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CASE BASED REVIEW

Clinical outcome of HCV-associated cryoglobulinemic glomerulonephritis following treatment with direct acting antiviral agents: a case-based review

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Abstract

Newer treatment protocols involving direct-acting antiviral agents (DAAs) have been associated with high rates of sustained virologic response (SVR) and clinical remission in patients with hepatitis C virus (HCV) associated cryoglobulinemic vasculitis (HCV-CV), but clinical response in those with renal involvement is less clear. Our goal was to evaluate the clinical course following DAA therapy in one of the largest cohorts of patients with HCV-associated cryoglobulinemic glomerulonephritis (HCV-GN) reported to date. This is an observational study of patients with chronic HCV infection and circulating cryoglobulins (CC) treated with DAAs in our department from January 2015 to January 2019. We identified a total of 67 patients with HCV and CC out of which nine patients fulfilled the criteria of HCV-GN and had adequate clinical follow-up time. We describe a cohort of nine patients with a mean age of 57 years and known duration of HCV infection ranging 3–20 years (four with evidence of compensated cirrhosis). All patients received the ritonavir-boosted paritaprevir/ombitasvir/dasabuvir regimen for 12 weeks and achieved SVR without subsequent viral relapse. Following DAAs completion, one patient developed "new-onset" cryoglobulinemic glomerulonephritis, six showed either persistent or worsening glomerulonephritis, and only two patients had a complete clinical response (CCR). Of the six patients with either persistent or worsening CV, 67% received additional immunosuppressive (IS) therapy for uncontrolled CV. Of the two patients that had a CCR, one patient received prior IS therapy while the other one improved without any additional intervention. Newer HCV treatment protocols involving DAAs are highly successful in eradication of HCV infection; however, in our experience, DAA treatment alone is insufficient in improving the renal outcomes of patients with HCV-GN and additional IS therapies should be considered.

Keywords Cryoglobulinemia · Direct-acting antiviral agents · Glomerulonephritis · Hepatitis C virus · Immunosuppression

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Introduction

Hepatitis C virus (HCV) infection affects more than 180 million individuals worldwide and is associated with significant morbidity and mortality by being an important cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma [1, 2]. HCV is both a hepatotropic and lymphotropic virus and represents the major antigenic stimulus for clonal B cell expansion with subsequent immunologic and lymphoproliferative complications [2]. Mixed cryoglobulins (either type II with monoclonal IgM/polyclonal IgG or type III with polyclonal IgM/IgG) can be detected in up to 50% of patients with chronic HCV infection while only 10–15% of them will eventually develop HCV-associated cryoglobulinemic vasculitis

(HCV-CV) [2]. Cryoglobulinemic glomerulonephritis occurs in approximately 20–30% of patients with HCV-CV and has been associated with the worst outcome, the 10-year overall survival and renal survival of these patients being 65–80% and 80%, respectively [3, 4].

The advent of direct-acting antiviral agents (DAAs) in the management of chronic HCV infection has been associated with high rates (over 90%) of sustained virologic response 12 weeks after the end of therapy (SVR12) [2]. As such, DAAs are now the standard-of-care for these patients [2, 5] and several studies have shown higher rates of complete clinical remission of HCV-CV following DAAs and a better side-effect profile than the formerly used INF-based regimens [2]. Nevertheless, up to 50% of patients that experience virologic response may have persistent circulating cryoglobulins and several case reports have shown persistence, relapsing and even new-onset HCV-CV following DAA therapy [2, 6]. What determines this divergent treatment response of HCV-CV to antiviral therapy in this subset of patients remains largely unknown. From a pathogenesis point of view, the mechanisms of clonal expansion in HCV infection, cryoglobulin production, renal deposition, and tissue injury remain incompletely understood, with experimental data being largely hypothesis generating [1, 4, 7–10]. Renal injury in HCV-CV occurs secondary to cryoglobulin deposition, complement activation with recruitment of inflammatory cells, and activation of resident glomerular cells [1]. An essential step in the pathogenesis of cryoglobulinemic glomerulonephritis appears to be mediated by infiltrating macrophages and their crosstalk with mesangial cells [7, 9]. In several cases, an incomplete suppression of the B cell clonal expansion, that drives a viral-independent cryoglobulin production, or an incomplete clearance of viral RNA from the cryoprecipitate could explain the persistent or relapsing vasculitis features, but this remains to be proven [11].

Despite the expanding body of literature focusing on the virologic, clinical, and immunological response to DAA therapies in HCV-infected individuals, very little is currently known about the subset of patients with HCV-associated cryoglobulinemic glomerulonephritis [2].

The goal of our study was to evaluate treatment response to DAA therapy in one of the largest series of patients with severe HCV-related cryoglobulinemic glomerulonephritis reported to date.

