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【 CASE REPORT 】

The Successful Treatment of a Case of HCV-associated Cryoglobulinemic Glomerulonephritis with Rituximab, Direct-acting Antiviral Agents, Plasmapheresis and Long-term Steroid Despite Serologically Persistent Cryoglobulinemia

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Abstract:

Novel treatments with rituximab or direct-acting antiviral agents (DAAs) were expected to improve the clinical outcomes of hepatitis C virus (HCV)-associated cryoglobulinemia in the last decade. Recently, however, persistent cases of cryoglobulinemia have been reported, and the ideal approach to treating such cases has not been established. We herein report a case of the successful treatment of HCV-associated cryoglobulinemic glomerulonephritis with rituximab, DAAs, occasional plasmapheresis and long-term steroid, with the patient's renal function and proteinuria improving over the long term despite serologically persistent cryoglobulinemia. This case suggests the efficacy of combination treatment with rituximab, DAAs, occasional plasmapheresis and long-term steroid for persistent cryoglobulinemia.

Key words: cryoglobulinemia, plasmapheresis, rituximab, plasma cell, hepacivirus, glomerulonephritis, membranoproliferative

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Introduction

Cryoglobulins are immunoglobulins that aggregate at low temperatures both *in vitro* and *in vivo*. In the human body, temperatures below normal body temperature cause them to accumulate in various organs (1); this is a disease state known as cryoglobulinemia. The clinical manifestations of cryoglobulinemia are hyperviscosity syndrome and systemic vasculitis. Renal involvement is common and manifests as rapidly progressive glomerulonephritis with membranous proliferative glomerulonephritis as the characteristic histological finding (1). Decades ago, treatments for cryoglobulinemia were similar to those for other forms of systemic vasculitis; in 1991, however, it was shown that about 90% of cases of mixed cryoglobulinemia are associated with hepatitis C virus (HCV) infection (2) and that chronic HCV infection triggers B-cell expansion, which results in the production of cryoglobulins (3).

Rituximab was reported as an efficacious treatment in 2003 (4, 5) and became the recommended immunosuppressive treatment for patients with histologically active HCV-associated glomerular disease who do not respond to antiviral therapy according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines in 2018 (6). In addition, direct-acting antiviral agents (DAAs) have been used for the last decade to improve the clearance of HCV (7). Although these treatments are expected to improve the clinical outcomes of cryoglobulinemia, several cases of treatment failure or relapse after rituximab and DAAs have recently been reported (8, 9).

We herein report a case of serologically persistent cryoglobulinemia in which glomerulonephritis was successfully treated with rituximab, DAAs, occasional plasmapheresis and long-term steroid.

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Case Report

A 68-year-old man was referred to our nephrology department because of progressive renal insufficiency and proteinuria. He had been diagnosed with chronic hepatitis C in his teens but had not been treated for it. He had been diagnosed with diabetes mellitus at 55 years old. He had a history of myocardial infarction at 59 and 63 years old. In addition, one year previously, he had been hospitalized due to worsening depression. One month before the present admission, his serum creatinine level had increased from 1.61 to 2.11 mg/dL. At that time, he had noted bilateral leg edema. He was admitted to our hospital to investigate the cause of his worsening renal function and edema.

