Discordance between anti-β_2-glycoprotein-I and anti-cardiolipin antibodies in patients with clinical criteria of antiphospholipid syndrome

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ABSTRACT

Objectives. *Clinical and immunological features of patients with clinical manifestations of the antiphospholipid syndrome (APS) with anti-beta$_2$-glycoprotein-I antibodies (anti-β_2-GP-I) but without anticardiolipin antibodies (aCL) or any other autoimmune condition are not well documented. We sought to determine the clinical significance of positive anti-β_2-GP-I with negative aCL.*

Methods. *From July 2002 through July 2003, 1,179 serum samples obtained in our hospital from the Community of Madrid were tested for anti-β_2-GP-I and aCL by enzyme–linked immunosorbent assay. Clinical records of patients with discordant anti-β_2-GP-I and aCL were retrospectively analysed.*

Results. *A total of 56 patients with discordant anti-β_2-GP-I and aCL were identified. By logistic regression analysis, after adjusting for age, sex and risk factors of thrombosis, the risk for developing APS criteria associated with anti-β_2-GP-I was significant [odds ratio 3.88; 95% confidence interval (CI): 1.05–14.27; p = 0.04). 15 out of 56 patients (26.8%) had positive anti-β_2-GP-I and negative aCL. 5 out of 15 anti-β_2-GP-I-positive patients had clinical APS without serological nor clinical evidence of any autoimmune disease.*

Conclusion. *Determination of anti-β_2-GP-I should be considered in individual cases with clinical manifestations of primary APS and repeated negative results on conventional antiphospholipid antibody test.*

Introduction

Antiphospholipid antibodies (aPL) are a family of autoantibodies that exhibit a broad range of target specificities and affinities, all recognizing various combinations of phospholipids and phospholipid binding proteins or both (1). Accumulating evidence indicates that beta$_2$-glycoprotein-I (β_2-GP-I) is the major target antigen for aPL, which play a crucial role in the pathogenesis of the antiphospholipid syndrome (APS) (2-4). An association of anti-β_2-GP-I antibodies with the clinical manifestations of APS has been demonstrated in patients with systemic lupus erythematosus (SLE) (5-10). The same studies have identified a subgroup of the SLE population with clinical features of APS who were negative for anticardiolipin antibodies (aCL) but positive for anti-β_2-GP-I (8-10). A small number of anecdotal reports of patients with clinical APS but no aPL or any other autoimmune condition, and who had anti-β_2-GP-I have been published (11,12). It is not rare that clinicians may encounter patients who meet the clinical criteria of APS but who do not exhibit the autoantibodies listed in the new diagnostic criteria set of APS (13).

We describe here a group of patients with clinical criteria of APS who had anti-β_2-GP-I but no detectable serum aCL or lupus anticoagulant (LAC) in the absence of any other autoimmune condition.

Patients and methods

Between July 2002 and July 2003, 1,179 samples were tested for aPL in the Immunology Department at the General University Hospital Gregorio Marañón in Madrid, Spain. We examined retrospectively the clinical and immunological features of patients in whom simultaneous assays for anti-β_2-GP-I and aCL were discordant. A discordance between anti-β_2-GP-I and aCL (positive for anti-β_2-GP-I but not for aCL or *vice-versa*) was defined when such a result was present on 2 or more occasions. The patients were admitted to the hospital or followed at the outpatient clinic for a variety of medical reasons, with clinical suspicion of APS. Medical histories of the patients with discordant aPLwere carefully revised by the same immunologist for any clinical criteria of APS or other autoimmune diseases, concomitant infections and other conditions known to be associated with aPL. Other immunological parameters that were collected included antinuclear antibodies (ANA), anti-DNA antibodies (anti-DNA), anti-extractable nuclear antigen antibodies (anti-ENA), rheumatoid factor (RF), complement factors 3 and 4 (C3 and C4) and immunoglobulin IgG, IgAand IgM levels.