Methods

Patient selection

This is an observational study of patients with chronic HCV infection and circulating cryoglobulins treated in our department with various DAA regimens from January 2015 to January 2019. Serological testing for cryoglobulins was done as previously reported and a serum cryocrit ≥ 2% was considered positive [1]. Patients were initially divided into two categories, as formerly described [11]: patients with asymptomatic circulating cryoglobulins (ACC) and patients with cryoglobulinemic vasculitis (HCV-CV). All patients that tested positive had mixed cryoglobulins, either type II or type III. The definition of HCV-CV was made according to the 2012 Revised Chapel Hill Consensus for the Nomenclature of Vasculitis [12]. Exclusion criteria were HIV or hepatitis B virus infection, presence of other autoimmune disorders, and/or lymphoma. Of the HCV-CV patients, we selected those with features of cryoglobulinemic glomerulonephritis that fulfilled one or more of the following criteria: acute nephritic syndrome and/or nephrotic syndrome, rapidly declining renal function, biopsy features of cryoglobulinemic glomerulonephritis with/without crescent formation, Birmingham Vasculitis Activity Score of at least 15, and/or treatment-resistant arterial hypertension. In addition, only patients with at least 6 months of follow-up period prior and post-DAA therapy were ultimately considered for study inclusion. The study was conducted after institutional approval (The Ethics Committee of Fundeni Clinical Institute, Registration Number, 23249) aligned with the provisions of the Declaration of Helsinki and written consent to participate in the study was provided by all participating patients.

Treatment

Patients with chronic HCV infection received various DAA regimens according to standard approved protocols and in agreement with the European Association for the Study of Liver recommendations for treatment of hepatitis C [13]. They consisted of either ritonavir-boosted paritaprevir/ombitasvir/dasabuvir (Viekirax/Exviera®, Abbvie), grazoprevir/elbasvir (Zepatier®, Merck), or sofosbuvir/ledipasvir (Harvoni®, Gilead). Sustained virologic response (SVR12) was defined as undetectable HCV-RNA assessed at 12 weeks after treatment completion. Serum HCV-RNA was quantified by real-time polymerase chain reaction. In addition to antiviral treatment, patients with uncontrolled cryoglobulinemic vasculitis (acute nephritic and/or nephrotic syndrome, rapidly progressive glomerulonephritis) were treated with steroids, immunosuppressive (IS) agents, and/or plasma exchange according to international guidelines and local protocols [6]. The induction treatment regimens consisted of the association of steroids (iv. methylprednisolone, 500–1000 mg/day, for 1–3 days, followed by oral prednisone at an initial dose of 1 mg/kg/day, subsequently tapered to a maintenance dose of 5–10 mg/day) and cyclophosphamide (either iv pulses of 500 mg, every 2 to 4 weeks, for a total of 6–8 pulses or oral at a dose of 2 mg/kg/day, for 2–4 months) or

rituximab (500 mg, two doses, 2 weeks apart). Maintenance treatment consisted of low-dose steroids (prednisone 5–10 mg/day) in association of either azathioprine (2 mg/kg/day), methotrexate (20–25 mg/week), or rituximab (500 mg, every 6 months), for a minimum of 18 months.

Clinical assessment

Clinical evaluation at baseline (considered at the time of antiviral treatment initiation) consisted of: demographic data (age, gender), presence or absence of cirrhosis, period of antiviral treatment, non-renal features of cryoglobulinemic vasculitis, and previous and post-DAA immunosuppression. Laboratory parameters followed included renal function (assessed by serum creatinine and glomerular filtration rate, estimated by CKD-EPI equation), 24-h proteinuria, hematuria, serum complement levels (C3 and C4), and rheumatoid factor. Clinical and laboratory assessments were reported at 6 and 3 months prior to DAA initiation, at the moment of DAA initiation and at 1, 3, 6, 12, and 15 months after treatment completion.

Complete clinical remission was defined as a BVAS score of 0 and an improvement of all affected organs. Partial clinical remission was defined as an improvement of at least half of the affected organs. Complete renal response was defined as proteinuria below 0.3 g/day, disappearance of hematuria, and eGFR improvement by at least 20%. Partial renal response was defined as at least 50% decrease in proteinuria and hematuria and less than 25% increase in serum creatinine from baseline.

Statistical analysis

Continuous variables were expressed as either mean ± standard deviation or median (interquartile range (IQR) 25th–75th percentiles) and categorical variables as percentages. Statistical analysis was performed using the XLSTAT (Addinsoft 2019, XLSTAT statistical and data analysis solution. https://www.xlstat.com. Boston, USA).

