

## Membranoproliferative glomerulonephritis associated with type II cryoglobulinaemia in a renal transplant patient with hepatitis C

### Glomerulonefrite membranoproliferativa em associação com crioglobulinemia tipo II em doente transplantado renal portador do vírus da hepatite C

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#### ■ ABSTRACT

The most common HCV-related nephropathy is membranoproliferative glomerulonephritis (MPGN), usually in the context of cryoglobulinaemia. The treatment of this entity is not consensual and represents a challenge to clinicians. We report a case of membranoproliferative glomerulonephritis associated with cryoglobulinaemia type II in a 46-year-old Caucasian male recipient of a deceased kidney transplant in 2010. His baseline serum creatinine (SCr) was 1.1 mg/dl. After three years post-transplantation, he presented with nephritic syndrome in association with renal function impairment (SCr – 2.1 mg/dl). The laboratory tests revealed positive rheumatoid factor, hypocomplementaemia and a positive cryocrit with type II cryoglobulinaemia. Antinuclear autoantibodies and anti-double stranded DNA antibodies were negative. Despite the presence of anti-HCV antibodies, the viral load remained undetectable. The allograft biopsy showed lesions compatible with membranoproliferative glomerulonephritis, with staining in the immunofluorescence for granular IgM and C3 and no C4d. He was treated with methylprednisolone pulses followed by oral prednisolone in association with rituximab. Two months after the last dose of rituximab, the SCr improved to 1.27 mg/dl, the proteinuria decreased and serum C3 levels normalized. Cryoglobulins and rheumatoid factor became negative and HCV RNA remained undetectable. The patient was lost for follow-up. In our case, the treatment with rituximab resulted in a favourable outcome, although a longer follow-up period may be needed to evaluate the clinical response, since other studies reported high relapse rates.

**Key Words:** Cryoglobulinaemia; hepatitis C; kidney transplant; membranoproliferative glomerulonephritis; rituximab.

## RESUMO

A glomerulonefrite membranoproliferativa com crioglobulinemia associa-se frequentemente à infecção pelo vírus da hepatite C. O tratamento desta entidade não é consensual e representa um desafio para os clínicos. Apresentamos um caso de glomerulonefrite membranoproliferativa associada a crioglobulinemia tipo II num doente do sexo masculino de 46 anos portador de enxerto renal de dador cadavérico desde 2010. O seu valor de creatinina sérico (SCr) era 1,1 mg/dl. Após 3 anos do transplante o paciente apresentou-se com síndrome nefrítico em associação com agravamento da função renal (SCr – 2,1 mg/dl). Os exames laboratoriais demonstraram: fator reumatoide positivo, crioglobulinemia tipo II, hipocomplementemia. Os anticorpos antinuclear e anti-double stranded DNA foram negativos. Apesar da presença de anticorpos anti-HCV a carga vírica era indetectável. A biópsia do enxerto renal demonstrou lesões compatíveis com glomerulonefrite membranoproliferativa com depósitos granulares para IgM e C3 e marcação com C4d negativa na imunofluorescência. Iniciou terapêutica com pulsos de metilprednisolona seguido posteriormente por prednisolona oral em associação com rituximab. Dois meses após a última dose de Rituximab o valor de creatinina melhorou para 1,27 mg/dl, a proteinúria diminuiu e os níveis de C3 normalizaram. O doseamento de crioglobulinas e de fator reumatoide foi negativo e a carga vírica do VHC permaneceu indetectável. Foi perdido o follow-up do doente. No nosso caso o tratamento com Rituximab associou-se a uma melhoria clínica. Um período de follow-up maior é necessário para a monitorização clínica uma vez que as taxas de recidiva são elevadas de acordo com estudos publicados na literatura.

