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ORIGINAL ARTICLE

# A prospective study of direct-acting antiviral effectiveness and relapse risk in HCV cryoglobulinemic vasculitis by the Italian PITER cohort

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**Abbreviations:** CG, cryoglobulin; CR, complete clinical response; CV, cryoglobulinemic vasculitis; DAA, direct-acting antiviral; EOT, end of treatment; FCR, full CR; FU, follow-up; MC, mixed cryoglobulinemia; NR, no clinical response; PITER, Italian Platform for the Study of Viral Hepatitis Therapy; PR, partial clinical response; RF, rheumatoid factor; SVR, sustained virologic response.

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## Abstract

**Background and Aims:** Mixed cryoglobulinemia is the most common HCV extrahepatic manifestation. We aimed to prospectively evaluate the cryoglobulinemic vasculitis (CV) clinical profile after a sustained virologic response (SVR) over a medium-term to long-term period.

**Approach and Results:** Direct-acting antiviral–treated cryoglobulinemic patients, consecutively enrolled in the multicentric Italian Platform for the Study of Viral Hepatitis Therapy cohort, were prospectively evaluated. Cumulative incidence Kaplan-Meier curves were reported for response, clinical deterioration, relapse and relapse-free survival rates. Cox regression analysis evaluated factors associated with different outcomes. A clinical response was reported in at least one follow-up point for 373 of 423 (88%) patients with CV who achieved SVR. Clinical response increased over time with a 76% improvement rate at month 12 after the end of treatment. A full complete response (FCR) was reached by 164 (38.8%) patients in at least one follow-up point. CV clinical response fluctuated, with some deterioration of the initial response in 49.6% of patients (median time of deterioration, 19 months). In patients who achieved FCR and had an available follow-up (137 patients) a relapse was observed in 13% and it was transient in 66.7% of patients. The rate of patients without any deterioration was 58% and 41% at 12 and 24 months, respectively. After achieving SVR, a clinical nonresponse was associated with older age and renal involvement; a clinical deterioration/relapse was associated with high pretreatment rheumatoid factor values, and FCR was inversely associated with age, neuropathy, and high cryocrit levels.

**Conclusion:** In patients with CV, HCV eradication may not correspond to a persistent clinical improvement, and clinical response may fluctuate. This implies an attentive approach to post-SVR evaluation through prognostic factors and tailored treatment.

## INTRODUCTION

HCV infection is the major cause of liver-related morbidity and is increasingly recognized as a trigger of B-cell lymphoproliferative disorders such as mixed cryoglobulinemia (MC) and non-Hodgkin's lymphoma.[1–5]

MC is characterized by intravascular immune complexes named cryoglobulins (CGs).[6] Mixed CGs are immune complexes that reversibly precipitate when the temperature goes below 37°C and consist of polyclonal IgGs and IgMs with rheumatoid factor (RF) activity.[7] IgMs are monoclonal or oligoclonal in type II MC or polyclonal in type III MC.[7–9]

MC may not show any clinical symptoms; however, it can cause a wide spectrum of clinical presentations including skin lesions, arthralgia, peripheral neuropathy, and single or multiple organ damage, which are the clinical manifestations of systemic vasculitis or

so-called cryoglobulinemic vasculitis (CV), involving small to medium-sized vessels.[1,3,10–12] CV is both an autoimmune and a lymphoproliferative disease. Even though it is clinically benign, it can evolve into lymphoma in a severe presentation.[4,6,13]

It has been recently reported that HCV is no longer the main cause of CV in France,[14] whereas HCV is still reported to be the etiologic agent in most patients with MC in different clinical centers in Italy. Most cryoglobulinemic patients are HCV-positive (70%–90%), and conversely 40%–60% of HCV-infected patients produce CGs, of whom 5%–30% have CV.[3] Due to these anomalies in the clinical manifestations and in the diagnostic criteria, MC and CV remain elusive conditions.

Although the therapeutic approach to patients with CV includes several options, such as steroids, plasmapheresis, and rituximab, etiological therapy is the first-line choice in patients with HCV-CV. In the past, this therapy was based on the use of interferon (IFN), despite frequent side effects and high rates of intolerance.[15,16] The treatment of HCV infection has undergone a substantial advancement with the introduction of new direct-acting antivirals (DAAs).

Patients with HCV-CV have high rates of clinical remission after treatment with DAAs,[17–20] although in some patients symptoms may persist or reappear after a transient clinical response.[21–27]

The aim of this study was to evaluate the clinical presentation of patients with MC in hepatology centers in Italy and prospectively evaluate medium-term to long-term clinical profiles in patients with CV in whom HCV was successfully eradicated. The final goal was to enhance our knowledge pertaining to the natural history of cryoglobulinemia after a sustained virological response (SVR) in order to better define patients in whom a continuous monitoring and treatment workup is required.

# METHODS

## Patients

The study population consisted of patients with chronic HCV infection consecutively enrolled in the Italian Platform for the Study of Viral Hepatitis Therapy (PITER) from about 60 hepatology centers distributed throughout the Italian territory,[28] which could be considered a representative sample of patients with chronic HCV infection in care in Italy. In this prospective analysis, all patients with cryoglobulinemia were consecutively enrolled, either with CV according to standard criteria[2,29] or without (only with laboratory findings, MC). We subsequently evaluated patients with CV following the DAA IFN-free regimen during the period from 2015 to 2019.

