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IgA antibodies against β2 glycoprotein I in hemodialysis patients are an independent risk factor for mortality

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Cardiovascular complications are the most important cause of death in patients on dialysis with end-stage renal disease. Antibodies reacting with β-glycoprotein I seem to play a pathogenic role in antiphospholipid syndrome and stroke and are involved in the origin of atherosclerosis. Here we evaluated the presence of anticardiolipin and anti-β-glycoprotein I antibodies together with other vascular risk factors and their relationship with mortality and cardiovascular morbidity in a cohort of 124 hemodialysis patients prospectively followed for 2 years. Of these, 41 patients were significantly positive for IgA anti-β-glycoprotein I, and the remaining had normal values. At 24 months, overall and cardiovascular mortality and thrombotic events were all significantly higher in patients with high anti-β-glycoprotein I antibodies. Multivariate analysis using Cox regression modeling found that age, hypoalbuminemia, use of dialysis catheters, and IgA β-glycoprotein I antibodies were independent risk factors for death. Thus, IgA antibodies to β-glycoprotein I are detrimental to the clinical outcome of hemodialysis patients.

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KEYWORDS: apolipoprotein H; autoantibodies; autoimmunity; hemodialysis; mortality; risk factor

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Cardiovascular disease in chronic kidney disease–treated patients has a higher prevalence than in the general population of the same age, and it is especially high in dialyzed patients.[1,2] Endothelial dysfunction is common in end-stage renal disease and may contribute to the development of both hypertension and atherosclerosis.[3] Cardiovascular complications rank first among the causes of death in patients with chronic kidney disease and end-stage renal disease on dialysis and include thrombotic episodes in cerebral and myocardial vessels.[4]

Antiphospholipid syndrome (APS) is an autoantibody-mediated disease in which antiphospholipid antibodies are directed against various combinations of phospholipids, phospholipid-binding proteins, or both.[5] The most commonly detected subgroups of antiphospholipid antibodies are lupus anticoagulant antibodies, anticardiolipin antibodies (aCL), and anti-β2 glycoprotein I antibodies (aB2GPI).[6] These antibodies bind to antigens localized on the membranes of cells involved in the coagulation cascade: platelets, monocytes, and endothelial cells.[7] Such interaction and interference with coagulation factors might be responsible for the hypercoagulability observed in APS.[8]

Several studies have demonstrated that antiphospholipid antibodies are more frequent in patients with chronic kidney disease than in the general population. However, its origin is unclear and the precise mechanisms involved in conferring possible cardiovascular risk to it are unknown.[9]

Although most of the aCL in the APS syndrome bind to the phospholipid-β2-glycoprotein I complex (B2GPI) as cofactor,[10] antibodies directed individually against B2GPI[11] have been described.

B2GPI, also known as apolipoprotein H, is a 50-kDa cationic plasma protein that presents a high affinity for anionic phospholipids. Its plasma concentration is 200 μg/ml, 40% of which are associated with lipids.[12] B2GPI has anticoagulant properties, modulates adenosine diphosphate-dependent activation of platelets,[13] and inhibits factor XII activation[14] and prothrombinase and tenase activity on platelets.[15] It has been demonstrated that aB2GPI antibodies