On admission, his height and weight were 160.7 cm and 61.7 kg, respectively. He did not notice any weight gain because he did not weigh himself regularly. His vital signs were as follows: blood pressure 153/89 mmHg, pulse 63 beats/min and temperature 36.3°C. A physical examination revealed bilateral leg pitting edema, no purpura, no cutaneous ulcer and no paresthesia, with otherwise normal findings. A complete blood count revealed a hemoglobin concentration of 8.8 g/dL. A laboratory examination showed aspartate aminotransferase of 44 U/L, alanine transaminase of 46 U/L, lactate dehydrogenase of 268 U/L, total protein of 5.0 g/dL, albumin of 2.3 g/dL, creatinine of 2.45 mg/dL and total cholesterol of 213 mg/dL (Table). Occult blood and proteinuria were positive on a urinalysis, and the 24-hour urinary protein excretion was 7.4 g/day. Hemoglobin A1c was 5.9%. Immunological tests revealed negative results for anti-nuclear antibody, anti-DNA antibody, proteinase-3 anti-neutrophil cytoplasmic antibody (ANCA), myeloperoxidase ANCA and anti-glomerular basement membrane antibody. Cryoglobulins were detected through a qualitative analysis, and cryocrit was 5%. Immunoglobulin M (IgM) was elevated to 692 mg/dL (reference range: 27-205 mg/dL). Rheumatoid factor (RF) was also elevated to 2018 IU/mL (reference range: 0-15.0 IU/mL). Complements were suppressed, as follows: complement C3 at 56.3 mg/dL (reference range: 70.5-125.6 mg/dL) and complement C4 at 2.6 mg/dL (reference range: 10.6-33.0 mg/dL). A virologic test showed serotype 1 HCV RNA of 7.0 LogIU/mL.

Based on these findings, the patient was diagnosed with rapidly progressive glomerulonephritis accompanied by nephrotic syndrome.

Renal biopsy findings

A renal biopsy performed on day 6 revealed mesangial expansion, double contour of the glomerular basement membrane in most of the glomeruli and global sclerosis in 30% of the glomeruli (Fig. 1A). Tufts were segmentally ballooned and filled with eosinophilic materials, which were immunohistochemically positive for IgM and thought to be hyaline thrombi (Fig. 1B, C). Immunofluorescent staining was strongly positive for IgG, IgM, and C3 in the glomerular capillary loops. Kappa and lambda light chains were equally positive in the glomerular capillary loops. Electron microscopy revealed abundant electron-dense deposits in the subendothelial areas, with cylinder-like structures visible at high magnification (Fig. 1D, E). Based on these findings, we made a diagnosis of HCV-associated cryoglobulinemic glomerulonephritis.

Clinical course

The clinical course during the patient's hospitalization is shown in Fig. 2. On day 9, he became oliguric, and his serum creatinine level increased to 3.08 mg/dL. Simultaneous hemodialysis and cryofiltration were performed on days 9 and 10. Subsequently, cryofiltration was performed weekly. On day 16, based on the diagnosis of cryoglobulinemic glomerulonephritis, 30 mg of prednisolone was initiated. Subsequently, proteinuria decreased significantly, but marked hyperglycemia appeared, and the patient was started on insulin before every meal. On day 21, glecaprevir-pibrentasvir was started for HCV infection. Because massive proteinuria (around 4 g/gCr) and microscopic hematuria persisted, 500 mg of methylprednisolone (mPSL) was administered for 3 consecutive days starting on day 27, and 375 mg/m^2 of weekly rituximab for 4 weeks was started after that. During the four weeks of rituximab administration, the dose of the oral steroid was tapered week by week. After oral mPSL was decreased to 8 mg, his proteinuria improved to 3.0 g/gCr, and his edema disappeared. HCV-RNA decreased to less than 1.2 LogIU/mL on day 49. He was discharged from our hospital on day 53.

Shortly after discharge (Fig. 3), the patient's proteinuria increased to >8 g/gCr. A test for HCV-RNA reported undetectable levels, and glecaprevir-pibrentasvir was withdrawn after the eight-week treatment. We suspected that cryoglobulin had persisted in his body and was worsening his glomerulonephritis based on his persistent high levels of RF. To treat this, we performed plasma exchange (PE). Although PE removed more than 60% of his cryoglobulins, his RF level increased after PE, and complement C3 continued to be consumed. When mPSL was tapered to 6 mg at 2 months after discharge, proteinuria increased, and PE was performed again. Subsequently he was maintained on 8 mg of mPSL and his proteinuria gradually declined. Six months after discharge, a final session of PE was performed because of the increasing proteinuria and decreasing complement C3.

