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# A new anatomo-clinical approach to an old disease

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## ■ CLINICAL PRESENTATION

In September 2012, in Cape Verde, a nephrotic syndrome was diagnosed in a 16-year-old boy. Empiric treatment was started with prednisolone 1mg/kg without achievement of remission.

In October 2013, the patient was brought to Portugal for treatment. On admission at our department

the patient presented normal renal function and a nephrotic syndrome with oedema, 16 g per day of proteinuria, hypoalbuminaemia (1.8 g/dl) and hypercholesterolaemia (290 mg/dl). Serum C3 and C4 were normal, ANA and anti-DNA antibodies were negative. The presence of hepatitis B, hepatitis C and HIV were excluded.

A kidney biopsy was done.

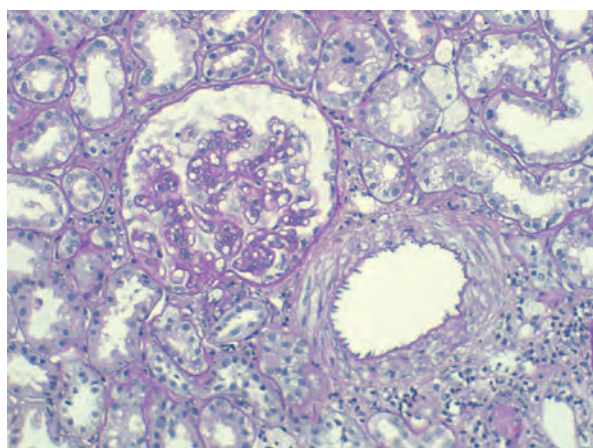


Figure 1  
Periodic acid-Schiff, x 200.

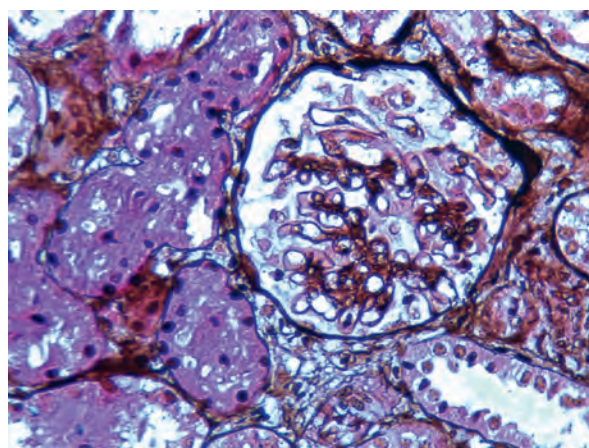


Figure 2  
Methenamine Silver, x 400.

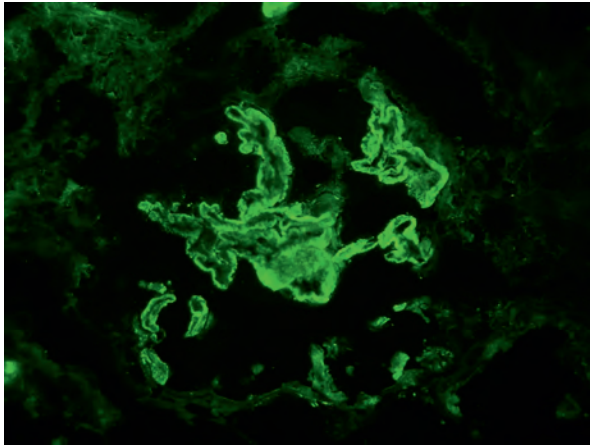


Figure 3

IgG, immunofluorescence, x 400.

## HISTOLOGY

In Fig. 1 we can observe a global and homogeneous thickening of the capillary walls of the glomerulus. No other changes are present. The tubule-interstitial area and the medium size artery are normal. This figure is representative of the entire biopsy fragment.