**Palavras-chave:** Crioglobulinemia; glomerulonefrite membranoproliferativa; hepatite C; rituximab; transplante renal.

## INTRODUCTION

Post-transplantation morbidity in renal transplant patients with hepatitis C virus (HCV) infection may be partially explained by the risk of *de novo* or recurrent HCV associated glomerulopathies<sup>1-5</sup>, which can lead to allograft dysfunction. The most common HCV-related nephropathy is membranoproliferative glomerulonephritis (MPGN), usually in the context of cryoglobulinaemia<sup>6,7</sup>. Other glomerular diseases have also been reported, such as membranous nephropathy, minimal change disease, thrombotic microangiopathy, acute transplant glomerulopathy and chronic transplant glomerulopathies<sup>1,4,5</sup>. Patients may have clinical manifestations of cryoglobulinaemia, including palpable purpura, arthralgias, neuropathy, and weakness, however the triad of purpura, asthenia and arthralgia is present in only 30% of the cases<sup>8,9</sup>. Renal manifestations include nephrotic or non-nephrotic proteinuria, haematuria and hypertension in 80% of the patients.

Treatment of this entity is a complex issue, non-consensual and represents a challenge to clinicians. Rituximab, a human mouse chimeric monoclonal antibody directed against CD20 antigen on B lymphocytes, has recently proved to be effective on the treatment of this entity, however, the ideal dosage of this drug has not yet been defined.

## CASE REPORT

A 46-year-old Caucasian male with a history of chronic kidney disease associated with HCV-related membranoproliferative glomerulonephritis (MPGN), had a cadaveric renal transplant in 2010. The patient did not receive any previous antiviral therapy. His baseline serum creatinine after transplantation was 1.1 mg/dl. He had a history of illicit drug abuse (cocaine and heroin) with methadone replacement therapy since 2006. His maintenance immunosuppressive treatment consisted of tacrolimus (Advagraf®) 5.5 mg/day, mycophenolate mofetil (MMF) 500 mg twice a day and prednisolone 5 mg/day. In September 2013, his SCr raised to 1.6 mg/dl, he had proteinuria in the nephrotic range (4g/day), active urinary sediment, legs oedema and severe *de novo* hypertension. He was admitted in the hospital for further investigation. Physical examination on admission revealed oedema of the legs and ankles and hypertension (180/100 mmHg), without palpable purpura, arthritis, cutaneous ulcers or peripheral neuropathy. Renal function progressively worsened to a maximum of SCr of 2.12 mg/dl. The other laboratory tests showed an elevated rheumatoid factor (16 IU/ml) and hypocomplementaemia with C3 and C4 reduction (23 mg/dl and 8.0 mg/dl, respectively). Antinuclear autoantibodies (ANAs) and anti-double stranded DNA (dsDNA) antibodies were negative. Cryocrit was positive

(21 mcg/mL) and cryoglobulin characterization revealed type II cryoglobulinaemia. The serological markers were negative for HIV and HBV infection, with positive anti-HCV antibodies. HCV viral load was undetectable ( $< 15$  UI/mL), however, HCV RNA was detected in cryoglobulin. Transaminases were normal.

An allograft biopsy was performed and showed lesions compatible with MPGN. Immunofluorescence staining showed granular IgM and C3 and trace deposits of C1q and IgG within the basal membrane. C4d staining was negative.

There were no other organ manifestations of cryoglobulinaemia. Immunosuppressive treatment was started with methylprednisolone pulses (500 mg/day for three days) followed by a rapid taper to 20 mg/day of oral prednisolone in association with intravenous Rituximab 375 mg/m<sup>2</sup> every 3 weeks (2 infusions). Two months after the last dose of rituximab, hypertension resolved, serum creatinine improved (1.27 mg/dl), proteinuria decreased (2g/day), serum C3 levels returned to normal while C4 levels had a discreet improvement (51 mg/dl; 9.0 mg/dl, respectively), rheumatoid factor became negative, cryoglobulins were cleared and HCV RNA remained undetectable. There were no infectious complications during the treatment with rituximab. After the last infusion, the patient restarted use of cocaine and was lost for follow-up.

## ■ DISCUSSION

Treatment of HCV-related MPGN associated with type II CG in a renal transplant patient is a complex issue and remains a challenge. The management is critical and the main purpose is to improve long-term allograft survival. The reported rate of recurrence of MPGN in this group of patients varies from 20-30% and the incidence of graft loss may exceed 15%<sup>10</sup>. The main goal of anti-retroviral therapy before transplantation is to achieve sustained viral response, because the risk of hepatic and extra hepatic complications is reduced. The HCV is an independent risk factor for graft loss and proteinuria after transplantation<sup>4,5,12</sup>. Higher HCV viral loads may result in more immune complexes and increased deposition of viral complexes in the kidney and a higher risk for HCV-related nephropathy<sup>1,13</sup> on the contrary, a sustained virological response (serum HCV-RNA undetectable) may reduce post-transplant recurrence of HCV

glomerulonephritis, as well as chronic allograft nephropathy<sup>1,13,14</sup>. Anti-HCV antibodies and HCV RNA have been detected in the great majority of patients with mixed cryoglobulinaemia, and the concentration of HCV RNA in cryoprecipitate is 1000 times higher than in serum<sup>7,15</sup>.

