ORIGINAL RESEARCH ARTICLE

β_2-Glycoprotein I/IgA Immune Complexes

A Marker to Predict Thrombosis After Renal Transplantation in Patients With Antiphospholipid Antibodies

BACKGROUND: Antiphospholipid syndrome is characterized by recurrent thrombosis and gestational morbidity in patients with antiphospholipid autoantibodies (aPLs). Predictive value of the presence of aPLs is low, and new markers are necessary to identify aPL carriers at higher risk and take preventive measures on them. The presence of circulating immune complexes of IgA bound to β_2-glycoprotein I (B2A-CIC) has been associated with occurrence of acute thrombotic events. In this work we study its possible predictive value for the appearance of acute thrombotic events in patients who are going to undergo transplant surgery, a well-known trigger of acute thrombotic events in aPL carriers.

METHODS: We performed a follow-up study based on the Magnum 12+12 Cohort of patients who received a kidney transplant (n=1339). Three groups were established: group 1 patients who were positive for IgA anti-β_2-glycoprotein I (aB2GP1) and B2A-CIC (n=125); group 2 patients who were positive only for IgA aB2GP1 (n=240); and control group, patients who were negative for IgA aB2GP1 (n=974). Levels of autoantibodies and B2A-CIC were quantified immediately before the transplant surgery and patients were followed up for 6 months.

RESULTS: In group 1, 46.4% of patients experienced any type of thrombosis versus 10.4% in group 2 ($P<0.001$) and 8.6% in the control group ($P<0.001$). The incidence of graft thrombosis in group 1 (31.2%) was significantly higher than that observed in group 2 (3.3%, $P<0.001$) and the control group (2.6%, $P<0.001$). In a multivariate analysis, the presence of B2A-CIC was an independent variable to experience any type of posttransplant thrombosis (hazard ratio, 6.72; 95% confidence interval, 4.81–9.37) and, prominently, for graft thrombosis (hazard ratio, 14.75; 95% confidence interval, 9.11–23.89). No significant differences were found between B2A-CIC–negative and control group patients.

CONCLUSIONS: The presence of B2A-CIC is a predictor of acute thrombotic events. Patients who were positive for IgA aB2GP1 only are at risk of experiencing thrombosis if they are B2A-CIC positive. If they are B2A-CIC–negative patients, they have the same risk as the control group. Treatments to prevent acute thrombotic events should focus on B2A-CIC–positive patients.

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Clinical Perspective

What Is New?

- The presence of circulating immune complexes of IgA bound to β_2-glycoprotein I (B2A-CIC) has been associated with acute thrombotic events in patients with IgA isotype antiphospholipid antibodies.
- The presence of B2A-CIC at pretransplant is the main independent risk factor to experience any type of posttransplant thrombosis in the first 6 months after kidney transplantation.
- The worst complications in the first weeks post-transplant, ie, graft thrombosis–mediated graft loss, were much more frequent in patients who were B2A-CIC positive.
- Immune complexes emerge as a biomarker that explores a new pathophysiological pathway of antiphospholipid syndrome.

What Are the Clinical Implications?

- Determination of immune complexes helps to identify which patients with antiphospholipid antibodies have high risk of developing thrombosis.
- Patients who were positive for IgA anti-β_2-glycoprotein I have a much higher risk of developing thrombotic events if they are B2A-CIC positive.
- B2A-CIC–negative patients have the same thrombosis risk as the control population.
- Treatment to prevent thrombosis should focus mainly on B2A-CIC–positive patients.

Antiphospholipid antibodies (aPLs) are a group of autoantibodies directed mainly against phospholipid-binding plasmatic proteins that can also be localized on membranes of endothelial cells and platelets.[1,2] The aPLs most frequently associated with vascular pathology are directed against β_2-glycoprotein 1 (B2GP1),[3] a plasma protein synthesized mainly in the liver, heart, and kidney.[4]

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by thrombosis or obstetric complications in patients having aPL.[5,6] Diagnosis of APS requires both clinical and laboratory criteria established in 2005. Clinical criteria include thrombosis (in arteries, veins, or small vessels) and miscarriages or fetal loss that must be diagnosed by objective methods such as imaging techniques or histopathology.[1,7] The presence of at least one of the following aPLs is considered by international consensus as a laboratory criterion for APS diagnosis: lupus anticoagulant, antibodies of IgG or IgM isotypes anticardiolipin, or anti-β_2-glycoprotein I (aB2GP1).[1,5]

The clinical relevance of antibodies aB2GP1 of IgA isotype (IgA-aB2GP1) has been increasing in recent years, especially in patients who are negative for other isotypes (IgA isolated-positive).[8–11] Therefore, in the 13th International Congress on Antiphospholipid Antibodies (2010, Galveston, TX), the task force recommended testing for the IgA-aB2GPI in cases in which other aPLs were negative (IgG and IgM isotypes) and APS was still suspected.[12]

The presence of aPLs is necessary, but not sufficient, to promote an APS event. Additional factors are needed to trigger thrombosis (second-hit hypothesis). Although it is known that the activation of the immune response and innate immunity, in the context of processes such as infection or surgery, play a key role and behave as second hits, the mechanisms that induce the thrombotic event remain unknown.[2]

A multicenter prospective study of 1000 patients with APS followed up for 10 years shows that ≈15% of patients developed a thrombotic event in the first 5 years. The predictive value of the presence of aPLs is insufficient to clearly identify the group having the highest risk of events, and new biomarkers are needed to identify which patients have a higher risk of thrombosis.[13]

We described the existence of circulating immune complexes (CICs) of IgA bound to B2GP1 (B2A-CIC) in the blood of patients who were positive for IgA-aB2GPI with a history of APS symptomatology.[14] In a recent work, we described that the presence of B2A-CIC is strongly associated with the occurrence of acute thrombotic events (odds ratio, 22.7; $P<0.001$).[15] This association was established through the quantification of B2A-CIC in serum samples obtained immediately after the occurrence of an acute thrombotic event because no serum samples before the occurrence of thrombotic events were available. Therefore, the possible predictive value of the presence of the B2A-CIC cannot be demonstrated.

