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Persistent Mixed Cryoglobulinemia Despite Successful Treatment of Hepatitis C, Aggressive B-Cell–Directed Therapies, and Long-term Plasma Exchanges

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INTRODUCTION

Cryoglobulins are Igs that precipitate in cold temperature. Three subsets have been described: type I consists of only monoclonal Ig, usually due to monoclonal gammopathy of undetermined significance, multiple myeloma, Waldenström macroglobulinemia, or chronic lymphocytic leukemia.[1] Type II cryoglobulins contain polyclonal IgG and a monoclonal IgM with rheumatoid factor (RF) activity directed against the IgG, whereas type III contains both polyclonal IgG and IgM RF.[2] Types II and III, also called mixed cryoglobulinemia, result in most cases from a B-cell proliferative process in the setting of persistent immune activation triggered by chronic hepatitis C virus (HCV) infection.[2]

HCV-associated cryoglobulinemia is initiated when HCV binds to the transmembrane protein CD81 on the surface of B cells, inducing clonal memory B-cell proliferation and reducing its activation threshold causing widespread autoantibody production, mostly IgM with RF activity toward anti-HCV IgG.[2,3] In a subset of patients, HCV also induces a gene translocation that prevents B cells from undergoing apoptosis, thus enhancing the clonal proliferation.[2,4] Monoclonal and polyclonal IgM bind to anti-HCV IgG to form circulating immune complexes that can deposit in small vessels, leading to complement activation and leukocyte recruitment, and ultimately clinical glomerulonephritis (GN) or vasculitis.[1]

The treatment of patients with cryoglobulinemic GN comprises different aspects: eradication of HCV infection with direct-acting antiviral agents (DAAs), immunosuppression including corticosteroids, therapies that target B-cell expansion, and plasma exchanges to remove cryoglobulins in severe cases.[1,3] Some patients will display persistent monoclonal B-cell proliferation and relapse despite a sustained virologic response with DAA therapy.[5] This persistent clonal B-cell proliferation can evolve into overt B-cell lymphoma.[2] Recently, Santoriello *et al.*[6] reported 3 cases of relapsing cryoglobulinemic GN after DAA therapy. Two had an identifiable serum M-spike before treatment, and although they did not have overt lymphoma, a monoclonal B-cell population was found in the renal parenchyma and bone marrow on follow-up, coining the phrase "monoclonal B-cell proliferations of renal significance."

We report a case of biopsy-proven refractory mixed cryoglobulinemic GN in a patient with sustained virologic response after DAA therapy for HCV infection, who subsequently developed a persistent serum IgM-κ M-spike only after sustained virologic response, without signs of hematologic malignancy. His clinical relapses and response to various therapies represent an unusual monoclonal gammopathy of renal significance.

CASE PRESENTATION

Clinical History and Initial Laboratory Data

A 47-year-old man presented in March 2015 with marked edema and purpuric rash of the lower extremities, nephrotic-range proteinuria, microscopic hematuria, and increased serum creatinine, which led to an extensive serologic workup and renal biopsy. Baseline clinical and laboratory characteristics are

Table 1. Relevant clinical and laboratory data at diagnosis and during follow-up

Laboratory test	At diagnosis	After HCV treatment (first relapse)	Second kidney biopsy	Last follow-up
Serum creatinine (mg/dl)	2.43	3.77	4.8	2.2
eGFR CKD-EPI (ml/min per 1.73 m^2)	31	18	13	34
Urine protein-to-creatinine ratio (g/g)	7.8	4.8	11.4	5.8
Hematuria	Yes	Yes	Yes	Yes
Hemoglobin (g/l)	118	89	84	96
Albumin (g/l)	19	28	29	43
C3 (g/l)	1.10	0.97	0.41	0.89
C4 (g/l)	< 0.08	0.01	<0.02	0.02
Rheumatoid factor (UI/ml)	147	129	475	571
Serum cryoglobulin	Positive	Positive	Positive	Positive
Serum M-spike (g/l)	Negative	0.7 (IgM-κ)	1.7 (IgM-κ)	0.7 (IgM-κ)
κ light chains (mg/l)	NA	433	447	78
λ light chains (mg/l)	NA	16	21	13
Light chains ratio (κ/λ)	NA	27	21	6
β2-microglobulin (mg/l)	NA	NA	15.2	3.2
HCV viral load/copies (UI/ml)	588 335	Negative	Negative	Negative
HCV genotype	1A	–	–	–
Extrarenal symptoms	Yes (purpura)	Yes (purpura)	Yes (purpura)	Yes (purpura)
HBsAg	Negative	–	–	–
Anti-HBc	Negative	–	–	–
HIV	Negative	–	–	–

anti-HBc, hepatitis B surface antibody; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; NA, not available.
Normal reference values for complement C3 are 0.90–2.30 g/l, and for complement C4 0.15–0.45 g/l.

provided in Table 1. Serologic testing revealed an active HCV infection (genotype 1A), complement activation (low C3 and C4), and positive RF. Serum cryoglobulins were present but no detectable M-spike was initially found on serum protein electrophoresis.

