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The importance of protocol biopsy in kidney transplantation – A clinical case to solve

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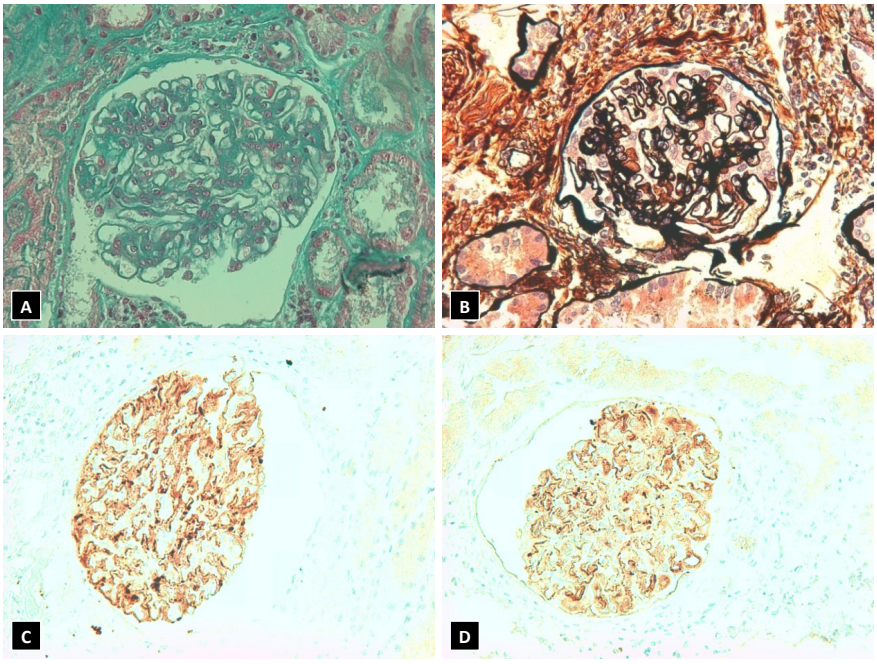
CLINICAL CASE

A 20-year-old man is referred to a nephrology consultation for a nephrotic syndrome. A kidney biopsy was performed **(Figure 1)**.

After exclusion of a secondary form of the disease, the patient was treated with steroids and oral cyclophosphamide without total remission and with frequent relapses. He was lost to medical attention at age 29 and started emergently haemodialysis at age 36.

At 39 years old, the patient received a deceased-donor allograft kidney with 5 mismatches. Thymoglobulin, methylprednisolone, tacrolimus and mycophenolate