For each patient, the main data regarding MC/CV were prospectively collected as reported by the prescribing clinicians following the different steps of DAA treatment in a dedicated section of the electronic case report form. Specifically, dedicated information regarding the main symptoms (i.e., purpura, asthenia, arthralgia, neuropathy, renal involvement, xerostomia/xerophthalmia, Raynaud phenomenon, and ulcers) was recorded. Laboratory findings (i.e., cryocrit levels, RF, and C4 complement values) were collected, when available, following DAA treatment (pretreatment, end of treatment [EOT], and at different points of the follow-up [FU], depending on the time of the real practice scheduled checkups at each clinical center). MC type (II or III) was also recorded, when available.

Liver damage was assessed by FibroScan (transient elastography).[30] The severity of liver disease was classified as “F0–F3” if the stiffness score was ≤12.5 kPa and as “F4 Cirrhosis” if it was >12.5 kPa or if there were signs of liver cirrhosis (signs of portal hypertension).[31] Patients coinfected with HIV or HBV were excluded.

## Outcomes

Following HCV eradication, an evaluation of clinical outcomes related to CV was prospectively conducted in patients with at least a 12-month FU after SVR.

The clinical outcome was defined as described.[17,18] Complete clinical response (CR) was assessed when all baseline clinical manifestations had improved, with the distinction of a full CR (FCR) when all the symptoms disappeared (*restitution ad integrum*), a partial clinical response (PR) with an improvement in at least half of the baseline symptoms, and no clinical response (NR) in the remaining cases. Therefore, the symptoms were recorded considering the modifications (improvement or worsening) compared to the previous checkup at every FU point. Starting from the EOT, in patients who achieved SVR, at least one FU was available up to 12 months; and then in a different subgroup analysis, an FU time after the first observation of a clinical response allowed for evaluation of the outcome behavior over time.

CV symptoms were assessed as has been reported.[17,18] Briefly, purpura was classified in four semiquantitative grades: 0, absence; +, limited and fluctuating on the lower limbs; ++, diffuse and persistent on the lower limbs; +++, involvement of the trunk and the lower limbs. Leg ulcer response was complete with complete healing, a reduction of at least 25% in diameter was considered an improvement, and a lower reduction or a worsening corresponded to a nonresponse. Arthralgia, weakness, and sicca syndrome were measured through a patient-scored visual analogue scale (VAS) (range, 0–100). Neuropathic symptoms, including both paraesthesias/pain and motor deficit, were also assessed by VAS and, when possible, by electromyography. Kidney function was evaluated according to serum creatinine and proteinuria.

The questionnaire was completed by 87% of patients with CV; among those remaining (13% of patients), the only qualitative evaluation (improvement or deterioration or no changes) was reported for each symptom at baseline and at each point of FU. The same specialist from each clinical center evaluated each patient during different FU appointments. The EOT evaluation was conducted having as reference, the pretreatment clinical data. Subsequently, during the follow up, the evaluation was made in view of the clinical data collected in the preceding visit. Differences in the scores were evaluated for each patient and reported as improved when an increasing positive value was detected and as deteriorated when a negative value was detected.

The term "clinical relapse" refers to the reappearance of the syndrome in patients who had previously reached FCR, whereas the terms "clinical deterioration" and "clinical improvement" denote a worsening or improvement in one or more symptoms, respectively.

## Statistical Analysis

Quantitative variables were reported as mean ± SD or median and interquartile range (Q1–Q3), while categorical variables were summarized by number and percentage. The chi-squared test or Fisher's exact test and a *t* test or Mann-Whitney test were used to compare, respectively, categorical and quantitative variables between symptomatic and nonsymptomatic patients.

Survival analysis was used to examine clinical response and clinical relapse after the first clinical response, during the FU. Patients who did not experience an event at the end of the period of observation were considered censored. The cumulative incidence curve was represented for clinical response and cumulative incidence, and relative 95% CIs were reported at different time points. The Kaplan-Meier curve was reported for clinical relapse and relapse-free survival rates, and relative 95% CIs were described at different time points.

Univariate Cox regression was used to evaluate factors associated with FCR without deterioration or relapse after the first clinical response. Only variables with $p < 0.15$ in univariate models were considered in the multivariable analysis. Stepwise Cox regression ($p$ entry and exit = 0.15) was implemented in the analysis of FCR without deterioration/relapse, considering the reduction of sample size and the number of events caused by missing values. HRs and relative 95% CIs were reported.

Mixed models were implemented to evaluate laboratory data over time, considering repeated measures for the same patient. Estimated mean and relative 95% CIs were reported. The $p$ value in comparison with the baseline value was adjusted by Dunnett's correction.

Logistic regression was implemented to evaluate the risk factor associated with NR at the EOT. Only variables with $p < 0.15$ in univariable models were considered in the multivariable analysis. ORs and relative 95% CIs were reported.

SAS 9.4 was used for all analyses, and $p < 0.05$ was considered statistically significant.

# RESULTS

## CV characteristics at enrollment

Out of 11,871 consecutively enrolled patients, the cryoglobulinemic status was only available in 28.5% of patients ($n$ = 3390). Among these, 1255 (37%) patients had circulating CGs at enrollment: these included 523 (41.7%) patients with symptoms, that is, patients with CV (as reported below); and the remaining 732 (58.3%) were symptomless (MC). CG type was available in 937 patients and consisted of type II in 67% and type III in 33%.

