Rituximab induces regression of hepatitis C virus-related membranoproliferative glomerulonephritis in a renal allograft

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\section*{Introduction}

Hepatitis C virus (HCV) infection complicates clinical outcome in liver and renal allografts [1]. Besides its important contribution to chronic liver disease, HCV infection is also a relevant cause of \textit{de novo} immune-mediated glomerulonephritis in both kidney and liver transplantation [2,3]. It has been shown that the development of \textit{de novo} membranous and especially membranoproliferative glomerulonephritis (MPGN) in renal allografts is strongly associated with pretransplant HCV-positive serology. It has also been demonstrated that type I MPGN is mediated by a very low level of nephritogenic type II cryoglobulins containing HCV-RNA [4]. Those lesions induce an accelerated loss of the graft [5].

Antiviral treatment for HCV-infected renal transplant candidates with interferon-\(\alpha\) (IFN-\(\alpha\)) prior to transplantation is strongly recommended, since the clearance of HCV-RNA is beneficial for post-transplant liver disease [6] and to prevent HCV-related glomerulonephritis [7]. Nevertheless, 30–50\% of patients do not tolerate or do not respond to IFN-\(\alpha\), thus continuing at risk for HCV-related glomerulonephritis. Furthermore, there is neither an effective nor a safe therapy for HCV-related glomerular lesions after transplantation; ribavirin-based therapy has shown some severe adverse events [8] and Interferon-alpha (INF-\(\alpha\)) can induce graft dysfunction or rejection [9]. Hence, it is important to explore new therapeutic approaches to treat this deleterious complication.

In this article, we report a case of HCV-related MPGN in a renal allograft treated with anti-CD20 monoclonal antibodies. We describe for the first time regression of glomerular subendothelial immune deposits and we discuss the virological and immunological changes induced by this treatment.

\section*{Clinical report}

In 1995, a 58-year-old woman was transferred to our transplant centre in order to receive a third renal allograft. She arrived with a summarized medical history of chronic renal failure caused by MPGN, having received the first renal allograft in 1981. A year later, in August 1982, she returned to haemodialysis because of chronic allograft nephropathy. In February 1983, she received a second renal allograft. Eleven years later, she developed severe proteinuria and progressive renal failure. A renal biopsy was performed, showing chronic allograft nephropathy and transplant glomerulopathy. Four months later, she restarted haemodialysis. At that moment, she arrived at our institution in order to receive the third allograft.

She was then diagnosed with HCV infection because of mild and persistent transaminitis (qualitative HCV-RNA). Unfortunately, neither the previous histological samples nor the virological parameters of the patient were available in order to reanalyse them more deeply.

Subsequently, a hepatic biopsy was performed, showing isolated steatosis; so INF-\(\alpha\) was not indicated. In 1998, she received the third renal transplant. Immunosuppression resulted from tacrolimus, mycophenolate mofetil (MMF) and steroids. Prednisolone was withdrawn after 1 year because of severe osteopenia. Five years later, nephrotic proteinuria (4.3 g/day), microhaematuria and hypoalbuminaemia appeared. Immunological, virological and histological studies were performed as described [4]. Relevant analytical findings are summarized in Table 1. Cryoglobulins (cryocite 5\%, IgM-\(\kappa\) monoclonal,
Table 1. Clinical and virological data

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 months</th>
<th>8 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryocrite (%)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Rheumatoid factor (Kint.u./l)</td>
<td>45</td>
<td>14.6</td>
<td>28</td>
<td>37</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>74</td>
<td>ND</td>
<td>76</td>
<td>69</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td>4.4</td>
<td>ND</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>C3PA (mg/dl)</td>
<td>28</td>
<td>ND</td>
<td>42</td>
<td>49</td>
</tr>
<tr>
<td>IgG (g/l)</td>
<td>5.2</td>
<td>4.4</td>
<td>3.37</td>
<td>5.73</td>
</tr>
<tr>
<td>IgM (g/l)</td>
<td>0.68</td>
<td>0.94</td>
<td>0.67</td>
<td>0.50</td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>0.48</td>
<td>0.81</td>
<td>0.86</td>
<td>0.41</td>
</tr>
<tr>
<td>ALT (µkat/l)</td>
<td>0.62</td>
<td>0.42</td>
<td>0.49</td>
<td>0.45</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>170</td>
<td>200*</td>
<td>150</td>
<td>184</td>
</tr>
<tr>
<td>Proteinuria (g/d)</td>
<td>4.2</td>
<td>0.76*</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Viral load (×10^6 UI/ml)</td>
<td>478</td>
<td>0</td>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>2.1</td>
<td>0</td>
<td>5.6</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Normal ranges are: rheumatoid factor (<16), C3 (75–140), C4 (10–34), C3PA (20–45), IgG (7–14), IgM (0.4–2.49), IgA (0.7–3.7), ALT (0.63), Creatinine (<86)

*R after recovery from radiocontrast-induced acute renal failure.

