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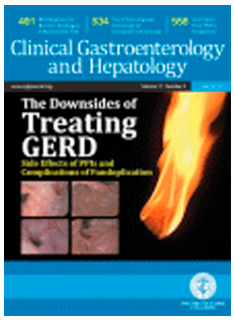

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Anti-HCV treatment in cryoglobulinemic patients

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Conflict of interest

XF received unrestricted grant support from Abbvie and has acted as advisor for Abbvie, Janssen, and Gilead. SL has acted as advisor for Abbvie, Janssen, and Gilead. MCL has acted as advisor for MSD, Janssen, BMS and Gilead. ZM has acted as advisor for BMS. MB, JMST, MCC, MRC and JHR have nothing to declare.

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Abbreviations: ACC, asymptomatic circulating cryoglobulins; ALT, alanine aminotransferase; BVAS.v3, Birmingham Vasculitis Activity Score version 3; C4, Complement 4 fraction; CH50, Total hemolytic complement fraction; CV, Cryoglobulinemic vasculitis; DAAs, Direct-acting antivirals; eGFR, glomerular filtration rate; HCV, Hepatitis C virus; HCV-CV, Hepatitis C associated cryoglobulinemic vasculitis; IFN, Interferon; RF, Rheumatoid Factor; RBV, Ribavirin; SOF, Sofosbuvir; SIM, Simeprevir; SVR, sustained virological response; SVR12, sustained virological response 12 weeks after end of therapy; TE, transient elastography; 3D regimen, Paritaprevir/Ritonavir, Ombitasvir, and Dasabuvir);

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Author contribution: MB, SL, XF and JHR have participated in the study design, analysis and interpretation of the data and drafting the manuscript. All authors have contributed to its critical revision for important intellectual content; have given final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article.

Abstract:

Background & Aims: Cryoglobulins (circulating immune complexes of polyclonal IgG, monoclonal IgM, and rheumatoid factor) are detected in the circulation of 40%–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 (DAA) treatment.

Methods: We performed a prospective study of 64 patients with HCV infection with circulating cryoglobulins receiving direct-acting anti-viral 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 (BVASv.3) was 0, or all affected organs improved 12 weeks after end of therapy. A complete immunological response (CIR) was defined as detection of no 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 (SVR12). Among patients with CV, the median BVASv.3 decreased from 9 (range, 2–31) to 3 (0–12) (P<.001). Twenty-five patients with CV (71%) achieved a complete clinical response. Immune-suppressive therapy was reduced for 4/13 patients and withdrawn for 6/13. Overall, 48% of patients achieved a CIR. A low baseline cryocrit level (below 2.7%) was the only factor associated with CIR (odds ratio, 9.8; 95% CI, 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 SVR12. A longer follow-up period after viral eradication might be necessary to ensure a normal immune response.

KEY WORDS: cryoglobulinemic vasculitis, hepatitis C, DAA, antiviral therapy

Introduction

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 is now known to account for more than 90% of all the 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 40-60% of patients with chronic HCV infection, although symptomatic vasculitis is observed only in around 10%[3].

CV has recently been 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].

As 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 to 1 mg/kg/day) may also 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 (Peg-INF)/ribavirin(RBV) 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 virological response (SVR), obtain clinical response and minimize the use of immunosuppressive therapy. Historically, the likelihood of attaining complete clinical response with Interferon (IFN)-based regimens was poor and usually followed by a high relapse rate[11]. The addition of the first protease inhibitors (Telaprevir and Boceprevir) increased response rates to about 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 immunological outcomes in HCV-CV patients are scarce due to the limited number of patients included in previous studies, sometimes the use of suboptimal antiviral regimens and inclusion of patients with moderate vasculitic activity[13,14,15,16,17].

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

Patients and methods

Patients

Prospective observational study of patients with chronic HCV infection and circulating cryoglobulins treated with DAA therapies between January 2014 and April 2016. Those who had detectable circulating cryoglobulins at least on two occasions were candidates to participate in the study. Patients were classified into 1) asymptomatic patients with circulating cryoglobulins (ACC) and 2) HCV-CV patients if they accomplished 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 after antiviral treatment were included in the current analysis.

Exclusion criteria were: 1) Patients with HIV or active HBV infection; 2) Prior history of liver transplantation; 3) Coexistence of autoimmune diseases

The study was approved by the Ethical Committee of Hospital Clínic 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 EASL guidelines[18] and inserted packages. Sustained virological response (SVR12) was defined as undetectable HCV-RNA levels 12 weeks after treatment cessation. Serum HCV RNA was quantified by real-time PCR assay (VERSANT 1.0, Siemens, lower limit of detection 15IU/mL). Data on all adverse events were prospectively collected during the follow-up.

