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Long-Term Effect of HCV Eradication in Patients With Mixed Cryoglobulinemia: A Prospective, Controlled, Open-Label, Cohort Study

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Limited data are available about the efficacy of antiviral treatment in hepatitis C virus (HCV)–associated mixed cryoglobulinemia (MC), especially concerning the long-term effects of HCV eradication. The aim of this study was to evaluate the influence of MC on the virological response and the long-term effects of viral eradication on MC. We prospectively enrolled 424 HCV$^+$ patients belonging to the following groups: MC syndrome (MCS)-HCV (121 patients with symptomatic MC), MC-HCV (132 patients with asymptomatic MC), and HCV (158 patients without MC). Pegylated interferon plus ribavirin treatment was administered according to standard protocols. Posttreatment follow-up ranged from 35 to 124 months (mean 92.5 months). A significant difference was observed in the rate of sustained virological response between the HCV group and both the MC-HCV ($P = 0.009$) and MC-HCV+MCS-HCV ($P = 0.014$) groups. Multivariate logistic regression analysis identified cryoglobulinemia as an independent prognostic factor of nonresponse. The clinical–immunological response in MCS-HCV correlated with the virological one. All patients with sustained virological response also experienced a sustained clinical response, either complete or partial. In the majority of sustained virological response patients all MCS symptoms persistently disappeared (36 patients, 57%); in only two (3%) did definite MCS persist. All virological nonresponders were also clinical nonresponders, in spite of a transient improvement in some cases. No evolution to lymphoma was observed. For the first time we have evaluated both the effects of interferon-based therapy on HCV patients with and without MC and with and without symptoms, as well as the long-term effects of viral eradication on MC. *Conclusion:* MC is a negative prognostic factor of virological response. Clearance of HCV led to persistent resolution or improvement of MCS, strongly suggesting the need for a next generation of highly effective antiviral drugs. (HEPATOLOGY 2015;61:1145-1153)

Mixed cryoglobulinemia (MC) is an autoimmune/lymphoproliferative disorder characterized by circulating immune complexes named cryoglobulins (CGs) that reversibly precipitate at low temperatures. The CGs are comprised of polyclonal immunoglobulin Gs (including anti–hepatitis C virus [HCV] immunoglobulin) and mono- or polyclonal immunoglobulin M with rheumatoid factor activity, sustained by the clonal expansion of rheumatoid factor B cells.[1-5] The clinical manifestations characterizing symptomatic MC (mixed cryoglobulinemia syndrome [MCS]) are secondary to systemic vasculitis of the small/medium vessels.[5,6]

Infection with HCV is present in 80%-90% of patients with MC.[4,7-9] Patients who are HCV-positive

Abbreviations: CG, cryoglobulin; EVR, early virological response; HCV-RNA, hepatitis C virus RNA; ITT, intention to treat; MaSVE, Center for the Systemic Manifestations of Hepatitis Viruses; MC, mixed cryoglobulinemia; MCS, mixed cryoglobulinemia syndrome; NHL, non-Hodgkin's lymphoma; PEG-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid virological response; SVR, sustained virological response.

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vary in geographical region, with a gradient from north to south. In most studies, 40%-60% of HCV patients show circulating CGs. The prevalence of MCS in the nonselected HCV population was reported to be less than 1% in North America and 2%-5% in some southern European countries. Interestingly, in some centers, including the Center for the Systemic Manifestations of Hepatitis Viruses (MaSVE), the percentage of MC-HCV patients with symptomatic MC vasculitis was up to 30%.[4,10,11] Although clinically benign, MC is a lymphoproliferative disorder that predisposes to B cell non-Hodgkin's lymphoma (NHL) in about 5%-10% of cases.[4,5] In fact, the overall risk of NHL in patients with MC is about 35 times higher than that in the general population.[12] Since HCV infects about 170 million individuals worldwide, the number of patients at risk for MC and its complications is substantial.