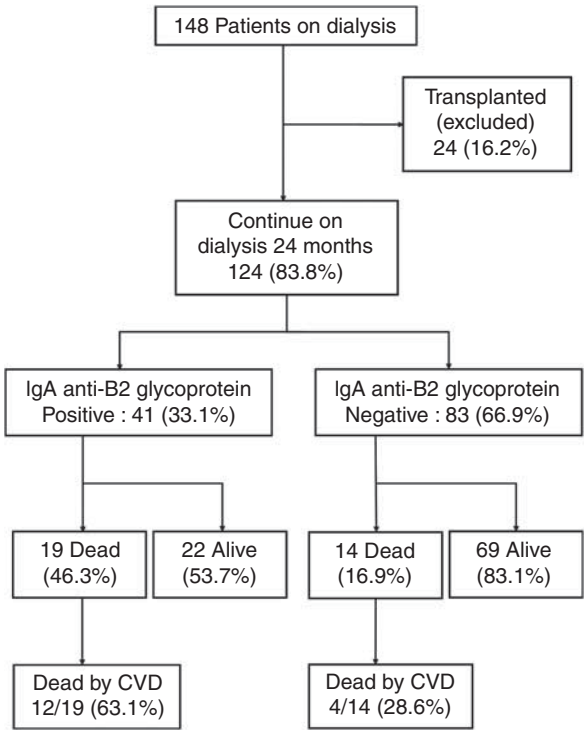

Figure 1 | Algorithm of disposition and outcomes. CVD, cardiovascular disease; IgA, immunoglobulin A.

also play a pathogenic role in APS,[8] stroke,[16] and in the origin of atherosclerosis.[17] Authors have referred to aB2GPI antibodies of immunoglobulin G (IgG) and IgM class in the pathogenesis of atherosclerosis and chronic vascular disease. However, the importance of IgA-aB2GPI has not been reported until recently.[18]

The purpose of this study has been to prospectively study end-stage renal disease patients on hemodialysis followed up for 2 years on the prevalence of aCL and aB2GPI and their influence on clinical outcomes.

RESULTS

A total of 148 patients on dialysis were included in the study and were prospectively followed up for 2 years. Of these, 24 (16.2%) received a renal transplant and were excluded, and hence 124 patients were finally included in the study (Figure 1).

Table 1 shows the demographics and clinical characteristics of patients; a prevalence of patients with IgA-aB2GPI (IgA-aB2GPI +) stands out.

Mortality

During the follow-up, 33 (26.6%) of the patients died. Compared with the surviving patients, those who died were older, and had a higher incidence of ischemic cardiomyopathy, central catheter, hypoalbuminemia, and positivity for the IgA-aB2GPI antibodies. Other antiphospholipid-associated antibodies were present in the 124 patients studied: anticardiolipin IgG (9.7%) anticardiolipin IgM (6.4%), and aB2GPI IgG (10.9%; Table 1), but they were not found to be related with mortality (Table 2).

Table 1 | Distribution of patients

	Studied Mean (s.d.)/ number (%)	Excluded Mean (s.d.)/ number (%)	*P*-value
Demographic characteristics			
Number of patients	124 (83.8%)	24 (16,2%)	
Time on hemodialysis before study (months)	37.3 (30)	36.9 (31)	0.915
Age (years at begin study)	64.3 (15.6)	61.7 (14.4)	0.766
Age range	20–90	25–82	
Men	82 (66.9%)	20 (83.3%)	0.110
Clinical measures			
Diabetes mellitus	47 (37.9%)	10 (41.7%)	0.729
Arterial hypertension	100 (79.8%)	19 (79.1%)	0.867
Hypoalbuminemia	35 (28.2%)	6 (25.0%)	0.746
Previous transplantation	27 (21.7%)	6 (25.0%)	0.728
Central venous catheter	52 (41.9%)	8 (33.3%)	0.432
Ischemic cardiomyopathy antecedents	39 (31.4%)	4 (16.7%)	0.144
Stroke/TIA antecedents	15 (12.0%)	4 (16.7%)	0.540
Coumarin treatment	22 (17.7%)	4 (16.7%)	0.899
Biochemical values			
Cholesterol (mg/dl)	147.9 (37.3)	151.0 (32.5)	0.989
LDL-C (mg/dl)	74.3 (30.2)	80.1 (25.7)	0.463
HDL-C (mg/dl)	44.4 (12.6)	44.1 (10.2)	0.761
Triglycerides (mg/dl)	152.7 (79.7)	134.5 (45.3)	0.345
Homocysteine (mg/dl)	32.4 (15.7)	36.9 (15.8)	0.627
Immunity and inflammation markers			
IgA-aB2GPI+	41 (33.1%)	5 (20.8%)	0.236
IgM-aB2GPI+	3 (2.4%)	0 (0%)	0.585
IgG-aB2GPI+	15 (12.1%)	2 (8.3%)	0.256
IgA-aCL+	2 (1.6%)	0 (0%)	0.701
IgM-aCL+	8 (6.4%)	3 (12.5%)	0.174
IgG-aCL+	12 (9.7%)	2 (8.3%)	0.299
CRP+	44 (35.4%)	8 (33.3%)	0.840

