Virologic, Clinical, and Immune Response Outcomes of Patients With Hepatitis C Virus–Associated Cryoglobulinemia Treated With Direct-Acting Antivirals

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BACKGROUND & AIMS: **Cryoglobulins (circulating immune complexes of polyclonal IgG, monoclonal IgM, and rheumatoid factor) are detected in the circulation of 40% to 60% of patients with chronic hepatitis C virus infection, and cryoglobulinemic vasculitis (CV) is observed in approximately 10% of patients. We aimed to assess the clinical and immune effects of direct-acting antiviral treatment.**

METHODS: **We performed a prospective study of 64 patients with HCV infection with circulating cryoglobulins receiving direct-acting antiviral therapy at a single center in Barcelona, Spain, from January 2014 through April 2016. Patients were classified as having CV (n = 35) or asymptomatic circulating cryoglobulins (ACC, n = 29). Clinical response was considered complete if a patient's Birmingham Vasculitis Activity Score (version 3) was 0, or if all affected organs improved 12 weeks after the end of therapy. A complete immunologic response (CIR) was defined as no detection of circulating cryoglobulins and normalized levels of complement and/or rheumatoid factor.**

RESULTS: **Clinical manifestations of CV included purpura (65%), weakness (70%), arthralgia (31%), myalgia (20%), peripheral neuropathy (50%), and renal involvement (20%). At baseline, patients with CV had significantly higher levels of rheumatoid factor and lower levels of C4 complement than patients with ACC, whereas cryocrits were similar between groups (3.2% vs 2.6%). Overall, 60 patients (94%) had a sustained viral response 12 weeks after therapy. Among patients with CV, the median Birmingham Vasculitis Activity Score (version 3) decreased from 9 (range, 2–31) to 3 (range, 0–12) ($P < .001$). Twenty-five patients with CV (71%) achieved a complete clinical response. Immune-suppressive therapy was reduced for 4 of 13 patients and withdrawn for 6 of 13. Overall, 48% of patients achieved a CIR. A low baseline cryocrit level (<2.7%) was the only factor associated with CIR (odds ratio, 9.8; 95% confidence interval, 2.2–44; $P = .03$).**

CONCLUSIONS: **Viral eradication was associated with clinical improvement in most patients with CV. Markers of immune activation, including circulating cryoglobulins, persisted in 52% of patients with CV or ACC, despite a sustained viral response 12 weeks after therapy. A longer follow-up period after viral eradication might be necessary to ensure a normal immune response.**

Keywords: Cryoglobulinemic Vasculitis; Hepatitis C; DAA; Antiviral Therapy.

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Abbreviations used in this paper: ACC, asymptomatic circulating cryoglobulins; BVAS.v3, Birmingham Vasculitis Activity Score version 3; C4, complement 4 fraction; CH50, 50% hemolytic complement activity; CV, cryoglobulinemic vasculitis; DAA, direct-acting antiviral; eGFR, glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; RF, rheumatoid factor; SVR, sustained virologic response; SVR12, sustained virologic response 12 weeks after end of therapy; TE, transient elastography.

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Approximately 180 million people worldwide are infected with the hepatitis C virus (HCV). Besides progression to cirrhosis and hepatocarcinoma, HCV patients are at risk of developing extrahepatic manifestations that include cryoglobulinemic vasculitis (CV). Indeed, HCV infection now is known to account for more than 90% of all cases of CV.[1,2] Circulating mixed cryoglobulins (immune complexes usually consisting of polyclonal IgG and monoclonal IgM with rheumatoid factor activity) are detected in 40% to 60% of patients with chronic HCV infection, although symptomatic vasculitis is observed in only approximately 10%.[3]

CV recently was classified as a secondary systemic vasculitis and named as HCV-associated CV (HCV-CV).[4] Main HCV-CV clinical manifestations include purpura, arthralgia, weakness, myalgia, polyneuropathy, glomerulonephritis, and intestinal ischemia.[5] Among them, the presence of renal, intestinal, cardiac, or central nervous system involvement confers a poor prognosis.[6]

Because HCV-CV activity usually correlates with viremia, treatment should be focused on targeting the potential causal agent. Therefore, antiviral agents are the mainstay of therapy for secondary vasculitis.[7] Prednisone (0.5–1 mg/kg/d) also may be useful to control disease activity, and depletion of B cells by rituximab may be necessary in severe HCV-CV cases.[8] Despite its side effects and poor tolerance, the combination of rituximab plus pegylated-interferon (IFN)/ribavirin compared with antiviral therapy alone has shown better short- and long-term results in patients with HCV-CV.[9,10]

The goals of antiviral treatment are to achieve sustained virologic response (SVR), obtain a clinical response, and minimize the use of immunosuppressive therapy. Historically, the likelihood of attaining a complete clinical response with IFN-based regimens was poor and usually was followed by a high relapse rate.[11] The addition of the first protease inhibitors (telaprevir and boceprevir) increased response rates to approximately 70%, but with a significant increase in side effects.[12] Currently, the advent of direct-acting antivirals (DAAs) has provided higher SVR rates and lower toxicity burden to HCV-infected patients. Nevertheless, data on the clinical and immunologic outcomes in HCV-CV patients are scarce because of the limited number of patients included in previous studies, sometimes the use of suboptimal antiviral regimens, and inclusion of patients with moderate vasculitic activity.[13–17]

The aim of this study was to analyze the virologic, clinical, and immunologic outcomes of patients with HCV-CV or asymptomatic circulating cryoglobulins (ACCs) treated with different DAA-based combinations.

