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David Saadoun has received consulting and lecturing fees from Medimmune, Abbvie, Bristol meyer squibb, Roche, Servier, Gilead, AstraZeneca and Glaxo Smith Kline. Si Nafa Si Ahmed received consulting and lecturing fees from Abbvie, Bristol Myers Squibb, Gilead, Janssen and Roche. Laurent Alric received consulting and lecturing fees from Gilead, Bristol Myers Squibb, Janssen, Abbvie, Merck Sharp Dhome, Roche. Christophe Hézode has been adviser and speaker for Abbvie, Bristol Myers Squibb, Gilead, Janssen, and Merck Sharp Dohme. Stanislas Pol, speaker : Glaxo Smith Kline, Bristol Myers Squibb, Boehringer Ingelheim, Janssen, Gilead, Roche, Merck Sharp Dohme, Sanofi, Novartis, Vertex, Abbvie; grants : Bristol Myers Squibb, Gilead, Roche, Merck Sharp Dohme; board member : Glaxo Smith Kline, Bristol Myers Squibb, Boehringer Ingelheim, Janssen, Gilead, Roche, Merck Sharp Dohme, Sanofi, Novartis, Vertex, and Abbvie. Thierry Poynard is the founder of BioPredictive marketing FibroTest (patent belong to Public Organization APHP). Patrice Cacoub has received consulting and lecturing fees from Abbvie, Astra Zeneca, Bristol-Myers Squibb, Gilead, Glaxo Smith Kline, Janssen, Merck Sharp Dohme, Roche, Servier and Vifor.

Contributorship

Study concept and design: DS, PC
Acquisition of data: DS, SP, YF, ASB, LA, CH, SNSA, CC, LDSM, LM, TP, PC
Analysis and interpretation of data: DS, MRR, PC
Drafting of the manuscript: DS, MRR, PC
Critical revision of the manuscript for important intellectual content: DS, SP, YF, ASB, LA, CH, SNSA, LDSM, LM, TP, MRR, PC

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Abbreviations used in this paper: DAA, direct acting antiviral ; HCV, hepatitis C virus; IFN, interferon; CryoVas, cryoglobulinemia vasculitis; Tregs, regulatory T cells; TFH, T follicular helper cells; SVR, sustained virological response; RF, rheumatoid factor; ALT, alanine aminotransferase.

Abstract

Abstract: Circulating mixed cryoglobulins are detected in 40%–60% of patients with hepatitis C virus (HCV) infection, and overt cryoglobulinemia vasculitis (CryoVas) develops in about 15% of patients. Remission of vasculitis has been associated with viral clearance, but few studies have reported the effectiveness of direct acting antiviral drugs in these patients. We performed open-label, prospective, multi-center study of the effectiveness and tolerance of an all-oral, interferon- and ribavirin-free regimen of sofosbuvir plus daclatasvir in patients with HCV-associated CryoVas. Forty-one consecutive patients with active HCV-associated CryoVas (median age, 56 years; 53.6% women) were recruited from hospitals in Paris, France from 2014 through 2016. They received sofosbuvir (400 mg/day) plus daclatasvir (60 mg/day) for 12 weeks (n=32) or 24 weeks (n=9) and evaluated every 4 weeks until week 24 and at week 36. Blood samples were analyzed for complete blood count, serum chemistry profile, level of alanine aminotransferase, rheumatoid factor activity, C4 fraction of complement, and cryoglobulin; peripheral blood mononuclear cells were isolated for flow cytometry analysis. Thirty-seven patients (90.2%) had a complete clinical response (defined by improvement of all the affected organs involved at baseline and no clinical relapse) after a median time of 12 weeks' therapy; all had a sustained virologic response (no detectable serum HCV RNA 12 weeks after the end of antiviral therapy). Patients' mean cryoglobulin level decreased from 0.56±0.18 at baseline to 0.21±0.14 g/L at week 36, and no cryoglobulin was detected in 50% of patients at this time point. After antiviral therapy, patients had increased numbers of T-regulatory cells, IgM+CD21–/low memory B cells, CD4+CXCR5+ IL21+ cells, and T-helper 17 cells, compared with before therapy. After a median follow-up period of 26 months (interquartile range, 20–30 months), no patients had a serious adverse event or relapse of vasculitis.

KEY WORDS: DAA, cryoglobulinemia vasculitis, autoimmunity, Treg cell

Circulating mixed cryoglobulins are detected in 40 to 60% of chronically infected hepatitis C virus (HCV) patients whereas overt cryoglobulinemia vasculitis (CryoVas) is observed in about 15% of cases [1-3]. The prognosis is variable and highly dependent on renal involvement or on the extent of vasculitis lesions [4].

