Journal of Autoimmunity 161 (2026) 103559

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

HCV-related cryoglobulinemic vasculitis (mixed cryoglobulinemia syndrome) in the era of direct-acting antivirals: A 10-year experience from Italian tertiary referral centers

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A R T I C L E I N F O

Keywords:
Cryoglobulinemic vasculitis
Mixed cryoglobulinemia
Cryoglobulinemia
Hepatitis C virus
HCV
Direct-acting antivirals
DAA

A B S T R A C T

Objective: To evaluate long-term clinico-serological response to direct-acting antivirals (DAAs) in patients with HCV-related cryoglobulinemic vasculitis (HCV-CV), and to compare the incidence of severe disease complications before/after antiviral therapy (AVT). The study also aimed to explore patterns of disease evolution following AVT.

Methods: This retrospective multicenter study included 161 HCV-CV patients (mean age 63.9 ± 11.1 years; 64.6% females) treated with DAAs and achieving sustained virological response. The mean observation period was 8.2 ± 5.2 years, including pre-AVT (2.2 ± 5.5) and post-AVT (5.6 ± 2.7) phases. Complete response (CR) was defined as full resolution of baseline CV symptoms, partial response (PR) as ≥50% improvement, all others were non-responders (NR).

Results: Post-AVT, significant reductions were observed in purpura (64% to 14%), fatigue (72% to 35%), arthralgias (55% to 26%), sicca syndrome (32% to 16%), skin ulcers (13% to 4%), and liver involvement (74% to 25%) (all $p < 0.01$), along with decreased cryocrit % (3.5 ± 4.6 to 0.5 ± 1.5, $p < 0.0001$) and increased C4 levels (9.6 ± 8.5 to 20.2 ± 11 mg/dl; $p < 0.0001$). CR was achieved in 74%, PR in 14%, NR in 12% of patients. Severe CV complications occurred more frequently pre-AVT than post-AVT (71% vs. 29%; $p < 0.0001$), particularly in NR patients (47% vs PR 26% and CR 12%; $p = 0.0006$), and those with persistent cryoglobulins (53% vs 12% without; $p < 0.0001$). Of note, NR patients showed mean higher cryocrit ($p = 0.0046$) and lower C4 ($p = 0.025$) levels evaluated within the last 12 months before DAAs.

Conclusion: DAAs significantly improved the clinical course of HCV-CV. Three post-treatment subsets can be identified: CR patients with minor residual symptoms, PR requiring careful monitoring, and NR group at high-risk for severe CV complications. Persistent serum cryoglobulins are predictors of long-term worse clinical course.

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https://doi.org/10.1016/j.jaut.2026.103559
Received 12 September 2025; Received in revised form 9 April 2026; Accepted 10 April 2026
Available online 27 April 2026

1. Introduction

The mixed cryoglobulinemia syndrome (MCs), also termed cryoglobulinemic vasculitis (CV), is an autoimmune systemic disorder currently classified among small-to medium-vessel vasculitides [1–4]. It is characterized by the clinical triad -purpura, fatigue, arthralgias-, leukocytoclastic vasculitis, and serum mixed cryoglobulins with low complement $C4^{5,6}$. Vasculitic lesions are secondary to intravascular deposition of cryo- and non-cryoprecipitable immune complexes, primarily mixed cryoglobulins [1–7], consisting of polyclonal IgGs (autoantigens) and IgMs (autoantibodies) with rheumatoid factor activity (RF), which is mono-/oligoclonal RF in MC type II and polyclonal in MC type III [1–5]. Both type II and type III MC are comparable with respect to clinical manifestations [3,4]. The underlying biological alteration of MCs is an indolent B-cell lymphoproliferation responsible for to cryoglobulin production. In a minority of cases, this condition may progress to overt B-cell non-Hodgkin's lymphoma (B-NHL) [3–5].

Numerous environmental triggers can cause MC often as occasional laboratory disorder; while the classical MCs develops in only a small percentage of individuals [2–5]. Following the discovery of the strong association with hepatitis C virus (HCV) [8–10], MCs is now recognized as the most common extrahepatic manifestation of HCV infection [3,4,7–14]. The virus may exert a chronic stimulus on the immune system leading to B-lymphocyte expansion and consequent autoantibody production including the mixed cryoglobulins [3,4,11,15].

The causative role of HCV infection has prompted the use of antiviral agents as a treatment option for HCV-related CV since the nineties [16–19]. The interferon-α (IFNα) was initially administered in mono-therapy as immunomodulating agent in the late eighties, already before the identification of HCV [19]. Successively, the antiviral treatment (AVT) with combined IFNα/Peg-IFNα and Ribavirin (RBV) increased the sustained virologic response (SVR) rate [17,19,20]. The introduction of the first-generation of direct-acting antiviral agents (DAAs), i.e. telaprevir and boceprevir, in combination with Peg-IFNα, significantly improved the SVR rates [21–24], although severe side effects developed in almost half of treated patients [21,23,24]. The introduction of second generation DAAs, administered in IFN-free regimens, drastically lowered the side effects and further improved the SVR rates [25].

Overall, the results of clinical trials as well the real-world experience [26–36] demonstrated that IFN-free DAAs may lead to both virological and clinico-immunological response in the majority of patients [27–29,33,35,37], while active MCs and/or disease clinical relapses can observed in a limited number of individuals [26,27,30,31,33,38,39]. However, the reports available in the literature are generally referred to CV patients followed for short follow-ups [28,30,33,37,40–43].

The present study aimed to evaluate: (i) the early and long-term effects of SVR achieved with DAAs on typical cryoglobulinemic manifestations; (ii) the incidence of severe and unpredictable cryoglobulinemic manifestations during the post-antiviral follow-up period compared with the corresponding pre-treatment interval; and (iii) the emergence of potentially novel clinical patterns in patients achieving SVR during a prolonged post-DAA follow-up.

2. Patients and methods

This retrospective multicenter study included a cohort of 161 diagnosed with HCV-CV, recruited from different Italian tertiary referral centers.

The present non-interventional study evaluating retrospectively anonymized data from patients' records did not require ethical committee approval in accordance with our institutional guidelines.

Clinical, serological, and virological data were collected from patient records; moreover, MC classification and patients' evaluation were performed according to previously described criteria [3,5,44].

Inclusion criteria were the presence of clinically overt MCs, ongoing HCV infection, and eligibility for DAA-based antiviral treatment (AVT) according to national and international treatment recommendations at the time [45,46].

The clinical parameters assessed included purpura, fatigue, arthralgia, peripheral sensory-motor neuropathy (presence of peripheral neuropathic symptoms: paresthesias, sensory loss, muscle impairments with/without paralysis evaluated by electromyography and nerve conduction study), and skin ulcers, as well as evidence of internal organ damage, namely renal (24h proteinuria and/or reduced glomerular filtration rate), pulmonary involvement (spirometric alterations >10% FVC reduction and x-ray interstitial involvement by HRCT in presence of respiratory symptoms, i.e. dyspnea and/or cough), as well as the CNS involvement (evaluated by magnetic resonance imaging in presence of dysarthria and hemiplegia and/or severely impaired cognitive function) according to previously reported methodologies [47–53].

