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Interferon-free therapy in hepatitis C virus mixed cryoglobulinaemia: a prospective, controlled, clinical and quality of life analysis

L. Gragnani[1] | G. Cerretelli[1] | S. Lorini[1] | C. Steidl[1] | A. Giovannelli[1] | M. Monti[1] | L. Petraccia[1] | S. Sadalla[1] | T. Urraro[1] | P. Caini[1] | A. Xheka[1] | A. Simone[1] | U. Arena[1] | M. Matucci-Cerinic[2] | D. Vergani[3] | G. Laffi[1] | A. L. Zignego[1]

[1]Department of Experimental and Clinical Medicine and Department of Oncology, Interdepartmental Center for Systemic Manifestations of Hepatitis Viruses (MASVE), Azienda Ospedaliero-Universitaria Careggi (AOUC), Florence, Italy

[2]Division of Rheumatology, Department of Geriatric Medicine, Azienda Ospedaliero-Universitaria Careggi (AOUC), Florence, Italy

[3]Institute of Liver Studies, King's College Hospital, London, UK

Correspondence
Prof. A L Zignego, Department of Experimental and Clinical Medicine and Department of Oncology, Interdepartmental Center for Systemic Manifestations of Hepatitis Viruses (MASVE), Azienda Ospedaliero-Universitaria Careggi (AOUC), Florence, Italy.
Email: a.zignego@dmi.unifi.it

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Summary

Background: Cryoglobulinaemic vasculitis (CV) is a lymphoproliferative disorder related to hepatitis C virus (HCV) infection; anti-viral therapy is the first therapeutic option. CV can be incapacitating, compromising the patients' quality of life (QoL). In a controlled study, interferon-based therapy was associated with a lower virological response in vasculitic patients than in patients without vasculitis. Limited, uncontrolled data on direct-acting anti-virals are available.

Aim: To evaluate safety, clinical efficacy, virological response and the impact of interferon-free treatment on QoL in HCV patients with and without mixed cryoglobulinaemia (MC).

Methods: We prospectively studied HCV patients with cryoglobulinaemia (with vasculitis-CV- and without vasculitis-MC-) and without cryoglobulinaemia (controls), treated with direct-acting anti-virals. Hepato-virological parameters, CV clinical response and impact on QoL were assessed.

Results: One hundred and eighty-two HCV patients were recruited (85 with CV, 54 with MC and 43 controls). A sustained virological response at 12 weeks (SVR12) was achieved in 166 (91.2%) patients (77/85 CV, 48/54 MC, 41/43 controls). In CV SVR patients, cryocrit levels progressively decreased and clinical response progressively improved, reaching 96.7%, 24 weeks after treatment. QoL, baseline physical and mental component summaries were lower in the CV group compared to the other groups ($P < 0.05$). Scores improved in all groups, and significantly in CV patients after SVR.

Conclusions: No significant differences in SVR rates were recorded between cryoglobulinaemic patients and controls and a high clinical and immunological efficacy was confirmed in CV, supporting the role of interferon-free therapy as the first therapeutic option. Interestingly, CV patients had worse baseline QoL than other HCV-positive groups and interferon-free therapy was effective in significantly increasing QoL, suggesting the important role of direct-acting anti-viral-based therapy in improving CV's individual and social burden.

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1 | INTRODUCTION

Hepatitis C virus (HCV) infection is considered a systemic disease,[1,2] frequently including lymphoproliferative disorders, mostly mixed cryoglobulinaemia (MC) and more rarely non-Hodgkin's lymphoma.[3-6] 80%-90% of MC patients are HCV-positive and a percentage ranging from 40% to 60% of HCV patients show cryoglobulinaemia; within patients with cryoglobulinaemia, 5%-30% develop symptomatic MC vasculitis (CV), also called MC syndrome.[4,5] Although CV is considered benign, it can lead to many complications, sometimes severe, which can affect the patient's quality of life.[7-11]

Aetiological therapy is the first-line option in HCV-CV patients and, for the past 15 y, pegylated-interferon (Peg-IFN) coupled with ribavirin (RBV) has represented the standard of care.[10,12-14] In the majority of cases, viral eradication leads to CV clinical remission.[2,13-15] The treatment of HCV infection experienced an important advancement with the introduction of new direct-acting anti-virals (DAAs), allowing IFN-free regimens, shorter treatments and minimising side effects.[16-18] Data regarding these new IFN-free regimens for the treatment of CV patients are still limited, and based on uncontrolled studies and short follow-ups. These studies are generally consistent with high rates of symptom remission in patients with sustained virological response (SVR), as well as a high tolerance of therapy.[19-25]

HCV was shown to have a tremendous impact on patient-reported outcomes, such as health-related quality of life (HQoL) and fatigue.[26,27] The value of IFN-free regimens in modifying such a negative impact can be fully appreciated by combining clinical outcomes and patient-reported outcomes.[28,29] Some extra-hepatic conditions such as CV could greatly affect HQoL, and the effect of treatment on this aspect of the disease should be considered as an important part of the therapy outcome.[30]

To our knowledge, this is the first prospective, controlled study evaluating patients with CV, patients with circulating cryoglobulins without vasculitis (MC) and patients with no evidence of MC/CV or other lymphoproliferative disorders (HCV controls), before, during and after treatment with IFN-free DAA regimens. Safety, clinical efficacy, virological response and the impact of treatment on HQoL were compared and evaluated among the 3 different groups of HCV-infected subjects.