Over the long-term, HCV-RNA remained undetectable throughout follow-up, and the CD20$^+$ B-cell count was as low as 0.4% at 22 months after discharge, yet cryoglobulin remained at detectable levels, and the cryocrit remained at 4% at 25 months after discharge. Finally, 27 months after discharge, the patient remained on 6 mg of mPSL, and his RF levels were still >1,800 IU/mL, but his proteinuria had decreased to around 0.3 g/gCr, and he had never exhibited any symptoms of cryoglobulinemic vasculitis. His serum creatinine level at this time was stable around 1.6 mg/dL, as it was before the illness (Table).

Table. Laboratory Data on Admission, at Discharge and 27 Months after Discharge.

Laboratory test	At diagnosis	At discharge	27 months after discharge
Complete Blood Count			
WBC (/μL)	6,990	6,010	11,970
RBC (/μL)	294	330	441
Hb (g/dL)	8.8	9.7	13.6
PLT ($\times10^4$/μL)	20.5	28.4	32.1
Blood Chemistry			
AST (U/L)	44	15	23
ALT (U/L)	46	11	23
LDH (U/L)	268	235	163
TP (g/dL)	5	5.2	6.7
Alb (g/dL)	2.3	3	4.1
CK (U/L)	102	28	53
BUN (mg/dL)	35	27	25
Cr (mg/dL)	2.45	2.34	1.66
eGFR (mL/min/1.73 m^2)	21.7	22.8	32.9
T-CHO (mg/dL)	213	199	120
Na (mEq/L)	140	141	132
K (mEq/L)	3.8	5.2	4.1
Cl (mEq/L)	110	110	94
Ca (mg/dL)	7.7	8.4	9.5
P (mg/dL)	3.9	3.4	2.5
Mg (mg/dL)	2	2.7	2.2
Immunological Examination			
CRP (mg/dL)	0.1	0.1>	0.1>
IgG (mg/dL)	600	449	792
IgA (mg/dL)	138	155	146
IgM (mg/dL)	692	445	479
C3 (mg/dL)	56.3	69.5	67.1
C4 (mg/dL)	2.6	2.7	2.8
CH50 (mg/dL)	10>	10>	16
RF (IU/mL)	2,018	1,727	1,832
HbA1c (%)	5.9		6.3
PR3-ANCA	negative		
MPO-ANCA	negative		
anti-nuclear antibody	negative		
anti-DNA antibody	negative		
anti-GBM antibody	negative		
Serum immunoelectrophoresis	IgM-κ		IgM-κ
Cryoglobulin	positive	positive	positive
Cryocrit (%)	5	4	4
HCV RNA (LogIU/mL)	7	1.2>	undetectable
Urinalysis			
RBC (/HPF)	30-49	100<	5-9
proteinuria (g/gCr)	9.4	3	0.35
24-hour urinary protein excretion (g/day)	7.4		
NAG (U/L)	54	9.9	13.4

WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, PLT: platelet, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, TP: total protein, Alb: albumin, CK: creatine kinase, BUN: blood urea nitrogen, Cr: creatinine, eGFR: estimated glomerular filtration rate, T-CHO: total cholesterol, Na: sodium, K: potasium, Cl: chlorine, Ca: calcium, P: phosphorus, Mg: magnesium, CRP: C-reactive protein, IgG: immunoglobulin G, IgA: immunoglobulin A, IgM, immunoglobulin M, C3: complement component 3, C4: complement component 4, RF: rheumatoid factor, HbA1c: hemoglobin A1c, PR3-ANCA: proteinase 3-anti-neutrophil cytoplasmic antibody, MPO-ANCA: myeloperoxidase-anti-neutrophil cytoplasmic antibody, anti-DNA antibody: anti-deoxyribonucleic acid antibody, anti-GBM antibody: anti-glomerular basement membrane antibody, HCV RNA: hepatitis C virus ribonucleic acid, NAG: N-acetyl-β-D-glucosaminidase

