Persistent Hepatitis C Virus–Associated Cryoglobulinemic Glomerulonephritis in Patients Successfully Treated With Direct-Acting Antiviral Therapy

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INTRODUCTION

Recent estimates suggest that 2.7 million to 3.9 million Americans are infected with hepatitis C virus (HCV). Mixed (type II) cryoglobulinemia can be detected in 25% to 30% of patients with chronic HCV, a minority (10%–15%) of whom develop symptoms of cryoglobulinemic vasculitis (CryoVas).[1] The pathogenesis of HCV-CryoVas involves viral-induced expansion of B-cell clones that produce IgM with rheumatoid factor (RF) activity. Monoclonal IgM binds to polyclonal IgG that recognizes hepatitis C nucleocapsid and core antigens. The resulting circulating immune complexes deposit in vascular beds, leading to complement activation, leukocyte recruitment, and, ultimately, glomerulonephritis or vasculitis.[2] HCV-associated cryoglobulinemic glomerulonephritis (HCV-CryoGN) occurs in 10% to 35% of patients with CryoVas.[1]

Viral eradication with pegylated interferon-α (IFN) and ribavirin was the mainstay of CryoVas therapy for many years[1] and showed variable efficacy in treating CryoGN.[3–7] However, in addition to the larger problem of viral resistance, persistent and/or recurrent HCV-CryoVas occurred in a small subset of patients with sustained virologic response (SVR).[8,9] B-cell depletion with rituximab provided added benefit in patients with CryoGN in whom viral eradication failed to induce clinical remission.[10,11] With the advent of IFN-free direct-acting antiviral (DAA) therapies, SVR rates now exceed 98%, with the added benefit of more favorable dosing schedules and side effect profiles.[12] DAAs also appear to be more effective in inducing SVR in the setting of CryoVas than their IFN-based predecessors.[13] Despite the fact that DAAs lack the immunomodulatory effects of IFN, they have shown promising results in the treatment of CryoVas and CryoGN and, in some cohorts, have even been associated with reduced use of immunosuppressive therapy.[13–18] Although cost concerns have limited broader use, multiple national and international associations have now established CryoVas as a priority indication for DAA therapy.[19,20]

Reports of HCV-CryoGN despite SVR after DAA therapy are rare. Sollima *et al*. reported a series that included 5 patients with CryoGN, only 1 of whom achieved a clinical response despite achieving SVR.[21] Ghosn *et al*. reported 2 patients with newly diagnosed HCV-CryoGN on kidney biopsy approximately 1 year after achieving SVR (1 patient with simeprevir and 1 patient with pegylated INF-α, ribavirin, and sofosbuvir).[22] Chowdhury and Tsen described a patient with recurrent HCV-CryoGN diagnosed approximately 2 months after achieving SVR with sofosbuvir and ribavirin.[23] We report herein 3 cases of biopsy-proven HCV-CryoGN diagnosed in patients who previously had achieved SVR with DAA therapy.

CASE PRESENTATON

Clinical Characteristics

Baseline clinical characteristics prior to DAA therapy are provided in Table 1. All patients had a known history of HCV infection, including 2 patients with genotype 1B and 1 patient with genotype 1A. Two

Table 1. Clinical characteristics of case patients prior to DAA therapy

Characteristic	Patient 1	Patient 2	Patient 3
Age (yr), sex	58, female	68, male	61, male
Year of HCV diagnosis	2015	1998	2004
HCV genotype	1A	1B	1B
Prior HCV therapy	No	PEG-INF + RBV	PEG-INF + RBV
HCV viral load by PCR (IU/ml)	3.6×10^6	3.2×10^6	1.1×10^6
Extrarenal symptoms	None	Arthralgias	None
Cryocrit (type)	Negative	2% (type II)	5% (type II)
Low C4	Yes	Yes	Yes
Positive RF	Yes	Yes	Yes
Serum M-spike	No	Yes (IgM-κ)	Yes (IgM-κ)
SCr (mg/dl)	0.7	2.9	2.6
UPCR prior (g/g)	5.4	3.0	11
Hematuria	Yes	Yes	Yes
Prior kidney biopsy	No	No	Yes (04/2014, MPGN)