Some MCs manifestations were classified as particularly severe and grouped as: i. sudden-onset sensory-motor peripheral neuropathy often as mononeuritis with foot or hand drop due to nerve injury responsible for acute sensory loss, muscle impairments and/or paralysis; ii. non-healing severe skin ulcers, with or without gangrene, persisting for more than 6 weeks despite standard treatment; iii. cryoglobulinemic glomerulonephritis, almost invariably an immune-complex-related membranoproliferative glomerulonephritis type I; and iv. B-cell NHL. The latter were classified in the main categories of indolent or aggressive B-cell lymphomas according to 2022 WHO Classification [54].

Data were collected at four time points: (a) at the time of referral, (b) immediately before the initiation of AVT (pre-DAAs), (c) in the 6 months after the completion of treatment (post-DAAs), and (d) at the end of follow-up.

From referral to the end of follow-up, patients were monitored for 8.2 ± 5.2 years, with a mean duration of 2.2 ± 5.5 years before and 5.6 ± 2.7 years after the administration of DAAs.

Clinical response to therapy was evaluated based on changes in the symptoms. A *complete response* (CR) was defined as the resolution of all the baseline typical MCs manifestations, i.e. purpura, fatigue, arthralgias, skin ulcers, sicca syndrome, and peripheral neuropathy. A *partial response* (PR) was defined as disappearance in at least 50% of baseline symptoms, while patients who did not meet criteria for CR or PR were classified as *non-responders* (NR).

Response to therapy was evaluated comparing pre-DAA (Table 1, column b) to post-DAA symptoms (Table 1, column c) and to the end of follow-up (Table 1, column d).

Furthermore, the clinical response to DAAs was also evaluated by means of a composite clinical and serological parameter variations (purpura, fatigue, arthralgias, skin ulcers, sicca syndrome, peripheral neuropathy, and serum cryoglobulin and C4 levels).

Finally, the incidence of above-mentioned particularly severe HCV-MCs complications was recorded during two distinct observation periods: the first spanning from patient referral to the start of DAAs, and the second extending from the end of AVT to the last follow-up visit.

3. Statistical analysis

The statistical analysis of the observed clinico-serological parameter variations included: (i) descriptive analysis, (ii) univariable and multivariable logistic regression analysis, and (iii) analysis of variance (ANOVA).

For descriptive analysis, categorical variables were presented as frequencies and percentages. Continuous variables, which were normally distributed as assessed by the Shapiro–Wilk test, were summarized as mean (standard deviation, SD). Differences in continuous variables were assessed using the paired Student's t-test for longitudinal comparisons between time points, while categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate.

Multivariable logistic regression analyses were performed considering both dependent and independent variables as specified in both

Results session and in Supplementary Table 1.

Analysis of variance (ANOVA) with Bonferroni–Dunn correction for multiple comparisons, was performed as detailed below.

Finally, data were analysed and plotted using GraphPad Prism Software (version 8.0; Graph-Pad Software Inc, San Diego, CA, USA). Statistical significance was set at a p-value <0.05. All tests were two-tailed, and results with $p < 0.05$ were considered statistically significant.

4. Results

The main characteristics of the cohort at study entry (referral) were summarized in Table 1, column (a); namely 161 MCs patients, 57 males (35%) and 104 female (65%); mean age of 63.9 ± 11.1SD years; average disease duration 5.4 ± 7.3SD years); the most typical clinical symptoms included purpura (61%), fatigue (68%), arthralgias (64%), peripheral sensory neuropathy (51%), and sicca syndrome (30%). While some organ involvement was very seldom recorded, namely mild bibasilar interstitial lung fibrosis in 10 cases (6%) complicated by pulmonary hemorrhage in one, symptoms of CNS involvement (dysarthria) in 3 (2%), and myositis in one (0.6%).

Active HCV infection was confirmed by the presence of serum HCV-RNA, determined using standard molecular diagnostic techniques.

Liver damage, evaluated based on the presence of HCV-related chronic hepatitis or cirrhosis, represented the most frequent visceral organ involvement (77% at referral, Table 1).

During the pre-DAAs time period, a total of 46 patients underwent IFN-based therapy; in addition, 28 patients were treated with immunosuppressors, namely anti-CD20 rituximab, in 12/28 of them within the last 12 months preceding the DAAs. Only few individuals (15 pts) underwent to one or more immunomodulators such as methotrexate, mycophenolate mofetil, cyclosporin A, salazopyrin, and/or plasmapheresis sessions.

Effects of AVT on cryoglobulinemic clinical features. All patients achieved sustained virological response (SVR) following DAAs.

The prevalence of typical clinical and serological features of CV did not show significant differences between the first (a) and the pre-DAAs patients' assessment (b) (Table 1). All HCV-MCs patients achieved an SVR following AVT.

While the effects of AVT, evaluated by comparing the clinical features observed before (time point b), with those recorded after DAA-therapy (time point c), showed a statistically significant reduction of purpura (64% vs 14%; $p < 0.0001$), fatigue (72% vs 35%; $p < 0.0001$), arthralgias (55% vs 26%; $p < 0.0001$), sicca syndrome (32% vs 16%; $p = 0.0064$), and skin ulcers (13% vs 4%; $p = 0.0096$). The positive effects on clinical feature remained stable at the end of follow-up (Table 1, column d) indicating a durable clinical benefit of AVT (Table 2; Fig. 1); With regards to the liver involvement, the presence of type C hepatitis or cirrhosis was detected in 119/161 (total 74%; with cirrhosis in 42/119, 35%) before DAAs; after AVT, the prevalence of liver involvement significantly decreased (58/161, 36%; $p < 0.001$) (Table 1).

Response to AVT by composite parameter evaluation. The analysis of key symptom (purpura, fatigue, arthralgias, skin ulcers, sicca syndrome, and peripheral neuropathy) outcomes after DAAs revealed a CR in 74% (119/161) of patients, a PR in 14% (23/161), while a minority of patients 12% (19/161) were classified as NR (Table 2, panel 2). The clinical improvement recorded after DAAs (Table 1, column c) was comparable with those observed at the end of follow-up (Table 1, column d), i.e. CR 74%, PR 14%, and NN 12% (Table 2, panel 2).

Moreover, by including the main serological parameters in the evaluation of response to DAAs, i.e. disappearance of serum cryoglobulins and normalization of C4 levels, a CR was observed in 48% (77/161) of patients, while 37% (60/161) exhibited a PR, and 15% (24/161) were classified as NR, respectively.