Figure 2 shows spike formation along the glomerular basal membrane (GBM). Figure 3 displays granular deposition of IgG globally along the GBM. In Fig. 4

subclasses of IgG (IgG1, IgG2, IgG3 and IgG4) were evaluated and only IgG4 is present along the GBM with the same pattern as IgG.

The presence of phospholipase A2 antigen was assessed by immunohistochemistry in formaldehyde fixed paraffin embedded kidney tissue (clone CL 0474). Figure 5-A (deceased-donor kidney) shows the normal presence of phospholipase A2 antigen in podocytes. In Fig. 5-B (our patient), the GBM shows brown, granular, deposits. These granules correspond to abnormal phospholipase A2 antigen and have the same pattern (co-localization) as the IgG and IgG4 deposits.

## Anatomico-clinical diagnosis

Circulating anti-phospholipase A2 antibodies were found before any other immunosuppression therapy was done. The combination of the clinical presentation, kidney histology and circulating anti-phospholipase A2 antibodies allows the diagnosis of idiopathic membranous glomerulopathy.

## TREATMENT AND EVOLUTION

Table 1 displays the treatment done over time and the evolution of proteinuria and circulating anti-phospholipase A2 antibodies.

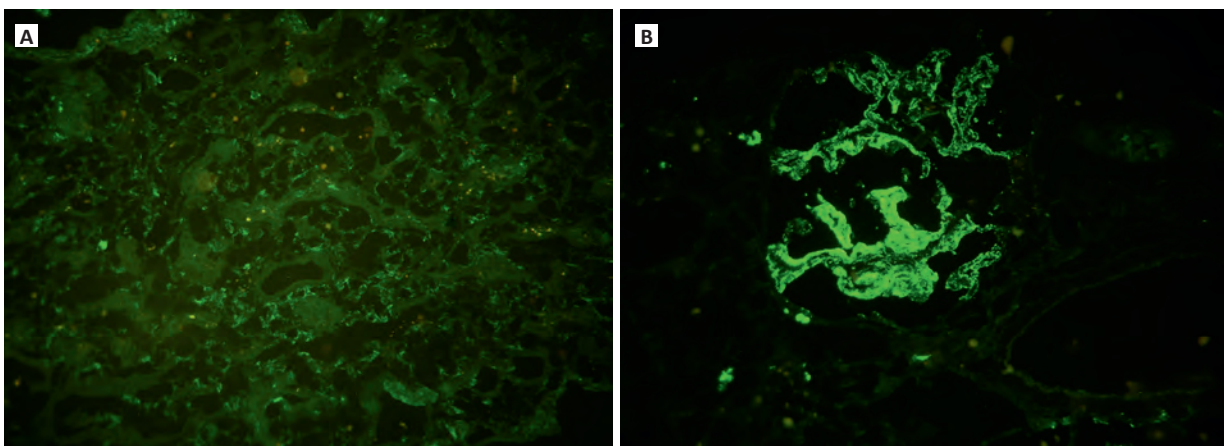
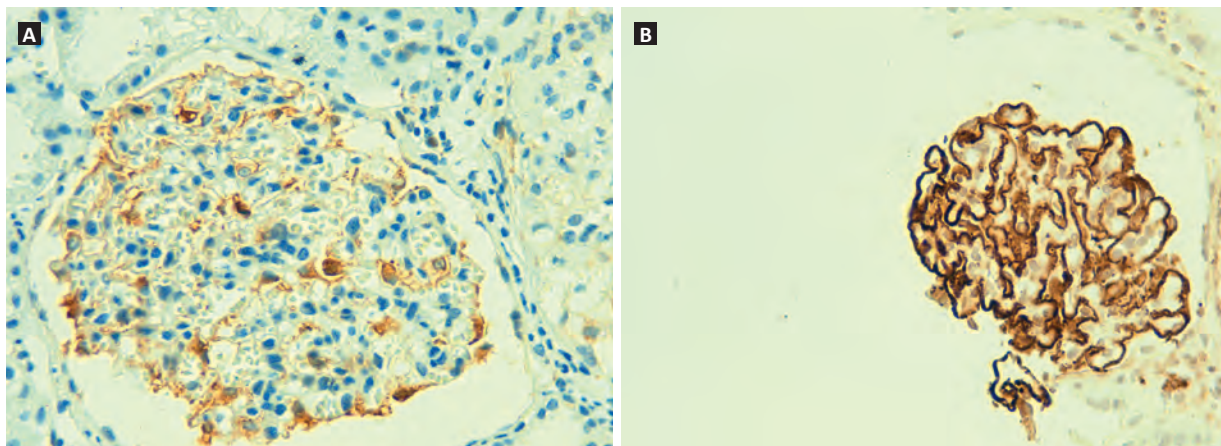


