Antiphospholipid Antibodies: A Risk Factor for Occlusive Retinal Vascular Disorders. Comparison with Ocular Inflammatory Diseases

JAVIER CARBONE, SILVIA SÁNCHEZ-RAMÓN, ROSARIO COBO-SORIANO, ELENA SEOANE, MARÍA JESÚS APARICIO, JOSÉ LUIS RUIZ-TÍSCAR, MARGARITA RODRÍGUEZ-MAHOU, JUAN JOSÉ RODRÍGUEZ-MOLINA, ELIZABETH SARMIENTO, CARLOS CORTÉS, and EDUARDO FERNÁNDEZ-CRUZ

ABSTRACT. ***Objective.*** To evaluate the prevalence of antiphospholipid antibodies (aPL) together with immunological characteristics of patients with occlusive retinal vascular disorders (ORVD) with and without risk factors (systemic arterial hypertension, diabetes mellitus, hyperlipidemia, and embolizing cardiac disease) for retinal occlusions compared to patients with ocular inflammatory diseases (OID) and healthy controls.

Methods. Sixty-eight patients with ORVD, 45 patients with OID, and 49 healthy persons were prospectively studied. Serologic studies included determination of anticardiolipin antibodies, lupus anticoagulant, antinuclear antibodies (ANA), levels of complement 4 and 3, total hemolytic complement (CH100), and circulating immune complexes (CIC).

Results. Elevated levels of aPL were detected in 16 (24%) patients with ORVD compared to 4 (9%) patients with OID (OR 3.15, $p < 0.05$) and 4 (8%) controls (OR 3.46, $p < 0.05$). No significant differences were seen in the prevalence of aPL comparing risk factor-positive patients with ORVD (8 of 33, 24%) to risk factor-free patients with ORVD (8 of 35, 23%). A higher frequency of positive ANA, elevated IgA, and increased CIC were detected in aPL positive patients with ORVD compared to patients with OID.

Conclusion. Detection of aPL in patients with ORVD may help determine which patients are eligible for prophylactic treatment. An immunologic profile characterized by high prevalence of ANA, CIC, and elevated IgA distinguishes ORVD patients with aPL from inflammatory ophthalmologic disorders. (J Rheumatol 2001;28:2437–41)

Key Indexing Terms:
ANTIPHOSPHOLIPID ANTIBODIES
UVEITIS
RETINAL OCCLUSION
AUTOIMMUNITY

Antiphospholipid antibodies (aPL) have been measured in occlusive retinal vascular disorders (ORVD)[1-8]. However, aPL retinopathy remains poorly defined clinically. It is necessary to establish the proper diagnosis of ORVD to predict both ocular and systemic consequences of the disease.

We designed a prospective immunological followup study of patients with ORVD, to investigate aPL as well as other immunological abnormalities. We wished to determine the true prevalence of aPL and to outline the immunological profile, if any, of aPL positive patients with ORVD. To our knowledge no previous prospective study has compared the prevalence of aPL and immunological alterations in ORVD with a cohort of patients with ocular inflammatory diseases (OID). As secondary vasculitis may coexist with aPL related disorders[9], we added the latter group to compensate for the possibility of inflammatory conditions inducing production of aPL[10]. The prevalence of aPL in patients without defined risk factors for thrombosis has been reported[6,8]. The present study includes patients with and without defined risk factors for retinal occlusions to more accurately establish the prevalence of aPL in patients with ORVD.

MATERIALS AND METHODS

We studied 68 patients with ORVD, 45 patients with OID (disease control group), and 49 healthy subjects (controls). Among the ORVD patients, 62 presented with retinal occlusions [46 cases (74%) were venous occlusions, 14 (23%) arterial occlusions, 2 (3%) combined arterial and venous occlusions] and 6 with anterior ischemic optic neuropathy. Seven ORVD patients presented with associated retinal vasculitis (5 venous occlusions, one patient with arterial occlusion, and another patient with ischemic optic neuropathy). Retinal vasculitis was defined as an inflammation of the retinal vessels affecting both the veins and capillary vessels. The diagnosis was established by ophthalmoscopic examination showing vascular sheathing, and by fluorescein angioscopy, where the coloring agent was seen to leak from the vessels. ORVD patients with concomitant vasculitis

From the Departments of Immunology and Ophthalmology, Hospital General Universitario Gregorio Marañón, Madrid, Spain.