Clinical assessment and immunological 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 > 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 ≥2kg in the last 6 months), cutaneous signs, sicca syndrome, peripheral and central nervous system involvement and renal disease (defined when at least two of the following parameters were present: glomerular filtration rate (eGFR) <60 ml/min/1.73m^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 by using the Birmingham Vasculitis Activity Score version 3 (BVAS.v3), which has shown to be useful in different systemic vasculitides[21] and it has previously demonstrated a 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.

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

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

Study Endpoints

Primary endpoints of the study were: 1) rate of SVR12, 2) clinical response in HCV-CV patients and 3) immunological response in all patients.

Clinical response was complete when BVAS.v3 score =0 or if improvement of all affected organs. Complete renal response was defined by decrease in proteinuria to <0.3 gr/24h, an improvement of at least 20% of eGFR when baseline value was <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. Partial clinical response was defined as BVAS.v3 <50% of the baseline score or improvement in at least half of the involved organs from baseline [13,23]. All other patients were clinical non-responders.

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

Secondary endpoints were: 1) immunosuppressant dose reduction or withdrawal in HCV-CV patients and 2) predictive factors associated to clinical or immunological response.

Statistical analysis

Continuous variables were reported as median and interquartile range (percentiles 25–75%) and categorical variables as absolute and relative frequencies. Groups were compared using the Mann–Whitney test for continuous variables and the Fisher's exact test for the categorical ones. Wilcoxon signed rank test was used for the comparison between two paired samples. Logistic regression analysis was used to estimate the odds ratio [OR (95%CI)] of predictive factors associated with the clinical and immunological response. The analysis was performed with SPSS version 20 (SPSS Inc, Chicago, IL). Significance was established at a two-sided p-value ≤0.05.

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 depicted in **Table 1**. Thirty-five patients (55%) accomplished the defined criteria of HCV-CV[4,24], while 29 patients (45%) had asymptomatic circulating cryoglobulins (ACC). A similar distribution of treatment-experienced patients and antiviral regimens was observed between the two 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 sex was more frequent in HCV-CV patients (74% vs. 48%; p=0.05) and ACC patients had significantly higher TE values (17 vs. 11.7 kPa; p=0.02).

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 (seven presented sensory polyneuropathy, five sensory-motor polyneuropathy, and four sensory-motor multiplex neuropathy). Among the seven

patients with renal involvement, five (71%) had a renal biopsy confirming a membrano-proliferative 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 (n=2) and intestinal involvement (n=1). Rituximab (375mg/m2/week x 4 weeks) was used in 3 subjects >6 months prior to DAAs due 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 one patient with renal involvement and neuropathy >12 months prior to DAAs.

All patients had type II cryoglobulins (usually polyclonal IgG/monoclonal IgM), except those with a cryocrit lower to 2% in whom immunoglobulin component could not be identified (37%). Overall, around 70% of patients showed 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 ($p<0.05$), and slightly higher cryoglobulins levels (3.2 *vs*. 2.6%, $p=0.10$). CH50 activity was similar in both groups (**Table 1**).

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 FU12 and one patient presented a breakthrough at week 4 of treatment; cirrhosis was present in 2 of the four patients with treatment failure.

Overall, antiviral treatment tolerance was excellent. Around 50% of patients referred no adverse events. Anemia was the most frequently reported adverse event (26.5%), followed by asthenia and headache. Only four patients treated with RBV needed erythropoietin due to a grade 3 anemia (hemoglobin < 8g/dl), whereas blood transfusion was not necessary in any case. No early discontinuation of antiviral therapy occurred. However, one (2.8%) HCV-CV cirrhotic patient died due to spontaneous bacterial peritonitis and multiorgan failure after end of treatment (**Table 3**).

Clinical response

The overall clinical response in HCV-CV patients was 86% (30/35). Purpura, myalgia, arthralgia and weakness resolved in 90% (28/32) of patients while 71% (5/7) of patients with renal involvement experienced complete recovery and neuropathic symptoms improved in 72%

(13/18). Two patients presenting Sicca syndrome and 1 with intestinal involvement were also asymptomatic at the end of the follow-up. Of note, although two HCV-CV patients did not clear the virus, one presented amelioration of symptoms (neuropathy) and the other 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 [0-12] points (p=0.001). Among the 25(71%) patients who experienced a complete clinical response (13 by BVAS.v3=0 and 12 by improvement of all affected organs), median BVAS.v3 decreased from 5 [2-31] to 0 [0-5] (p=0.001). In addition, in the five (14%) patients experiencing partial clinical response, BVAS.v3 score decreased from 12 [9-12] to 6 [1-6] (p= 0.06) **(Supplementary Table 1)**.