Clinical manifestations of APS were

considered according to the 1999 Sapporo revised criteria for APS (13). An underlying autoimmune disease was considered when the patients met specific criteria including: SLE, rheumatoid arthritis and systemic vasculitis classified according to the American College of Rheumatology (ACR) criteria (14-16). For those patients who did not fulfil criteria of a defined autoimmune disease, the presence of the following exclusion conditions were considered: malar rash, discoid rash, oral or pharyngeal ulceration (excluding nasal septum ulceration or perforation), arthritis, photosensitivity, pleuritis (in the absence of pulmonary embolism or left-sided heart failure), pericarditis in the absence of myocardial infarction or uraemia, persistent proteinuria greater than 0.5 g per day, biopsy-proven immune complex-related glomerulonephritis, lymphopenia less than 1,000/μl, anti-DNA, anti-ENA, ANA, rheumatoid factor and treatment with drugs known to induce aPL. Among the major clinical manifestations of APS, deep vein thrombosis (DVT) was confirmed by Doppler studies; peripheral arterial thrombosis by arteriography; stroke by computed tomography scan and pulmonary thromboembolism (PT) by ventilation/perfusion pulmonary scintigraphy.

Measurement of IgG and IgM anti-β_2-GP-I was performed by enzyme–linked immunosorbent assay (ELISA, Orgentec Diagnostika GmbH, Mainz, Germany). Wells of γ-irradiated polysterene microtitre plates were coated with highy purified β_2-GP-I. The best cut-off levels of anti-β_2-GP-I and aCLwere determined by ROC curves in 100 consecutive patients with thrombosis and suspected antiphospholipid syndrome that were studied for aCL and anti-β_2-GP-I by ELISA. Positive results [> 8 U/ml for IgG and IgM, respectively] were reported. IgG and IgM aCL were measured by ELISAon microtitre plates (Orgentec Diagnostika GmbH, Mainz, Germany). Plates were coated with highly purified cardiolipin and saturated with human β_2-GP-I, which provide results independent of endogeneous β_2-GP-I. The results for IgG and IgM aCL were reported as negative [IgG < 10 (GPL units) and IgM < 7 (MPLunits)], low-positive [IgG, 10-20 (GPL units) and IgM, 7-20 (MPL units)], moderate [IgG, 20-80 (GPL units) and IgM, 20-60 (MPL units)] and high [IgG > 80 (GPL units) and IgM > 60 (MPL units)].

LAC was performed by coagulimetric tests in the Haematology Department. Briefly, coagulation studies included thrombin time, prothrombin time, activated partial thromboplastin time (APTT) and diluted Russell viper venom time (dRVVT) were measured in every sample according to established methods previously described (17). The APTT was carried out using a reagent highly sensitive to LA (IZASA Lab, Barcelona, Spain). A lyophilized fraction of Russell viper venom®, in cephalin, containing factor X activating enzyme (American Diagnostics, USA) was used for the dRVVTtest. The inhibitory effect on both APTTand dRVVT was assessed performing mixing studies on 1:1 mixtures with normal plasma. Rosner's APTTindex and dRVVT-index were calculated in order to assign the inhibitory status of the samples.(18) LA confirmatory tests were carried out in all samples, and *also on 1:1* mixtures with normal plasma.

Statistical analysis was performed using Fisher exact test and a Student t test as indicated. The p values were determined by chi-squared tests. A logistic regression analysis was used to study associations between the prevalence of anti-β_2-GP-I, aCL and the presence of clinical criteria of APS. Statistical computations were performed using the Statistical Package for the Social Sciences (SPSS).

Results

A total of 56 patients with discordant anti-β_2-GP-I and aCL were identified. 15 patients (26.8%) had positive anti-β_2-GP-I and negative aCL (anti-β_2-GP-I-group) and 41 (73.2%) patients had negative anti-β_2-GP-I and positive aCL (aCL-group). Clinical characteristics of the patients are shown in Table I. The IgG isotype of anti-β_2-GP-I was detected in 8 (53.3%, mean titres 15 U/ml), IgM anti-β_2-GP-I in 6 (40%, mean titres 15 U/ml), and IgG plus IgM anti-β_2-GP-I in 1 (6.7%, IgG=15 U/ml, IgM =14 U/ml) patients of the anti-β_2-GP-I-group. The IgG isotype of aCLwas de-

Table I. Demographic and clinical data of patients with discordant anti-2-GP-I and aCL antibodies.