The symptoms of patients with CV at enrollment (regardless of previous symptomatic status) were mostly represented, as expected, by components of the classical triad—purpura, asthenia, and arthralgia—followed by neuropathy and sicca syndrome (xerostomia/xerophthalmia); each symptom was present in a percentage of patients ranging from 69% to 95%. Sixty-four (12.2%) patients had renal involvement (from proteinuria and hematuria at urinalysis to a frank reduction in glomerular filtration rate).

## DAA treatment virological response

Among the 1255 cryoglobulinemic patients, 1204 (96%) achieved SVR to a first-line DAA therapy. Out of the 1255 evaluated patients, 51 (4%) did not appear to be responders (relapse or breakthrough during the first-line DAA therapy), 19 of whom (1.5 %) underwent a second-line DAA therapy, while the remaining 32 (2.5%) patients were lost upon FU. Patients who failed to eradicate HCV by the first-line DAA therapy underwent a second-line DAA therapy, and all but 2 out of 19 (10.5%) achieved SVR. Overall, considering first- and second-line therapy, 1221 reached SVR.

There were no differences in the SVR rate achieved from the first-line DAA therapy between patients with CV and those with MC (data not shown).

## Demographic and clinical characteristics of patients with MC

Out of 1221 patients who achieved SVR (after the first-line or second-line DAA treatment), the main pretreatment demographic and clinical data of CV and MC patients are shown in Table 1.

**TABLE 1** Demographic and clinical characteristics of patients with HCV-related MC who achieved SVR

| | Patients with CV ($n$ = 423)[a] | Patients with asymptomatic MC ($n$ = 655) | $p$ |
|---|---|---|---|
| Age (years) (mean ± SD) | 62.7 ± 12.1 | 62.2 ± 12.6 | 0.444 |
| Sex (%, no./patients) | | | |
| Male | 35.9% (152/423) | 47.3% (310/655) | <0.001 |
| Female | 64.1% (271/423) | 52.7% (345/655) | |
| Fibrosis distribution (%, no./patients) | | | 0.013 |
| F0–F1 | 42.8% (167/390) | 33.9% (204/602) | |
| F2 | 10.5% (41/390) | 9.1% (55/602) | |
| F3 | 9.2% (36/390) | 9.8% (59/602) | |
| F4-cirrhosis | 37.4% (146/390) | 47.2% (284/602) | |

[a]Among patients with CV who achieved SVR, clinical data regarding the cryoglobulinemic syndrome were missing for 100 patients.

No difference in age was observed between the two groups. Female gender was more prevalent among patients with CV. The distribution of fibrosis stage was different between patients with CV and those with MC ($p$ = 0.013), and the prevalence of cirrhosis was higher in patients with MC.

## CV clinical response

A flowchart of the evaluated patients in each subanalysis is shown in Figure 1. Data concerning clinical response following SVR were available for 423 patients with CV. The median (Q1–Q3) follow-up time was 15 (13–27) months. At the EOT, among 423 patients who achieved SVR, 57% reached a clinical response (28% PR, 17% CR, and 12% FCR), and 43% were classified as NR.

It was observed that a CV clinical response (FCR + CR + PR) was reported during at least one point of the FU in 373 out of 423 (88%) patients with CV; and, in particular, FCR (complete disappearance of all the manifestations) was reached, during at least one point of the FU, by 164 (38.8%) patients.

Figure 2A shows the curve of the first time in which a clinical response (either CR, FCR, or PR) was observed. After the SVR assessment, the clinical response rate at month 12 of the FU was 76%, and the median time of the first clinical response was 3 months. Pertaining to the different degrees of clinical response, improvements increased over time (second and third years of the FU).

Considering the 288 SVR patients with data corresponding to more than one FU point after the first observation of a clinical response (and consequently allowing for evaluation of the outcome behavior over

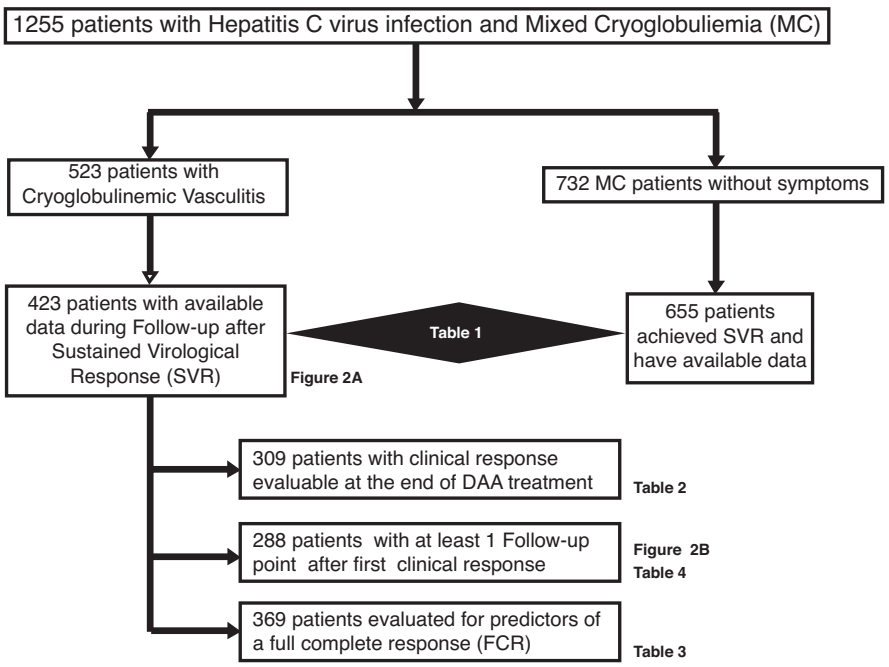


**FIGURE 1** Flowchart of evaluated patients in each subanalysis of clinical response following SVR in patients with HCV-related CV