Patients and Methods

Patients

We performed a prospective observational study of patients with chronic HCV infection and circulating cryoglobulins treated with DAA therapies between January 2014 and April 2016. Patients who had detectable circulating cryoglobulins on at least 2 occasions were candidates to participate in the study. Patients were classified as follows: ACC patients, and HCV-CV patients if they met the definition described at the 2012 Chapel Hill updated consensus for the nomenclature of vasculitides.[4] In addition, only patients with at least 12 weeks of follow-up evaluation after antiviral treatment were included in the current analysis.

Exclusion criteria were as follows: (1) patients with human immunodeficiency virus or active hepatitis B virus infection; (2) prior history of liver transplantation; (3) co-existence of autoimmune diseases.

This study was approved by the Ethical Committee of the Hospital Clínic at the University of Barcelona and was performed according to the Declaration of Helsinki.

Antiviral Therapy

Different therapy combinations with the new licensed DAAs in Spain were used according to the European Association for the Study of the Liver guidelines[18] and package inserts. Sustained virologic response was defined as undetectable HCV RNA levels 12 weeks after treatment cessation (SVR12). Serum HCV RNA was quantified by real-time polymerase chain reaction assay (VERSANT 1.0; Siemens, Munich, Germany; lower limit of detection, 15 IU/mL). Data on all adverse events were collected prospectively during the follow-up evaluation.

Clinical Assessment and Immunologic Markers

Baseline clinical evaluation included demographic and laboratory data, involvement of organs affected by HCV-CV, and the use of glucocorticoids or other immunosuppressive agents. Liver fibrosis was assessed by transient elastography (TE). Cirrhosis was diagnosed by liver biopsy, a TE value greater than 14 kPa,[19] the presence of esophageal varices, or ultrasonographic signs of cirrhosis (nodular liver surface, splenomegaly, and/or ascites).[20]

Clinical features attributed to HCV-CV included general symptoms (fever, myalgia, arthralgia, weight loss $\geq$2 kg in the past 6 months), cutaneous signs, sicca syndrome, peripheral and central nervous system involvement, and renal disease (defined when at least 2 of the following parameters were present: glomerular filtration rate [eGFR] $<$60 mL/min/1.73 m^2, hematuria and/or proteinuria $>$0.3 g/24 hours, or when cryoglobulinemic membranoproliferative glomerulonephritis was confirmed in a kidney biopsy).

Disease activity was evaluated using the Birmingham Vasculitis Activity Score version 3 (BVAS.v3), which has been shown to be useful in different systemic vasculitides[21] and it was shown previously to have good correlation with initial disease severity and response to

treatment in HCV-CV patients.[22] Of note, BVAS.v3 is a clinical index of disease activity with a weighted score ranging from 0 to 63 points based on symptoms in separate organ systems.

Immunologic markers included rheumatoid factor (RF), C4 complement fraction (C4), and 50% hemolytic complement activity (CH50). Circulating cryoglobulins were quantified from blood samples stored at 37°C for 30 minutes before serum separation. The serum was centrifuged later at 37°C for 10 minutes and the resulting sample was incubated at 4°C for 7 days before cryoprecipitate examination.

All variables were determined at baseline and 12 weeks after antiviral treatment.

Study End Points

Primary end points of the study were as follows: (1) rate of SVR12, (2) clinical response in HCV-CV patients, and (3) immunologic response in all patients.

Clinical response was complete when the BVAS.v3 score was 0 or if there was improvement of all affected organs. Complete renal response was defined by a decrease in proteinuria to less than 0.3 g/24 h, improvement of at least 20% of eGFR when the baseline value was less than 60 mL/min/1.73 m^2, and hematuria resolution. Neuropathy improvement (paresthesia and motor deficit) was evaluated clinically by visual analogue scale and confirmed electrophysiologically when necessary. A partial clinical response was defined as a BVAS.v3 less than 50% of the baseline score or improvement in at least half of the involved organs from baseline.[13,23] All other patients were clinical nonresponders.

The immunologic response was complete when circulating cryoglobulins became negative along with complement and/or RF normalization, and partial when any improvement of immunologic parameters was detected.

Secondary end points were an immunosuppressant dose reduction or withdrawal in HCV-CV patients, and predictive factors associated with clinical or immunologic response.

Statistical Analysis

Continuous variables were reported as medians and interquartile ranges (percentiles 25%–75%), and categoric variables as absolute and relative frequencies. Groups were compared using the Mann–Whitney test for continuous variables and the Fisher exact test for categoric variables. The Wilcoxon signed-rank test was used for the comparison between 2 paired samples. Logistic regression analysis was used to estimate the odds ratio and 95% confidence interval of predictive factors associated with the clinical and immunologic response. The analysis was performed with SPSS version 20 (SPSS, Inc, Chicago, IL). Significance was established at a 2-sided P value of .05 or less.

Results

Baseline Characteristics

Approximately 700 chronic HCV-infected patients received DAA-based antiviral therapy in our center during the study period. Of those, a total of 64 HCV-infected patients had circulating cryoglobulins. The baseline characteristics of the study cohort are shown in Table 1. Thirty-five patients (55%) accomplished the defined criteria of HCV-CV,[4,24] and 29 patients (45%) had ACC. A similar distribution of treatment-experienced patients and antiviral regimens was observed between the 2 groups. Ten patients (16%) received an IFN-based DAA combination (5 patients in each group), and the remaining 54 received IFN-free therapy. There were no differences in baseline characteristics between groups with IFN-based or IFN-free therapies.

Comparing biological baseline features between groups (Table 1), asymptomatic and vasculitic patients did not differ regarding laboratory parameters. Nevertheless, female gender was more frequent in HCV-CV patients (74% vs 48%; $P = .05$) and ACC patients had significantly higher TE values (17 vs 11.7 kPa; $P = .02$).