2 | PATIENTS AND METHODS

2.1 | Patients

Between January 2015 and March 2016, we enrolled consecutive patients with detectable levels of serum HCV-RNA and eligibility for IFN-free anti-viral treatment, at the out-patient clinic of the Interdepartmental Center for Systemic Manifestations of Hepatitis Viruses (MaSVE), University of Florence, Florence, Italy, according to previously described inclusion criteria.[13]

The study included 3 different patient groups: Group "CV": HCV patients with cryoglobulinaemic vasculitis; Group "MC": HCV patients with circulating cryoglobulins without symptoms; Group "HCV": patients without CV, cryoglobulins or any other autoimmune/lymphoproliferative disorders. Subjects with uncertain classification were excluded from the study. Exclusion criteria also included co-infection with HIV or HBV, presence of severe comorbidities not related to MC (ie hepatocellular carcinoma) and the administration of rituximab or plasma exchange cycles during the 24 weeks prior to the study. Patients taking low-medium doses of corticosteroids (0.1-0.5 mg/kg/d) or symptomatic drugs/measures (ie colchicine/low antigen diet) were included. Demographical information, treatment history, HCV genotype data and laboratory evaluations were obtained from the records of the MaSVE out-patient clinic.

2.2 | Ethical guidelines

The study was conducted according to the 1975 Declaration of Helsinki; all treatments were open-label and therefore ethical approval was not required for administration, when provided by the National Health Care System. Therapy administered for compassionate use required individual approval (named patient programme) by the institutional review board.

The study received institutional review board approval (evaluation code: SPE 14.084_ AOUC); informed consent was obtained from all patients.

2.3 | Treatment

Patients were treated with IFN-free DAA combinations (Table 1) according to the recommendations of the European Association for the Study of the Liver,[31] on which the Italian Association for the Study of the Liver and the Italian Medicines Agency guidelines are based, and were partly administered on compassionate use.

2.4 | Laboratory and clinical assessments

All evaluations were performed at baseline, at week 4 of treatment, at the end of treatment (EOT) and at weeks 12 and 24 of the post-therapy follow-up.

2.4.1 | Hepato-virological laboratory and clinical parameters

HCV infection was diagnosed by detecting serum HCV-RNA (AMPLICOR® HCV Test, v2.0, Roche Diagnostics, Alameda, CA, USA). HCV genotype was determined by a diagnostic test (VERSANT HCV Genotype 2.0, Siemens Healthcare Diagnostics, Deerfield, IL, USA). Liver-related biochemical parameters (alanine aminotransferase, aspartate aminotransferase, total and direct bilirubin) were also evaluated through routine tests.

Liver disease stage was assessed by non-invasive methods including liver elastography using FibroScan (Echosens, Paris, France), imaging, clinical presentation and laboratory data; the METAVIR score and Child-Pugh scoring system for cirrhotic patients were used to assess the severity of liver damage.

2.4.2 | MC laboratory and clinical parameters

To assess MC diagnosis, circulating cryoglobulins were determined in at least 3 metachronous samples.[32] The percentage of packed cryoglobulins in the serum, the cryocrit, was assessed following the same time-points as the other laboratory evaluations. The other immunological parameters for evaluating MC response (measurement of the C4 complement fraction and rheumatoid factor [RF]) were performed as routine diagnostic tests.

Estimated glomerular filtration rate (eGFR) was calculated with the MDRD GFR equation, based on creatinine and patient characteristics.

Clinical evaluation of CV was performed at EOT and at weeks 12 and 24 of the post-therapy follow-up in relation to previous studies[33] and to the current classification criteria.[5,34-36]

In detail, purpura was assessed as previously shown[33] with minor modifications and classified according to 4 semi-quantitative grades: 0 (no purpura), + (limited or fluctuating involvement of the lower limbs), ++ (diffuse and persistent involvement of the lower limbs), +++ (diffuse and persistent involvement of the trunk and the lower limbs); leg ulcer response was considered as complete when all the ulcers completely healed, as improved with a reduction of at least 25% in diameter and as no response with the reduction of less than 25% in ulcer diameter, or if they worsened.

Arthralgia, weakness and sicca syndrome were measured through a patient-scored Visual Analog Scale (VAS) (range, 0-100); treatment response was defined as follows: complete (disappearance of symptom/s), improvement (at least 25% VAS reduction), no response (less than 25% VAS reduction or worsening). Neuropathic symptoms, including both paraesthesias/pain and clinically evident motor deficit,

TABLE 1 Main demographical, hepatovirological and clinical baseline characteristics of 182 HCV patients stratified in the 3 study groups

	HCV-ctrs (n = 43)	MC (n = 54)	CV (n = 85)	*P*
Age (y)[b]	59.8 (±1.73)	60.4 (±1.7)	65 (±1)	0.0084 HCV-ctrs vs CV 0.0254 MC vs CV
Gender (male/female)	25/18	30/24	29/56	0.0066 HCV-ctrs vs CV 0.0088 MC vs CV
Metavir score[a]				
F0-F1	0	0	33	
F2	0	0	7	
F3	19	23	5	
F4	24	31	40	
Child-Pugh score (A/B/C)	20/4/0	29/2/0	29/10/1	
ALT (U/L)[b]	105 (±12)	120.4 (±10)	84.3 (±8)	0.0430 MC vs CV
AST (U/L)[c]	76.3 (±7.8)	94 (±8)	73 (±9.1)	NS
Bilirubin Tot[d]	0.88 (±0.07)	0.94 (±0.05)	0.8 (±0.04)	0.02 MC vs CV
Bilirubin Dir[e]	0.33 (±0.04)	0.32 (±0.02)	0.29 (±0.04)	NS
HCV-RNA (IU/mL × 10^6)	2.4 (±0.38)	2.7 (±0.48)	2.9 (±0.43)	NS
HCV genotype				
1a	6 (13.95%)	8 (14.8%)	8 (9.4%)	
1b	25 (58%)	33 (61.1%)	48 (56.5%)	
2	6 (13.95%)	4 (7.4%)	18 (21.2%)	
3	3 (7%)	7 (13%)	7 (8.2%)	
4	3 (7%)	2 (3.7%)	2 (2.35%)	
Mixed (1b+2)	—	—	2 (2.35%)	
Previous AVT response				
Naïve	24	25	46	
Experienced	19	29	39	
DAA treatment				
3D[f]	8	13	16	
3D+RBV	1	5	4	
Sofosbuvir+Daclatasvir	4	2	9	
Sofosbuvir+Daclatasvir+RBV	5	11	7	
Sofosbuvir+Ledipasvir	6	8	12	