Treatment of HCV-CryoVas is challenging [5-7]. Sustained virological response is the main goal in these patients since clinical remission of vasculitis is closely associated with viral clearance [8]. Few studies have reported promising results on the effectiveness and tolerance of direct acting antiviral drugs (DAA), either in association with interferon (IFN) or using IFN-free regimens [8-11]. The aim of the present study was to evaluate effectiveness and tolerance of an all oral IFN- and ribavirin-free regimen with sofosbuvir plus daclatasvir in patients with cryoglobulinemia vasculitis.

Main clinical features included purpura (75.6%), arthralgia (63.4%), peripheral neuropathy (51.2%), skin ulcers (17.1%), glomerulonephritis (12.2%), and gut involvement and myocarditis (2.4%).

At week 24, thirty-seven (90.2%) patients were complete clinical responders and four (9.8%) were partial responders (**Table 1**). Partial response was due to persistent kidney insufficiency (n=2) or peripheral neuropathy (n=2) while skin and joint involvement disappeared. Median time to achieve a clinical response was twelve weeks. The incidence of complete remission at 3, 6 and 12 months was 85% (74-96), 90% (81-99), and 90% (81-99), respectively. The event free survival at 2 years was 100%. A sustained virological response at week 12 after treatment was noted in all cases. The alanine aminotransferase level decreased from 55.3 ± 6.4 to 20.4 ± 2.0 IU/L, ($p<0.0001$). Disappearance of cryoglobulin was evidenced in fifty percent of cases.

Regulatory T cells deficiency (Tregs), $IgM^{+}CD21^{-/low}$ memory B cells expansion, T follicular helper cells (TFH, $CD4^{+}CXCR5^{+}IL21^{+}$) and Th17 cells ($CD4^{+}IL17^{+}$) expansion

significantly reverted after DAA therapy (1.7% ± 0.3% versus 2.3% ± 0.16%; 28.4% ± 5.5% versus 18.7% ± 6.5%; 2.7% ± 0.5% versus 1.1% ± 0.2%; and 2.9% ± 0.9% versus 1.6% ± 0.5%, respectively; $p<0.05$ for all) (**Figure 1**). We did not observe significant difference in frequency of $CD4^{+}IFN\gamma^{+}$ before versus after DAA therapy (27% ± 2.9% versus 25.9% ± 2.8%, P = 0.91).

Seven patients (17%) experienced at least one side effect. Main side effects of antiviral therapy included fatigue (12.2%), nausea (7.3%), and vertigo and insomnia (2.4%). No serious adverse event was reported. After a median follow-up of 26 (20-30) months, all patients were alive and no relapse of vasculitis was observed. One patient with cirrhosis developed hepatocellular carcinoma four months after the end of antiviral therapy.

The present study demonstrates a rapid virological response and clinical improvement of HCV-CryoVas. The HCV viral load dropped dramatically within four weeks of treatment. Ninety percent of patients achieved a complete clinical response of vasculitis with very few side effects. A close correlation was found between clinical and virological response as rapid clinical improvement of symptoms of vasculitis was simultaneously achieved. By comparison, treatment with sofosbuvir and ribavirin had previously shown 87% of complete clinical response and 58% of side effects (of whom 8% were serious). A recent Italian study found similar rate of adverse events (i.e. 59%), including 30% of anemia, likely due to 64% use of ribavirin in 44 HCV-CryoVas patients treated by DAA[9].

The second-generation DAAs have determined a further increase of antiviral efficacy while IFN-free regimens minimized side effects rates in patients with CryoVas [8-12]. Sise et al also reported efficacy of DAA therapy in active glomerulonephritis in a small series of patients whose onset of proteinuria was recent [10]. Gragnani et al have recently reported forty four patients treated by sofosbuvir based IFN-free regimen which included ribavirin in twenty eight patients [9]. All patients were virological responders and 93% improved

clinically (of whom 66% were complete clinical responders). However, 59% experienced side effects of whom 85% had more than one adverse event. Bonacci et al reported 71% of complete clinical response, 48% of cryoglobulin clearance and 94% of SVR in their series of 35 CryoVas patients treated by DAA [11]. Sollima et al reported persistent vasculitis despite SVR in 6 out of 7 patients [12].

Clearance of cryoglobulin is an important goal of the treatment of HCV-CryoVas. Indeed, vasculitis relapse does occur in some HCV patients despite SVR and is usually associated with persistent cryoglobulinemia and with the occurrence of a B cell lymphoma [13]. A complete immunological response (i.e. disappearance of cryoglobulin) was achieved in 45 to 60% of CryoVas patients treated with DAAs [8, 11, 14]. Interestingly, we found that sofosbuvir plus daclatasvir therapy is able to revert Tregs deficiency and expansion of $IgM^{+}CD21^{-/low}$ memory B cells, TFH cells and Th17 cells in patients. Such results appear as favourable to obtain a complete and sustained clearance of mixed cryoglobulinemia.