The close association between MC and HCV dramatically modifies the therapeutic approach with the introduction of an etiological perspective.[13] The improvement or resolution of MCS in some patients after HCV eradication suggests such a therapeutic approach as the first option in the treatment of HCV-related mild to moderate MCS.[14-18] Since MC is an elusive condition, there is a chance that retrospective studies may actually include MC patients in the negative control group, meaning that prospective studies would be useful. Those performed so far are frequently characterized by their retrospective nature, limited size of populations, absence of appropriate controls, variability in therapeutic protocols, and limited posttreatment follow-up.[14] This hampers a correct evaluation of the influence of MC (symptomatic or not) on the virological response to anti-HCV treatments.

Therefore, the aim of this study was to prospectively analyze a large cohort of HCV patients with or without MC, symptomatic or not, treated with pegylated interferon (PEG-IFN) and ribavirin (RBV) according to standard criteria in order to evaluate (1) whether the presence of MC influences the virological response and (2) the long-term effects of sustained virological response (SVR) on MCS.

Patients and Methods

Study Design. This was a prospective, open-label, controlled cohort study.

Patients. Patients were referred to the outpatient clinic of the MaSVE, University of Florence, Italy, and prospectively entered the study according to the following inclusion criteria: detectable levels of serum HCV-RNA and eligibility for antiviral treatment with PEG-IFN and RBV according to the international standard of care.[19,20] Demographic information, treatment history, HCV genotype data, and laboratory evaluations were obtained from the records of the MaSVE outpatient clinic.

Patients were grouped into three different cohorts: (1) MCS-HCV group, patients with active cryoglobulinemic vasculitis ("definite" MCS);[21] (2) MC-HCV group, patients with circulating CGs but without MCS; (3) HCV group (control), patients without MC or any other autoimmune/lymphoproliferative disorder. Subjects with uncertain classification were excluded from the study. Exclusion criteria also included severe cryoglobulinemic vasculitis (i.e., progressive renal involvement, mononeuritis multiplex, skin ulcer or distal necrosis, rapidly progressive nephritis, motor neuropathy, digestive and/or pulmonary involvement, and other life-threatening complications), according to current Italian guidelines.[14]

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The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the local ethics committee. Patients gave written informed consent.

Infection with HCV was proven by detecting circulating anti-HCV antibodies (EIA-2 and RIBA-2; Ortho Diagnostic Systems, Raritan, NJ) and HCV-RNA (AMPLICOR HCV Test, v2.0; Roche Diagnostics, Alameda, CA). The HCV genotype was determined by a diagnostic test (VERSANT HCV Genotype 2.0; Siemens Healthcare Diagnostics, Deerfield, IL).

Mixed cryoglobulinemia was assessed by circulating CGs found in at least three metachronous samples. All patients with MCS satisfied available classification criteria.[22,23] Both clinical and laboratory parameters (including CG levels and characterization, complement fraction levels, rheumatoid factor, and autoantibodies) were evaluated according to standard methodologies as previously described.[24,25]

Treatment. We administered PEG-IFN alpha-2a (180 μg) or alpha-2b (1.5 μg/kg) in combination with RBV (800-1200 mg, weight-based dose).[19,20] In no case did patients receive corticosteroids or other drugs usually administered for MCS.[14] Antiviral therapy was considered complete when patients observed the 80/80/80 rule (continued prescription of at least 80% of IFN doses and 80% of RBV doses for at least 80% of the planned treatment duration), referred to as the gold standard of HCV treatment adherence.[26]

Efficacy Assessments. All patients were evaluated for the main hepatovirological and clinical–immunological parameters at least every 3 months during the treatment and every 6 months during the posttreatment follow-up. Analysis of clinical and virological efficacy included all patients who received one or more dose of study medication (intent to treat [ITT]). Patients with missing values were considered nonresponders. For ethical reasons, symptomatic MC patients who were nonresponders were treated with different therapeutic options, including the use of rituximab (alone or in combination with antiviral therapy [AT]), plasma exchange, corticosteroids, as well as, more recently, new AT (direct acting antiviral) and, consequently, no longer part of the follow-up study.

