Membranous nephropathy PLA2R+ associated with Chagas disease

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ABSTRACT

Chagas disease (CD) — a tropical parasitic disease caused by the protozoan Trypanosoma cruzi — is a major health problem in Latin America. The immune response against the parasite is responsible for chronic CD lesions. Currently, there are no reports of an association between CD and membranous nephropathy (MN). The detection of the phospholipase A2 receptor (PLA2R) as a target antigen in idiopathic MN can improve the differential diagnosis of primary and secondary forms of MN. The authors report the case of a male patient with positive serology for CD who presented sudden death and underwent autopsy. Histological sections of the heart showed multifocal inflammatory infiltrate composed mainly of mononuclear cells, leading to myocardiocytes necrosis and interstitial fibrosis. The kidneys showed a MN with positive expression for PLA2R. As far as we know, this is the first report of a case of primary MN in a patient with CD, with severe chronic cardiomyopathy and heart failure.

Keywords
Chagas Disease; Glomerulonephritis, Membranous; Receptors, Phospholipase A2

INTRODUCTION

Chagas disease (CD) is a major health problem in Latin America. There are an estimated 15-17 million people infected by Trypanosoma cruzi and 90-100 million are exposed to it. The infection is mainly transmitted through insect bites by inoculation of the feces of infected triatomine through the punctured skin.

Morbidity is related to the intensity of parasitism, the biological characteristics of the infecting strains and clones, the number of re-infections, and the host response. It is characterized by an acute phase with intense parasitism with or without symptoms, followed by a chronic phase, which usually presents as an asymptomatic undetermined form that may evolve to cardiac and/or digestive forms.

About one-quarter of infected individuals develop chronic chagasic cardiomyopathy, which is the most severe form of the disease. The pathogenesis is unknown and some authors suggest an immune response to persistent parasites or parasite antigens. The lack of correlation of rare tissue parasitism in the chronic phase and the intensity of the inflammatory process supports the hypothesis of a break of immunological tolerance to heart antigens by T. cruzi infection with an autoimmune response.
Several endemic infectious diseases are associated with glomerulopathies in Brazil. The most frequent are proliferative forms of glomerulonephritis associated with hepatoesplenic schistosomiasis in northeast Brazil. There are no reports of glomerulonephritis associated with CD in humans. Costa et al. demonstrated that mice infected with T. cruzi can develop a mesangial glomerulopathy mediated by immune complexes containing parasite antigens and the rheumatoid factor during the chronic phase of the disease. There is no known association of membranous nephropathy (MN) with CD described in the literature. Primary MN, a common cause of the nephrotic syndrome in adults, is an organ-specific autoimmune disease with involvement of the M-type phospholipase A2 receptor (PLA2R) as a major target antigen. The assessment of both the circulating PLA2R autoantibody and PLA2R in the biopsy samples has been valuable for the differential diagnosis between primary and secondary forms of MN with prognostic and therapeutic implications.

The aim of this report is to discuss a case of idiopathic MN, with enhanced glomerular expression of PLA2R, in a patient with a severe form of chagasic cardiomyopathy.

**CASE REPORT**

A 58-year-old male bricklayer who was born in northeast Brazil had a diagnosis of CD presenting a positive reaction for anti-T. cruzi antibodies. An electrocardiogram showed right bundle branch and left anterior fascicular blocks, which are common electrocardiographic abnormalities of Chagas cardiomyopathy (Figure 1).

Anti-HCV, anti-HBs, AgHBs, anti HIV 1 and 2, and anti-HTLV I/II were negative. Anti-HBC was positive. The patient had been treated for tuberculosis 19 years ago and was a tobacco smoker. Clinical follow-up was lost soon after he started the treatment for congestive heart failure. He died suddenly and the autopsy was performed.

**AUTOPSY FINDINGS**

The ectoscopy revealed a mulatto, slightly overweight, tall man. His heart weighed 500 g (reference value [RV]: 350 g) and showed myocardial hypertrophy and dilation of all cardiac chambers without other typical findings of CD. On histological examination, there was interstitial and perivascular inflammatory infiltration by mononuclear cells with many plasma cells, foci of myocardial cell necrosis, and interstitial fibrosis (Figure 2). There was chronic passive congestion of the lungs, liver, and spleen (weights: lungs 1.760 g [RV: 800-1000 g]; liver 1.865 g [RV: 1400-1600 g]; spleen 335 g [RV: 150 g]). Megaesophagus and megacolon were not found.

The kidneys gross findings revealed no abnormalities. The histological examination showed an MN characterized by uniform and diffuse thickening of the glomerular capillary walls, discrete tubular

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**Figure 1.** Electrocardiogram showing sinus bradycardia (with normal PR interval), right bundle branch, and left anterior fascicular blocks.

**Figure 2.** Photomicrography of the heart showing interstitial and perivascular lymphomononuclear inflammatory infiltrate with many plasma cells (H&E, 400X).
atrophy, and interstitial fibrosis (Figure 3). There were no suspected signs or symptoms of glomerular disease in the patient’s clinical record; it was an incidental autopsy finding.

Electron microscopy analysis, performed on fixed paraffin-embedded tissue obtained from the autopsy, showed intense thickening of the glomerular basement membrane due to electron-dense deposits intermingled with lucent areas of dissolution of these deposits (Figure 4).

Immunofluorescence for PLA2R, performed on formalin-fixed paraffin-embedded renal tissue by Hanna Debiec (Institut National de la Santé et de la Recherche Médicale),\textsuperscript{10,11} was positive (Figure 5).

