Long-term effects of the new direct antiviral agents (DAAs) therapy for HCV-related mixed cryoglobulinaemia without renal involvement: a multicentre open-label study

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ABSTRACT

Objective. *To investigate the long-term effects and safety of new direct antiviral agents (DAAs) in patients with hepatitis C virus (HCV)-related mixed cryoglobulinaemia (MC) without renal involvement.*

Methods. *The study enrolled 22 consecutive patients, 19 received sofosbuvir-based regimen and three patients received other DAAs, individually tailored according to latest guidelines. As of December 2016, the median length of follow-up was 17 months (range 13-21).*

Results. *Extra-hepatic manifestations at enrollment were: purpura and arthralgia (12 cases), peripheral neuropathy (10 cases) and marginal zone B-lymphomas (2 cases). After a four-week DAA therapy, all patients became HCV-negative. Moreover, after 48 weeks since the beginning of DAA treatment, sustained regression of purpura and arthralgias was observed respectively in eight and in nine cases; peripheral neuropathy improved in seven cases, and cryocrit median values decreased from three (1-20) at baseline to two (1-12) after 48 weeks. Two cases with indolent marginal zone lymphomas did not show any haematological response: size and number of the involved nodes remained unchanged. In addition, the monoclonal B-cell population found in the peripheral blood in four cases did not disappear after recovery from HCV-RNA. Mild side effects occurred in nine patients, but six patients developed ribavirin-related anaemia requiring reduction of ribavirin dose.*

Conclusion. *DAA therapy is safe and effective to eradicate HCV in MC, but seems associated with satisfactory clinical response in mild or moderate cryoglobulinaemic vasculitis and no response in B-NHL.*

Introduction

It is well-known that hepatitis C virus (HCV) can determine not only acute and chronic hepatitis but also several extra-hepatic disorders, including autoimmune diseases, mixed cryoglobulinaemia (MC) and non-Hodgkin lymphomas (NHL) (1, 2). A large body of clinical, epidemiological, biological, and molecular data suggests the association between HCV and monoclonal lymphoproliferative disorders and supports a causal relationship (3-6). Many authors have demonstrated that both MC and NHL could disappear together with HCV after antiviral therapy, though with a response rate largely unsatisfactory as compared to HCV cases without extra-hepatic manifestations (7-10). Antiviral therapies have significantly improved in recent years; the association of pegylated interferon (Peg-IFN) and ribavirin (RBV), able to eradicate the viral infection in near 50% of the patients, has now been abandoned after the introduction of the new direct antiviral agents (DAAs) (11-13). Several studies published on these new DAAs have reported a remarkable eradication rate, ranging from 90% to 100% regardless of HCV genotype (14, 15). Presently, numerous published reports have documented novel DAAs regimens showing a comparable efficacy in patients with MC concerning viral eradication, while a definite clinical improvement has been observed only in a fraction of patients who obtained a sustained virological response (SVR) (16-23).

A clinical assessment of MC patients is often difficult because a fraction of them continues to assume steroids or other immunosuppressive agents and because of the considerable clinical heterogeneity of the disease, showing

several pathological conditions and multi-organ involvement within the same patient. Furthermore, the follow-up of these cases is challenging, involving several specialists, and each haematological or hepatological centre enrols only few cases, which prevents carrying out randomised trials. The American Association for the Study of Liver Diseases as well as the European Association for the Study of the Liver (EASL) suggest the use of the new DAAs for treatment of patients affected by symptomatic MC even in absence of a chronic liver disease (24, 25). According to these suggestions, we treated a group of patients affected by HCV-associated MC vasculitis with DAAs therapy individually tailored based on previous treatment(s), HCV-genotype, and severity of the underlying liver disease. Safety, biological and clinical efficacy, and virological response were evaluated during a 48-week follow-up.