In recent years, APS has been associated with many clinical situations including transplant surgery.[16] Graft thrombosis is the main cause of graft loss in the first weeks after kidney transplantation.[17,18] Our group recently described the association of pretransplant presence of IgA aB2GP1 antibodies with graft thrombosis and graft loss in the first weeks after kidney transplantation.[19] Although most patients who have experienced thrombosis have had IgA-aB2GP1, the predictive value of the presence of IgA-aB2GP1 is insufficient to clearly identify the group having the highest risk of events, and additional markers are needed to better identify patients who require preventive treatment.[20]

In this work, we analyze the presence of B2A-CIC in pretransplant serum from a group of patients who are going to undergo a known trigger for APS events, transplant surgery, and its relationship with the occurrence of thrombotic events and graft loss by thrombosis in the first 6 months after transplantation.

METHODS

Study Design

We performed a historical-cohort follow-up study based on the Magnum 12+12 Cohort that included all patients who had received a kidney transplant in the Hospital "12 de Octubre" (Madrid, Spain) in a 12-year period from January 1, 2000 to December 31, 2011.[20] Sera used for the analysis were collected in the 24 hours before the transplant surgery.

Aim

This study aimed to determine the pretransplant prevalence of B2A-CIC in patients who are positive for IgA-aB2GP1 and investigate their possible association with thrombosis, graft thrombosis, and graft loss in the first 6 months after transplant. The main end points were thrombosis, graft loss, causes of graft loss and graft survival at 6 months, the period of greater risk of posttransplant thrombotic events.[21,22]

Ethical Issues

The study was submitted to the Ethics Committee for Clinical Research of Hospital 12 de Octubre and received a favorable report (Reference Number CEIC-15/008). No informed consent was required.

Patients

All patients in the Magnum 12+12 Cohort (N=1375) were included in the study and were studied as long as they maintained a functional graft and up to the end of the observation time. Patients left the study when they lost the graft or died.

Two subcohorts were formed: the control cohort that includes the patients who were negative for IgA-aB2GP1 (n=974) and the positive cohort that includes all the patients who were positive for IgA-aB2GP1. A total of 36 patients who were positive for IgA-aB2GP1 (9%) were excluded from the positive cohort because their pretransplant serum samples were unavailable. There were no significant differences between the pretransplant clinical characteristics and posttransplant evolution of the 36 excluded patients and the patients of the original complete group of patients who were positive for IgA-aB2GP1 (online-only Data Supplement Table I).

Patients in the positive cohort (n=365) were divided into 2 groups: group 1, positive for the presence of B2A-CIC; and group 2, negative for the presence of B2A-CIC.

Immunosuppressive Treatment

The most used immunosuppressive protocol was based on tacrolimus associated with steroids and mycophenolate mofetil. Immunosuppression regimen in patients >60 years, who received kidneys from >60-year-old donors, was based on cyclosporine A (until 2003) or tacrolimus, steroids, and mycophenolate mofetil with or without induction. Only 15 patients were treated with sirolimus/everolimus associated with cyclosporine A or tacrolimus.

Thymoglobulin (rabbit antithymocyte globulin) 1.5 mg/kg for 4 to 7 days was also administered in hyperimmunized patients and patients with a transplantation from non–heart-bearing donors. In elderly people (>60 years of age), basiliximab (monoclonal antibodies anti-IL2R) (20 mg day 0 and 4) with steroids, cyclosporine A (10 $mg \cdot kg^{-1} \cdot d^{-1}$) and mycophenolate mofetil (2 g/d) were administered until 2003. After that and until the end of this study, tacrolimus (0.1 $mg \cdot kg^{-1} \cdot d^{-1}$) was chosen as a calcineurin inhibitor.

Preventive Antithrombotic Treatment

Preventive treatment with antithrombotic agents was administered to patients with vascular morbidity and with risk for thrombosis during the pretransplant period. Treatment was interrupted in all the patients immediately before their surgery, and those treated with anticoagulation received subcutaneous heparin 5000 U twice daily during 1 week in the immediate posttransplant period. All patients who had been treated pretransplantation with anticoagulation or an antiplatelet agent received the same preventive treatment after transplantation.

Definitions

Thrombotic Events

Thrombotic events were defined in accordance with the International Consensus Statement for Antiphospholipid Syndrome[1] as venous thrombosis, arterial thrombosis, pulmonary thromboembolism, and graft thrombosis diagnosed clinically and confirmed by imaging techniques or histopathology study.[17]

Graft Loss by Thrombosis

Graft loss by thrombosis was considered in patients who had lost their graft and presented graft vein/arterial thrombosis. Graft thrombosis was considered only in patients without surgery-related complications or acute rejection diagnostic criteria.

Patients With Thrombosis Not Related to Graft Loss

This included patients who had experienced at least 1 episode of any type of thrombosis, excluding graft loss–related thrombosis.

Patients With Any Type of Thrombosis

This included the patients who have had at least 1 episode of any type of thrombosis, including graft loss by thrombosis.

Transplant-Related Mortality

This was defined as mortality attributable to any cause within 6 months of transplantation.

Long-Term Outputs

These were defined as those that occur beyond the first transplant semester.

Cytomegalovirus Infection

This was defined in accordance with the evidence of cytomegalovirus replication determined with a polymerase chain reaction–based quantitative nucleic acid amplification test.

APS Events

These include thrombotic events, myocardial infarction, and stroke according to the International Consensus Statement.[1,23] Gestational morbidity was not considered in this work.

Acute Rejection

Acute rejection was defined as acute deterioration in allograft function with acute rejection–specific histopathologic changes in the graft.

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Nonfunctioning Graft

This was defined as a graft that never functioned, after having ruled out infections, surgical complications, accelerated or hyperacute rejection, clear signs of massive thrombosis, vascular complications, and urinary tract obstruction.

Laboratory Determinations

IgA-aB2GPIs were quantified by enzyme-linked immunosorbent assays using QUANTA Lite β_2 GPI IgA (INOVA Diagnostics Inc). Cutoff was established at 20 U/mL with the 99th percentile of a healthy normal population[8,12] that coincided with the manufacturer's recommendation.

B2A-CIC levels were quantified as previously described. Sera with values of B2A-CIC higher than 20.9 AU were considered positive.[21] All the immunoassays were performed in a Triturus Analyzer (Diagnostics Grifols, S.A.).

Statistical Methods

Results are expressed as absolute frequency and percentage or medians with interquartile ranges. Association between qualitative variables was determined with the Pearson χ^2 test (or Fisher exact test, when appropriate). Data were expressed as number and percentage. The Mann-Whitney *U* test was used for comparisons in scaled variables with 2 categories.