The main clinical manifestations in HCV-CV patients included purpura (65%), weakness (70%), peripheral neuropathy (50%), arthralgia (31%), myalgia (20%), and renal involvement (20%). Sixteen of the 18 (89%) patients with neuropathic symptoms had an electromyography confirming peripheral neuropathy (7 presented with sensory polyneuropathy, 5 with sensory-motor polyneuropathy, and 4 with sensory-motor multiplex neuropathy). Among the 7 patients with renal involvement, 5 (71%) had a renal biopsy confirming a membranoproliferative glomerulonephritis. Regarding immunosuppressive therapy at the time of starting DAA agents, 13 (37%) HCV-CV patients were on glucocorticoid therapy. The main manifestation for being treated was purpura (n = 6), severe peripheral neuropathy (n = 4), renal involvement (n = 2), and intestinal involvement (n = 1). Rituximab (375 mg/m2/week × 4 weeks) was used in 3 subjects more than 6 months before DAAs owing to cryoglobulinemic glomerulonephritis (n = 2) and severe peripheral neuropathy (n = 1), but none of the patients was on rituximab at the time of antiviral therapy. Plasma exchange was performed in 1 patient with renal involvement and neuropathy more than 12 months before DAAs.

All patients had type II cryoglobulins (usually polyclonal IgG/monoclonal IgM), except those with a cryocrit lower than 2% in whom the immunoglobulin component could not be identified (37%). Overall, approximately 70% of patients showed a decreased C4 fraction and CH50 activity and most patients in the CV group had a positive RF. HCV-CV patients showed significantly lower C4 and higher RF levels ($P < .05$), and slightly higher cryoglobulin levels (3.2 vs 2.6%; $P = .10$). CH50 activity was similar in both groups (Table 1).

Table 1. Baseline Characteristics of the 64 Patients Included in the Study

Baseline parameters	All patients (n = 64)	Patients with cryoglobulinemic vasculitis (n = 35)	Asymptomatic patients with circulating cryoglobulins (n = 29)	*P*
Age, y	61 (52–69)	61 (53–70)	63 (51–68)	.96
Female sex, n (%)	40 (63)	26 (74)	14 (48)	.05
Race, n (%)				-
Caucasian	64 (100)	35 (100)	29 (100)	
Clinical manifestations, n (%)				
Purpura		23 (66)		-
Arthralgia/arthritis		11 (31)		
Weakness		25 (71)		
Polyneuropathy		18 (51)		
Renal involvement		7 (20)		
Sicca syndrome		2 (6)		
Abdominal involvement		1 (3)		
Viral parameters				
HCV genotype, n (%)				
1a	7 (11)	5 (14)	2 (7)	.29
1b	53 (83)	28 (80)	25 (86)	
2	1 (1.5)	-	1 (3.5)	
3	1 (1.5)	-	1 (3.5)	
4	2 (3)	2 (6)	-	
Baseline HCV RNA level, log_{10} *IU/mL*	6.1 (5.5–6.4)	5.9 (5.5–6.5)	6.1 (5.4–6.3)	.69
General laboratory				
ALT level, *IU/mL*	68 (43–137)	64 (34–115)	79 (51–166)	.09
Platelets, $\times 10^9$/*L*	123 (81–172)	150 (84–183)	119 (75–155)	.22
Creatinine, *mg/dL*	0.71 (0.64–0.90)	0.78 (0.62–1.04)	0.7 (0.65–0.85)	.26
Transient elastography, *kPa*	13.6 (9.7–27)	11.7 (7.5–20.9)	17 (12–34)	.02
Cirrhosis, n (%)	37 (57)	15 (44)	20 (69)	.06
MELD score	7.5 (6–10)	7 (6–9)	9 (6–10)	.30
Immunologic parameters				
Cryocrit (%)	2.7 (1.3–5)	3.2 (1.5–5.7)	2.6 (1.2–3)	.10
C4, *g/L*	0.08 (0.02–0.14)	0.02 (0.01–0.11)	0.09 (0.06–0.18)	.02
CH50, *IU/mL*	13 (11–28)	14 (12–29)	12 (10–26)	.11
Rheumatoid factor, IU/mL	20 (10–97)	80 (10–200)	10 (10–20)	.01
Treatment, n (%)				
Naive	26 (41)	13 (37)	13 (45)	.62
Null responder	38 (59)	22 (63)	16 (55)	
DAA treatment regimens, n (%)				.60
3D	20 (31)	10 (29)	10 (34)	
SOF+LDV	18 (28)	10 (29)	8 (28)	
SOF+SIM	4 (6)	2 (6)	2 (7)	
SIM+DAC	4 (6)	3 (8)	1 (3)	
SOF+DAC	3 (5)	2 (6)	1 (4)	
PegIFN+DAAs	10 (16)	5 (14)	5 (17)	
Others[a]	5 (8)	3 (8)	2 (7)	
Use of RBV, n (%)	45 (70)	24 (69)	21 (72)	.62
Treatment duration 12/24 wk, n (%)	44 (69)/20 (31)	23 (66)/12 (34)	21 (72)/8 (28)	.56
Immunosuppressive therapy, n (%)				
Corticosteroids		13 (37)		
Rituximab		3 (8)		

NOTE. Continuous data are expressed as medians (interquartile range). Local laboratory normal ranges: C4, 0.110–1 g/L; CH50, 28–60 IU/mL; RF, <20 IU/mL. ALT, alanine aminotransferase; DAC, daclatasvir; LDV, ledipasvir; MELD, model for end-stage liver disease; PegINF, pegylated interferon; RBV, ribavirin; 3D, paritaprevir/ritonavir/ombitasvir/dasabusvir; SIM, simeprevir; SOF, sofosbuvir.

