

Treatment of hepatitis C-virus-related glomerulonephritis

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Membranoproliferative glomerulonephritis (MPGN) associated with type II cryoglobulinemia is the predominant type of hepatitis C virus (HCV)-related glomerulonephritis. The blockade of the renin-angiotensin system, as well as a combined anti-HCV therapy that associates standard or pegylated α -interferon with ribavirin, are mandatory in all patients experiencing an HCV-related glomerulonephritis. In patients with nephrotic-range proteinuria and/or progressive renal failure, immunosuppressive therapy is necessary. Rituximab, the monoclonal anti-CD20 antibody that selectively targets the B cells, seems to be as least as efficient as cyclophosphamide. Because it is also better tolerated, it should be preferred to cyclophosphamide. During the acute phase, plasmapheresis and steroid pulses can be used. However, future prospective, controlled, and randomized studies are still required to establish evidence-based guidelines to treat HCV-related glomerulopathies.

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Hepatitis C virus (HCV) infection leads to chronic liver disease, but also to extra-hepatic manifestations.¹ These include mixed cryoglobulinemia, lymphoproliferative disorders and renal disease. HCV infection has been reported in association with distinct histological patterns of glomerulonephritis in native kidneys. Membranoproliferative glomerulonephritis (MPGN) associated with type II cryoglobulinemia is the predominant type of HCV-related glomerulonephritis.² Less common glomerulonephritis have also been reported in HCV-infected patients. Of these, MPGN without cryoglobulinemia,³ membranous glomerulonephritis,³ focal segmental glomerular sclerosis,⁴ proliferative glomerulonephritis,^{3,5} renal thrombotic microangiopathy associated with anticardiolipin antibodies,⁶ and fibrillary and immunotactoid glomerulopathies.⁷ In this mini-review, we focus on the treatment of HCV-related cryoglobulinemic glomerulonephritis.

CRYOGLOBULINEMIC MPGN

More than 80% of patients with mixed cryoglobulinemia are infected by HCV, and cryoglobulinemia is found in all patients with HCV-related MPGN.² Cryoglobulins are immunoglobulins (Igs) that precipitate at cold temperature. HCV-associated cryoglobulinemic glomerulonephritis seems to be related to the deposition in the glomerulus of immune complexes made by the HCV antigen, anti-HCV Ig G antibodies, and a rheumatoid factor, which is an IgM kappa. Clinically, patients present with proteinuria and microscopic hematuria. Nephrotic syndrome and acute nephritic syndrome, with rapid deterioration of renal function, are observed in, respectively, 20 and 25% of patients.^{2,8} Fifty percent of patients have moderate renal insufficiency,² and hypertension is present in 80% of patients.⁸ HCV-related cryoglobulinemic renal disease is often associated with extra-renal manifestations. The most frequently observed are purpura, arthralgia and peripheral neuropathy.⁹

Laboratory parameters reveal the presence of circulating cryoglobulins, which are most commonly type II cryoglobulins in which the rheumatoid factor is an IgM kappa. The complement components, C4 and C1q, are usually low. Serum C3 level is also moderately decreased. Serum anti-HCV antibodies and HCV RNA are detected in both the serum and the cryoprecipitate. HCV RNA concentrates in cryoprecipitate (1000-fold higher than in the serum).

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Histological examination of kidney biopsies usually reveals the presence of glomerular infiltration by activated macrophages. The glomerular basement membrane shows double contours, which are caused by the interposition of monocytes between the basement membrane and the endothelium. Immunofluorescence exhibits subendothelial deposits of IgM, IgG, and the complement components. On electron microscopy, large subendothelial deposits are present. Vasculitis of small renal arteries is present in 30% of cases. In most cases, the renal prognosis is good;⁸ however, renal disease remain the first cause of morbid-mortality in mixed cryoglobulinemia.⁸

TREATMENT OF HCV-RELATED GLOMERULONEPHRITIS

Symptomatic therapy

Blood pressure control, diuretics, the blockade of the renin-angiotensin system using either angiotensin-converting enzyme inhibitor alone or combined with an angiotensin receptor antagonist, as well as the treatment of hyperlipidemia, are of proven benefit.¹⁰