Materials and methods

Twenty-two consecutive patients affected by HCV-related MC were included in this study. All cases were enrolled between February 2015 and November 2015 at the Clinical and Experimental Onco-Haematology Unit, CRO Aviano; the Department of Internal Medicine, Pordenone General Hospital; Rheumatology Clinic, Department of Medical and Biological Sciences, University of Udine, Department of Internal Medicine, University of Bologna and Department of Clinical and Surgical Sciences, University of Trieste, Italy. The inclusion criterion of this observational study was the presence of detectable serum levels of HCV-RNA associated with MC usual clinical symptoms (*i.e.,* purpura, asthenia, arthralgia) and with measurable levels of cryoglobulins (cryocrit >1% on at least two occasions). All patients who tested positive to hepatitis B surface antigen (HBsAg) or anti-human immune deficiency virus (HIV) antibodies were excluded. Since all cases met the eligibility criteria for treatment with DAAs according to the Italian Medicine Agency indications (Agenzia Italiana del Farmaco, AIFA: http://www.agenziafarmaco.gov.it), treatments were administered on-label, therefore not requiring ethical approval. Since MC patients with renal involvement (*i.e.*, cases with nephrotic-range proteinuria) were followed at another Department, they were not included in this paper, but will be described in a forthcoming publication.

Patients' clinical and biological data were recorded at baseline, every four weeks until the end of treatment (EOT, either 12 or 24 weeks), and every two months after EOT. All laboratory testing were carried out using standard methods, including liver function, kidney function, haematological parameters, determination of complement components, rheumatoid factor (RF), and cryoglobulin serum levels. MC was classified as Type II in presence of monoclonal IgM complex with polyclonal IgG, and Type III in presence of polyclonal immunoglobulins. Liver biopsies had been previously performed (often many years earlier) in a small fraction of the patients showing clinical, biochemical and ultrasonographic signs of chronic liver disease. However, the grading for liver fibrosis was checked in all cases by liver elastography using FibroScan before entering this study. We considered a patient as a carrier of liver cirrhosis based on liver biopsy. In absence of a liver biopsy, presence of two of the following findings were considered indicative of liver cirrhosis: 1) a platelet count <100.000/mmc; 2) presence of oesophageal varices on oesophagogastroduodenoscopy; 3) evidence of cirrhosis and/or portal hypertension and/or ascites by imaging studies; 4) liver elastography (FibroScan) compatible with stage 4 fibrosis (26, 27).

The peripheral blood flow cytometric analysis verifying the presence of monoclonal B-cell population was performed at baseline, at EOT, and at the 48th week after the EOT. The multiparameter analysis, based on antigen expression and morphologic properties defined by forward and side scatter, was performed with an FACSCalibur (BD Biosciences) flow cytometer with standard software in use in each laboratory. B-cell malignancy was diagnosed in presence of an abnormal pattern of antigen expression and light chain restriction (28).

Peripheral neuropathy was determined by electromyography. Long duration, large amplitude and polyphasic motor unit potentials were considered as diagnostic parameters for chronic axonal neuropathies.

Treatment

Nineteen patients were treated with different sofosbuvir-based DAAs therapy and three patients received other DAAs (Table I) according to EASL and AIFA recommendations (25). Drug dosages were: sofosbuvir 400 mg daily, simeprevir 150 mg daily, ledipasvir 90 mg daily, ombitasvir, paritaprevir, ritonavir 12.5/75/50 mg co-formulated tablet daily and dasabuvir 250 mg daily, ribavirin A 800-1200 mg daily based on body weight.

Table II shows details on treatment schedules. The administration of anti-CD20 antibodies or other immunosuppressive therapy during and after antiviral therapy was not allowed, but one patient with severe purpura (grade 3) underwent steroids therapy during the antiviral treatment and follow-up. The responses were evaluated at EOT (12th or 24th week), and at the 24th and 48th week after EOT (29).

Outcome measures

As previously reported (30), the following four types of response to treatment were initially defined (*i.e.,* virological, biochemical, immunological, and clinical). Virological response, *i.e.,* the effect of treatment on HCV-RNA. SVR12: loss of HCV-RNA at 12 weeks and SVR24 loss of HCV-RNA at 24 weeks. Relapse: loss of HCV-RNA at EOT but positivity at the 24th or 48th week after EOT. No response: persistent positivity of HCV-RNA during therapy and follow-up. Biochemical response, *i.e.*, the effect of therapy on alanine aminotransferase (ALT); 0-36 IU/L was considered normal value. Complete response, *i.e.,* normalisation of the serum ALT level during treatment followed by normal ALT values lasting for 24 weeks after discontinuation of therapy. No response: ALT out of normal value during treatment and follow-up. Relapse: normalisation of the serum ALT level during treatment followed by the return

Table I. Baseline, clinical, biochemical and histological features of 22 patients with HCV-related mixed cryoglobulinaemia.