(Continues)

TABLE 1 (Continued)

	HCV-ctrs (n = 43)	MC (n = 54)	CV (n = 85)	*P*
Sofosbuvir+Ledipasvir+RBV	9	5	7	
Sofosbuvir+Simeprevir	5	3	7	
Sofosbuvir+Simeprevir+RBV	1	2	—	
Sofosbuvir+RBV	4	5	23	
Laboratory				
Cryocrit (%)	0	1.2 (±0.2)	3.7 (±0.8)	0.0017 MC vs CV
RF[g]	19.8 (±0.7)	20.5 (±1.7)	221(±107)	0.0026 HCV-ctrs vs CV 0.026 MC vs CV
C4[h]	0.20 (±0.03)	0.15 (±0.02)	0.09 (±0.008)	0.0007 HCV-ctrs vs CV 0.0057 MC vs CV

HCV-ctrs, HCV controls; MC, mixed cryoglobulinaemia without vasculitis; CV, cryoglobulinaemic vasculitis; AVT, anti-viral treatment. Data are expressed as mean ± standard error.
[a]Based on liver stiffness assessed by FibroScan.
[b]Alanine aminotransferase (ALT) normal range: 12-65 U/L.
[c]Aspartate aminotransferase (AST) normal range: 15-37 U/L.
[d]Total bilirubin normal range: 0.2-1 mg/dL.
[e]Direct bilirubin normal range: 0-0.2 mg/dL.
[f]3D: ombitasvir, paritaprevir+ritonavir, dasabuvir.
[g]Rheumatoid factor (RF) normal values: <20 IU/mL.
[h]C4 normal values: 0.1-0.4 g/L.

were also assessed by VAS and, when possible, by electromyographical analysis. Kidney function was evaluated according to serum creatinine and urinalysis with the evaluation of proteinuria. A complete response was defined as a combination of normalisation of renal function when abnormal (serum creatinine) and proteinuria of 0.5 g/d or less. An improvement was defined as stable or ameliorated renal function, and/or persisting for at least a 50% reduction of proteinuria. No response was defined as worsening of renal function not attributable to different causes and/or proteinuria increase or persistence higher than 0.5 g/d.

Raynaud phenomenon response was evaluated considering the attack number in a 2-week period and severity according to the VAS scale (0-100), as previously suggested.[36] On this basis, we considered the disappearance of attacks as a complete response, the reduction of at least 30% frequency and severity of the attacks as an improvement and, a reduction of less than 30% as a non-response.

2.5 | Efficacy assessments

SVR was defined as an undetectable serum HCV-RNA at week 12 (SVR12) after therapy cessation; a HCV-RNA evaluation was performed at week 24 of the post-treatment follow-up (SVR24).

Clinical and immunological response was assessed following previously used criteria.[22,37] Briefly, we considered "full-complete responders" patients with the disappearance of all baseline symptoms, and "complete responders" those with improvement of all the baseline symptoms, "partial responders" those with the disappearance or improvement of at least half of the baseline symptoms, and "non-responders" patients with the disappearance or improvement of fewer than half of the baseline symptoms.

MC immunological response was "complete" in the case of the disappearance of cryoglobulins, "partial" for a decrease of cryocrit to <50% of baseline level, and "null" in all other cases, following previously accepted criteria.[22,37]

2.6 | Measurements and analysis of the HQoL

Patients were interviewed with a patient-reported outcome questionnaire at baseline, EOT and at weeks 12 and 24 of the post-treatment follow-up; we focused on a widely used generic/validated instrument for HQoL evaluation, the Short Form-36 version 2 (SF-36v2).[38] It assesses 8 HQoL scales (scores ranging from 0 to 100 with higher values corresponding to better health status): physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional and mental health along with 2 summary scores summarising the physical (physical component summary score [PCS]) and mental health (mental component summary score [MCS]) components of SF-36.[39]

2.7 | Safety assessment

Patients were monitored for the occurrence of adverse events (AEs) during regularly scheduled check-ups and additional unscheduled appointments were made to ascertain the presence and/or the severity of the AE reported by telephone.

2.8 | Statistical analysis

Quantitative variables were analysed using one-way analysis of variance (nonparametric ANOVA) or with *t* test for unpaired samples. Categorical variables were analysed with the χ^2 test and Fisher's

exact test when necessary. All tests were two-sided at a 0.05 significance level. Analyses were performed by Stata v.9.0 (StataCorpLP, College Station, TX, USA). Scores were obtained from the SF-36v2 questionnaire from dedicated software and then statistical analysis was performed.

