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Abstract:

Background: Direct-acting antiviral agents (DAAs) have modified the management of chronic hepatitis C virus (HCV) infection, including HCV-related mixed cryoglobulinemia vasculitis (CryoVas). However, patients might experience vasculitis relapse. No reliable predictors of CryoVas relapse following sustained virological response (SVR) have been established. Herein, we aimed to describe HCV-CryoVas relapse rates as well as the factors associated with it.

Methods: In this international multicenter study, patients with HCV-CryoVas treated with DAA in 3 countries (Egypt, France and Italy) were analyzed retrospectively. The main outcome consisted of the assessment of CryoVas relapse rates. Factors associated with relapse-free survival were evaluated in a multivariate adjusted model.

Results: 913 patients were evaluated, 911 (99.8%) of whom obtained SVR. After a median follow-up of 35 months, 798 patients (87.4%) had sustained remission of vasculitis, while 115 (12.6%) experienced CryoVas relapse. By the time of relapse, patients presented skin involvement in 100%, renal involvement in 85.2%, and peripheral neuropathy in 81.7%. Relapses were treated with glucocorticoids alone in 90.9%, or were associated with plasma exchange, cyclophosphamide or rituximab in 50%, 37.3% and 6.4%, respectively. Death occurred in 11 relapsers, mainly due to infections. The cumulative incidence of CryoVas relapse or death was 0.9% (95% CI 0.4-1.7), 12.9% (95% CI 10.8-15.3) and 14 % (95% CI 11.8-16.5), at 12, 24 and 36 months after the end of DAA treatment, respectively. Independent baseline risk factors associated with CryoVas relapse or death were male sex, arterial hypertension, skin ulcers/necrotizing vasculitis, peripheral neuropathy and kidney involvement.

Conclusions: A substantial proportion of CryoVas patients experience relapse after DAA-induced SVR. Relapses are moderate to severe, and they impact survival after 24 months, mainly due to infections. Independent risk factors for relapse or death have been found.

1. Introduction

Mixed cryoglobulinemia vasculitis (CryoVas) is a small-vessel systemic vasculitis in which clinical manifestations range from purpura, arthralgia and fatigue to more serious and life-threatening complications with neurologic and renal involvement [1]. Circulating mixed cryoglobulins are detected in 40% to 60% of patients chronically infected with hepatitis C virus (HCV), while HCV-CryoVas occurs in approximately 15% of HCV-infected patients [2-5]. Based on the role of chronic HCV antigenic stimulation that provides expansion of rheumatoid factor (RF) B cells responsible for the production of cryoglobulins [6], early interferon-based therapies showed that sustained virologic response (SVR) was associated with CryoVas clinical response [7]. However, such treatment was limited by its poor tolerability and low antiviral efficacy (SVR rate < 50%). Recently approved direct-acting antiviral agents (DAAs) that are able to induce long-term viral clearance in almost all HCV patients are now considered to be first-line anti-HCV therapy. Despite the very good virologic and clinical outcomes, all DAA studies in patients with HCV-CryoVas found a significant proportion of patients who were still positive for cryoglobulins, and vasculitis relapses were also reported [3-5]. The persistence of a few copies of HCV-RNA in liver cells, macrophages, or lymphocytes, or the persistence of pathogenic B-cell clones are potential explanations for such relapses [8, 9, 10]. It has been suggested that conditions characterized by the production of immune complexes, such as infections, cancer, and vaccination, may be associated with vasculitis relapse due to the capacity of these immune complexes to bind to rheumatoid factor (RF)-producing B cells, thereby favoring their reactivation and cryoglobulin production [5, 11, 12]. To date, long-term studies have failed to find reliable markers that are predictive of either response or relapse of CryoVas following sustained viral clearance [3, 4].

This study offers a large cohort of patients with HCV-CryoVas treated with DAAs and aimed to evaluate CryoVas characteristics and relapse rate as well as factors associated with it.

2. Patients and methods

a. Study design

We performed an international, multi-center, retrospective study with patients who met validated criteria for the diagnosis of HCV-CryoVas with positive serum HCV-RNA, circulating cryoglobulins and symptoms and signs of active vasculitis (i.e., skin, joint, renal, peripheral nerve, central neurological, pulmonary, and/or cardiac involvement) [13]. Data were obtained from the following four centers: 1) Nephrology Unit, Internal Medicine Department, Cairo University, Egypt; 2) Rheumatology and Clinical Immunology Unit, Internal Medicine Department, Cairo University, Egypt; 3) Department of Internal Medicine and Clinical Immunology, National Reference Center for Autoimmune Systemic Rare Diseases, Hospital La Pitié-Salpêtrière, Paris, France; and 4) Referral Centre for Mixed Cryoglobulinemia, Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy. All cases were treated between 2015 and 2019 with IFN-free DAAs, consisting of sofosbuvir-based protocols containing either simeprevir, daclatasvir, ledipasvir, ribavirin or the combination of glecaprevir/pibrentasvir. Only cases achieving sustained virological response after DAA were considered for analysis. All patients were negative for hepatitis B surface antigen as well as anti-HBs, anti-HBc and anti-human immunodeficiency virus antibodies. Patients treated with IFN-based therapeutic regimens were excluded. These exclusion criteria aimed to avoid biases inherent to other viruses' clinical manifestations or immunological effects of interferon-based therapies.