Light microscopy (Figure 1) showed 20 glomeruli with mesangial expansion by matrix and hypercellularity, and double contours of the glomerular basement membrane. Glomeruli also exhibited diffuse endocapillary hypercellularity with numerous infiltrating macrophages and intraluminal pseudothrombi positive to periodic acid–Schiff stain. No vasculitis or crescent was noted. There was no significant interstitial fibrosis or tubular atrophy. Immunofluorescence showed glomerular intraluminal staining for IgG and IgM (2–3+) and κ and λ light chains (2+) (Figure 1). Electron microscopy showed subendothelial and intraluminal electron-dense deposits with a focal microtubular appearance (Figure 1).

Diagnosis

The initial diagnosis was HCV-associated mixed cryoglobulinemic membranoproliferative glomerulonephritis.

Clinical Follow-up and Additional Investigations

The patient received corticosteroids (i.v. methylprednisolone followed by oral prednisone with taper) and a course of rituximab (4 weekly doses of 325 mg/m^2), resulting in a decline in serum creatinine from 2.43 to 1.51 mg/dl (estimated glomerular filtration rate 31 to 54 ml/min per 1.73 m^2) and a marked reduction in urine protein-to-creatinine ratio from 7.8 to 0.4 g/g. He then completed 3 months of antiviral therapy with sofosbuvir + ledipasvir and achieved a sustained virologic response.

The patient relapsed in November 2015, 9 months after the initial presentation (Figure 2). He presented again with progressive lower extremity edema and purpura, and worsening proteinuria and renal function (Table 1, Figure 2). Although HCV RNA remained undetectable in the serum, his RF levels increased and a new IgM-κ M-spike (IgM 0.7 g/L, κ 433 mg/L, κ/λ ratio 27) was detected on serum protein electrophoresis and immunofixation. He received a second course of rituximab (1 g i.v., repeated 2 weeks later), which only resulted in a transient stabilization of his renal function and proteinuria. In early 2016, his renal function and proteinuria deteriorated again, and he gained more than 15 kg of edema despite a maximal dosage of furosemide (Table 1). The rising IgM-κ M-spike (IgM 1.7 g/L, κ 447 mg/L, κ/λ ratio 20) prompted a bone marrow biopsy. The bone marrow aspirate showed no signs of myeloma or B-cell lymphoma, and flow cytometry analysis showed marked B-cell lymphopenia, likely secondary to rituximab received 3 months earlier. A total-body computed tomography scan showed no lymphadenopathy. In light of a glomerular filtration rate of only 13 ml/min per 1.73 m^2, a second renal biopsy was performed showing a persistent membranoproliferative glomerulonephritis pattern of injury with intraluminal pseudothrombi with only discrete fibrosis and tubular atrophy. Immunofluorescence showed again intraluminal staining for IgG, IgM (2–3+), and both κ and λ light chains (2+). Given the sustained virologic response and an identifiable serum M-spike, the final diagnosis was type II cryoglobulinemia membranoproliferative glomerulonephritis secondary to monoclonal gammopathy of renal significance.

The patient was then treated aggressively with corticosteroids, i.v. cyclophosphamide, and plasma exchanges (PLEX) according to American Society for Apheresis guidelines[7] (10 treatments with albumin every 2 days). His condition rapidly improved, his edema subsided, and furosemide was tapered to 80 mg p.o. twice a day. However, as soon as PLEX treatments were stopped, his renal function further deteriorated.