ND, not determined.

IgG polyclonal) and complement activation by the classical pathway were present. HCV (genotype 1b) was found in serum and in cryoprecipitate. A renal allograft biopsy was done, producing 10 glomeruli, showing duplication of the glomerular basement membrane with subendothelial deposits (Figure 1A). Significant mesangial glomerular hypercellularity was also present (Figure 1A, C). Signs of chronic allograft nephropathy (Banff grade I) were also evident. Immunofluorescence microscopy showed granular staining of IgM and C3a along the glomerular capillary walls (Figure 1E). Mild C4d staining in glomerular capillaries was also present. It is noteworthy that C4d staining in peritubular capillaries was negative. The electron microscopy study corroborated subendothelial electron-dense deposits (Figure 1G), without ultrastructural evidence of transplant glomerulopathy. There were other organ manifestations of systemic cryoglobulinaemia. Therefore, diagnosis of HCV-related type 1 MPGN was made.

Rituximab treatment was proposed to the patient, who gave written informed consent. After the approval of The Ministerio de Sanidad and our local Ethics Committee, an intravenous infusion of 375 mg/m² of rituximab (MABTHERE®, Roche, Switzerland), was given once a week for four consecutive weeks. Two months after the last rituximab dose, the patient suffered from a chest pain episode. Cardiological studies included a coronarography, leading to radiocontrast-induced acute renal failure requiring haemodialysis. An echocardiography was performed, showing mild pericardial effusion with a severe mitral regurgitation but preserved left ventricular function. The final diagnosis was idiopathic pericarditis. In order to minimize renal damage and to try to recover renal function, tacrolimus was withdrawn, yielding to progressive renal function recovery (Table 1).

There was also a progressive clinical and biological remission of the nephrotic proteinuria without the elevation of transaminases. Cryoglobulins disappeared and complement fractions normalized. Lymphocyte subset monitoring showed that B-lymphocytes (CD20+ and CD19+) were sustainly depleted during 1 year (Figure 2, Table 2) when B-cells reappeared.

After 1 year of follow-up, renal function worsened and proteinuria increased. Plasma type II cryoglobulins, rheumatoid factor and complement activation by the classic pathway appeared again. A new renal biopsy was performed, showing glomerular basement membrane 'double contours' (Figure 1B and D) with negative immunohistochemical results for IgG, IgM, IgA and C3. C4d deposition in glomerular and, importantly, in peritubular capillaries was present (Figure 1F). Electron microscopy revealed reduplication of the glomerular basement membrane with a subendothelial electron-lucent zone (transplant glomerulopathy, Figure 1H). Also, a Banff borderline acute rejection was diagnosed. Anti-human leucocyte antigens (Anti-HLA) antibodies were investigated (Flow PRA, One Lambda Inc. CA, USA). Before rituximab treatment, the antibodies against class I major histocompatibility complex (MHC) molecules were present reacting to 12% of beads, increasing to 21% after the treatment, whereas the antibodies recognizing class II MHC molecules were initially negative and only detected (5%) at 1 year (Table 2). The cellular acute rejection was treated with three pulses of 500 mg of methylprednisolone. Renal function partially recovered although with persistent proteinuria. Afterwards, renal function declined progressively and finally, 3 months later, haemodialysis therapy had to be restarted due to severe congestive cardiac insufficiency and irreversible graft failure.

Discussion

In renal transplantation, diagnosis of HCV-related MPGN is associated with poor graft outcome. Although there have been several attempts to treat this glomerulonephritis, solely, pre-transplant IFN-alpha administration has demonstrated benefit [7]. Indeed, most transplant physicians have had frustrating experiences either with HCV-related graft loss or waiting for graft loss in order to safely initiate IFN-alpha. Thus, treatment of HCV-related MPGN remains an old challenge for transplant teams.

Oligoclonal non-neoplastic B-cell proliferation appears to be the key feature of HCV-related and non-related mixed cryoglobulinaemia [10,11]. Probably, HCV-lymphotropism causes production of autoantibodies such as monoclonal rheumatoid factor as well as non-Hodgkin’s B-cell lymphoma. This feature has led us to investigate new therapeutic strategies in order to reduce or deplete the B-cell clonal expansion. Rituximab is a humanized murine monoclonal antibody (IgG1κ), directed against CD20 antigen, a transmembrane protein present during different
steps in the maturation of B-lymphocytes [12]. Although primary indication for rituximab is non-Hodgkin’s lymphoma [13], encouraging results have been observed in some autoimmune diseases [14,15] and in essential mixed cryoglobulinaemia [16–18], even resistant to conventional treatment [19]. In renal transplantation, rituximab has also been proved to be an effective and safe therapy for refractory acute humoral rejection [20] and to prevent acute rejection in highly sensitized patients in the waiting list [21,22], being an encouraging new therapy for antibody-mediated pathology with a safe profile. Furthermore, rituximab therapy is also useful in transplanted patients with post-transplant lymphoproliferative disease [23]. Therefore, rituximab could theoretically offer a therapeutic opportunity to renal-transplant patients with HCV-related MPGN since, on one hand, rituximab has shown efficacy in essential mixed
cryoglobulinaemia and HCV-related MPGN in non-transplanted patients while, on the other hand, encouraging experiences have also appeared in renal transplantation. This background let us test rituximab in our patient.