Graft loss by thrombosis, graft-survival probabilities, and patient-survival probabilities were calculated using the Kaplan-Meier method and differences between the survival distributions were assessed with the log-rank test. The relative measure of a condition on survival was expressed as hazard ratio (HR).

Multivariate analyses were performed using Cox regression (proportional-hazards model). The relative measure of an effect was expressed as hazard ratio.

Receiver operating characteristic (ROC) curve analysis was performed to determine the cutoff value of the assays. The optimum cutoff point was established using the maximum value of the Youden index (J value).

Probabilities <0.05 were considered significant. For multiple comparisons *P* values were adjusted with the Bonferroni correction.

Donor age was not evaluated in the statistical analysis because it is a recipient age–dependent variable. (When selecting the donor recipients, an attempt was made to match up their ages as much as possible.)

Data were processed and analyzed using Medcalc for Windows version 16.8 (MedCalc Software). The adjustment of *P* values for multiple comparisons were obtained by the Bonferroni method using the p.adjust function of the "R" program language (R Foundation for Statistical Computing).

RESULTS

A total of 125 (34%) of the 365 patients who were IgA-aB2GP1 positive had B2A-CIC values above the cutoff (group 1), whereas 240 were negative (group 2). Table 1 shows the pretransplant characteristics of the 1339 patients studied: group 1, group 2, and the control group. Levels and distribution of positive patients for each group of other aPLs (IgG and IgM) are described in online-only Data Supplement Table II.

In comparison of the pretransplant clinical characteristics (Table 1) of patients in group 1 versus the control group, group 1 patients were older (median, age 62 versus 52 years; *P*<0.001), had higher cold ischemia time (median, 22 versus 20 hours; *P*<0.001), and experienced more pretransplant thrombotic events (21.6% versus 11.0%, *P*<0.001). On analyzing the clinical characteristics of the group 2 patients, it was found that, in comparison with the control group, they were older (median age, 60 versus 52 years; *P*<0.001) and the proportion of men was significantly lower (47.9% versus 61.9%; *P*<0.001).

Graft Loss Caused by Graft Thrombosis

A total of 72 patients had graft loss caused by graft thrombosis (GL-GT) representing the main cause of graft loss: 51.4% of the 140 patients who had lost their graft in the first semester.

The percentage of patients with GL-GT for the control group was 2.6% (n=25), 31.2% (n= 39) for group 1 and 3.3% (n=8) for group 2. Most of the cases of graft thrombosis (54.2%) occurred in the patients in group 1. The percentage of patients who were positive for B2A-CIC was 82.9% in patients with GL-GT who were positive for IgA-aB2GP1 (39/47, Figure 1).

The relative risk for GL-GT for group 1 patients (B2A-CIC positive) versus the control group was 12.16 (95% confidence interval [CI], 5.94–29.39; *P*<0.001). The relative risk for the incidence of GL-GT in patients in group 1 versus group 2 was 9.36 (95% CI, 4.51–19.41; *P*<0.001). No significant differences in GL-GT were observed between patients in group 2 and the control group (Table 2).

The Kaplan-Meier survival analysis (Figure 2A) showed significantly higher GL-GT rates in group 1 than in the control group (HR, 14.6; 95% CI, 6.0–32.0; *P*<0.001) and in comparison with group 2 (HR, 10.9; 95% CI, 4.1–28.8; *P*<0.001). Differences between group 2 and the control group were not significant.

When patients with GL-GT were compared with the rest of the patients, they were older (median age, 61.5 versus 54 years; *P*=0.010), they had a higher proportion of type 2 diabetes mellitus (30.6% versus 16.8%; *P*=0.005), and most were positive for IgA-aB2GP1 (65.3% versus 25.1%; *P*<0.001). Furthermore, patients with GL-GT had significantly higher levels of B2A-CIC than patients without graft thrombosis (median, 35.1 versus 13.6 AU/mL; *P*<0.001; Figure 2B) and most were positive for B2A-CIC (54.2% versus 6.8%; *P*<0.001; online-only Data Supplement Table III).

Graft Survival at 6 Months Was Significantly Lower in Group 1 Patients

The patients in group 1 also had the highest number of cases of graft loss (for all the causes) in the first 6 months after transplantation.

Table 1. Pretransplant Characteristics of Patients in the 3 Groups

Condition	B2A-CIC (+) n=125		B2A-CIC (–) n=240		Control Group n=974		*P* Value*		
	Number or median	% or IQR	Number or median	% or IQR	Number or median	% or IQR	B2A-CIC (+) vs B2A-CIC (–)	B2A-CIC (+) vs Control Group	B2A-CIC (–) vs Control Group
Male sex	75	60	115	47.9	603	61.9	0.111	1.000	<0.001
Age, y	62	50–71	60	46.5–69	52	40–64	0.360	<0.001	<0.001
Time on dialysis, mo	17.5	8.2–32.4	17.5	89-36.1	17.5	8.7–34.5	1.000	1.000	1.000
Panel reactive antibody at time of transplant >50%	3	2.4	4	1.7	22	2.3	1.000	1.000	1.000
Cold ischemia	22	18–24.1	21	15–24	20	16–23	0.177	<0.001	0.618
Previous kidney transplant	16	12.8	28	11.6	170	17.5	1.000	0.714	0.114
Associated pathologies									
Diabetes mellitus	32	25.6	46	19.1	188	19.3	0.594	0.372	1.000
Type 1	3	2.4	2	0.8	26	2.7	1.000	1.000	0.435
Type 2	28	22.4	44	18.3	163	16.6	1.000	0.444	1.000
Myocardial infarction	4	3.2	6	2.5	29	3	1.000	1.000	1.000
Stroke	9	7.2	15	6.2	48	4.9	1.000	1.000	1.000
Thrombosis antecedents (patients)†	27	21.6	30	12.4	107	11	0.102	<0.001	1.000
Arterial thrombosis	1	0.8	5	2.1	2	0.2	1.000	0.912	0.012
Pulmonary thromboembolism	1	0.8	4	1.7	13	1.3	1.000	1.000	1.000
Venous thrombosis	26	20.8	24	10	99	10.2	0.021	<0.001	1.000
Causes end-stage renal disease									
Chronic glomerulonephritis	13	10.4	28	11.6	140	14.4	1.000	0.852	0.975
Interstitial kidney disease	10	8	21	8.7	84	8.6	1.000	1.000	1.000
IgA nephropathy	9	7.2	16	6.6	47	4.8	1.000	1.000	0.969
Polycystic kidney disease	12	9.6	27	11.2	126	12.9	1.000	1.000	1.000
Nephroangiosclerosis	11	8.8	24	10	100	10.3	1.000	1.000	1.000
Diabetes mellitus	26	20.8	36	15	127	13	0.630	0.078	1.000
Lupus erythematosus	2	1.6	4	1.7	12	1.2	1.000	1.000	1.000
Vesicoureteral reflux	5	4	4	1.7	40	4.1	0.939	1.000	0.318
Unknown	21	16.8	40	16.6	134	13.8	1.000	1.000	0.882
Other	16	12.8	40	16.6	164	16.8	1.000	0.924	1.000
Donor origin									
Brain death	113	90.4	202	83.5	796	81.7	0.414	0.066	1.000
Living donor	2	1.6	9	3.7	49	5	1.000	0.408	1.000
Non–heart-beating	10	8	30	12.4	129	13.2	0.777	0.387	1.000
Double renal transplant	5	4	9	3.7	22	2.3	1.000	1.000	0.837
Preventive antithrombotic treatment									
Coumarin (isolated)	9	(7.2)	8	(3.3)	36	(3.7)	0.289	0.188	1.000
Coumarin (comb)	2	(1.6)	2	(0.8)	7	(0.7)	1.000	0.910	1.000
Low-dose aspirin	29	(23.2)	28	(11.7)	145	(14.9)	0.012	0.050	0.603
Clopidogrel (isolated)	4	(3.2)	4	(1.7)	27	(2.8)	1.000	1.000	0.993