Abbreviations: aB2GPI+, patients with elevated titers of anti-β glycoprotein I of any isotype; aCL+, patients with elevated titers of anticardiolipin of any isotype; CRP+, patients with elevated values of C-reactive protein (>1 mg/dl); HDL-C, high-density lipoprotein cholesterol; Ig, immunoglobulin; LDL-C, low-density lipoprotein cholesterol; TIA, transient ischemic attack.

Patients with IgA-aB2GPI

A total of 41 patients were IgA-aB2GPI + (33.1%) at the beginning and remained positive until the end of follow-up (group 1). In addition, 83 (66.9%) were always IgA-aB2GPI− (group 2). Mortality was higher in group 1 (46.3% vs. 16.8%, $P<0.001$). Patients included in group 1 were older (but not significantly, $P=0.088$) and had more thrombotic events (46.3% vs. 12.0%, $P<0.001$) and more thrombosis on vascular access (24.4% vs. 7.2%, $P=0.007$) in the follow-up study. In addition, incidence of atherosclerosis-related vascular events (stroke, acute myocardial infarction, and transient ischemic attack) was higher in group 1 (12.2% vs. 7.2%, $P=0.033$). Other variables showed no differences (Table 3).

Table 2 | Parameters on alive and dead patients

	Alive	Dead	
	Mean (s.d.)/ number (%)	Mean (s.d.)/ number (%)	*P*-value
Number of patients	91 (73.4%)	33 (26.6%)	
Time on dialysis (months)	36.0 (32.4)	38.4 (21.6)	0.343
Age (years at begin study)	61.58 (15.8)	71.6 (12.6)	<0.001
Age range	20–87	39–90	
Men	61 (67.0%)	21 (63.7%)	0.638
Diabetes mellitus	31 (34.1%)	16 (48.4%)	0.143
Arterial hypertension	71 (78.0%)	29 (87.9%)	0.216
Hypoalbuminemia	18 (19.8%)	17 (51.5%)	<0.001
Previous transplantation	22 (24.1%)	5 (15.1%)	0.281
Central venous catheter	29 (31.9%)	23 (69.7%)	<0.001
Incidence of thrombotic processes	20 (22.0%)	9 (27.3%)	0.538
Ischemic cardiomyopathy antecedents	22 (24.1%)	17 (51.5%)	0.004
Stroke/TIA antecedents	8 (8.7%)	7 (18.1%)	0.061
Coumarin treatment	15 (16.5%)	7 (21.2%)	0.542
Cholesterol (mg/dl)	148.8 (33.8)	149.4 (47.6)	0.588
LDL-C (mg/dl)	76.3 (32.2)	68.1 (24.4)	0.358
HDL-C (mg/dl)	46.0 (12.1)	43.2 (14.0)	0.571
Triglycerides (mg/dl)	151.6 (73.2)	153.7 (98.0)	0.297
Homocysteine (mg/dl)	35.7 (16.5)	28.2 (14.6)	0.341
IgA-aB2GPI+	22 (24.1%)	19 (57.5%)	<0.001
IgM-aB2GPI+	1 (1.1%)	2 (6.0%)	0.154
IgG-aB2GPI+	10 (10.9%)	5 (15.1%)	0.191
IgA-aCL+	2 (2.1%)	0 (0%)	0.537
IgM-aCL+	8 (8.7%)	0 (0%)	0.077
IgG-aCL+	11 (12.1%)	1 (3.0%)	0.098
CRP+	30 (33.0%)	14 (42.4%)	0.331

Abbreviations: aB2GPI+, patients with elevated titers of anti-β glycoprotein I of any isotype; aCL+, patients with elevated titers of anticardiolipin of any isotype; CRP+, patients with elevated values of C-reactive protein (>1 mg/dl); HDL-C, high-density lipoprotein cholesterol; Ig, immunoglobulin; LDL-C, low-density lipoprotein cholesterol; TIA, transient ischemic attack.