Results

We reviewed the records of all patients ($n = 413$) with chronic HCV infection treated with DAA therapy in our department and we identified 67 patients with circulating cryoglobulins (Fig. 1). Of these, 36 patients had asymptomatic cryoglobulinemia and 31 patients had features of cryoglobulinemic vasculitis. From the 31 patients with HCV-CV, we identified nine patients that fulfilled the criteria of cryoglobulinemic glomerulonephritis. All cryoglobulinemic patients had HCV genotype 1b, were treated with DAAs for 12 weeks, and achieved SVR12, while none had viral relapse during the follow-up period. The majority of patients ($n = 63$) were treated with the ritonavir-boosted paritaprevir/ombitasvir/dasabuvir regimen, while three and one patients had received sofosbuvir/ledipasvir and grazoprevir/elbasvir regimen, respectively. Following DAA therapy, 69% of the entire cohort of patients had cleared their cryoglobulins, while 31% had persistent circulating cryoglobulins. Of the 22 patients with HCV-CV without renal involvement, 19 showed complete resolution of symptoms and the other three patients showed partial improvement of their baseline symptoms.

Within the group of patients with cryoglobulins, we then focused on the patients with cryoglobulinemic glomerulonephritis. Their baseline demographic and clinical characteristics are shown in Table 1. The mean age was 57 ± 8 years, majority (67%) were females, they had a known duration of chronic HCV infection ranging 3–20 years and four of them had evidence of compensated cirrhosis (Child-Pugh Class A). All patients received the ritonavir-boosted paritaprevir/ombitasvir/dasabuvir regimen for 12 weeks and achieved SVR12 without subsequent viral relapse. Eight out of nine patients had previous manifestations of cryoglobulinemic glomerulonephritis and six of them had received, in the 6 months preceding the initiation of DAA therapy, various regimens of immunosuppressive therapy (Table 1). Patient 1 had no evidence of vasculitis or circulating cryoglobulins prior to DAA therapy having a "new-onset" cryoglobulinemic vasculitis at 4 months after treatment.

Five patients underwent renal biopsy, all of them showing a membranoproliferative pattern of glomerulonephritis with mesangial expansion due to increased cellularity and matrix accumulation, glomerular basement membrane duplication, and severe endocapillary hypercellularity. Three patients had intracapillary thrombi and four patients had extracapillary hypercellularity (ranging 5 to 50% of the examined glomeruli) (Table 1). The degree of interstitial fibrosis and tubular atrophy (IFTA) was mild in most patients (less than 10%), except for patient 2 that had moderate IFTA (25%). Immunofluorescence studies were consistent with HCV-GN showing an IgM-κ dominant and weaker staining for IgG and λ with additional mesangial and capillary wall staining for C3 and C1q. Electron microscopy showed in all patients' extensive subendothelial and mesangial deposits. The remaining four patients had contraindications and did not undergo a kidney biopsy (uncontrolled arterial hypertension, thrombocytopenia).

Renal involvement was characterized by severe nephrotic syndrome in one patient, acute nephritic syndrome in the remaining eight patients. Progressive renal function decline at various time-points during the follow-up period was seen in six patients. Except for patient 1, with the "new-onset" CV, all patients had previous evidences of immunological activity such as presence of circulating cryoglobulins (100%), increased rheumatoid factor (100%), and decreased serum C4 (100%) and C3 (50%). At the moment of DAA initiation, 75%