Anti-HCV therapy

Because a link has been established between HCV infection and the occurrence of cryoglobulinemic MPGN, an anti-viral therapy has been used in HCV-positive patients presenting with a glomerulonephritis in order to achieve clearance of HCV from the serum and, consequently, to have a beneficial effect on renal injury. In the early 1990s, standard α -interferon (α -IFN) has been used alone. Unfortunately, the results were disappointing. In 15 patients who had a complete clearance of HCV RNA after α -IFN therapy, Misiani *et al.*¹¹ reported an improvement in renal function. In contrast, there was no effect on proteinuria. All patients relapsed after α -IFN therapy was stopped. Later, Johnson *et al.*⁵ reported the results of a prospective uncontrolled study where 14 patients experiencing an HCV-related glomerulonephritis were treated with α -IFN for 6–12 months. Overall, proteinuria significantly decreased, whereas renal function remained stable. In 11 patients, sera were tested for HCV RNA while on this therapy. Patients who became cleared of HCV RNA ($n=6$) had a better outcome compared to those who remained HCV RNA positive ($n=5$). However, virological and renal relapses were observed after completing the therapy. In this study, the use of oral prednisone, in addition to α -IFN, in five patients had no effect on renal function. In contrast, steroid pulses had a beneficial effect in two patients. Finally, the use of cytotoxic agents, with or without plasma exchange, was associated with a high rate of death and a flare up in HCV viremia.⁵

During the last few years, a combined therapy of α -IFN, especially pegylated IFN, with ribavirin has become the golden standard of HCV treatment because it has been found to be more effective than α -IFN alone. This has prompted physicians to treat HCV-related glomerulonephritis with this combination. However, published case reports and uncontrolled studies have only included small numbers of patients

so far. In a prospective uncontrolled study, Sabry *et al.*¹² treated 20 patients presenting with MPGN ($n=17$), membranous glomerulonephritis ($n=2$) and mesangioproliferative glomerulonephritis ($n=1$) with α -IFN and either with or without ribavirin. All patients were given α -IFN 3 million units (MU) three times weekly. In cases of persistent HCV RNA at 3 months, ribavirin was added at the daily dose of 15 mg/kg. The treatment was continued for 12 months. Four out of the 20 patients became HCV RNA negative within the first 3 months and, consequently, did not receive ribavirin therapy. Only one out of the 16 remaining patients, who additionally received ribavirin, became cleared of HCV RNA within the serum. Seven patients underwent a ribavirin dose reduction because of adverse events, mainly hemolytic anemia. Overall, both HCV RNA concentration and proteinuria decreased significantly. Serum albumin level, as well as both C3 and C4 levels, increased significantly. Renal function remained stable. In this study by Sabry *et al.*, no data are provided regarding the outcome of renal disease after cessation of anti-HCV therapy. In a retrospective study, Bruchfeld *et al.*¹³ report on their first treatment with concentration-controlled ribavirin plus α -IFN therapy in HCV-related glomerular disease. The intended trough ribavirin plasma concentration was 10–15 mmol/l. Four patients received standard α -IFN, two received pegylated α -IFN and ribavirin, and one patient received ribavirin monotherapy because of bad tolerance of α -IFN. Five of the patients had a sustained virological response 6–32 months after anti-viral therapy was stopped. One patient relapsed 3 months after completing therapy, whereas one patient who was on ribavirin monotherapy did not had a virological response. Serum albumin level normalized in all patients. Proteinuria decreased in all patients. Glomerular filtration rate improved in three patients and remained stable in four other patients. Despite monitoring ribavirin plasma concentration, the main side effect observed was ribavirin-induced hemolytic anemia, which required ribavirin dose reduction, low-dose iron and systematic erythropoietin support. Later, Rossi *et al.*¹⁴ reported a decrease in proteinuria and an improvement in renal histology.