Original diseases as causes of end-stage renal disease were not different among groups (Table 3).

Survival at 2 years

Therefore, patient survival at 2 years was significantly lower in group 1 (Figure 2) and, interestingly, cardiovascular disease was significantly higher in group 1 as a cause of death compared with group 2. Out of the total 19 deceased patients in group 1, the cause of death in 12 (63.1%) was cardiovascular disease, whereas in group 2, only 4 of 14 (28.5%) died because of cardiovascular disease ($P = 0.043$, Table 4).

IgA-anti-B2GPI is an independent risk factor for death

The hazard ratio of anti-IgA-aB2GPI + for mortality without adjusting for other risk factors was 3.274 (95% confidence interval, $P < 0.001$). Notably, the hazard ratio of anti-IgA-aB2GPI + continued to be clearly significant (2.544; 95% confidence interval 1.264–5.119, $P = 0.009$) in the Cox regression model adjusted for other important variables to predict mortality such as patient age, presence of hypoalbuminemia, and use of catheter for dialysis (Table 5).

DISCUSSION

Our study is the first prospective study that has shown that the presence of IgA-aB2GPI is an independent risk factor for death in hemodialysis patients together with other well-known factors such as patient age, hypoalbuminemia, and the use of central catheter for dialysis.

It has been described that the mortality rate in patients on maintenance hemodialysis is >20% per year and cardiovascular disease is the first cause of death. Age, inadequacy of dialysis, inflammation, high levels of parathyroid hormone, vascular access, hypoalbuminemia, and ischemic cardiomyopathy, together with biomarkers associated with inflammation, cardiovascular risk factors, and protein-energy wasting, have been related with mortality in patients on hemodialysis.[19]

Mortality in our units was 26.6% in 2 years. As expected, cardiovascular disease was the most important cause of death. Patients who died were more frequently older (mean age of 72 years standing out), had a background of ischemic cardiomyopathy and hypoalbuminemia, and had used a central catheter for dialysis. The presence of IgA-aB2GPI antibodies was also a significant risk factor for death. In addition, cardiovascular mortality was significantly higher in IgA-aB2GPI + versus IgA-aB2GPI− patients (12/19, 63.1% vs. 4/14, 28.5%, $P = 0.043$). In group 1, no patients died from cancer, whereas cancer-caused mortality in group 2 (negative patients) was similar to the general population ($P = 0.024$ for cancer-caused mortality in group 1 versus 2). This finding should be confirmed in bigger samples of patients and requires further investigation.

Antibodies of IgA isotype directed against B2GPI have been previously associated with a higher risk for vascular disease, including peripheral vascular disease, cerebral ischemia, stroke, myocardial infarction, and with thromboembolic events,[16,20,21] especially in patients with systemic lupus erythematosus.[22] Our results support that laboratory criteria for APS[23] might be revised to include IgA-aB2GPI antibodies, as also suggested by other authors.[21]

Elevated titers of aCL and aB2GPI of IgG and IgM class in chronic kidney disease–dialyzed subjects have been previously described,[24] but the presence of IgA-aB2GPI antibodies has not been reported.

Interestingly, dialyzed patients who were IgA-aB2GPI + were negative for IgA-aCL antibodies. This suggests that IgA-aB2GPI antibodies recognize epitopes available in free B2GPI but not in the phospholipid-bound form. aB2GPI antibodies of IgA isotype, but not aCL, have been associated with macrovascular disease and mortality in scleroderma patients.[25] Two B2GPI conformations exist in serum, lineal and circular.[26] Further immunochemical studies are needed to define the precise epitope target of IgA-aB2GPI antibodies.

