New Biomarker to Predict Thrombosis in Patients with Antiphospholipid Antibodies: Immune Complexes of Beta 2 Glycoprotein 1

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Antiphospholipid antibodies (aPL) are a heterogeneous group of autoantibodies against phospholipids and phospholipid-binding proteins. aPL-recognized antigens present in the plasma or in the membranes of endothelial cells, platelets, and other cells involved in the coagulation cascade [1]. Beta 2 glycoprotein I (B2GPI) is one of the major antigens for aPL. It is a 50-kDa protein synthesized by the liver, kidney, and heart. The physiological function of B2GPI is not yet clear. However, B2GPI has an important role promoting an anticoagulation state. The aPL against B2GPI are mostly associated with vascular pathology.

Antiphospholipid syndrome (APS) is a multisystemic autoimmune disorder characterized by recurrent thromboses and/or gestational morbidity in patients with aPL antibodies. Diagnosis of APS requires the presence of at least one of the clinical criteria (vascular thromboses or pregnancy morbidity) and one of the laboratory criteria: lupus anticoagulant, anticardiolipin antibodies of immunoglobulin G (IgG) and/or immunoglobulin M (IgM) isotypes, or anti-B2GPI (aB2GPI) antibodies of IgG and/or IgM isotypes [2].

IgA aB2GPI antibodies are not included in the diagnostic criteria of APS. The controversy is mainly because diagnostic kits with differences in sensitivity and specificity are used [3,4]. However, in the past few years much of the attention has been focused on the diagnosis and pathogenic roles of IgA isotypes. These antibodies have been associated with APS [5] and are an independent risk factor for acute cerebral ischemia, acute myocardial infarction, cardiovascular diseases, and atherosclerotic diseases [6]. IgA aB2GPI testing is useful for the diagnosis of patients with thrombotic events. Thus, the international consensus guidelines from the 13th International Congress on Antiphospholipid Antibodies [7] recommended testing IgA aB2GPI antibodies in cases of clinical suspicion and negative "consensus" antibodies.

The pathogenic role of IgA aB2GPI is not only reported in the APS population. IgA aB2GPI prevalence is higher in patients with chronic kidney disease than in the general population (30% vs. 1.5%) and is an independent risk factor for cardiovascular morbidity and mortality [8]. Currently it is unknown how the immune response of IgA antibodies against B2GPI is produced. Recently the presence of complexes of HLA class II with misfolded B2GPI was described. They can act as novel autoantigens in APS [9]. A possible explanation for the high prevalence of aPL in this group of patients is kidney dysfunction that may result in the production of a misfolded B2GPI. As a result, it will benefit the exposure of protein epitopes that in physiological conforma-

tion are unknown. The exposure of these epitopes in the context of mucosal infection may trigger an autoimmunity response against B2GPI mediated by the production of pathogenic aPL with a molecular mimicry mechanism [9-12]. The presence of pathogenic aPL in patients with chronic dysfunction of the heart and liver (both also produce physiologically B2GPI) support this hypothesis [13].

The presence of pretransplant IgA aB2GPI antibodies has been associated with increased risk of thrombosis [14] and specifically with early graft thrombosis in transplanted patients [15]. The presence of aPL antibodies is required but, in many cases, is not sufficient to develop a thrombotic event. Meroni [16] introduced a hypothesis that suggests that it is the "second hit" involving both the proinflammatory environment and the immune system that is necessary to trigger the thrombosis formation [17]. Surgery is considered an important risk factor of thrombosis and it may also be a second trigger for thrombosis production in positive IgA aB2GPI patients undergoing transplant surgery. However, the predictive value of IgA aB2GPI is incomplete to identify the patients with thrombotic risk. Of patients who are positive for IgA aB2GPI, 80% will not have any thrombotic event [15]. New biomarkers for characterizing the patients with highest thrombotic risk are needed.

Recently, circulating immune complexes between IgA and B2GPI (B2A-CIC) have been described in the blood of patients with thrombotic clinic of APS [18]. The association between B2A-CIC and

acute thrombosis ($P < 0.001$) was established in a group of patients with recent thrombotic events [19]. Quantification of B2A-CIC levels in serum samples that were obtained immediately after the occurrence of a thrombotic event demonstrated a strong association between this new biomarker and the acute thrombotic event. Positive B2A-CIC patients have lower levels of platelets than negative patients. These data suggest the possibility that B2A-CIC induce platelet activation and aggregation. Consequently, B2A-CIC are an additional risk factor for development and present a prothrombotic environment that promotes the thrombus formation.

The association between B2A-CIC and thrombotic events gave rise to the idea to measure the levels of this new biomarker in patients with end-stage renal disease (ESRD) treated by kidney transplantation. In a recent work we analyzed a cohort of patients with chronic kidney diseases, positive and negative for B2A-CIC, who were planning to undergo transplant surgery. We found that 46% of patients positive for IgA aB2GPI and B2A-CIC in pre-transplant serum samples suffered thrombosis after kidney transplantation vs. 10.4% of patients positive for IgA aB2GPI and negative for B2A-CIC ($P < 0.001$) and 8.6% in the control group ($P < 0.001$). Incidence of graft thrombosis in the group of patients positive for both antibodies and immune complexes (31.2%) is significantly higher than in the group of patients who are negative for the immune complexes (3.3%, $P < 0.001$) and the control group (2.6%, $P < 0.001$). In a multivariable analysis, the presence of B2A-CIC was an independent risk factor for post-transplant thrombosis, hazard ratio (HR) 6.72; 95% confidence interval (CI) 4.81–9.37, and prominently for graft thrombosis, HR 14.75; 95%CI 9.11–23.89. No significant differences were found between the B2A-CIC negative group and the control [20]. Patients who are positive for both IgA aB2GPI and B2A-CIC before transplantation have the highest risk for thrombotic events after transplant surgery. The association between graft loss after kidney transplantation and the presence of B2A-CIC in IgA aB2GPI positive patients in pre-transplant serum samples is statistically significant. Consequently, B2A-CIC are a very important risk factor for graft thrombotic and any thromboembolic event after kidney transplantation.

One possible explanation for these recent findings is that the antibodies incorporated into B2A-CIC would be directed against epitopes that are only present in some conformations of the protein. These epitopes may be associated with pathological situations and be more related with anticoagulant function. The knowledge that epitopes of B2GPI are involved in B2A-CIC formation is necessary to understand the biological mechanism underlying this finding.

CONCLUSIONS

In summary, the presence of B2A-CIC in patients positive for IgA aB2GPI is associated with a thrombotic risk. B2A-CIC emerges as a new biomarker to predict which patients are at risk of thrombosis events following kidney transplantation and may lead to early prophylactic treatment. It is mandatory to analyze the presence and pathogenic role of IgG-CIC and IgM-CIC to confirm the importance of immune complexes in APS pathogenic.

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