obstetric history

The number of pregnancies was 95, 142, and 111 in the groups with PAPS, unexplained pregnancy morbidity and SLE, respectively. Obstetric history included 30 miscarriages, 25 fetal deaths, and 7 cases of prematurity in the group with PAPS; 55 miscarriages, 27 fetal deaths, and 1 case of prematurity in the group with unexplained pregnancy morbidity; and 38 miscarriages, 24 fetal deaths, and 6 cases of prematurity in the group with SLE.

Methods

aCL ELISA

All IgG and IgM aCL were determined in our laboratory according to the standardised aCL enzyme linked immunosorbent assay (ELISA).¹⁴ IgA aCL were tested as previously reported.⁹

a β_2 GPI ELISA

IgG and IgM a β_2 GPI were detected by ELISA using irradiated ELISA plates (Nunc Maxisorp, Denmark) as previously described.¹⁵

IgA a β_2 GPI were detected by an in house ELISA. Briefly, microtitre ELISA plates (Maxisorp, Nunc, Denmark) were coated with 4 μ g/ml human β_2 GPI (Yamasa Co, Choshi, Japan) in phosphate buffered saline (PBS) or PBS alone and incubated overnight at 4°C. After blocking with 1% bovine serum albumin (BSA; Sigma), 0.1% Tween 20 (Sigma) in PBS (1%BSA-0.1%Tween-PBS), serum diluted 1:100 in 1%BSA-0.1%Tween-PBS was added in duplicate. After incubation and washes with PBS-0.1%Tween, alkaline phosphatase conjugated goat antihuman IgA was added in the appropriate dilution. Colour was developed by adding 100 μ l of 1 mg/ml of *p*-nitrophenylphosphate disodium in 1 M diethanolamine buffer (pH 9.8). The IgA a β_2 GPI titre of each sample was derived from the standard curve according to the dilutions of a positive IgA control which showed high IgA binding to β_2 GPI but low binding to a control well without antigen, suggesting

that IgA antibody was appropriately detected, and converted to units. The cut off point for the IgA a β_2 GPI assays was established as the mean + 5SD of 100 controls.

Lupus anticoagulant

Data for LA were those historically present in the patients' clinical records before starting anticoagulation treatment. LA was screened using activated partial thromboplastin time (aPTT) and dilute Russell's viper venom time (dRVVT), and confirmed according to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid dependent Antibodies.¹⁶

Statistical analysis

Statistical analysis was performed using the SPSS 7.5 program. Differences between medians were analysed by Mann-Whitney test. Categorical comparisons between patient groups were expressed as relative risk with its 95% confidence interval. All *p* values were determined by Fisher's exact test. A *p* value of <0.05 was considered significant.

RESULTS

Obstetric characteristics

Patients from the group with unexplained pregnancy morbidity had a significantly higher number of pregnancies than those with PAPS (median 4 (range 1–17) *v* median 3 (range 1–7), *p*=0.04). No differences in the number of pregnancies were found between the groups with PAPS and SLE (median 3 (range 1–7) *v* median 3 (range 1–15), *p*=0.9) and between the groups with unexplained pregnancy morbidity and SLE (median 4 (range 1–17) *v* median 3 (range 1–15), *p*=0.06). Patients from the group with unexplained pregnancy morbidity had a higher number of miscarriages (median 1.5 (range 0–10)) than patients from the groups with PAPS or SLE (median 0 (range 0–6) and median 0 (range 0–7), respectively) but the differences were not statistically significant. The numbers of fetal deaths and cases of prematurity did not differ between groups. Table 3 summarises all these data.

IgA aPL and association with other isotypes

Overall, IgA aCL were present in 32/84 (38%) patients with pregnancy morbidity. These antibodies were found in 12, 6, and 14 patients from the groups with PAPS, unexplained pregnancy morbidity and SLE, respectively. Although the prevalence of IgA aCL was higher in patients with PAPS (43%) and SLE (50%) than in the group with unexplained pregnancy

Table 3 Obstetric history of patients with PAPS, patients with unexplained pregnancy morbidity, and patients with SLE

	Pregnancies No (median (range))	Miscarriages No (median (range))	Fetal death No (median (range))	Prematurity No
PAPS	95 (3 [1–7])	30 (0 [0–6])	25 (1 [0–1])	7
Unexplained pregnancy morbidity	142 (4 [1–17])	55 (1.5 [0–10])	27 (1 [0–1])	1
SLE	111 (3 [1–15])	38 (0 [0–7])	24 (1 [0–1])	6

PAPS, primary antiphospholipid syndrome; SLE, systemic lupus erythematosus.

All numbers given are total number of events. Obstetric history was defined according to 1999 Sapporo criteria for APS.