The treatment of this entity is a complex, non-consensual issue and represents a challenge to clinicians. The general initial approach includes immunosuppression with corticosteroids in association with cyclophosphamide or Rituximab or/and plasmapheresis, followed by treatment of the underlying disease. Antiviral therapy is not routinely recommended in a renal transplant patient because of concerns regarding allograft rejection. According to KDIGO clinical practice guidelines of 2008, treatment with interferon should be reserved to patients with fibrosing cholestatic hepatitis or life-threatening vasculitis<sup>16</sup>. Ribavirin monotherapy seems to improve liver enzymes level, but is not associated with beneficial effect on liver histology neither in virological clearance. The IFN in combination with ribavirin is effective in two-thirds of patients after a minimum therapy of six months, but it is poorly tolerated and results in graft dysfunction in a significant number of patients<sup>16,17</sup>.

Protease inhibitors (telaprevir and boceprevir) are emerging as a third feature of combination therapy with peg INF- $\alpha$  and ribavirin. Two major trials (SPRINT- 2 and ADVANCE), demonstrated the efficacy in achieving higher sustained viral response (75% – telaprevir; 69% – boceprevir) in patients with genotype 1<sup>18,19</sup>. To date, there are no studies of this therapy in renal transplant and randomized controlled trials are needed<sup>20</sup>. Its use is not recommended in patients with renal transplant.

The use of Rituximab (the monoclonal anti-CD 20 antibody) therapy in the setting of renal transplantation is multiple and includes desensitization and ABO-incompatible transplantation, treatment of humoral rejection, post-transplant lymphoproliferative disorders and recurrent or *de novo* glomerular diseases. There have been several case reports and studies showing the effectiveness of Rituximab in the treatment of glomerular diseases, however, more randomized studies are needed<sup>7,8,12,21,22</sup>.

We describe a case of membranoproliferative glomerulonephritis associated with type II cryoglobulinaemia in a renal transplant patient with hepatitis C. The association of this virus with mixed cryoglobulinaemia in our

case may be explained by the presence of circulating anti-HCV antibodies, and the presence of HCV-RNA in the cryoprecipitate, suggesting that HCV exerted a pathogenic role in the formation of cryoglobulins. This diagnostic was surprising since the patient presented with sustained negative viral load, but there are other reported cases in the literature of HCV-related glomerulonephritis in patients with undetectable HCV-RNA<sup>23</sup>.

In our case, the patient presented with a severe renal disease that demanded a more aggressive approach. Recently Rituximab had been used in a few studies of cryoglobulinaemic MPGN associated with HCV infection and the results were encouraging. The IgM autoantibody producing B lymphocytes appears to be depleted preferentially, which may explain the efficacy of this drug in this disease<sup>24</sup>. Rituximab seems to be as least as efficient as cyclophosphamide, is also better tolerated and, in contrast to cyclophosphamide, does not enhance HCV replication<sup>7</sup>. For these reasons we decided to treat the patient with this drug. The treatment with Rituximab allowed an improvement in renal function. However, a longer follow-up period post-therapy (12 months) is needed to evaluate clinical response, since the relapse rate may be high. Rituximab therapy can induce a clinical response in 80% of patients (not transplanted) for refractory mixed cryoglobulinaemia secondary to hepatitis C virus infection<sup>22</sup>. The schedule and dosage recommended for this drug in renal transplant is not yet defined. The EUDRACT study (that did not include renal transplant patients)<sup>22</sup> demonstrated that the low dose regimen (2 weekly doses of 250 mg/m<sup>2</sup>) is as efficacious as the high dose regimen (4 weekly doses of 350 mg/m<sup>2</sup>) and is associated with less complications<sup>22</sup>.

**Conflict of interest statement:** None declared.

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