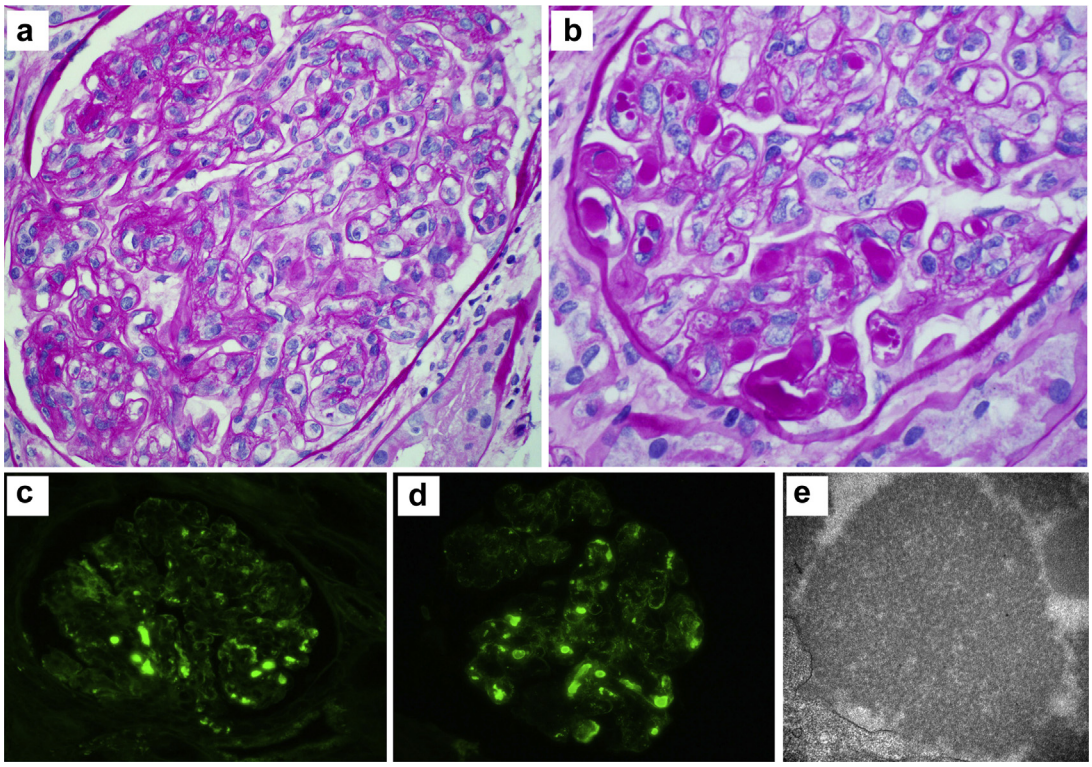

Figure 1. Initial renal biopsy. (a) Membranoproliferative glomerulonephritis pattern of injury and endocapillary hypercellularity by infiltrating monocytes. Periodic acid–Schiff stain, original magnification ×400. (b) Numerous intraluminal periodic acid–Schiff–positive pseudothrombi. Periodic acid–Schiff stain, original magnification ×600. On immunofluorescence, there was bright glomerular intracapillary positivity for (c) IgG and (d) IgM. Intracapillary staining for Kappa, Lambda, C3, and C1q was also present (not shown). (e) Electron microscopy showing intraluminal deposits composed of ill-defined microtubules. Original magnification ×40,000.

A second round of PLEX accompanied with a third cycle of rituximab (4 doses of 375 mg/m^2) was performed in July and August 2016 with a significant but transient improvement in renal function that relapsed as soon as PLEX was stopped. Since September 2016, he has received weekly PLEX. Attempts to space treatments every 10 days have been unsuccessful.

In September 2016, RF reached 1545 UI/ml with an IgM-κ M-spike of 3.3 g/l and a κ/λ ratio of 18. A liver biopsy was performed to eliminate persistent HCV infection, because hepatocytes can act as an HCV reservoir despite absent plasma HCV RNA. Hepatologic parenchyma was normal and HCV RNA was negative on tissue biopsy (316 ng/μl liver RNA analyzed using the Cobas AmpliPrep/Cobas TaqMan HCV qualitative test v2.0; Roche Diagnostics, Basel, Switzerland). A positron emission tomography scan showed no sign of lymphoma. A second bone marrow aspiration and biopsy showed no sign of myeloma or B-cell lymphoma. In collaboration with hematologists, a myeloma-based regimen targeting the progressive IgM-κ clone was attempted. The patient received 2 cycles of bortezomib + cyclophosphamide-dexamethasone regimen, with no response.

Given his resistance to rituximab, cyclophosphamide, bortezomib, and chronic corticosteroid use, azathioprine was added in April 2017. Thiopurine S-methyltransferase activity was tested prior to eliminate a deficiency, which would increase the risk of toxicity. Reactive thiopurine metabolites were monitored to guide therapy dosing and avoid adverse effects due to thiopurine toxicity (targeted 6-thioguanine, levels between 230 and 450 pmol/8 × 10^8 erythrocytes, and 6-methylmercaptopurine, levels <5700 pmol/8 × 10^8 erythrocytes to avoid hepatotoxicity).[8] The azathioprine dose was progressively increased to 350 mg daily (2.8 mg/kg), respecting these thresholds and under this therapy, his glomerular filtration rate stabilized and his RF and proteinuria remained low (Figure 2); however, he never normalized his C4 and remained PLEX dependent, as evidenced by his recurrent purpura. Interestingly, β2-microglobulin, which was initially 15.2 mg/l, fell to 3.2 mg/l at last follow-up (Table 1). During his disease, he experienced only 1 infection treated with oral antibiotics as an outpatient and showed no signs of liver injury.