B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I; and IQR, interquartile range.

**P* values were adjusted by the Bonferroni method for multiple comparisons.

†A patient may have >1 thrombotic event.

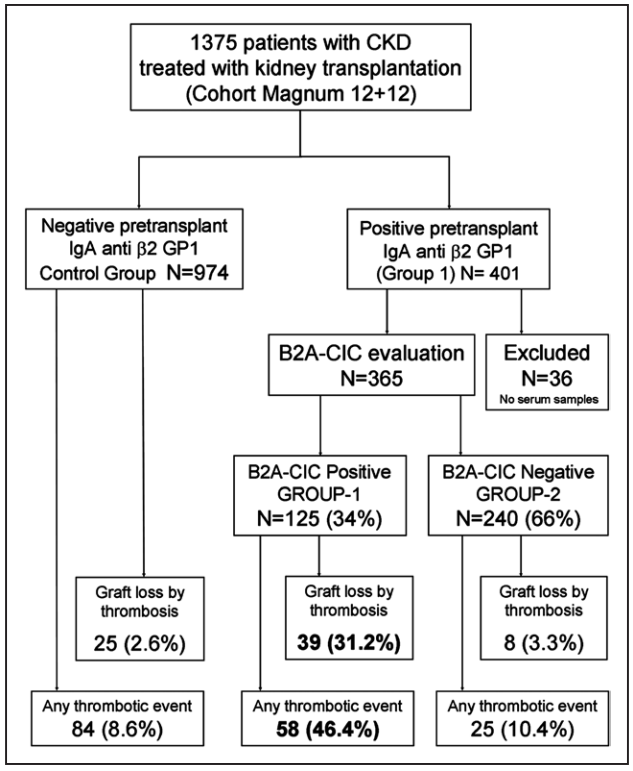

Figure 1. Description of the study.
Distribution of patients and main outcomes. B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I; CKD, chronic kidney disease; and GP1, glycoprotein I.

In a Kaplan-Meier analysis (Figure 2C), graft loss (for any cause) was significantly higher in group 1 versus the control group (HR, 6.46; 95% CI, 3.47–12.03; *P*<0.001) and versus group 2 (HR, 5.08; 95% CI, 2.51–10.31; *P*<0.001). Differences between group 2 and the control group were not significant (Table 2).

It is notable that the proportion of patients with a nonfunctioning graft (kidney that never functioned without experiencing rejection or thrombosis) was significantly higher (*P*=0.006) in group 1 than in the control group (4% versus 0.6%; *P*=0.006; relative risk, 6.36; 95% CI, 1.97–20.54). The differences between the groups were not significant in the remaining causes of graft loss (Table 2).

Other Thrombotic Events

In addition to the patients with GL-GT, 95 patients without graft loss (7.1%) presented thrombotic events. This thrombosis in patients with non–graft thrombosis was more frequent in patients in group 1 (15.2%) than in the control group (6.1%; *P*<0.001; Table 2). When patients who had experienced any type of thrombotic event (including GL-GT and other thrombosis) were considered, it was found that posttransplant thrombosis was significantly more frequent in the group 1 patients (46.4%), this frequency being significantly higher than that observed in group 2 (10.4%; relative risk, 4.45; *P*<0.001) and in the control group (8.6%; relative risk, 5.38; *P*<0.001).

There were no differences concerning posttransplant thrombosis among patients who received preventive treatment and those who did not (data not shown), with the exception of patients treated with low-dose aspirin who had a higher incidence of graft thrombosis (8.4% versus 4.8%; *P*=0.038).

Multivariate Analysis

Factors that showed an association with GL-GT with a *P* value of <0.150 (online-only Data Supplement Table III) were subjected to a Cox proportional hazards multivariate analysis (Table 3). The presence of B2A-CIC (HR, 14.75; 95% CI, 9.11–23.89; *P*<0.001), type 2 diabetes mellitus (HR, 1.97; 95% CI, 1.15–3.37; *P*=0.014), and transplant from a non–heart-beating donor (HR, 2.55; 95% CI, 1.38–4.71; *P*=0.001) were identified as independent variables for graft loss by thrombosis. Pretransplant presence of arterial hypertension was identified as a significant protection factor (HR, 0.40; 95% CI, 0.23–0.68; *P*<0.001).

A univariate analysis of the factors associated with any type of thrombosis was also performed (online-only Data Supplement Table IV).

The variables that were identified with a *P* value of <0.150 were subjected to a Cox multivariate analysis. Presence of B2A-CIC (HR, 6.72; 95% CI, 4.81–9.37; *P*<0.001) was identified as the independent variable for thrombosis having a stronger association. Sex, age, type 2 diabetes mellitus, and non–heart-beating donor were also identified as independent variables. Again, it was found that the presence of hypertension before transplantation acts as a significant protective factor against the incidence of thrombotic events (Table 3).

Transplant-Related Mortality

Patients in group 1 had a significantly higher mortality (*P*<0.001) in the first 6 months after transplant than that observed in group 2 (8% versus 1.8%) and the control group (8% versus 2.9%; Table 2 and Figure 3A).