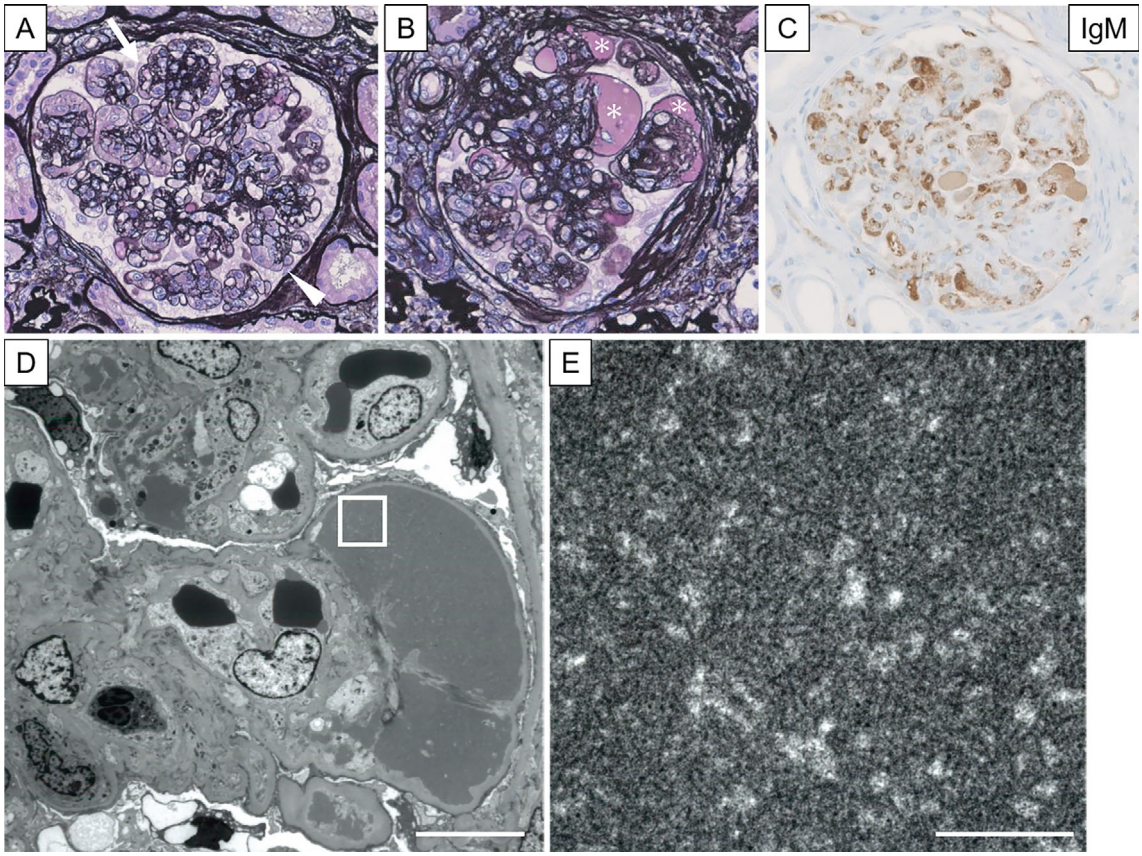

Figure 1. **Renal pathological findings. Double contour of glomerular basement membrane (arrow), mesangial interposition and endocapillary hypercellularity (arrowhead) were globally observed (periodic acid-methenamine-silver; PAM stain, original magnification ×200) (A). Hyaline thrombi (*) were also detected as immunohistochemically positive for IgM (B, C). Electron microscopy showed abundant electron-dense deposits in subendothelial areas (D) (scale bar 10 μm). High magnification of the area shown in the box revealed cylinder-like structures (E) (scale bar 1.0 μm).**

Discussion

In the present case, cryoglobulinemia persisted, and glomerulonephritis appeared imminent despite treatment with rituximab, DAAs, a high-dose steroid and plasmapheresis. As the patient's proteinuria began to worsen, we continued low-dose steroid treatment and performed PE occasionally. Over the long term, his proteinuria gradually improved. Finally, the patient achieved remission for glomerulonephritis, although his cryoglobulinemia persisted.