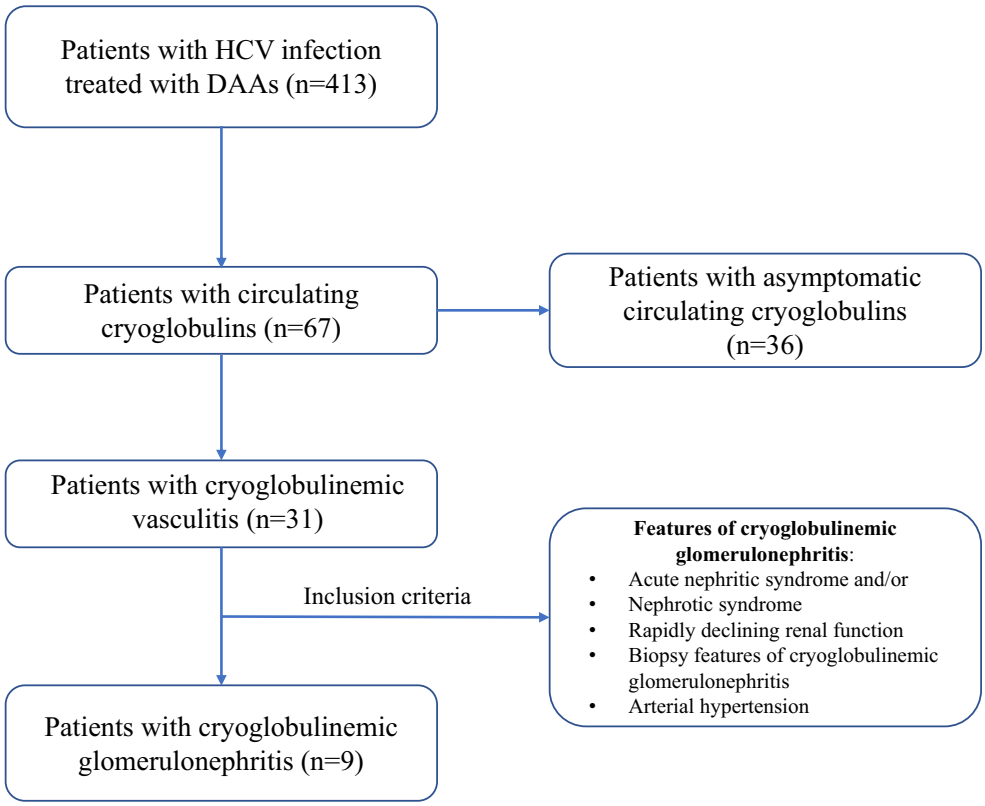

Fig. 1 Patient selection

and 25% still had decreased serum levels of C4 and C3, respectively, and 100% had positive rheumatoid factor (RF), despite that 75% had received prior immunosuppression. The median level of C3, C4, and RF was 97 (IQR; 95–121) mg/dl, 6.4 (IQR; 3.2–12) mg/dl, and 147 (IQR; 110–290) UI/l, respectively. Median 24-h proteinuria and hematuria were 1 (IQR; 0.5–1.2) g/day and 41 (6–76) cells/mmc, respectively.

Following antiviral treatment completion, one patient developed "new-onset" cryoglobulinemic vasculitis with constitutional, cutaneous, and renal manifestations. Patient 1, with the "new-onset" CV, received an induction treatment with oral steroids (0.5 mg/kg/day) and iv pulses of cyclophosphamide (500 mg, biweekly), initially achieved a partial clinical remission, but subsequently relapsed and initiated on a similar reinduction regimen (Table 2). Six patients showed either persistent or worsening of glomerulonephritis and four of them received additional IS therapy. Three patients required reinduction IS therapy due to uncontrolled nephritis, while patient 5 had a worsening nephritis shortly after DAA completion while on maintenance treatment. Of these, only one patient achieved a partial renal response. The remaining two patients had a complete clinical response (CCR) following DAAs, one patient receiving prior induction and current maintenance immunosuppression, while the other one improved without any additional intervention.

The temporal trends of renal manifestations and immunological activity are shown in Fig. 2. Overall, serum creatinine peaked at 3 months post-DAA therapy and showed a tendency to decline afterwards. The level of proteinuria peaked at 6 months following DAA cessation and improved afterwards, while hematuria showed a progressively increasing level after 3 months and until the end of follow-up. From the immunological standpoint, serum C4 and RF remained persistently abnormal throughout the study period, while serum C3 showed a decline at month 6 and improved afterwards. At the last follow-up visit, 55% of patients still had abnormal renal function, 67% had persistent hematuria and proteinuria, 55% had persistent low-levels of C4, and 78% had positive RF.

Discussion

Hepatitis C virus-related cryoglobulinemic vasculitis is an immune-mediated disorder commonly occurring with longstanding infection and is associated with significant morbidity and mortality [14]. In this paper, we focused on clinical course of patients with HCV-related cryoglobulinemic glomerulonephritis in the new era of HCV treatment with direct-acting antiviral agents.

The first-line therapy for HCV-related cryoglobulinemic vasculitis remains treatment of HCV with antiviral agents, but, as illustrated in our series and previous literature, in cases with cryoglobulinemic flares,

Table 1 Characteristics of the study cohort and laboratory data at the initiation of antiviral therapy

	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt. 6	Pt. 7	Pt. 8	Pt. 9
Age (years)	57	51	54	67	49	70	46	66	56
Gender	M	M	F	F	M	F	F	F	F
DAA treatment period	04/2016 – 06/2016	08/2016 – 11/2016	10/2017 – 01/2018	12/2017 – 03/2018	03/2018 – 06/2018	03/2018 – 06/2018	10/2017 – 01/2018	12/2017 – 03/2018	10/2015 – 01/2016
HCV genotype	1b	1b	1b	1b	1b	1b	1b	1b	1b
Viral load (UI/ml)	732.000	851.000	2.210.000	60.900	11.200.000	270.000	1.340.000	103.000	652.000
DAA regimen	3D regimen	3D regimen	3D regimen	3D regimen	3D regimen	3D regimen	3D regimen	3D regimen	3D regimen
Cryoglobulinemia	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Previous IS	None	CS, RTX	CS, Aza	CS, RTX, PLEX	CS, CF	None	CS, MXT, Aza, PLEX	CS, CF	None
Kidney biopsy	MPGN 20% crescents	MPGN 50% crescents	Not done	MPGN 16% crescents	MPGN 5% crescents	Not done	Not done	MPGN no crescents	Not done
Biopsy date	11/2016	03/2017	Not done	03/2013	07/2017	Not done	Not done	10/2014	Not done
BVAS	16	15	23	16	25	15	16	16	15
Serum creatinine (mg/dl)[1]	0.99	1.5	1.99	1.73	1.6	1.2	0.93	0.79	0.7
eGFR (ml/min/1.73m^2)[1]	84	53	37	40	50	61	74	94	105
C3 level (mg/dl)[1] (NR; 90–180 mg/dl)	95	125	121	60	158	78	97	111	97
C4 level (mg/dl)[1] (NR:10–40 mg/dl)	12	2.3	23	3	6.8	5	2	6.4	12
Hematuria (cells/mmc)[1]	0	258	6	41	76	5	311	70	30
Proteinuria (g/d)[1]	0	11.8	1	1.2	1.6	0.5	0.6	0.5	0.4
Rheumatoid factor (UI/ml)[1] (NR; < 15 UI/ml)	15	284	1100	110	147	5180	194	1120	90