Figure 3. Photomicrography of the kidney. A - (HE, 400X); B - (PAS, 400X) – Membranous nephropathy (MN) characterized by uniform and diffuse thickening of the glomerular capillary walls; C - Irregular basement membrane with spiked and holey appearance (Churg stage II-III) (Methenamine silver stain 400X).

Figure 4. Thickened glomerular basement membrane (GBM) by incorporated heterogeneous deposits (stage III to IV MGN). Some of the deposits are electron-dense (D) and the lucent areas are consistent with resorbed deposits (L); segmental foot process effacement (P) (EM bar scale 500nm).

Figure 5. Glomerular expression of the M-type phospholipase A2 receptor (PLA2R- 400X).
Fibrosis with a distortion of pulmonary architecture and fibrous pleuritis in the apical area of the right lung was present, which could be a consequence of the previously treated tuberculosis. Severe centrilobular emphysema was also found. In addition, there was mild systemic atherosclerosis and benign prostatic hyperplasia.

**DISCUSSION**

Worldwide, MN is a common cause of nephrotic syndrome in adults. In approximately two-thirds of cases, the etiology is unknown and is referred to as primary. Some examples of the causes of secondary cases are chronic viral, bacterial, and parasitic infections; neoplasia; drugs; and other autoimmune diseases. Experimental studies have shown that in the primary form there is a reaction between a circulating antibody and an intrinsic antigen in the subepithelial side of the glomerular basement membrane with the formation of in situ immune complexes. Complement activation with the generation of membrane attack complex C5b-9 can explain the podocyte injury, morphological changes, and proteinuria. Recent evidence has shown that up to 70% of patients with primary MN have non-complement-fixing IgG4 autoantibodies to PLA2R, a target antigen located at the podocytes. MN, like most immune glomerular diseases, is a manifestation of an immune response to self-antigens expressed on the podocyte cell membrane.

The presence of circulating anti-PLA2R autoantibodies shows the specificity of 57–89% for the diagnosis of primary MN. However, some of these studies tested PLA2R only in groups of patients with primary MN, excluding the cases with secondary MN. Studies that tested PLA2R in cases with secondary MN sampled a small number of patients, most of whom had autoimmune diseases and neoplasia, but not infectious diseases. Qin et al. measured serum anti-PLA2R autoantibodies in 60 primary MN patients and 46 secondary MN patients, 16 of whom had hepatitis B virus (HBV)-associated MN. Anti-PLA2R antibodies were detected in only three patients with HBV. The patients did not have clinical remission with antiviral treatment, and the authors inferred that these patients had a coexistence of two diseases.

Taking all of these considerations into account, the detection of PLA2R circulating antibodies still does not rule out a secondary cause, the investigation of which is expensive and time-consuming in the routine practice of MN.

Old studies have shown that high levels of proteinuria, initial increased serum creatinine determination, and interstitial fibrosis were significant predictors of a poor outcome of MN. However, these markers are not adequate to guide and evaluate treatment efficacy. Spontaneous remission can occur in patients with heavy proteinuria. The positive correlation between the presence of circulating anti-PLA2R and immunological activity makes the PLA2R test useful to evaluate the activity of the disease, and is a source of information for treatment. High levels of anti-PLA2R are present in association with severe proteinuria, which decreases during remission and increases with a relapse of the disease. It has been suggested that the serum levels of anti-PLA2R can be used for the diagnosis and monitoring of the treatment of primary MN.

In this case, there was no blood sample to evaluate the activity of the disease; MN was discovered incidentally during autopsy. The kidneys showed morphologically glomerular basement membrane thickening stage II to III, with minimal tubular atrophy and interstitial fibrosis, and a strong positivity for PLA2R in the glomeruli. Notwithstanding, a positive correlation between PLA2R glomerular deposits and anti-PLA2R serum levels is not the rule. Debiec and Ronco studied 42 patients with MN and found 10 with PLA2R glomerular deposits but anti-PLA2R serum negativity. The serum clearing of anti-PLA2R antibodies can occur because of renal tissue deposition. Svobodova et al. in a retrospective cohort of 65 patients with primary MN, found 6 patients negative for circulating anti-PLA2R antibodies but with positive kidney biopsies for PLA2R antigen during active disease. At the time of remission 37 patients were biopsied again and 15 of them were negative for anti-PLA2R circulating antibodies, but the antigen was found in the corresponding biopsies. In summary, detection of PLA2R in biopsies is an important diagnostic tool in the
absence of serum samples. It can make a differential diagnosis with secondary MN—mainly lupus MN—but is useless in providing any information on the activity of the disease. The case presented herein could have been in an initial phase of active disease, or even in partial or complete remission.

Most authors agree that patients who have elevated initial serum creatinine evolve more frequently to end-stage renal failure. In order to assess the relationship between anti-PLA2R and clinical outcome, Oh et al. tested autoantibodies in serum samples at the time of biopsy in 100 patients with primary MN, but neither the presence of, nor the levels of, the anti-PLA2R antibody showed a significant correlation with the clinical outcome.

In conclusion, this case report shows a patient who presented, concomitantly, two unrelated diseases: primary MN PLA2R positive and Chagas myocarditis.

The detection of circulating anti-PLA2R autoantibodies is very useful in monitoring the activity of the disease and the treatment efficacy. Further studies with a larger sample of secondary forms of MN are needed to better understand the prevalence of the PLA2R test in the serum and in the kidney biopsies of primary MN.

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