Hepatovirological Efficacy. To assess the hepatovirological efficacy, serum HCV-RNA was determined at regular intervals during the study. The primary end point was the evaluation of SVR, defined as undetectable serum HCV-RNA levels 24 weeks after treatment cessation. Determination of HCV viremia at weeks 4 and 12 allowed evaluation of rapid virological response (RVR) and early virological response (EVR).[19]

Liver disease severity was evaluated at least twice by transient elastography, according to several studies.[27] Briefly, liver stiffness values were measured using FibroScan (Echosens, Paris, France) and reflected the METAVIR fibrosis stage, according to published cutoffs for absent, significant, and severe fibrosis as well as cirrhosis.[28]

Genotyping of IL28B was performed as described[29]; all genotyping results were consistent with the Hardy-Weinberg equilibrium.

Clinical–Immunological Efficacy. The main MC-related parameters were evaluated as previously described to assess the clinical–immunological efficacy in MCS patients.[21,30,31] A complete clinical response was defined as improvement in all baseline clinical manifestations and a partial clinical response, as improvement in at least half of the baseline symptoms. All other patients were classified as clinical nonresponders. Arthralgia and neuropathy, including paresthesia/pain and clinically evident motor deficit, were measured through a patient-scored visual analog scale (range 0-100). Renal function was evaluated according to serum creatinine and proteinuria/24 hours. A complete response was defined as the combination of normalization of renal function when abnormal (serum creatinine) and proteinuria of 0.5 g/day or less. A partial response was defined as a stable or improved renal function and/or a reduction of at least 50% of proteinuria. No response was defined as worsening of renal function not attributable to other causes and/or proteinuria increase or a reduction insufficient for the definition of complete or partial response.

Statistical Methods. Continuous variables were summarized by descriptive statistics, and categorical variables were summarized using patient counts and percentages. Comparisons between groups were carried out using the chi-squared test or, where appropriate, Fisher's exact test for qualitative variables and analysis of variance for quantitative variables. Logistic regression analysis was used to assess the factors associated with SVR; odds ratios with 95% confidence intervals were derived from the model using Wald's method.

Patients with a missing HCV-RNA value for any reason at the end of follow-up were considered to be nonresponders.

Data analyses were performed on the ITT population. Virological response analysis was also repeated in the per protocol population—defined as all patients who completed the treatment—as confirmation of the ITT population results.

All statistical tests were performed at the $P \leq 0.05$ level (two-sided). Statistical analyses were carried out using the SAS System, version 9.2.

Table 1. Patient Baseline Characteristics: Values Are Expressed as Mean ± Standard Deviation