b. Data collection

Clinical and biological data were recorded for each patient at baseline (i.e., before starting DAA), at the end of DAA treatment (EOT), 3 months after EOT (for SVR), 12 months after EOT, and when relapse occurred using a standardized form. The following data were collected at baseline: sex, age, body mass index (BMI), CryoVas manifestations, such as constitutional (fever, asthenia, anorexia),

cutaneous (purpura, distal ulcers, necrotizing vasculitis), rheumatologic (inflammatory arthralgia, arthritis, myalgia), renal (proteinuria, hematuria, serum creatinine level), and neurologic symptoms (polyneuropathy, multiple mononeuritis, central nervous system), as well as comorbidities and the presence and type of underlying hematologic disorders, such as non-Hodgkin lymphoma (NHL). Laboratory evaluation included HCV viremia (low viremia: less than 200,000 IU/mL; moderate viremia: between 200,000 to 2,000,000 IU/mL; and high viremia: more than 2,000,000 IU/mL), levels of serum complement (C3 and C4), cryoglobulins, serum creatinine levels, urinalysis (hematuria) and a 24-hour proteinuria, albuminemia and serum electrophoresis. Cryoglobulinemia was defined as either positive or negative due to the lack of homogeneous methodology for quantifying it across centers (e.g., cryocrit percentage, g/L). Liver disease was evaluated by non-invasive methods including liver elastography, imaging, clinical presentation and laboratory data; the METAVIR score corresponding to liver stiffness measurement was used to assess the degree of liver fibrosis.

c. Outcomes

The main outcomes of the present study consisted of a description of CryoVas relapse characteristics, rates and factors associated with it. Secondary outcomes included the evaluation of the relapse severity, the sustained CryoVas clinical and immunological response and relapse-free and overall survival.

The clinical response of CryoVas was evaluated by the resolution of clinical manifestations as follows. Constitutional response was defined as the disappearance of fever, asthenia and anorexia. Skin and joint response was defined as the disappearance of purpura and/or ulcers and disappearance of inflammatory arthralgias and/or arthritis, respectively. Neurological response was defined by clinical improvement of pain, paresthesia and/or muscle strength with improvement of the electromyogram (when available) as compared with baseline. Renal response was defined by proteinuria <0.5 g/day or a protein/creatinine ratio <50 mg/mmol, disappearance of hematuria and

improvement of the estimated glomerular filtration rate (eGFR) >20% compared with the baseline value (in case of baseline eGFR < 60 mL/min).

Complete response corresponded to the resolution of all baseline clinical manifestations, and partial response to an improvement of at least half of baseline clinical manifestations. Refractory disease was defined as the persistence or worsening of either constitutional symptoms, active skin lesions, inflammatory arthralgia and/or arthritis, renal or neurological involvement. Relapse was defined as a) the recrudescence of any of the baseline manifestations unequivocally attributable to CryoVas at any time after DAA therapy in patients with previous complete or partial response, or b) the occurrence of any unseen symptom attributable to CryoVas, even if it was not present before.

d. Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) or median (interquartile range [IQR]), and categorical variables as numbers (percentage). Time to relapse was defined as the time between the date of EOT and the date of relapse diagnosis or last follow-up, whichever occurred first. Relapse-free survival function was estimated using the Kaplan-Meier estimator. Factors associated with relapse-free survival were evaluated in Cox regression models in univariate analysis. Log linearity for continuous variables and proportional hazards assumptions were checked. A multivariate adjusted model was selected using a stepwise selection procedure on Akaike's criterion (AIC) from variables achieving $p < 0.10$ in univariate analyses. All tests were two-sided at a 5% significance level. Results of Cox regression models are expressed as hazard ratios (HRs) [95% confidence intervals (95% CIs)]. Analyses were performed on R statistical platform software, version 4.0.1.

3. Results

a. Baseline characteristics of HCV-CryoVas patients

A total of 954 patients were initially included, 761 (79.8%) from Egypt, 129 (13.5%) from France and 64 (6.7%) from Italy. Thirty-seven patients were excluded because of missing follow-up data after DAA completion, and four other patients experiencing CryoVas relapse after DAA therapy were excluded due to missing date of relapse onset. In total, the study cohort for analyses was comprised of 913 patients (**Figure 1**). The median follow-up was 35 months (IQR 28-41), with 224 (24.5%) patients being followed over 41 months.

The demographic, clinical and biological characteristics of the study population are reported in **Table 1**. The median age was 42 years [IQR 27-89], 588 patients (64.4%) were female, 21 (2.3%) had a BMI > 30 kg/m^2, and 43 (4.7%) had cirrhosis (estimated METAVIR score = F4). The main baseline CryoVas manifestations were purpura (93.5%), kidney disease (36.4%), peripheral neuropathy (12.4%), skin ulcers (3.7%) and necrotizing vasculitis (3.4%). Twenty patients (2.2%) had concomitant low-grade B-cell NHL. Serum cryoglobulins were found in 901 (98.9%) out of 911 patients tested. RF was present in 98.1% of patients, and the median C4 level was 8 mg/dL [7-12]. There were no differences in baseline characteristics with respect to the country of origin.. Along with DAA therapy, most patients received simultaneous treatment with steroids (85.7%). Cyclophosphamide and rituximab were concomitantly prescribed with DAAs in 2.5% and 1.6% of patients, respectively. Plasma exchange was indicated in 0.9% of patients with life-threatening clinical manifestations.