J. Carbone, MD; S. Sánchez-Ramón, MD; E. Seoane, MD; J.L. Ruiz-Tíscar, MD; M. Rodríguez-Mahou, PhD; J.J. Rodríguez-Molina, PhD; E. Sarmiento, MD; E. Fernández-Cruz, MD, PhD, Department of Immunology; R. Cobo-Soriano, MD, PhD; M.J. Aparicio, MD; C. Cortes, MD, PhD, Department of Ophthalmology.

Address reprint requests to Dr. J. Carbone, Department of Immunology, University General Hospital Gregorio Marañón, Doctor Esquerdo 46, 28007 Madrid, Spain. E-mail: carbone@teleline.es

Submitted October 24, 2000; revision accepted June 8, 2001.

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were prone to have chronic vascular sheathing. Central vein occlusion (4 cases) and major branch vein occlusion (one case) together with peripheral eye vasculitis were associated with venous occlusion. Branch retinal artery occlusion in the eye with associated arterial occlusion was observed.

We included 33 ORVD patients with risk factors for vessel occlusion (mean age 58 ± 12 yrs). Risk factors were systemic arterial hypertension (n = 7), diabetes mellitus (n = 2), hyperlipidemia (n = 12), embolizing cardiac disease (n = 1), and a combination of more than one of the above (n = 11). We also included 35 consecutive outpatients with ORVD without risk factors (mean age 54 ± 12 yrs). Four of the total of 68 patients with ORVD reported a history of arterial or venous thrombosis: 2 intracranial vessel thrombosis and 2 deep vein thrombosis. Other symptoms classically associated with aPL were reported: livedo reticularis, n = 4 (11%); unexplained repeated abortions, 3 (9%); and thrombocytopenia, 4 (11%), in the 35 risk factor-free patients with ORVD. In risk factor-positive patients with ORVD (n = 33) we observed: repeated abortions, n = 2 (6%) and thrombocytopenia, n = 6 (18%).

The OID group (n = 45) comprised: 24 patients diagnosed with uveitis (anterior uveitis, n = 9; intermediate uveitis, 2; posterior uveitis, 8; birdshot retinochoroidopathy, 2; Vogt-Koyanagui-Harada syndrome, 2; and panuveitis, 1), 16 with retinal vasculitis, 2 optic neuritis, 2 episcleritis, and one with pemphigoid. Retinal vasculitis due to secondary causes such as infectious diseases or systemic disease as well as concomitant ocular disease was ruled out in all patients with vasculitis, all being considered to have primary retinal vasculitis. Patients with primary retinal vasculitis tended to have peripheral vascular sheathing, macular edema, and diffuse capillary leakage with adjacent hemorrhage in the absence of ischemia. Healthy controls were randomly selected volunteers with no history of ophthalmopathy or other disease that could explain the presence of aPL or other immunological abnormalities.

We performed a prospective case-controlled followup study during 3.5 years (May 1996 – October 1999). Patients were referred to the Clinical Immunology Unit for immunological evaluation and followup from the Retina Unit as well as other outpatient clinics from our hospital in Madrid. During the followup study each patient was scheduled for visits every 3–6 months, where laboratory tests, clinical examination, and completion of a questionnaire were carried out. Patients voluntarily entered the study at different times. As a result the length of followup differs among patients. The mean followup time was 26 ± 8 months (range 8–42). All patients and controls were carefully investigated for history of connective tissue disease or thrombophilia, including venous and arterial thrombotic events, abortions, or the presence of any clinical symptoms such as joint pain or swelling, photosensitive rash, etc., and for the presence of risk factors for thrombosis.

Exclusion criteria included the presence of infectious diseases, the use of drugs known to be associated with aPL, and diagnosis of known connective tissue diseases at the time of enrollment. For the disease control group, patients with systemic connective tissue diseases or with immunological ocular diseases known to be associated with thrombosis, such as Behçet's disease, were excluded.