	HCV (n = 158)	MC-HCV (n = 132)	MCS-HCV (n = 121)	*P*
Mean age (years)	49.3 ± 13.3	51.1 ± 12.9	55.0 ± 10.4	0.0002 HCV vs. MCS-HCV0.0131 MC-HCV vs. MCS-HCV
Sex (male/female)	112/46	64/68	45/76	<0.0001 HCV vs. MC/MCS-HCV
Histology				
Chronic hepatitis (%)	128 (81)	102 (78)	83 (68.6)	0.02 HCV vs. MCS-HCV
Cirrhosis (%)	28 (17.7)	28 (21.2)	35 (28.9)	
nd (%)	2 (1.3)	2 (0.8)	3 (2.5)	
Number of treatments (SoC)	142 (89.9)	106 (80.3)	96 (79.3)	0.014 HCV vs. MCS-HCV
1 (%)	16 (10.1)	26 (19.7)	25 (20.7)	0.018 HCV vs. MC-HCV
>1 (%)				
ALT (ULN)	3.85 ± 2.4	3.62 ± 3.8	3.43 ± 2.2	ns
Viral titer (IU/mL × 10^6)	2.4 ± 4.9	2.5 ± 4.7	2.5 ± 5.01	ns
HCV genotype 1 (%)	78 (49.4)	73 (55.3)	55 (45.5)	ns
2 (%)	49 (31.0)	30 (22.7)	43 (35.5)	
3 (%)	23 (14.5)	21 (15.9)	17 (14.0)	
4 (%)	8 (5.1)	6 (4.5)	3 (2.5)	
5 (%)	–	1 (0.8)	–	
nd (%)	–	1 (0.8)	3 (2.5)	
Mean cryocrit (%)	0	3.5 ± 5.4	8.2 ± 7.2	<0.0005 HCV vs. MC/MCS-HCV
Mean C3* (mg/dL)	115.3 ± 63.2	109.5 ± 58.9	104.5 ± 61.5	ns
Mean C4† (mg/dL)	91.6 ± 45.7	13.7 ± 27.5	10.5 ± 11.3	<0.0005 HCV vs. MC/MCS-HCV
Mean RF‡ (IU/mL)	16.7 ± 8.0	226.3 ± 155.3	380.5 ± 292.4	<0.0005 HCV vs. MC/MCS-HCV

*Complement C3, normal values 83-177 mg/dL.
†Complement C4, normal values 20-150 mg/dL.
‡Rheumatoid factor, normal values <25 IU/mL.
Abbreviations: SoC, standard of care; ALT, alanine aminotransferase; ULN, upper limit of normal; ns, not significant; IU, international units; nd, not determined; HCV, hepatitis C virus; MC, mixed cryoglobulinemia; MCS, mixed cryoglobulinemia syndrome.

Results

Baseline Characteristics of Patients. From July 2003 to July 2010, 424 HCV-infected Caucasian patients (226 males, mean age 51.3±12.6 years) referred to the MaSVE outpatient clinic were prospectively recruited according to the inclusion criteria. Patients were enrolled in the different cohorts as follows: (1) 121 patients (45 [37.2%] males, mean age 55.0 ± 10.4 years) in the MCS-HCV group; (2) 132 patients (64 [48.4%] males, mean age 51.1 ± 12.8 years) in the MC-HCV group; and (3) 158 patients (112 [70.9%] males, mean age 49.3 ± 13.3 years) in the HCV group. Thirteen patients were excluded for uncertain classification.

The mean duration of HCV infection was approximately 169 months (range 75-366 months).

Post-treatment follow-up ranged from 35 to 124 months (mean 92.5 months).

Out of 411 patients, 67 (16.3%) experienced at least one previous antiviral treatment and were relapsed, nonresponders, or breakthrough (treatment-experienced patients) (Table 1).

Baseline characteristics of the 411 enrolled patients are summarized in Table 1. The most relevant clinical MCS manifestations were purpura, arthralgia, and weakness (Meltzer and Franklin triad). According to the exclusion criteria, no patients had severe or life-threatening MC vasculitis (inclusion of only mild/moderate MCS).[14] The number of MCS patients who during the study period were excluded due to a severe MC vasculitis was comparable to the number of included patients and represented by subjects requiring also treatment with rituximab and/or plasma exchange and/or other immunomodulating therapies (data not shown).

The study groups were comparable as to viremia titers, viral genotype, or IL28B single-nucleotide polymorphism (rs12979860) allele distribution, whereas, as expected, female sex was significantly more represented in the MCS-HCV and MC-HCV groups ($P < 0.001$). The MCS patients were older and had more severe liver disease (Table 1). The mean duration of MCS was 85 months (range 24-170 months). There was a higher number of treatment-experienced patients in the MCS and MC groups than in controls ($P = 0.014$ and $P = 0.018$, respectively).