b. Response of HCV-CryoVas manifestations

A sustained virologic response was obtained in 911 out of the 913 patients (99.8%). After follow-up, 798 patients (87.4%) had CryoVas sustained remission, while 115 (12.6%) experienced vasculitis relapse. **Table 2** summarizes the clinical and laboratory outcomes at EOT of the main cryoglobulinemia vasculitis manifestations following DAAs. Purpura and constitutional symptoms – found in almost all patients at baseline – resolved best after antiviral therapy, being found in 1.2 and 2.5% of patients at EOT, respectively. Severe cutaneous manifestations, such as skin ulcers and

necrotizing vasculitis also resolved in most patients, persisting in 0.9% and 0.3% of patients, respectively. Kidney involvement and peripheral neuropathy were present in 8.2% and 8.3% of patients at the EOT, respectively. Cryoglobulins disappeared in 91.4% of patients, and C4 levels normalized in 84.2%. RF was present in only 5.7% of patients after DAAs.

c. Relapse of CryoVas manifestations in patients with SVR post-DAA

Of the 913 patients, 115 (12.6%) relapsed after SVR with DAAs, with a median time to relapse of 15 months (IQR 14-17) after EOT. Clinical and laboratory characteristics of patients experiencing relapse of vasculitis are described in **Table 3**. All patients presented skin involvement by the time of relapse, either with ulcers or necrotizing vasculitis (101/115; 88%) or purpura (65/115; 57%). Renal disease was the second most frequent manifestation at relapse, being found in 98/115 patients (85.2%). Most patients (94/115; 81.7%) had signs and symptoms of peripheral neuropathy at relapse, while this was clinically present at baseline in only 18 (15.7%) of them. By the time of relapse, almost all patients presented with positive cryoglobulins (93.4%) and RF (99.1%) and a low C4 level (median 6.2 mg/dL). CryoVas relapses were treated with glucocorticoids alone (100/115; 90.9%) or in association with plasma exchange (55/115; 50%), cyclophosphamide (41/115; 37.3%) or rituximab (7/115; 6.4%). Improvement of skin manifestations was achieved in most patients (97/115; 89%), whereas renal disease improved in about half of them (50/115; 52.1%) (**Table 3**).

Of the 20 (2.2%) patients who died during follow-up, 11 were Egyptians relapsers. Death among relapsers occurred after a median of 3 months [1-17] after DAA and was caused mainly by infections in the context of immunosuppressive therapies (10/11; 90.9%). The proportion of death in this group was higher as compared with non-relapsers [11/115 (9.6%) vs. 9/798 (1.1%), respectively]. The cumulative incidence of CryoVas relapse or death (without CryoVas relapse) is illustrated for all patients (**Figure 2A**) and according to the center of enrollment (Egypt, France, or Italy) **(Figure 2B)**. The overall cumulative incidence of CryoVas relapse or death was 0.9% (95%

CI 0.4; 1.7), 12.9% (10.8; 15.3) and 14 % (11.8; 16.5), at 12, 24 and 36 months after the end of DAA treatment, respectively.

d. Risk factors associated with death or relapse-free survival of CryoVas

In univariate analyses (**Supplementary Table 1)**, male sex, age, BMI >30 kg/m^2, arterial hypertension, skin ulcers, necrotizing vasculitis, kidney disease and peripheral neuropathy at baseline were significantly associated with an increased risk of CryoVas relapse. Accordingly, the need for more aggressive therapeutic strategies, such as plasma exchange done before DAAs, were associated with increased risk of relapse. No laboratory features were associated with increased risk of relapse. Although patients in Italy showed the highest relapse rate (16%) as compared to Egypt (13%) and France (5%), the enrollment center did not constitute a factor associated with relapse. Multivariable analyses retained the presence of arterial hypertension, skin ulcers/necrotizing vasculitis, male sex, peripheral neuropathy and kidney disease at baseline as independent risk factors associated with CryoVas relapse or death (**Table 4**).

4. Discussion

The present study is the largest multicenter international study assessing the extent of relapse in HCV-CryoVas patients following SVR obtained from IFN-free DAA regimens. We analyzed a cohort of 913 patients from four centers in three different geographical locations and found an overall relapse rate of vasculitis of 12.6%. Relapses consisted mostly of moderate to severe forms of vasculitis characterized by skin ulcers/necrotizing vasculitis, renal involvement and peripheral sensorimotor neuropathy. They required aggressive treatment strategies with plasma exchange in half of the cases and cyclophosphamide in a third of patients. The death rate was higher in relapsers as compared with the remaining cohort, being mostly attributed to infections.