3 | RESULTS

From January 2015 to March 2016, 182 consecutive HCV-infected patients (84 males and 98 females, mean age 63 ± 0.84 years) matched the inclusion criteria and were prospectively enrolled in the study. Twenty-six patients were treated on a compassionate basis, while drugs were provided for the others by the national health care system. Among the 182 HCV-positive patients, 139 (76%) had circulating cryoglobulins, 85/139 (61%) with symptoms/vasculitis (CV) and 54 (39%) without symptoms (MC). The remaining 43/182 (24%) subjects displayed no evidence of signs/symptoms of MC (pathological controls "HCV-ctrs"). Among the CV group, 14 of 85 subjects were included in a previous uncontrolled study.[22] The flow chart of the 3 study groups is reported in Data S1. The main baseline demographical, clinical and virological characteristics of the study population are reported in Table 1. The groups were homogeneous for the majority of the characteristics and parameters we evaluated, with, as expected, higher mean age and number of women in the CV population compared to the other groups (Table 1); in addition, the Italian criteria of DAA eligibility at the time of enrolment permitted us to treat F0-F2 patients only when clinically significant mixed cryoglobulinaemia or lymphoma was diagnosed and, therefore, some liver-related parameters resulted as less altered in patients belonging to the CV group compared to the others (Table 1).

DAA-based IFN-free regimens were administered combined with RBV in 20/43 (46.5%) HCV-ctrs, in 28/54 (52%) MC patients and in 59/85 (69%) CV patients.

All but 3 patients reached EOT; among the 179 patients who completed the treatment, 6 experienced a relapse by week 4 of the post-therapy follow-up.

Patients were followed up for a median of 16.4 months (range 9-23) after the completion of therapy.

As a further 7 patients were lost to follow-up after the EOT, 166 patients (41/43 HCV-ctrs; 48/54 MC; 77/85 CV) were monitored until at least week 24 after the EOT.

3.1 | Virological and hepatological response

An "intention to treat" analysis was possible on 182 patients and a "per protocol analysis" on 179 patients in regard to virological outcome.

Following the intention to treat analysis, SVR12, confirmed by SVR24, was obtained in 166 (91.2%) patients, and stratified as follows: 41/43 (95.3%), 48/54 (88.9%) and 77/85 (90.6%) from the HCV-ctrs, the MC and the CV groups respectively. The per protocol analysis showed a SVR12 rate of 92.7%, stratified as follows: 41/42 (97.6%), 48/54 (88.9%) and 77/83 (92.8%) from the HCV-ctrs, the MC and the CV groups respectively.

Three patients interrupted treatment (Table 2). One CV patient was hospitalised for the appearance of jaundice during therapy; another patient, with severe CV, voluntarily interrupted treatment reporting a subjective worsening of a previous sensorimotor neuropathy not supported by 2 different neurological check-ups. The last patient interrupted therapy at week 9 when she underwent a liver transplant (Table 2); to note, this patient remained HCV-RNA negative after the liver transplant.

Among the 6 patients who relapsed by week 4 of the post-therapy follow-up, 1 poly-transfused patient was identified with an unrecognised mixed genotype infection (genotype 1b+2, Table 2) and responded to a second treatment tailored for genotype 2 (data not shown). All the other patients tested as negative in the test for

TABLE 2 Features of the 9 out of 182 patients who relapsed or prematurely interrupted therapy

Patient	Age/gender	Group	HCV genotype	Liver disease	Previous AVT	DAA/scheduled weeks of therapy	Reason of treatment failure
1	53/M	HCV-ctrs	4	F4	N	SOF+SMV+RBV/12	Relapse at week 4 of follow-up
2	59/M	MC	4	F4	I	SOF+LED/24	Relapse at week 4 of follow-up
3	76/F	MC	1b	F3	I	SOF+LED/12	Relapse at week 4 of follow-up
4	70/F	CV	1b	F3/F4	I	3D/12	Relapse at week 4 of follow-up
5	58/M	CV	1a	F4	N	SOF+RBV/48	Relapse at week 4 of follow-up
6	43/M	CV	1b+2	F3	N	3D+RBV/24	Apparent relapse, unrecognised mixed genotype infection
7	69/F	CV	1b	F4	N	3D/24	Interruption for hyperbilirubinaemia at week 1
8	56/F	CV	1b	F1/F2	I	3D/24	Voluntary interruption[a] at week 4
9	49/F	HCV-ctrs	1a	F4	N	3D/24	Interruption due to liver transplant[b]

HCV-ctrs, HCV controls; MC, mixed cryoglobulinaemia without vasculitis; CV, cryoglobulinaemic vasculitis. The stage of liver disease is expressed by METAVIR score; N, treatment naïve; I, interruption of IFN-based treatment for intolerance; SOF, sofosvubir; SMV, simeprevir; LED, ledipasvir; RBV, ribavirin; 3D, ombitasvir, paritaprevir+ritonavir, dasabuvir.

[a]The patient voluntarily interrupted treatment after 4 wk reporting a subjective worsening of a previous sensorimotor neuropathy.

[b]This patient interrupted treatment at week 9 due to a liver transplant, and maintained a negative viraemia.

resistance-associated variants (NS3, NS5A or NS5B variants, depending on the drug combination).

According to previously published results,[22,23] of the 166 patients who completed the post-therapy follow-up, the available liver function parameters showed an overall improvement (Table S1).

3.2 | CV clinical response

The 77 CV patients who experienced SVR24 were evaluated for clinical response at different time points of the post therapy follow-up (Figure 1). At EOT, 1.7%, 56.7%, 23.3% and 18.3% of CV patients were full complete-, complete-, partial-, and non-responders respectively. Improvement was evident at SVR12 with 20.7%, 43.1%, 29.3% and 6.9% full complete-, complete-, partial- and nonresponders respectively. At SVR24 clinical efficacy further increased with 25% and 60% of CV patients experiencing a full-complete response and a complete response, respectively, while the percentage of partial responders decreased to 11.7% and to 3.3% for the nonresponders.