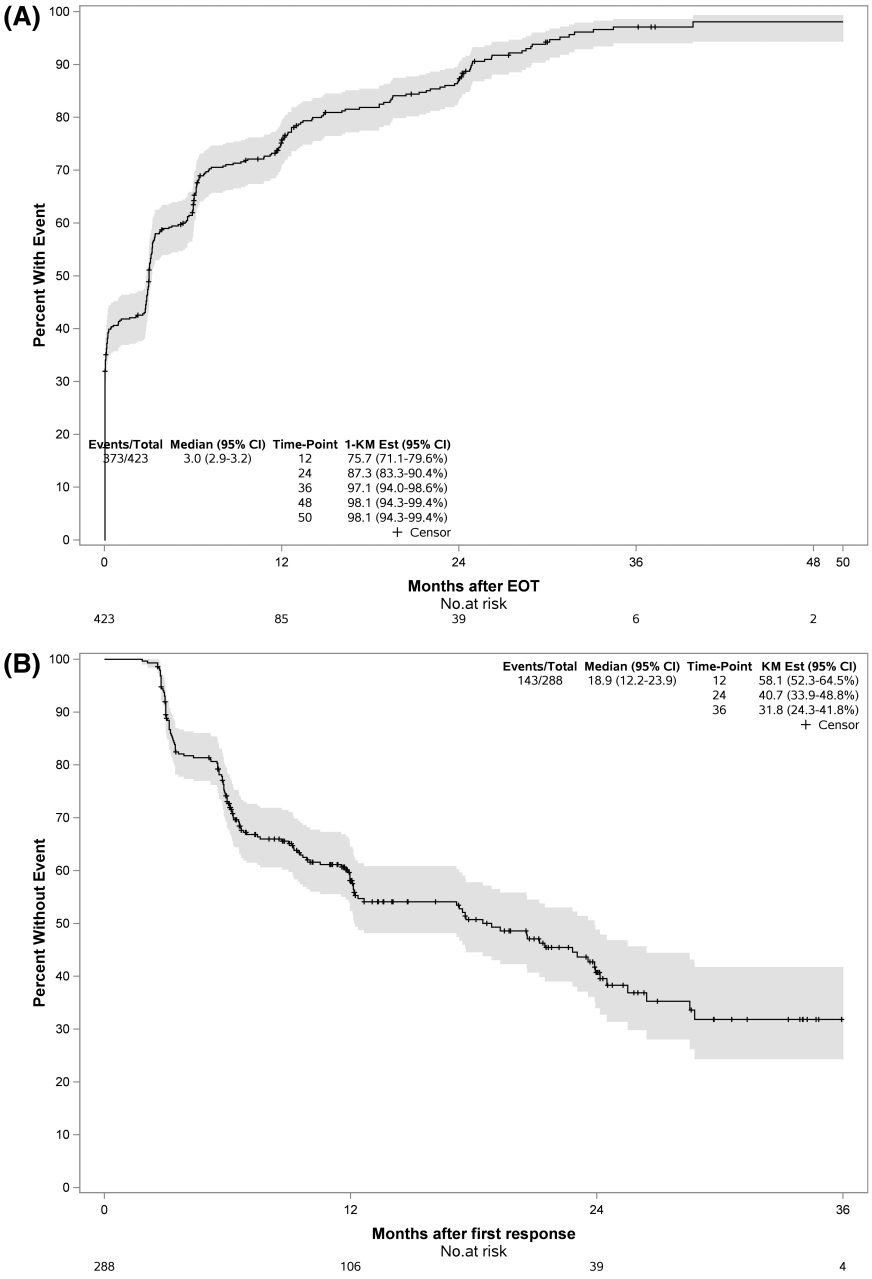


**FIGURE 2** (A) Curve describing the first time in which a clinical response was observed (either CR, FCR, or PR) after end of DAA treatment in patients with CV. (B) Curve of CV clinical deterioration or relapse occurring during the FU after the first clinical response following HCV eradication

time), a clinical deterioration of the initial response or relapse was recorded in 143 patients (49.6%). In patients with fluctuations in the clinical pattern, the median time of deterioration in the clinical status was 19 months. The rate of patients without deterioration was 58% at 12 months and 41% at 24 months (Figure 2B).

Symptoms that persisted more frequently 2 years after viral eradication were arthralgia (45%), fatigue (41%), neuropathy (38%), and sicca syndrome (37%).