Effects of AVT on laboratory parameters. By focusing on CV

Table 1
Clinical and laboratory findings at patient's referral, before/after DAAs, and at the end of FU in 161 HCV-CV.

	first evaluation a	before DAA treatment b	after DAA treatment c	end of FU d	a vs b	a vs c	a vs d	b vs c	b vs d	c vs d
Clinical symptoms, n (%)										
Purpura	99/161 (61%)	103/161 (64%)	22/161 (14%)	18/161 (11%)	p = 0.789	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**	p = 0.4772
Fatigue	110/161 (68%)	116/161 (72%)	56/161 (35%)	55/161 (34%)	p = 0.5723	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**	p > 0.9999
Arthralgias	87/161 (54%)	88/161 (55%)	42/161 (26%)	34/161 (21%)	p = 0.8966	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p<0.0001**	p = 0.3404
Peripheral s. neuropathy	82/161 (51%)	89/161 (55%)	68/161 (42%)	63/161 (39%)	p = 0.6026	p = 0.1375	**p = 0.0407**	p = 0.0638	**p = 0.0176**	p = 0.6355
Peripheral m. neuropathy,	29/161 (18%)	32/161 (20%)	29/161 (18%)	28/161 (17%)	p = 0.6169	p > 0.9999	>0.9999	p = 0.7349	p = 0.6106	p = 0.8794
CNS	3/161 (2%)	3/161 (2%)	5/161 (3%)	5/161 (3%)	p > 0.9999	p = 0.7109	p = 0.4878	p > 0.9999	p = 0.7104	p > 0.9999
Myositis	1/161 (0.6%)	1/161 (1%)	1/161 (1%)	2/161 (1%)	p > 0.9999	p > 0.9999	p > 0.9999	p > 0.9999	p > 0.9999	p > 0.9999
Sicca syndrome	49/161 (30%)	51/161 (32%)	25/161 (16%)	33/161 (20%)	p = 0.8885	**p = 0.003**	**p = 0.0372**	**p = 0.0064**	**p = 0.0464**	p = 0.3016
Skin ulcers	19/161 (12%)	21/161 (13%)	6/161 (4%)	8/161 (5%)	p = 0.8441	**p = 0.0096**	**p = 0.025**	**p = 0.0096**	**p = 0.0274**	p = 0.7707
Liver involvement	124/161 (77%)	119/161 (74%)	58/161 (36%)	40/161 (25%)	p = 0.6946	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	p = 0.0845
Kidney involvement	24/161 (15%)	27/161 (17%)	29/161 (18%)	34/161 (21%)	p = 0.723	p = 0.4431	p = 0.1801	p = 0.8628	p = 0.5056	p = 0.5568
Lung involvement	10/161 (6%)	10/161 (6%)	10/161 (6%)	13/161 (8%)	p > 0.9999	p > 0.9999	p = 0.6587	p > 0.9999	p = 0.8029	p = 0.8177
Laboratory findings										
Cryocrit %, mean ± SD	4.2 ± 6.0	3.5 ± 4.6	1.6 ± 4.3	0.5 ± 1.5	p = 0.23	**p<0.0001**	**p<0.0001**	**P = 0.0021**	**p<0.0001**	**p<0.0001**
C3 mg/dl, mean ± SD	96.4 ± 31.8	100.2 ± 45.2	110.7 ± 31.8	123.4 ± 32.5	p = 0.81	**p<0.0001**	**p<0.0001**	**p = 0.0006**	**p<0.0001**	**p = 0.0011**
C4 mg/dl, mean ± SD	11.4 ± 8.8	9.6 ± 8.5	15.0 ± 8.7	20.2 ± 11.0	p = 0.12	**p = 0.0004**	**p<0.0001**	**p<0.0001**	**p<0.0001**	**p = 0.0002**

Table 2
Incidence of severe MC manifestations and response to DAAs treatment in 161 HCV-MC patients.

Severe MC manifestations before/after DAAs

	Severe MC manifestations	Pre-DAAs	Post-DAAs	p
A	Non-healing SU ± cangrene **17%** (28/161)	**14%** (22/161)	4% (6/161)	**0.0024**
	Sudden onset s-m neuropathy **21%** (34/161)	**14%** (22/161)	**7%** (12/161)	**0.015**
	Cryoglobulinemic G-nephritis **14%** (22/161)	**10%** (16/161)	**4%** (6/161)	**0.0446**
	B-cell lymphoma **10%** (16/161)	**7%** (11/161)	**3%** (5/161)	0.1985
	Total 100	**71%** (71/100)	**29%** (29/100)	**0.001**

Clinical response to DAAs and severe MC manifestations

	Clinical response to DAAs	Severe manifestations after DAAs	p
B	*Complete response* **74%** (119/161)	**13%** (15/119)	**0.001**
	Partial response **14%** (23/161)	**22%** (5/23)	
	Non-responders **12%** (19/161)	**47%** (9/19)	

Persistence of serum cryoglobulins and severe MC manifestations

	Serum cryogl. after DAAs	Severe manifestations after DAAs	p
C	*Persistent* **25%** (40/161)	**38%** (15/40)	**0.001**
	Absent **75%** (121/161)	**12%** (14/121)	

Panel A: prevalence of incident severe manifestations before/after DAA regimens; MC: mixed cryoglobulinemis syndrome;
Panel B: response to DAA regimens evaluated on the basis of clinical symptoms (purpura, fatigue, arthralgias, skin ulcers, peripheral neuropathy, sicca syndrome) and incidence of severe manifestations in patients' subsets with different responses to DAAs.
Panel C: percentages of patients with persistent presence (25%) or absence (75%) of serum cryoglobulins after DAAs, and incidence of severe manifestations in patients' subsets with/without serum cryoglobulins post-DAAs follow-up period.
Severe manifestations included: non-healing SU (skin ulcer that persists for more than 6 weeks and shows no signs of healing despite standard treatments); sudden onset s-m (sensory-motor) peripheral neuropathy with acute nerve injury responsible for sensory loss, muscle impairments and potentially paralysis, often as mononeuritis with foot drop or hand weakness; cryoglobulinemic glomerulonephritis; and B-cell lymphoma (see text).