Immunosuppressive agents are typically indicated for HCV-CryoVas patients with severe disease manifestations such as membranoproliferative glomerulonephritis, severe neuropathy and life-threatening complications. In the present study, only 4.8% of patients required the use of rituximab and glucocorticosteroids associated with antiviral therapy, which is similar to the 4.5% rate recently reported by Gragnani et al [9]. Such very low rates of immunosuppressant use with IFN-free regimen compare favorably with the 17% and 43% reported with sofosbuvir plus ribavirin [8] or first generation protease inhibitors (telaprevir or boceprevir) plus peginterferon and ribavirin [15], respectively.

In conclusion, the present study demonstrates that a combination of an interferon- and ribavirin-free, antiviral therapy with sofosbuvir plus daclatasvir can induce a quick clinical and virological response. Up to 90% of CryoVas patients achieved a complete clinical response. The tolerance was satisfactory with no serious adverse events. In

addition, less than five percent of patients required the use of rituximab and glucocorticosteroids associated with antiviral therapy. The follow up of HCV-CryoVas patients with still detectable cryoglobulinemia is mandatory and prospective studies are needed to evaluate the long term risk of clonal expansion and lymphoma. Further studies are warranted to assess whether there is still a place for glucocorticosteroids and immunosuppressants in HCV-CryoVas patients in the era of DAA therapy.

Competing interests

None

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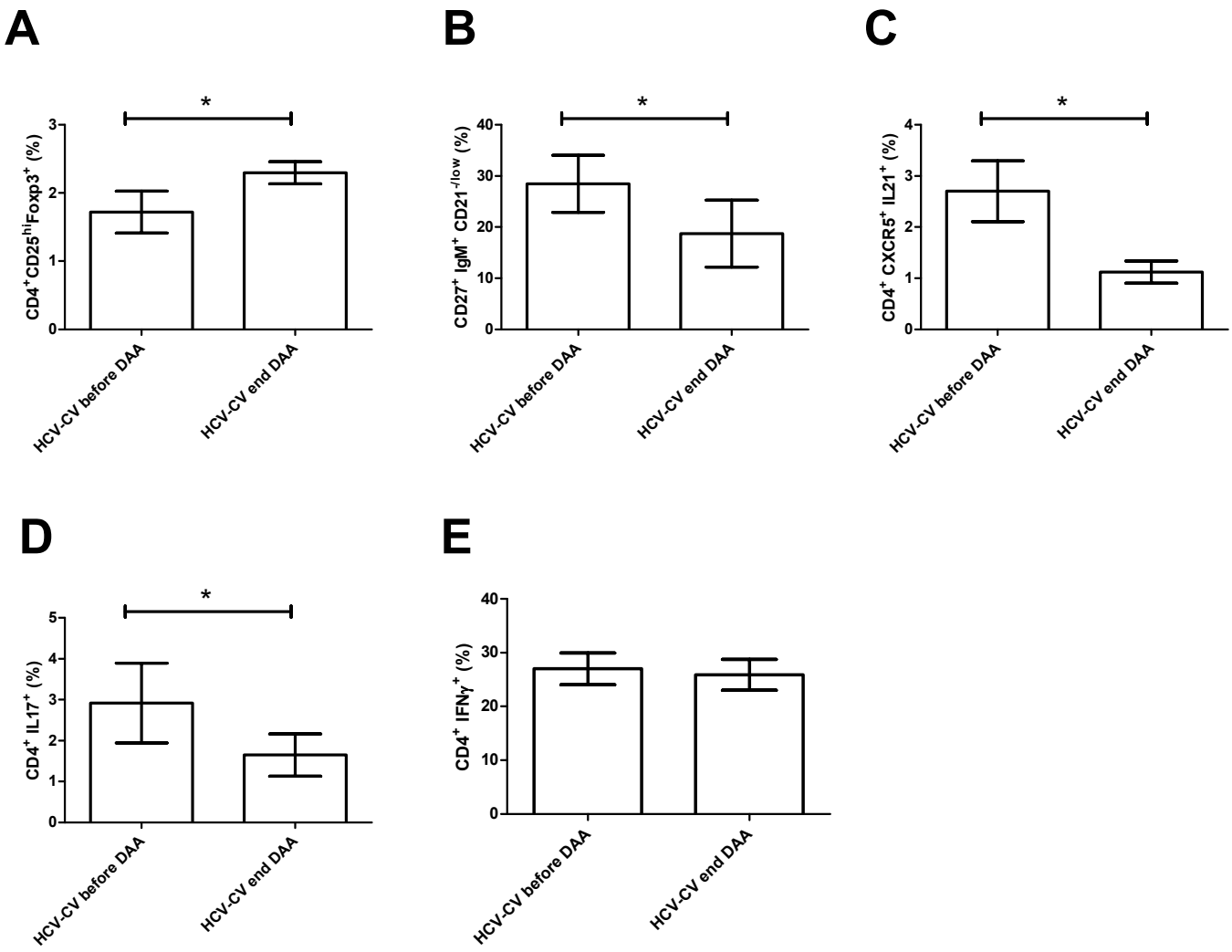
Figure Legends