Recent long-term studies have reported clinical relapse of CryoVas in 4% to 18% of HCV-cured patients treated with DAAs [4,5,14,15]. A prospective cohort of 148 patients with a 15-month

median follow-up found no relapse and reported 4 deaths (2.8%) unrelated to CryoVas [3]. In another prospective study comprising 46 patients with a 24-month median follow-up, relapse caused severe organ damage in 2 out of 5 patients and death in one [4]. In the recent study of Colantuono S. et al., 4 out of 11 CryoVas relapsers had severe or life-threatening manifestations and, remarkably, further treatment with rituximab in two of them was ineffective [14]. We found that a more severe baseline form of vasculitis – with renal disease, skin ulcers, necrotizing vasculitis and neuropathy – was associated with clinical relapse. Multivariate analysis retained kidney disease and arterial hypertension as independent risk factors for relapse. Poor prognosis of renal involvement in CryoVas has been reported [16]. In this light, renal involvement, whether as a direct consequence of vasculitis or not (i.e., hypertension), seems to play a critical role in CryoVas prognosis, and should be primarily considered in the management of these patients.In our cohort of patients, we found a discrepancy with previous studies regarding the prevalence of peripheral sensorimotor neuropathy. At baseline, only 12.4% of our patients presented with peripheral neurological involvement, a picture that is reported to range from 41% to 76% in other CryoVas patients [3-5, 15]. Probably most of these patients had worsening of preexisting peripheral neuropathy that at baseline was either mild or not considered to be related to active vasculitis. Another possible reason might be due to the younger age (median = 42 years) and the diverse ethnicity of our patients compared with other series. The prevalence of neuropathy has been reported to increase significantly with age in HCV patients [17], and African-Caribbean diabetic patients were reported to have a lower incidence of neuropathy as compared with White patients [18].

The increased rate of HCV-CryoVas relapses in males as compared with females is intriguing. The presence of many immune-related genes on the X chromosome, especially the TLR7 gene, is believed to cause, together with estradiol, the higher prevalence of autoimmune diseases in females [19]. However, some immune responses (e.g., to certain vaccines) and inflammation are reported to

be higher in elderly males than in females [19]. Stimulation of TLR7 and/or TLR9 by microbial nucleic acids, concomitantly with the engagement of BCR by immune complexes, is a possible mechanism for reactivation of pathogenic RF-expressing B cells in HCV-cured CryoVas patients [10]. It is likely that the pressure on TLR9 by bacterial DNA from the microbiome [20] and by endogenous DNA from apoptotic cells is more intense and persistent than that on TLR7 by single-stranded RNA from viruses. The breaking of immune tolerance in CryoVas via TLR9 (i.e., independent of viral action) [10] together with the more pronounced inflammatory milieu seen in males [19] could explain the persistence of B clones leading to vasculitis even after viral clearance.

Severe forms of CryoVas at baseline constituted independent risk factors for vasculitis relapse after viral cure, confirming what was observed in the largest cohort of CryoVas patients treated with DAA to date [3]. This further highlights the need for more aggressive therapies towards this patient profile. In contrast to what has been previously noted [25], B-cell NHL was not associated with vasculitis recurrence in our study. Although large, our study had a limited number of patients with B-cell NHL, and the indolent and often subclinical course of this lymphoma may have caused it to be underreported. We were also unable to find an association of laboratory variables, such as cryoglobulin, RF, C4 level or others, with vasculitis relapse, maybe due to the lack of standardization of quantification methods across centers. High levels of BAFF and APRIL are known to stimulate B cell survival. A recent study showed an increased expression of BAFF and APRIL in HCV-CryoVas patients at EOT compared with their pre-treatment levels. BAFF and APRIL levels continued to increase throughout the follow-up period. These cytokines may be the basis for further CryoVas relapse [15] and may represent more reliable biomarkers in predicting the progression and relapse of CryoVas.

We acknowledge some limitations of the present study. First, the retrospective nature of the study brings inherent biases regarding missing data or the homogeneous manner in which they are reported. However, we tried to limit these as much as possible and use only variables of clinical

relevance whose values were widely available. Furthermore, recruitment imbalance between centers could limit their external validity. Yet, it should be acknowledged that with the advancement of highly effective anti-HCV therapies, the large sample size presented here is extremely timely in predicting factors associated with vasculitis relapse on a large scale.

In conclusion, more than 10% of CryoVas patients may experience vasculitis relapse even after DAA-induced SVR. Vasculitis relapses are usually moderate to severe, requiring intensive treatment, and substantially impact survival after 24 months, mostly due to infection. Male sex, arterial hypertension and severe baseline CryoVas manifestations represented independent risk factors for relapse and should guide physicians towards a closer follow-up for this special population. It is possible that treatments acting on B cell clones that are responsible for persistent cryoglobulin production, such as rituximab and belimumab, might be able to reduce relapse rates. Also, a more tailored immunosuppressant strategy should be sought, given the infectious risk presented by these patients. Further studies are needed to design the best approach for patients with HCV-CryoVas relapse.

Legends to figures

Figure 1. Flowchart of the survey

Study's flow chart and the respective proportions of patients experiencing either sustained vasculitis remission or relapse. Of the 913 patients included in the analyses, 911 (99.8%) obtained sustained virologic response following DAA.

CryoVas = cryoglobulinemic vasculitis, DAA = direct-acting antiviral and HCV = hepatitis C virus.

Figure 2. Cumulative incidence of relapse or death in a large international cohort of HCV-cryoglobulinemia vasculitis, in the whole cohort (top panel, A) and according to country (bottom panel, B).

References

1. Cacoub P, Saadoun D. Extrahepatic Manifestations of Chronic HCV Infection. N Engl J Med. 2021 Mar 18;384(11):1038-1052.

2. Gragnani L, Visentini M, Fognani E, Urraro T, De Santis A, Petraccia L, et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. Hepatology, 2016. 64(5): p. 1473-1482.