[1] Laboratory data shown were recorded at the initiation of antiviral treatment. *DAA*, direct acting antiviral agents; *IS*, immunosuppression; *eGFR*, estimated glomerular filtration rate; *RF*, rheumatoid factor; *BVAS*, Birmingham Vasculitis Activity Score; *M*, male; *F*, female; *CS*, corticosteroids; *RTX*, rituximab; *CF*, cyclophosphamide; *PLEX*, plasma exchange; *MPGN*, membranoproliferative glomerulonephritis; *3D regimen*, ritonavir-boosted paritaprevir/ombitasvir/dasabuvir; *NR*, normal range; *HCV*, hepatitis C virus; *Aza*, azathioprine; *MXT*, methotrexate

Table 2 Clinical evolution of the study cohort following DAA therapy

	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt. 6	Pt. 7	Pt. 8	Pt. 9
Post-DAA evolution	New-onset vasculitis	Worsening vasculitis	Worsening vasculitis	Persistent vasculitis	Worsening vasculitis	Worsening vasculitis	Worsening vasculitis	Improved vasculitis	Improved vasculitis
Post-DAA additional IS	CF Steroids	CF Rituximab	CF Steroids Rituximab	None	Aza Steroids	None	PLEX Rituximab Steroids	Aza	None
IS period	11/2016 – present	01/2017 – present	01/2018 – present	None	06/2018 – present	None	02/2018 – present	03/2018 – present	None
Evolution at 6 months post-DAA									
Serum creatinine (mg/dl)	1.8	1.9	2.52	1.3	0.94	1.9	1.5	0.66	0.9
C3 level (mg/dl) (NR; 90–180 mg/dl)	42	110	60	63	120	102	65	142	89
C4 level (mg/dl) (NR; 10–40 mg/dl)	1.6	5.1	4	2.5	6.8	7	2	19	12
Hematuria (cells/mmc)	3914	120	154	63	76	16	267	45	10
Proteinuria (g/d)	4.2	12.8	5	0.7	1.8	0.5	2	0.6	0.3
RF (UI/ml) (NR; < 15 UI/ml)	15	318	261	19	311	4000	315	720	35
Last follow-up									
Serum creatinine (mg/dl)[1]	0.9	1.45	2	1.4	1	1.9	1.34	0.7	0.89
C3 level (mg/dl)[1] (NR; 90–180 mg/dl)	105	138	103	63	120	110	97	130	95
C4 level (mg/dl)[1] (NR; 10–40 mg/dl)	1.6	6.8	16	3	14	7.5	2	20	9
Hematuria (cells/mmc)[1]	400	100	40	60	50	20	311	0	0
Proteinuria (g/d)[1]	0.4	1.6	3.5	0.6	1.8	0.5	0.3	0.3	0.3
RF (UI/ml)[1] (NR; < 15 UI/ml)	10	154	260	20	320	4000	194	600	11
Clinical response at last follow-up visit	Partial renal response	Partial renal response	No remission	No remission	No remission	No remission	No remission	Complete clinical remission	Complete clinical remission
Follow-up post-DAA (mo)	19	15	13	10	7	7	13	10	24

DAA, direct acting antiviral agents; *IS*, immunosuppression; *RF*, rheumatoid factor; *CF*, cyclophosphamide; *PLEX*, plasma exchange; *Aza*, azathioprine; *NR*, normal range; *mo*, months; *RF*, rheumatoid factor