Parameter (NV)	Median (Range)	n (%)
Males		11 (50)
Age, years	69 39-74)	
HCV genotype		
1a		1 (5)
1b		11 (50)
2		6 (27)
3		2 (9)
4		2 (9)
HCV-RNA (IU/ml)	629,100 (20,200-10,006,995)	
AST (0-36 U/L)	35 (20-197)	
ALT (0-45 U/L)	72 (12-173)	
Creatinine (0.6-1.0 mg/dl)	1.0 (0.6-1.3)	
Type II cryoglobulinaemia		17 (77)
Cryocrit (%)	3 (1-20)	
Rheumatoid factor (<25 IU/mL)	111 (20-1120)	
C4 (10-40 mg/dl)	7 (1-17)	
Clinical manifestations		
Purpura		12 (55)
Arthralgias		12 (55)
Peripheral neuropathy		10 (45)
Raynaud's phenomenon		4 (18)
Sicca syndrome		2 (9)
Non-Hodgkin lymphoma		2 (9)
Metavir liver fibrosis score		
F 1		3 (14)
F 2		4 (18)
F 3		2 (9)
F 4		13 (59)
ChildPugh-score (A/B)		13/1 (93/7)
Previous antiviral treatment response:		
Naïve		14 (64)
No response		3 (14)
Relapse		5 (22)
DAA treatment		
Sofosbuvir based regiment		19 (86)
Other DAAs regimens		3 (14)

C4: complement component four; ALT: alanino-aminostrasferase; NV normal value.

to abnormal values during follow-up. In some patients, this parameter was not considered because of normal ALT level at the beginning of treatment. Immune response, *i.e.*, the effect of therapy on serum RF concentration, C4 and cryocrit level. Complete response: normalisation of serum RF concentration, disappearance of circulating cryoglobulins and return of C4 to normal levels. Partial response: reduction (but not normalisation) of RF and cryoglobulins >50%, and C4 under normal levels. No response: reduction of RF and cryocrit levels below 50% or stable levels of all parameters including C4. Relapse: partial or complete normalisation of serum RF and cryoglobulins during therapy followed by the return to higher values during follow-up.

Clinical response, *i.e.*, the effect of therapy on clinical manifestations of the disease (including purpura, arthralgia and weakness). Complete response: the disappearance of all clinical signs of disease. Partial response: improvement of the clinical symptoms; reduction of the purpura score >50% and reduction of at least half of the baseline arthralgias and weakness. No response: improvement of fewer than half of baseline symptoms or stable disease. Relapse: partial or complete normalisation of clinical symptoms during therapy followed by the return to higher scores after EOT.

Follow-up

Biochemical, virological (HCV-RNA), immunological and clinical parameters were determined each month during therapy and every two months during follow-up, up to 48 weeks after the EOT. As of December 2016, the median length of follow-up was 17 months (range 13–21).

Statistical analysis

Descriptive statistics of relevant variables were performed using median and range values. Mean and standard deviation were poorly informative and not presented because of the limited number of patients.

Results

Patient characteristics

The main clinical, biochemical, histological characteristics of patients, and the distribution of HCV genotypes are shown in Tables I and II. The study enrolled 22 patients (11 males, 50%), median age 69 (range 39-–4 years) with HCV-related cryoglobulinaemic vasculitis. Twelve patients (55%) presented purpura on the legs, and 12 (55%) had arthralgia. Peripheral neuropathy was found in ten cases (45%), Raynaud's phenomenon in four cases (18%), sicca syndrome in two cases (9%), and indolent NHL (marginal zone lymphoma) in two cases (9%), both showing monoclonal lymphocytes in the bone marrow (therefore considered as pathological stage IV); one of them showed mediastinal pathological nodes and the other case showed several lumbo-aortic and mesenteric pathological nodes. Seventeen cases (77%) had type II MC, and five cases (23%) type III MC. Cryocrit ranged from 1% to 20% (median 3%), serum complement C4 levels were low in most cases (91%, median 7; range: 1–17 mg/dl), and serum levels of RF were high in 19 cases (86%), median 111 (range: 20–1120 IU/L). ALT was elevated in 15 cases (68 %) with median 72 U/L (range: 12–173 U/L), serum creatinine levels were normal (<1.0 mg/dl) in all cases. Three patients (14%) had stage 1 fibrosis, five patients (23%) had stage 2–3 fibrosis, and 14 (64%) had stage 4 fibrosis, four of them with portal hypertension (oesophageal varices). Four patients showed a monoclonal B-cell clone in peripheral blood. The immune phenotype of the clonal

Table II. Main clinical characteristics of 22 patients with HCV-related mixed cryoglobulinaemia and antiviral treatments.