Out of 164 patients who achieved FCR (at one point of FU), 137 had an available FU after achievement of the FCR. A relapse was observed in 13% of patients (18 patients) at least at one point of the FU after FCR. Interestingly, 9 out of these 18 patients were further evaluated after the clinical relapse, demonstrating that the latter was transient in most cases (6/9, 66.7%), with only 3 out of 9 (33.3%) patients who maintained the NR degree of clinical response during the whole FU.

## CV immunological response

The modifications of the main CV laboratory data before and after antiviral therapy are reported in Figure 3. A

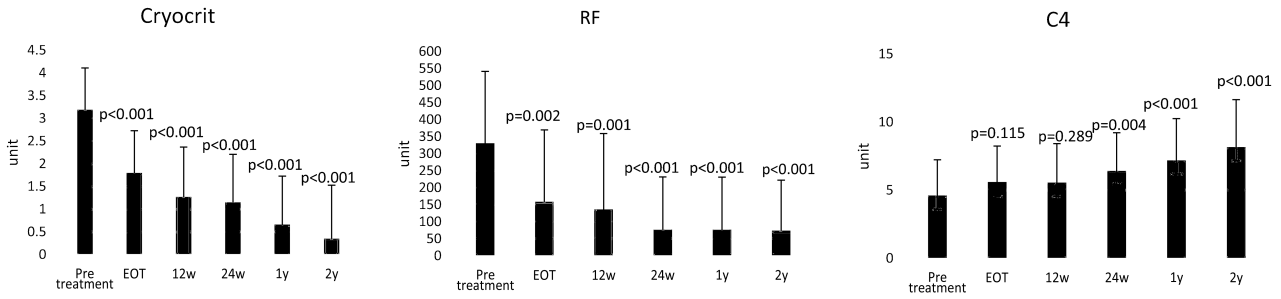


**FIGURE 3** Comparison between cryocrit, RF, and C4 complement values before and after DAA treatment at different time points. Estimated means by mixed model (cryocrit, $n$ = 144; RF, $n$ = 42; C4, $n$ = 22) with at least a 1-year FU; cryocrit, RF, or C4 complement data at EOT; and at least one other value during the FU ($p$ value compared with baseline adjusted by Dunnett's correction)

significant improvement was observed at 24 weeks after the EOT, increasing even further during the long-term FU.

## Pretreatment factors potentially associated with a clinical response at the EOT

Out of 309 patients for whom it was possible to assess clinical response at the end of DAA treatment, factors independently associated with a nonclinical response at the EOT from the multivariate analysis included age (OR, 1.02; 95% CI, 1–1.04; $p$ = 0.039) and renal involvement (OR, 1.79; 95% CI, 0.96–3.36; $p$ = 0.05) (Table 2).

## Pretreatment factors potentially associated with FCR without clinical deterioration or relapse

To evaluate FCR without relapse, out of the 423 patients, 54 reached FCR but did not undergo a clinical evaluation after this endpoint. Among the 369 patients evaluated, 62 (16.8%) reached a persistent FCR without clinical deterioration during the FU. Female sex, advanced age, purpura at the time of admission to the study (pretreatment), arthralgia, neuropathy, and higher cryocrit values lowered the probability of maintaining an FCR without clinical deterioration or relapse (Table 3). After stepwise regression, age, neuropathy, and high cryocrit levels remained in the model as independently inversely associated with the outcome (maintaining the FCR without a clinical deterioration) (Table 3).

## Pretreatment factors associated with a clinical deterioration or relapse after clinical response

Out of 288 patients who achieved a clinical response (FCR + CR + PR) and had at least one FU point after the clinical response, 143 (49.6%) showed a successive clinical deterioration or relapse. By univariate Cox regression analysis, a significant association between clinical deterioration or relapse and RF values and the presence of sicca syndrome before treatment was observed (Table 4). The multivariable model showed that pretreatment high RF values represented an independent prognostic index of clinical deterioration or relapse during the FU. The distribution of pretreatment RF values in patients with CV with or without clinical relapse after DAA-based therapy is reported in Figure 4.

# DISCUSSION

This is a nationwide, multicentric, prospective study, evaluating HCV cryoglobulinemic patients both before and after DAA-based treatment, distinguishing patients with and without vasculitis. This may be of interest especially to hepatologists involved in the treatment and monitoring of all cryoglobulinemic patients with HCV, ranging from 40% to 60% of HCV-positive patients.[3] In fact, a dedicated approach focusing on the diagnosis and impact of MC treatment has been included in PITER, which is a prospective cohort of consecutive patients admitted to the most important Italian hepatology centers as inpatients or outpatients prior to antiviral treatment.[27]

The underestimation of MC status confirms previous ad interim data collected during the PITER enrollment phase, showing a high real-life variability in the diagnostic approach to MC.[32] Most of the enrolling hepatology centers used to perform MC tests only when CV was clinically evident. Furthermore, cryo testing, requiring special blood sample management, is frequently inadequate.