Virological Response. The rates of virological response in the ITT analysis are outlined in Fig. 1. Univariate analysis indicated that SVR rates were lower in patients with cryoglobulinemia than in those without. There was a significant difference in SVR rates

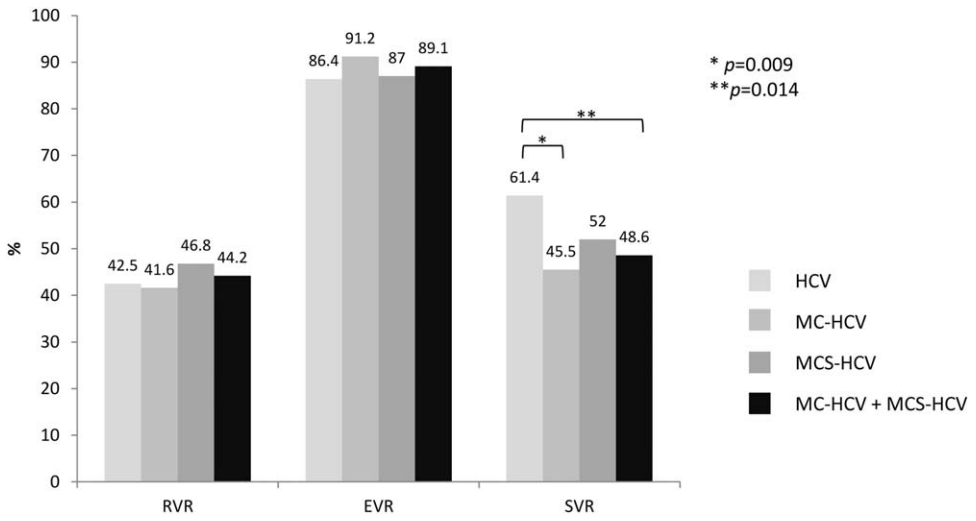

Fig. 1. Rates of virological responses in the intention-to-treat population.

between HCV and MC-HCV patients and between HCV and MC-HCV+MCS-HCV patients (Fig. 1). Differences in rates of RVR and EVR (both complete and partial EVR) in the three groups did not reach statistical significance.

None of the patients who had not achieved EVR reached SVR. As expected, a significant association in each group was found between the RVR and EVR rates and HCV genotype (genotype 1 or 4 versus genotype 2 or 3, $P < 0.0001$).

Other factors previously shown to have predictive value for SVR were evaluated, including sex, age (<50 versus ≥50 years), HCV genotype (genotype 1 and 4 versus genotype 2 or 3), liver disease severity (chronic hepatitis versus cirrhosis), IL28B genotype (C/C versus C/T+T/T), HCV-RNA level (<500,000 versus ≥500,000 IU/mL), and previous antiviral treatment (naive versus experienced). Significantly lower SVR rates were observed in patients infected with HCV genotype 1 or 4, with severe liver disease, with unfavorable genotype of IL28B (C/T or T/T), and with previous antiviral treatments ($P < 0.0001$). Furthermore, patients over 50 years old or with an HCV-RNA level >500,000 IU/mL showed lower rates of SVR ($P = 0.010$ and $P = 0.0002$, respectively) (Table 2). The mean duration or total cumulative dose of IFN or ribavirin did not differ significantly according to response (data not shown).

Multivariate logistic regression analysis identified the presence of cryoglobulinemia as an independent prognostic factor of nonresponse to antiviral therapy. This was obtained when considering MCS patients only and MC+MCS patients. The predictive value of previously identified factors of response was confirmed, and it included viral genotype, severity of liver disease, IL28B genotype, viral load, and previous anti-HCV treatment (Table 3).

We also performed a per protocol analysis, in which we included only patients who completed the therapy, were SVR, or experienced a breakthrough, nonresponse, or relapse. We therefore excluded from the study 19 HCV, 23 MC-HCV, and 10 MCS-HCV patients who did not complete the therapy because of dropout or adverse events. The rates of virological response according to per protocol analysis are presented in Supporting Information Fig. S1.