Patients	Age/sex	HCV type	Clinical symptoms at baseline	Liver disease	Previous therapies	Previous IFN-based therapies	DAA therapy and weeks of treatment
1	73/F	2	Purpura, arthralgias, neuropathy	C	None	N	SOF+RBV, 24
2	69/F	2	Arthralgias	C	Steroids	REL	SOF+RBV,24
3	63/M	1b	Purpura, arthralgias	C	None	NR	SOF+SIM +RBV , 12
4	70/M	2	Purpura, arthralgias, neuropathy	C	Colchicine	N	SOF+RBV, 24
5	70/M	1b	Raynaud, sicca s.	C	None	REL	SOF+SIM +RBV, 12
6	55/M	2	Purpura, arthralgias, neuropathy	CH	Steroids, Cyclosporine	N	SOF+RBV,12
7	72/F	1b	Sicca s.	CH	None	N	PAR+OMB+RTV+DAS, 12
8	57/M	4c	Arthralgias, Raynaud	CH	Steroids	R	PAR+OMB+RTV+RBV, 12
9	70/F	4c	Raynaud	C	None	N	PAR+OMB+RTV+RBV, 24
10	55/M	3a	Arthralgias, neuropathy	C	None	N	SOF+RBV, 24
11	73/F	2	Arthralgias, neuropathy	CH	Steroids, Plasmaferesis, RTX	N	SOF+RBV, 12
12	69/M	1b	Purpura, arthralgias, neuropathy	CH	Steroids ,RTX	NR	SOF+SIM, 12
13	62/M	1b	Purpura, neuropathy	C	Steroids, Plasmaferesis, RTX	N	SOF+RBV, 24
14	74/F	2a/2c	Purpura, arthralgias	CH	Steroids, Colchicina Cyclosporine	N	SOF+RBV,12
15	63/F	1b	Neuropathy	CH	None	N	SOF+LED,12
16	54/F	3a	Purpura, arthralgias	CH	Steroids, Cyclosporine	N	SOF+RBV, 24
17	67/F	1b	Purpura, NHL	C	Steroids	N	SOF+RBV,24
18	39/M	1a	Purpura, arthralgias	C	Steroids	N	SOF+LED +RBV,12
19	70/M	1b	NHL	C	Steroids	N	SOF+ LED+RBV , 12
20	71/M	1b	Purpura	C	Steroids	NR	SOF+LED , 24
21	74/F	1b	Purpura, Raynaud, neuropathy	CH	None	REL	SOF+LED, 12
22	65/F	1b	Neuropathy	C	RTX	REL	SOF+SIM, 24

C: cirrhosis; CH: chronic hepatitis; NHL: non-Hodgkin's lymphoma; RTX: rituximab; N: treatment naïve; REL: Relapse; NR: non responder; SOF: sofosbuvir; RBV: ribavirin; SIM: simeprevir; PAR+OMB+RTV+DAS: Paritenavir+ Ombitasvir+ Ritonavir+ Dasabuvir: LED: ledipasvir.

B lymphocytes (CD3-; CD4-; CD5-; CD8-; CD10; CD19+; CD20+, CD22+; CD23-; CD43-; CD79a+; CiclinD1-) indicated the presence of circulating marginal zone lymphoma cells.

Previous treatments

Most cases had been previously treated with steroids due to purpura and arthralgias. Two patients received prednisone (50 mg/day progressively tapered) followed by plasmapheresis (six sessions) and, subsequently, rituximab (once weekly for four weeks, at 375 mg/sqm) for severe peripheral neuropathy. One case had been treated with corticosteroid (10 mg/day), colchicine (1 mg/day) and cyclosporine (18 mg/kg/day) for eight weeks due to a severe purpura on the leg and arthralgias. Fourteen patients (64%) were naïve for antiviral therapy, and the remaining eight cases (36%) were virological non-responders or relapsers to a previous Peg-IFN+RBV antiviral therapy. Details on treatment schedules with DAAs and main virological, hepatological and clinical data before treatment are provided in Table II.