Figure 1: Sofosbuvir plus daclatasvir revert Tregs deficiency and expansion of $IgM^{+}CD21^{-/low}$ memory B cells, T follicular helper (TFH) cells and Th17 cells in HCV-cryoglobulinemia vasculitis patients.

Flow cytometry figures showing the increase in $CD4^{+}CD25^{hi}FoxP3^{+}$ regulatory T cells (A), the decrease in $IgM^{+}CD21^{-/low}$ memory B cells (B) and $CD4^{+}CXCR5^{+}IL21^{+}$ TFH cells (C) and $CD4^{+}$ $IL\text{-}17^{+}$ (D) following sofosbuvir plus daclatasvir therapy in patients with HCV-cryoglobulinemia vasculitis. No significant change was found in $CD4^{+}$ $IFN\gamma^{+}$ (E) cells. Data are shown for all HCV-cryoglobulinemia vasculitis patients for whom assessments were available. $*p < 0.05$.

Table 1. Course of the main symptoms of HCV cryoglobulinemia vasculitis

			Week 24			Week 36 (SVR 12-24)	
Symptoms	**Baseline**	**Disappearance**	**Improvement**	**Persistence**	**Disappearance**	**Improvement**	**Persistence**
	n	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Purpura	31	31 (100)	_	_	31 (100)	_	_
Arthralgias	26	26 (100)	_	_	26 (100)	_	_
Peripheral neuropathy	21	2 (9.5)	17 (80.1)	2 (9.5)	8 (38.1)	11 (52.4)	2 (9.5)
Skin ulcers	7	7 (100)	_	_	7 (100)	_	_
Renal involvement	5	2 (40)	1 (20)	2 (40)	3 (60)	1 (20)	1 (20)
Myocarditis	1	1 (100)	_	_	1 (100)	_	_
Gut involvement	1	1 (100)	_	_	1 (100)	_	_


A
B
C
D
E
*
CD4+CD25hiFoxp3+ (%)
CD27+ IgM+ CD21-/low (%)
CD4+ CXCR5+ IL21+ (%)
CD4+ IL17+ (%)
CD4+ IFNγ+ (%)
HCV-CV before DAA
HCV-CV end DAA

Patients and Methods

Patients

The VASCUVALDIC 2 study is an open label prospective multicentre study including 41 consecutive patients with active HCV-CryoVas, recruited between 2014 and 2016. To be eligible, the patient must have been at least 18 years of age or older, without any upper age limit, informed, and present an active HCV-vasculitis defined by a clinically active vasculitis with skin, joint, renal, peripheral nerve, central neurological, digestive, pulmonary and/or cardiac involvement (no histological evidence needed if patient had purpura), and a chronic active HCV infection (positive HCV RNA). Patients with active HCV-vasculitis were included irrespective of the HCV genotype, previous antiviral therapies received or response to these previous therapies. Exclusion criteria included non-active CryoVas, human immunodeficiency virus or hepatitis B virus active infection, and current decompensated cirrhosis.

The baseline clinical evaluation included age, gender, any recent weight loss, neurologic involvement (peripheral and/or central nervous system), cutaneous involvement (Raynaud's phenomenon, purpura, distal ulcers), arthralgia, myalgia, gastrointestinal tract involvement, renal involvement [proteinuria, hematuria and abnormal reduction of glomerular filtration rate (GFR)], and clinical signs of hepatic insufficiency and/or portal hypertension. All patients had the same follow up visits every 4 weeks until week 24, and at week 36. The diagnosis of lymphoid neoplasms was based on the World Health Organization (WHO) criteria. The study was performed according to the Helsinki declaration and was approved by the ethic committee of La Pitié-Salpêtrière hospital.