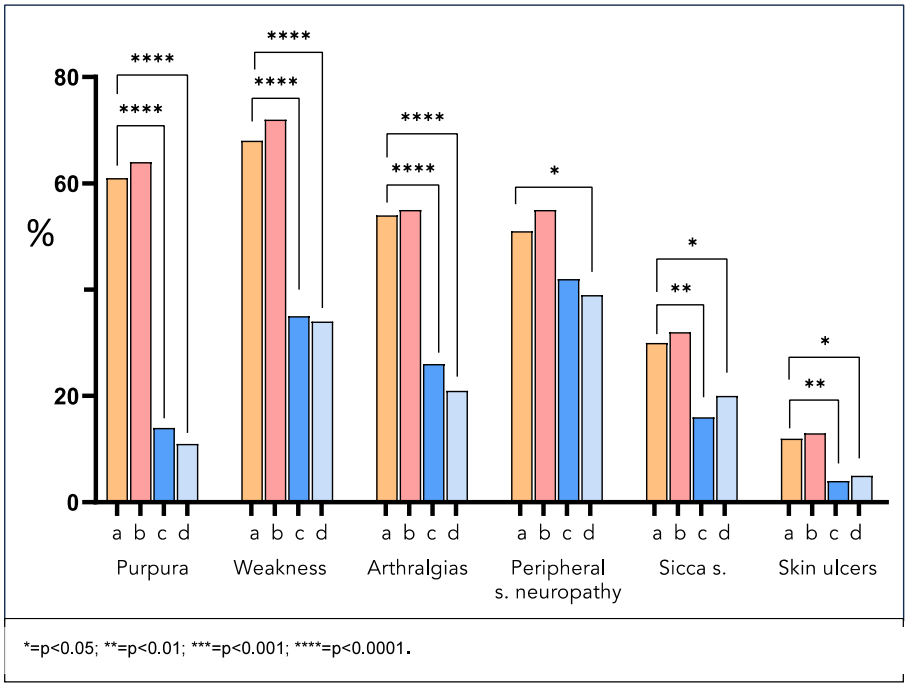

Fig. 1. Prevalence of typical cryoglobulinemic vasculitis symptoms at referral, before/after DAAs, end of follow-up
The prevalence of typical HCV-CV symptoms remained quite stable during the period preceding the antiviral treatment (time points a & b); while they showed a statistically significant reduction after DAA treatment (time points c & d). $p < 0.05$; **$p < 0.01$; ***$p < 0.0001$.

serological parameter variations, a significant improvement was also observed after DAAs regimens; namely, cryocrit significantly decreased from 3.5 ± 4.6 to 1.6 ± 4.3 ($p = 0.0021$), along with the normalization of C4 levels (from 9.6 ± 8.5 mg/dl to 15.0 ± 8.7 mg/dl; normal values > 12 mg/dl; $p < 0.0001$). The significant reduction of cryocrit and recovery of C4 observed after DAAs regimens were further strengthened at the end of the follow-up, time point d (cryocrit from 1.6 ± 4.3 to 0.5 ± 1.5; $p < 0.0001$; C4 from 15.0 ± 8.7 mg/dl to 20.2 ± 11.0 mg/dl; $p = 0.0002$; Table 1, Fig. 2).

The majority of individuals showed a complete disappearance of serum cryoglobulins (cryocrit 0%) during the long-term period following AVT (75%, 121/161 vs 25%, 40/161; $p < 0.0001$). The persistence of serum cryoglobulins, with/without low C4, following AVT was observed in 25% of patients (40/161; Table 2, panel 3); among them 17% had cryocrit values $> 0.5\%$, while 8% had only trace amounts of cryoglobulins (cryocrit $\leq 0.5\%$); Interestingly, patients non-responder to antiviral treatment showed medially higher cryocrit levels within the last 12 months before DAAs regimens (Anova, $p = 0.0046$) as well as a mean lower serum C4 levels (Anova, $p = 0.025$) (Fig. 3).

Fig. 4 describes the long-term clinical courses of two representative patients with divergent outcomes despite comparable baseline clinical and serological profiles.

Incidence and timing of MCs severe complications. During the follow-up, a total of 100 incident severe MC-related complications affecting 64 pts were recorded (see Patients & Methods; Table 2, panel 1). Notably, 71 of these events occurred prior to DAAs (involving 57 pts), while 29 events were recorded after treatment completion (involving 29 pts). Therefore, the incidence of severe complications was significantly higher in the pre-treatment period (71%) compared to the post-treatment period (29%) ($p < 0.0001$; Table 2, panel 1). With regard to the incidence of B-cell lymphomas, before DAA regimens 11 patients had developed lymphoma (3 indolent, 8 aggressive). During the subsequent follow-up period after DAA therapy, 5 additional patients developed B-cell lymphoma, specifically 2 indolent and 3 aggressive subtypes, respectively.

The frequency of these events during the post-DAAs period varied significantly according to a different response to AVT, i.e. CR, PR, or NR, evaluated at time-point (d) (Table 2, panel 2).

Taking into account the clinical response including key symptoms behavior, the 47% (9/19) NR individuals experienced one or more complications, compared to 22% (5/23) of PR and 13% (15/119) of CR patients ($p = 0.001$) (Table 2, panel 2). While considering the composite response including both clinical and serological parameters evaluation, 38% (9/24) of NR individuals developed one or more severe complications, compared to 17% (10/60) of PR and only 4% (3/77) of CR patients ($p < 0.0001$).

Finally, the patients with persistent serum cryoglobulins after DAAs (25%, 40/161) (Table 2, panel 3) exhibited a significantly higher event rate compared to those without serum cryoglobulins following AVT (53%, 21/40, vs 12%, 14/121, respectively; $p < 0.001$).

Overall MCs patients outcomes. The clinical course of MCs was generally mild to moderate during the pre-DAA period and frequently asymptomatic after antiviral therapy (ATV), as reflected by the significantly lower incidence of severe disease manifestations observed across the two follow-up periods (Table 2, panel 1).

In our retrospective analysis, multivariable logistic regression was performed with response to DAA therapy (CR, PR, and NR) as the dependent nominal variable, and patients' age (continuous), sex (nominal), and prior treatments (interferon-based and/or anti-CD20) as independent variables. The analysis did not show any significant association between these variables and the clinical response to DAA regimens [coefficient, −2.946; Exp(coef), 0.053; 95% lower, 0.002; 95% upper, 1.837; $P = 0.1042$] (see also Supplementary Table 1).

Similarly, multivariable logistic regression analysis did not reveal any significant association between response to DAA therapy (dependent nominal variable) and duration of post-DAA follow-up (independent continuous variable, expressed in months), as well as patients' age and sex (independent variables) [coefficient, −2.515; Exp (coef), 0.081; 95% lower, 0.003; 95% upper, 2.471; $P = 0.1494$].

No patients were lost to follow-up, whereas 15 of 161 patients (9%) died during the post-DAA follow-up. Deaths were directly attributable to MCs in 2 patients. Ten additional deaths were due to malignancies (8