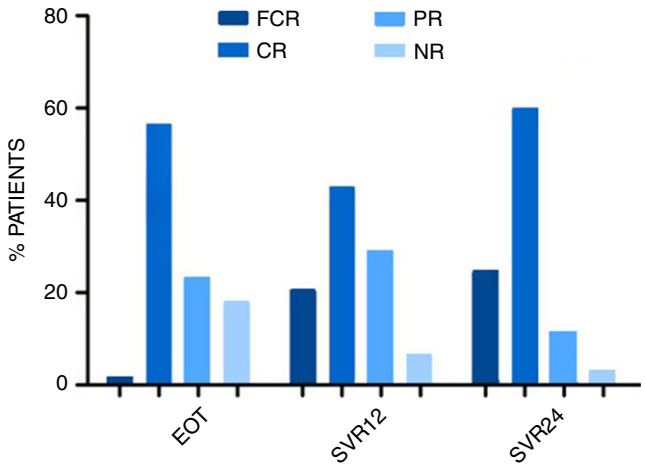

FIGURE 1 Clinical response of patients with cryoglobulinaemic vasculitis. FCR: full complete responders; CR, complete responders; PR, partial responders; NR, nonresponders; EOT, end of treatment; SVR, sustained virological response

CV SVR patients were stratified, according to baseline severity, in patients with (1) mild/moderate manifestations (66/77) and (2) with severe manifestations (11/77), as recently stated.[14]

Pre-therapy grade b severity was characterised as 3/3 (100%) for clinical nonresponders, 6/9 (67, 7%) for partial-responders, 1/46 (2%) for complete responders, and 1/19 (5.3%) for full-complete clinical response.

A total of 7/85 CV patients did not achieve SVR and clinical response; these included 3 patients with severe CV: a patient with severe sensorimotor neuropathy with a history of CNS involvement (transient ischaemic attack-like syndrome) and who interrupted treatment early, and 2 with rapidly progressive glomerulonephritis previously treated with rituximab (Table 2), including a case of virological relapse after transient response. In the latter case, kidney function, was moderately reduced before and after treatment (chronic kidney disease stage 3B), after the recurrence of HCV replication rapidly worsened (chronic kidney disease stage 5, requiring haemodialysis).

Among the 85 CV patients who initiated treatment, the 3 patients with grade C severity[14] had to be included in the virological failure group (2 of them interrupted therapy and 1 experienced a virological relapse, Table 2).

The behaviour of the main symptoms is reported in Table 3. In accordance with previous reports,[22] we noted that while the improvement of some symptoms was rapid (ie purpura and skin ulcers), others such as fatigue, sicca syndrome and peripheral neuropathy were slow to respond (Table 3).

The behaviour of eGFR in the 9 HCV-CV patients with kidney involvement who reached an SVR and completed the follow-up until SVR24 are reported in Table S2. None of these patients had baseline eGFR levels <30 mL/min and showed a long-lasting eGFR worsening;

TABLE 3 Behaviour of the main CV symptoms after therapy in CV patients who achieved viral eradication

BSL		EOT			SVR12			SVR24		
Symptoms	n	Disappearance n (%)	Improvement n (%)	Persistence n (%)	Disappearance n (%)	Improvement n (%)	Persistence n (%)	Disappearance n (%)	Improvement n (%)	Persistence n (%)
Purpura	58	36 (62%)	5 (9%)	17 (29%)	46 (79%)	2 (3%)	10 (17%)	48 (83%)	5 (9%)	5 (9%)
Arthralgias	41	12 (29%)	12 (29%)	17 (41%)	14 (34%)	7 (17%)	20 (49%)	12 (29%)	12 (29%)	17 (41%)
Weakness	52	11 (21%)	16 (31%)	25 (48%)	14 (27%)	18 (35%)	20 (38%)	19 (37%)	20 (38%)	13 (25%)
Peripheral neuropathy	37	6 (16%)	10 (27%)	21 (57%)	16 (43%)	7 (19%)	14 (38%)	18 (49%)	6 (16%)	13 (35%)
Raynaud phenomenon	17	9 (53%)	2 (12%)	6 (35%)	10 (59%)	1 (6%)	6 (35%)	13 (76%)	1 (6%)	3 (18%)
Sicca syndrome	34	12 (35%)	14 (41%)	8 (24%)	11 (32%)	15 (44%)	8 (24%)	10 (29%)	15 (44%)	9 (26%)
Ulcer	7	1 (14%)	1 (14%)	5 (71%)	5 (71%)	1 (14%)	1 (14%)	7 (100%)	—	—
Proteinuria	9	1 (11%)	1 (11%)	7 (78%)	3 (33%)	1 (11%)	5 (56%)	3 (33%)	—	6 (67%)

CV, cryoglobulinaemic vasculitis; BSL, baseline; EOT, end of treatment; SVR, sustained virological response.

in some cases there was a light decrease in eGFR during and after treatment, while SVR24 eGFR levels were maintained or slightly increased compared to baseline values.

During the post-therapy follow-up, no patients died or developed haematological malignancies.

3.3 | Immunological response

The mean cryocrit progressively decreased in MC patients from 1.2 ± 0.2 at baseline to EOT (0.63 ± 0.12), slightly increased at SVR12 (0.65 ± 0.15) and lowered significantly at SVR24 (0.45 ± 0.14; $P < 0.05$ compared to baseline and to week 4) (Figure S2, panel A). The immunological response at SVR12 was complete in 39.4% of cases, partial in 18.2%, and null in 42.4% of patients. At SVR24 there were about 76% responders (66.7% complete 9% partial responders) versus 24.3% null responders. The levels of serum RF slightly decreased and C4 complement component slightly increased from baseline to SVR24 in MC patients (Figure S2, panels B and C).