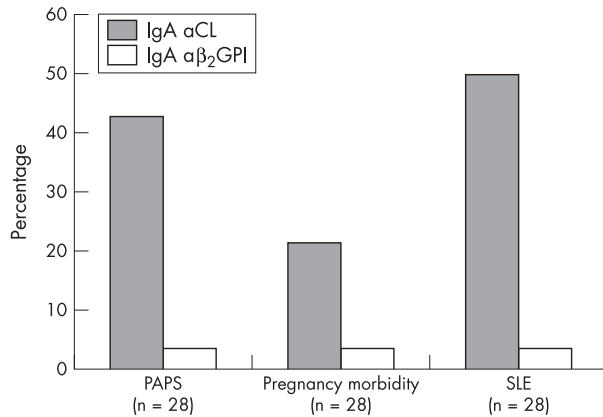


Figure 1 Prevalence of IgA aCL and IgA aβ₂GPI in patients from the groups with PAPS, unexplained pregnancy morbidity, and SLE.

morbidity (21%), the differences were not statistically significant. All 12 patients from the group with PAPS had IgA aCL together with IgG and/or IgM isotypes. Three of the six patients from the group with unexplained pregnancy morbidity who presented IgA aCL were found to be also positive for the IgG and/or IgM isotype. Two of 14 patients from the SLE group had IgA aCL as the sole aPL. Figure 1 shows the prevalence of IgA aCL and IgA aβ₂GPI.

IgA aβ₂GPI antibodies were present in one patient from each group. In all the cases, IgA aβ₂GPI were present together with IgG and/or IgM aβ₂GPI and aCL.

DISCUSSION

In this study we evaluated the prevalence and clinical significance of IgA aCL and IgA aβ₂GPI in a cohort of patients with pregnancy morbidity distributed in three groups of patients: patients with well defined PAPS *v* patients with unexplained pregnancy morbidity *v* patients with SLE. Overall, our data showed the presence of IgA aCL in 38% of the 84 patients with pregnancy morbidity. Some studies of the prevalence and clinical associations of IgA aCL have been carried out, but only a few focused on pregnancy morbidity. Gharavi *et al* reported the presence of IgA aCL in 21/40 patients with “aPL associated clinical complications”.¹⁷ Although their study was not intended primarily to analyse pregnancy morbidity because they studied not only patients with fetal loss but also with thrombosis and thrombocytopenia, these authors were the first to suggest that testing for IgA aCL might be useful to identify occasional patients with APS. Later, Kalunian *et al* studied 85 patients with SLE, suggesting that measurement of all isotypes of aCL, including IgA, should be performed in patients with SLE considering pregnancy, to identify those with a high risk of fetal loss.¹⁸

In our study IgA aCL were usually present together with either IgG and/or IgM isotypes except in three patients with unexplained pregnancy morbidity and two patients with SLE, raising the question as to whether IgA aCL might be important in the pathogenesis of pregnancy morbidity.

Traditionally, obstetric complications are thought to be due to placental and/or spiral artery thrombosis,¹⁹ a mechanism supported by some but not all biopsy findings.²⁰ Although aPL may induce thrombosis in several ways (that is, endothelial cell activation²¹), some experimental work suggests that IgA aCL are as prothrombotic as the IgG or IgM isotypes.²²

Although first reports failed to show an association between IgA aβ₂GPI and recurrent fetal loss⁶ or recurrent spontaneous abortions and unexplained fetal deaths,²³ subsequent studies showed that IgA aβ₂GPI were significantly raised in women with pregnancy morbidity,^{10,12} suggesting that testing for these antibodies may help in identifying additional women with APS who are not identified by traditional testing.

Yamada *et al* screened 36 patients with unexplained recurrent spontaneous abortion.¹² IgA aβ₂GPI levels were higher in these patients than in the healthy non-pregnant controls. They also found that the frequency of IgA aβ₂GPI was higher in patients with recurrent spontaneous abortion (13.9%) than in the controls (0%). Lee *et al* showed that IgA aβ₂GPI were more frequent in women with recurrent spontaneous abortion or fetal death than in fertile controls.¹⁰ In a recent study we evaluated the prevalence and clinical significance of IgA aCL, aβ₂GPI, and antiprothrombin antibodies as alternative additive risk factors for the well established IgG and/or IgM aCL and LA in a large cohort of patients with SLE.⁹ However, we failed to demonstrate an association between the presence of IgA aβ₂GPI and arterial/venous thrombosis or pregnancy loss in that cohort.

In this study IgA aβ₂GPI were present in three (4%) of the entire pregnancy morbidity group of 84 patients, with no differences in the distribution between groups (one patient from each group was found to be positive for IgA aβ₂GPI). Moreover, these antibodies were present together with IgG and/or IgM aβ₂GPI in all cases.

From our data we can conclude that the prevalence of IgA aCL is high in patients with pregnancy morbidity. Prospective, case-control studies may clarify the significance of IgA aCL and contribute to the better management of such patients. As the frequency of IgA aβ₂GPI in patients with pregnancy morbidity was low and these antibodies were usually present together with IgG and/or IgM isotypes, our data do not support routine testing for IgA aβ₂GPI as they are not useful in identifying additional women with APS.

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REFERENCES

- 1 Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC, *et al*. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999;42:1309–11.