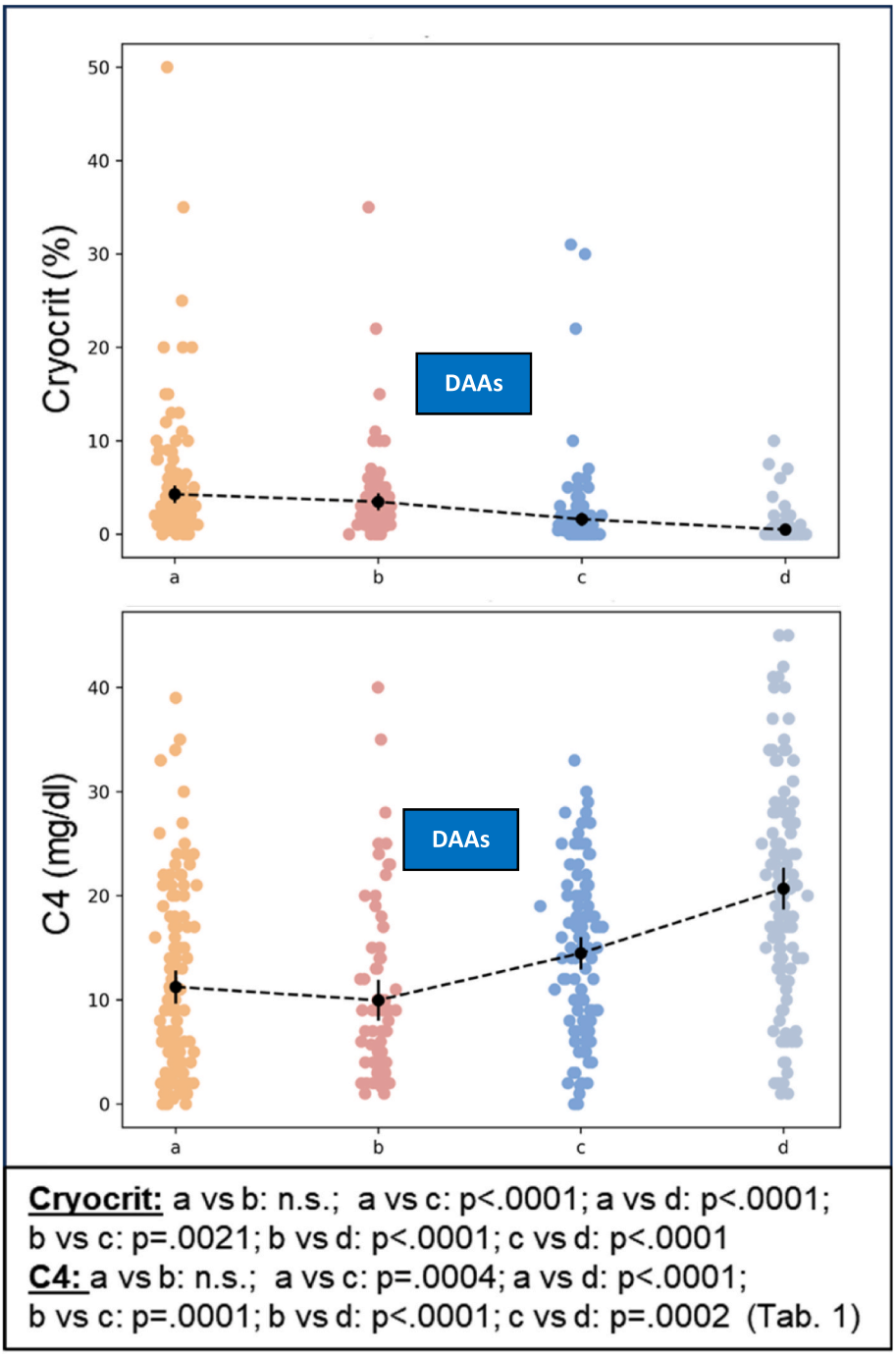

Fig. 2. Cryocrit and C4 levels in 161 patients with cryoglobulinemic vasculitis at referral, before/after DAAs, end of follow-up
The figure represents the trend of two main serological hallmarks of CV at four time points (a and b: before DAAs regimens; c and d: after DAAs). Cryocrit % significantly decreased after DAAs from the initial mean values of 3.5 ± 4.6 SD (b) to the final 0.5 ± 1.5 SD % (d). The majority of individuals (75%) showed a persistent absence of serum mixed cryoglobulins during the post-DAAs follow-up period (see text). Cryocrit: a vs b: n.s.; a vs c: $p < 0.0001$ a vs d: $p < 0.0001$ b vs c: $p = 0.0021$ b vs d: $p < 0.0001$ c vs d: $p < 0.0001$). Conversely, the C4 levels, abnormally reduced before DAA treatment (b: 9.6 ± 8.5SD mg/dl, normal values > 12 mg/dl), showed a statistically significant recovery during the post-DAAs period (d: 20.2 ± 11.0SD mg/dl). C4 a vs b: n.s.; a vs c: $p = 0.0004$ a vs d: $p < 0.0001$; b vs c: $p = 0.0001$ b vs d: $p < 0.0001$ c vs d: $p = 0.0002$.

patients: thyroid, colon, basal cell, breast, lung, and hepatocellular carcinoma, seminoma, and osteosarcoma), heart failure (1 patient), or COVID-19–pneumonia (1 patient); in 2 cases, the cause of death could not be ascertained.

Mild-to-moderate COVID-19 infection was documented in 27 of 161 patients (17%), whereas only one patient required hospitalization for severe COVID-19 pneumonia, which was responsible for death.

Overall, no association was observed between mortality and either the type of clinical response to DAA therapy (CR, PR, NR) or previous immunosuppressive treatments.

4.1. MC clinical course and patients' monitoring after AVT

On the basis of the above-described variations in clinical/laboratory parameters following SVR (Tables 1 and 2), a novel MC clinical course may be hypothesized (Fig. 5). In this context, the disease could evolve

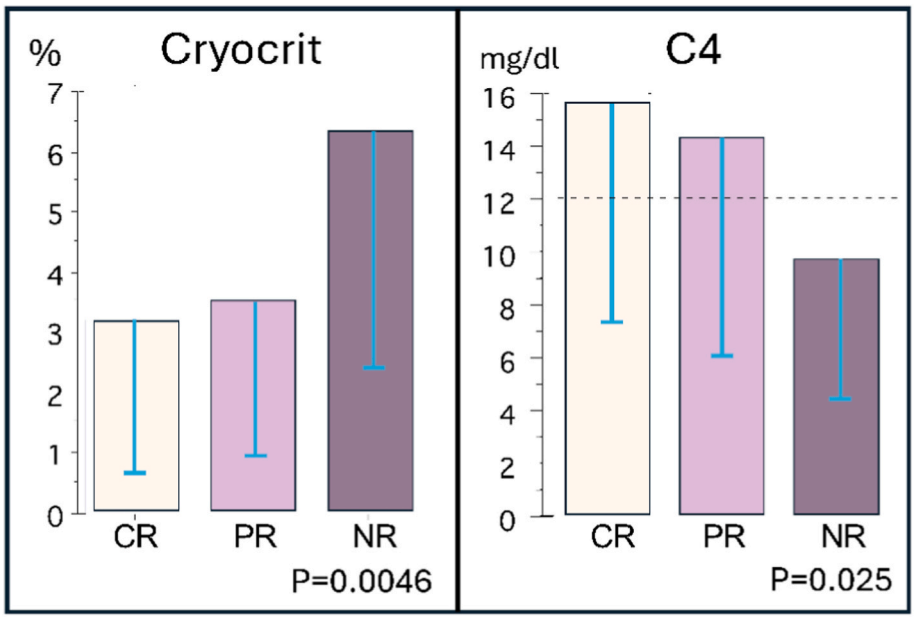

Fig: 3. Cryocrit and complement C4 levels evaluated within the last 12 months before DAAs regimens and response to interferon-free treatments. Patients non-responder (NR) compared to those with complete (CR) or partial response (PR) showed medially higher cryocrit (Anova, p = 0.0046) as well as medially lower serum C4 levels (Anova, p = 0.025). Dotted line: lower limit of C4 normal values.

into three main clinicopathological subsets, reflecting the heterogeneity of individual responses to ATV. Patients achieving a CR may be frequently asymptomatic or may present with residual manifestations attributable to pre-existing, stable organ damage, such as peripheral neuropathy–related nerve injury, sequelae of prior long-term corticosteroid exposure, and/or advanced age. In contrast, patients with PR may continue to experience active manifestations of MCs and therefore require closer clinical monitoring. The NR subgroup may represent a small proportion of patients who, despite achieving SVR, display clinical and serological features and a disease course comparable to those observed in the pre–DAA era.