3. Cacoub P, Si Ahmed SN, Ferfar Y, Pol S, Thabut D, Hezode C, et al. Long-term Efficacy of Interferon-Free Antiviral Treatment Regimens in Patients With Hepatitis C Virus-Associated Cryoglobulinemia Vasculitis. Clin Gastroenterol Hepatol, 2019. 17(3): p. 518-526.

4. Bonacci M, Lens S, Londoño M-C, Mariño Z, Cid MC, Ramos-Casals M, et al. Long-Term Outcomes of Patients With HCV-Associated Cryoglobulinemic Vasculitis After Virologic Cure. Gastroenterology, 2018. 155(2): p. 311-315.e6.

5. Visentini M, Del Padre M, Colantuono S, Yang B, Minafò YA, Antonini S, et al. Long-lasting persistence of large B-cell clones in hepatitis C virus-cured patients with complete response of mixed cryoglobulinaemia vasculitis. Liver Int, 2019. 39(4): p. 628-632.

6. Carbonari M, Caprini E, Tedesco T, Mazzetta F, Tocco V, Casato M, et al. Hepatitis C virus drives the unconstrained monoclonal expansion of VH1-69-expressing memory B cells in type II cryoglobulinemia: a model of infection-driven lymphomagenesis. J Immunol, 2005. 174(10): p. 6532-9.

7. Gragnani L, Fognani E, Piluso A, Boldrini B, Urraro T, Fabbrizzi A, et al. Long-term effect of HCV eradication in patients with mixed cryoglobulinemia: a prospective, controlled, open-label, cohort study. Hepatology, 2015. 61(4): p. 1145-53.

8. Del Padre M, Todi L, Mitrevski M, Marrapodi R, Colantuono S, Fiorilli M et al. Reversion of anergy signatures in clonal CD21(low) B cells of mixed cryoglobulinemia after clearance of HCV viremia. Blood, 2017. 130(1): p. 35-38.

9. Comarmond C, Garrido M, Pol S, Desbois AC, Costopoulos M, Le Garff-Tavernier M et al. Direct-Acting Antiviral Therapy Restores Immune Tolerance to Patients With Hepatitis C Virus-Induced Cryoglobulinemia Vasculitis. Gastroenterology. 2017 Jun;152(8):2052-2062.e2.

10. Comarmond C, Lorin V, Marques C, Maciejewski-Duval A, Joher N, et al. TLR9 signalling in HCV-associated atypical memory B cells triggers Th1 and rheumatoid factor autoantibody responses. J Hepatol. 2019 Nov;71(5):908-919.

11. Sollima S, Milazzo L, Vassalini P, Antinori S, Galli M. Recurrence of mixed cryoglobulinaemia vasculitis following influenza vaccination despite clearance of hepatitis C virus infection. Clin Exp Rheumatol, 2018. 36 Suppl 111(2): p. 161-162.

12. Visentini M, Quartuccio L, Del Padre M, Colantuono S, Minafò YA, Fiorilli M, et al. Late relapses of hepatitis C virus-cured mixed cryoglobulinaemia associated with infection or cancer. Rheumatology (Oxford), 2018. 57(10): p. 1870-1871.

13. Quartuccio L, Isola M, Corazza L, Ramos-Casals M, Retamozo S, Ragab GM, et al. Validation of the classification criteria for cryoglobulinaemic vasculitis. Rheumatology (Oxford), 2014. 53(12): p. 2209-13.

14. Colantuono S, Mitrevski M, Yang B, Tola J, Carlesimo M, De Sanctis GM, et al. Efficacy and safety of long-term treatment with low-dose rituximab for relapsing mixed cryoglobulinemia vasculitis. Clinical Rheumatology, 2017. 36(3): p. 617-623.

15. Hegazy MT, Allam WR, Hussein MA, Zoheir N, Quartuccio L, El-Khamisy SF, et al. Increased genomic instability following treatment with direct acting anti-hepatitis C virus drugs. EBioMedicine, 2018. 35: p. 106-113.

16. Mazzaro C, Maso LD, Mauro E, Gattei V, Ghersetti M, Bulian P, et al. Survival and Prognostic Factors in Mixed Cryoglobulinemia: Data from 246 Cases. Diseases. 2018; 6(2):35.

17. Santoro L, Manganelli F, Briani C, Giannini F, Benedetti L, Vitelli E, et al. Prevalence and characteristics of peripheral neuropathy in hepatitis C virus population. J Neurol Neurosurg Psychiatry. 2006;77(5): 626-629.

18. Zitouni K, Tinworth L, Earle KA. Ethnic differences in the +405 and −460 vascular endothelial growth factor polymorphisms and peripheral neuropathy in patients with diabetes residing in a North London, community in the United Kingdom. BMC Neurology, 2017. 17(1): p. 125.

19. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016 Oct;16(10):626-38.

20. Kreuk LS, Koch MA, Slayden LC, Koch MA, Slayden LC, Lind NA, et al. B cell receptor and Toll-like receptor signaling coordinate to control distinct B-1 responses to both self and the microbiota. Elife.2019;8:e47015. https://doi.org/10.5061/dryad.660js04

21. Torcia MG, Nencioni L, Clemente AM, Civitelli L, Celestino I, Limongi D, et al. Sex differences in the response to viral infections: TLR8 and TLR9 ligand stimulation induce higher IL10 production in males. PLoS One. 2012;7(6):e39853.