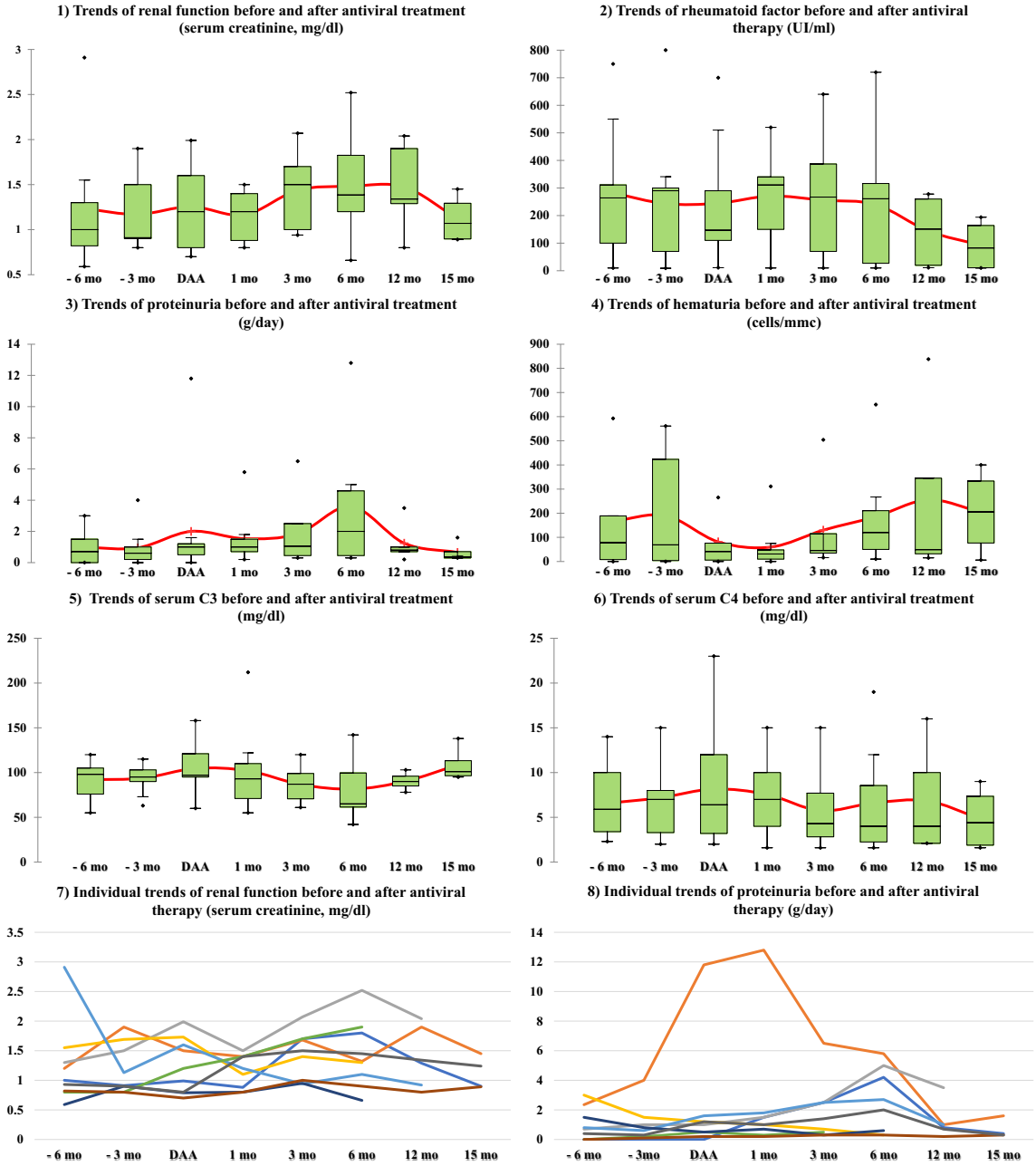

Fig. 2 Evolution of the main parameters monitored for 24 months (6 months prior to DAA therapy, DAA treatment period, and up to 15 months following DAA cessation)

nephrotic syndrome or rapidly progressive kidney failure additional immunosuppressive therapy should be considered [5, 14]. The goal of treatment of HCV-CV is to achieve HCV clearance potentially allowing a decreased need for additional IS therapy and its risks [15]. However, formerly used interferon-based regimens were associated with modest SVR12 and clinical response rates (40–60%), with an overall poor tolerance and high treatment discontinuation rate [14]. In such patients, addition of rituximab to pegylated-interferon/ribavirin has shown better short- and long-term outcomes of HCV-CV as compared with antiviral therapy alone [16].

Since 2013, the approval of direct-acting antiviral agents led to major improvement in the management of patients with chronic HCV infection [14, 15]. Overall, DAA treatment with various agent combinations leads to close to universal rates of SVR12, with an additional benefit of much better side effect profile than their predecessors [2, 14].