No statistically significant differences were observed among the groups for RVR or EVR rates, but a significantly higher rate of SVR was observed in HCV patients (69.8%) when compared to MC-HCV (55%, $P = 0.017$) and MCS-HCV (56.8%, $P = 0.033$) patients.

Clinical–Immunological Response in MCS Patients. The clinical–immunological response in MCS patients was strictly related to the virological one. The main clinical and laboratory features of 63

Table 2. Results of Univariate Analyses Examining Effects of Negative Prognostic Factors on Sustained Virologic Response, According to Intention-To-Treat Analysis

Factors	P
Presence of cryoglobulinemia	0.014
Male sex	0.469
Age ≥50 years	0.010
HCV genotypes 1 and 4	<0.0001
Cirrhosis	<0.0001
C/T or T/T IL28B genotype	<0.0001
HCV RNA level >500,000 IU	0.0002
Previous anti-HCV treatment	<0.0001

Table 3. Multivariate Logistic Regression Analysis of Negative Predictive Factors of Response to Anti-HCV Therapy

	HCV vs. MCS-HCV			HCV vs. MC-HCV+MCS-HCV		
Factors	**Odds Ratio**	**95% CI**	***P***	**Odds Ratio**	**95% CI**	***P***
Age ≥50 years	1.33	0.65-2.72	0.4376	1.62	0.91-2.89	0.0995
Male sex	1.02	0.48-2.17	0.9613	1.22	0.68-2.20	0.5064
HCV genotypes 1 and 4	7.23	3.59-14.56	<0.0001	8.63	4.82-15.48	<0.0001
Cirrhosis	2.61	1.19-5.72	0.0167	3.41	1.74-6.67	0.0003
C/T or T/T IL28B genotype	4.10	2.04-9.12	<0.0001	4.17	2.34-7.41	<0.0001
HCV RNA level >500,000 IU	3.86	1.76-8.45	0.0007	3.64	1.94-6.84	<0.0001
Previous anti-HCV treatment	3.41	1.28-9.12	0.0143	2.50	1.14-5.47	0.0217
Presence of cryoglobulinemia	2.25	1.07-4.73	0.0139	2.03	1.12-3.68	0.0204

SVR MCS patients, before treatment and at the end of a 6-month follow-up, are summarized in Table 4. All MCS patients who experienced SVR also experienced a complete clinical response except for two. This response was maintained during the entire follow-up period. There were 36 SVR patients (57%) who showed a complete and persistent disappearance of all initial MCS signs and symptoms; 25 patients (40%) had milder and usually isolated symptoms or signs, but a progressive worsening did not occur in any of them. A "definite" MCS persisted in the remaining two (3%) patients, though it was milder than before the treatment. Both these patients were characterized by a long-lasting vasculitis, previously treated for more than two decades with various combinations of nonetiological therapies without consistent results. All MCS patients who did not achieve SVR were clinical nonresponders. Transient improvement in MCS was observed together with a viremia decrease in some of these patients.

Patients with MCS were characterized by more frequent adverse events than controls. In fact, at least one adverse event was observed in 35 (28.9%) MCS-HCV patients and in 17 (10.7%) HCV patients ($P = 0.009$). These adverse events were mostly hematological, especially anemia (24 [19.8%] MCS-HCV versus 14 [8.9%] HCV, $P = 0.06$) and neutropenia (16 [13.2%] in MCS-HCV versus 11 [6.9%] in HCV, $P = 0.2$). Other adverse events were more frequent in the HCV-MCS group than in controls but did not reach statistical significance, including pruritus (8 [6.6%] MCS versus 5 [3.2%] HCV), depression (7 [5.8%] MCS-HCV versus 4 [2.5%] HCV), and weight loss (7 [5.8%] MCS-HCV versus 4 [2.5%] HCV). No patients died as a result of therapy, and no significant differences were observed between the MCS-HCV and HCV groups regarding dropout rates due to intolerance (data not shown). No SVR patients evolved to NHL during the long-term follow-up after therapy.