5. Discussion

This study provides a comprehensive evaluation of the long-term impact of DAA therapy on disease course in a large HCV-CV cohort, assessed a decade after the introduction of interferon-free regimens. It represents the longest follow-up of post-SVR patients reported to date and shows a significant reduction in major CV clinical manifestations (purpura, fatigue, arthralgia, skin ulcers, sicca syndrome, and peripheral neuropathy) after ATV. In parallel, we observed significant improvement in key biological markers, including reduced serum mixed cryoglobulins and normalization of complement C4. Notably, both the marked decline in circulating cryoglobulins—reflecting the underlying lymphoproliferative disorder [3,4,11]—and the normalization of C4 are rarely observed in the natural course of CV including the phases of disease remission [3,4,11,55].

The long-term benefits of DAAs on peripheral neuropathy were comparatively less pronounced; this outcome aligns with previous clinical observations indicating that peripheral neuropathy often exhibits limited responsiveness to various targeted treatments in the routine clinical practice [28,30,32,36,40,43], possibly due to pathogenetic peculiarities of this complication. Cryoglobulinemic peripheral neuropathy often follows an insidious course, with early irreversible nerve damage that is difficult to manage from onset. Symptoms may persist and progressively worsen over time, even in the absence of key etiopathogenic factors such as HCV and/or cryo-immune complexes [27,31,32,36].

Despite the complete response evaluated by composite symptom panel observed in the majority of CV patients, approximately 12% of them were consistently classified as non-responders. As regards the persistence of circulating cryoglobulins in a significant number of cases (25%), it is in line with previous observations collected in shorter post-SVR follow-up studies.

The duration of post-AVT follow-up we reported is the longest described so far. In fact, previous studies on wide cohorts of patients reported long-term effects of DAAs on CV, with a medium duration of 2 years [27], 2 years and 7 months [32], and 35 months [56]. The only study reporting comparable follow-up length (median: 5 years) was recently although the sample was small and only 21 individuals showed MC symptoms [57].

Considering the pattern of clinical response, the first studies after the introduction of DAA-based IFN-free therapies, even if performed in smaller settings with a shorter post-treatment observation time-frame, already showed very good to high (from 62 to 100% of response rate) clinical efficacy [26,28,33,40] then confirmed in further analyses [31,32,36].

The percentage of NR observed during long-term follow-up is consistent with previous reports. Hegazy et al. reported 12.6% NR at the end of follow-up [56], while Kondili et al. described a 12% NR rate 24 months after EOT, despite transient relapses in responder patients [27]. Indeed, symptom reappearance or worsening was transient in 70% of cases and less frequent in patients with complete resolution of MC manifestations [27].

This aligns with our finding of a higher frequency of severe complications in NR compared to CR and PR. Moreover, cryocrit levels were inversely correlated with maintenance of clinical response [27], supporting our observation that persistent cryocrit is associated with severe complications during post-treatment follow-up.

The overall impact of AVT should be interpreted in the context of the natural history of CV in large cohorts from the pre-DAA era. Most patients exhibit a mild-to-moderate symptom profile (purpura, fatigue, arthralgia, skin ulcers, peripheral neuropathy, sicca syndrome), with variable cryoglobulin levels and persistently low C4, typically following a fluctuating long-term course. However, a substantial subset develops severe complications with unpredictable onset and potentially poor outcomes if not promptly treated.