Regarding the CV group, the baseline cryocrit levels were higher than those observed in the MC group, with a mean value of 3.7 ± 0.8 (range 38-1.5), which halved rapidly at week 4 of treatment (1.65 ± 0.3; $P < 0.001$ vs baseline), and progressively decreased until SVR24 (Figure S3, panel A). The immunological response was complete, partial and null at SVR12, in 46.2%, 32.3% and 21.5% of cases respectively. At SVR24, the percentage of CV patients with a complete immunological response increased to 69.2% while partial and null response both decreased to 18.5% and 12.3% respectively.

The serum RF and C4 values rapidly and significantly improved during treatment and the post-therapy follow-up (Figure S3, panels B and C).

3.4 | Evaluation of HQoL

HQoL evaluation was available for 15/41 HCV-ctrs, 18/48 MC and 26/77 CV patients. The scores of the 8 items related to SF-36, stratified for each group and collected at EOT, SVR12 and SVR24, are provided in Table S3. Considering the single items, improvement reached significance for some items solely in the CV patient group (Table S3).

The behaviour of the 2 summary scores PCS and MCS, calculated at the same points in time, for the 3 studied populations, is reported in Figure 2.

At baseline, CV patients were characterised by a more considerable impairment in both scores (PCS: 51 ± 4; MCS: 55.2 ± 3.4) compared to HCV-ctrs (PCS: 68.5 ± 10; MCS: 68.2 ± 4.7) and MC (PCS: 69.2 ± 6; MCS: 66 ± 7.2) patients (CV PCS vs HCV-ctrs PCS: $P < 0.05$; CV PCS vs MC PCS: $P < 0.01$; CV MCS vs HCV-ctrs MCS: $P < 0.05$). In all 3 groups, we observed a decrease in MCS and PCS at the EOT as well as a gradual increase in the 2 scores during the post-therapy follow-up (Figure 2).

3.5 | Treatment safety

Among the 182 patients who initiated DAA therapy, AEs occurred in 99 (54.4%); stratifying for the 3 study groups, AEs were reported by 15/43 (34.9%) HCV patients, by 27/54 (50%) MC patients and by 57/85 (67%) CV patients. On the whole, 26/182 subjects experienced more than 1 AE. As shown in detail in Table S4, the most frequent AE was anaemia, which was related to ribavirin administration. In the majority of cases, the decrease in haemoglobin required an intervention (Table S4), even if a blood transfusion was not needed in any instance.

One case of hyperbilirubinaemia was the most severe AE and the only one requiring therapy interruption and hospitalisation. As previously mentioned, this patient discontinued treatment after 1 week of administration as the total bilirubin score reached 24 mg/dL (normal range 0.2-1 mg/dL). A patient complained of worsening neuropathy and voluntarily interrupted therapy; however, as previously mentioned, electroneurography and 2 different neurological consultations did not find any evidence of such worsening.

All the other AEs, reported in Table S4, were mild and usually well tolerated.

4 | DISCUSSION

To our knowledge, this is the first prospective and controlled analysis of DAA-treated patients with HCV-related cryoglobulinaemia, either symptomatic (CV) or asymptomatic (MC). As described in a study performed in the IFN-based era,[13] controls (HCV-ctrs) were represented by HCV patients without cryoglobulinaemia or other autoimmune/lymphoproliferative disorders. We evaluated virological and clinical-immunological response and the effect of treatment on the HQoL in these HCV-patient subsets, consecutively treated with IFN-free therapy.

According to previous results and well-known demographical characteristics of mixed cryoglobulinaemia subjects,[13] CV patients were older and with a female over-representation compared to MC and HCV-ctrs groups. Conversely, the less severe degree of liver disease in CV than in patients without vasculitis observed in this study, but not in other reports,[13] was probably influenced by the Italian criteria of DAA eligibility at the time of enrolment. This allowed us to only treat patients with severe liver damage (≥F3) with the exception of patients with clinically significant mixed cryoglobulinaemia, (or lymphoma), even with mild/absent liver fibrosis. A previous controlled analysis in similar patient categories, treated with peg-IFN and RBV, showed a significant difference in the rate of SVR between HCV-ctrs and both MC and CV groups.[13] Thus, the presence of cryoglobulinaemia was a prognostic factor of non-response independent from the severity of liver disease, or other well-known prognostic factors.[13] In this study, both intention to treat and per protocol analyses confirmed the excellent virological outcome reported by previous studies on CV or MC patients treated with DAAs,[20,22-25,40]