Figure 4

A – IgG3, immunofluorescence, x 400. B – IgG4, immunofluorescence, x 400.

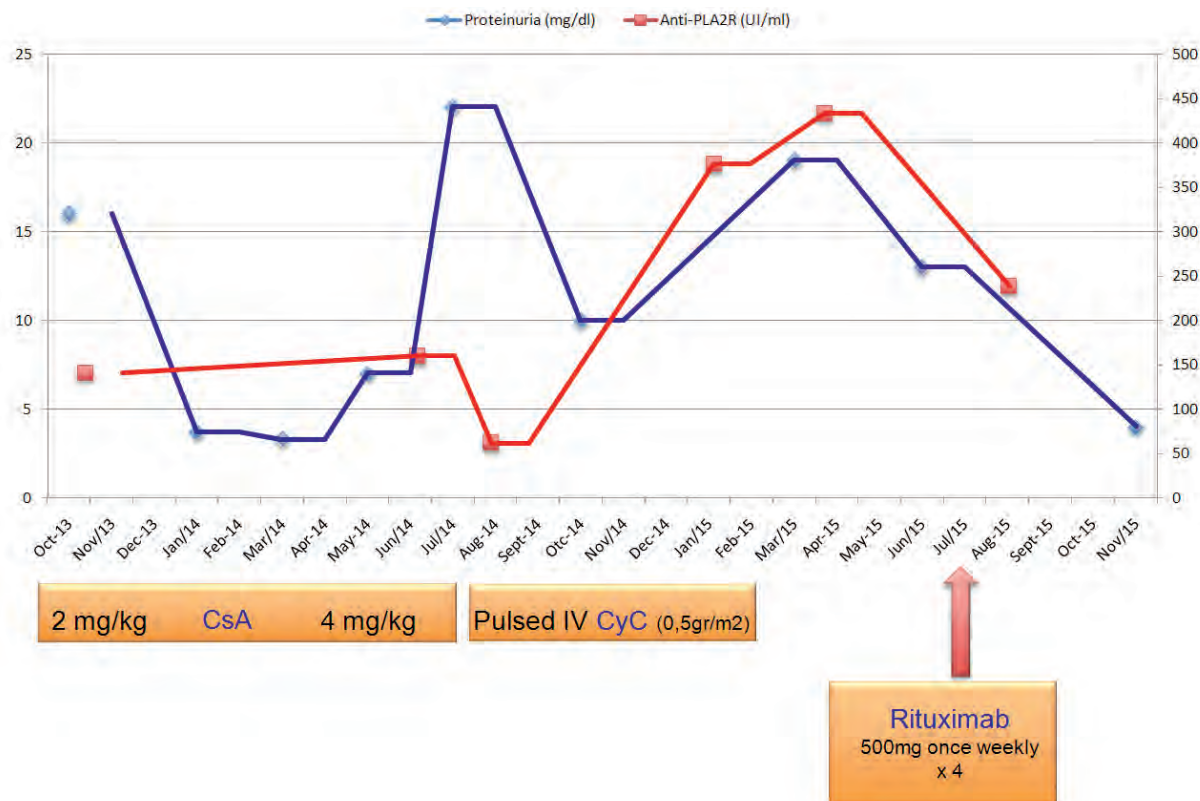


**Figure 5**

A – PLA2R antigen in glomerulus of a deceased-donor kidney; immunohistochemistry, x 400. B – PLA2R antigen in glomerulus of the patient's kidney, immunohistochemistry, x 400.