22. Mingomataj EÇ, Bakiri AH. Regulator Versus Effector Paradigm: Interleukin-10 as Indicator of the Switching Response. Clin Rev Allergy Immunol. 2016 Feb;50(1):97-113.

23. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Critical reviews in immunology vol. 32,1 (2012): 23-63.

24. Levy G, Solt I. The Human Microbiome and Gender Medicine. Gender and the Genome. October 2018:123-127.

25. Landau DA, Saadoun D, Halfon P, Martinot-Peignoux M, Marcellin P, Fois E, et al. Relapse of hepatitis C virus-associated mixed cryoglobulinemia vasculitis in patients with sustained viral response. Arthritis Rheum, 2008. 58(2): p. 604-11.

Table 1. Demographic, clinical and biological variables of patients with HCV-CryoVas who achieved a sustained virologic response after direct-acting antivirals, according to presence of absence of relapse.

	All patients (n = 913)	**No relapse (n = 798)**	**Relapse (n = 115)**
Center			
France	93 (10.2)	88 (11.0)	5 (4.3)
Egypt	756 (82.8)	656 (82.2)	100 (87.0)
Italy	64 (7.0)	54 (6.8)	10 (8.7)
Female sex	588 (64.4)	530 (66.4)	58 (50.4)
Age, years	42 [35-46]	43 [35-46]	40 [35-45]
BMI, kg/m^2			
18 - 24	458 (50)	402 (51)	56 (48)
25 - 29	424 (47)	370 (47)	54 (47)
≥ 30 - 34	21 (2)	17 (2)	4 (2)
NA	10	9	1
Diabetes	13 (1.4)	11 (1.4)	2 (1.7)
Arterial hypertension	50 (5.5)	41 (5.1)	9 (7.8)
Smoking (n = 911)	377 (41.4)	327 (41.1)	50 (43.5)
Estimated liver fibrosis degree*			
F0	13 (1.4)	13 (1.6)	0 (0.0)
F1	64 (7.0)	53 (6.6)	11 (9.6)
F2	481 (52.7)	416 (52.2)	65 (56.5)

F3	311 (34.1)	273 (34.3)	38 (33.0)
F4	43 (4.7)	42 (5.3)	1 (0.9)
NA	1	1	0
Child-Pugh classification			
A	41 (4.5)	40 (5.0)	1 (0.8)
B	2 (0.2)	2 (0.3)	0 (0.0)
Clinical manifestations			
Purpura	854 (93.5)	739 (92.6)	115 (100.0)
Constitutional symptoms	813 (89.0)	706 (88.5)	107 (93.0)
Kidney involvement	332 (36.4)	275 (34.5)	57 (49.6)
Peripheral neuropathy	113 (12.4)	95 (11.9)	18 (15.7)
Joint involvement	105 (11.5)	90 (11.3)	15 (13.0)
Raynaud's phenomenon	45 (4.9)	38 (4.8)	7 (6.1)
Skin ulcers	34 (3.7)	26 (3.3)	8 (7.0)
Lymphoma	20 (2.2)	19 (2.4)	1 (0.9)
Albumin, g/dL	3.8 [3.5-4.0]	3.8 [3.5-4.0]	3.8 [3.4-4.0]
NA	11	11	0
Positive cryoglobulin	901 (98.9)	786 (98.7)	115 (100.0)
NA	2	2	0
Positive rheumatoid factor	813 (98.1)	701 (97.9)	112 (99.1)
NA	84	82	2
Serum C4, mg/dL	8 [7-10]	8 [7-9]	8 [6-14]

NA	48	45	3
Monoclonal component	58 (6.5)	52 (6.7)	6 (5.2)
NA	26	26	0
eGFR, ml/min	87 [72-111]	87 [72-111]	95 [77-113]
NA	8	8	0
CKD Stage			
1	412 (45.8)	350 (44.5)	62 (55.4)
2	401 (44.6)	361 (45.9)	40 (35.7)
3	81 (9.1)	71 (9.0)	10 (8.9)
4	4 (0.4)	4 (0.5)	0 (0.0)
5	1 (0.1)	1 (0.1)	0 (0.0)
NA	14	11	3
HCV viremia**			
Low	97 (10.7)	84 (10.6)	13 (11.5)
Moderate	560 (61.9)	487 (61.5)	73 (64.6)
High	248 (27.4)	221 (27.9)	27 (23.9)
NA	8	6	2

Results are presented as number of patients (%) or median [interquartile range].

Joint involvement regards inflammatory arthralgia, arthritis and/or myalgia, whereas renal involvement corresponds to proteinuria, hematuria and/or elevated serum creatinine level. CKD stages correspond to the respective glomerular filtration rate (GFR) as follows: > 90 mL/min for stage 1, 60-89 mL/min for stage 2, 30-59 mL/min for stage 3, 15-29 mL/min for stage 4 and < 15 mL/min for stage 5. The normal range for C4 is 10-40 mg/dL.

* Liver fibrosis degree was estimated through liver stiffness measurement from liver transient elastography and its corresponding METAVIR score.