Immunologic and virological markers

HCV viral load was quantified using Abbott HCV RealTime assay (Abbott, Rungis, France) with a lower limit of detection of 12 IU/mL. HCV genotyping was performed by NS5b gene sequencing according to the previously validated consensual method [1]. Laboratory evaluation included a complete blood count, serum chemistry profile, alanine aminotransferase (ALT), rheumatoid factor activity, C4 fraction of complement and cryoglobulin. Cryoglobulins were measured as previously described [2] and classified according to the method described by Brouet et al. [3]. The estimation of glomerular filtration rate (GFR) was determined by modification of diet in renal disease (MDRD) study equations. Urine collection was also performed in order to quantify protein excretion. Liver fibrosis was evaluated (by liver biopsy and/or non-invasive tests) according to the previously validated Metavir scoring system [4].

Flow cytometry

PBMCs were obtained by density-gradient centrifugation. PBMCs were stained with the following monoclonal antibodies (at predetermined optimal dilutions) for 30 minutes at 4°C: Fluorescein isothiocyanate (FITC)–conjugated CD21, PE–conjugated CD27 and CXCR5, alexa fluor 450–conjugated CD3, allophycocyanin (APC)–conjugated IgM, energy-coupled dye (ECD)–conjugated CD4 and CD19, PerCP–Cy7–conjugated CD25, vioblue–conjugated IgD, and brillant violet 510–conjugated CD127 (BioLegend). Detection of intracellular FoxP3 was accomplished using fixed and permeabilized cells according to the manufacturer's instructions (eBioscience), and incubated with APC–conjugated FoxP3. PBMCs from HCV-CryoVas patients were stimulated for 4 hours with 50 ng/ml phorbol myristate acetate (PMA) and 1 mM ionomycin (Sigma-Aldrich) in the presence of brefeldin A (BD PharMingen). Cells cultured in the presence of brefeldin A were stained for cell surface markers and then permeabilized with Cytofix/Cytoperm buffer (BD PharMingen) and stained with FITC–conjugated IFNγ (BD PharMingen), Alexa Fluor 647–conjugated IL-17A (eBioscience), and Alexa Fluor 647–conjugated IL-21 (Bio-Legend).

FACS analyses were performed on a Navios flow cytometer using Kaluza analysis software (Beckman Coulter).

Study Design

All patients received antiviral therapy with sofosbuvir (400mg daily) plus daclatasvir (60mg daily) for 12 (n=32) or 24 (n=9) weeks. Duration of antiviral therapy was based on extent of liver disease and patient's prior antiviral treatment. **Patients who received antiviral therapy for 24 weeks had severe liver fibrosis (METAVIR stage 3 or 4) and non response to previous antiviral treatment including sofosbuvir plus ribavirin or Peg-interferon-α/ribavirin plus protease inhibitor (i.e. telaprevir or boceprevir).**

Endpoints

The primary endpoint was a complete clinical response of CryoVas at week 24 and the sustained virological response at week 12 post antiviral treatment (SVR12). The complete clinical response was defined by improvement of all the affected organs involved at baseline and the absence of clinical relapse. The skin and articular improvement were evaluated clinically (i.e. disappearance of purpura and/or ulcers and/or skin necrosis, disappearance of arthralgia and/or arthritis). Renal improvement was evaluated biologically (i.e. proteinuria <0.3g/24h, disappearance of hematuria and improvement of GFR > 20% at week 24 if GFR < 60 m**L**/min/1.73 m² at diagnosis). Peripheral neurological improvement was evaluated clinically (i.e. improvement of pains and paraesthesia by visual analogue scales, improvement of muscular testing in case of motor impairment at baseline) and/or electrophysiologically (i.e. improvement of electromyogram abnormalities at week 24 compared to baseline). The neuropathy total symptom score-6 (NTSS-6) was applied to evaluate individual neuropathy sensory symptoms [5]. Patients defined as partial clinical responders at week 24 had an

improvement in some but not all organs involved at baseline. Patients with no clinical improvement at week 24 were defined as treatment failure.

The secondary endpoints included: (i) the course of cryoglobulinemia, C4 complement fraction and alanine aminotransferase, and (ii) the rate and type of side-effects during the total thirty six weeks of follow-up. A sustained virological response was defined by the absence of detectable serum HCV RNA twelve weeks after the end of antiviral therapy; the remaining patients were classified as virological failures.

Safety assessments

Data on all adverse events were collected prospectively during the follow-up.

Statistical analysis

Data are expressed as the mean, standard error of mean (SEM) or median [interquartile range (IQR)] for quantitative data and counts, and percent for categorical data. This study was not designed to evaluate formal statistical hypotheses, and no sample-size calculations were performed. Analyses of effectiveness, tolerance and baseline patient characteristics were performed on the intention-to-treat population, defined as all enrolled patients who received at least one dose of study drugs (sofosbuvir, daclatasvir).