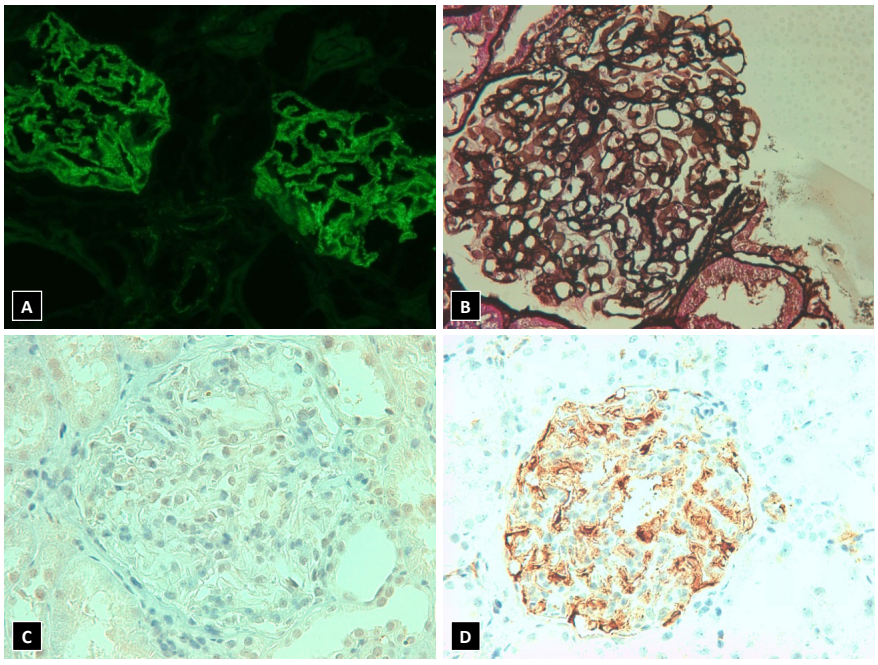
Figure 1
Native kidney biopsy



A – Masson' Trichrome, 400X. B – Silver Methenamine, 400X. C – IgG4, IHC in paraffin tissue, 400X. D – PLA2R, IHC in paraffin tissue, 400X.

Figure 2

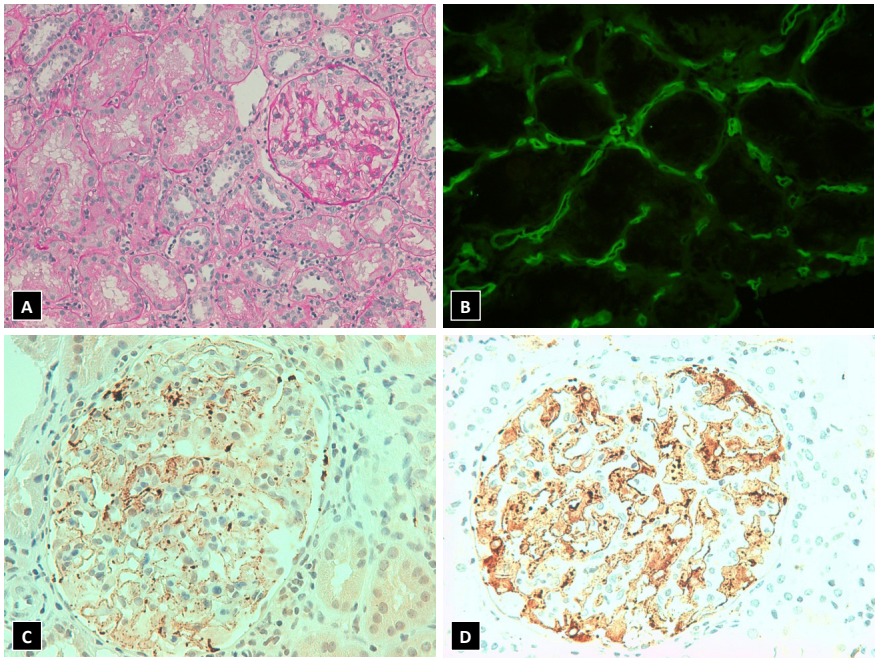
First allograft kidney biopsy, 16 days after transplantation



A – IgG4, IMF in frozen tissue, 200X. B – Silver Methenamine, 400X. C – IgG4, IHC in paraffin tissue, 400X. D – PLA2R, IHC in paraffin tissue, 400X.

Figure 3

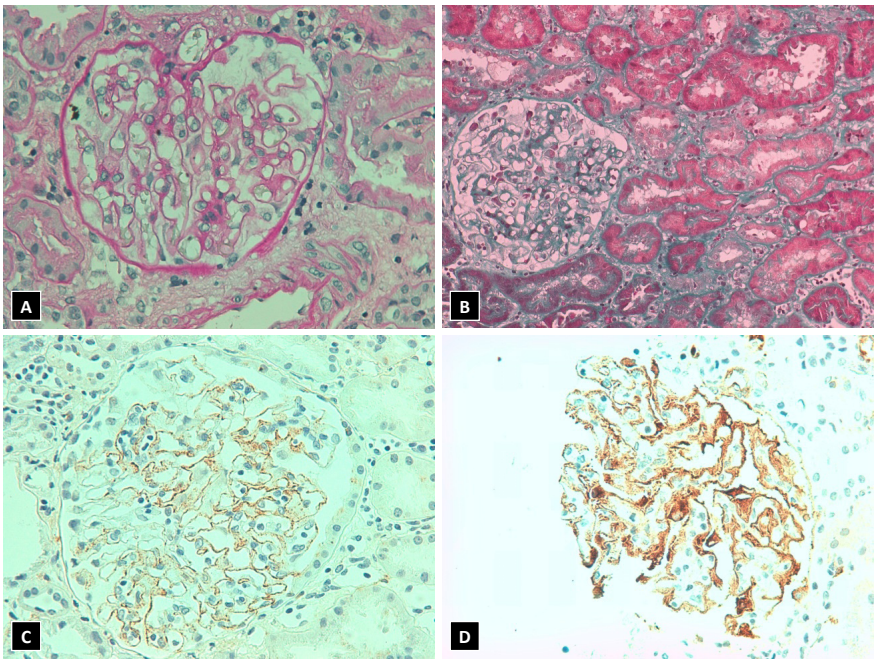
Second allograft kidney biopsy, 10 months after transplantation