[a]Grazoprevir + elbasvir (n = 3), faldaprevir + deleobuvir (n = 2).

Antiviral Therapy: Efficacy and Tolerance

In all, 60 (94%) patients achieved SVR12; no significant differences in SVR12 rates were observed between ACC and HCV-CV patients (93 and 94%, respectively). An overall improvement was observed for the main liver parameters (Table 2). Three patients relapsed at the 12-week follow-up evaluation and 1 patient presented a breakthrough at week 4 of treatment; cirrhosis was present in 2 of the 4 patients with treatment failure.

Overall, antiviral treatment tolerance was excellent. Approximately 50% of patients referred no adverse events. Anemia was the most frequently reported adverse event (26.5%). Only 4 patients treated with ribavirin needed erythropoietin owing to grade 3 anemia (hemoglobin level, <8 g/dL), and blood transfusion was not necessary in any case. No early discontinuation of antiviral therapy occurred. However, 1 (2.8%) HCV-CV cirrhotic patient died of spontaneous bacterial peritonitis and multiorgan failure after end of treatment (Table 3).

Clinical Response

The overall clinical response in HCV-CV patients was 86% (30 of 35), with 25 patients (71%) achieving complete clinical response. Purpura, myalgia, arthralgia, and weakness resolved in 90% (28 of 32) of patients, 71% (5 of 7) of patients with renal involvement experienced complete recovery, and neuropathic symptoms improved in 72% (13 of 18). Two patients presenting with Sicca syndrome and 1 patient presenting with intestinal involvement also were asymptomatic at the end of the follow-up period. Of note, although 2 HCV-CV patients did not clear the virus, 1 patient presented with amelioration of symptoms (neuropathy) and the other patient achieved a complete clinical response (Meltzer triad: purpura, weakness, and arthralgia).[25]

When assessing clinical improvement by BVAS.v3, the score decreased significantly from a median of 9 (range, 2–31) to 3 (range, 0–12) points ($P = .001$). Among the 25 (71%) patients who experienced a complete clinical response (13 by BVAS.v3 score of 0, and 12 by improvement of all affected organs), the median BVAS.v3 decreased from 5 (range, 2–31) to 0 (range, 0–5) ($P = .001$). In addition, in the 5 (14%) patients experiencing a partial clinical response, BVAS.v3 score decreased from 12 (range, 9–12) to 6 (range, 1–6) ($P = .06$) (Supplementary Table 1).

When specifically analyzing the subgroup of patients with vasculitic nephropathy (n = 7; median eGFR, 40 mL/min), 100% of patients accomplished viral

Table 2. Clinical, Biological, and Immunologic Features Before and After DAA Therapy

Parameters	Cryoglobulinemic vasculitis (n = 35)			Asymptomatic patients (n = 29)		
	Pretreatment	Follow-up period	*P*	Pretreatment	Follow-up period	*P*
SVR rate, n (%)	-	33 (94)	-		27 (93)	-
Cryocrit (%)	3.2 (1.5–5.7)	0.5 (0–1.4)	.01	2.6 (1.2–3)	0 (0–1.1)	.01
Circulating cryoglobulins, n (%)	35 (100)	19 (55)	-	29 (100)	11 (38)	-
C4 complement fraction, *g/L*	0.02 (0.01–0.11)	0.12 (0.05–0.16)	.01	0.09 (0.06–0.18)	0.12 (0.08-0.17)	.02
Reduced C4 fraction, n (%)	26 (74)	15 (43)	-	18 (62)	12 (41)	-
CH50 activity, *U/mL*	14 (12–29)	44 (25–53)	.01	12 (10–26)	31 (14–46)	.01
Reduced CH50 activity, n (%)	28 (80)	8 (22)	-	22 (76)	13 (45)	-
Rheumatoid factor level, *IU/mL*	80 (10–200)	20 (10–95)	.01	10 (10–20)	10 (10–10)	.04
Positive rheumatoid factor, n (%)	24 (69)	17 (49)	-	9 (31)	6 (20)	-
ALT level, *IU/mL*	64 (34–115)	24 (17–28)	.01	79 (51–166)	20 (16–27)	.01
Platelets, $\times 10^9$/L	123 (81–172)	159 (107–229)	.19	119 (75–155)	118 (67–170)	.98
MELD score	7 (6–9)	6 (6–8.5)	.24	9 (6–10)	8 (6–10)	.25
Creatinine level, *mg/dL*	1.5 (1–1.7)	1.25 (1.1–2.1)	.12	0.7 (0.65–0.85)	0.7 (0.62–0.082)	.86
eGFR, *mL/min/1.73 m²*	90 (53–90)	90 (65–90)	.20	90 (81–90)	90 (83–90)	.46
Prednisone, *mg/d*	10 (5–30)	0 (0–3.7)	.01	-	-	
Complete clinical response, n (%)	-	25 (71)				
BVAS v3 score	9 (4–12)	3 (0–6)	.001	-	-	
Clinical manifestations, n (%)						
Purpura	23 (65)	2 (6)	.01			
Arthralgia	11 (31)	1 (3)	.01			
Weakness	25 (70)	1 (2)	.01			
Polyneuropathy	18 (50)	5 (14)	.01			
Renal involvement	7 (20)	2 (5)	.02			
Hematuria >10 RBCs/hpf, n (%)	5 (71)	2 (25)	.03			
Median eGFR, *mL/min/1.73 m²*	40 (31–44)	54 (36–60)	.03			
Proteinuria, *g/L*	1.4 (1.1–1.9)	0.17 (0.9–1.8)	.73			
Creatinine level, *mg/dL*	1.6 (1–1.7)	1.27 (1.1–2)	.89			

NOTE. Continuous data are expressed as medians (interquartile range), except BVAS.v3 score (median and range).
ALT, alanine aminotransferase; MELD, model for end-stage liver disease; RBC, red blood cell.