**Low viremia is less than 200,000 IU/mL, moderate viremia between 200,000 to 2,000,000 IU/mL, and high viremia more than 2,000,000 IU/mL.

CKD: chronic kidney disease. CryoVas: cryoglobulinemia vasculitis. HCV: hepatitis C virus. NA: not available.

Table 2. Clinical and laboratory outcomes in patients with HCV-CryoVas at the end of treatment by direct-acting antivirals, according to the presence or absence of vasculitis relapse.

	All patients (n = 913)	**No relapse (n = 798)**	**Relapse (n = 115)**
Clinical manifestations			
Peripheral neuropathy	76 (8.3)	60 (7.5)	16 (13.9)
Kidney involvement	75 (8.2)	67 (8.4)	8 (7.0)
Joint involvement	44 (4.8)	38 (4.8)	6 (5.2)
Raynaud's phenomenon	37 (4.1)	34 (4.3)	3 (2.6)
Constitutional symptoms	23 (2.5)	19 (2.4)	4 (3.5)
Purpura	11 (1.2)	11 (1.4)	0 (0.0)
Skin ulcer	8 (0.9)	7 (0.9)	1 (0.9)
Necrotizing vasculitis	3 (0.3)	3 (0.4)	0 (0.0)
Laboratory features			
Cryoglobulinemia	77 (8.6)	68 (8.7)	9 (8.0)
NA	14	12	2
Cryoglobulinemia manifestations response			
Worsened	6 (0.7)	3 (0.4)	3 (2.7)
No response	31 (3.5)	29 (3.7)	2 (1.8)
Partial response	37 (4.2)	33 (4.3)	4 (3.5)
Complete response	814 (91.7)	710 (91.6)	104 (92.0)
NA	25	23	2
C4 level, mg/dL	22.0 [16.0-26.0]	22.0 [16.0-26.0]	21.0 [15.0-24.8]

NA	69	64	5
C4 response			
Partial or no response	128 (15.8)	113 (16.1)	15 (13.8
Complete response	683 (84.2)	589 (83.9)	94 (86.2)
NA	102	96	6
Rheumatoid factor	46 (5.7)	35 (5.0)	11 (10.2)
NA	105	98	7
Titer, IU/ml	37.9 [12.0-72.0]	26.0 [12.0-56.0]	92.5 [31.4-167.5]
NA	122	14	3
Rheumatoid factor response			
Partial or no response	44 (5.6)	33 (4.8)	11 (10.3)
Complete response	747 (94.4)	651 (95.2)	96 (89.7)
NA	122	114	8
Monoclonal component	41 (4.7)	35 (4.7)	6 (5.2)
NA	46	46	0

Results are presented as number of patients (%) or median [interquarile range].

Joint involvement regards inflammatory arthralgia, arthritis and/or myalgia, whereas renal involvement corresponds to proteinuria, hematuria and/or elevated serum creatinine level.

CryoVas: cryoglobulinemia vasculitis. HCV: hepatitis C virus. NA: not available.

The normal range for C4 is 10-40 mg/dL.

Table 3. Clinical characteristics, outcomes and treatments of 115 relapsing patients with HCV-CryoVas after sustained viral response following direct-acting antivirals.

	n = 115
Clinical manifestations, n (%)	
Skin ulcers or necrotizing vasculitis	101 (88)
Kidney involvement	98 (85.2)
Neuropathy	94 (81.7)
Constitutional symptoms	67 (58.3)
Purpura	65 (57.0)
Joint involvement	48 (41.7)
Laboratory features, n (%) or median [IQR]	
Serum albumin gr/L (n = 113)	3.0 [2.7-3.2]
Hemoglobin gr/dL (n = 114)	12.8 [10.5-13.7]
Proteinuria, g/24h (n = 114)	3.0 [2.4-3.5]
Hematuria (n = 114)	73 (64.0)
Serum C3, mg/dL (n = 107)	77.0 [54.6-101.0]
Serum C4, mg/dL (n = 113)	6.2 [6.0-8.0]
Circulating cryoglobulins	108 (93.9)
Rheumatoid factor (n = 108)	107 (99.1)
Monoclonal component	9 (7.8)
Median time to relapse, months	
Onset of renal relapse	16 [14-18]
Onset of skin relapse	17 [15-19]
Death	

Cause of death, n (%)	
Infection	10 (90.9)
Unknown	1 (9.1)
Median time to death, months	3 [1-17]
Treatment of relapses (n = 110), n (%)	
Glucocorticoids	100 (90.9)
Plasma exchange	55 (50.0)
Cyclophosphamide	41 (37.3)
Rituximab	7 (6.4)
Improvement after treatment of relapse, n (%)	
Renal improvement (n = 114)	50 (52.1)
Skin improvement (n = 109)	97 (89.0)

Results are presented as number of patients (%) or median [IQR].

CryoVas: cryoglobulinemia vasculitis. HCV: hepatitis C virus. IQR: interquartile range.

Table 4. Multivariate analysis of baseline factors associated with relapse-free survival of HCV-CryoVas after sustained virological response post-direct acting antivirals.