Recently, one case of de novo HCV-related MPGN and two cases of essential mixed cryoglobulinaemia recurrence after renal transplantation treated with rituximab therapy were reported by Basse et al. [24]. The authors described a 4-month follow-up after rituximab administration. Interestingly, they showed clinical and biological regression of parameters such as proteinuria and cryoglobulinaemia, as previously described in non-transplanted patients [16,17]. However, unexpected severe infectious complications appeared in two cases; they speculate that rituximab, together with xenobiotic immunosuppressants that target T- and B-cell functions, may induce a defect in the response to recall antigen humoral immunity, yielding to an increased risk of life-threatening infections. In previous experiences using rituximab in renal transplantation, as well as in our case, no major infectious diseases have been described. Nevertheless, we cannot exclude that, in our case, reduction of baseline immunosuppression could contribute to avoid infectious complications.

Some authors are reluctant to use rituximab in patients with viral hepatitis because of the initial reports of fulminant hepatitis after rituximab treatment in hepatitis-B infected patients [25]. Sansonno et al. [16] found increased serum HCV-RNA after rituximab treatment, in concordance with disappearance of cryoglobulins. This enhanced HCV-viraemia could reflect virus shedding through rituximab-induced B-cell cytotoxicity or even a decrease of the immune pressure on HCV. However, other authors [18] did not find any change in HCV viral load and IgG titers after treatment with rituximab, despite effective clinical response. These discrepancies let us investigate whether rituximab could aggravate liver disease and increase HCV-viraemia in our patient. In our case, serum transaminases were within the normal range during the whole 1-year follow-up. Also, before treatment, HCV viral load was mainly concentrated in the cryoprecipitate, as previously described [4]. Rituximab induced an initial and transient slight reduction of viraemia although, after a period of 1 year, in coincidence with the recurrence of cryoglobulinaemia, most of the virus was again captured in the cryoprecipitate. Thus, 4-dose rituximab seems not to increase the HCV replication in our patient.

After treatment, cryoglobulins disappeared, rheumatoid factor activity reduced and activation of the classical complement’s pathway vanished. The clinical response paralleled the immunological one. Thus, reduction of proteinuria and renal function stabilization was observed for 8 months. However, at 1 year there was a recurrence of cryoglobulinaemia together with renal function deterioration, and so a new renal allograft biopsy was done. No features of HCV-related MPGN were observed, although there were histological signs of transplant glomerulopathy and Banff borderline acute rejection with C4d staining in glomerular and peritubular capillaries. The new renal findings were thus consistent with both chronic humoral and acute cellular rejections. Accordingly, we found circulating anti-HLA antibodies, especially recognizing class I MHC molecules that probably account for the C4d staining in peritubular capillaries. Some experimental data suggest that anti-CD20 monoclonal antibodies may influence T-cell responses. Selenko et al. [26] reported that treatment with anti-CD20 not only kills lymphoma cells but also promotes uptake and cross-presentation of lymphoma-cell-derived peptides by antigen-presenting dendritic cells, inducing maturation and allowing generation of...
specific cytotoxic T-lymphocytes. Moreover, treatment with anti-CD20 can improve in vitro mitogenic responses with phytohaemagglutinin and challenge antigens as tetanus toxoid and allogeneic stimulus [27]. A similar mechanism could contribute not only to the clearance of HCV, promoting the activation of specific antiviral cytotoxic T-lymphocytes, but also to allograft rejection. However, we cannot exclude the fact that tacrolimus withdrawal had played a role, at least in part, in these effects. We can speculate that rituximab treatment in our patient could promote overexpression of HLA molecules, which could secondarily enhance anti-HLA alloantibody secretion. Indirect evidence of T-cell activation after rituximab treatment was provided by the observation of an important increase in activated non-B-, DR+ peripheral lymphocytes.

Besides all these virological and immunological findings, there was the noteworthy evidence that cryoglobulin-mediated glomerular deposits completely disappeared after anti-CD20 treatment. This renal structural change was consequently translated into clinical and biological effects, as regression of the nephrotic syndrome was reached. Therefore, this new therapeutic approach, although with caution, opens a brand-new opportunity to these patients, whose graft-survival expectation has been deeply impaired until now.

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Conflict of interest statement. None declared.

References


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