Table 3. Summary of Adverse Events During Antiviral Therapy

Adverse event[a]	HCV-CV patients (n = 35)	ACC patients (n = 29)
No adverse event	18 (51.4%)	18 (62%)
Anemia, hemoglobin level, <10 g/dL	11 (31.4%)	6 (21%)
Headache	2 (5.7%)	0
Pruritus	2 (5.7%)	0
Insomnia	1 (2.8%)	1 (3.4%)
Rash	1 (2.8%)	0
Infection	1 (2.8%)	0

[a]Some patients had more than 1 adverse event.

eradication. Moreover, these excellent SVR rates were followed by complete renal response in 71% (5 of 7) of subjects with a significant improvement in eGFR (median, 40 to 54 mL/min/1.73 m^2; $P = .03$), decrease of proteinuria, and disappearance of hematuria. These findings also were consistent with a significant decrease in BVAS.v3 score from a median of 16 to 3 points ($P = .01$).

Cirrhosis stage did not have an influence on the achievement of clinical response. Indeed, a total of 47% cirrhotic patients and 56% noncirrhotic patients achieved a clinical response ($P = .7$), and change in BVAS score did not differ between both groups.

Regarding immunosuppressive therapy, glucocorticoid doses could be reduced in 4 of 13 (30%) patients, and withdrawn in 6 (46%). Neither rituximab nor plasma exchange sessions were needed in any patient during the study period.

Immunologic Response

All immunologic parameters improved in both groups 12 weeks after therapy. Circulating cryoglobulins became undetectable in 45% and 62% of patients in the HCV-CV and ACC groups, respectively (Table 2). Among HCV-CV patients, 42%, 71%, and 29% of patients presented normalization of C4, CH50, and RF levels, respectively. For the ACC patients, the figures were 33%, 41%, and 33%. Overall, 30 patients (48%) achieved a complete immunologic response, 43% (15 of 35) of those with HCV-CV and 53% (15 of 29) of those with ACC ($P = .20$) (Figure 1). Despite failure to achieve SVR12, 1 patient achieved a complete clinical and immunologic response after treatment with cryoglobulin negativization.

The relationship between clinical and immunologic response among HCV-CV patients was assessed. Interestingly, the majority (73%) of immunologic responders also presented clinical improvement, whereas immunologic parameters normalized only in 37% of patients with clinical response (for detailed information on antiviral therapy and clinical response, see Supplementary Table 1).

Predictive Factors of Clinical and Immunologic Response

We aimed to assess if there were any baseline predictors of clinical and immunologic response. Among HCV-CV patients, we failed to find an association between clinical response and relevant variables such as treatment regimen and duration, cirrhosis, cryocrit levels, or BVAS.v3 score, which probably can be explained by the small number of clinical nonresponders.

At the immunologic level, the variables associated with complete immunologic response for both HCV-CV and ACC patients were as follows: IFN-based therapy, antiviral treatment duration, baseline cryocrit, and RF. Of note, patients treated for 24 weeks had a higher rate of cryocrit negativization (70% vs 44%; $P = .05$), C4 improvement (75% vs 48%; $P = .05$), and complete immunologic response (70% [14 of 20] vs 37% [16 of 44]; $P = .01$) compared with those treated for 12 weeks. However, when a multivariate analysis was performed, only the presence of a baseline cryocrit less than 2.7% (median value) was associated independently with the achievement of complete immunologic response (odds ratio, 9.8; 95% confidence interval, 2.2–44; $P = .03$) (Table 4).

Discussion

The landscape of antiviral therapy in HCV-infected patients has changed dramatically with the new DAAs.[18] However, few data are available regarding the safety and efficacy of the new drugs in CV, particularly in patients with ACCs, who are at risk of developing CV. Recent studies in CV patients have included only patients treated with sofosbuvir-based regimens.[13,14,17] Therefore, a broader experience of different DAA combinations is needed to elucidate unresolved issues, particularly the efficacy of these regimens in achieving and maintaining clinical and immunologic response.

The results of our study showed a greater immunologic activation (higher circulating cryoglobulins, lower C4 and CH50, and increased RF) in HCV-CV patients compared with ACC. This finding is explained by a correlation between CV damage and the amount and structure of the circulating cryoglobulins, particularly by the presence of RF, which can activate the complement cascade.[26] Although these findings already were described in HCV-CV patients,[26] our data show an immunologic activation in patients with ACC as well.

The goals of antiviral treatment in patients with HCV-CV are not only achieving SVR, but also symptomatic response of CV and minimization of the use of immunosuppressive therapies. Antiviral treatment