A trial of bendamustine was attempted with the intention of eliminating the B-cell clone responsible for his RF, as given for Waldenström macroglobulinemia. Azathioprine was withheld immediately before administration. Unfortunately, within a month, his RF increased from 251 to 1462, proteinuria climbed from

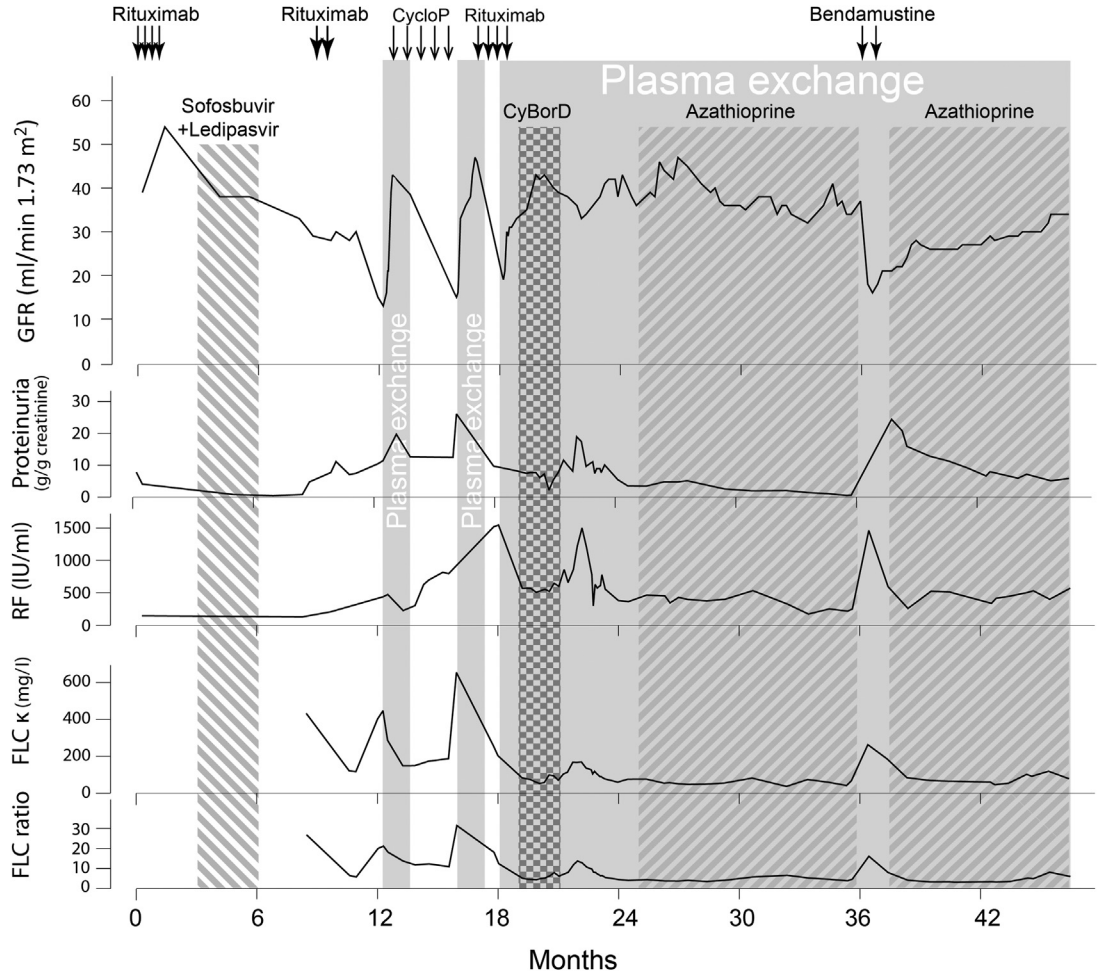

Figure 2. Timeline of the clinical course from diagnosis to last follow-up. CyBorD, bortezomib + cyclophosphamide-dexamethasone; cycloP, cyclophosphamide; FLC, free light chains; GFR, glomerular filtration rate; RF, rheumatoid factor.