These complications include severe skin ulcers with or without

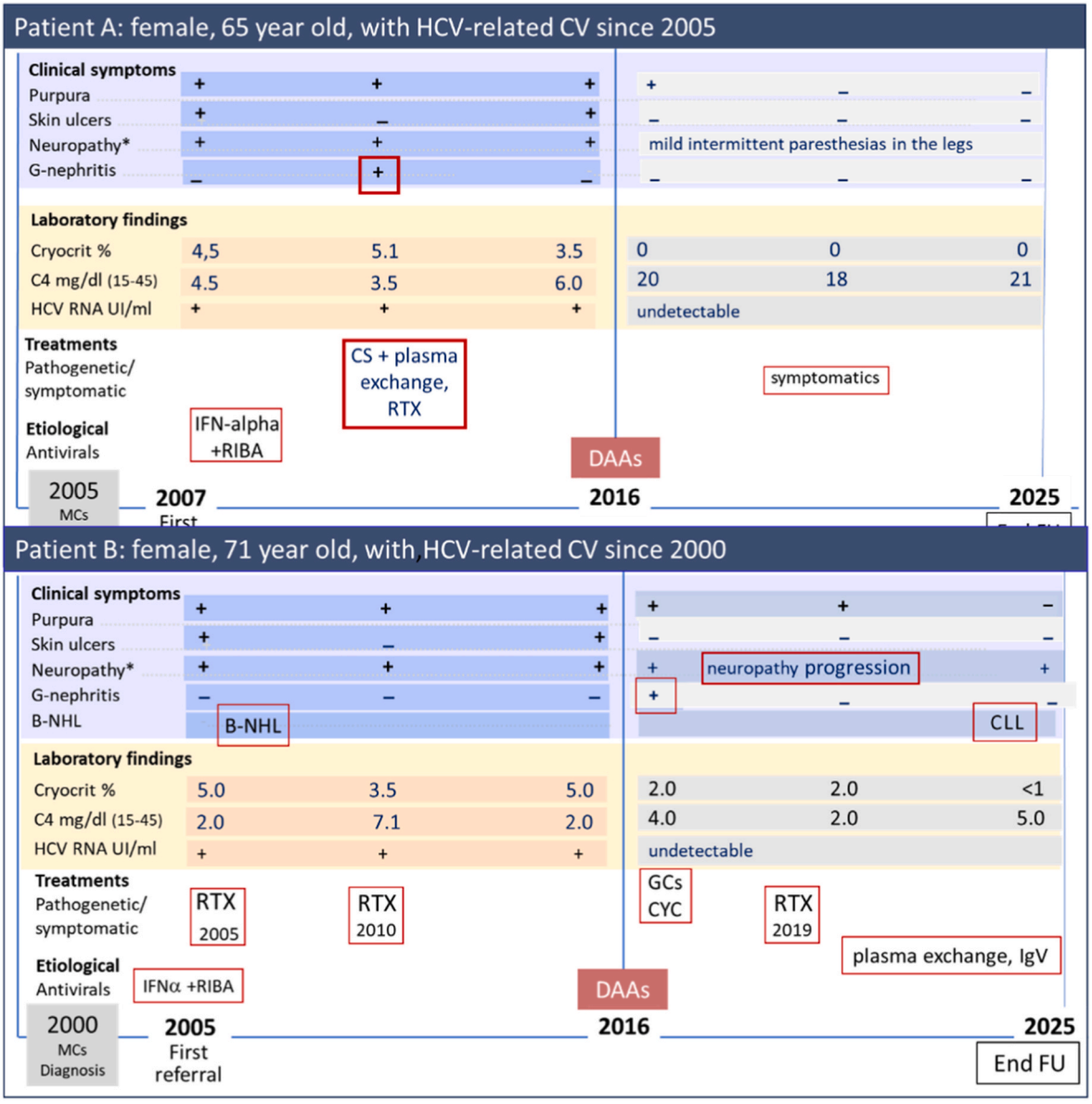

Fig. 4. Long-term clinical follow-up of two patients with divergent clinical course of CV after antiviral treatment by means of DAAs regimens.
The figure describes the long-term clinical courses of two representative patients with divergent outcomes despite comparable baseline clinical and serological profiles.
Patient A: 65-year-old female with HCV-CV since 2005, characterized by purpura, arthralgias, fatigue, skin ulcers, peripheral sensory neuropathy*, mixed cryoglobulinemia, and low C4. Interferon-alpha + ribavirin (IFNa/RIBA) did not affect the HCV viremia. A severe episode of cryoglobulinemic glomerulonephritis (2012) was successfully treated with combined corticosteroids (CS), plasmapheresis, and rituximab.
DAAs regimen (Ombitasvir + Paritaprevir + Ritonavir) led to SVR (2016) with stable remission of clinical/serological features.
During long-term follow-up, only mild reactivation of peripheral sensory neuropathy (treated with pregabalin) was observed.
Patient B: 71-year-old female with chronic hepatitis since the 1980s was diagnosed with HCV-CV (2000) based on purpura, arthralgias, fatigue, peripheral neuropathy, mixed cryoglobulinemia. Two attempts at AVT with IFNa/RIBA did not affect the HCV viremia.
She was usefully treated with rituximab for active cryo-vasculitis and low-grade B-cell non-Hodgkin lymphoma (B-NHL) (2005-2010).
DAAs (Ombitasvir/Paritaprevir + Ritonavir/Dasabuvir) was followed by sudden onset severe cryoglobulinemic glomerulonephritis successfully treated with CS and cyclophosphamide treatment.
Subsequently, disabling peripheral sensory-motor neuropathy progressively worsened; it was treated with multiple plasmapheretic and IgV sessions with partial clinical improvement. More recently (2004), the B-NHL evolved towards chronic lymphocytic leukemia (CLL) by the same original B-lymphocyte clone. This complication remains still asymptomatic and is currently under clinical monitoring.

gangrene, acute sensory-motor peripheral neuropathy, cryoglobulinemic glomerulonephritis, and neoplastic disorders. In our CV series, their incidence was significantly higher in the pre-AVT phase compared with the longer post-AVT period.

Notably, assessment of DAA response using a composite panel of clinical and serological features (purpura, fatigue, arthralgia, skin ulcers, sicca syndrome, peripheral neuropathy, serum cryoglobulins, and C4 levels) identified a lower proportion of CR patients, who nonetheless showed a very low rate of severe complications during long-term post-AVT follow-up. Thus, a broader panel capturing key CV features allows identification of patients with minimal risk of serious complications. This finding underscores the pathogenetic relevance of residual

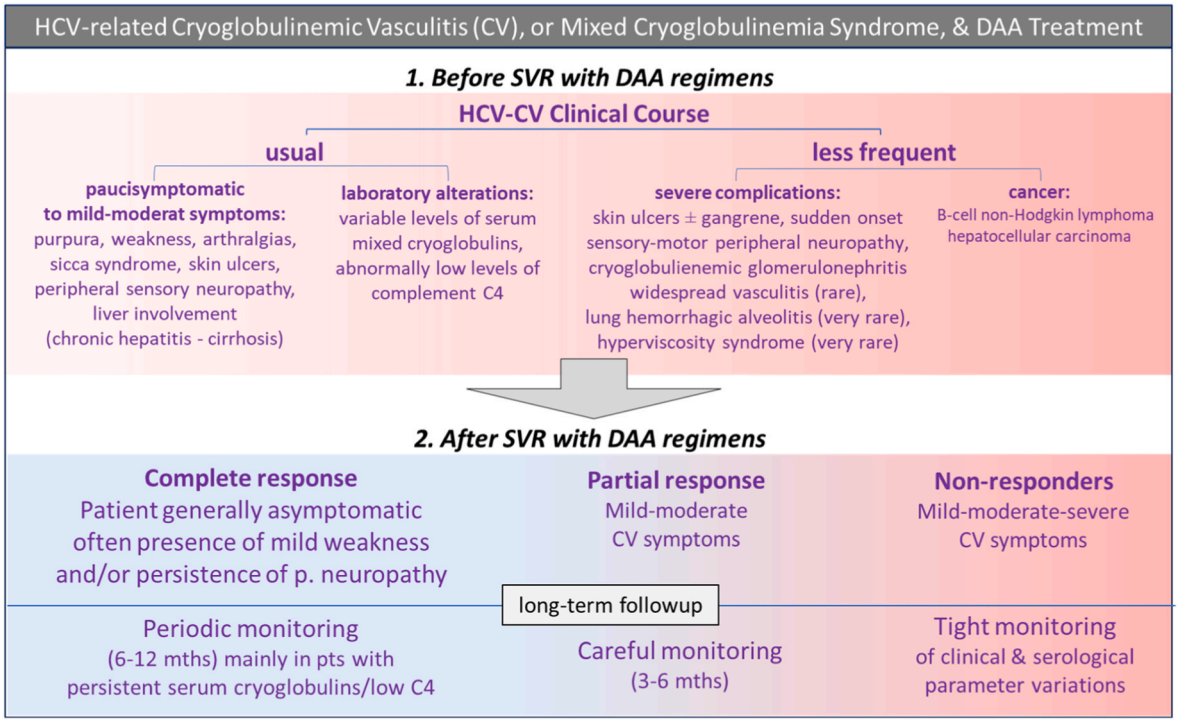

Fig. 5. Proposed conceptual framework of HCV-related cryoglobulinemic vasculitis clinical course pre- and post-SVR with DAAs
1: HCV-related cryoglobulinemic vasculitis (CV), or mixed cryoglobulinemia syndrome (MCs), is characterized by mild-to-moderate clinical symptoms in the majority of individuals, often punctuated by one or more severe manifestations requiring timely and aggressive treatments (from left to right). Clinical course of this multifaceted disorder is completely unpredictable; moreover, CV may favor the development of various comorbidities that may contribute to worse disease outcome.
2: A possible model of CV after DAA-induced SVR might encompass at least three main clinico-pathological subsets:
-*Complete response* may be observed in the majority of patients, frequently asymptomatic or with residual symptoms of previous organ damages, as neuropathic manifestations of previous immune-mediated peripheral neuropathy; moreover, long-term corticosteroid therapy, cardiovascular/metabolic comorbidities, and/or older age may contribute to persistent symptomatology. Periodic monitoring of these patients may be advisable, especially in individuals with persistent cryoglobulinemia.
-*Partial response* regards a clinically heterogeneous subgroup requiring careful monitoring during the follow-up.
-*Non-responders regards* a minority of patients showing the same clinical/serological characteristics and a clinical-prognostic course as in the pre-DAAs period. In these cases, symptomatic/pathogenetic treatments tailored according to the disease course, as well as tight clinical and laboratory monitoring, are mandatory to timely detect the most severe CV complications.
In any case, the subgroup of patients with persistent serum mixed cryoglobulins, with/without low C4, should be more carefully monitored.

cryoglobulinemia in the post-DAA setting.

