CASE REPORT

A case of rapid amelioration of hepatitis C virus-associated cryoglobulinemic membranoproliferative glomerulonephritis treated by interferon-free directly acting antivirals for HCV in the absence of immunosuppressant

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Abstract Mixed cryoglobulinemic syndrome, which is a systemic vasculitis characterized by the immune complex deposition in small- and medium-sized arteries and most often due to chronic hepatitis C virus (HCV) infection, sometimes clinically manifests as refractory glomerulonephritis or nephritic syndrome. Patients with mixed cryoglobulinemic nephropathy who have a rapidly progressive glomerulonephritis should receive immunosuppressive therapy. After disease stabilization, patients should receive concurrent therapy for the underlying HCV infection. The standard therapy of a chronic HCV infection is IFN monotherapy or IFN combined with ribavirin; however, after the introduction of direct-acting antivirals (DAAs), the standard therapy for patients with HCV genotype 1 has dramatically changed. We report a case of HCV-associated cryoglobulinemic membranoproliferative glomerulonephritis (MPGN) successfully treated by daclatasvir and asunaprevir, which are IFN-free DAAs for HCV, in combination with angiotensin II receptor blocker without immunosuppressive therapy. The patient developed severe nephrotic syndrome with progressive kidney dysfunction. Blood examination revealed a high copy number of HCV-RNA (6.4 log IU/mL, type 1),

cryoglobulinemia, paraproteinemia of IgM-κ, and hypocomplementemia. Histological analysis showed MPGN type 1. These findings were compatible with those observed in HCV-associated cryoglobulinemic MPGN. This case offers original evidence for the application of newer generation of IFN-free DAAs in the treatment of HCV-associated cryoglobulinemic nephropathy.

Keywords Hepatitis C virus · Cryoglobulinemic membranoproliferative glomerulonephritis · Interferon-free direct-acting antiviral agents

Introduction

Patients with long-standing hepatitis C virus (HCV) infection can develop chronic hepatitis, hepatocellular carcinoma, and liver cirrhosis. Several extrahepatic manifestations are also associated with HCV infection, including hematologic, renal, and autoimmune disorders [1]. Mixed cryoglobulinemia (MC) syndrome, which is a systemic vasculitis characterized by multiple organ involvement due to immune complex deposition in small-sized vessels, often occurs in patients with HCV infection. Cryoglobulinemic membranoproliferative glomerulonephritis (MPGN) is one of the most common causes of HCV-associated cryoglobulinemic nephropathy. Treatment of HCV-associated cryoglobulinemic nephropathy with severe organ involvement remains difficult and targets either the viral trigger or the downstream B cells. Immunosuppressive therapy, with glucocorticoid combined with rituximab or, if unavailable, cyclophosphamide, is

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provided as initial therapy in patients with rapidly progressive glomerulonephritis (RPGN). Patients should receive concurrent therapy for the underlying HCV infection after glomerulonephritis stabilization. However, poor outcomes have been reported in most adult patients with HCV-associated MPGN, with progression to renal failure due to a high recurrence rate after discontinuation of immunosuppressive therapy [2, 3]. Treatment of the underlying HCV infection has recently rapidly evolved with the introduction of direct-acting antivirals (DAAs), wherein curative sustained viral response (SVR) rates have been achieved for HCV genotype 1, with the added benefit of fewer drug adverse effects [4–6].

Here, we describe a case of a patient with HCV-associated cryoglobulinemic MPGN clinically manifested as RPGN, in whom SVR of HCV and complete remission of glomerulonephritis were achieved after treatment with interferon (IFN)-free DAAs and angiotensin II receptor blocker (ARB). To the best of our knowledge, this is the first report of the successful use of a double therapy without immunosuppressant in patients with HCV-associated cryoglobulinemic MPGN.