Virological response to DAAs

The main treatment related data are summarised in Table III. All patients (100%) had undetectable HCV-RNA viremia after one month of therapy and remained undetectable until the 48th week of follow-up in all cases but one (5%), who relapsed four months after EOT. This patient had genotype 1b, was naïve for antiviral interferon-free regimens but showed a grade 4 liver fibrosis.

Clinical response to DAAs

At EOT, we observed improvement of the purpura, in seven patients (58%), and a complete regression of the purpura in eight patients (67%) at the 12th week. At the 48th week, the number of patients showing clinical improvement of the purpura remained unchanged. The behaviour of the arthralgias showed a different pattern, since nine cases (75%) obtained a fast relief from symptoms at EOT. At the 48th week, no additional patient showed improvement of arthralgias. Symptoms relief of peripheral neuropathy was observed in seven cases (50%) at EOT, and remained in complete response at the 12th and at the 48th week, while three cases did not show any improvement of neuropathic pain and paresthesias. Two cases with indolent NHL (marginal zone) did not show any clinical response (purpura and arthralgias) and any radiological improvement of number and size of nodes. At the 48th week, no improvement emerged for four cases (33%) of purpura, three (25%) of arthralgias, and three (33%) of neuropathy; therefore, they were considered as "non-responders." Based on the above-reported criteria, the remaining patients (75%) obtained a complete clinical response.

Immunological response

The treatment induced a reduction of cryocrit levels in 15 cases (68%), but only one (5%) showed undetectable levels of cryoglobulins 48 weeks after EOT. Among the seven other patients, the cryocrit remained unchanged at EOT, at the 24th and at the 48th week. The RF levels decreased in 16 patients (72%), but a normal RF level was observed in one case only at 24 weeks (5%). A restoration of C4 normal levels of the C4 to normal levels was noticed in four cases (18%) at 24 weeks. On the basis of the above-reported criteria, only one patient (5%) obtained the complete immunological response,

Table III. Clinical, biochemical and immunological features after DAAs therapy in 22 patients with HCV-related mixed cryoglobulinaemia.

Symptoms, biochemical and virological features	Baseline	At week 4	EOT	SVR12	SVR24	SVR48
Purpura	12 (55%)	12 (55%)	5 (23%)	4 (18%)	-	4 (18%)
Arthralgias	12 (55%)	12 (55%)	3 (14%)	3 (14%)	-	3 (14%)
Peripheral neuropathy	10 (45%)	10 (45%)	3 (14%)	3 (14%)	-	3 (14%)
NHL	2 (9%)	2 (9%)	2 (9%)	2 (9%)	-	2 (9%)
Median ALT level (U/L)						
NV < 36 U/L	72 (12-173)	22 (7-71)	20 (10-44)	21 (10-35)	23 (10-85)	27 (9-95)
Cryocrit (%)	3 (1-20)	3 (1-19)	2 (1-12)	2 (1-12)	2 (0-6)	2 (0-6)
Rheumatoid Factor (IU/mL)						
NV < 25 U/L	111 (22-1,120)	Not available	66 (12-550)	72 (10-460)	74 (12-444)	83 (12-859)
Median C4 (mg/dl)						
NV> 10 (mg/dl)	7 (1-17)	Not available	9 (3-20)	9 (2-22)	9 (3-22)	-
HCV-RNA positivity (%)	22 (100%)	0	0	0	1 (5%)	1 (5%)

SVR: sustained virological response; NV: normal value; NHL: non Hodgkin's lymphoma.

while ten patients (45%) obtained a partial response.

Biochemical Response to DAAs

Twelve patients had normal ALT at baseline, hence they were excluded from this assessment. After four weeks of treatment, DAAs induced normalisation of the ALT serum levels in all the remaining ten patients (100%), the achievement of ALT normal levels was confirmed at EOT and at 24 weeks. ALT levels decreased from a median of 72 U/L (12-173) pretreatment to 20 U/L (10-44) at EOT and to 25 U/L 10-85) after 24 weeks (Table III). One patient relapsed at four months after EOT.

Side effects of the therapy

During therapy, adverse events related to DAAs were observed in 11 cases (50%). Table IV summarises the main adverse reactions of DAAs. Main side effects of DAAs were mild, and no case interrupted the therapy. Six patients presented anaemia secondary to RBV treatment. Four of them required reduction of RBV dose and they completed the treatment at reduced RBV dosage (Table IV).