A – Periodic acid shift, 200X. B – C4d, IMF in frozen tissue, 400X. C – IgG4, IHC in paraffin tissue, 400X. D – PLA2R, IHC in paraffin tissue, 400X

Figure 4

Third allograft kidney biopsy, 12 months after transplantation



A – Periodic acid shift, 400X. B – Masson's Trichrome, 200X. C – IgG4, IHC in paraffin tissue, 400X. D – PLA2R, IHC in paraffin tissue, 400X.

mofetil were used as induction therapy. The immunosuppressive therapy was maintained with prednisolone, tacrolimus and mycophenolate mofetil with a good evolution.

At the 16th day post-transplantation a protocol biopsy was performed **(Figure 2)**. No alteration was made in therapy and the patient was discharged from the hospital with a creatinine of 1.7 mg/dl and with no proteinuria **(Figure 5)**.

At the 10th month post-discharge, a second allograft biopsy was performed to evaluate *de novo* donor specific antibodies (DSA) associated to a proteinuria of 3.7 mg/day with a stable allograft function **(Figure 3)**. The patient was treated with intravenous immunoglobulin (IvIg) and rituximab **(Figure 5).**

After therapy, the proteinuria declined to 1.4 mg/day; DSA did not decrease and serum creatinine was steady. A third allograft biopsy was performed at the 12th month post-transplant **(Figure 4)**. The patient was treated with plasmapheresis, IvIg and rituximab **(Figure 5).**

At 15th months post-transplant, a fourth biopsy was performed and showed similar findings to the third biopsy. Rituximab was added to patient's therapeutic regime.

Currently, at 35 months post-transplant, the patient presents a proteinuria of 700 mg/day with a creatinine of 2.1 mg/dl **(Figure 5).**