Case report

A 70-year-old man was referred to our hospital in May 2015 for evaluation of nephritic syndrome from a community hospital. He was diagnosed with diabetes mellitus, hypertension, and HCV genotype 1 infection 10 years before the first visit. He was treated with Peg-IFN and ribavirin (RBV) for HCV infection; however, the treatment was discontinued because he developed IFN-induced depression. He developed anasarca and gained 5 kg a month before the first visit. He was diagnosed in the community hospital with nephritic and nephrotic syndromes, which were characterized by severe proteinuria and hematuria. In addition, blood tests revealed a high copy number of HCV-RNA (6.4 log IU/mL), cryoglobulinemia, paraproteinemia of IgM- κ , and hypocomplementemia. These findings were compatible with those observed in HCV-associated cryoglobulinemic nephropathy. Therefore, DAA therapy with daclatasvir (DCV, 60 mg p.o. per day) and asunaprevir (ASV, 200 mg p.o. per day) was initiated 2 weeks before the first visit to treat HCV infection for 6 months.

Edema in the extremities, but no sign of purpura, arthritis, and neuropathy, was noted upon physical examination at the initial visit. Urinalysis showed heavy microscopic hematuria and nephrotic-range proteinuria of 9.61 g/gCr. Blood test results revealed impaired kidney function with a creatinine level of 1.47 mg/dL, hypocomplementemia with a C3 level of 54 mg/dL and a C4 level of

9 mg/dL, elevated rheumatoid factor, paraproteinemia of IgM- κ , and cryoglobulinemia (Table 1). Bone marrow aspiration showed no evidence of multiple myeloma or lymphoproliferative disorder. Immunoelectrophoresis revealed monoclonal IgM- κ , polyclonal IgG, and polyclonal IgA in precipitated cryoglobulin, which suggests type II cryoglobulin. Histological analysis of the kidney showed 32 glomeruli, 17 of which were diffusely and uniformly abnormal with features characteristic of MPGN: mesangial interposition, double-contour appearance of the capillary wall, mesangial cell proliferation, and mesangial matrix expansion with lobular appearance. Global sclerosis was observed in 15 glomeruli. Mild interstitial fibrosis, diffuse tubulointerstitial damage, and severe atherosclerosis were also noted. Immunofluorescence study showed segmental deposits of IgG, C3, IgM, and κ along the glomerular capillary wall, although the signal intensity of IgG and κ was slightly weak. Electron microscopy revealed dense deposits in the subendothelial and mesangial areas with mesangial cell interposition (Fig. 1). A definitive diagnosis of HCV-associated cryoglobulinemic MPGN was made based on these findings.

His clinical course is shown in Fig. 2. HCV in the circulation cleared, and the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) normalized within 2 weeks after the initiation of DAA therapy. Hematuria and proteinuria dramatically decreased without immunosuppressive therapy, followed by a slow improvement in the serum levels of creatinine, total protein, and complements. Persistent proteinuria with a urinary protein–creatinine ratio of 1 g/gCr was normalized by adding azilsartan (20 mg p.o. per day). Semi-quantified cryoglobulin value decreased in parallel with the serum levels of IgM and RF, following HCV eradication. SVR of HCV and complete remission of MPGN were maintained despite persistent cryoglobulinemia, 17 months after initiating DAA therapy.

Discussion

MC MPGN is characterized by the deposition of antigen–antibody complexes in the glomerular capillaries and small arterioles, which leads to vasculitis associated with the deposition of immunoglobulins and complements. In patients with HCV infection, the immune complex consists of HCV, anti-HCV IgG, and IgM anti-IgG with rheumatoid factor activity, and is detected as cryoglobulins. Chronic HCV infection triggers B-cell expansion, which results in the secretion of these monoclonal or polyclonal antibodies in the majority of cases [7]. Treatment of active HCV-associated cryoglobulinemic nephropathy targets HCV virus and B-cell suppression