Regarding the characteristics of cryoglobulinemic patients, the higher prevalence of the female sex and the high median age confirmed previously reported data.[10,29,33,34]

Although the association between HCV and cryoglobulinemia is widely recognized, its relationship with liver disease is still unclear; and there are some contradictory results between the data reported in this analysis and previously published data.[35] In this

**TABLE 2** Factors associated with clinical response in patients with HCV-related CV at the end of antiviral treatment: Univariate and multivariate analyses

| | | Univariate analysis[a] (*n* = 309) | | Multivariate analysis (*n* = 309) | |
|---|---|---|---|---|---|
| | | OR (95% CI) | *p* | OR (95% CI) | *p* |
| Age (years) | | 1.02 (1.00–1.04) | 0.052 | 1.02 (1.00–1.04) | 0.039 |
| Sex | Male | 1 | | | |
| | Female | 0.90 (0.56–1.43) | 0.646 | | |
| Purpura | No | 1 | | | |
| | Yes | 1.01 (0.59–1.75) | 0.964 | | |
| Asthenia | No | 1 | | | |
| | Yes | 1.33 (0.77–2.30) | 0.308 | | |
| Arthralgia | No | 1 | | | |
| | Yes | 1.05 (0.64–1.70) | 0.853 | | |
| Neuropathy | No | 1 | | | |
| | Yes | 1.15 (0.73–1.81) | 0.557 | | |
| Renal involvement | No | 1 | | | |
| | Yes | 1.70 (0.92–3.16) | 0.093 | 1.79 (0.96–3.36) | 0.058 |
| Xerostomia/xerophthalmia | No | 1 | | | |
| | Yes | 1.27 (0.80–2.01) | 0.308 | | |
| Raynaud | No | 1 | | | |
| | Yes | 0.93 (0.53–1.65) | 0.811 | | |
| Ulcer | No | 1 | | | |
| | Yes | 0.88 (0.24–3.18) | 0.843 | | |
| Pretreatment cryocrit | | 1.01 (0.96–1.06) | 0.68 | | |
| Pretreatment RF | | 1.00 (1.00–1.00) | 0.559 | | |
| Pretreatment C4 | | 1.03 (0.88–1.21) | 0.694 | | |
| Rituximab | Yes | | | | |
| | No | 1.65 (0.69–3.93) | 0.262 | | |

[a]Out of 423 patients with CV, 309 were evaluated for the clinical response at the end of the FU; the remaining were evaluated in different FU time points.



study, including both patients with CV and patients with MC, advanced fibrosis was associated with MC. Some differences in the clinical characteristics of patients referred to different specialists (e.g., rheumatologists, hematologists, and nephrologists) could play a role in determining the differences in liver fibrosis stage distribution.

Pertinent to virological response, the SVR rate observed in patients with MC was similar to those observed by other authors[18,21,36] during the same time frame in noncryoglobulinemic patients with HCV from the PITER cohort.[37]

Concerning the clinical outcome, the overly enthusiastic attitude of the first studies on outcomes following DAA therapy has been hampered by recent studies with longer post-SVR monitoring revealing that the clinical management of patients with SVR-CV is more complex than expected.[17–20,23,25,38–40] In fact, the persistence of symptoms and/or a later recurrence after a transient clinical response have recently been described by other authors.[19,21–23,25,27,40]

Therefore, in our study, special attention was paid to the characterization of CV clinical response over time following SVR, with the distinction of 3 degrees of a clinical response, and the evaluation of the kinetics of clinical improvement/deterioration during different FU points.

A clinical response to some degree (FCR, CR, and PR) was observed in 57% of patients at the EOT, which increased to 88% at one point of the FU, whereas 12% remained NR as no improvement in at least half of the symptoms was observed at the end of the second to third years of the FU.

The design of the present study was also meant to indicate the time when the first clinical response could be expected. This is clinically relevant because patients with CV often did not show a clinical improvement at EOT but did later, with the first amelioration starting about 3 months after EOT (median time, 9 months). In addition, about 50% of patients experienced a further improvement in the first and second years after viral eradication. However, a deterioration in the initial

**TABLE 3** Pretreatment factors associated with FCR without clinical deterioration or relapse in patients with HCV-related CV: Cox regression analysis

| | | Univariate analysis ($n$ = 369) | | Multivariate analysis ($n$ = 278) | |
|---|---|---|---|---|---|
| **Variable** | | **HR (95% CI)** | ***p*** | **HR (95% CI)** | ***p*** |
| Age (years) | | 0.96 (0.94–0.98) | <0.0001 | 0.96 (0.94–0.99) | 0.002 |
| Sex | Male | 1 | | | |
| | Female | 0.42 (0.25–0.69) | 0.001 | | |
| Purpura | No | 1 | | | |
| | Yes | 0.32 (0.14–0.74) | 0.008 | | |
| Asthenia | No | 1 | | | |
| | Yes | 0.41 (0.25–0.68) | 0.001 | 0.53 (0.26–1.10) | 0.088 |
| Arthralgia | No | 1 | | | |
| | Yes | 0.44 (0.27–0.72) | 0.001 | | |
| Neuropathy | No | 1 | | | |
| | Yes | 0.4 (0.23–0.69) | 0.001 | 0.4 (0.18–0.87) | 0.022 |
| Renal involvement | No | 1 | | | |
| | Yes | 0.75 (0.37–1.53) | 0.434 | | |
| Xerostomia/xerophthalmia | No | 1 | | | |
| | Yes | 0.6 (0.36–1.00) | 0.051 | | |
| Raynaud | No | 1 | | | |
| | Yes | 0.54 (0.25–1.19) | 0.128 | | |
| Ulcers | | 1.04 (0.25–4.24) | 0.960 | | |
| Pretreatment cryocrit | | 0.81 (0.67–0.99) | 0.041 | 0.81 (0.66–0.98) | 0.03 |
| Pretreatment RF | | 1 (0.99–1) | 0.202 | | |
| Pretreatment C4 | | 1.2 (0.97–1.48) | 0.090 | | |

response was also observed in 49.6% of patients in a median time of around 19 months.