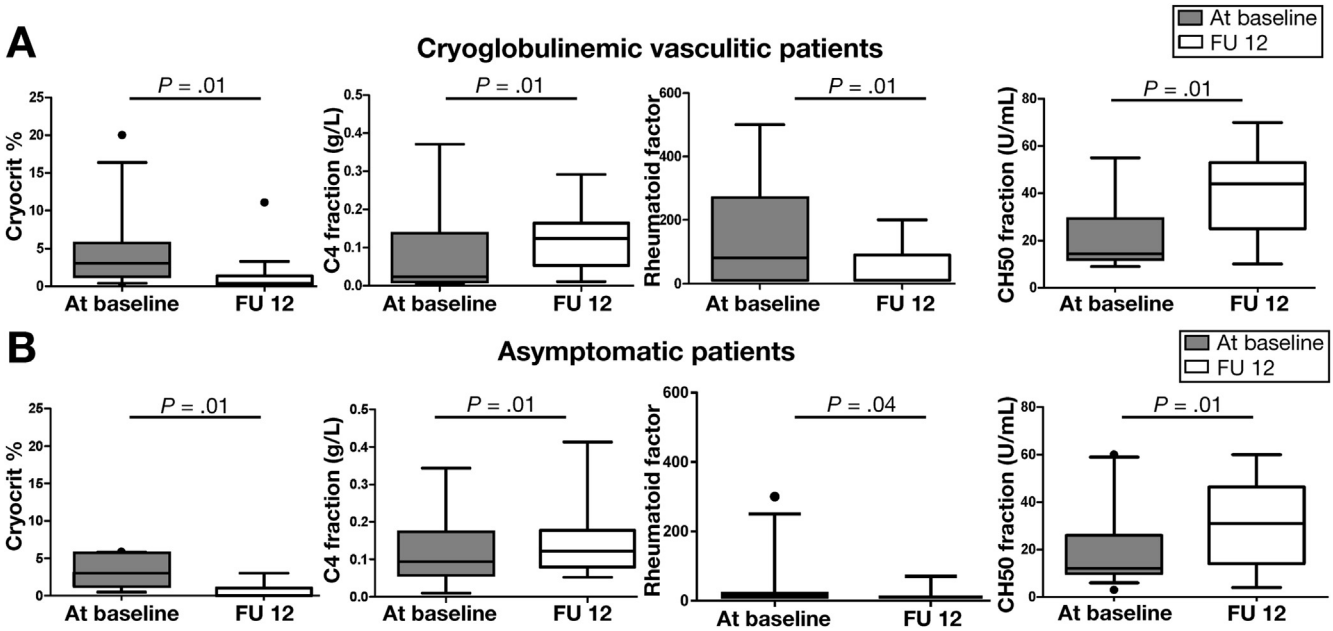

Figure 1. Immunologic parameters in (*A*) HCV-CV and (*B*) ACC patients at baseline and 12 weeks after DAA regimens. FU, follow-up weeks.

efficacy was high in both groups (94% SVR12) regardless of the type of antiviral regimen. Disappearance of purpura, arthralgia, myalgia, and weakness was reported in nearly 90% of CV subjects. The BVAS.v3 score, a generic tool designed for all types of vasculitis, was used to assess the clinical disease status before and after HCV therapy. BVAS.v3 not only can anticipate disease damage, but also is predictive of lower survival rates when the score is greater than 8 points,[21,27] which occurred in more than 50% (n = 19) of the HCV-CV patients. Despite this high value, a complete clinical response rate of 71% was achieved, reinforced by an overall BVAS.v3 decrease to a median of 3 points. Although the rate of clinical response was similar to that reported in a recent study by Gragnani et al,[17] the CV patients in our study showed higher baseline vasculitis activity disease, depicted by a mean BVAS.v3 score of 9 vs 5.4 points,[17] respectively.

Kidney involvement has been frequently associated with unfavorable virologic and clinical response in HCV-CV patients.[28,29] However, all of our patients with vasculitic nephropathy achieved SVR12, and 71% also had a complete clinical response, suggesting that vasculitic nephropathy no longer would be a pitfall for viral eradication with new DAAs.

Our study also showed that glucocorticoids could be either tapered or stopped in most patients after viral clearance with DAA agents, including those with CV nephropathy and neuropathy. Rituximab was not necessary for any of the HCV-CV patients. These excellent outcomes might suggest that immunosuppressive therapy from here onward could be necessary only in those HCV-CV patients with immediate life-threatening situations.

The clinical response in HCV-CV patients was associated with an immunologic improvement in almost all patients, but this response was complete in only one third of them. The asymptomatic immunologic activation in ACC patients also improved after therapy. However, some degree of immunologic activation still was present in nearly 50% of patients at the end of follow-up

Table 4. Baseline Factors Associated With the Achievement of Complete Immunologic Response in the Whole Cohort

Variable	Univariate analysis,[a] OR (95% CI)	*P* value	Multivariate analysis, OR (95% CI)	*P* value
Cirrhosis	0.4 (0.15–1.1)	.08		
IFN-based therapy	5.4 (1.01–27)	.04		
24 vs 12 wk	3.7 (1.2–11)	.02		
Cryocrite, <2.7 (%)	6.6 (2.2–19)	<.01	9.8 (2.2–44)	.003
C4, *g/L*	265 (4–999)	.09		
Rheumatoid factor level	0.99 (0.98–0.99)	.03		

CI, confidence interval; OR, odds ratio.
[a]Only variables with a *P* value < .10 in univariate analysis are shown.

evaluation. Similar results have been reported previously with both IFN and DAA regimens.[16,17,30] Although a possible explanation of this finding might be that B-cell clonal expansion continues expanding in a virus-independent fashion, the presence of an overt B-cell lymphoma was excluded clinically as well.[31,32] These results suggest that clinical recovery occurs first after viral clearance, and immunologic response seems to arise later, probably depending on the time required for reversion of the B-lymphocyte clonal expansion producing immunologic changes.[3,33] Indeed, a longer treatment course, which implies a longer virus-free period, appears to favor an immunologic response. In the Gragnani et al[17] study, the rate of complete immunologic response increased slightly from 32% at week 12 to 39% at week 24, (although not all patients had been assessed at this time point). Thus, a longer observational period probably would allow us to draw a stronger conclusion regarding immunologic and clinical response.