Quantitation of aPL. IgG and IgM isotypes of aCL were detected using a commercial standardized ELISA (Fresenius, Gull Diagnostic, Bad Homburg, Germany). The results were reported as negative (IgG < 5 GPL units and IgM < 5 MPL units), low positive (IgG 5–15 GPL units, IgM 5–15 MPL units), moderate (IgG 15–60 GPL units, IgM 15–60 MPL units), and high (IgG > 60 GPL units, IgM > 60 MPL units). Rapid plasma reagin test (RPR) was performed by a standard procedure. A fluorescent treponemal antibody confirmatory test was performed on all patients who had positive RPR results. Activated partial thromboplastin time (aPTT), tissue thromboplastin inhibition (Izaza Laboratory, Barcelona, Spain), and a simplified dilute Russell's viper venom time (DRVVT; American Diagnostics, Greenwich, CT, USA) were measured for each patient. Correction of aPTT by mixture with control plasma at 50% dilution, platelet neutralization test (Boehringer, Mannheim, Germany), and confirmatory DRVVT were performed to confirm the presence of lupus anticoagulant (LAC). aPL prevalence data include those obtained through the followup studies.

Other immunological tests. Serum immunoglobulin levels (IgG, IgA, IgM), complement factors C3, C4, and Factor B were measured by nephelometry (Beckman, San Jose, CA, USA), rheumatoid factor (RF) and C-reactive protein (CRP) by nephelometry (Behring, Marburg, Germany). Circulating immune complexes (CIC) were measured by a C1q dependent nephelometric assay (Nephelometer Analyser II, Behring). Classical pathway evaluation (CH100) was measured by radial immunodiffusion (Sanofi-Pasteur, Paris, France). Antinuclear antibodies (ANA) were detected by indirect immunofluorescence (IIF) on rat liver, stomach, and kidney sections and human laryngeal epithelial carcinoma (HEp-2) substrate (Bios, Munich, Germany).

Antibodies to double stranded DNA (anti-dsDNA) were detected by ELISA. Antiextractable nuclear antibodies (ENA) including anti-Sm, anti-Ro, and anti-La were detected by IIF and ELISA. Serum samples were also screened by IIF on neutrophil preparations to detect neutrophil cytoplasmic antibodies (ANCA). The reported data of autoimmune related abnormalities were those encountered at enrollment and subsequently confirmed in at least 2 other determinations.

Statistical analysis was performed using Fisher's exact test and Student t test as indicated. Logistic regression analysis was used to study associations between the prevalence of aPL and occlusive retinal vascular events. Computations were performed using SPSS.

RESULTS

In the ORVD group, aPL were detected in 16 of 68 (24%) patients in a significantly higher proportion than in either the OID group (4 of 45, 9%; $p < 0.05$) or healthy controls (4 of 49, 8%; $p < 0.05$). Four out of 16 (25%) patients with ORVD tested negative for aPL at enrollment but changed to positive later in the study. From the logistic regression analysis, the risk associated with aPL was significant ($p < 0.05$). Comparing the ORVD and OID groups, the odds ratio was 3.15 ($p < 0.05$) for aPL positivity. A statistically significant increased risk ($p < 0.05$) was found when comparing ORVD patients and healthy controls (OR 3.46). The distribution of the different aPL was as follows: the IgG isotype of aCL was detected in 10 (62%), IgM aCL in one (6%), IgG plus IgM aCL in 2 (13%), LAC (negative for aCL) in 2 (13%), and IgG plus IgM aCL plus LAC in one (6%) patient with ORVD. Eleven out of 16 (69%) patients with positive aCL had moderate to high levels of aCL. The vasoocclusive retinopathy observed in patients with only aCL did not differ between patients with aCL plus LAC and patients positive for LAC only. A false positive RPR was detected in only one patient who was also positive for the LAC test. Interestingly, no significant differences were noted in the prevalence of aPL between the group of ORVD patients with the defined risk factors for thrombosis (8 of 33, 24%) and patients without risk factors (8 of 35, 23%). Six of 16 (38%) aPL positive ORVD patients had a history of aPL related manifestations.