- 2 **Matsuura E**, Igarashi Y, Yasuda T, Triplett DA, Koike T. Anticardiolipin antibodies recognize beta 2-glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J Exp Med* 1994;179:457–62.
- 3 **Oosting JD**, Derksen RH, Bobbink IW, Hackeng TM, Bouma BN, de Groot PG. Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: an explanation for their pathogenic mechanism? *Blood* 1993;81:2618–25.
- 4 **Beyers EM**, Galli M, Barbui T, Comfurius P, Zwaal RF. Lupus anticoagulant IgGs (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb Haemost* 1991;66:629–32.
- 5 **Galli M**. Non beta 2-glycoprotein I cofactors for antiphospholipid antibodies. *Lupus* 1996;5:388–92.
- 6 **Tsutsumi A**, Matsuura E, Ichikawa K, Fujisaku A, Mukai M, Koike T. IgA class anti-beta2-glycoprotein I in patients with systemic lupus erythematosus. *J Rheumatol* 1998;25:74–8.
- 7 **Fanopoulos D**, Teodorescu MR, Varga J, Teodorescu M. High frequency of abnormal levels of IgA anti-beta2-glycoprotein I antibodies in patients with systemic lupus erythematosus: relationship with antiphospholipid syndrome. *J Rheumatol* 1998;25:675–80.
- 8 **Selva-O'Callaghan A**, Ordi-Ros J, Monegal-Ferran F, Martinez N, Cortes-Hernandez F, Vilardell-Tarres M. IgA anticardiolipin antibodies—relation with other antiphospholipid antibodies and clinical significance. *Thromb Haemost* 1998;79:282–5.
- 9 **Bertolaccini ML**, Atsumi T, Escudero-Contreras A, Khamashta MA, Hughes GRV. The value of IgA antiphospholipid testing for the diagnosis of antiphospholipid (Hughes) syndrome in systemic lupus erythematosus. *J Rheumatol* 2001;28:2637–43.
- 10 **Lee RM**, Branch DW, Oshiro BT, Rittenhouse L, Orcutt A, Silver RM. IgA β 2 glycoprotein-I antibodies are elevated in women with unexplained recurrent spontaneous abortion and unexplained fetal death [abstract]. *J Autoimmun* 2000;15:A63.
- 11 **Lee RM**, Branch DW, Silver RM. Immunoglobulin A anti-beta2-glycoprotein antibodies in women who experience unexplained recurrent spontaneous abortion and unexplained fetal death. *Am J Obstet Gynecol* 2001;185:748–53.
- 12 **Yamada H**, Tsutsumi A, Ichikawa K, Kato EH, Koike T, Fujimoto S. IgA-class anti-beta2-glycoprotein I in women with unexplained recurrent spontaneous abortion. *Arthritis Rheum* 1999;42:2727–8.
- 13 **Tan EM**, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
- 14 **Harris EN**, Pierangeli S, Birch D. Anticardiolipin wet workshop report. Fifth International Symposium on antiphospholipid antibodies. *Am J Clin Pathol* 1994;101:616–24.
- 15 **Amengual O**, Atsumi T, Khamashta MA, Koike T, Hughes GRV. Specificity of ELISA for antibody to beta 2-glycoprotein I in patients with antiphospholipid syndrome. *Br J Rheumatol* 1996;35:1239–43.
- 16 **Brandt JT**, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb Haemost* 1995;74:1185–90.
- 17 **Gharavi AE**, Harris EN, Asherson RA, Hughes GR. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. *Ann Rheum Dis* 1987;46:1–6.
- 18 **Kalunian KC**, Peter JB, Middlekauff HR, Sayre J, Ando DG, Mangotich M, *et al*. Clinical significance of a single test for anti-cardiolipin antibodies in patients with systemic lupus erythematosus. *Am J Med* 1988;85:602–8.
- 19 **Greaves M**. Antiphospholipid antibodies and thrombosis. *Lancet* 1999;353:1348–53.
- 20 **Out HJ**, Kooijman CD, Bruinse HW, Derksen RH. Histopathological findings in placentae from patients with intra-uterine fetal death and anti-phospholipid antibodies. *Eur J Obstet Gynecol Reprod Biol* 1991;41:179–86.
- 21 **Del Papa N**, Guidali L, Sala A, Buccellati C, Khamashta MA, Ichikawa K, *et al*. Endothelial cells as target for antiphospholipid antibodies. Human polyclonal and monoclonal anti-beta 2-glycoprotein I antibodies react in vitro with endothelial cells through adherent beta 2-glycoprotein I and induce endothelial activation. *Arthritis Rheum* 1997;40:551–61.
- 22 **Pierangeli SS**, Liu XW, Barker JH, Anderson G, Harris EN. Induction of thrombosis in a mouse model by IgG, IgM and IgA immunoglobulins from patients with the antiphospholipid syndrome. *Thromb Haemost* 1995;74:1361–7.
- 23 **Lee RM**, Emlen W, Scott JR, Branch DW, Silver RM. Anti-beta2-glycoprotein I antibodies in women with recurrent spontaneous abortion, unexplained fetal death, and antiphospholipid syndrome. *Am J Obstet Gynecol* 1999;181:642–8.