No. of subjects (%)	Anti-β_2-GP-I group (N = 15)	Anti-CL group (N = 41)	p
Age, mean ± SD	2 (13.3)	50± 14.7	0.95
Gender			
Male	49±16.5	15 (36.6)	0.085
Female	13 (86.7)	26 (63.4)	0.085
Risk factors of thrombosis	4 (26.7)	10 (24.4)	0.558
Clinical APS criteria	11 (73.3)	17 (41.5)	0.034
History of thrombosis	7 (46.7)	14 (34.1)	0.29
Stroke	1 (6.7)	2 (4.9)	0.615
DVT, PT	4 (26.7)	6 (14.6)	0.431
RT	2 (13.3)	6 (14.6)	0.637
History of miscarriages	4 (26.7)	3 (7.3)	0.123
Thrombocytopaenia	2 (13.3)	6 (14.6)	0.637
Underlying disease	4 (26.6)	20 (48.7)	0.223
SLE	0 (0)	8 (19.5)	0.09
Other autoimmune diseases	3 (20)	4 (9.8)	0.056
Malignancies	1 (6.7)	2 (4.9)	0.615
Chronic infection	0 (0)	4 (9.8)	0.56
Transplantation	0 (0)	2 (4.9)	1

DVT: deep vein thrombosis; PT: pulmonary thromboembolism; RT: retinal thrombosis; SD: standard deviation.

Table II. Immunological data of patients with discordant anti-2-GP-I and aCLantibodies

No. of subjects (%)	anti-β_2-GP-I-group (N = 15)	aCL-group (N = 41)	p
Positive ANA	4 (26.7)	16 (39)	0.299
Positive anti-DNA	2 (13.3)	7 (17.1)	0.547
Positive RF	2 (13.3)	2 (4.9)	0.289
C3 hypocomplementemia	3 (20)	4 (9.8)	0.273
C4 hypocomplementemia	5 (33.3)	4 (9.8)	0.048
Positive LAC	5 (33.3)	4 (9.8)	0.048

tected in 20 (48.8%, mean titres 18 GPL units), IgM aCL in 14 (34.1%, mean titres 13 MPL units), IgG plus IgM aCL in 7 (17.1%, mean titres IgG 17 GPL, IgM 15 MPL) patients of the aCL-group. There was not a statistical predominance of any anti-β_2-GP-I isotype (Table I). 9 patients of the aCL-group (21.9%) had moderate titers of aCL. When dividing patients according to age, younger and older than 50 years, into 2 groups, there where no significant differences regarding prevalences of thrombotic events and aPL were observed between both groups (data not shown).

A statistically significant higher frequency of patients with clinical APS developed in the anti-β_2-GP-I-group compared to the aCL-group ($p < 0.05$) (Table I). By logistic regression analysis, after adjusting for age, sex and risk factors of thrombosis, the risk for developing APS criteria associated with anti-β_2-GP-I antibodies was significant. When we compared anti-β_2-GP-I and aCL groups, the odds ratio (OR) was 3.88 (95% confidence interval: 1.05-14.27; $p = 0.04$) for anti-β_2-GP-I positivity. Table II represents the distribution of immunological abnormalities in patients according to the presence of anti-β_2-GP-I and aCLantibodies. When compared anti-β_2-GP-I-group versus aCL-group, the only single immunological abnormalities significantly increased in the anti-β_2-GP-I-group, were the presence of C4 hypocomplementemia ($n = 5$) and of positive LAC ($n = 4$).