Distribution of aPL in the 4 OID aPL positive patients showed: 3 IgG aCL (in one patient with optic neuritis, another with intermediate uveitis, and one with posterior uveitis) and one IgM aCL in a patient with retinal vasculitis. aCL showed in the 4 healthy controls: 3 of the IgM class and one IgG aCL.

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Table 1. Distribution of immunological abnormalities in patients with ORVD, OID, and healthy controls.

	ORVD				
	aPL Positive, n = 16	aPL Negative, n = 52	OID, n = 45	Controls n = 49	p
ANA positive	9 (56)	14 (27)	8 (18)	1 (2)	0.03[a], 0.007[b], 0.0011[c]
Anti-dsDNA	2 (13)	0	1 (2)	0	NS[abc]
ENA	2 (13)	1 (2)	0	0	NS[abc]
C3 low	3 (19)	5 (10)	6 (13)	5 (10)	NS[abc]
Elevated CIC	12 (75)	17 (32)	19 (42)	14 (29)	0.004[a], 0.027[b], 0.003[c]
C4 low	8 (50)	13 (25)	12 (27)	8 (16)	0.05[a], 0.08[b], 0.01[c]
CH100 low	7 (44)	14 (26)	13 (29)	13 (27)	NS[abc]
Elevated IgA	4 (25)	12 (23)	3 (7)	5 (10)	0.49[a], 0.03[b], 0.20[c]

Data in parentheses are percentages. [a] aPL positive patients with ORVD vs aPL negative patients. [b] aPL positive patients with ORVD vs patients with OID. [c] aPL positive patients with ORVD vs healthy controls. CIC: circulating immune complexes. ENA: antiextractable nuclear antigens.

Table 1 gives the distribution of immunological abnormalities in patients with ORVD (according to the presence of aPL), OID, and healthy controls. ANA at titers ranging from 1:40 to 1:640 were found in 23 out of 68 (34%) patients with ORVD. Twenty-nine (43%) patients with ORVD had elevated CIC. Twenty-one (31%) patients with ORVD had low levels of CH100. Low levels of C4 (29%) and C3 (12%) were found in ORVD patients.

Comparing ORVD versus OID patients and healthy controls, the ANA test was the only single abnormal autoimmune test significantly increased in patients with ORVD ($p < 0.05$). The pattern of immunological abnormalities present in aPL positive patients with ORVD compared to aPL negative patients, OID patients, and healthy controls (Table 1) showed that the only abnormal immunological test significantly increased in these patients was the presence of positive ANA test together with increased CIC. aPL positive patients with ORVD showed a higher frequency of polyclonal IgA hypergammaglobulinemia than patients with OID. aPL positive patients with ORVD showed other immunological abnormalities — anti-dsDNA 13%, ENA 13%, ANCA 6%, RF (13%); low levels of C3 (19%), C4 (50%) and CH100 (44%); and elevated levels of CRP (19%), erythrocyte sedimentation rate (ESR, 6%), IgM (6%), and IgG (6%) — although these were not statistically significant compared to aPL negative patients with ORVD and control groups. However, the prevalences of anti-dsDNA, ENA, and low levels of C4 and CH100 were higher in aPL positive patients with ORVD. When we compared aPL positive ORVD patients with the group of idiopathic retinal vasculitis alone (n = 16) (data not shown), the only abnormal immunological tests significantly increased in terms of frequency were the positive ANA test ($p < 0.005$), increased CIC ($p < 0.05$), and elevated IgA ($p < 0.05$).

No significant differences were seen in the prevalence of overall immunological alterations between the group of ORVD patients with risk factors for thrombosis and ORVD patients without risk factors (Table 2). Sixty-three percent of the aCL positive patients with no risk factors for thrombosis were less than 50 years old (mean age 51 yrs, range 31–75). We found that splitting the patients with ORVD into 2 groups according to age (younger and older than 50 years) revealed no significant differences between the groups regarding prevalence of aPL or other immunologic abnormalities.

Four of the ORVD patients with coincidental vasculitis had aPL, one patient had positive ANCA test, and the other 2 patients showed positive ANA and ENA antibodies during followup.

