Evolution of HCV-Associated Cryoglobulinemic Vasculitis after Treatment with Direct-Acting Antivirals

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ABSTRACT

Background: Circulating cryoglobulins are detected in 40-60% of patients with HCV chronic infection, and cryoglobulinemic vasculitis is observed in 15% of cases.

Methods: We included 24 patients with HCV-related mixed cryoglobulinemia (MC) and 8 patients with asymptomatic HCV cryoglobulinemia, between 2016-2019. All patients received DAA therapy: 22 patients with ombitasvir/paritaprevir/ritonavir and dasabuvir and 10 patients with ledipasvir/sofosbuvir. The primary endpoint was complete clinical remission of vasculitis at week 24.

Results: All 5 patients with renal involvement received immunosuppressive therapy; complete clinical remission occurred in 3 of these cases. SVR was achieved in 91.6% of patients with vasculitis, compared to 100% in the asymptomatic group ($p=0.01$). Purpura, myalgia, arthralgia and muscular weakness resolved in 91.6% of patients after SVR. Neurological symptoms improved in 75% of cases. All immunological parameters improved after therapy. Circulating cryoglobulins became undetectable in 54.2% of patients with vasculitis and in 62.4% of the asymptomatic group. The predictive factors for clinical and immunological response were: level of fibrosis, cryocrit and C4 levels, rheumatoid factor activity, and BVASv3.

Conclusions: Direct antiviral therapy generates a virological response of over 95% in patients with HCV cryoglobulinemic vasculitis, and is associated with increased rates of complete clinical response and moderate immunological response.

Key words: cryoglobulins, vasculitis, hepatitis C virus, direct-acting antivirals

INTRODUCTION

Mixed circulating cryoglobulins are detected in 40-60% of patients with chronic hepatitis C virus (HCV) infection, while cryoglobulinemic vasculitis is observed in 15% of cases. Prognosis is variable, and depends on the degree of renal injury or the extent of vasculitic lesions (1,2).

Patients with mild or moderate forms of HCV-associated glomerulonephritis (stable renal function and/or non-nephrotic proteinuria) should be treated with direct-acting antivirals (DAAs).

In patients with HCV-associated renal disease with resistance to DAA therapy, immunosuppressive therapy should be associated.

Received: 23.07.2021

Accepted: 27.09.2021

The DAA regimens which are used are very efficient, with an SVR rate of around 95%, and with adverse effects occurring in less than 10% of cases (3).

Treatment of HCV vasculitis is difficult. Sustained virological response is the main goal in these patients, because clinical remission of vasculitis is closely linked to viral clearance. Studies regarding treatment with direct antivirals have shown a remarkable eradication rate, of 90-100%, depending on HCV genotype (4,5).

The use of ledipasvir/sofosbuvir should be avoided in the treatment of patients with cryoglobulinemic nephropathy and eGFR ≤ 30 ml/min, due to the risk of accumulation of the active metabolite GS-331007, which deteriorates renal function. In these cases, therapeutic regimens such as ritonavir/paritaprevir/ombitasvir and dasabuvir (as is the case in our study), elbasvir/grazoprevir or glecaprevir/pibrentavir should be used.

MATERIAL AND METHOD

We included 24 patients with HCV-related mixed cryoglobulinemia (MC) in a prospective study, performed in the Internal Medicine Department, Fundeni Clinical Institute, between 2016-2019. Patients were at least 18 years old, with no upper age limit, and presented active HCV vasculitis, defined by vasculitic lesions of the skin, joints, kidneys, peripheral nerves and/or cerebral, digestive, pulmonary and/or cardiac involvement (Chapel Hill criteria, 2012) (6).

8 patients with asymptomatic HCV cryoglobulinemia were also included.

Exclusion criteria were non-active cryoglobulinemic vasculitis, HIV or HBV infection, decompensated liver cirrhosis.

Clinical evaluation at study entry included age, gender, neurological involvement (peripheral and/or central), skin involvement (Raynaud phenomenon, purpura, distal ulcers, skin necrosis), arthralgia, myalgia, bowel movement disorders, kidney injury (proteinuria, hematuria and glomerular filtration rate) and clinical signs of liver disease.

All patients were evaluated every 4 weeks, up to 24 weeks.

Disease activity and therapeutic response were evaluated using the Birmingham vasculitis activity score (7).

Viral load was determined using Abbot HCV Real Time Assay, with a lower limit of detection of 12 IU/mL. HCV genotyping was performed using the sequence of the NS5b gene. Laboratory evaluation included complete blood count, serological biochemical profile,

rheumatoid factor, quantitative IgM, total complement and C4 fraction, cryoglobulins (cryocrit over 1% in at least 2 determinations and characterization of cryoprecipitate by immunofixation).