Table 1 Laboratory test at the initial visit

<i>Peripheral blood</i>		<i>Chemical analysis</i>		<i>Blood coagulation</i>	
WBC	4900/mL	Amy	44 U/L	PT	11.4 s
Seg.	71.5%	T-Cho	194 mg/dL	APTT	35.0 s
Lymph.	17.5%	HDL-Cho	41 mg/dL	Fib	477 mg/dL
Mono.	6.5%	TG	139 mg/dL	Bleeding time	1.0 min
Eos.	3.5%	Blood sugar	190 mg/dL	<i>Urinalysis</i>	
RBC	341×10^4 /mL	HbA1c(NGSP)	6.4%	pH	6.0
Hb	11.4 g/dL	<i>Serology</i>		Protein	4+
Ht	31.7%	CRP	0.06 mg/dL	Occult blood	3+
Pit	22.5×10^4 /mL	IgG	642 mg/dL	Sugar sediment	–
				RBC	>100/HPF
<i>Chemical analysis</i>		IgA	211 mg/dL	<i>Dysmorphic</i>	
AST	17 U/L	IgM	458 mg/dL	WBC	10–19/HPF
ALT	9 U/L	IgE	68.9 IU/mL	RBC cast	0/LPF
LDH	231 U/L	RF	439 IU/mL	Granular cast	0/LPF
gGTP	26 U/L	ANA	Negative	Fatty cast	2/LPF
ALP	142 U/L	C3	54 mg/dL	<i>Urine chemistry</i>	
T-bil	1.3 mg/dL	C4	3 mg/dL	NAG	77.7 U/L
TP	5.9 g/dL	CH50	17 U/mL	b2MG	6567 mg/mL
Albumin	3.1 g/dL	PR3-ANCA	<1.0 U/mL	Protein	1223 mg/dL
Uric acid	8.2 mg/dL	MPO-ANCA	<1.0 U/mL	Creatinine	127.2 mg/dL
BUN	20 mg/dL	Anti-GBM Ab	<2.0 U/mL	BJP	Negative
Creatinin	1.47 mg/dL	Monoclonal protein	IgMk		
Na	140 mEq/L	Cryoglobulin	+		
K	4.1 mEq/L	Syphilis (RPR)	Negative		
Cl	102 mEq/L	Syphilis (TP)	Negative		
Ca	8.3 mg/dL	HBsAg	Negative		
IP	3.6 mg/dL	HCVAb	Positive		
Mg	2.0 mg/dL				

based on the pathophysiology [8, 9]. Many papers reported the effectiveness of anti-viral therapy with IFN and RBV in patients with HCV-related cryoglobulinemic vasculitis; however, SVR was achieved only in 40–80% of patients. The response of MC has been closely linked to the antiviral response, which suggests the importance of HCV as an antigen [10–12]. Importantly, HCV genotype 1 or 4 and high viral load can be viral factors predictive of a poor response to IFN and RBV [13]. IFN-free DAA therapy with DCV and ASV without immunosuppressive therapy resulted in a rapid virological response in the present case, followed by an improvement in hematuria and proteinuria despite persistent cryoglobulinemia and hypocomplementemia. The close association between persistent clearance of HCV viremia and improvement in glomerulonephritis suggests the primary etiologic role of HCV in circulation in the activation of immune complex-mediated vasculitis. We considered that the reason of favorable clinical course is a rapid and sustained viral eradication due to the high efficacy of DCV and ASV for HCV genotype 1.

The pathophysiological role of cryoglobulins remains unclear in HCV-associated cryoglobulinemic nephropathy. Some papers reported that cryoglobulins are deposited in the glomerular capillary and induce endotheliitis via complement activation and anti-endothelial antibody activity, leading to VCAM-1 overexpression and subsequent platelet aggregation [14]. Recent papers have provided evidence that the deletion of B-cell proliferation by rituximab results in improvement in MC, accompanied by cryoglobulin reduction [8, 9]. Evidence suggests that HCV-infected B cells and cryoglobulin consisting of B-cell-derived immunoglobulins are involved in the development of MC vasculitis. However, most HCV-infected patients who have circulating cryoglobulins do not develop vasculitis. Yamabe reported the cases of HCV-associated MPGN, wherein circulating cryoglobulin was absent and immunofluorescence showed no deposition of IgG and IgA in glomeruli [15]. Barsoum described that the immunoglobulin class involved in the immune complex has a considerable impact on glomerulonephritis in MC [16]. The experimental model of cryoglobulin provides