Variables	**HR (95% CI)**	***p* value**
Sex		
Female	1	
Male	1.89 (1.32-2.71)	0.0005
Hypertension		
No	1	
Yes	2.24 (1.24-4.03)	0.007
Skin ulcers		
No	1	
Yes	1.93 (0.99-3.78)	0.054
Kidney involvement		
No	1	
Yes	1.48 (1.03-2.12)	0.033
Peripheral neuropathy		
No	1	
Yes	1.80 (1.07-3.04)	0.028

Supplementary Table 1: Univariate analysis of baseline characteristics and treatments prescribed for HCV-CryoVas relapse.

	N events / N total	HR (95% CI)	*p* value
Center			
France	12/93	1	
Egypt	100/756	0.81 (0.44 to 1.48)	0.49
Italy	12/64	1.05 (0.47 to 2.34)	0.90
Sex			
Female	60/588	1	
Male	64/325	2.07 (1.45 to 2.94)	**<0.0001**
Age		1.01 (1.00 to 1.03)	**0.042**
BMI, kg/m^2			
< 25	61/458	1	
25 – 29	57/424	1.00 (0.69 to 1.43)	0.99
≥ 30	5/21	2.70 (1.08 to 6.78)	**0.034**
Diabetes			
No	122/900	1	

Yes	2/13	1.31 (0.32 to 5.30)	0.71
Arterial hypertension			
No	109/863	1	
Yes	15/50	2.99 (1.74 to 5.16)	**<0.0001**
Smoking			
No	70/534	1	
Yes	54/377	1.12 (0.78 to 1.60)	0.54
Estimated liver fibrosis degree			
0-2	81/558	1	
3-4	43/354	0.83 (0.57 to 1.20)	0.33
Esophageal varices			
No	120/902	1	
Yes	4/11	3.60 (1.32 to 9.80)	**0.012**
Skin purpura			
No	3/59	1	
Yes	121/854	2.50 (0.79 to 7.90)	0.12
Skin ulcer			
No	114/879	1	

Yes	10/34	2.71 (1.42 to 5.18)	**0.003**
Skin necrotizing vasculitis			
No	116/882	1	
Yes	8/31	2.35 (1.15 to 4.82)	**0.019**
Raynaud's phenomenon			
No	116/868	1	
Yes	8/45	1.31 (0.64 to 2.68)	0.46
Peripheral neuropathy			
No	101/800	1	
Yes	23/113	1.75 (1.11 to 2.77)	**0.017**
Joint involvement			
No	109/808	1	
Yes	15/105	1.16 (0.67 to 2.00)	0.60
Kidney involvement			
No	63/581	1	
Yes	61/332	1.68 (1.18 to 2.38)	**0.004**
Lymphoma			
No	119/893	1	

Yes	5/20	1.84 (0.75 to 4.53)	0.18
Constitutional symptoms			
No	15/100	1	
Yes	109/813	0.83 (0.48 to 1.45)	0.52
Albuminemia (HR per g/dL)		0.90 (0.56 to 1.46)	0.67
Cryoglobulin			
No	1/10		
Yes	123/901		0.99*
Rheumatoid factor			
No	2/16	1	
Yes	116/813	0.82 (0.20 to 3.30)	0.78
Serum C4		1.01 (0.99 to 1.03)	0.41
Presence of M component			
No	110/829	1	
Yes	12/58	1.61 (0.88 to 2.94)	0.13
Serum creatinine		1.32 (0.84 to 2.07)	0.23
eGFR		1.00 (1.00 to 1.01)	0.25

CKD stage			
1-2	110/819	1	
3-5	13/86	1.07 (0.60 to 1.90)	0.83
HCV viremia			
Low	15/97	1	
Moderate	75/560	0.80 (0.46 to 1.39)	0.43
High	32/248	0.79 (0.43 to 1.46)	0.46
Time from symptoms to DAA > 5 years		1.35 (0.68 to 2.69)	0.39
Treatments			
Duration of DAA treatment			
12 weeks	108/838	1	
24 weeks	12/60	1.75 (0.96 to 3.17)	0.067
Other	2/13	1.18 (0.29 to 4.80)	0.81
Glucocorticoids or immunosuppressant			
No	17/127	1	
Yes	107/786	0.90 (0.54 to 1.52)	0.71
Glucocorticoids			
No	20/131	1	

Yes	104/782	0.77 (0.47 to 1.25)	0.28
Immunosuppressant			
No	119/875	1	
Yes	5/38	0.99 (0.40 to 2.42)	0.98
Erythropoietin			
No	119/895	1	
Yes	5/18	2.49 (1.02 to 6.11)	**0.046**
Blood transfusion			
No	123/910	1	
Yes	1/3	2.49 (0.34 to 18.3)	0.37
Cyclophosphamide			
No	124/890		
Yes	0/23		0.065*
Rituximab			
No	122/898	1	
Yes	2/15	0.93 (0.23 to 3.77)	0.91
Plasma exchange			
No	120/905	1	
Yes	4/8	3.88 (1.43 to 10.5)	**0.008**

Angiotensin converting enzyme blocker			
No	112/871	1	
Yes	12/42	2.63 (1.44 to 4.79)	**0.002**

Joint involvement regards inflammatory arthralgia, arthritis and/or myalgia, whereas renal involvement corresponds to proteinuria, hematuria and/or elevated serum creatinine level. Liver fibrosis degree was estimated through liver stiffness measurement from liver transient elastography and its corresponding METAVIR score.

BMI: body mass index; CI: confidence interval; eGFR: estimated glomerular filtration rate.

* P-value obtained from log-rank test