Notably, this is a rare case of persistent cryoglobulinemia after B-cell depletion had been achieved and the virologic response had been maintained with rituximab and DAAs. There have been several previous reports of persistent cryoglobulinemia after HCV eradication by DAAs (10-13). These can be explained by the presence of persistent B-cell clones producing cryoglobulins after HCV eradication (10). Therefore, B-cell depletion therapy is needed to treat these cases of cryoglobulinemia. Rituximab is a B-cell-depleting monoclonal antibody targeting CD20 that is widely expressed among B-cell-lineage cells, except for plasmablasts and plasma cells. Thus, plasmablasts and plasma cells can still produce cryoglobulins after rituximab treatment until they are eradicated by other means. Indeed, in previous studies of HCV-associated cryoglobulinemic glomerulonephritis treated with rituximab, it took a few months for the RF and IgM levels to decrease after rituximab treatment (4, 14). In the present case, however, the RF and IgM levels remained high, almost as high as they had been at the diagnosis, even 27 months after treatment. This persistent elevation of cryoglobulinemic hallmarks can be explained by the presence of long-lived plasma cells (LLPCs), which are observed in the bone marrow or spleens of patients with immune thrombocytopenia for up to six months following the start of rituximab treatment (15, 16). LLPCs have special gene profiles that are programmed for long life and are characterized by $CD19^{-}$ $CD38^{hi}$ $CD138^{+}$ cells by flow cytometry (17). We did not confirm the presence of LLPCs in our patient, but we speculate that LLPCs might have continued to produce the cryoglobulins that persisted in our patient.

Importantly, we should emphasize that this patient's cryoglobulinemic glomerulonephritis was successfully treated despite the persistence of cryoglobulinemia. We believe that occasional plasmapheresis after rituximab treatment, admin-