During followup, 2 of 16 (13%) aPL positive patients with ORVD subsequently developed features of lupus-like disease. No patient evolved to full blown lupus during the followup. In addition, 2 of 16 patients with aPL (13%) developed further episodes of arterial intracranial thrombosis.

No patient with OID and autoimmune related abnormalities evolved to a systemic connective tissue disease. No healthy person with aCL developed aPL related symptoms.

Table 2. Distribution of immunological abnormalities in patients with ORVD according to presence of risk factors for thrombosis.

	ORVD		
	With Risk Factors, n = 33	Without Risk Factors, n = 35	p
aPL positive	8 (24)	8 (23)	0.55
ANA positive	10 (30)	13 (37)	0.61
Elevated CIC	12 (36)	17 (49)	0.33
C4 low	12 (36)	9 (26)	0.43
C3 low	3 (9)	5 (14)	0.71
CH100 low	9 (27)	12 (34)	0.60

Data in parentheses are percentages.

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DISCUSSION

The ophthalmologic vasoocclusive phenomena associated with aPL include anterior ischemic optic neuropathy, branch and central artery occlusions, cilioretinal artery occlusions, amaurosis fugax, and vein occlusions[1-8]. We found a higher prevalence of aPL positive patients than reported in other studies among patients with ORVD[1-7]. In our study it was important to retest patients for aPL, as 25% of the aPL positive patients had evolved from a negative test for aPL at the beginning of the study. While a previous study[8] found a high prevalence of aPL in patients with retinal occlusions without risk factors for developing thrombosis, in the current study we observed that in patients with conditions where thrombotic tendency was a feature (systemic arterial hypertension, diabetes mellitus, hyperlipidemia, and embolizing cardiac disease), aPL of all isotypes were present in a prevalence similar to those encountered in patients without those risk factors.

Although the association between aPL and ORVD in our patients does not necessarily imply a cause–effect relation, it is unlikely that aPL simply represent an epiphenomenon. An increased incidence of aPL in diabetic patients compared with control subjects has recently been described[11]. It has been suggested that aCL might be involved in the vascular complications of type 1 diabetes mellitus[12]. Several studies indicate that aPL may also play a role in the development of atherosclerosis by targeting some of the sequential steps that constitute early atherogenesis[13]. Antibodies to oxidized low density lipoproteins seem to be involved in the inflammation of the vessel wall in atherosclerosis and in vasculitis phenomena[14]. Further, aCL have been identified as independent risk factors for first ischemic stroke and for myocardial infarction[15,16].

Patients with retinal occlusions with identifiable thrombogenic risk factors and positive aPL might have a higher risk of developing occlusive vascular disease, as they have an additional thrombophilic factor known to induce intravascular clotting. In this regard, it has been suggested that, once confirmed by large scale clinical trials, the proatherogenic properties of aPL may merit screening and intervention programs in selected populations[13].

aPL positive patients with ORVD exhibited a high frequency of ANA, low C4 levels, low CH100 activity, elevated IgA, and increased circulating immune complexes. In a prospective study in women with idiopathic pregnancy loss and antiphospholipid syndrome we found a similar immunologic profile. We suggested that those patients could define a subset prone to evolve to another systemic autoimmune disease such as systemic lupus erythematosus (SLE)[17]. Up to 30–40% of patients with aPL and stroke may have a "lupus-like" syndrome with positive ANA or increased ESR[18]. We believe these findings suggest that some immunological features — namely positive ANA and increased CIC — in aPL positive patients with ORVD distinguish those individuals from immunologic ocular diseases. These patients should be carefully monitored for development of connective tissue diseases.

Further research with larger numbers of cases is needed to confirm the clinical usefulness of these nonspecific humoral factors. Patients with aPL who subsequently may develop SLE may thus be identified, and the pathogenic mechanisms that may participate in the vasoocclusive retinopathy associated with aPL may be clarified.

ACKNOWLEDGMENT

We thank Dr. M. Rabadán, Dr. A. Rodríguez, and Dr. C. Pascual from the Haematology Service for performing the LAC tests. We also acknowledge Dr. Gregorio Garrido (Department of Preventive Medicine) for helpful discussions, and Dr. Mónica Moreno for her contributions to the English translation.

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