More recently, we treated 18 patients who had HCV-related cryoglobulinemic MPGN with a combined therapy of standard or pegylated IFN and ribavirin.¹⁵ After a symptomatic treatment of nephrotic-range proteinuria by furosemide, with or without angiotensin-converting enzyme inhibitors, and with or without plasma exchange that was or was not associated with steroids, 18 patients received anti-HCV therapy, whereas seven other patients did not receive any anti-viral therapy. Fourteen out of the 18 were treated with standard α -IFN 3 MU three times weekly plus ribavirin at 600 to 1000 mg/day, and the four other patients received pegylated IFN at 1.5 μ g/kg weekly with the same dose of ribavirin. The mean duration of anti-HCV therapy was 18 ± 10 months (range 6–24 months). The mean duration of follow-up after completing anti-HCV treatment was 16.7 ± 17.7 months (range 6–30 months). A sustained

virological response was observed in 67% of patients. Pegylated IFN was given in three virological responders and one non-responder. After anti-HCV treatment, proteinuria and cryoglobulins levels decreased. In addition, serum albumin level increased significantly in virological responders compared to non-responders who were receiving the combination therapy as well as the control patients who were receiving any anti-viral treatment. In contrast, serum creatinine level remained stable in all three groups. Our data suggest that the anti-viral therapy should be given for a long period. We recommend to treat the patients for at least 48 weeks, and to continue the anti-viral therapy even in the absence of a decrease in HCV RNA concentration of 2 log at week 12. After HCV RNA clearance, cryoglobulinemia persists for a long period.

Despite the absence of a complete and sustained virological response, ribavirin monotherapy has been shown to have a beneficial effect on HCV-related glomerulopathy in immunocompetent patients,¹⁶ as well as in renal-¹⁷ and liver-transplant patients.¹⁸ However, in patients with impaired renal function, ribavirin should be given with caution because its clearance is correlated with creatinine clearance.¹⁹ The accumulation of ribavirin induces hemolytic anemia. Severe chronic hemolysis is responsible for iron overload, liver iron deposition and an acceleration in liver fibrosis progression.²⁰

Immunosuppressive therapy

In the past, patients with mixed cryoglobulinemia, with or without renal involvement, were treated by plasma exchange to remove circulating cryoglobulins from the plasma and, consequently, to diminish the deposition of immune complexes in the kidney.⁹ Cyclophosphamide was also used to improve renal disease by suppressing B-lymphocyte stimulation and cryoglobulins production.⁹ Steroid pulses were administered to treat glomerular infiltration abnormalities.⁹ Low doses of oral steroids were also given in some patients.⁹ Previous uncontrolled studies that included small number of patients treated with these therapies showed that this regime often controlled the acute phase of the disease, but was often poorly tolerated. The flare-up of HCV RNA concentration observed during immunosuppressive therapy may be harmful on HCV-related liver disease. A prospective, multicenter, randomized, controlled trial of treatment for HCV-associated glomerulonephritis with steroids and cyclophosphamide, which is followed or not with α -IFN, is ongoing in Italy. Its aim is to evaluate the consequences of increasing HCV replication by immunosuppressive therapy on the efficacy of α -IFN therapy.²

Recently, rituximab, a human-mouse chimeric monoclonal antibody that reacts with the CD20 antigen, thus directly and selectively targeting the B cells, has proved effective and very well tolerated in patients with B-cell non-Hodgkin's lymphomas. Hence, it was used to treat mixed cryoglobulinemia and HCV-related cryoglobulinemic MPGN. Zaja *et al.*²¹ treated patients with rituximab at four weekly doses