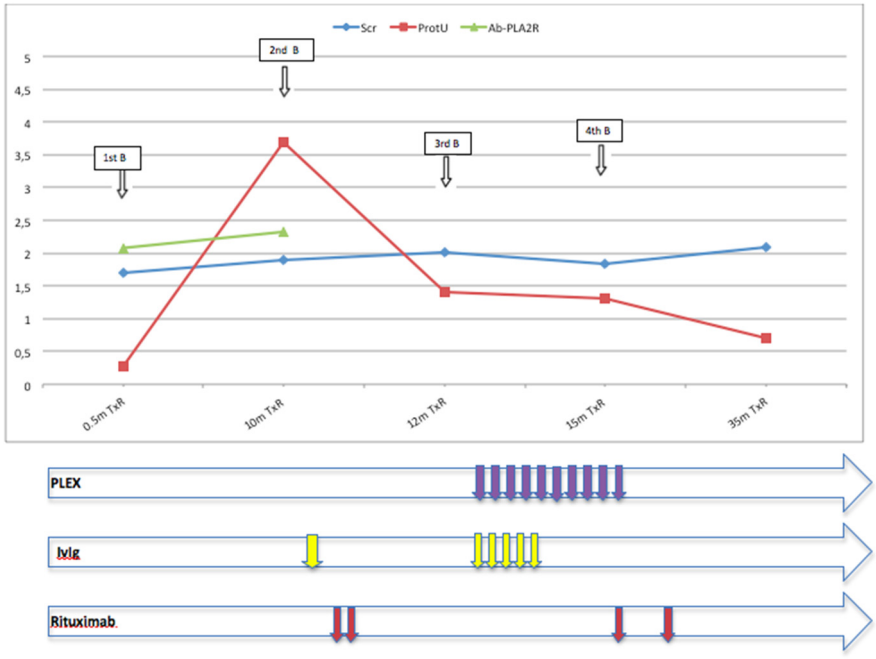
HISTOLOGY

Figure 1 shows the native kidney tissue and demonstrates a thickened glomerular basal membrane (GBM) in Masson's Trichrome and the presence of spikes in methenamine silver. Co-location of granular deposits of IgG4 and phospholipase A2 receptor (PLA2R) along GBM is demonstrated by immunochemistry (IMC) in paraffin tissue.

Figure 2 represents a protocol allograft biopsy performed 16 days after transplantation, and shows a normal glomerulus by methenamine silver. The immunofluorescence (IMF) in frozen tissue reveals granular deposition of IgG with predominance of IgG4 along GBM. IMC in paraffin tissue presents granular PLA2R along GBM. The IgG4 was negative by this technique.

Figure 5

Treatment and evolution



Abbreviations: Scr – Serum creatinine, mg/dl. ProtU – Proteinuria, g/day. Ab-PLA2R – antibodies anti-PLA2R. PLEX – plasmapheresis. Ivig – Intravenous immunoglobulin

The PAS in **Figure 3** shows thickened GBM in glomerulus but also the presence of mononuclear cells in glomeruli (glomerulitis) and peritubular capillaries (capillaritis). IMF in frozen tissue reveals the presence of C4d in peritubular capillaries. The IHC shows IgG4 and PLA2R granular co-location along GBM.

PAS and Masson's trichrome in **Figure 4** confirm the thickening of GBM and the reduction/disappearance of glomerulitis and capillaritis. The IgG4 and PLA2R are present along GBM. The C4d in peritubular capillaries is negative in this biopsy.

CLINICAL-ANATOMICAL DIAGNOSIS

■ **Figure 1; native kidney biopsy:**
Idiopathic Membranous Glomerulopathy- stage 2

The presence of thickened GBM, with spike formation in methenamine silver and deposition of IgG4 along GBM in a nephrotic patient leads to the diagnosis of membranous glomerulopathy (MG). The clinical exclusion of a second form of the disease and the presence of granular PLA2R along GBM suggests an idiopathic form of MG[1,2].The serum anti-PLA2R test was not available at that time.

■ **Figure 2; 16 days after transplantation:**
Subclinical Recurrent Idiopathic Membranous Glomerulopathy- stage 1

The normal light microscopy and the presence of IgG4 along GBM in an asymptomatic patient with previous membranous glomerulopathy in native kidney allow the diagnosis of subclinical recurrent membranous glomerulopathy (RMG) in stage 1. The early recurrence and the presence of PLA2R co-located with IgG4 confirm the diagnosis of recurrent idiopathic membranous glomerulopathy[1,2]. The serum anti-PLA2R test at transplantation time was negative-Figure 5.

■ **Figure 3; 10 months after transplantation:**
Recurrent Idiopathic Membranous Glomerulopathy – stage 2 and Acute Humoral Rejection.

At this time, the patient presented nephrotic proteinuria. Global thickening of GBM and spike formation along GBM are visible in light microscopy which demonstrate the evolution of MG from stage 1 to 2[1,2]. The serum anti-PLA2R test remained negative **(Figure 5)**.

The presence of DSA, glomerulitis, capillaritis and C4d in peritubular capillaries allows the diagnosis of acute humoral rejection[1,2] without deterioration of allograft function.

