Interferon mono-therapy for symptomatic HCV-associated mixed cryoglobulinemia : meta-analysis of clinical studies

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Abstract

Objective : **Mixed cryoglobulinemia (MC) is an important complication of hepatitis C virus (HCV) infection. Antiviral therapy is now an important approach for symptomatic HCV-MC ; some information exists on IFN mono-therapy for symptomatic HCV-MC in the non-transplant setting, but its efficacy is still unclear.**

Methods : **We evaluated efficacy and safety of mono-therapy with standard or pegylated interferon (IFN) for symptomatic HCV-associated MC in non-immunosuppressed individuals by performing a systematic review of the literature with a meta-analysis of clinical studies. We used the random-effects model of DerSimonian and Laird with heterogeneity and sensitivity analyses. The primary outcome was sustained viral response (SVR, as a measure of efficacy), and the secondary outcome was the drop-out rate due to side-effects (as a measure of tolerability).**

Results : **We identified eleven clinical studies ($n = 235$ unique patients) ; the rate of baseline kidney involvement ranged between 11% and 74%. The summary estimate of frequency of sustained viral response was 0.15 with a 95% Confidence Interval (CI) of 0.08 ; 0.22 (random-effects model). Significant heterogeneity occurred ($P = 0.001$; $Chi^2 = 28.9\%$). Stratified analysis did not meaningfully change the results. The frequency of patients stopping antiviral agents was 3.4% ; most patients experienced minor side effects which did not require interruption of therapy. Baseline cirrhosis ($P < 0.04$), kidney involvement ($P < 0.07$), and arthralgias ($P < 0.04$) showed negative impact on viral response. We found an excellent relationship between viral and clinical response [weighted $K = 0.72$ (95% CI, 0.54 ; 0.89)], by an evaluation at individual level on a subset of reports ($n = 65$ unique patients).**

Conclusions : **This meta-analysis of clinical studies shows that antiviral therapy with standard or pegylated IFN alone for symptomatic MC associated with HCV gives satisfactory response in a minority of patients only. Clinical trials based on combination therapy (pegylated interferon plus ribavirin) or novel immunosuppressive agents are under way in order to improve efficacy and safety of symptomatic HCV-MC.** (Acta gastroenterol. belg., **2013**, 76, **363-371**).

Key words : hepatitis C virus, mixed cryoglobulinemia, interferon, meta-analysis.

Introduction

HCV infection is associated with a number of extrahepatic manifestations, including mixed cryoglobulinemia. Mixed cryoglobulinemia is a systemic vasculitis that mainly affects the small and, less frequently, medium sized arteries and veins. It is characterized by the deposition of immune complexes containing rheumatoid factor (RF), IgG, HCV RNA, and complement on endothelial surfaces, eliciting vascular inflammation. MC is also characterized by the proliferation of B-cell clones producing pathogenic IgM with RF activity, and represents an example of immune complex vasculitis. MC leads to clinical manifestations ranging from the MC syndrome (purpura, arthralgias, asthenia) to more serious lesions with neurologic and kidney involvement.

The association between hepatitis C virus (HCV) infection and mixed cryoglobulinemia was reported first in 1990 (*1*) and subsequently confirmed in several studies (*2-4*). It was calculated that around 80-90% of patients with MC are HCV-infected (*5*). The documented link between HCV and MC has suggested the opportunity to control HCV-MC by antiviral therapy based on the belief that the underlying infection is driving immune complex formation and resultant vasculitis. The standard of care (SoC) for HCV is currently combination antiviral therapy (pegylated IFN plus ribavirin) (*6-7*) but evidence on this approach in HCV-MC population is extremely limited. On the other hand, kidney involvement is frequent in HCV-MC patients and clinical guidelines have repeatedly suggested ribavirin avoidance in chronic kidney disease (CKD) due to ribavirin accumulation with its adverse consequences (i.e., haemolytic anaemia) (*6-8*).

The information in the medical literature about IFN mono-therapy for HCV-MC is not abundant and the relationship between clinical improvement and viral response is still unclear. The primary goal of this study was to synthesize the available evidence on efficacy and safety of antiviral treatment (standard or pegylated IFN alone) for symptomatic HCV-MC in the non-transplant setting by performing a meta-analysis of published studies.