Discussion

Since the early 1990s, several studies have shown the close correlation between virological and clinical response in HCV-related MC. However, these studies frequently used modified antiviral protocols and did not include adequate numbers of patients and/or controls. Furthermore, follow-up was often short. To the best of our knowledge, the response of patients with definite MCS has never been compared to that of patients with MC and without MC/MCS. These limitations were justified by the rarity of MCS, suggesting the need for long-term, prospective studies.

In the present study, for the first time, a very large population of MC patients with or without symptoms was consecutively enrolled over a decade and the effects of antiviral treatment were compared with those observed in a control population of HCV patients without MC or other autoimmune/lymphoproliferative disorders; all patients were treated with the same protocol. The unique design of the study provides a

Table 4. Main Mixed Cryoglobulinemia Syndrome Manifestations (Clinical and Laboratory) Diagnosed Before Treatment and at the End of a 6-Month Follow-Up in the 63 MCS-HCV Patients Who Achieved a Sustained Virological Response

MCS Manifestations	Pretreatment (%)	End of Follow-up (%)
Clinical		
Purpura	48 (78.6)	2 (3.3)
Arthralgias	51 (83.6)	7 (11.4)
Weakness	55 (90.1)	13 (21.1)
Neuropathic symptoms	46 (75.5)	8 (13.1)
Renal involvement	9 (14.7)	0
Skin ulcers	8 (13.1)	0
Sicca syndrome	28 (45.9)	11 (18.0)
Laboratory		
Cryoglobulins	61 (100)	2 (3.3)
Rheumatoid factor*	59 (96.7)	19 (31.1)
Reduced C4†	53 (86.8)	6 (9.8)

*Elevated rheumatoid factor upper normal value (<25 IU/mL).
†Complement C4 levels below normal values (20-150 mg/dL).

prospective evaluation of both the virological and the clinical–immunological responses. The prospective approach and long-term follow-up made the inclusion in each cohort very reliable, as needed for a correctly controlled analysis.

The usefulness of anti-HCV therapy in MC has been widely discussed. Early studies using IFN monotherapy showed that, in spite of a clinical–immunological response during treatment, both infection and vasculitis generally relapsed after treatment.[32-35] The MC response improved with the increased efficacy of antiviral therapy, passing from recombinant IFN[32] to PEG-IFN plus RBV. This latter combination, in some studies, was able to lead to a clinical response ranging from 62.5% to 78% of patients,[17,36] suggesting that it should be the first therapeutic option for patients with HCV-MCS.[14]

In our study, the majority of patients with MCS who reached SVR also experienced a complete and persistent clinical–immunological response according to the study criteria. All symptoms and laboratory alterations (i.e., cryocrit, rheumatoid factor, C4 consumption) disappeared in most of the MCS-SVR patients; the remaining MCS patients had only isolated symptoms (i.e., sicca syndrome, peripheral paresthesia, arthralgia) and/or laboratory data altered, although improved compared to baseline. Only two MCS-SVR patients (3%) maintained a definite syndrome according to the established criteria,[23] although all the clinical manifestations improved compared to initial symptoms.

On the contrary, none of the patients who did not achieve SVR experienced persistent improvement in the syndrome during follow-up, despite transient improvement seen in some subjects at the end of therapy, probably due to the antiproliferative effect of IFN.

None of the SVR patients developed a frank lymphoma during the long follow-up. This observation is meaningful, considering that it was previously demonstrated that MC patients have a 35-fold increased risk of developing NHL compared to the general population.[12] A Japanese study[37] reported a 2.6% rate of NHL development after a 15-year follow-up in a cohort of chronically HCV-infected patients who underwent IFN-based treatment and did not achieve viral eradication; this rate dropped to 0% in SVR patients treated with the same protocol. Even if not designed for this kind of evaluation, our study confirms the observation of the Japanese study. In our study, we could not compare the evolution to malignancy between SVR and non-SVR MCS patients since non-SVR MCS patients underwent other treatments.