Fig. 1 Pathological findings. Pathological findings show the characteristic features of an MPGN type I. Mesangial expansion with lobular formations (**a**), mesangial interposition (arrow in **b**), and double-contour appearance of the capillary wall (arrow head in **b**) in glomeruli were observed by PASM-HE stain. Electron micrograph

showed subendothelial electron-dense deposits (arrow in **c** and **d**) and expansion of subendothelial space (arrowhead in **f**). High magnification picture **d** and **f** are square in **c** and **e**, respectively. Immunofluorescence showed segmental deposits of IgG, IgM, C3, and κ along the glomerular capillary walls

interesting evidence. In a transgenic mouse model expressing the heavy chain alone, or both heavy and light chains, Kikuchi demonstrated that the pathologic activity of cryoglobulin depends on a unique immunoglobulin combination [17]. In our case, cryoglobulin level in parallel with IgM with RF activity decreased, following HCV eradication despite sustained cryoglobulinemia after complete remission of glomerulonephritis. In sum, the component and/or amount of cryoglobulin in the circulation or in glomerulus may be crucial in MC vasculitis pathophysiology in relation to antigen.

Treatments for HCV infection have drastically improved recently by the development of DAAs, although the previous standard therapy of chronic HCV infection was IFN monotherapy or IFN combined with RBV. DAAs directly inhibit the functions of HCV-related nonstructural proteins (NS3/4A, NS5A, or NS5B). DAAs strongly suppress HCV replication, and DAA-based therapy leads to

HCV eradication with high success rates [18–22]. Telaprevir and boceprevir, the first generation of HCV-NS3/NS4A serine protease inhibitors, were approved in 2011 for treatment of genotype 1 HCV, combined with IFN and RBV. IFN and RBV can often cause adverse effects in patients with kidney disease. Dual oral therapy using DCV and ASV, IFN-free DAAs, was approved for chronic HCV infection treatment in Japan in 2014. Many case reports described the improvement of HCV-associated cryoglobulinemic MPGN by various drug regimens. Stine et al. found variable responses in three cases of chronic HCV infection associated with MC when sofosbuvir (a DAA) was combined with Peg-IFN and RBV (cryoglobulinemia resolved in two cases, but MC did not resolve in the third case) [23]. Urraro et al. reported complete remission in a case of MC after SVR following the addition of boceprevir (DAA) to the combination of rituximab, Peg-IFN, and RBV [24]. Another study by Cornella et al. described the

Fig. 2 Clinical course. A couple of months prior to the first visit, abnormal urinary findings started to emerge, followed by the elevation of serum creatinine. HCV in the circulation cleared and AST normalized within two weeks after the initiation of DAA therapy, and hematuria and proteinuria dramatically improved with subsequent improvement of kidney function despite sustained cryoglobulinemia and hypocomplementemia. Semi-quantified cryoglobulin value decreased in parallel with the serum levels of IgM and RF, following HCV eradication. *ND* not detected

persistence of MC in five cases despite completion of triple therapy with oral antiviral agents (boceprevir, telaprevir, or sofosbuvir), particularly in cirrhosis cases, wherein longer duration may be needed [25]. Our case is the first report wherein IFN-free DAAs without immunosuppressants have been able to successfully treat HCV-associated MPGN manifested as severe nephritic syndrome complicated with kidney dysfunction. KDIGO recommended that all patients with renal impairment and HCV infection should be evaluated for antiviral therapy. The decision whether to treat or not should be based on the potential benefits and risks of the therapy.

The newer generation of DAAs is associated with increased SVR and good safety profiles without the need for IFN and RBV. However, data for evaluating the safety and efficacy of the treatments in patients with kidney disease are scarce. Our experience with this patient leads us to believe that DCV and ASV combination therapy is a useful alternative treatment for the management of HCV-induced MPGN. A major concern is the lack of long-term studies on patient/kidney outcomes after treatment of HCV-associated nephropathy. Further studies with more patients should be conducted to determine the true benefit of this therapy.

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Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB Approval Number 680-1) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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