The nonparametric Mann Withney test was used to compare continuous variables. All tests are 2-sided at the 0.05 level. Statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software, San Diego, Calif).

Results

Characteristics of HCV-CryoVas patients

Main patient characteristics are detailed in **Supplementary Table 1**. Forty one HCV-CryoVas patients, with a median (IQR) age of 56 (50-62) years were included. Among the twenty one patients with peripheral neuropathy, eight had sensory-motor polyneuropathy, six

had sensory polyneuropathy, and seven had sensory-motor multiplex neuropathy. Among the five patients with kidney involvement, four had a renal biopsy showing membrano-proliferative glomerulonephritis. Mean (±SEM) cryoglobulin level was of 0.56 ± 0.18g/**L**. Eighty nine percent of patients had a type II mixed cryoglobulin. Mean C4 serum level was of 0.08 ± 0.02g/**L**. Eighteen (43.9%) patients had a Child-Pugh class A cirrhosis. At time of DAA initiation, two (4.8%) patients were treated with immunosuppressive therapy [rituximab infusions (weekly infusions of 375 mg/m2 for 4 weeks) and prednisone (60mg/day progressively tapered) in 2 cases] and/or plasmapheresis (n=3), due to severe neuropathy (n=3) and/or skin ulcer (n=1). Nineteen (46.3%) patients were antiviral treatment naïve and the remaining twenty four (53.7%) were virological non-responders to a previous antiviral therapy (**Supplementary Table 1**).

Treatment Efficacy

The main treatment-related data are summarized in **Table 1, Supplementary Table 2 and Supplementary Figures 1 and 2**. Purpura, skin ulcers and arthralgia disappeared in all cases. Peripheral neuropathy improved in 17 out of 21 cases. Median BVAS [6] decreased from 8 (4-17) to 0 (0-5) ($p<0.01$). The mean Neuropathy Total Symptom Score-6 (NTSS-6) decreased from 12.4 ± 3.5 to 3.2 ± 2.1. Motor symptoms improved in 10 out of 15 patients. HCV viral load dropped from 5.6 to 1.18 IU/mL at week 4 ($p<0.0001$) (**Supplementary Figure 1**). Mean creatinine level was 116±23 µmol/L at baseline and 76±6 µmol/**L** at week 24, while mean GFR was 88±32 and 89±9 m**L**/min/1.73/m², respectively (**Supplementary Figure 2**). Proteinuria decreased from 0.9±0.4 to 0.2±0.1 g/24h. Hematuria disappeared in four out of five (80%) cases at week 24. Kidney involvement improved in all five of whom 80% had complete renal response. The mean cryoglobulin level decreased from 0.56 ± 0.18 at baseline to 0.21 ± 0.14 g/L at week 36 ($p<0.05$). The C4 serum level increased from 0.08 ± 0.02 to 0.14 ± 0.02 g/L at week 36 ($p<0.05$). One patient had a stage IV marginal zone B cell

lymphoma with villous lymphocytes associated with HCV-CryoVas. He was considered in complete remission of his lymphoma and in partial remission of CryoVas at the end of antiviral therapy. He still had persistent circulating B cell clone and a type II mixed cryoglobulinemia IgG/IgMk at the end of follow up.

Safety

Seven patients (17%) experienced at least one side effect. One patient with cirrhosis developed hepatocellular carcinoma four months after the end of antiviral therapy. His liver nodule was resected and he was still alive and in remission at the end of follow up.

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Supplementary Figure 1: Kinetics of cryoglobulinemia (A), C4 complement level (B), rheumatoid factor activity (C), and HCV RNA (D).

Data are shown for all HCV-cryoglobulinemia vasculitis patients for whom assessments were available.

Supplementary Figure 2: Outcome of kidney parameters

A. Proteinuria; B. Serum creatinine level; C. Glomerular filtration rate (GFR).

Data are shown for all HCV-cryoglobulinemia vasculitis patients for whom assessments were available [proteinuria (n=16), serum creatinine level (n=20), glomerular filtration rate (n=19)].