**Table 1**

Treatment and evolution



CSA – cyclosporine; Cyc – cyclophosphamide.



## ■ DISCUSSION

The thickening of the glomerular basement membrane (GBM) with spike formation by light microscopy (Figs. 1 and 2), with fine granular deposits of IgG and complement C3 (Fig. 3) by immunofluorescence allows the diagnosis of membranous glomerulopathy (MG).

The electron microscopy, not done in this case and not essential for the diagnosis, is characterized by the presence of sub-epithelial electron-dense deposits. This histopathological pattern does not refer to a unique disease entity: 80% of cases without any identified aetiology are referred to as idiopathic; the remaining 20% occurring in patients with an identified associated clinical condition are classified as secondary<sup>1</sup>.

Over the last 10 years, substantial advances have been made in the understanding of the molecular pathogenic mechanism of idiopathic MG (iMG). Circulating autoantibodies to phospholipase A2 receptors (anti-PLA2R) were detected in about 70% of patients with iMG<sup>2</sup>. Circulating autoantibodies against thrombospondin type-1 domain-containing 7A (THSD7A) were detected in 5–10% of the iMG anti-PLA2R negative patients<sup>3</sup>. PLA2R and THSD7A were detected in podocytes of normal human glomeruli, and both antigens are co-localized with IgG4 deposits on the outer aspect of the glomerular basement membrane (sub-epithelial) .

### ■ Serological diagnosis

Phospholipase A2 receptor (PLA2R) discovery was very quickly translated into clinical practice. Simple serologic assays, such as the indirect immunofluorescence test and ELISA, provide specific, sensitive, and quantitative measurements of circulating anti-PLA2R antibodies. Anti-PLA2R antibodies are detected in the serum of patients with MG, but not in the serum of patients with other nephropathies, with autoimmune diseases, or of healthy individuals, being highly specific for MG<sup>4</sup>.

In a prospective study<sup>5</sup>, we found that detection of anti-PLA2 antibodies had a sensitivity of 57.6% (CI 39.2-74.5) and a specificity of 100% (CI 47.8-100) [AUC 0.788;  $p < 0.0001$ ] for the diagnosis of iMG in a sample

of patients who had distinct **disease stages (active or in remission)**. Hu *et al.*, in a recent meta-analysis<sup>6</sup>, estimated that serum anti-PLA2R level sensitivity and specificity for the diagnosis of iMG in the active stage of the disease were 74.0 and 95.0 %, respectively.

A number of recent studies<sup>4</sup> showed that circulating levels of anti-PLA2R antibodies were good prognostic biomarkers and enabled precise monitoring of the response to immunosuppressive treatment (Table I).

### ■ Diagnosis based on kidney tissue

The PLA2R is weakly expressed in podocytes under normal conditions (Fig. 5A) but it is not accessible to T cells. Detection of PLA2R antigen in kidney tissue (Fig. 5-B) is also an important clue to the diagnosis of iMG, and PLA2R is often associated with predominant or exclusive IgG4 deposits (Fig. 4)<sup>7</sup>.

However, presence of PLA2R antigen is commonly seen in MG secondary to sarcoidosis or hepatitis B infection, and the presence of PLA2R antigen can be detected in the absence of circulating anti-PLA2R antibodies<sup>8</sup>. Conversely, in some patients, circulating anti-PLA2R antibodies are not associated with PLA2R antigen<sup>7</sup>.

These results led to the recommendation for using a combined serological (antibody) and biopsy specimen (antigen) analysis in all patients with MG<sup>8</sup>.

**Disclosure of potential conflicts of interest:** None declared

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