Cryoglobulins have been classified by Brouet et al (8) as type II in the presence of monoclonal IgM and polyclonal IgG, and as type III in forms with polyclonal immunoglobulins.

Liver fibrosis was evaluated by non-invasive methods (fibroscan, fibromax).

All patients were eligible for DAA treatment: 22 patients with ombitasvir/paritaprevir/ritonavir and dasabuvir (3D regimen) and 10 patients with ledipasvir/sofosbuvir (LDV/SOF).

The primary end-point was complete clinical remission of vasculitis at week 24. Complete clinical response was defined as improvement of the functions of all initially affected organs, and by the absence of clinical relapse.

Skin and joint improvement were evaluated clinically (remission of purpura and/or ulcers; remission of arthralgia or arthritis). Renal injury was evaluated biologically (proteinuria below 0.3 gr/24h, remission of hematuria, and improvement of GFR > 20% at week 24, if GFR < 60 mL/min at initiation).

Improvement of peripheral neurological affection was evaluated clinically (improvement of pain and of paresthesia using the visual analogue scale, improvement of muscular contraction and of initial motor affection) and/or electrophysiologically (improvement of electromyogram anomalies at week 24, compared to the initial aspect). Neuropathy total symptoms score-6 (NTSS-6) was applied for evaluating symptoms of sensory neuropathy.

Partial clinical response at week 24 was defined as an improvement in the functions of some of the initially affected organs. Patients without clinical response at week 24 were defined as therapeutic failure.

Secondary endpoints were:

- Virological response at week 12 (end of treatment - EOT) and 24 (SVR).
- Evolution of cryoglobulinemia and of the C4 fraction of the complement.
- Secondary effects of therapy.

Statistical analysis

Median and range values were used for relevant variables. The groups were compared using the Mann-Whitney test for continuous variables, and the Fisher test for categorical variables. The Wilcoxon rank test

was used for comparing two samples. Logistical regressive analysis was used to identify predictive factors for clinical and immunological response.

RESULTS

Our study included 32 patients with HCV infection and cryoglobulinemia, who received direct antiviral therapy. The clinical, biochemical and immunological

characteristics of patients are presented in *table 1*. Of the 32 patients, 24 presented characteristics of cryoglobulinemic vasculitis, while 8 had asymptomatic cryoglobulinemia.

By comparing the 2 groups, we remarked the predominance of female gender among patients with vasculitis, as well as lower C4 levels in this group (0.03 g/L vs 0.10 g/L, $p=0.05$) and higher RF (96 IU/mL vs 11 IU/mL $p=0.01$) and cryocrit levels (3.4% vs 2.3%).

Table 1 - Baseline characteristics of the 64 patients included in the study

Baseline parameters	All patients (n=32)	Patients with cryoglobulinemic vasculitis (n=24)	Asymptomatic patients with circulating cryoglobulins (n=8)	P
Age, y	58 (38-76)	57 (42-72)	64 (56-76)	0.91
Female gender, n (%)	19 (59.3%)	16 (66.6%)	3 (37.5%)	0.04
Clinical manifestations, n (%)				
Purpura	22 (68.7)			
Arthralgia/arthritis	9 (28.1)			
Weakness	26 (81.2)			
Polyneuropathy	18 (56.2)			
Renal injury	5 (15.6)			
Sicca syndrome	3 (9.3)			
Abdominal involvement	2 (6.2)			
Raynaud phenomem	5 (15.5)			
Viral parameters				
HCV genotype, n (%)				
1a	3 (9.3)		-	
1b	29 (90.6)		100	
2				
3				
4				
Baseline HCV RNA level, IU/mL	860.200 (14600-10.880.000)	910.000 (26.860-10.880.000)	210.800 (14.600-630.000)	0.02
General laboratory				
ALT level, IU/mL	76 (41-188)	81 (40-191)	76 (41-153)	0.08
Platelets, $\times 10^9$ /L	178 (108-246)	127 (74-159)	151 (88-176)	0.05
Transient elastography, kPa	14 (9.2-30)	19 (11.9-37)	12.8 (8.2-24)	0.01
Immunologic parameters				
Cryocrit (%)	2.5 (1.2-5.1)	3.4 (1.7-5.9)	2.3 (0.9-3)	0.04
C4, g/L	0.07 (0.02-0.15)	0.03 (0.02-0.12)	0.10 (0.07-0.19)	0.03
CH 50, IU/mL	15 (11-29)	13 (11-29)	14 (10-25)	0.06
Rheumatoid factor, IU/mL	31 (10-118)	96 (10-193)	11 (10-26)	0.01
Treatment, n (%)				
Naive	20	12	8	
Null responder	12	12	0	
DAA treatment regimens, n (%)				
3D	22	12	8	
LDV/SOF	10	10		
Use of RBV, n (%)				
SVR 12	(30/32) 93.7%	(22/24) 91.6 %	(8/8) 100%	0.01
Immunosuppressive therapy, n (%)				
Corticosteroids		8 (33.3)		
Corticosteroids+cyclophosphamide		4 (16.6)		
Plasmapheresis		3 (12.5)		
Liver fibrosis, n (%)				
F1	4 (12.6)	2 (8.3)	2 (25%)	
F2	5 (15.6)	2 (8.3)	3 (37.5%)	
F3	7 (21.8)	5 (20.8)	2 (25%)	
F4	16 (50)	15 (62.6)	1 (12.5%)	