Supplementary Table 1. Main characteristics of HCV cryoglobulinemia vasculitis patients

	N=41
Age, (years)	56 (50-62)
Female gender (n, %)	22 (53.6)
HCV infection	
HCV genotype	
1	25 (61)
2	2 (4.9)
3	9 (21.9)
4	3 (7.3)
5	2 (4.9)
Metavir liver fibrosis score	
Stage 1	12 (29.3)
Stage 2	3 (7.3)
Stage 3	8 (19.5)
Stage 4	18 (43.9)
Mean baseline HCV RNA (log10 IU/m**L**)	5.9 ± 0.2
Mean ALT level (IU/L)	55.3 ± 6.4
Haematologic variables	
Mean hemoglobin level (g/dL)	12.5 ± 0.7
Mean neutrophil count (/mm3)	3361 ± 68
Mean platelet count (/mm3)	181 ± 57
Previous response to antiviral therapy	
Naive	19 (46.3)
No response	10 (24.4)
Partial response	4 (9.7)
Relapse	8 (19.5)
Mixed cryoglobulinemia related	
Mean serum cryoglobulin level (g/L)	0.56 ± 0.18
Mean serum C4 level (g/L)	0.08 ± 0.02
Median serum RF levels (IU/mL)	47 ±18
Vasculitis	
Purpura	31 (75.6)
Arthralgia	26 (63.4)
Polyneuropathy	21 (51.2)
Skin ulcer	7 (17.1)
Kidney involvement	5 (12.2)
Clinical features of vasculitis	
1	2 (4.8)
2	31 (75.6)

≥3	8 (19.5)

HCV viral load was quantified using Abbott HCV RealTime assay (Abbott, Rungis, France) with a lower limit of detection of 12 IU/mL. The lower limit of detection of cryoglobulinemia was 0.05 g/L. The lower limit of detection of rheumatoid factor was 20 IU/mL).
The normal values and range of hemoglobin level was (11.5-16 g/dL), of neutrophil count was (1.8-7.5/mm^3), of platelet count was (150-500/mm^3), of ALT was (11-26 IU/mL), and of C4 complement level was (0.16-0.39 g/L).

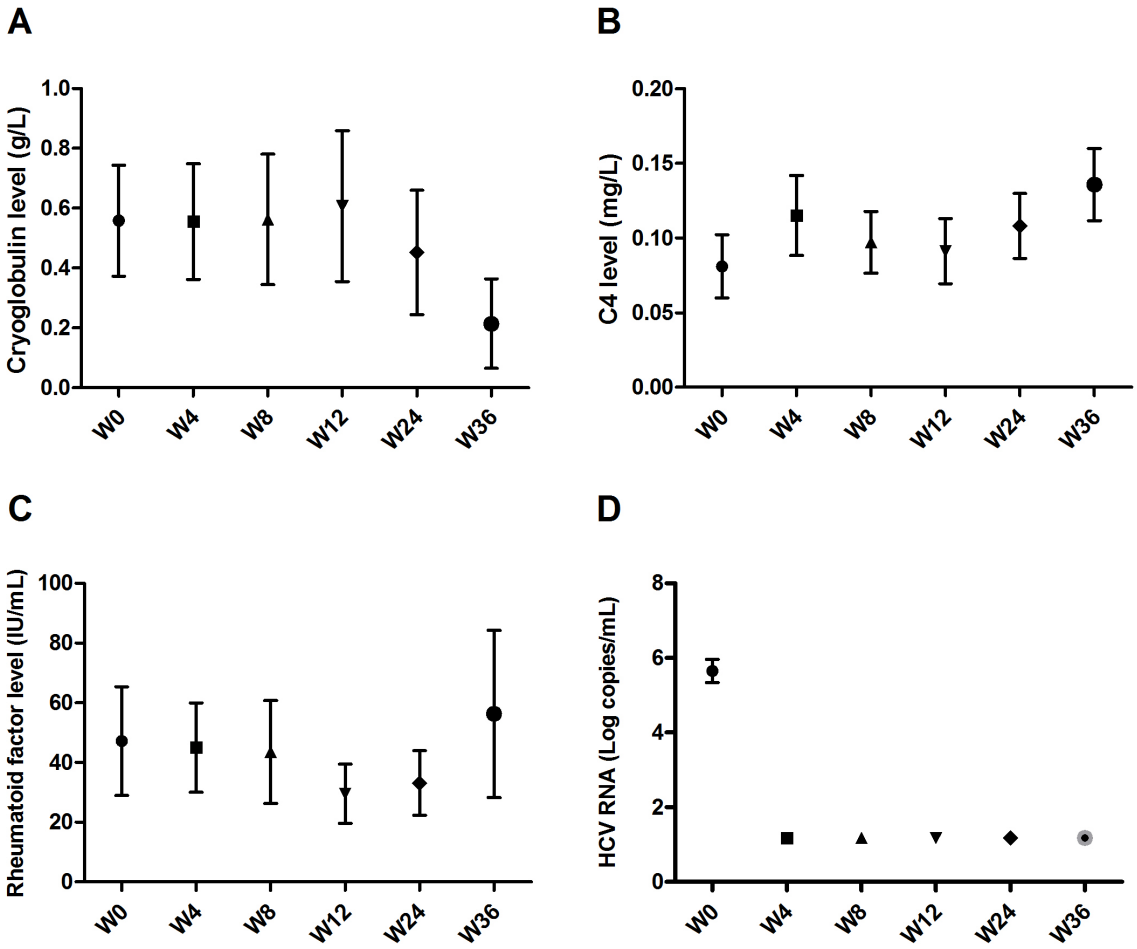
Supplementary Table 2. Main characteristics of HCV cryoglobulinemia vasculitis patients