Validation of the B2A-CIC Levels Cutoff

The values of IgA-based CIC units (AU) were analyzed by using the receiver operating characteristic curve (ROC curve) to validate whether B2A-CIC cutoff previously described for patients with non–transplant-related APS symptoms was also suitable for patients with posttransplant thrombosis. For GL-GT, an area under the ROC curve of 0.752 (95% CI, 0.704–0.795; *P*<0.001; Figure 4A) was obtained. The optimal cutoff (defined with the maximum value of the Youden J index) was 20.83 AU (sensitivity, 85.1%; specificity, 73.0%).

Table 2. Outcomes in the First Semester After Transplant in Patients in the 3 Groups

Condition	B2A-CIC (+) n=125		B2A-CIC (–) n=240		B2A-CIC (–) n=240		*P* Value*		
	n	%	n	%	n	%	B2A-CIC (+) vs B2A-CIC (–)	B2A-CIC (+) vs Control Group	B2A-CIC (–) vs Control Group
Patients with thrombotic events in the first semester									
Including graft loss by thrombosis†	58	46.4	25	10.4	84	8.6	<0.001	<0.001	1.000
Excluding graft loss by thrombosis†	19	15.2	17	7.1	59	6.1	0.066	<0.001	1.000
Deep venous thrombosis	12	9.6	15	6.3	44	4.5	1.000	0.081	1.000
Pulmonary thromboembolism	2	1.6	4	1.7	8	0.8	1.000	1.000	1.000
Arterial thrombosis	6	4.8	2	0.8	11	1.1	0.114	0.018	1.000
Myocardial infarction	1	0.8	2	0.8	5	0.5	1.000	1.000	1.000
Stroke	1	0.8	3	1.3	4	0.4	1.000	1.000	0.864
Any antiphospholipid syndrome event ‡	59	47.2	33	13.8	112	11.5	<0.001	<0.001	1.000
Graft loss in the first 6 mo									
Total graft losses	48	38.4	22	9.2	70	7.2	<0.001	<0.001	1.000
Acute rejection	1	0.8	4	1.7	8	0.8	1.000	1.000	1.000
Nonfunctioning graft	5	4	3	1.3	6	0.6	0.555	0.006	1.000
Death	0	0	2	0.8	11	1.1	1.000	1.000	1.000
Graft thrombosis	39	31.2	8	3.3	25	2.6	<0.001	<0.001	1.000
Surgery related	0	0	2	0.8	2	0.2	1.000	1.000	1.000
Others	3	2.4	3	1.3	18	1.8	1.000	1.000	1.000
Mortality in the first 6 mo	10	8	7	2.9	18	1.8	0.087	<0.001	0.888
Cardiovascular diseases	5	4	4	1.7	8	0.8	0.519	0.006	0.708
Infections	3	2.4	3	1.3	7	0.7	1.000	0.186	1.000
Cancer	1	0.8	0	0	0	0	0.495	0.342	—
Others	1	0.8	0	0	3	0.3	0.495	1.000	1.000
Patients with cytomegalovirus infection	9	7.2	21	8.8	75	7.7	1.000	1.000	1.000

B2A-CIC, circulating immune complexes of IgA bound to β_2-glycoprotein I; and —, not calculated (zero elements).
**P* values were adjusted by the Bonferroni method for multiple comparisons.
†A patient may have >1 type of thrombotic event.
‡It includes thrombosis, myocardial infarction, and stroke.

The cutoff was also reevaluated using the presence of any type of thrombosis as a classification variable, obtaining an area under the ROC curve of 0.709 (95% CI, 0.659–0.755; Figure 4B) and practically the same optimum cutoff: 20.86 AU (sensitivity, 70.7%; specificity, 76.1%). Adopting 20.83 AU as a cutoff for the independent variable, the univariate odds ratio for graft loss by graft thrombosis was 16.23 (95% CI, 9.71–27.10; *P*<0.001) and for any thrombosis in the 6 months after transplant was 8.78 (95% CI, 5.87–13.13; *P*<0.001).

An additional ROC curve analysis of levels of B2A-CIC (AU) in patients with graft loss in the first 6 months after transplant with the exclusion of patients with GL-GT can be seen in the online-only Data Supplement Figure I.

Long-Term Outputs

The incidence of thrombotic events, graft losses, and mortality in patients who surpassed the first semester after transplant was low; significant differences were not found in the 3 groups of patients (online-only Data Supplement Table V and Figure 3B). There were also no significant differences in the causes of graft loss or death, with the exception of a slightly more significant mortal-