Table IV. Adverse events in 22 patients in DAA (IFN-free) therapy.

Events	n (%)
Common adverse events	
Fatigue	5 (23)
Rash	3 (14)
Pruritus	2 (9)
Nausea	2 (9)
Insomnia	2 (9)
Irritability	2 (9)
Photosensitivity	2 (9)
Ribavirin induced anaemia	
Grade 1	4 (5)
Grade 2	2 (14)
Grade 3	0 (0)
Grade 4	0 (0)

Discussion

The association of PEG-IFN plus RBV has been considered, for over a decade, the standard antiviral therapy in HCV infections and HCV-related MC (11, 12, 31, 32). In MC patients, this therapy yielded an overall SVR rate significantly lower than that observed in patients with chronic hepatitis without MC (11-13, 31). The comparison was difficult since a large amount of chronic hepatitis faced a very limited number of MC cases. In addition, most MC cases came from Italy and France, determining an additional bias in comparing the two populations. The negative effect of MC in HCV patients had been previously observed also in cases that had undergone triple therapy (Peg-IFN+RBV plus boceprevir or telaprevir). However, triple therapy in MC had achieved a slight SVR improvement, but it determined serious adverse events (33, 34). When DAAs became available, their efficacy and safety were checked in MC. Preliminary studies reported a SVR of 74% (16) and of 83% (17) in 24 and 12 HCV-related MC patients, respectively. These studies, including a relatively small number of patients, had adopted suboptimal treatment schedules using sofosbuvir plus RBV in a cohort of patients infected with various HCV genotypes. However, a recent paper by Gragnani *et al*. (19) observed 100% SVR12 rate in 44 patients, suggesting that updated treatment protocols may produce comparable virological responses in MC patients and in HCV patients without MC (35). Confirming these observations, DAAs treatment determined a rapid undetectable HCV-RNA viremia after the fourth week also in all cases, while the SVR48 rate was 95% with a single relapsing patient. By these results, unlike with IFN-based therapies, the presence of MC vasculitis did not still seem to represent a risk factor for virological non-response. In fact, the DAAs therapies showed a comparable SVR (near to 100%) in HCV chronic hepatitis as well as in MC, with a very limited number of adverse events (14, 15). Accordingly, even in this study no serious adverse reactions causing treatment suspension were observed. The most common side effects were fatigue, insomnia, and anaemia in patients receiving RBV, as in other recently published series of patients (16-19).

Clinical and immunological responses seemed less satisfactory than the virological response. The clinical responses, achieved in combination with the disappearing of the viral load, were obtained both in naïve patients and in non-responders/relapsers to previous antiviral therapy with PEG-IFN plus RBV. However, despite the rapid and

definitive elimination of the virus, the cryoglobulin and RF production persisted in most cases, and, in a minority of patients, together with the clinical symptoms. C4 consumption also was unchanged in most cases. These findings indicated that the immunological interactions between complement and cryoglobulins persisted even after the disappearing of HCV-RNA. It is likely that in these cases the B-cell proliferation and cryoglobulins production became independent of virus replication. Artemova *et al*. (36) have observed the same behaviour in a small cohort of patients affected by HCV-positive cryoglobulinaemic vasculitis; in fact, they have not found a correlation between virological response and cryoglobulin production. The follow-up of these cases was rather short (20 weeks), but also our cases followed-up for 48 weeks showed similar results. It is possible that the immunological alterations induced by HCV replication could disappear years after viral eradication. Our first cryoglobulinaemic patient (not included in this study) treated with DAAs showed the normalisation of C4 level about 24 months after EOT; therefore, to draw definitive conclusions, our cases, as well as other published records, should be reconsidered after one or two years of follow-up. Furthermore, we observed an incomplete response of symptoms of sicca syndrome and of peripheral neuropathy. These results are similar to those of previous studies (16-18); indeed, also Gragnani *et al*. (19) have observed the persistence of sicca syndrome and peripheral neuropathy in most affected patients. It is likely that the irreversible damage of the salivary gland or of the peripheral nerves may be responsible for the lack of response (37). Sise *et al*. (17) and Gragnani *et al*. (19) have both observed rapid responses even in cases affected by cryoglobulinaemic glomerulo-nephritis. These responses are relevant since renal involvement in MC is associated with a very poor prognosis, as previously reported (38, 39). We cannot confirm those observations since the present study did not enroll cases with renal involvement. However, no cases of severe vasculitis were included in our series of patients; therefore, the efficacy of DAAs in this clinical setting is still to be proven. In a recent paper (40), Emery *et al*. checked new DAAs in a small number of life-threatening vasculitis with unsatisfactory results despite viral eradication, all cases required additional treatment such as plasmapheresis or rituximab.