0.6 to 24.4 g/g of creatinine, and glomerular filtration rate dropped from 37 to 16 ml/min per 1.73 m^2 (Figure 2). Since this coincided at the moment azathioprine was stopped, we concluded that this drug was effective and restarted it. Nephrotoxicity from bendamustine seemed less likely given the severity of the proteinuria.

DISCUSSION

Here we report a case of biopsy-proven refractory mixed cryoglobulinemic membranoproliferative glomerulonephritis in a patient with sustained virologic response after DAA therapy for HCV infection. The patient showed evidence of persistent cryoglobulins and monoclonal IgM κ RF, suggesting a B-cell clonal disorder independent of the initial viral trigger. Had there been no cryoglobulin with monoclonality, we would have considered a lupus-like immune complex GN, also reported following DAA.[9] Finally, each relapse occurred concomitantly with an increase in serum free κ light chains and RF level, supporting a pathogenic role.

Multiple studies have highlighted the utility of B-cell–depleting therapy such as rituximab in cases of mixed cryoglobulinemic GN, mostly in the setting of HCV, to stop immune complex–mediated organ damage.[1,3] Although our patient initially responded to rituximab therapy, he rapidly became resistant. His dramatic response on 3 separate trials of PLEX (the third one being long-term) emphasizes its efficacy. Unlike free light chains and other Igs, cryoglobulins and IgM are confined to the intravascular space, making them ideal targets for PLEX. These benefits are clearly superior to those observed in other renal diseases, such as antineutrophil cytoplasmic antibody–associated vasculitis and myeloma cast nephropathy.

Underlying hematologic malignant disease has been associated with relapsing type II cryoglobulinemia.[1] The frequent relapses and the progressive IgM-κ M-spike in our patient prompted an extensive hematologic evaluation, but no signs of overt myeloma or B-cell lymphoma were found. Rituximab therapy may have masked a small clone on the first bone marrow biopsy and flow cytometry, but this was unlikely at the

Table 2. Teaching points

- Plasma exchanges efficiently remove cryoglobulins and should be used in severe cryoglobulinemia with renal impairment (MPGN).
- Recurrence of cryoglobulinemia in patients in whom a sustained viral response has been achieved should prompt an exhaustive evaluation for B-cell lymphoproliferation.
- Type II cryoglobulinemia is an MGRS-associated disease.
- Azathioprine can be used in cryoglobulinemic GN. Reactive thiopurine metabolites must be monitored to optimize dosing and avoid adverse effects due to thiopurine toxicity (targeted 6TG levels between 230 and 450 pmol/8 $\times$ 10^8 erythrocytes to reduce hematotoxicity and 6-MMP below 5700 pmol/8 $\times$ 10^8 erythrocytes to reduce the risk of hepatotoxicity).

GN, glomerulonephritis; MGRS, monoclonal gammopathy of renal significance; MPGN, membranoproliferative glomerulonephritis; 6-MMP, 6-methylmercaptopurine; 6TG, 6-thioguanine.

time of the second biopsy. Type II cryoglobulinemic GN is included in the spectrum of monoclonal gammopathy of renal significance–associated renal diseases.[10] Despite its polytypic appearance by immunofluorescence, reflecting the composition of the cryoglobulins, type II cryoglobulinemia is associated with IgM-secreting B-cell lymphoproliferative disorders.[1,2] It is reported that monoclonal diseases are less responsive to conventional immunosuppression and require instead clone-directed chemotherapies.[10] However, our patient also failed bortezomib and bendamustine and yet clearly had a partial response to azathioprine, because his disease reappeared drastically within a month of its discontinuation.

Azathioprine is a derivative of mercaptopurine, and its metabolite 6-thioguanine is responsible of its immunosuppressive effects by blocking purine synthesis. Close monitoring of 6-thioguanine and 6-methylmercaptopurine levels is very helpful to guide therapy dosing and avoid toxicity.[8] Without these measurements, we would have been hesitant to increase the dosage up to 350 mg daily. Nevertheless, chronic use of azathioprine in patients with inflammatory bowel disease has been associated with an increased risk of lymphoma and we are now considering third-generation B-cell–depleting monoclonal antibodies.

In conclusion, cryoglobulinemic GN can relapse despite sustained viral response in the setting of HCV infection due to persistent disorder of memory B-cell clones. Monoclonal IgM-κ protein seems to be responsible for kidney disease in this setting. Combined therapy with immunosuppression and PLEX can help preserve renal function in these patients (Table 2). Many B-cell–targeted therapies exist.[3] With the help of hematologists, nephrologists should consider the different options available to treat dysproteinemias.

DISCLOSURE

All the authors declared no competing interests.

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