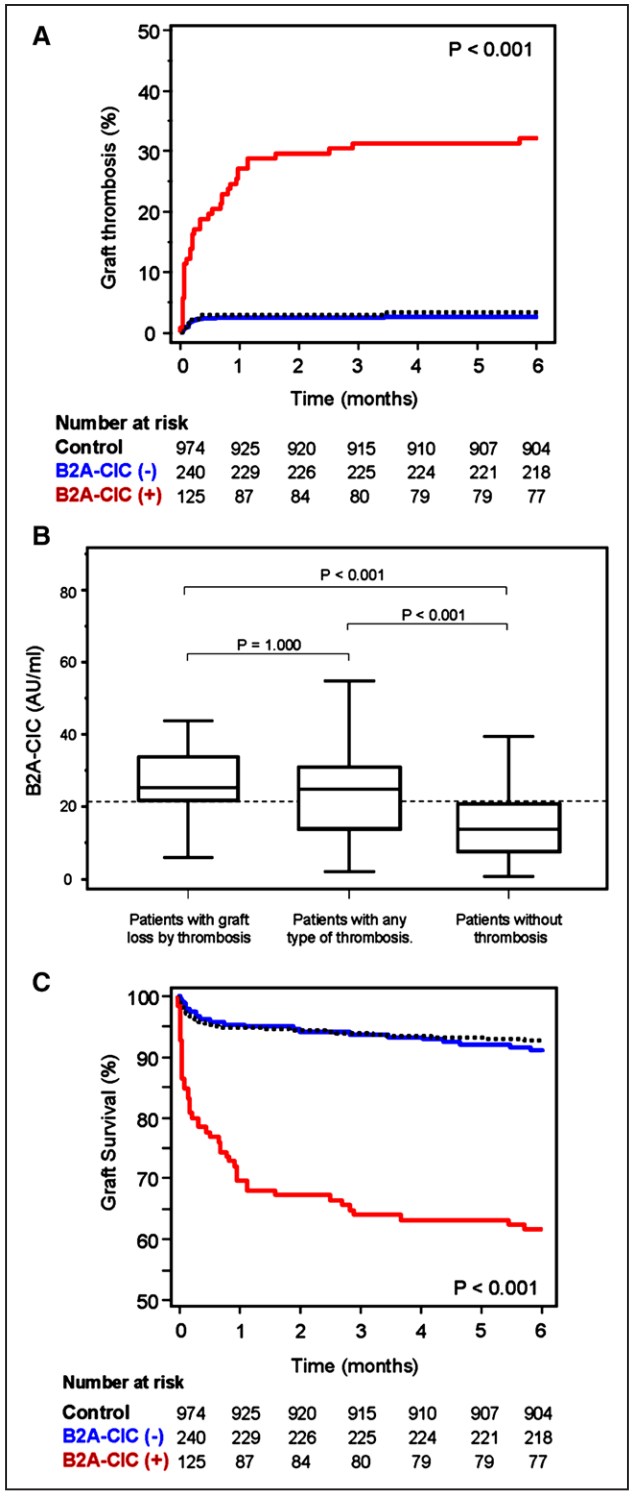

Figure 2. Graft survival and pretransplant serum levels of circulating immune complexes of IgA bound to β_2-glycoprotein I (B2A-CIC).
A, Incidence of graft thrombosis in the 3 groups during the 6 months of the follow-up. Patients in group 1 (B2A-CIC positive, red line) had significantly higher incidence of graft thrombosis survival (Kaplan-Meier survival analysis) than did the control group (black dotted line) (hazard ratio, 14.06; 95% confidence interval, 6.0–33.0) and group 2 (B2A-CIC negative, blue line) (hazard ratio, 10.9; 95% confidence interval, 4.1–28.8). (*Continued*)

ity from cardiovascular causes in B2A-CIC–negative patients than in controls (1.8% versus 0.2%; *P*=0.045; online-only Data Supplement Table V).

DISCUSSION

In this work, we are describing for the first time that the pretransplant presence of B2A-CIC identifies a subgroup of patients prone to develop posttransplant thrombosis, thus behaving as a predictive biomarker.

The condition of the patients studied in this work is special: end-stage renal disease treated with a kidney transplant. Patients with end-stage renal disease who are going to receive transplants make up an ideal model to study the occurrence of APS events, because they include a high percentage of asymptomatic patients with aPL (IgA-aB2GP1), and all of them are subjected to a well-known second hit, that is, transplant surgery.

Although this population may seem very complex for the study of APS events because they have an additional disease, the work focuses on exploring a new pathophysiological pathway of APS[24] that could help to better understand its pathogenesis and to establish new therapeutic strategies. It should be considered that most studies on APS also are based on groups of patients with concomitant conditions (autoimmune diseases). In this way, the cutoff for B2A-CIC obtained in this study with patients with end-stage renal disease is practically identical to that obtained with patients with non–transplant-related APS,[15] suggesting that both patients with end-stage renal disease APS and patients with conventional APS would be equivalent regarding B2A-CIC thrombotic risk.

IgA-aB2GPI antibodies behave as an independent risk factor for APS events, especially graft thrombosis. However, the presence of IgA-aB2GP1 is not sufficient to identify the population that is potentially at risk of throm-

Figure 2 Continued. The differences between B2A-CIC–negative patients and the control group were not significant. **B,** Pretransplant serum levels of B2A-CIC. The levels were significantly higher (*P*<0.001) in patients who lost the kidney by graft thrombosis (median, 25.1; interquartile range, 21.7–33.7) and in patients with any type of thrombosis in the first 6 months after transplantation (median, 24.9; interquartile range, 13.9–30.9) than in those patients who did not have any thrombosis in the first 6 months after transplantation (median, 13.6; interquartile range, 7.7–20.6). The *P* values were adjusted by the Bonferroni method for multiple comparisons. **C,** Graft survival at 6 months of follow-up (including all causes of graft loss). Patients who were B2A-CIC positive (red line) had a significantly lower graft survival (Kaplan-Meier survival analysis) than did patients in the control group (black dotted line; hazard ratio, 6.46; 95% confidence interval, 3.47–12.03) and B2A-CIC negative (blue line) (hazard ratio, 5.08; 95% confidence interval, 2.51–10.31). Differences between B2A-CIC negative and the control group were not significant.

Table 3. Multivariate Analysis (Cox Regression) of Outcomes in the First 6 Months After Transplantation

Variable	Univariate			Multivariate		
	Hazard Ratio	95% Confidence Interval	*P* Value	Hazard Ratio	95% Confidence Interval	*P* Value
Graft loss by graft thrombosis in the first 6 mo*						
B2A-CIC positive	13.35	8.39–21.24	<0.001	14.75	9.11–23.89	<0.001
Age, y	1.02	1.00–1.04	0.011	1.01	0.99–1.03	0.227
Type 2 diabetes mellitus	2.15	1.30–3.55	0.003	1.97	1.15–3.37	0.014
Non–heart-beating donor	1.69	0.94–3.03	0.078	2.55	1.38–4.71	0.003
Hypertension	0.62	0.37–1.03	0.066	0.40	0.23–0.68	<0.001
Any thrombosis in the first 6 mo†						
B2A-CIC positive	6.56	4.76–9.05	<0.001	6.72	4.81–9.37	<0.001
Male sex	0.64	0.47–0.87	0.004	0.63	0.46–0.85	0.003
Age, y	1.03	1.02–1.04	<0.001	1.03	1.02–1.04	<0.001
Type 2 diabetes mellitus	1.78	1.26– 2.51	0.001	1.42	0.99–2.04	0.057
Hypertension	0.67	0.47–0.95	0.023	0.46	0.32–0.65	<0.001
Non–heart-beating donor	1.37	0.91–2.07	0.133	2.09	1.35–3.24	0.001

B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I.

*Risk factors associated with graft loss by graft thrombosis in the first 6 months.

†Risk factors associated with any type of thrombosis, except for age (year) and cold ischemia time (hour). The remaining variables are dichotomous.

bosis, because only a small proportion of patients who were positive for these antibodies develop thrombotic events.[9,20] This situation is similar to the situation observed with other aPL of IgG and IgM isotypes.[6]

In this work, we have shown that patients who are positive for IgA-aB2GP1 and also present B2A-CIC are those having the highest risk of thrombotic events when they are subjected to a situation capable of triggering the occurrence of thrombotic events such as transplant surgery. Those who are negative for B2A-CIC have a risk similar to that found in the control population.