Moreover, the significantly higher cryocrit and lower C4 levels observed before DAA therapy in NR patients, compared with CR and PR, are consistent with this interpretation.

Notably, the persistence or reappearance of serum cryoglobulins after SVR does not necessarily reflect ongoing virus-driven immune stimulation. A recent prospective study of DAA-treated HCV patients showed detectable cryoglobulins in ~20% of cases up to 96 weeks post-therapy, with dynamic fluctuations and transient reappearance in previously negative individuals [58]. These data suggest that, once established, B-cell dysregulation may become partially independent of viral replication.

In this context, the persistence of cryoglobulins after SVR should be interpreted with caution. On one hand, their reappearance may reflect intercurrent immunological triggers, such as infections, neoplasms, or other inflammatory events, as previously reported [32,39,59]. On the other hand, their stable persistence—especially when associated with elevated RF and low C4 levels—may represent the serological expression of a residual clonal B-cell expansion, potentially progressing toward overt lymphoproliferative disorders [40,60,61].

Recent mechanistic evidence supports this hypothesis. Single-cell and genomic analyses of HCV-related cryoglobulinemic vasculitis show that pathogenic B-cell clones may persist despite viral clearance and harbor somatic mutations, including lymphoma-associated drivers [62]. These findings provide a biological basis for why, in some patients, cryoglobulins and vasculitic manifestations persist after SVR, suggesting that a "point of no return" in virus-driven B-cell clonal evolution may be reached [27,31,32,36]. This condition is in keeping with the persistence of cryo-immune-complexes after DAA regimens, generally achieved at a late stage of the disease, no longer susceptible to regress despite SVR. In accordance with this hypothesis, the two patients described here (Fig. 3) are quite representative of divergent long-term disease outcomes after DAAs.

A key aspect in evaluating CV after etiological treatment is the identification of pre-AVT response predictors. Outcome definitions are influenced by several factors, including disease severity, timing of assessment, and the parameters evaluated, which partly explains the heterogeneity reported in the literature. Overall, disease severity is the strongest predictor of poorer response, particularly in the presence of irreversible organ damage (such as neuropathy [28,36,38,40] or sicca syndrome [28]) or particularly severe symptoms [28,33,36–38,40]. Laboratory alterations, such as elevated levels of RF [27,31] have also been reported as potential predictors of response. Other studies have investigated markers or surrogates of monoclonality [26,28,31,38], e.g., monoclonal B-lymphocytosis (MBL), (14; 18) translocation, κ/λ free light chain ratio [31], genetic determinants [31], or correlated response with a composite severity index [32]. Notably, MBL and an altered κ/λ ratio were identified as predictors of poor outcome in the early post-DAA follow-up [31].

In summary, the introduction of DAAs has revolutionized the

management of HCV-related MC, similarly to B-cell depletion therapy. Together, these approaches have profoundly impacted the clinical and pathogenetic course of the disease. The high efficacy of DAAs in achieving viral clearance minimizes the risk of MC relapse related to persistent infection, a limitation of interferon-based regimens despite their immunomodulatory effects. However, a subset of patients continues to produce cryoglobulins, with or without complement consumption and/or elevated RF. These patients require prolonged follow-up after viral clearance, as also recommended by the European Association for the Study of the Liver [18].

Particular attention should be paid to recurrent and new-onset MC, by investigating etiopathogenetic factors other than HCV that may underlie cryoglobulinemic vasculitis. In the post-DAA era, prospective studies are needed to assess the incidence of CV , additional etiological factors, and novel biomarkers to identify patients at risk of relapse or progression despite viral eradication.

The present study does not provide definitive evidence of actual changes in the pathomorphosis of MC. Addressing this issue would require comparative analyses across different antiviral regimens, which remain challenging due to the rarity of the disease.

At present, we can only tentatively hypothesize that DAA therapy may give rise to a novel disease framework, based on the clinical and serological evolution of MC over long-term follow-up. This hypothesis is supported by laboratory findings suggesting that, in a small subset of patients, virus-driven B-cell expansion may have reached an irreversible state no longer responsive to SVR. This may explain a limited but significant group of true non-responders, representing a relevant clinical condition in the post-DAA era.

Therefore, we may propose as a working hypothesis that the post-DAA setting includes at least three clinical MC subsets: (i) patients achieving CR, generally asymptomatic or presenting only with sequelae of prior organ damage and/or long-term corticosteroid therapy, particularly in older individuals; periodic monitoring in this subgroup is advisable; (ii) patients with partial response to DAAs, requiring closer monitoring due to an increased risk of developing more severe complications needing symptomatic and/or pathogenetic interventions; and (iii) non-responder patients, a small but non-negligible proportion of individuals whose clinical course resembles the pre-DAA period. In all cases, patients with persistent serum mixed cryoglobulins require careful monitoring, as this likely reflects ongoing B-cell lymphoproliferation, often difficult to assess in routine practice and independent of clinical response. Identification of patient subsets with lower (complete responders) or higher (non-responders) risk of complications may be clinically relevant; however, careful long-term follow-up, tailored to individual clinical conditions, remains strongly advisable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Clodoveo Ferri: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Marcella Visentini:** Writing – original draft, Data curation, Conceptualization. **Caterina Vacchi:** Writing – review & editing, Data curation. **Gianfranco Lauletta:** Writing – review & editing, Data curation. **Caterina Naclerio:** Writing – review & editing, Data curation. **Irene De Giorgi:** Investigation, Formal analysis. **Teresita Caruso:** Formal analysis, Data curation. **Annalisa Villa:** Investigation, Conceptualization. **Matteo D'Ambrosi:** Investigation, Data curation. **Francesca D'Arcangelo:** Investigation, Conceptualization. **Silvia Marri:** Data curation. **Poupak Fallhai:** Writing – review & editing. **Alessandro Antonelli:** Writing – review & editing. **Anna Linda Zignego:** Writing – review & editing, Methodology. **Dilia Giuggioli:** Methodology, Conceptualization. **Laura Gragnani:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Milvia Casato:** Writing – original draft, Investigation, Conceptualization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2026.103559.

Data availability

Data will be made available on request.

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