B2GPI is expressed in platelets and endothelial cells,[27] and has been found in atheroma plates.[28] aB2GPI antibodies, mostly of the IgA isotype, have been described as an independent risk factor associated with acute coronary syndromes in young patients.[29] The exact mechanism of

Table 3 | Differences between patients positive (group 1) and negative (group 2) for IgA-anti-B2GPI

	Group 1 IgA-aB2GPI positive Mean (s.d.)/ number (%)	Group 2 IgA-aB2GPI negative Mean (s.d.)/number (%)	*P*-value
Number of patients	41 (33.1%)	83 (66.9%)	
Time on dialysis (months)	39.6 (20.4)	35.7 (21.6)	0.141
Dead in 24 months	19 (46.3%)	14 (16.8%)	<0.001
Men	27 (65.8%)	55 (66.2%)	0.963
Age (years)	67.7 (12.2)	62.6 (17.1)	0.088
Age range	39–83	20–90	
Diabetes mellitus	20 (48.7%)	27 (32.5%)	0.079
Arterial hypertension	34 (82.9%)	66 (79.5%)	0.651
Central venous catheter	20 (48.8%)	32 (38.5%)	0.277
Previous transplantation	8 (19.5%)	19 (22.9%)	0.668
Hypoalbuminemia	16 (39.0%)	19 (22.9%)	0.060
Ischemic cardiomyopathy antecedents	13 (31.7%)	26 (31.3%)	0.966
Stroke/TIA antecedents	6 (14.6%)	9 (10.8%)	0.542
Coumarin treatment	10 (24.4%)	12 (14.5%)	0.173
Cholesterol (mg/dl)	154.9 (36.6)	144.2 (32.9)	0.089
Triglycerides (mg/dl)	146.7 (81.0)	154.8 (79.6)	0.297
Homocysteine (mg/dl)	31.3 (18.0)	33.4 (17.4)	0.341
CRP+	15 (36.6%)	31 (37.3%)	0.934
Incidence of thrombotic processes	19 (46.3%)	10 (12.0%)	<0.001
AVF thrombosis (alone or with other thrombosis)	10 (24.4%)	6 (7.2%)	0.007
Non-AVF venous or arterial thrombosis	9 (22.8%)	4 (4.8%)	0.003
Atherosclerosis-related diseases (stroke, TIA, AMI)	5 (12.2%)	2 (2.4%)	0.033
IgM-aB2GPI+	2 (4.9%)	1 (1.2%)	0.219
IgG-aB2GPI+	8 (19.5%)	7 (8.4%)	0.075
IgA-aCL+	2 (4.9%)	0 (0%)	0.107
IgM-aCL+	2 (4.9%)	6 (7.2%)	0.616
IgG-aCL+	2 (4.9%)	10 (12.0%)	0.204
Etiology of end-stage renal disease			
Glomerular disease	8 (19.5%)	17 (20.4%)	0.899
Nephroangiosclerosis	6 (14.6%)	17 (20.4%)	0.430
Diabetic nephropathy	15 (36.5%)	19 (22.8%)	0.107
Tubulointerstitial nephritis	2 (4.8%)	10 (12%)	0.203
Others (obstructive, metabolic, and so on)	5 (12.1%)	12 (14.5%)	0.730
Undetermined	5 (12.1%)	8 (9.6%)	0.662

Abbreviations: aB2GPI+, patients with elevated titers of anti-β2 glycoprotein I of any isotype; aCL+, patients with elevated titers of anticardiolipin of any isotype; AMI, acute myocardial infarction; AVF, arteriovenous fistula; CRP+, patients with elevated values of C-reactive protein (>1 mg/dl); Ig, immunoglobulin; TIA, transient ischemic attack.

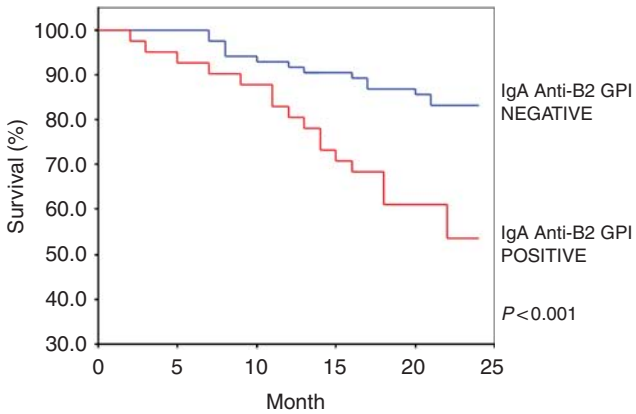

Figure 2 | Survival curves at 24 months in patients positive (group 1) and negative (group 2) for immunoglobulin A (IgA) anti-β2 glycoprotein I (anti-B2GPI) antibodies.