A statistically significant association exists between the presence of B2A-CIC with graft losses attributable to nonfunctioning grafts. A significant majority of these patients did not undergo nephrectomy because they had no serious complications. For this reason, a complete histopathologic study is not available for them. On the basis of the presence of B2A-CIC and the symptoms, we have been able to speculate that some of these patients might have experienced some form of silent and progressive thrombotic microangiopathy that would harm the organ by annulling its function, but which had not had serious systemic implications that required medical attention and nephrectomy.

In addition to GL-GT, the presence of B2A-CIC is also associated with other thrombotic events. Renal transplant recipients are at high risk of thromboembolic events in the first months posttransplant.[25] Although some of these events can be related to the transplant surgery, treatments, or time of hospitalization,[24] the presence of B2A-CIC can be considered the most important risk factor.

The biological significance of the presence of B2A-CIC is uncertain. APS is an autoimmune disease having a special situation because the antigen and antibody are present in the blood at the same time. Given the abundance of the antigen (B2GP1), we would have expected that all the antibodies would be permanently bound to their antigen and could not be detected in the laboratory, because the laboratory assays are only designed to evaluate unbound antibodies (the free form).

However, in reality, the antibodies exist in free form and are detected in the diagnostic test. One possible explanation is that antibodies found in blood in free form could have low affinity or be directed against B2GP1 epitopes that are not accessible in physiological conditions.

The biological behavior of the antibodies incorporated into B2A-CIC would be different from that of the free-form antibodies, not only because they have greater affinity, but also because they would be directed against epitopes that are only present in some conformations of the protein that would be more related to their anticoagulant function. The search for epitopes recognized by the antibodies integrated in the B2A-CIC and their possible association with the different physiological functions of B2GP1 should be studied in future research.

It is striking that patients with hypertension at pretransplant have a significantly lower risk of thrombosis. Some antihypertensive treatments might provide vascular protection by reversing endothelial dysfunction and pro-

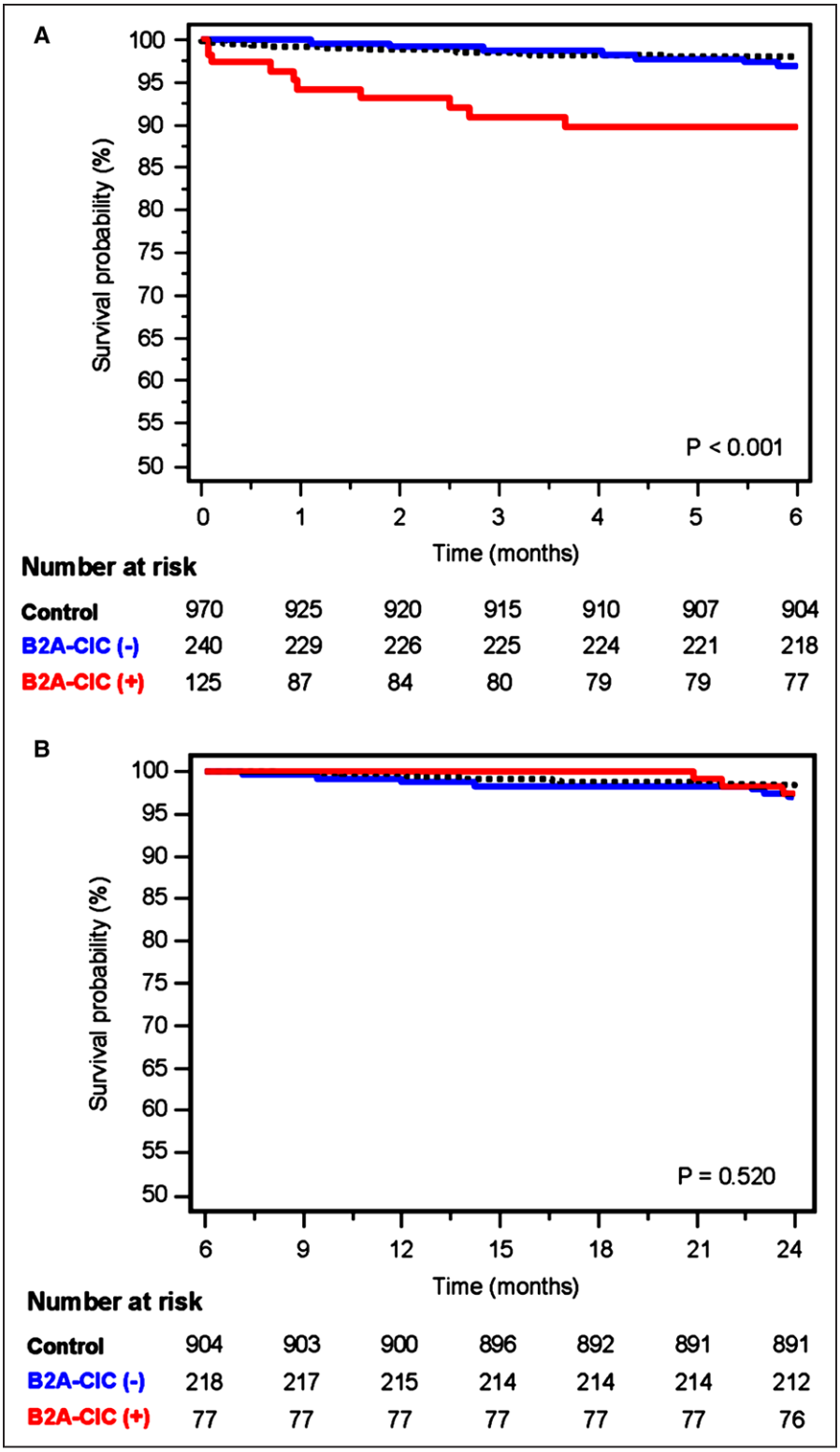

Figure 3. Patient survival analysis.
A, Survival in the first 6 months after transplantation (Kaplan-Meier survival analysis). Patients who were B2A-CIC positive had a higher mortality than did patients in the control group (hazard ratio, 4.48; 95% confidence interval, 1.39–14.42). In comparison with the B2A-CIC negative, the differences are not enough to be significant (hazard ratio, 2.85; 95% confidence interval, 0.74–11.00). **B,** Survival analysis up to 24 months after transplantation of patients who remained in the study at the end of the first semester. There were no significant differences among the 3 groups of patients. B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I.

thrombotic abnormalities, contributing to a reduction in thrombosis-related complications.[26] In this way, hemodialyzed patients treated with antihypertensive drugs have a significantly longer period of vascular access permeability (without vascular thrombosis) than do those who were not treated with them,[27] and renal transplant recipients treated with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers associated with vitamin D have a 60% lower rate risk of venous thromboembolism.[28] Therefore, it can be speculated that antihypertensive treatment before transplantation could have an endothelium-protecting role and could explain, at least in part, a lower risk of thrombosis. This possible protective effect should be investigated in further studies.