Patients	HCV genotype	Liver fibrosis	Previous antiviral therapy*	HCV RNA	ALT (IU/L)	Duration of DAA (Weeks)	Virological response	Clinical response
1	3	1	PegIFN/RBV (R)	6.51	116	12	SVR	CR
2	2	3	0	6.63	NA	12	SVR	CR
3	3	4	PegIFN/RBV (R)	4.22	38	12	SVR	CR
4	1b	4	Sof/RBV (NR)	5.8	151	24	SVR	CR
5	4	4	0	5.55	37	12	SVR	CR
6	1b	3	0	4.62	52	12	SVR	CR
7	1a	4	PegIFN/RBV (PR)	5.87	27	12	SVR	PR
8	1a	4	PegIFN/RBV (PR)	NA	NA	12	SVR	CR
9	1b	3	0	6.2	23	12	SVR	CR
10	1a	1	PegIFN/RBV (R)	6.92	57	12	SVR	CR
11	2	4	PegIFN/RBV (PR)	6.44	19	12	SVR	CR
12	1b	1	0	5.85	7	12	SVR	CR
13	1b	4	0	5.82	NA	12	SVR	CR
14	1b	4	Sof/RBV (NR)	6.62	NA	24	SVR	CR
15	4	4	PegIFN/RBV (R)	3.54	86	12	SVR	CR
16	1a	2	0	5.62	18	12	SVR	CR
17	1b	4	PegIFN/RBV (R)	6.94	29	12	SVR	CR
18	3	4	PegIFN/RBV/PI (NR)	4.66	131	24	SVR	CR
19	5	1	0	7.1	28	12	SVR	CR
20	1b	1	0	8.7	25	12	SVR	CR
21	1a	4	PegIFN/RBV/PI (NR)	5.04	60	24	SVR	CR
22	1a	3	PegIFN/RBV (R)	5.14	81	12	SVR	CR
23	4	4	PegIFN/RBV (PR)	6.36	67	12	SVR	CR
24	1b	3	PegIFN/RBV (R)	5.47	22	12	SVR	CR
25	1	4	0	5.76	24	12	SVR	CR
26	1b	1	0	3.53	13	12	SVR	CR
27	3	3	0	6.04	101	12	SVR	CR
28	3a	2	PegIFN/RBV (R)	5.15	62	12	SVR	CR
29	1b	4	PegIFN/RBV/PI (NR)	6.5	70	24	SVR	CR
30	1b	4	Sof/RBV (NR)	6.03	46	24	SVR	PR
31	1a	1	IFN (NR)	6.67	48	12	SVR	CR
32	5a	1	0	5.56	147	12	SVR	CR
33	3a	4	0	3.21	74	12	SVR	CR
34	3a	3	PegIFN/RBV/PI (NR)	7.05	91	24	SVR	PR
35	1a	1	0	6.66	36	12	SVR	CR
36	1	4	Sof/RBV (NR)	6.51	120	24	SVR	CR
37	1a	1	0	7	21	12	SVR	CR
38	3	1	0	6.5	20	12	SVR	CR
39	3	2	0	5.79	50	12	SVR	CR
40	1	3	Sof/RBV (NR)	6.52	30	24	SVR	CR
41	1	1	0	6.57	20	12	SVR	PR

Liver fibrosis was assessed according to METAVIR score. NA, not available; **PegIFN/RBV, pegylated interferon-α plus ribavirin; IFN, interferon-α; PegIFN/RBV/PI, pegylated interferon-α plus ribavirin plus protease inhibitor; Sof/RBV, sofosbuvir plus ribavirin**; HCV RNA in Log/mL; ALT, alanine aminotransferase; SVR, sustained virological response; CR, complete remission of vasculitis; PR, partial remission of vasculitis.

*** Previous response to antiviral therapy was given in brackets. NR, no response; PR, partial response; R, relapse.**

Supplementary Figure 1: Kinetics of cryoglobulinemia (A), C4 complement level (B), rheumatoid factor activity (C), and HCV RNA (D).



Supplementary Figure 2: Outcome of kidney parameters