Five out of 15 (33.3%) patients in the anti-β_2-GP-I-group had clinical APS without serological nor clinical evidence of any autoimmune disease. Serologic exclusion criteria for clinical primary APS in this subgroup of patients included positive anti-DNA ($n = 2$), ANA more than 1:320 ($n = 1$) and rheumatoid factor ($n = 1$). Clinical exclusion criteria included, autoimmune thyroiditis ($n = 1$) and photosensitivity ($n = 1$). Clinical details of the patients are shown in Table III, none had a family history of clotting disorders. All patients tested had normal levels of protein C, protein S and antithrombin III, as well as repeatedly normal PT and APTT. 4 patients had the IgG isotype of anti-β_2-GP-I (mean titres 16 U/ml) and the other patient IgM anti-β_2-GP-I (19 U/ml). During a mean follow-up of 31.2 months the patients had repeated determinations of aCL and LAC for at least 3 determinations separated in time, all which have been negative. None of the patients with clinical APS and anti-β_2-GP-I antibodies had malignancies or other clinical conditions. When we compared anti-β_2-GP-I-subgroup with aCL-positive patients without serological, no clinical evidence of any autoimmune disease and no significant differences were demonstrated in the prevalence of overall demographic and clinical parameters. However, use of prophylactic aspirin or oral anticoagulants were given to only 1 anti-β_2-GP-I-patient (20%) in comparison with 8 out of 9 aCL-patients (88.8%, $p < 0.05$). The only abnormal immunological test observed in the subgroup of anti-β_2-GP-I-patients was the findings of IgM hypergammaglobulinemia in 1 patient.

Discussion

We have identified a small group of patients that developed clinical manifestations of primary APS during evolution, who had repeatedly positive anti-β_2-GP-I, but with negative aCL by conventional assays. It is now widely accepted that β_2-GP-I is an absolute requirement for the binding of aPLAbs purified from patients with autoimmune disease when assayed using anionic phospholipid ELISAs. β_2-GP-I is known to bind to negatively charged surfaces as well as to activated platelets and to act as an inhibitor of the intrinsic blood coagulation pathway in vitro. Although there has been considerable controversy as to the exact nature of the antigenic epitope to which aPL Abs bind, it has become clear that β_2-GP-I

Table III. Selected characteristics of patients with clinical APS and anti-β_2-GP-I antibodiesAand no serological nor clinical evidence of any autoimmune disease.

Patient no.	Age (yr)	Gender	Thrombotic events	F-up (ms)	Other clinical features	Other immunological abnormalities	Prophylaxis for thrombosis[B]
1	54	M	DVTs	48	None	None	None
2	50	F	RTs	12	LR, Tr	IgM	None
3	68	F	DVTs, PT	24	None	None	None
4	58	F	DVTs, PT, BO	60	None	None	Acenocoumarol
5	72	F	DVTs	12	None	None	None

A: without aCL; B: at the time of study; BO: femoral-popliteal bypass obstruction; DVTs: deep vein thrombosis; F-up: follow-up from the first event of APS (months); IgM: immunoglobulin M high levels; LR: livedo reticularis; PT: pulmonary thromboembolism; RTs: retinal thrombosis; Tr: thrombocytopenia.

is the most common and best-characterized antigenic target. In fact, the binding of β_2-GP-I to aPL induces a conformational change in β_2-GP-I, thus exposing a cryptic epitope on β_2-GP-I for the autoantibodies to bind (18). The discordance between anti-β_2-GP-I and aCL observed in these patients likely reflects the underlying heterogeneity of aPL (1). However, given the low titres of anti-β_2-GP-I, it may be reflecting the recognition of different epitopes when the β_2-GP-I is isolated than when is binded to cardiolipin (19).

A study by Cabral *et al*. on 6 patients with recurrent arterial and/or venous thrombosis, reported that none of them had aPL but all had serum anti-β_2-GP-I referring to anti-cofactor syndrome (11). On the contrary, Amengual *et al*. (20) found that all patients with SLE (with or without APS) or primary APS with anti-β_2-GP-I, were also positive for aCL. The cases presented here further support the concept that in individual cases, anti-β_2-GP-I autoantibodies might provide additional information to β_2-GP-I-dependent aCL ELISA tests. In this regard, it has been reported that anti-β_2-GP-I antibodies are a predictor of arterial thrombosis in patients with APS (21). Recent efforts to better characterize the specificity of aPL have resulted in the identification of some anti-β_2-GP-I-related peptides associated with specific clinical manifestations of the disease (22). However, there is still controversy regarding issues such as the utility of detecting antibodies to β_2-GP-I or phospholipids other than cardiolipin. These autoantibodies are not listed in the new diagnostic criteria set because their usefulness needs to be validated and their detection standardised. Nevertheless, we suggest that detection of anti-β_2-GP-I may be helpful as part of the laboratory evaluation for APS of individual cases when the APS syndrome is strongly suspected despite negative aCLor LAC.

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