Table 4 | Causes of death in patients positive (group 1) and negative (group 2) for IgA-aB2GPI

Causes of death	Group 1 IgA-aB2GP+ *N*=19	Group 2 IgA-aB2GP− *N*=14	*P*-value (Fisher)
Vascular pathology	12 (63.1%)	4 (28.5%)	0.043
Stroke	4	1	
Myocardial infarction	4	3	
Heart failure	1	0	
Intestinal thrombosis	2	0	
Pulmonary embolism	1	0	
Neoplasia	0 (0%)	4 (28.5%)	0.024
Infections	2 (10.5%)	1 (7.1%)	0.438
Others	5 (26.3%)	5 (35.7%)	0.251

Abbreviations: aB2GPI, anti-β2 glycoprotein I; IgA, immunoglobulin A.

aB2GPI antibodies causing thrombosis and vascular disease remains unknown, although inhibition of B2GPI anti-coagulant activity has been suggested as a pathogenic mechanism.[30] It is not known how the immune response against B2GPI is generated. Some infections (*Helicobacter pylori*, *Actinomyces*, or yeast) have been claimed to trigger an

Table 5 | Multivariate analysis of factors associated with death statistically significant in the univariate analysis

Variable	Hazard ratio	95% Confidence limits	*P*-value
IgA-anti-B2GPI positive	2.544	1.264–5.119	0.009
Access by catheter	2.208	1.036–4.704	0.040
Age	1.027	0.998–1.057	0.073
Hypoalbuminemia	2.153	1.072–4.326	0.031

Abbreviations: aB2GPI, anti-β2 glycoprotein I; IgA, immunoglobulin A.
The association is quantified using the hazard ratio, bringing its 95% confidence interval and its statistical significance. Positivity of IgA-aβ2GPI on univariate analysis had a hazard ratio of 3.274, with a 95% interval of 1.640-6.536 and *P*-value of <0.001.

immune response, biased toward production of IgA, by molecular mimicry between pathogens and B2GPI epitopes.[31]

In hemodialysis patients, it can be speculated that antigens released from endothelial cell membranes interact with dialysis membranes forming hapten-carriers complexes. Under the immunodeficiency state induced by uremia, these complexes would stimulate the formation of aB2GPI antibodies that would promote hypercoagulability and favor the progression of atherosclerosis, which is, in turn, one of the more frequent complications in these patients.[32]

In conclusion, we present an original finding, that is, the presence of anti-IgA-aB2GPI antibodies in hemodialysis patients could be associated with mortality and incidence of thrombotic processes. On the basis of this study, we suggest that testing IgA-aB2GPI in patients on dialysis may be important to identify those at risk of thrombosis and vascular disease. Then, a better and quick management could be established. However, before recommending an effective therapy in patients with IgA-aB2GPI, prospective and multicenter studies with an elevated number of patients demonstrating the usefulness of IgA-aB2GPI as a biomarker of vascular disease in dialysis patients are mandatory.

MATERIALS AND METHODS

Design of the study

This work is a historical cohort study with follow-up (from 1 January 2007 to 31 December 2008). The study was approved by the Institutional Review Board of the Hospital 12 de Octubre (disposition algorithm and outcomes, Figure 1).

Patients

A total of 148 patients with end-stage renal disease who had been on chronic hemodialysis for at least 9 months were included in the study. The patients were recruited from two different dialysis units, the Hospital 12 de Octubre and Clinica San Luciano, both units belonging to the same Nephrology Department. Enrollment included the total unscreened population. The patients were prospectively followed up for 2 years. Of these patients, 24 (16%) received a renal transplantation and were therefore excluded from the study. The characteristics of these 24 patients did not differ from the 124 who completed the follow-up and who were finally analyzed (Table 1). Demographic data of the 124 patients studied were: 121 Caucasian, 1 Asian, and 2 East African.