When specifically analyzing the subgroup of patients with vasculitic nephropathy (n=7, median eGFR of 40ml/min), 100 % of patients accomplished viral eradication. Moreover, these excellent SVR rates were followed by complete renal response in 71% (5/7) of subjects with a significant improvement of eGFR [median= 40 to 54 ml/min/1.73m^{2,} p=0.03], decrease of proteinuria and disappearance of hematuria. These findings were also consistent with a significantly BVAS.v3 scored decrease from a median of 16 to 3 points (p=0.01).

Cirrhosis stage did not have an influence on the achievement of clinical response. Indeed, a total of 47% cirrhotic patients and 56% non-cirrhotic patients achieved a clinical response (p=0.7) and change in BVAS score did not differ between both groups.

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

Immunological response

All immunological 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 group, 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 immunological response, 43% (15/35) of HCV-CV and 53% (15/29) ACC (p=0.20) (**Figure 1**). Despite failure to achieve SVR12, one patient achieved a complete clinical and immunological response after treatment with cryoglobulin negativization.

The relationship between clinical and immunological response among HCV-CV patients was assessed. Interestingly, the majority (73%) of immunological responders also presented clinical improvement, whereas immunological 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 immunological response

We aimed to assess if there were any baseline predictors of clinical and immunological 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 might be probably explained by the small number of clinical non-responders.

At the immunological level, the variables associated with complete immunological response for both, HCV-CV and ACC patients were: 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=0.05), C4 improvement (75% *vs*. 48%; p=0.05) and higher rate of complete immunological response [70% (14/20) *vs*. 37% (16/44); p=0.01)] compared to those treated for 12 weeks. However, when a multivariate analysis was performed, only the presence of a baseline cryocrit <2.7% (median value) was independently associated to the achievement of complete immunological response (OR =9.8 [2.2-44], p=0.03) **(Table 4).**

Discussion

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

The results of our study showed a greater immunological activation (higher circulating cryoglobulins, lower C4 and CH50 and raised RF) in HCV-CV patients compared to 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 had been already described in HCV-CV patients [26], our data show an immunological 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 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. BVAS.v3 score, a generic tool designed for all types of vasculitis, was used in order to assess the clinical disease status before and after HCV therapy. BVAS.v3 not only can anticipate disease damage but is also predictive of lower survival when the score is >8 points [21,27] ,which occurred in >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 is similar to that reported in a recent study of Gragnani et al[17]; 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 frequently been associated with unfavorable virological and clinical response in HCV-CV patients[28,29]. However, all of our patients with vasculitic nephropathy achieved SVR12, and 71% also a complete clinical response, suggesting that vasculitic nephropathy would no longer be a pitfall for viral eradication with new DAAs.

This is the largest study showing 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 onwards 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 immunological improvement in almost all patients, but this response was complete in only 1/3 of them. The asymptomatic immunological activation in ACC patients also improved after therapy. However, some degree of immunological activation was still present in nearly 50% of patients at the end of follow-up. Similar results have been previously reported with both, IFN and DAAs 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 clinically excluded as well [31,32]. These results suggest that clinical recovery occurs first after viral clearance, and immunological response seems to arise later, probably depending on the

time required for reversion of the B-lymphocyte clonal expansion producing immunological changes[3,33] . Indeed, a longer treatment course which implies a longer virus-free period appears to favor an immunological response. In Gragnani study, the rate of complete immunological response increased slightly from 32% at week 12 to 39% at week 24, (although not all patients had been assessed at this time-point)[17]. Thus, a longer observational period would probably allow us to draw a stronger conclusion regarding immunological and clinical response.

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

The results of our study show that SVR correlates with clinical and immunological improvement in most patients. Indeed, an abrupt decay of HCV-RNA with current DAAs is associated with a 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 period of time.

Figure legends

Figure 1 Immunological Parameters in HCV-CV (Panel A) and ACC patients (Panel B) at baseline and 12 weeks after DAAs regimens.