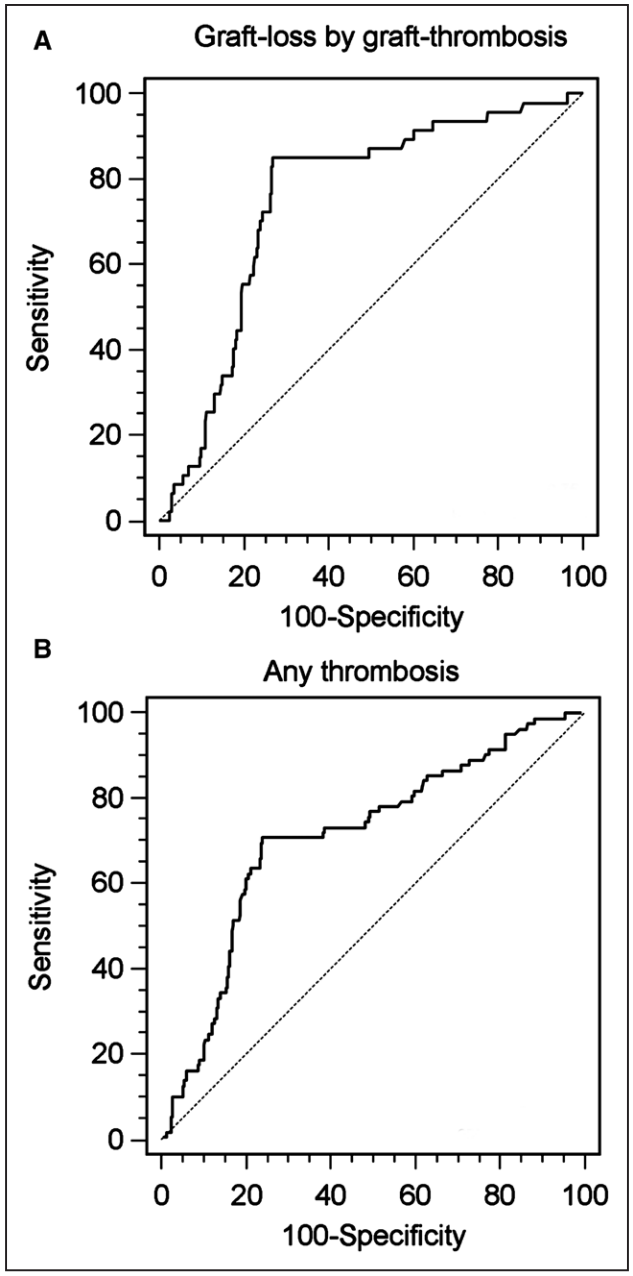

Figure 4. Receiver operating characteristic curves analysis for B2A-CIC evaluation.
A, Receiver operating characteristic curve of levels of B2A-CIC (AU/mL) for risk of graft loss by graft thrombosis in the first 6 months after transplantation (P<0.001). Area under the the receiver operating characteristic curve, 0.752 (95% confidence interval, 0.704–0.795). **B,** Receiver operating characteristic curve of levels of B2A-CIC (AU/mL) in patients with any type of thrombosis in the first 6 months after transplantation (P<0.001). Area under the receiver operating characteristic curve, 0.709 (95% confidence interval, 0.680–0.774). B2A-CIC indicates circulating immune complexes of IgA bound to β_2-glycoprotein I.

This study has several limitations. Only CIC formed by B2GP1 bound to antibodies of IgA isotype were evaluated. The CIC integrated by antibodies of IgG and IgM isotypes were not evaluated because, in this cohort of patients, the prevalence of IgG and IgM aB2GP1 was very low and no association with thrombosis or graft loss was found.[20] Another weakness of the study is that, although it has collected the experience of 12 years, it is a single-center study with patients who have undergone transplantation. Thus, multicenter studies to confirm these findings, both with patients who have undergone transplantation and including other APS-associated situations, are mandatory.

B2A-CIC determination could help clinicians to distinguish which patients could receive preventive therapy. According to our results, positive CIC patients have a high risk for thrombosis and therefore could receive preventive therapy for posttransplant thrombosis. Low-dose aspirin was used most frequently as preventive treatment. Patients of group 1 exhibited a high frequency of this treatment in comparison with those of group 2 and the control group, because the group 1 patients presented more thrombotic antecedents before the transplantation. Patients preventively treated with low-dose aspirin had a significantly higher incidence of graft thrombosis than did other patients, which suggests that the low-dose aspirin therapy did not appear to be sufficiently preventive in our cohort. This coincides with others studies published in aPL carriers in which aPL-positive individuals did not benefit from low-dose aspirin for primary thrombosis prophylaxis.[29,30] Modern anticoagulation (with X factor inhibitors) and mainly hydroxychloroquine could be the therapy of choice for thrombosis prevention.[31,32] Patients who are positive for IgA-aB2GP1 and negative for CIC should receive therapy only if there are concurrent risk factors for thrombosis such as diabetes mellitus with severe cardiovascular disease. However, multicenter and randomized trials with this approach are mandatory before the use of this therapy can be established.

In summary, the presence of pretransplant B2A-CIC in patients who were positive for IgA-aB2GP1 is associated with thrombotic risk and can thus be considered as a biomarker of thrombotic complications. The presence of IgA-aB2GP1 without B2A-CIC implies a thrombosis risk similar to the general population. B2A-CIC determination could help clinicians to distinguish which patients could receive preventive therapy.

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the database and the statistical analysis. Drs Morales, García, González, Daniel Pleguezuelo, and Paz-Artal were responsible for the patient care and clinical data collection. All authors contributed to the data interpretation and report preparation.

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DISCLOSURES

None.

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FOOTNOTES

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