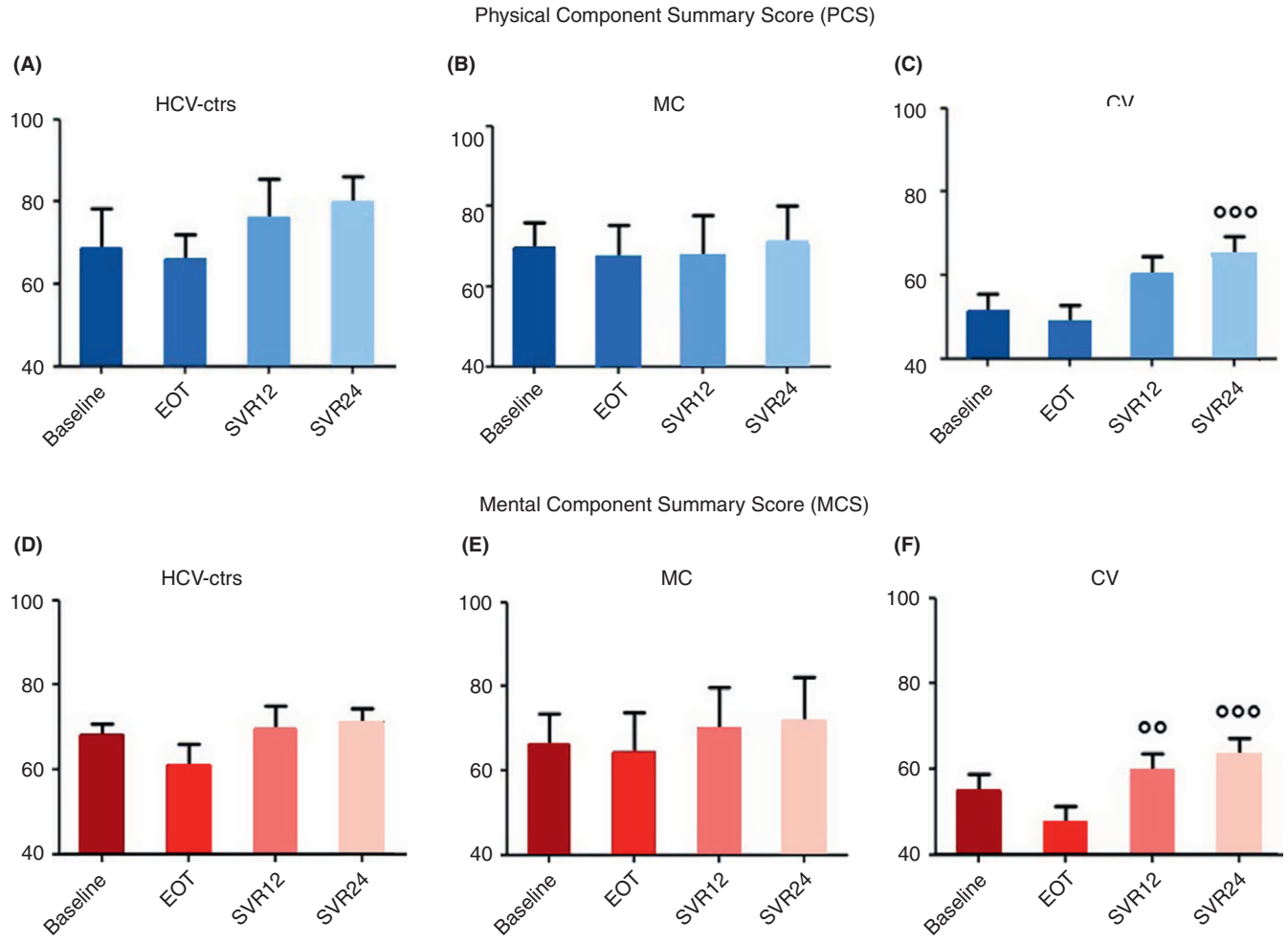

FIGURE 2 Behaviour of the 2 summary scores, physical component summary score (PCS) and mental component summary score (MCS), in the 3 patient groups. (A) PCS in HCV patients without MC; (B) PCS in MC patients; (C) PCS in CV patients; (D) MCS in HCV patients without MC; (E) MCS in MC patients; (F) MCS in CV patients; $^{**}P < 0.01$ compared to baseline scores; $^{\circ\circ}P < 0.01$ and $^{\circ\circ\circ}P < 0.001$ compared to EOT scores. HCV-ctrs, HCV-controls; CV, cryoglobulinaemic vasculitis; MC, mixed cryoglobulinaemia; EOT, end of treatment; SVR, sustained virological response

hindering a valuable logistic regression analysis to identify outcome predictors. In other words, using DAAs, it was not possible to find significant differences in SVR rates between cryoglobulinaemic patients and controls. However, we observed that the few cases of treatment failure (patients who interrupted treatment or relapsed) generally belonged to the MC or CV groups and were advanced cases; interestingly, in one of the cases, with kidney involvement, viral relapse was followed by a consistent worsening of the cryoglobulinaemic nephropathy. This appears to be in agreement with the study by Kondo et al., where the incidence of treatment discontinuation among patients with chronic kidney disease was significantly higher than that among patients without renal damage.[41] In our study, among patients with renal involvement experiencing viral eradication, we did not observe a persistent worsening of eGFR. In detail, CV patients with renal involvement (baseline eGFR>30) who reached week 24 of post-therapy follow-up maintained or slightly improved the pre-treatment eGFR levels. Longer follow-ups will be useful to ascertain the real effect of viral eradication on kidney damage.

According to previous results,[20,22,24] independent of the presence or absence of mixed cryoglobulinaemia, liver function parameters showed an overall improvement in all SVR patients.

We observed a high rate of improvement of CV clinical indices following the virological responses (85% considering both full complete/complete response), which went on improving during a longer follow-up. However, patients with severe manifestations had a worse clinical outcome during the mid-term post-therapy follow-up. All the patients who did not experience clinical improvement and a relevant percentage of partial responders had grade b CV severity,[14] and all 3 patients with grade c CV experienced both virological and clinical failure. Conversely, a full complete clinical response was recorded in only 1 grade b patient. This is in accordance with observations by Emery et al.[24] that reviewed 18 HCV patients with symptomatic CV and observed a lower clinical response in those with

severe vasculitis. In particular, of 7 patients with mild-moderate CV and 7 patients with severe CV, 6 patients (85.7%) and 1 patient (14.2%), respectively, achieved a complete clinical response. None of the 4 patients with fulminant CV achieved a complete clinical response at last follow-up despite achievement of SVR12 in all cases.