In summary, this was a prospective single-center study in which patients were followed up homogeneously and assessed the immunologic impact of DAA-based therapy at SVR12 in a considerable number of patients with asymptomatic cryoglobulinemia, who are at risk of developing overt CV.

The results of our study show that SVR correlates with clinical and immunologic improvement in most patients. Indeed, an abrupt decay of HCV RNA with current DAAs is associated with rapid control of clinical vasculitis manifestations, allowing reduction and cessation of traditional immunosuppressive therapy. A complete normalization of the immune activation status seems to take longer after HCV clearance. However, these promising results deserve further evaluation in larger series, followed by a longer follow-up period.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2016.09.158.

References

1. Ramos-Casals M, Stone JH, Cid MC, et al. The cryoglobulinaemias. Lancet 2012;379:348–360.
2. Retamozo S, Brito-Zerón P, Bosch X, et al. Cryoglobulinemic disease. Oncology (Williston Park) 2013;27:1098–1105, 1110–1116.
3. Santer DM, Ma MM, Hockman D, et al. Enhanced activation of memory, but not naïve, B cells in chronic hepatitis c virus-infected patients with cryoglobulinemia and advanced liver fibrosis. PLoS One 2013;8:e68308.
4. Jennette JC, Falk RJ, Bacon PA, et al. 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013;65:1–11.
5. Cacoub P, Comarmond C, Domont F, et al. Cryoglobulinemia vasculitis. Am J Med 2015;128:950–955.
6. Ferri C, Sebastiani M, Giuggioli D, et al. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. Semin Arthritis Rheum 2004;33:355–374.
7. Hernandez-Rodriguez J, Alba MA, Prieto-Gonzalez S, et al. Diagnosis and classification of polyarteritis nodosa. J Autoimmun 2014;48–49:84–89.
8. De Vita S, Quartuccio L, Isola M, et al. A randomized controlled trial of rituximab for the treatment of severe cryoglobulinemic vasculitis. Arthritis Rheum 2012;64:843–853.
9. Saadoun D, Rigon MR, Sene D, et al. Rituximab plus Peg-interferon-α/ribavirin compared with Peg-interferon-α/ribavirin in hepatitis C–related mixed cryoglobulinemia. Blood 2010;116:326–334.
10. Dammacco F, Tucci FA, Lauletta G, et al. Pegylated interferon-α, ribavirin, and rituximab combined therapy of hepatitis C virus–related mixed cryoglobulinemia: a long-term study. Blood 2010;116:343–353.
11. Saadoun D, Resche-Rigon M, Thibault V, et al. Antiviral therapy for hepatitis C virus–associated mixed cryoglobulinemia vasculitis: a long-term follow-up study. Arthritis Rheum 2006;54:3696–3706.
12. Saadoun D, Resche Rigon M, Pol S, et al. PegIFNα/ribavirin/protease inhibitor combination in severe hepatitis C virus-associated mixed cryoglobulinemia vasculitis. J Hepatol 2015;62:24–30.
13. Saadoun D, Thibault V, Si Ahmed SN, et al. Sofosbuvir plus ribavirin for hepatitis C virus-associated cryoglobulinemia vasculitis: VASCUVALDIC study. Ann Rheum Dis 2016;75:1777–1782.
14. Sise ME, Bloom AK, Wisocky J, et al. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with sofosbuvir-based direct-acting antiviral agents. Hepatology 2016;63:408–417.
15. Makara M, Sulyok M, Csacsovszki O, et al. Successful treatment of HCV-associated cryoglobulinemia with ombitasvir/paritaprevir/ritonavir, dasabuvir and ribavirin: a case report. J Clin Virol 2015;72:66–68.
16. Cornella SL, Stine JG, Kelly V, et al. Persistence of mixed cryoglobulinemia despite cure of hepatitis C with new oral antiviral therapy including direct-acting antiviral sofosbuvir: a case series. Postgrad Med 2015;127:413–417.
17. Gragnani L, Visentini M, Fognani E, et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. Hepatology 2016;64:1473–1482.
18. Pawlotsky JM, Aghemo A, Back D, et al. European Association for Study of Liver. EASL recommendations on treatment of hepatitis C 2015. J Hepatol 2015;63:199–236.
19. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835–847.
20. Berzigotti A, Castera L. Update on ultrasound imaging of liver fibrosis. J Hepatol 2013;59:180–182.
21. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). Ann Rheum Dis 2009;68:1827–1832.
22. Lamprecht P, Moosig F, Gause A, et al. Birmingham vasculitis activity score, disease extent index and complement factor C3c reflect disease activity best in hepatitis C virus-associated cryoglobulinemic vasculitis. Clin Exp Rheumatol 2000;18:319–325.
23. Gragnani L, Fognani E, Piluso A, et al. Long-term effect of HCV eradication in patients with mixed cryoglobulinemia: a