DAA, direct-acting antiviral; HCV, hepatitis C virus; MPGN, membranoproliferative glomerulonephritis; PEG-INF, pegylated interferon; PCR, polymerase chain reaction; RBV, ribavirin; RF, rheumatoid factor; SCr, serum creatinine; UPCR, urine protein to creatinine ratio.

patients (patients 2 and 3) had failed prior antiviral therapy with pegylated INF-α and ribavirin. The other patient (patient 1) was treatment naive. All 3 patients had features of immunological activation prior to receiving DAA therapy, including low C4 (3/3), positive RF (3/3), and positive serum cryoglobulin testing (2/3). Two patients had a detectable M-spike on serum protein electrophoresis, which was characterized as IgM-κ on serum immunofixation electrophoresis. All 3 patients had evidence of renal disease prior to receiving DAA therapy, including hematuria and nephrotic-range proteinuria, and 1 patient (patient 3) had undergone a kidney biopsy approximately 5 months prior to completing DAA therapy, the results of which showed HCV-CryoGN and vasculitis. One patient reported arthralgias, but no patient had a history of purpuric skin rash or neuropathy.

Clinical characteristics at the time of the index kidney biopsy (i.e., after completion of DAA therapy) are provided in Table 2. All 3 patients achieved SVR after treatment with ledipasvir + sofosbuvir (Harvoni; Gilead Sciences Inc., Foster City, CA) and had undetectable HCV viral loads at the time of the index kidney biopsy. The time between completion of DAA therapy to kidney biopsy ranged from 2 to 10 months. The duration of SVR preceding kidney biopsy ranged from 38 to 318 days. The median serum creatinine (SCr) at the time of biopsy was 2.8 mg/dl, and all patients had persistent nephrotic-range proteinuria and microhematuria. No patients had extrarenal manifestations of cryoglobulinemic vasculitis at the time of the index biopsy. Two patients (patients 2 and 3) had features of persistent immunological activation after completing DAA therapy, including low C4 (2/3), positive RF (2/3),

Table 2. Clinical characteristics after DAA therapy at time of kidney biopsy

Characteristic	Patient 1	Patient 2	Patient 3
DAA therapy	Ledipasvir + sofosbuvir	Ledipasvir + sofosbuvir	Ledipasvir + sofosbuvir
Interval (mo) between completion of DAA therapy and renal biopsy	2	5	10
In SVR?	Yes	Yes	Yes
Duration of SVR (d)	38	154	318
SCr (mg/dl)	1.2	4.4	2.8
UPCR (g/g)	3.6	3.5	9.0
Hematuria	Yes	Yes	Yes
Extrarenal symptoms	No	No	No
Cryocrit (type)	Negative	3% (type II)	1% (type II)
Low C4	No	Yes	Yes
Positive RF	No	Yes	Yes
Serum M-spike	No	Yes (IgM-κ)	Yes (IgM-κ)

DAA, direct-acting antiviral; RF, rheumatoid factor; SCr, serum creatinine; SVR, sustained virologic response; UPCR, urine protein to creatinine ratio.

or detectable serum cryoglobulin (2/2). An IgM-κ M-spike remained detectable in patients 2 and 3, prompting subsequent hematologic–oncologic evaluation for overt B-cell lymphoma, which was negative in patient 2. A small (<1%) monoclonal B-cell population was identified by flow cytometry performed on bone marrow aspirate in patient 3; however, no overt lymphoma was detected.

Pathology

A summary of the renal biopsy findings is provided in Table 3. All 3 patients exhibited a membranoproliferative pattern of glomerulonephritis (MPGN) characterized by mesangial expansion by increased cells and matrix and duplication of the glomerular basement membrane with cellular interposition (Figure 1). Glomeruli also exhibited mild endocapillary proliferation with infiltrating mononuclear leukocytes and rare neutrophils. Interestingly, large, intracapillary immune thrombi typical of HCV-CryoGN were inconspicuous or absent. The degree of tubular atrophy and interstitial fibrosis varied from mild (2 biopsy samples) to moderate (1 biopsy sample). One biopsy sample showed focal endarteritis. By immunofluorescence, all 3 biopsy samples showed IgM-κ–dominant staining, with weaker-intensity staining for IgG and λ, characteristic of the type II cryoglobulin deposits seen in the setting of HCV infection. Electron microsopy confirmed the presence of relatively sparse mesangial and subendothelial electron dense deposits, which appeared granular and lacked an organized annular tubular substructure. One patient (patient 3) had a kidney biopsy prior to DAA therapy. Notably, both the intensity of staining by immunofluorescence and the burden of detectable