***Author names in bold (Martin Bonacci and Sabela Lens) designate shared co-first authorship**

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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 (years)	61(52-69)	61(53-70)	63(51-68)	0.96
Female sex n(%)	40(63)	26 (74)	14 (48)	0.05
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)	0.29
1b2	53 (83)1	28 (80)	25 (86) 1(3.5)	
3	1	-	1(3.5)	
4	2	-	-	
		2(6)		
Baseline HCV RNA(log_{10}IU/mL)	6.1 (5.5-6.4)	5.9 (5.5-6.5)	6.1 (5.4-6.3)	0.69
General laboratory				
ALT level (IU/mL)	68 (43-137)	64 (34-115)	79 (51-166)	0.09
Platelets(x10^9/L)	123 (81-172)	150 (84-183)	119 (75-155)	0.22
Creatinine(mg/dl)	0.71 (0.64-0.90)	0.78 (0.62-1.04)	0.7 (0.65-0.85)	0.26
Transient Elastography (kPa)	13.6 (9.7-27)	11.7 (7.5-20.9)	17 (12-34)	0.02
Cirrhosis n(%)	37 (57)	15 (44)	20 (69)	0.06
MELD score	7.5 (6-10)	7 (6-9)	9 (6-10)	0.30
Immunological parameters				
Cryocrit (%)	2.7 (1.3-5)	3.2 (1.5-5.7)	2.6 (1.2-3)	0.10
C4 (g/L)	0.08 (0.02-0.14)	0.02 (0.01-0.11)	0.09 (0.06-0.18)	0.02
CH50 IU/mL	13(11-28)	14(12-29)	12(10-26)	0.11
Rheumatoid Factor (IU/mL)	20 (10-97)	80 (10-200)	10 (10-20)	0.01
Treatment n(%)				
Naïve	26 (41)	13 (37)	13(45)	0.62
Null responder	38 (59)	22 (63)	16 (55)	
DAA Treatment regimens n (%)				0.60
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)	
PegINF+DAAs	10 (16)	5 (14)	5 (17)	
Others**	5 (8)	3 (8)	2 (7)	
Use of RBV, n(%)	45(70)	24(69)	21(72)	0.62
Treatment duration 12/24w n(%)	44 (69)/20 (31)	23 (66)/12 (34)	21 (72)/8 (28)	0.56
Immunosuppressive therapy n(%)				
Corticosteroids		13(37)		
Rituximab		3(8)		

Continuous data are expressed as median (interquartile range).

PegINF: Pegylated interferon; RBV, Ribavirin; SOF, Sofosbuvir; SIM, Simeprevir; DAC, Daclatasvir ; 3D, Paritaprevir/Ritonavir/Ombitasvir/Dasabusvir ;LDV,Ledipasvir; ALT, Alaninoaminotransferase; GGT, Gamma glutamil transpeptidase ; MELD,Model for End-Stage Liver disease. ** Grazoprevir+Elbasvir(n=3), Faldaprevir+ Deleobuvir(n=2)

Local laboratory normal range : C4 complement fraction (C4) 0.110-1 g/L; 50% hemolytic complement activity (CH50) 28-60 IU/mL; Rheumatoid Factor (RF) <20 IU/mL.

Table 2. Clinical, biological and immunological features before and after DAA therapy

Parameters	Cryoglobulinemic Vasculitis n=35			Asymptomatic patients n=29		
	Pretreatment	Follow-up	p	Pretreatment	Follow-up	p
SVR rate n(%)	-	33(94)	-		27(93)	-
Cryocrit (%)	3.2 (1.5-5.7)	0.5 (0-1.4)	0.01	2.6 (1.2-3)	0 (0-1.1)	0.01
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)	0.01	0.09 (0.06-0.18)	0.12 (0.08-0.17)	0.02
Reduced C4 fraction n(%)	26(74)	15(43)	-	18(62)	12(41)	-
CH50 activity U/mL	14(12-29)	44(25-53)	0.01	12(10-26)	31(14-46)	0.01
Reduced CH50 activity n(%)	28(80)	8(22)	-	22(76)	13(45)	-
Rheumatoid factor level (IU/mL)	80 (10-200)	20(10-95)	0.01	10 (10-20)	10 (10-10)	0.04
Positive Rheumatoid factor n(%)	24(69)	17(49)	-	9(31)	6(20)	-
ALT level (IU/mL)	64 (34-115)	24(17-28)	0.01	79 (51-166)	20(16-27)	0.01
Platelets (x10^9/L)	123 (81-172)	159(107-229)	0.19	119 (75-155)	118(67-170)	0.98
MELD score	7 (6-9)	6(6-8.5)	0.24	9 (6-10)	8(6-10)	0.25
Creatinine level (mg/dl)	1.5 (1-1.7)	1.25 (1.1-2.1)	0.12	0.7 (0.65-0.85)	0.7 (0.62-.082)	0.86
eGFR(ml/min/1.73m^2)	90(53-90)	90(65-90)	0.20	90 (81-90)	90 (83-90)	0.46
Prednisone (mg/day)	10(5-30)	0 (0-3.7)	0.01	-	-	
Complete clinical remission n(%)	-	25(71)				
BVAS v3 score	9 (4-12)	3 (0-6)	0.001	-	-	
Clinical manifestations n(%)						
Purpura	23(65)	2(6)	0.01			
Arthralgia	11(31)	1(3)	0.01			
Weakness	25(70)	1(2)	0.01			
BVAS.v3 score	4(2-31)	0(0-6)	0.01			
Cryocrit (%)	3.9(2.2-6.2)	0.7(0-1.6)	0.01			
Polyneuropathy	18(50)	5(14)	0.01			
BVAS.v3 score	10.5(2-31)	3(0-12)	0.01			
Cryocrit (%)	5.6(1.2-10)	0.3(0-1.4)	0.04			
Renal involvement	7(20)	2(5)	0.02			
Hematuria >10 RBCs/hpf(n,%)	5(71)	2(25)	0.03			
Median eGFR(ml/min/1.73m^2)	40(31-44)	54(36-60)	0.03			
Proteinuria (g/l)	1.4(1.1-1.9)	0.17(0.9-1.8)	0.73			
Creatinine levels (mg/dl)	1.6(1-1.7)	1.27(1.1-2)	0.89			
BVAS.v3 score	16(2-31)	3(0-12)	0.03			
Cryocrit (%)	6(1.2-15.5)	0(0-1.3)	0.01			