Interestingly, the FCR rate was persistent without deterioration only in 16.8% of patients who achieved FCR during the FU. This implies that, after viral eradication, the persistence of some or most pretreatment symptoms should be considered as not infrequent.

Apart from modulations of the clinical responses, as worsening or improvement during the FU, we also observed a consistent percentage (about 13%) of clinical relapses in patients who had achieved FCR. This percentage was not far from previous observations, even if performed in different settings.[22,24,26,41] This study made it possible to highlight that relapse was transitory in about 70% of cases. This stresses the usefulness of accurate monitoring over time for these patients, possibly with the aid of prognostic predictors in order to avoid inappropriate therapeutic strategies.

Concerning laboratory data, a close association between SVR and cryocrit lowering, RF decreasing, as well as an increase in C4 values was observed after treatment, thus confirming previous studies.[17,18,20,38]

Regarding potential clinical outcome predictors, at the EOT, a multivariate logistic analysis showed that age and the presence of nephropathy were independent prognostic factors of NR. This observation, already suggested by previous studies,[18,21,25,39] may be correlated with more advanced vasculitis and with nonreversible organ damage.

Focusing on the factors potentially associated with FCR after treatment, age and neuropathy emerged as independent negative prognostic factors, while among laboratory data, a higher baseline cryocrit level was an independent predictor of NR, stressing the need to treat asymptomatic patients before the development of organ damage (e.g., neurological and/or renal).

In relation to the risk of long-term clinical deterioration or relapse in patients who achieved a clinical response, high baseline RF values emerged as an independent prognostic index for clinical relapse during the FU, confirming previous results in less numerous cohorts.[27] This finding has a pathophysiological meaning as RF molecules are produced by the same B cells whose clonal expansion is considered the basis of cryoglobulinemia.[27,42] It is also an indirect confirmation of the possible maintenance of the pathogenic clones in patients with CV after SVR.[27,43] Data relating to RF are clinically relevant, taking into account that it can easily be assessed through a noninvasive peripheral blood draw, whereas performing a reliable cryocrit is difficult in clinical practice.[44]

Apart from the risk of clinical relapse, a long-term FU of patients with CV maintaining circulating cryoglobulins

**TABLE 4** Pretreatment factors associated with relapse after clinical response in patients with HCV-related CV: Cox regression analysis

| | | Univariate analysis ($n$ = 288) | | Multivariate analysis ($n$ = 94) | |
|---|---|---|---|---|---|
| **Variable** | | **HR (95% CI)** | ***p*** | **HR (95% CI)** | ***p*** |
| Age (years) | | 1.01 (1.00–1.02) | 0.178 | | |
| Sex | Male | 1 | | | |
| | Female | 1.01 (0.71–1.44) | 0.937 | | |
| Purpura | No | 1 | | | |
| | Yes | 0.67 (0.45–1.01) | 0.055 | 0.75 (0.41–1.37) | 0.349 |
| Asthenia | No | 1 | | | |
| | Yes | 1.08 (0.71–1.64) | 0.730 | | |
| Arthralgia | No | 1 | | | |
| | Yes | 0.89 (0.63–1.26) | 0.507 | | |
| Neuropathy | No | 1 | | | |
| | Yes | 1.34 (0.95–1.88) | 0.092 | 1.38 (0.74–2.56) | 0.313 |
| Renal involvement | No | 1 | | | |
| | Yes | 0.91 (0.58–1.41) | 0.672 | | |
| Xerostomia/xerophthalmia | No | 1 | | | |
| | Yes | 1.41 (1.01–1.99) | 0.047 | 0.84 (0.52–1.70) | 0.841 |
| Raynaud | No | 1 | | | |
| | Yes | 0.87 (0.57–1.32) | 0.512 | | |
| Ulcer | No | 1 | | | |
| | Yes | 0.43 (0.11–1.72) | 0.232 | | |
| Pretreatment cryocrit | | 0.99 (0.94–1.03) | 0.514 | | |
| Pretreatment RF | | 1 (1.00–1.001) | 0.017 | 1 (1.00–1.001) | 0.021 |
| Pretreatment C4 | | 0.99 (0.89–1.09) | 0.786 | | |
| Rituximab | No | 1 | | | |
| | Yes | 0.65 (0.29–1.48) | 0.303 | | |



is useful when considering the possible evolution into a frank non-Hodgkin lymphoma.

Our study has some limitations. We acknowledge the potential center-related underestimation of MC prevalence among individuals with chronic HCV infection in Italy. In addition, the potential nonuniformity in reporting the subjective data from different centers and for all enrolled patients could have impacted the evaluation of clinical response; however, the semiquantitative evaluation of the main symptoms could have reduced this bias.