of 375 mg/m². Two of these patients, with HCV-related cryoglobulinemic MPGN, were unresponsive to conventional treatments, including α -IFN, plasmapheresis, steroids, and either cyclophosphamide or 2-chlorodeoxyadenosine. A rapid response, and the disappearance of proteinuria and inactive urinary sediment, was observed in one patient who had a recent onset of nephritis. No improvement was noticed in the second patient when rituximab was interrupted after two infusions due to a thrombosis of the retinal artery. In the patient who completed rituximab therapy, HCV RNA concentration showed minimal fluctuations.²¹ Later, Roccatello *et al.*²² treated six patients who had HCV-related cryoglobulinemic glomerulonephritis with rituximab. One patient received the standard four weekly doses, and the five other patients were treated by the standard protocol, plus two additional infusions at 1 and 2 months later. Proteinuria was decreased in all patients. Serum creatinine level decreased in two patients, increased in one patient and remained stable in the three other patients. Interestingly, HCV viral load decreased or remained stable in all patients. Very recently, a renal-transplant patient who was experiencing a *de novo* HCV-related type III cryoglobulinemic MPGN was treated with rituximab therapy: he had a clearance of cryoglobulinemia, a decrease in proteinuria and no change in serum creatinine or HCV RNA.²³ We have also found that rituximab is efficient in treating *de novo* cryoglobulinemic MPGN in HCV-positive or -negative renal-transplant patients, but is associated with a high rate of infectious complications, which might be related to the impairment of T- and B-cell functions that these patients experience.²³ Further controlled randomized studies are required to define the exact indications of rituximab, the dose of rituximab required, as well as its long-term effect on HCV liver disease.

After controlling the nephrotic syndrome by either plasmapheresis or rituximab, a combined anti-viral therapy associating standard or pegylated IFN with or without ribavirin should be set up. The efficacy of the anti-viral therapy after a deep immunosuppression induced by rituximab is not established. Prospective studies using rituximab followed by anti-viral therapy are required.

IN SUMMARY

Cryoglobulinemic membranoproliferative glomeronephritis is the most frequent HCV-related renal injury (Table 1). All patients should be treated with angiotensin-converting enzyme inhibitor in association or not with angiotensin receptor antagonist, as well as with anti-HCV therapy. The latter relies on a combined anti-viral therapy of standard or pegylated IFN and ribavirin for at least 48 weeks. Ribavirin doses should be adapted according to creatinine clearance in order to avoid its main side effect, that is, hemolytic anemia. Combined anti-viral and immunosuppressive therapies may be the treatment of choice for patients with severe renal disease, that is, nephrotic syndrome and/or progressive renal failure, or diseases that are refractory to anti-HCV therapy alone. During the acute phase, plasmapheresis and steroid

Table 1 | Treatment of HCV-related glomerulonephritis

Patients with moderate proteinuria and non-rapid but progressive renal failure:

Symptomatic treatment

Anti-HCV therapy for at least 12 months

Standard α -IFN 3 MU three times/week or pegylated α -IFN 1.5 μ g/kg/week

Ribavirin: dose adapted to the creatinine clearance or to a trough plasma concentration of 10–15 mmol/l with or without erythropoietin support

Patients with nephrotic-range proteinuria and/or progressive renal failure:

Symptomatic therapy: furosemide, ACEI alone or combined with an ARA

Plasma exchange: 3 l of plasma three times/week for 2 or 3 weeks

Rituximab: 375 mg/m²/week for 4 weeks^a or cyclophosphamide:

2 mg/kg/day for 2–4 months

Methylprednisolone pulses: 0.5–1 g/day for three consecutive days

Anti-HCV therapy (see above)

^aAdditional infusions of rituximab might be given in cases of early relapse after conventional therapy.

pulses might be used to, respectively, remove circulating cryoglobulins from the plasma and to treat the glomerular infiltration abnormalities. Even in the absence of controlled randomized studies, anti-CD20 monoclonal antibodies should be preferred to cyclophosphamide. Rituximab was found to be at least as efficient as cyclophosphamide in blocking cryoglobulins production. It is also better tolerated and seems, in contrast to cyclophosphamide, not to enhance HCV replication. Future prospective, controlled and randomized studies are still required to establish evidence-based guidelines to treat HCV-related glomerulopathies.