The main clinical manifestations of cryoglobulinemic vasculitis were: purpura (68.7%), arthralgia/arthritis (28.1%), weakness (81.2%), polyneuropathy (56.2%), and kidney injury (15.6%).

One of the 5 patients with kidney injury received immunosuppressive therapy associated with plasma exchange. Complete clinical remission was obtained in 3 cases (60%), with a significant improvement of kidney failure (creatinine clearance > 60 mL/min) and with SVR at 12 weeks, in all 3 cases. Nephrotic syndrome and creatinine clearance did not improve significantly in one patient, while the fifth patient presented an improvement of proteinuria (< 1 gr/24 hrs) and of creatinine clearance (from 34 to 55 mL/min).

16 of 18 patients with neurological symptoms were evaluated by electromyography, which confirmed peripheral polyneuropathy: 6 with neuropathy multiplex, 7 with sensory polyneuropathy, and 3 with sensorimotor polyneuropathy.

Kidney biopsy was performed in all 5 patients with kidney injury, and confirmed membrano-proliferative glomerulonephritis, 3 of these patients being on treatment with glucocorticoid plus monthly cyclophosphamide pulse therapy, while 2 were receiving only glucocorticoid.

Plasma exchange was performed in 3 patients, one

with kidney injury and 2 with neuropathy multiplex.

All patients presented type 2 cryoglobulins (polyclonal IgG/monoclonal IgM).

81.2% (26 of 32) of patients presented a decrease of C4 and of CH 50 activity, and all patients with vasculitis were positive for rheumatoid factor.

SVR at 12 weeks after the end of antiviral therapy was obtained in 91.6% (22 of 24) of patients in the vasculitis group, compared to 100% in the asymptomatic group ($p=0.01$).

Purpura, myalgia, arthralgia and muscle weakness remitted in 91.6% of patients after SVR; of the 5 patients with kidney injury, 4 obtained remission with the resolution of nephrotic syndrome and improvement of glomerular filtration rate (43 vs 57 mL/min /1.73 m²).

Neurological symptoms improved in 75% of cases. 3 patients with sicca syndrome and 2 with intestinal involvement were asymptomatic at the end of the follow-up period.

Clinical improvement was also documented by the statistically significant decrease of BVASvs3 from a mean initial value of 8 points (3-27) to a value of 3 points (0-11), $p<0.001$.

All immunological parameters improved at 12 weeks after the end of therapy. Circulating cryoglobu-

Table 2 - Clinical, biological and immunological evolution after antiviral therapy