To better understand our results and place them in a larger clinical practice perspective, we performed a thorough

Table 3 Clinical and virologic response after direct-acting antiviral therapy in patients with hepatitis C virus–associated cryoglobulinemic vasculitis

Author	Number of pts	No. of pts. with GN	IS (no. pts.)	SVR12 (%)	CCR (%)	Partial CR (%)	Clinical response of GN	CIR (%)/ PIR (%)	CV relapse	New-onset CV
Makara (2015) [17]	1 (CV)	None	1 (RTX, PLEX)	100%	100%	–	–	100%	NR	No
Saadoun (2015) [18]	24 (CV)	5	7 (RTX, PLEX, CF)	74%	86.9%	12.5%	80%	46.1% (CIR)	NR	No
Cornella (2015) [19]	5 (CV)	3	3 (RTX)	100%	None	100%	Improvement, but no CCR	60/40%	NR	No
Obata (2016) [20]	1 (CV)	1	None	100%	100%	–	100%	100% (CIR)	NR	No
Gragnani (2016) [21]	44 (CV)	4	2 (RTX)	100%	77%	23%	100%	40/34%	NR	No
Cacoub (2016) [22]	27 (CV)	7	9 (RTX) 6 (PLEX)	75%	56%	40%	Not reported	63/26%	NR	No
Sise (2016) [15]	12 (CV)	7	6 (RTX, PLEX)	83%	33%	33%	100% (43% CCR)	44/45%	NR	No
Tsuge (2016) [23]	1 (CV)	1	None	100%	100%	–	100%	100% (CIR)	NR	No
Sollima (2016) [24]	7 (CV)	5	6 (RTX)	100%	14% (1/7)	14% (1/7)	20%	14% (CIR)	14%	No
Chowdhury (2016) [25]	1 (CV)	1	CF, RTX, PLEX	100%	–	–	100%	100% (CIR)	100%	No
Bonacci (2017) [26]	35 (CV) 29 (ACC)	7	3 (RTX)	94%	71%	15%	71% (CCR)	43% (CIR)	NR	No
Lauletta (2017) [27]	22 (CV)	4	None	100%	63%	23%	75% (1 pt. with worsened GN)	58%/ 18%	NR	No
Shimada (2017) [28]	1 (CV)	1	CS	100%	100%	–	100%	0%	NR	No
Saadoun (2017) [29]	41 (CV)	5	2 (RTX, PLEX)	100%	90.2%	9.8%	100% (60% CCR)	50% (CIR)	NR	No
Artemova (2017) [30]	5 (CV) 4 (ACC)	N/A	None	100%	60% showed improvement		Not reported	Not reported	1 relapsed	No
Emery (2017) [31]	18 (CV) 65 (ACC)	10	4 (RTX, PLEX)	89%	39%	22%	20% CCR	29%/ 47%	NR	No
Comarmond (2017) [32]	27 (CV)	7	N/A	81.%	89%	Not reported	Not reported	37% (CIR)	NR	No
Ghosn (2017) [33]	2	None	None	100%	–	–	–	–	NR	2
Bonacci (2018) [11]	46 (CV) 42 (ACC)	9	3 (RTX, PLEX)	100%	80%	11%	66.6% (CCR)	66% (CIR)	5 relapsed	No
Santoriello (2018) [34]	3 (CV)	3	2 (RTX, PLEX)	100%	33%	33%	66% (1 with CCR, 1 progressed to ESRD)	100%	NR	No

GN, glomerulonephritis; *IS*, immunosuppression; *SVR12*, sustained virologic response 12 weeks after the end of therapy; *CCR*, complete clinical remission; *CR*, clinical remission; *CIR*, complete immunological remission; *PIR*, partial immunological remission; *CV*, cryoglobulinemic vasculitis; *ACC*, asymptomatic circulating cryoglobulins; *RTX*, rituximab; *PLEX*, plasma exchange; *CF*, cyclophosphamide; *ESRD*, end-stage renal disease; *CS*, corticosteroids; *NR*, no relapse; *N/A*, not available

literature review of the use of DAAs in the management of HCV-CV available to date and reported it in Table 3. Similarly with our report, most data originates from small-cohort studies and there is a consistent observation that DAA therapy is associated with SVR12 in vast majority cases, ranging from 74 to 100% of treated HCV-CV patients. Correspondingly, in our cohort, we observed a SVR12 of 100% and a complete immunological response in 69% of patients, while 31% of them still had persistent circulating cryoglobulins at last follow-up visit. Moreover, of those patients without renal involvement, a complete clinical remission was achieved in 86% of cases, while the rest of them showed a partial clinical remission. These data are in line with those reported in the literature (Table 3). In addition to the high rate of virologic and clinical response seen in HCV-CV, steroids and/or other immunosuppressive agents were able to be tapered or even discontinued following DAA therapy [26]. Moreover, Cacoub et al showed that, despite increasing costs of DAA therapy compared with IFN-based regimens, the costs related to hospitalization and non-antiviral therapy have significantly decreased with the advent of these newer agents [22].