SVR, Sustained virological response; CH50, 50% hemolytic complement activity (CH50); BVAS.v3, Birmingham Vasculitis Activity Score; eGFR,glomerular filtration rate.

* Continuous data are expressed as median (interquartile range) except BVAS.v3 score (median and range)

Table 3. Summary of adverse events during antiviral therapy.

Adverse Event*	HCV-CV patients (n=35)	ACC patients (n=29)
No Adverse Event	18 (51.4%)	18 (62%)
Anemia (Hb <10 g/dl)	11 (31.4%)	6 (21%)
Asthenia	1 (2.8%)	4 (13.8%)
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

* Some patients had more than one adverse event.

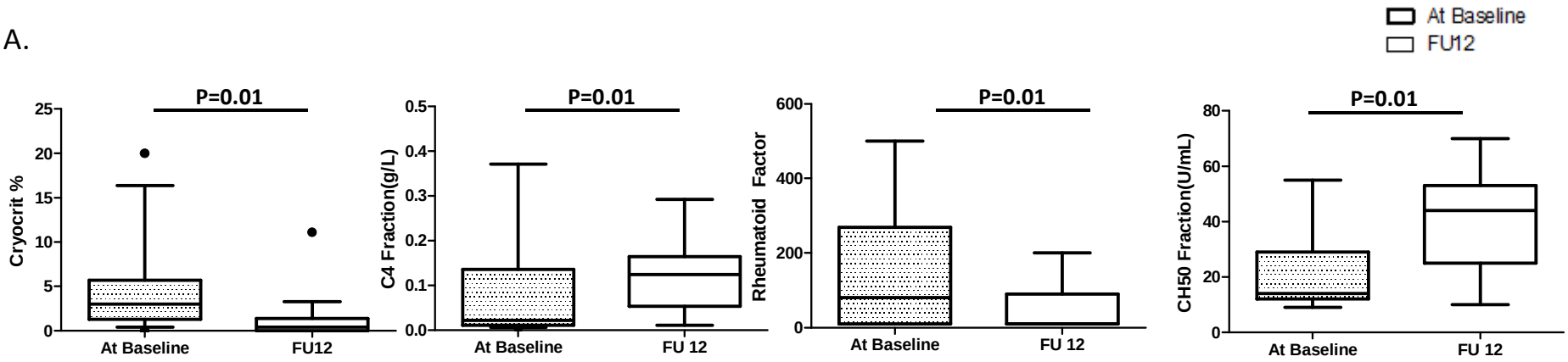
Table 4. Baseline factors associated to the achievement of complete immunological response in the whole cohort.

Variable	Univariate analysis* OR [CI 95%]	P value	Multivariate analysis OR [CI 95%]	P value
Cirrhosis	0.4 [0.15-1.1]	0.08		
IFN-based therapy	5.4 [1.01-27]	0.04		
24 vs 12 weeks	3.7 [1.2-11]	0.02		
Cryocrite (%) <2.7	6.6 [2.2-19]	<0.01	9.8 [2.2-44]	0.003
C4 (g/l)	265 [4-999]	0.09		
Rheumatoid factor levels	0.99 [0.98-0.99]	0.03		

Only variables with a p value <0.10 in univariate analysis are displayed.

Cryoglobulinemic Vasculitic Patients

A.



Asymptomatic Patients

B.