	Cryoglobulinemic vasculitis n=24			Asymptomatic patients n=8		
	Pretreatment	Posttreatment	P	Pretreatment	Posttreatment	P
SVR		93.7 %			100 %	
Cryoglobulin (n, %)	24 (100)	11 (45.8)	0.001	8 (100)	3 (37.5)	0.001
Cryocrit (%)	3.4 (1.7-5.9)	0.3 (0-1.5)	0.01	2.3 (0.9-3)	0.1 (0-1.1)	0.01
Decrease of C4 (n, %)	20 (83.3%)	5 (20.8 %)	0.001	6 (75 %)	3 (37.5 %)	0.001
Decrease of C4 fraction, g/L	0.03 (0.02-0.12)	0.14 (0.09-0.19)	0.01	0.10 (0.07-0.19)	0.14 (0.07-0.20)	0.02
Decrease of CH 50 U/mL activity (n, %)	19 (79.1 %)	8 (33.3 %)	0.001	6 (75%)	2 (25%)	0.001
CH 50 U/mL activity	13 (11-29)	36 (22-51)	0.01	14 (10-25)	33 (15-47)	0.01
Positive rheumatoid factor (n, %)	17 (70.8%)	11 (45.8%)	0.001	4 (50%)	2 (25%)	0.01
Rheumatoid factor level UI/mL	96 (10-193)	26 (11-89)	0.01	11 (10-26)	10 (10-14)	0.03
ALT	81 (46-191)	25 (18-30)	0.01	76 (41-153)	21 (16-28)	0.01
Platelets x 10 ⁹ /l	127 (74-159)	149 (91-210)	0.21	151 (88-186)	152 (90-196)	0.98
GFR mL/min/1.73 m ²	93 (62-93)	94 (65-94)	0.20			
Clinical manifestations (n, %)						
Purpura	22 (68.7)	1 (4.1)	0.01			
Arthralgia	9 (28.1)	1 (4.1)	0.01			
Weakness	26 (81.2)	2 (8.3)	0.01			
Polyneuropathy	18 (56.2)	6 (25)	0.01			
Neural affection	5 (15.6)	1 (4.1)	0.01			
Hematuria	5 (15.6)	1 (4.1)	0.01			
GFR mL/min/1.73 m ²	43 (40-47)	57 (42-61)	0.02			
Proteinuria g/L	1.7 (1.1-2.1)	0.32 (0.1-1.9)	0.62			
BV AS	9 (4-18)	0 (0-5)	0.01			

lins became undetectable in 54.2% of patients with vasculitis and in 62.4% of patients from the asymptomatic group.

Post-therapeutical normalization of CH 50, C4 and RF levels was obtained in 79.2%, 66.7% and 54.2% respectively in the vasculitis group, compared to 64.5%, 75% and 75% respectively in the asymptomatic group.

Most of the patients with immunological response (19 pts, representing 79.1%) presented an improvement of clinical manifestations.

Predictive factors for clinical and immunological response, as shown by logistic regression analysis, were: degree of fibrosis, cryocrit level, C4 level, RF activity and BVASv3 (table 3).

The patients included in our study presented the following comorbidities: essential arterial hypertension in 4 cases, renal parenchymal hypertension in 3 patients with cryoglobulinemic chronic glomerulonephritis, chronic bronchitis in one patient, type 2 insulin dependent diabetes mellitus in 1 case, and minor depressive disorder in one case. Arterial hypertension was treated with calcium channel blockers (amlodipine), sartans (irbesartan), beta-blockers (metoprolol) and loop diuretics (furosemide), drugs that have minor interactions with the antiviral medication. The depressive disorder was treated with benzodiazepines (lorazepam 2 mg). Patients with arthralgia/arthritis received intermittent treatment with non-steroidal anti-inflammatory drugs (diclofenac 50-100 mg/day).

The patients with these comorbidities presented a favorable clinical, virological and immunological evolution.

DISCUSSIONS

HCV cryoglobulinemic vasculitis is a severe disease and a difficult-to-treat entity, with a 10-year mortality rate of 40%, and with a 35-fold increase in the risk of non-Hodgkin lymphoma, compared to the general population. The main manifestations of HCV cryoglobulinemic vasculitis include purpura and peripheral neuropathy (68.7%), arthralgia/arthritis (28.1%), kidney

injury (15.6%), Raynaud phenomenon (15.5%). (9)

Sustained virological response at 12 weeks from the end of treatment with direct antivirals was 91.6%, while complete clinical remission in patients with vasculitis was 91.6%. (5)

In the VASCUVALDIC study, which enrolled 24 patients with HCV cryoglobulinemic vasculitis, treated with sofosbuvir and ribavirin, complete clinical response at week 24 was 87.5%. (10)

In comparison, treatment with peginterferon and ribavirin, and subsequently with first-generation protease inhibitors, showed a response rate of under 30%, as well as very frequent adverse effects. (11,12)

In our study, treatment with ombitasvir/ritonavir/paritaprevir (viekirax) plus dasabuvir (exviera) – 22 pts, and with sofosbuvir/ledipasvir (harvoni) – 10 pts, determined an SVR rate of 91.6% in patients with cryoglobulinemic vasculitis and of 100% in patients with asymptomatic cryoglobulinemia. The favorable virological evolution was associated with a favorable clinical evolution. The five patients with kidney injury were also treated with immunosuppressive medication (corticosteroids, cyclophosphamide).

Almost all studies have shown that a complete or partial reduction of clinical symptoms, during and after administration of DAAs, was correlated with SVR. A complete clinical response was defined as an improvement of all affected organs and/or a Birmingham vasculitis activity score (version 3) of 0. The best response rate was reported in the prospective study performed by Saadoun et al (13), in which all patients (41 cases) presented SVR, as well as complete and partial clinical response rates of 90% and 10%, respectively, after 12 or 24 weeks of sofosbuvir/daclatasvir.