■ **Figure 4; 12 months after transplantation:** Recurrent Idiopathic Membranous Glomerulopathy-stage 2

In this biopsy, glomerulitis, capillaritis, C4d in peritubular capillaries disappeared. Transplant glomerulopathy, interstitial fibrosis and tubular atrophy were not present.

DISCUSSION

Recurrence of glomerulonephritis in allograft kidney

Briganti *et al*[3] analysed biopsies of all patients transplanted in Australia during a 10-year period (1988-1997) and found that recurrent glomerulonephritis was the third most frequent cause of allograft loss among patients, after chronic rejection and death with a functioning allograft. A large retrospective study demonstrates that recurrence disease causes >15% of allograft loss[4].

Information on the cause of end-stage renal disease is essential to the diagnosis of recurrence. The early diagnosis of recurrent glomerular disease usually requires IMF and/or electronic microscopy[1].

Diagnosis of the early stage of recurrent disease is allowed by biopsies performed for other indications or in protocol biopsies[1].

Protocol or surveillance biopsies, which are performed irrespective of graft function, are valuable. They allow detection of subclinical rejection, immunosuppressive toxicity and glomerulonephritis recurrence[5]. Asymptomatic histological recurrence in renal allograft may be missed if protocol biopsies are not available.

The study of recurrent glomerulonephritis will contribute to not only improving long-term graft survival, but also to clarifying the pathogenesis of glomerulonephritis[6].

Our early diagnosis in this patient was allowed by the existence of a native kidney biopsy and by the performance of protocol biopsy in all patients before discharge.

Recurrence of membranous glomerulopathy

The majority of recurrence of membranous glomerulopathy is *de novo.* They occur late (about 5 years post-transplant), are associated with features of chronic humoral rejection and are PLA2R negative[7].

According to the large protocol biopsy study of Dabade *et al*[8] idiopathic MG recurred in about 40% of transplanted patients. The median time to recurrence is about 13 months but can be 1 to 2 weeks after transplantation. Protocol biopsies can reveal the recurrence before the development of proteinuria. The initial clinical manifestations and light microscopy changes of recurrent MG are subtle or absent.

Serum anti-PLA2R can help distinction between *de novo* and recurrent MG. In the Debiec *et al* study[7] serum anti-PLA2R was always negative in *de novo* MG and was positive in 50% of recurrent MG. The presence of anti-PLA2R at the time of transplantation was associated with disease recurrence in allograft and patients with positive pre-transplant anti-PLA2R should be monitored closely for recurrent MG[9]. Collins *et al*[10] believed that co-location of IgG4 and PLA2R was indeed a manifestation of anti-PLA2R antibodies, which in some patients may be undetectable in the circulation due to adsorption in the glomerulus or cessation of antibody production.

Our patient does not present proteinuria or serum anti-PLAR2 and light microscopy is normal. The IHC and IMF studies of native and allograft kidney allowed the diagnosis of a very early recurrence of idiopathic MG.

Acute humoral rejection

In this case, the performance of a biopsy when our patient developed nephrotic proteinuria allowed the diagnosis of an acute humoral rejection before the deterioration of allograft function. The timely rejection treatment probably explains the absence of chronic lesions in the consecutive biopsy and the stable allograft function.

EVOLUTION AND TREATMENT

Protocol biopsies allow early diagnosis of subclinical recurrent MG[4]. The disease is often progressive and about 30% develops symptoms within 6 months. Recurrent MG causes 10% of graft losses within 4.2±2.7 years after transplantation[8].

The high rate of recurrence and the progressive nature of recurrent membranous glomerulopathy post-transplant suggest that these patients are unlikely to have spontaneously remission[11].

El-Zoghby *et al*[11] demonstrated that the rate of remission of recurrent MG with rituximab treatment appears to be higher than in native MG. They found that 75% of patient treated with rituximab had either partial or complete remission at 12 months.

Our patient experienced nephrotic proteinuria at 10 months. He was treated with rituximab and presents a proteinuria of 700mg/day currently at 35 months **(Figure 5)**.

Disclosure of potential conflicts of interest: none declared

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