prospective, controlled, open-label, cohort study. Hepatology 2015;61:1145–1153.
24. De Vita S, Soldano F, Isola M, et al. Preliminary classification criteria for the cryoglobulinaemic vasculitis. Ann Rheum Dis 2011;70:1183–1190.
25. Meltzer M, Franklin EC, Elias K, et al. Cryoglobulinemia: a study of twenty-nine patients. I. IgG and IgM cryoglobulins and factors affecting cryoprecipitability. Am J Med 1996;40:837–856.
26. Lauletta G, Russi S, Conteduca V, et al. Impact of cryoglobulinemic syndrome on the outcome of chronic hepatitis C virus infection: a 15-year prospective study. Medicine (Baltimore) 2013;92:245–256.
27. Suppiah R, Mukhtyar C, Flossmann O, et al. A cross-sectional study of the Birmingham vasculitis activity score version 3 in systemic vasculitis. Rheumatology 2011;50:899–905.
28. Landau DA, Scerra S, Sene D, et al. Causes and predictive factors of mortality in a cohort of patients with hepatitis C virus-related cryoglobulinemic vasculitis treated with antiviral therapy. J Rheumatol 2010;37:615–621.
29. Fabrizi F. Hepatitis C virus, cryoglobulinemia, and kidney: novel evidence. Scientifica (Cairo) 2012;2012:128382.
30. D'Amico E, Chincoli C, Cacciatore P, et al. Effects of combined antiviral therapy on asymptomatic mixed cryoglobulinemia in naive patients with chronic hepatitis C virus infection: a preliminary study. Dig Dis Sci 2005;50:2344–2347.
31. Landau D-A, Saadoun D, Halfon P, et al. Relapse of hepatitis C virus–associated mixed cryoglobulinemia vasculitis in patients with sustained viral response. Arthritis Rheum 2008; 58:604–611.
32. Terrier B, Semoun O, Saadoun D, et al. Prognostic factors in patients with hepatitis C virus infection and systemic vasculitis. Arthritis Rheum 2011;63:1748–1757.
33. Reyes-Avilés E, Kostadinova L, Rusterholtz A, et al. Presence of rheumatoid factor during chronic HCV infection is associated with expansion of mature activated memory B-cells that are hypo-responsive to B-cell receptor stimulation and persist during the early stage of IFN free therapy. PLoS One 2015;10:1–16.

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Conflicts of interest
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Supplementary Table 1. Clinical Outcomes in Vasculitic Patients at Follow-Up 12 Weeks After DAAs Therapy Completion

		BVAS.v3		Meltzer triad		Nephropathy		Neuropathy		Clinical response
Number	Treatment, *wk*	Baseline	FU12	Baseline	FU12	Baseline	FU12	Baseline	FU12	FU12
1	3D, 12	6	6	-	-	-	-	Yes	Yes	Null
2	3D, 12	18	3	Yes	No	-	-	Yes	No	Complete
3	3D, 12	8	3	Yes	No	-	-	Yes	No	Complete
4	Simeprevir + daclatasvir, 24	12	12	-	-	Yes	Yes	-	-	Null
5	Faldaprevir + deleobuvir, 24	10	9	-	-	-	-	Yes	Yes	Null
6	Sofosbuvir + daclatasvir, 24	23	5	Yes	No	Yes	No	Yes	No	Complete
7	Sofosbuvir + ledipasvir, 24[a]	31	3	Yes	Yes	Yes	No	Yes	No	Complete
8	Sofosbuvir + ledipasvir, 12	11	3	Yes	No	-	-	Yes	No	Complete
9	Simeprevir + daclatasvir, 12	3	0	Yes	No	-	-	-	-	Complete
10	Elbasvir + grazoprevir, 12	10	9	-	-	-	-	Yes	Yes	Null
11	3D, 12	6	3	-	-	-	-	Yes	No	Complete
12	Sofosbuvir + ledipasvir, 12	10	3	Yes	No	-	-	Yes	No	Complete
13	Sofosbuvir + ledipasvir, 12	3	0	Yes	No	-	-	-	-	Complete
14	Elbasvir + grazoprevir, 12	3	0	Yes	No	-	-	-	-	Complete
15	Sofosbuvir + daclatasvir, 24[b]	9	6	Yes	No	-	-	Yes	Yes	Partial
16	Sofosbuvir + ledipasvir, 12	12	6	-	-	-	-	Yes	No	Partial
17	Sofosbuvir + simeprevir, 24	11	10	-	-	Yes	Yes	-	-	Null
18	3D, 12	4	0	Yes	No	-	-	-	-	Complete
19	Sofosbuvir + ledipasvir, 24	4	0	Yes	No	-	-	-	-	Complete
20	Simeprevir + daclatasvir, 12	12	1	Yes	Yes	Yes	No	-	-	Partial
21	3D, 12	11	6	Yes	No	-	-	Yes	Yes	Partial
22	3D, 12	4	0	Yes	No	-	-	-	-	Complete
23	3D, 24	11	3	Yes	No	-	-	Yes	No	Complete
24	Sofosbuvir + simeprevir, 24	2	0	Yes	No	-	-	-	-	Complete
25	Peg + sofosbuvir + ribavirin	8	0	Yes	No	-	-	Yes	No	Complete
26	Peg + ribavirin + daclatasvir + asunaprevir, 24	22	3	Yes	No	Yes	No	Yes	No	Complete
27	Peg + ribavirin + daclatasvir + asunaprevir, 24	16	0	-	-	Yes	No	Yes	No	Complete
28	Sofosbuvir + ledipasvir, 12	10	4	Yes	No	-	-	Yes	No	Complete
29	3D, 12	2	0	Yes	-	-	-	-	-	Complete
30	Peg + ribavirin + simeprevir, 24[b]	4	1	Yes	No	-	-	-	-	Complete
31	Sofobuvir + ledipasvir, 12	3	0	Yes	No	-	-	-	-	Complete
32	Peg + ribavirin + simeprevir, 24	4	0	Yes	No	-	-	-	-	Complete
33	3D, 12	3	0	Yes	No	-	-	-	-	Complete
34	Sofosbuvir + ledipasvir, 12[c]									Partial
35	Sofosbuvir + ledipasvir, 24[c]									Complete

FU12, follow-up 12 weeks after DAAs therapy completion; PegINF, pegylated interferon 3D, paritaprevir/ritonavir/ombitasvir/dasabusvir.
[a]Patient with intestinal involvement.
[b]Patients with non-SVR.
[c]Patients with Sicca syndrome.