Similar results were reported in the study published by Gragnani et al (14), in which 93% of patients (41 of 44) presented a complete or partial response of vasculitis, with an SVR rate of 100%.

However, Sollima et al (15) reported that patients with cryoglobulinemic vasculitis treated with DAAs and who obtained SVR, may present the persistence of symptoms or may relapse. Moreover, this study

Table 3 - Main characteristics associated with complete immunological response

Variable	Universal analysis OR (95% CI)	P	Multivariable analysis OR (95% CI)	P
F1-2	0.8 (0.21-1.9)	0.05		
Cryocrit < 2,2 %	7.2 (2.8-21)	0.01	9.4 (2.8-38)	0.002
C4 > 0.25 g/L	6.7 (2.3-24)	0.03	8.2 (2.3-31)	0.004
RF < 20 IU/L	1.14 (0.87-1.14)	0.07		
BVAS < 6	3.53 (1.18 -10.59)	0.027	4.68 (2.24-11.43)	0.003

suggests that long-term follow-up is necessary, especially in patients with advanced stages of vasculitis, and especially in patients with kidney injury.

Regarding the therapeutical regimens that associate ribavirin (RBV), the rate of cryoglobulin clearance is similar (47% vs 48%), but with a lower SVR rate compared to RBV-free regimens.

It has been shown that DAAs reestablish homeostasis of B and T cells (16). However, clinical symptoms of cryoglobulinemic vasculitis have variable remission rates. In our study, purpura, arthritis/arthralgia and polyneuropathy improved in 62.6%, 24% and 31.2% of cases, respectively. The immunological changes and especially the levels of cryoglobulin after anti-HCV treatment dictate the persistence of vasculitic activity.

In our study, cryocrit level decreased from 3.4 (1.7-5.9) before therapy to 0.3 (0-1.5) post-therapy.

Bonacci et al (17) found that a baseline cryocrit below 2.7% is independently associated with complete immunological response, defined as the absence of circulating cryoglobulins and normalization of complement and/or RF levels. Similarly, our study showed that the decrease of cryocrit levels below 2.2% correlated with a favorable clinical evolution and normalization of complement activity. Normalization of complement activity was observed in 66.7% of cases, and was accompanied by normalization of the C4 fraction of the complement in 79.2% and with the disappearance of RF in 54.2% of cases.

The pathogenic mechanism for the persistence of cryoglobulin production and of its clinical manifestations, after HCV clearance, is unclear. In cryoglobulinemic vasculitis, B cell proliferation may enter an independent, autonomous phase, as shown by the persistence of t mutation (14:18) in B cell clones, and the presence of small amounts of HCV-RNA in the lymphatic system after obtaining SVR (18,19). Patients who still present manifestations of cryoglobulinemic vasculitis after HCV clearance should be followed-up, due to the increased risk of B cell NHL (20,21).

Regarding kidney injury in cryoglobulinemic vasculitis, it is considered a life-threatening complication.

Before the introduction of DAAs, treatment relied especially on PegIFN and immunosuppressive therapy, with rituximab being the most efficient. In patients treated with first-generation protease inhibitors (telaprevir or boceprevir) plus PegIFN and ribavirin, the use of rituximab was necessary in 43% of cases, compared to those treated with sofosbuvir and ribavirin, where rituximab was associated in 17% of cases (10).

In our study, five patients presented histologically documented glomerular injury, with clinical remission

and significant improvement of creatinine clearance (>60 mL/min) in 60% of cases; in these patients, corticosteroids and cyclophosphamide were associated to antiviral therapy, and results were maintained 12 months post-therapy.

Our results indicate the fact that inhibition of viral replication per se is essential for inducing the clinical remission of cryoglobulinemic vasculitis.

Multivariable analysis of the main characteristics associated with complete immunological response revealed the following parameters: cryocrit<2.2%, C4>0.25 g/L, and BVAS<6.

Adverse effects consisted in fatigue, insomnia, nausea, pruritus, and irritability in 46.8% of patients. These adverse effects were of low intensity, did not require the interruption of antiviral therapy, and improved under symptomatic medication.

CONCLUSIONS

Direct interferon-free and ribavirin-free antiviral therapy generates a virological response in over 90% of patients with HCV cryoglobulinemic vasculitis, and is associated with high rates of complete clinical response, with moderate immunological response and with a low rate of adverse effects.

Our study reveals the importance of initiating treatment in early stages of HCV infection.

Conflict of interest

The authors declare that they have no conflicts of interests.

Ethical approval

For performing this study ethical approval was obtained.

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