Table 3. Pathology findings

Finding	Patient 1	Patient 2	Patient 3
Total glomeruli/GS glomeruli	36/1	22/5	16/4
Primary LM pattern	MPGN	MPGN	MPGN
Glomerular monocyte infiltration	Mild	Mild	Mild
Cellular/fibrocellular crescents	2	0	0
Segmental scars	1	0	4
Immune thrombi	No	Rare	No
TAIF (%)	20	25	40
Endovasculitis	No	Yes	No
Immunofluorescence			
IgG	1+	Trace	1+
IgM	2+	1+	2+
IgA	Negative	Trace	Negative
C3	2+	1+	2+
C1	2+	Negative	1+
Kappa (κ)	2+	1+	2+
Lambda (λ)	1+	Trace	1+
Electron microscopy	Mes, subendo	Mes, subendo	Mes, subendo
Annular-tubular substructure	No	No	No
Foot process effacement (%)	50	60	95
Other findings	None	Clonal B-cell infiltrate	None

GS, globally sclerotic; MPGN, membranoproliferative glomerulonephritis; Mes, mesangial; subendo, subendothelial; TAIF, tubular atrophy and interstitial fibrosis.

immune deposits at the ultrastructural level had decreased on the kidney biopsy performed after completion of DAA therapy.

The biopsy sample from patient 2 was notable for the presence of an atypical B-cell interstitial infiltrate with lymphoplasmacytic differentiation involving <10% of the cortical parenchyma. By immunohistochemistry, the cells were PAX5 positive and aberrantly expressed bcl-2. *In situ* hybridization highlighted an excess of κ-positive cells (κ:λ ratio, 6:1). Ig heavy-chain gene rearrangement studies performed by fluorescent polymerase chain reaction confirmed the presence of a clonal lymphoid population within the renal parenchyma.

Treatment and Outcomes

Treatment and outcome data are provided in Table 4, and a summary of the patients' clinical courses is presented in Figure 2. All 3 patients achieved SVR. Patient 1 did not receive immunosuppressive therapy. At last follow up 11-months postkidney biopsy, the patient had a persistent immunologic response (defined by the disappearance or marked reduction of circulating cryoglobulins and normalization of RF and C4 levels), serum creatinine had improved from 1.2 to 0.7 mg/dl, and the proteinuria and hematuria had resolved. Patient 2 received a course of rituximab, resulting in a persistent immunologic response, a decline in serum creatinine from 4.4 to 2.5 mg/dl, and a marked reduction in urine protein to creatinine ratio from 3.5 to 0.5 g/g. A repeat kidney biopsy was performed 10 months after the index biopsy, was negative for HCV-CryoGN, and showed chronic changes of mesangial sclerosis, tubular atrophy, and interstitial fibrosis (Figure 1). Patient 3 was treated aggressively with corticosteroids, rituximab, and plasmapheresis, but remained dialysis dependent and, at the time of writing, is awaiting a kidney transplant. Because repeat testing for cryoglobulin titers, C4, and RF were not performed, it is unclear whether this patient achieved an immunologic response after DAA therapy.

DISCUSSION

DAAs are highly effective in the treatment of HCV infection, leading to SVR in the overwhelming majority of patients, but are less consistent in treating CryoVas, with rates of clinical response ranging from 64% to 96% and rates of immunological response (defined by the disappearance or marked reduction of circulating cryoglobulins and normalization of RF and C4 levels) ranging from 48% to 89%.[13–18] Herein we report 3