In our study, two cases with indolent marginal zone lymphomas did not show any haematological response: size and number of the involved nodes remained unchanged. In addition, the monoclonal B-cell population found in the peripheral blood in four cases did not disappear after recovery from HCV-RNA. Since several previous studies had observed the efficacy of antiviral treatment with IFN-based regimens in HCV-related B-cell NHL (8-10), there was convincing evidence that HCV-lymphoproliferative diseases was associated with HCV replications and that the viral eradication was associated with the disappearing of the clonal B cells. In fact, many authors observed the contemporary behavior of the HCV-RNA and the of B-cell clonality. The dissociated response of HCV-RNA and B-cell clonality in the IFN-free antiviral treatments is difficult to explain. However, alfa-IFN, given its powerful antiproliferative activity, had been used for years in the treatment of several lymph- and myelo-proliferative diseases, including chronic myeloid leukaemia, primary thrombocythemia, myeloma and indolent non-Hodgkin's lymphomas. Therefore, it is likely that the Alfa-IFN together with the elimination of HCV-RNA (and the correlated antigen stimulation) could counteract both clonal expansion and cryoglobulin production (7, 10, 41). Conversely, the DAAs, able to eliminate the viral replication much more efficiently than the IFN-based regimens, are not endowed with anti-proliferative properties, and, therefore, are less efficient to counteract B-cell monoclonal proliferation and the cryoglobulins production (19). However, Comarmond *et al*. reported a recovery of the immunological alterations due to HCV infection after DAAs therapy in MC (42). Previous case reports have observed the efficacy of IFN-fee DAAs therapy in HCV-associated lymphomas (43-50). Among these publications, it is worth mentioning the excellent paper by Arcaini *et al*. (47) who described the efficacy and safety of the new DAAs therapy in a significant number of indolent NHL cases collected in several centres in Italy and France. The report has confirmed the high efficacy of DAAs on HCV since the totality of the patients (except one with decompensated cirrhosis) obtained SVR, even those cases that had undergone previous chemotherapy and/or previous interferon-based antiviral treatments. The haematological response seemed less satisfactory, since only a fraction of cases (32%) obtained complete remission of NHL, while most cases, after a partial remission, relapsed or had a disease progression requiring chemo- or immunotherapy. Although the methods for determining the responses are lacking, the results are very interesting, as they confirm a rather good response rate of DAAs in HCV-positive marginal zone lymphomas (MZL), both nodal and extra-nodal. Conversely, no response was observed in the four cases of small lymphocyte lymphoma/chronic lymphocytic leukaemia (SLL/CLL) or in two follicular lymphomas and in two cases affected by lymphoplasmacytic lymphomas. These findings support the hypothesis of different pathophysiologic events between SLL/CLL and MZL. In previous papers, our group (10), Arcaini (8), and other authors (9) have shown that the combination therapy (IFN or PEG-IFN and RBV) is effective in HCV-positive indolent NHL of different histology including lymphocytic lymphoma, marginal-zone lymphoma, lymphoplasmacytic lymphomas and even follicular lymphomas. It is likely that in those cases the anti-proliferative properties of IFN play a leading role in the cure of lymphoproliferative diseases. In fact, it is well known that rituximab as single agent can induce a complete response in a large fraction of low-tumour-burden follicular lymphoma (51), and other indolent lymphomas, including MZL.

In conclusion, our study demonstrated that IFN-free DAAs therapy in HCV-related cryoglobulinaemic vasculitis

yields high virological, satisfactory clinical (in mild to moderate vasculitis), and low immunological responses. The therapy showed optimal tolerance with only minor adverse events. The results obtained with IFN-free treatment in HCV-related MC open up new opportunities to treat difficult cases of severe vasculitis, or autoimmune diseases (52). Moreover, this study highlights the importance of early eradication of HCV, before the damage the organ becomes irreversible and before the lymphoproliferative disease becomes independent of viral replication.

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