REFERENCES

1. Cacoub P, Costedoat-Chalumeau N, Lidove O, Alric L. Cryoglobulinemia vasculitis. *Curr Opin Rheumatol* 2002; **14**: 29–35.
2. D'Amico G. Renal involvement in hepatitis C infection: cryoglobulinemic glomerulonephritis. *Kidney Int* 1998; **54**: 650–671.
3. Morales J, Morales E, Andr s A, Praga M. Glomerulonephritis associated with hepatitis C virus infection. *Curr Opin Nephrol Hypertens* 1999; **8**: 205–211.
4. Stehman-Breen C, Alpers CE, Fleet WP, Johnson RJ. Focal segmental glomerular sclerosis among patients infected with hepatitis C virus. *Nephron* 1999; **81**: 37–40.
5. Johnson RJ, Gretch DR, Couser WG et al. Hepatitis C virus-associated glomerulonephritis. Effect of alpha-interferon therapy. *Kidney Int* 1994; **46**: 1700–1704.
6. Baid S, Pascual M, Williams Jr WW et al. Renal thrombotic microangiopathy associated with anticardiolipin antibodies in hepatitis C-positive renal allograft recipients. *J Am Soc Nephrol* 1999; **10**: 146–153.
7. Markowitz GS, Cheng JT, Colvin RB et al. Hepatitis C viral infection is associated with fibrillary glomerulonephritis and immunotactoid glomerulopathy. *J Am Soc Nephrol* 1998; **9**: 2244–2252.
8. Tarantino A, Campise M, Banfi G et al. Long-term predictors of survival in essential mixed cryoglobulinemic glomerulonephritis. *Kidney Int* 1995; **47**: 618–623.
9. Fabrizi F, Colucci P, Ponticelli C, Locatelli F. Kidney and liver involvement in cryoglobulinemia. *Semin Nephrol* 2002; **22**: 309–318.
10. Chadban SJ, Atkins RC. Glomerulonephritis. *Lancet* 2005; **365**: 1797–1806.
11. Misiani R, Bellavita P, Fenili D et al. Interferon alfa-2a therapy in cryoglobulinemia associated with hepatitis C virus. *N Engl J Med* 1994; **330**: 751–756.
12. Sabry AA, Sobh MA, Sheaashaa HA et al. Effect of combination therapy (ribavirin and interferon) in HCV-related glomerulopathy. *Nephrol Dial Transplant* 2002; **17**: 1924–1930.
13. Bruchfeld A, Lindahl K, Stahle L et al. Interferon and ribavirin treatment in patients with hepatitis C-associated renal disease and renal insufficiency. *Nephrol Dial Transplant* 2003; **18**: 1573–1580.
14. Rossi P, Bertani T, Baio P et al. Hepatitis C virus-related cryoglobulinemic glomerulonephritis: long-term remission after antiviral therapy. *Kidney Int* 2003; **63**: 2236–2241.
15. Alric L, Plaisier E, Thebault S et al. Influence of antiviral therapy in hepatitis C virus-associated cryoglobulinemic MPGN. *Am J Kidney Dis* 2004; **43**: 617–623.
16. Hu SL, Jaber BL. Ribavirin monotherapy for hepatitis C virus-associated membranous nephropathy. *Clin Nephrol* 2005; **63**: 41–45.
17. Kamar N, Sandres-Saune K, Selves J et al. Long-term ribavirin therapy in hepatitis C virus-positive renal transplant patients: effects on renal function and liver histology. *Am J Kidney Dis* 2003; **42**: 184–192.
18. Pham HP, Feray C, Samuel D et al. Effects of ribavirin on hepatitis C-associated nephrotic syndrome in four liver transplant recipients. *Kidney Int* 1998; **54**: 1311–1319.
19. Kamar N, Chatelut E, Manolis E et al. Ribavirin pharmacokinetics in renal and liver transplant patients: evidence that it depends on renal function. *Am J Kidney Dis* 2004; **43**: 140–146.
20. Kamar N, Boulestin A, Selves J et al. Factors accelerating liver fibrosis progression in renal transplant patients receiving ribavirin monotherapy for chronic hepatitis C. *J Med Virol* 2005; **76**: 61–68.
21. Zaja F, De Vita S, Mazzaro C et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood* 2003; **101**: 3827–3834.
22. Roccatello D, Baldovino S, Rossi D et al. Long-term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. *Nephrol Dial Transplant* 2004; **19**: 3054–3061.
23. Basse G, Ribes D, Kamar N et al. Rituximab therapy for *de novo* mixed cryoglobulinemia in renal-transplant patients. *Transplantation* (in press).