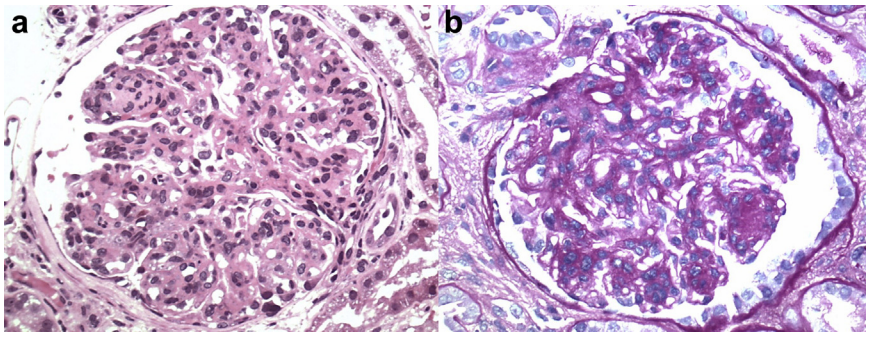

Figure 1. (a) Patient 2 underwent kidney biopsy 5 months after completion of direct-acting antiviral (DAA) therapy. Glomeruli were hyperlobulated owing to increased mesangial cells and matrix, thickening and duplication of glomerular basement membranes, and segmental endocapillary leukocyte infiltration, consistent with a membranoproliferative pattern of glomerulonephritis (hematoxylin and eosin, original magnification ×210). (b) Ten months after completion of DAA therapy, and following a course of treatment with rituximab, patient 2 underwent a second kidney biopsy. Glomeruli exhibited a mild increase in the mesangial matrix, correlating with the history of hypertension and tobacco use, but were normocellular, consistent with the resolution of active glomerulonephritis (period acid–Schiff, original magnification ×210).

Table 4. Treatment and follow-up

Treatment/follow-up	Patient 1	Patient 2	Patient 3
Immunosuppressive therapy	None	RTX	Steroids + RTX + PLEX
Interval (mo) between kidney biopsy and last follow-up	11	10	23
SCr (mg/dl)	0.7	2.5	4.7 (HD-dependent)
UPCR (g/g)	0.13	0.5	4
Hematuria	No	No	N/A
Extrarenal symptoms	No	No	No
Cryocrit (type)	Negative	Negative	N/A
Low C4	No	No	N/A
Positive RF	Negative	Negative	N/A
Serum M-spike	No	No	N/A

HD, hemodialysis; N/A, not available; PLEX, plasma exchange; RF, rheumatoid factor; RTX, rituximab; SCr, serum creatinine; UPCR, urine protein to creatinine ratio.

patients with HCV-CryoGN diagnosed on kidney biopsy after successful completion of DAA therapy, 1 of whom lacked evidence of persistent immune activation but had stable renal disease (patient 1), and 2 of whom had evidence of persistent immune activation and either worsening (patient 2) or stable (patient 3) renal disease. Others have previously reported that persistent (n = 6), recurrent (n = 1), and even *de novo* (n = 2) HCV-CryoGN can occur despite achievement of SVR.[21–23] HCV-CryoGN in the setting of SVR may be an indication for immunosuppressive therapy.

The pathogenesis of HCV-CryoGN involves a number of disturbances in immune homeostasis, particularly the HCV-driven expansion of memory B-cell clones that are responsible for the production of pathogenic IgM with RF activity.[13,24,25] Restoration of peripheral B-cell and T-cell homeostasis appears to be an important factor in achieving clinical remission of HCV-CryoVas and Cryo-GN. Patients who achieve a clinical response with DAA therapy alone have decreased proportions of autoreactive memory B-cell clones, as well as increased numbers of T_{reg} cells and decreased numbers of pro-inflammatory T_h1 and T_h17 subsets.[25] Clinical evidence of immunological response (characterized by the disappearance or marked reduction of circulating cryoglobulins and normalization of RF and C4) also appears to correlate with clinical improvement, and may help to stratify patients at risk for persistent CryoGN despite achieving SVR.[14]

Some studies have shown, however, that immunological improvement may lag behind SVR in the first 12 weeks after DAA therapy.[16,26] Therefore, at least in the early posttreatment phase, longer follow-up may result in higher immunological and clinical response rates in some patients, obviating the need for immunosuppressive therapy. This is illustrated by the clinical course of patient 1, who underwent kidney biopsy only 38 days