Dialysis schedule. All patients underwent bicarbonate dialysis using polyamide or polysulfone membranes thrice weekly. A total of 52 patients (42%) were dialyzed through a permanent tunneled catheter.

Dialysis adequacy was quantified using fractional clearance of urea, which is commonly expressed as Kt/V. Efficient clearance was considered when clearance values were >1.2 Kt/V. The dialysis schedule was modified to obtain a Kt/V of >1.2 in all the cases.

Definitions

Cardiovascular risk factors. Arterial hypertension was defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg on repeated measurements or when the patient was taking any antihypertensive treatment. Diabetes mellitus was considered to be present if the patient was on insulin, oral hypoglycemic agents, or diabetic diet, or if the patient's fasting glucose was >126 mg/dl.

Cardiovascular events. A history of cardiovascular disease was defined as ischemic cardiomyopathy (including myocardial infarction, coronary heart disease, coronary revascularization, history of angina with abnormal coronarography, or myocardial scintigraphy), cerebrovascular disease (acute strokes and transient ischemic attack) or a history of carotid endarterectomy.

Thrombotic events. A past history of thrombotic event was defined as when the patient had a history of deep venous thrombosis, arterial thrombosis, pulmonary embolism, arteriovenous fistula, or vascular access thrombosis.

We have considered thrombotic episodes to exist in the 24 months of study when there was acute stroke, transient ischemic attack, pulmonary thromboembolism, deep venous thrombosis, or acute arterial thrombotic episodes in lower limbs. Arteriovenous fistula thrombosis was considered independently.

Laboratory determinations

Routine laboratory determinations were performed every month, including hematological data and biochemical serum values (creatinine, urea, liver enzymes, glucose, cholesterol and triglycerides, uric acid, calcium, phosphate, total proteins and albumin, alkaline phosphatase, sodium, potassium, chloride, bicarbonate, and pH).

C-reactive protein was measured along with the determination of autoantibodies. Patients were considered as positive for C-reactive protein when its levels were >1 mg/dl in all of the measurements.

Parathyroid hormone was measured every 3 months. For this analysis, parathyroid hormone levels were considered at the beginning and at the end of the study. The last available parathyroid hormone measurement was used in the patients who had died during the study.

Hypoalbuminemia was considered to exist if the serum albumin was <4 g/dl in $\geqslant 3$ measurements.

aCL and aBGPI antibodies

aCL and aBGPI antibodies were measured at the beginning of the study and at 6 and 18 months. Autoantibodies were quantified by enzyme-linked immunosorbent assays. The kit used to determine IgG-aCL, IgM-aCL, IgA-aCL, IgG-aB2GPI, IgM-aB2GPI, and IgA-aB2GPI was QUANTA Lite (INOVA Diagnostics, San Diego, CA). Levels of antibodies >20 U/ml were considered positive (for IgG, IgA, or IgM), according to the manufacturer's guidelines. In the first

determination, IgA-aB2GPI positivity or negativity was confirmed using another different enzyme-linked immunosorbent assay (ORGENTEC Diagnostika GmbH, Mainz, Germany).

Patients were considered aCL + or aBGPI + if the average value of three extractions was >20 IU/ml with at least two positive samples.

Statistical methods

Results are expressed as mean ± s.d. or absolute frequency and percentage. Association between qualitative variables was determined with χ^2 or Fisher's exact test, when >25% of expected values were <5. In variables with two categories, comparisons were performed using Student's *t*-test for independent samples.

Survival was calculated using the Kaplan–Meier Method and differences between the distributions of survival were assessed by log-rank test.

Death risk was also estimated by multivariate analysis using the Cox regression model. Those mortality risk factors that were significant in the univariate analysis (Table 2) and whose probabilities were ⩽0.001 were included in the multivariate analysis. Hazard ratios with probabilities <0.050 were considered significant.

The statistical program STATA 11 (StataCorp LP, College Station, TX) was used for processing and data analysis.

DISCLOSURE

All the authors declared no competing interests.

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