Despite the proved effectiveness of DAAs for the treatment of HCV-CV, their efficacy in the management of severe HCV-related cryoglobulinemic glomerulonephritis is less well established. We undertook a literature review of reported study cohorts and identified only a few patients with HCV-CV and renal involvement, the vast majority having an incomplete characterization of clinico-pathological features and post-DAA period (Table 3). To better understand the evolution of these patients, we specifically selected from the entire cohort of patients with HCV-CV only those that showed cryoglobulinemic glomerulonephritis and had an adequate follow-up time prior and post-DAA therapy. By comparison to previous reported literature, our study cohort had the particularity of worsening nephritis following DAA therapy, despite that most patients had received previous aggressive immunosuppression (Table 1). Although it is generally agreed that severe forms of HCV-related cryoglobulinemic glomerulonephritis will require dual antiviral/immunosuppressive therapy to control vasculitis symptoms, initial case reports showed that these patients can achieve complete renal remission only with antiviral therapy. Obata et al reported a case of rapidly progressive cryoglobulinemic glomerulonephritis with complete clinical remission following DAAs without any additional IS therapy [20]. Bonacci et al showed that six of their nine patients with glomerulonephritis achieved complete renal response without additional immunosuppression, while Gragnani et al noted an improvement of nephritis in all of their four patients [11, 21]. In our cohort, only two out of nine patients had a complete clinical remission following DAA therapy. One patient received a standard induction regimen consisting of cyclophosphamide and corticosteroids, followed by DAAs and maintenance treatment with azathioprine, while the other one achieved a complete renal response without any additional IS therapy. Nevertheless, six out of nine patients showed either persistent or worsened vasculitis, despite that 83% had received prior IS. Of these, only patient 2 obtained a partial renal response after reinduction therapy and more than 12 months following the end of antiviral therapy. Sollima et al reported the persistence of nephritis in four out of five patients, despite receiving additional B cell-depleting agents [24]. However, cases of worsening nephritis have only been rarely described. Santoriello et al reported that one of their three patients had worsening nephritis following DAAs and did not recover kidney function despite additional immunosuppression, remaining dialysis dependent [34]. In addition to patients with worsening nephritis, we describe one patient without any previous features of cryoglobulinemia that developed a "new-onset" cryoglobulinemic vasculitis. Given the rarity of this situation, this case adds up to the previous two cases of new-onset CV following DAA therapy [33] and emphasizes the need to continuously follow these patients despite maintaining SVR and clinical response.

A potential explanation for our findings of worsening nephritis following DAAs in a subset of patients can only be hypothesized. From a pathophysiology standpoint, the immune dysregulation seen in HCV-CV patients is characterized by an abnormal B cell phenotype [35, 36], with increased levels of IgM+ memory B cells, possibly driven by an overexpression of BAFF (BLYS) [37], that are accompanied by higher levels of T helper cells and lower levels of T regulatory cells [32, 35]. Renal injury in HCV-CV occurs secondary to cryoglobulin deposition, complements activation with recruitment of inflammatory cells, and activation resident glomerular cells [1].

In patients that achieve clinical remission of vasculitis, Comarmond et al has shown that, after DAA therapy, these dysregulations of humoral and cellular immunity are reversed and underlie the clinical response of vasculitis [32]. However, a subset of patients may show persistent serum cryoglobulins and a longer follow-up period may identify those that ultimately relapse despite having undetectable viral load [11, 24, 25]. In a potential alternative explanation, Chowdhury et al noted the persistence of viral RNA in the cryoprecipitate in a relapsing CV patient despite undetectable viral load suggestive of possibility of subliminal residual HCV infection [25]. However, what determines this severe outcome of patients with cryoglobulinemic glomerulonephritis remains incompletely understood. It has been shown that patients with lupus nephritis in complete clinical remission show residual histological activity that could represent a risk factor for a subsequent relapse [38]. Similarly, it can be hypothesized that in such cases of HCV-related cryoglobulinemic glomerulonephritis, viral clearance and/or immunosuppression treatment attenuate inflammatory lesions in the kidney but are ineffective in complete clearance of cryoglobulin deposits.

Moreover, our patients had persistent signs of immunologic activity throughout the study period that could account for ongoing tissue deposition of cryoglobulins. Ongoing immunologic activity could be driven by persistent RF-expressing B cells, that have undergone clonal expansion and cryoglobulin production in a viral independent manner [34]. In these patients, prolonged IS therapy might be needed to control the autoimmune process while follow-up kidney biopsies may permit to adequately assess the immunological and histological activity.

In conclusion, newer treatment protocols involving DAAs are being used to treat HCV infection, but their efficacy remains unproven for kidney disease. Our cohort emphasizes the severity of renal involvement in HCV-CV and the fact that despite the viral eradication, as a trigger for the autoimmune processes, renal immune-mediated injury persists or may newly manifest in certain patients. Clinicians treating HCV-CV patients should be ongoingly aware of the need for clinical monitoring and consideration of additional immunosuppressive options aimed at control the renal damage.

Compliance with ethical standards The study was conducted after institutional approval (The Ethics Committee of Fundeni Clinical Institute, Registration Number, 23249) aligned with the provisions of the Declaration of Helsinki and written consent to participate in the study was provided by all participating patients.

Disclosures None.

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