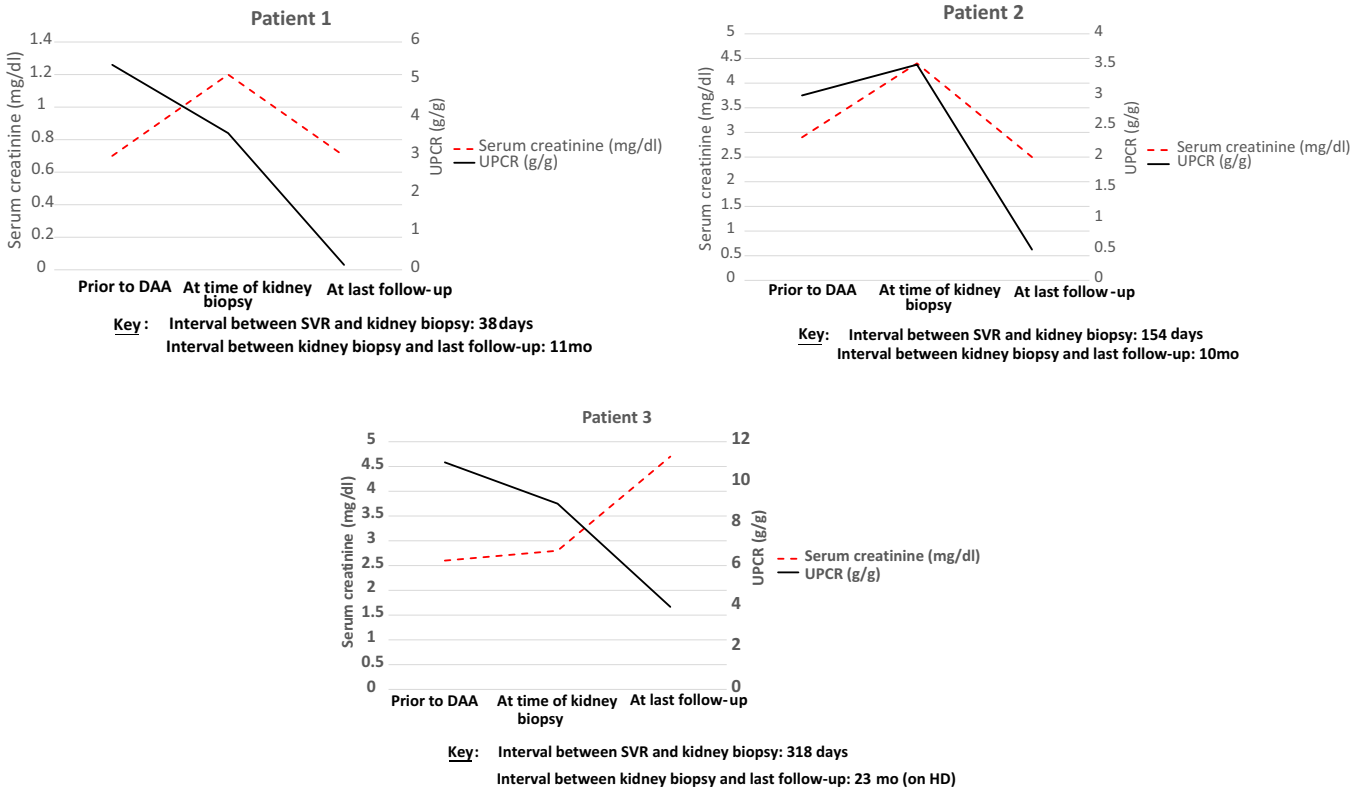

Figure 2. For each of our 3 patients, serum creatinine and urine protein to creatinine ratio (UPCR) are provided at 3 time points: last known values prior to direct-acting antiviral (DAA) therapy, at the time of kidney biopsy, and at last known follow-up. Also indicated in the key is the duration of sustained virologic response (SVR) at the time of kidney biopsy and the duration of follow-up. HD, hemodialysis.

after achieving SVR and lacked evidence of persistent immune activation, and subsequently achieved a complete renal response without requiring immunosuppressive therapy. As such, kidney biopsy may not be necessary in the early posttreatment phase in the absence of rapidly worsening renal indices, and may even lead to unnecessary use of immunosuppression. The other 2 patients, however, showed evidence of persistent immune activation at the time of kidney biopsy, and had clinical and pathologic evidence of severe CryoGN 154 and 318 days after achieving SVR, respectively. Therefore, markers of persistent immune activation later in the posttreatment period appear to correlate with ongoing clinical activity and should prompt kidney biopsy in patients with evidence of renal disease.