Our data also indicate that the probability of viral eradication in patients with MC varies when compared with controls without MC. The multivariate logistic analysis considering the main factors already identified as influencing the virological response in HCV patients indicated the presence of cryoglobulinemia as an independent prognostic factor of nonresponse to antiviral therapy. Interestingly, this was obtained when considering both only MCS patients and MC+MCS patients. Finally, the per protocol analysis also showed a significantly higher rate of SVR in HCV patients when compared to both MC-HCV and MCS-HCV ones. This shows that the difference in SVR rates cannot be completely attributed to a lower tolerance in MC patients to IFN-based therapy. A possible explanation is that these subjects' resistance to IFN could be attributed to a higher involvement of the lymphatic compartment by the viral infection itself. This has been shown in several studies, ranging from the initial demonstration of higher rates of peripheral blood mononuclear cell infection[38] and of bone marrow mononuclear cells in MC.[39] In addition, although HCV can be completely eradicated with antiviral therapy, a longer persistence in peripheral blood mononuclear cells has been demonstrated in numerous studies.[4,40,41] Furthermore, a major involvement of B cells by viral infection was shown, and several studies have suggested the compartmentalization of viral quasi-species in peripheral blood mononuclear cells,[42] with consequent higher diversification of viral sequences, which is inversely correlated with the sensitivity to IFN-mediated eradication.[43,44] The hypothesis of the key role played by a longer persistence of HCV in lymphatic reservoirs also agrees with the lack of different serum HCV-RNA early kinetics in the different groups, in spite of significantly different SVR rates. Other factors that possibly play a role include high levels in HCV-MC patients of some chemokines[45] previously shown to be correlated with a reduced ability to respond to IFN-based therapy, such as CXCL10,[46,47] whose levels have been shown to be higher in these patients compared to healthy controls, mostly in the presence of active vasculitis.[48]

Patients with MC also experienced adverse events more frequently than controls, especially hematological ones, thus confirming previous observations.[49] However, in our population, these adverse events were never very severe or fatal, and their occurrence did not significantly affect the dropout rate when compared to controls. The differences with previous reports were probably related to the exclusion from treatment of patients with severe vasculitis.

Interestingly, none of our patients who did not experience SVR and maintained HCV infection had a long-

lasting remission of symptoms, showing that complete viral eradication is required for permanent improvement or disappearance of the disease. This was an additional confirmation of the strict correlation between viral eradication and clinical response in MCS. A previous study showed a higher percentage of clinical–immunological than virological responses[49]; but in this case the follow-up was only 6 months, and it is conceivable that the antiproliferative effect of IFN per se as well as the transient inhibition of viral replication explain this transient improvement. The use of the new potent direct acting antivirals in such patients appears to be very promising, as also suggested by pioneer studies.[15,50]

In conclusion, this wide, prospective, and controlled study for the first time provides an evaluation of the long-term effects of viral eradication on HCV-MC patients as well as a comparison of the effects of PEG-IFN+RBV therapy on HCV patients with MCS, MC, and without MC (clinical and/or laboratory symptoms). Our observations confirm that (1) the great majority of patients with MCS achieving SVR also experience sustained clinical and immunological response and the persistence of MCS in SVR patients is a rare event (most of these patients persistently lost all previous signs and symptoms of the syndrome, and the remaining patients had milder, generally isolated symptoms, with no patients evolving to lymphoma); (2) MC patients were less likely to respond virologically to anti-HCV therapy than patients without MC; (3) MC patients experienced hematological side effects more frequently than MC-negative controls; and 4) persistent remission of MCS was never observed in MC patients who had not achieved SVR.

On the whole, this study definitively confirms the key importance of viral eradication in allowing persistent resolution or consistent improvement of HCV-MCS, strongly suggesting the need for a next generation of highly effective antiviral drugs.

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