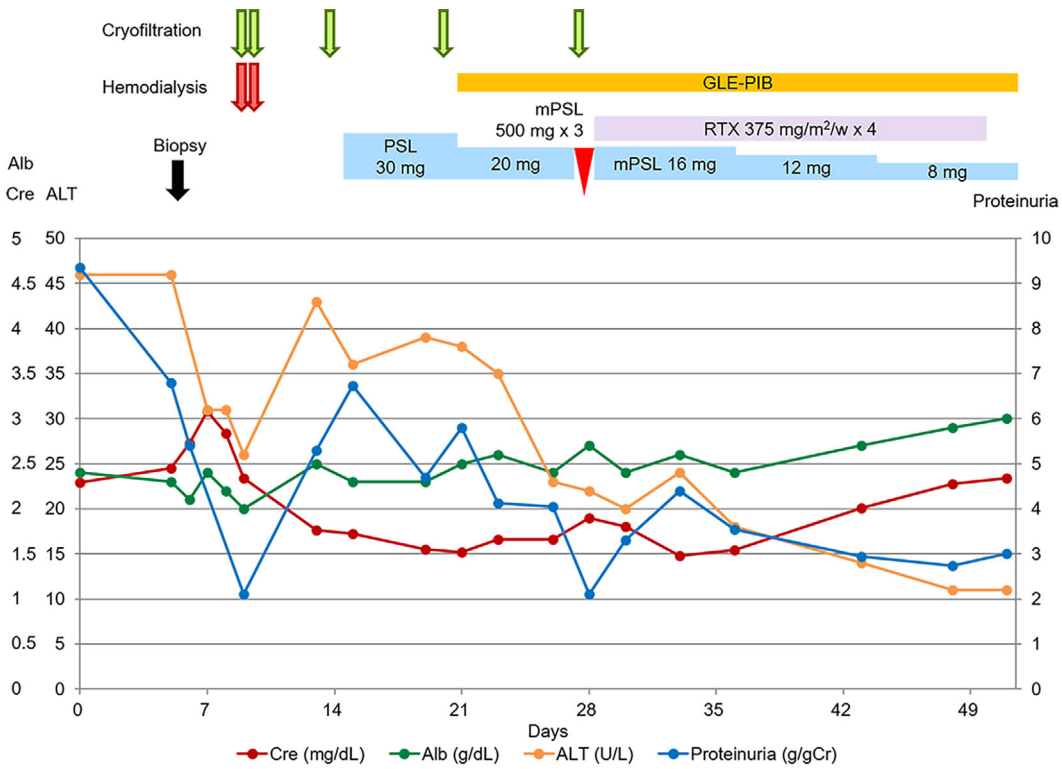

Figure 2. **The patient's clinical course during hospitalization. Before the diagnosis with cryoglobulinemic glomerulonephritis, the patient needed hemodialysis twice. Cryofiltration was also performed. After the diagnosis, prednisolone, glecaprevir-pibrentasvir and rituximab were started on a weekly basis. Prednisolone was tapered early because of worsening hyperglycemia. On the 53rd day of hospitalization, he was discharged, although proteinuria persisted at around 3 g/gCr. PSL: prednisolone, mPSL: methylprednisolone, RTX: rituximab, GLE: glecaprevir, PIB: pibrentasvir**

istered in response to worsening proteinuria or decreasing C 3, was an effective means of controlling his glomerulonephritis. Plasmapheresis is generally effective in urgent, life-threatening cryoglobulinemia cases in advance of the initiation of fundamental treatment (1). However, we also consider it a reasonable means of removing cryoglobulins that may still be produced by LLPCs even after rituximab therapy. Indeed, there is one reported case in which long-term plasmapheresis after rituximab improved cutaneous lesions of cryoglobulinemia (18). Our case indicates that plasmapheresis after rituximab is also effective for cryoglobulinemic glomerulonephritis. We selected PE as the modality for plasmapheresis after rituximab in order to supply immunoglobulin at the same time. A variety of plasmapheresis protocols have been reported, and further studies will be needed in order to identify more effective protocols for conducting plasmapheresis in combination with rituximab.

In addition, continuous low-dose steroid administration after rituximab was effective for our patient. In previous reports, steroids were withdrawn early or avoided in rituximab protocols for cryoglobulinemia (4, 14). In our case, however, when mPSL was tapered to 6 mg, glomerulonephritis deteriorated, so we continued 8 mg of mPSL for long-term maintenance. This may be due to the difference in the baseline activities of cryoglobulinemic glomerulonephritis between the previous report and our present case (proteinuria: previous report 2.3±2.1 g/day vs. our case 7.4 g/day; C3, previous report 125.7±60.8 mg/dL vs. our case 56.3 mg/dL) (14). As steroid is a conventional immunosuppressive agent, its effectiveness in treating cryoglobulinemia has long been recognized, and various mechanisms have been proposed. In the present case, we suspect a putative mechanism associated with complement C5 activation, which is reported to play a prominent role in the pathogenesis of cryoglobulinemic glomerulonephritis (19). Low-dose steroid can prevent complement C5 activation caused by cryoglobulins, which might have improved the cryoglobulinemic glomerulonephritis, although cryoglobulinemia was persistent. In addition, another putative mechanism of steroid is related to LLPCs, a special population of plasma cells which is mentioned above. LLPCs are reportedly surrounded by $CD4^+$ T-cells, which are assumed to produce survival signals for LLPCs (20). Low-dose steroid can suppress $CD4^+$ T-cell activities and LLPC proliferation, which might enhance the efficacy of rituximab. In this way, a low-dose steroid may be effective in combination with rituximab for suppressing C5 activation and LLPC proliferation, especially in patients with persistent cryoglobulinemia.