Altogether, data concerning both virological and clinical/immunological response suggest that there is an opportunity to treat cryoglobulinaemic patients early preventing the evolution to severe disease forms.

The finding of more altered immunological parameters in the CV than in the MC group is consistent with previous reports[13,23,24] and suggests a minor involvement/activation of the immune system in MC, possibly corresponding to an early disease stage. Emery et al. showed a better immunological response in MC than CV patients, even if the difference in clearing cryoglobulins was not significant.[24] Similar data were described by Bonacci et al. reporting, for MC patients, a cryoglobulin clearance of 62% and a complete immunological response of 53% compared to 45% of cryoglobulin clearance and 43% of complete immunological response in CV patient reports.[23] Our analysis partially confirmed these findings, even though we observed a higher rate of complete immunological response in CV patients. In SVR24 CV patients, the rate of complete immunological response was lower than that of complete clinical response; this discrepancy was previously reported.[13,23,24,42] Longer follow-ups will be useful to assess the percentage of persistent CV stigmata.[13]

Results of particular interest were obtained by the comparative HQoL evaluation among the 3 different groups of patients that was performed using the SF-36 questionnaire to obtain PCS and MCS components through specific analysis. The potential impact on HQoL of extra-hepatic manifestations and its consequences on indirect costs of HCV management were highlighted in a meta-analysis by Younossi et al.[43] The French "VASCUVALDIC" study showed data regarding increasing HQoL scores in 24 CV patients treated with Sofosbuvir plus RBV, analysing the baseline values vs EOT and SVR12.[20] The authors reported a progressive improvement of scores at EOT and SVR12. Conversely, considering the evolution of HQoL, we observed a decrease in MCS and PCS at EOT in all 3 groups, despite a gradual increase in the 2 scores during the post-therapy follow-up. Conceivably this could have been caused by side effects induced by RBV administration in the majority of patients who underwent the SF-36 interview. Even if side effects were generally mild and well tolerated, they could have had a negative impact on aspects of everyday life. The HQoL decline was transient and the benefits on physical and emotional states were evident from SVR12. To the best of our knowledge, no previous comparative analyses on HQoL between CV vs non-CV HCV patients were available. We could show for the first time that patients with CV had lower PCS and MCS before treatment and, therefore, a worse HQoL, compared to MC and HCV-ctrs. Using a scientifically validated questionnaire, we were able to confirm our previous observations stemming from clinical practice. Even if the improvement of PCS and MCS was clear in all 3 groups, for CV patients the SVR12 and SVR24 values were significantly higher, compared to baseline scores. This means that DAA treatment could be highly beneficial for CV patients regarding HQoL, which is an important goal of therapy. These aspects, taken into account by pharmacoeconomic analyses, seem to have important implications on public expenditure, not only through direct medical costs but also through indirect costs due to decreased work productivity. This represents a relevant issue especially in countries with a high prevalence of HCV.[27,44]

Regarding therapy safety and tolerance, all the AEs were generally mild and well tolerated; however, we recorded a higher percentage of AEs in MC and CV than in HCV-ctrs. This is in accordance with a large controlled study performed on CV, MC and HCV-ctrs patients in the era of IFN-based therapy,[13] suggesting the key role played by RBV use in influencing the observed differences. The percentage of AEs in the CV group (57%) was very similar to the one reported by other authors.[13,20,23]

In summary, our prospective/controlled analysis, including a wide population of cryoglobulinaemic patients, confirmed the high efficacy and safety of DAAs in patients with HCV-related cryoglobulinaemia. The high SVR rates did not allow for a multivariate logistic regression analysis and no statistical differences in SVR rates were observed. However, patients who interrupted treatment or relapsed were mostly those with cryoglobulinaemia and with advanced, long-lasting vasculitis. Similar correlations were observed concerning the CV clinical outcome; despite the remarkable success rate, patients with severe CV did not experience a complete response. Collectively, these data emphasise the need to identify cryoglobulinaemic patients and to treat them as soon as possible to obtain the best virological and clinical benefits. This is only feasible when a correct diagnostic approach to HCV patients has been used, so as not to underestimate the condition.[45]

More interestingly, this study shows that among patients with chronic HCV infection, those with CV have significantly worse HQoL and DAA therapy is effective in increasing both physical and mental scores; this additional benefit of IFN-free treatments should be taken into account as an important goal of modern anti-viral therapy.

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AUTHORSHIP

Guarantor of the article: Anna Linda Zignego.

Author contributions: Laura Gragnani worked on study conception and design, acquisition of data, data analysis and interpretation of manuscript writing. Guia Cerretelli collected data, performed statistical analysis and revised the paper. Antonella Simone performed cryocrits. Serena Lorini performed cryocrits, collected data and helped in the statistical analysis. Andrea Giovannelli and Adela Xheka collected data. Carolina Steidl collected data and revised the paper. Monica Monti collected medical data and revised the paper. Patrizio Caini, Teresa Urraro and Diego Vergani critically revised the manuscript. Luisa Petraccia Sinan Sadalla and Umberto Arena collected patients' medical records and revised the paper. Giacomo Laffi and Marco Matucci-Cerinic revised the paper. Anna Linda Zignego worked on study conception and design, performed data analysis and interpretation, and critically revised the paper. All authors approved the final version of the manuscript and the authorship list.

ORCID

L. Gragnani https://orcid.org/0000-0001-6800-9149
A. L. Zignego http://orcid.org/0000-0002-8552-4166

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

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