Persistent immunological and clinical activity presumably is due to persistence of the RF-producing memory B-cell clones, which have been shown to persist for at least 24 weeks in some patients successfully treated with DAAs.[27,28] In the pre-DAA era, it was shown that resolution of CryoVas after viral eradication was associated with regression of these RF-producing B-cell clones, and that B-cell depletion with rituximab provided added benefit in patients with HCV-CryoVas in whom antiviral therapies alone failed to induce clinical remission.[10,11] DAAs have been shown to be effective in reducing the frequency of RF-producing B-cell clones in the peripheral blood of HCV-infected patients; however, monoclonal populations can persist after viral eradication.[29] It is interesting to note that the 2 patients (patients 2 and 3) with evidence of immunologic and clinical activity both had IgM-κ M-spikes in the serum and identifiable monoclonal B-cell populations (in the renal parenchyma and bone marrow, respectively). Given the lack of evidence of overt lymphoma in these patients, these could be considered as "monoclonal B-cell proliferations of renal significance." Patient 2 responded well to rituximab, highlighting the utility of B-cell–depleting therapy in cases of refractory or *de novo* CryoGN in the setting of SVR.

Additional factors lend complexity to the relationship between DAAs, HCV SVR, and persistent HCV CryoGN. Renal response can lag behind viral clearance due to impaired clearance of cryoglobulin-containing immune complexes, particularly in cirrhotic patients. Multiple studies have demonstrated that HCV-RNA levels in the cryoprecipitate can be up to 100- to 1000-fold higher than in the serum and, in rare cases, may result in false-negative serum polymerase chain reaction for HCV RNA.[30–32] HCV viral particles were detected in the cryoprecipitate of the patient who developed recurrent HCV-CryoGN 2 months after achieving SVR, reported by Chowdhury and Tsen.[23]

Table 5. Teaching points

- The pathogenesis of hepatitis C virus (HCV)–associated cryoglobulinemic glomerulonephritis (HCV-CryoGN) involves the HCV-driven expansion of memory B-cells clones that are responsible for the production of pathogenic monoclonal IgM with rheumatoid factor (RF) activity. Patients who achieve sustained virologic response (SVR) with direct-acting antiviral (DAA) therapy have decreased proportions of these autoreactive memory B-cell clones.
- In a subset of patients, HCV-CryoGN can persist despite achievement of SVR (i.e., in the absence of detectable HCV viremia), likely due to residual RF-producing B-cell clones.
- Clinical evidence of continued immunological activity, characterized by persistent circulating cryoglobulins, RF seropositiviy, and/or low C4, may help to stratify patients who are at risk for persistent CryoGN despite achieving SVR.
- B-cell depletion with rituximab may provide added benefit in patients with HCV-CryoGN in whom antiviral therapies alone fail to induce clinical remission.

This is not a standard laboratory study and was not performed in any of our patients. Therefore, quantitative polymerase chain reaction for HCV-RNA on the cryoprecipitate may be worthwhile in this setting to exclude the much less likely possibility of minimal residual viral replication.

In conclusion, nephrologists and renal pathologists should be aware that HCV-CryoGN can occur in the absence of ongoing viral replication due to persistence of RF-producing memory B-cell clones (Table 5). Evidence of renal disease in the setting of persistent immunological activity (defined by the detectable circulating cryoglobulins, elevated RF levels, and/or depressed C4 levels), even when associated with SVR, should prompt kidney biopsy to assess the need for immunosuppressive therapy. Future studies may help to determine whether longer follow-up will increase immunological and clinical response rates in patients who receive DAA therapy alone, particularly those with renal involvement. Earlier initiation of DAAs, before the occurrence of severe organ damage, may also improve clinical outcomes, and may even eliminate or reduce the need for immunosuppression in some patients.

DISCLOSURE

All the authors declared no competing interests.

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