In conclusion, the prospective analysis of DAA-treated cryoglobulinemic patients consecutively enrolled in a nationwide cohort, including the majority of hepatology Italian centers, clearly shows that clinical response frequently fluctuates. Indeed, the clinical manifestation pattern may change and reappear, either persistently or transiently; and this implies an attentive approach to post-SVR monitoring of patients with CV, especially when they show symptom maintenance or recurrence. In this light, the accurate evaluation of both clinical and laboratory factors that represent prognostic indexes will aid in predicting different clinical evolutions. This could permit us to tailor the frequency and quality of FU appointments, as recommended for HCV-related liver damage, which will assist in the selection of the best therapeutic approach following HCV eradication.

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## CONFLICT OF INTEREST

Dr. Kondili consults for, is on the speakers' bureau for, and received grants from AbbVie. She consults for and is on the speakers' bureau for Gilead. Dr. Blanc is on the speakers' bureau for and received grants from Gilead. He is on the speakers' bureau for AbbVie, Menarini, and Viiv. Dr. Gentile consults

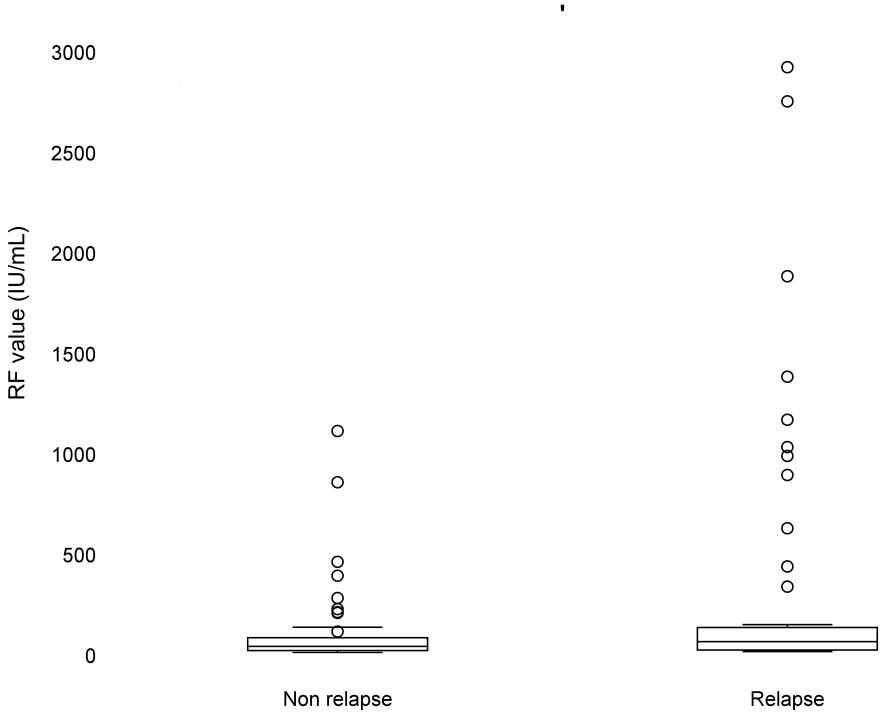


**FIGURE 4** Pretreatment RF values in CV patients without (non relapse) or with clinical relapse (relapse) following the SVR after DAA therapy



for and received grants from Gilead. He consults for MSD, AbbVie, and Angelini. He advises Abbott. He is on the speakers' bureau for Nordic, Advanz, and Pfizer. Dr. Puoti advises, is on the speakers' bureau for, and received grants from Gilead and AbbVie. He advises and is on the speakers' bureau for MSD. Dr. Verucchi consults for, advises, is on the speakers' bureau for, and received grants from Gilead and AbbVie. Dr. Ciancio is on the speakers' bureau for and received grants from Gilead. She is on the speakers' bureau for AbbVie and Alpha Sigma.

## ETHICS

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of good clinical practice. The study protocol was approved by the ethics committee of the Istituto Superiore di Sanità (protocol CE/13/389-23/07/2013) and by the local ethics committees from each clinical center. Patients' data were evaluated through an anonymous analysis, adopting codes generated from the electronic case report forms. All patients provided written informed consent to participate in the study.

## AUTHOR CONTRIBUTIONS

*Conceptualization*: Loreta A. Kondili and Anna Linda Zignego. *Data curation*: Valentina Panetta and Maria Giovanna Quaranta. *Formal analysis*: Valentina Panetta. *Resources*: Monica Monti, Giuseppina Brancaccio, Cesare Mazzaro, Marcello Persico, Mario Masarone, Ivan Gentile, Pietro Andreone, Salvatore Madonia, Elisa Biliotti, Roberto Filomia, Massimo Puoti, Anna Ludovica Fracanzani, Diletta Laccabue, Donatella Ieluzzi, Carmine Coppola, Maria Grazia Rumi, Antonio Benedetti, Gabriella Verucchi, Barbara Coco, Liliana Chemello, Andrea Iannone, Alessia Ciancio, Francesco Paolo Russo, Francesco Barbaro, Filomena Morisco, Luchino Chessa, Marco Massari, Pierluigi Blanc, and Anna Linda Zignego. *Writing–original draft*: Loreta A. Kondili, Anna Linda Zignego, and Laura Gragnani. *Writing–review and editing*: Loreta A. Kondili, Anna Linda Zignego, Laura Gragnani, and Maria Giovanna Quaranta. *Supervision*: Loreta A. Kondili and Anna Linda Zignego. All the authors have read and approved the final manuscript.

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