In conclusion, we described a case of the successful treatment of HCV-associated cryoglobulinemia with serologically persistent cryoglobulinemia using rituximab, DAAs, occasional plasmapheresis and a long-term steroid. When pa-

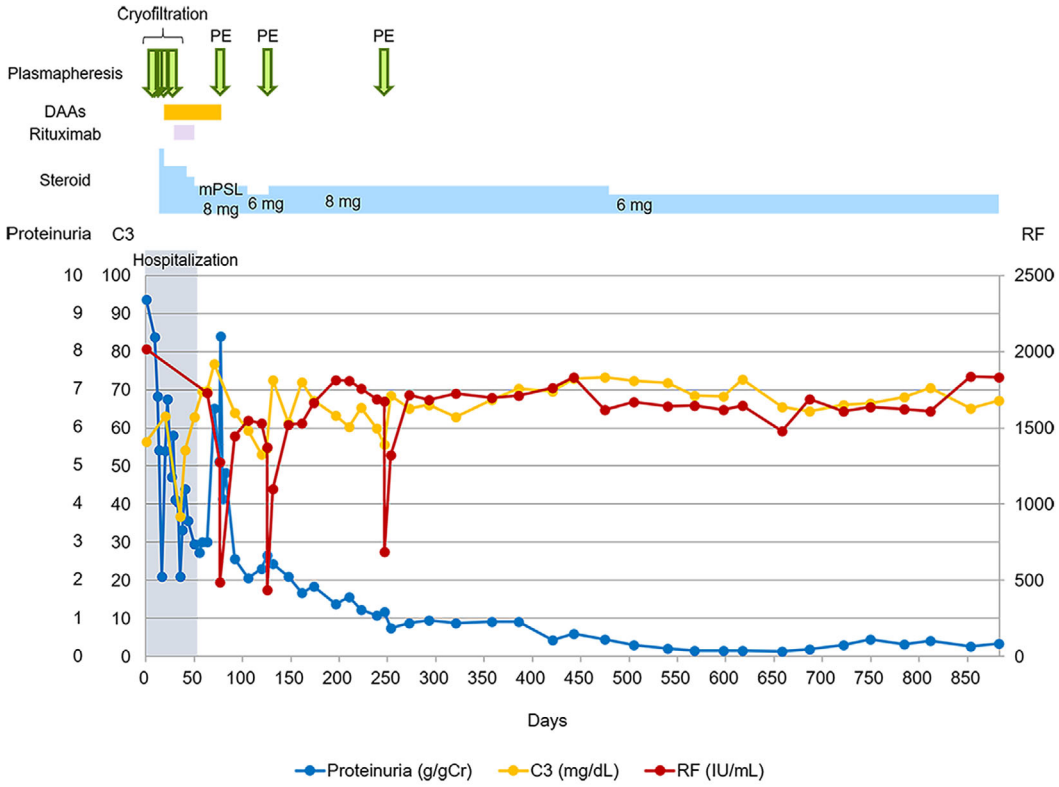

Figure 3. **Long-term follow-up showing cryoglobulinemic hallmarks. Soon after discharge, proteinuria was exacerbated, but one session of plasma exchange relieved this symptom. Later, proteinuria had ameliorated, but the RF level had rebounded, and complement C3 was still being consumed. After the steroid dosage was decreased, proteinuria increased again. Accordingly, plasma exchange was performed again, and his mPSL dosage was increased, leading to decreased proteinuria. Although the patient's RF level remained high throughout the clinical course, the consumption of C3 was diminished, and proteinuria eventually fell to around 0.3 g/gCr. For details concerning the patient's clinical course during hospitalization, please see Figure 2 above. RF: rheumatoid factor, DAAs: direct-acting antiviral agents, PE: plasma exchange**

tients show persistent cryoglobulinemia after rituximab treatment, the presence of LLPCs should be considered. Occasional plasmapheresis is a reasonable treatment even after rituximab for such patients. Low-dose steroid administration can also suppress glomerulonephritis and improve the efficacy of rituximab, especially in patients with persistent cryoglobulinemia. Our ability to treat HCV-associated cryoglobulinemia is continuously improving through the emergence of new protocols for rituximab and DAA administration. Thus far, however, little evidence has been established for new treatments. Further investigations will be needed to establish an optimal regimen.

Author's disclosure of potential Conflicts of Interest (COI).
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