Patients and methods

Search strategy and data extraction

Electronic searches of the National Library of Medicine's MEDLINE database, Current Contents and manual searches of selected specialty journals were performed to identify all pertinent literature. It has been previously demonstrated that an electronic search alone may not be sensitive enough (*9*). Various MEDLINE

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database engines (Ovid, PubMed and GratefulMed), and Embase were used. The key-words 'hepatitis C virus', 'mixed cryoglobulinemia', and 'interferon', were used. Reference lists from qualitative topic reviews and published clinical trials were also searched. Our search was limited to human studies that were published in the English literature. Data extraction was conducted independently by two investigators (F.F., V.D.) and consensus was achieved for all data. Studies were compared to eliminate duplicate reports for the same patients, which included contact with investigators when necessary. Eligibility and exclusion criteria were pre-specified.

Criteria for inclusion

To be included in this meta-analysis a clinical study had to fulfil certain criteria : it had to be published as a peer-reviewed article, report the results of mono-therapy with (conventional or pegylated) IFN, and use the viral response (SVR) as a clinical end-point. The decision as to the inclusion or exclusion of clinical studies was not related to results. Patients who underwent primary antiviral therapy (naïve patients) or those who had already completed an antiviral course (non-responder or relapser patients) were enrolled.

Only studies evaluating patients with HCV-associated symptomatic MC in the non-transplant setting were included. Both case-control and cohort studies were considered eligible for inclusion in the analysis. To be considered for inclusion in our meta-analysis, studies had to define HCV infection by detecting anti-HCV antibody and HCV viraemia (HCV RNA) in serum. Information on HCV status had to be registered at the time of enrolment.

Ineligible studies

Studies were excluded if they reported inadequate data on treatment or measures of response, or included patients with coexisting diseases such as infection with human immunodeficiency virus, haemophilia, or other specific aetiology of liver disease such as hepatitis B, hepatitis A, Epstein-Barr virus, cytomegalovirus, alcoholic hepatitis/cirrhosis, autoimmune hepatitis, hepatocellular carcinoma, Wilson's disease, haemochromatosis, and alpha-1 antitrypsin deficiency. Reports that were only published as abstracts or as interim reports were excluded ; letters and review articles were not considered for this analysis. Studies that involved renal or liver transplant recipients were not included. Studies reporting viral response rates by methods other than polymerase chain reaction (PCR) (e.g. bDNA assay) were excluded.

Definitions and end-points of interest

The primary outcome of interest in this systematic review was sustained viral response (SVR) as a measure of efficacy. SVR was defined as disappearance of HCV viraemia (HCV RNA) by PCR at least 6 months after completion of therapy. Secondary end-points included the frequency of patients interrupting antiviral therapy due to side-effects. These definitions are now standards.

Stastistical methods

Outcomes were analysed on an intention-to-treat basis, i.e., all patients included in these studies were considered for the calculation of the response rate, while patients without the end-point were considered as failures. When not given in the publication, the response rate according to the intention-to-treat method was calculated by the data abstractors (F.F., and V.D.). Quantitative, summary (or pooled) estimates of the SVR and drop-out rate across individual studies were generated using the random-effects-model of DerSimonian and Laird (*10*). Confidence intervals for point estimates were computed using nonparametric resampling (bootstrap) methods. The estimate for each study was weighted inversely to its squared standard error when computing the overall estimate and its confidence interval. The confidence intervals for the random effects model were quoted, since the standard errors under the fixed effects model may be misleading, and the test for homogeneity was rejected. Chi-square statistics were used to test for homogeneity across studies (*11*). The inter-rater agreement by weighted kappa statistics was used to evaluate the relationship between clinical and virological response ; to this purpose two investigators (F.F., V.D.) extracted published individual patient data from studies giving information on it. Pearson's correlation coefficient was used to assess the association between outcomes of interest and variables thought to be potential sources of heterogeneity (*12*). Specifically, the effect of various subject and trial characteristics of interest on the reported size of the estimated intervention benefits (SVR) and drop-out rate were assessed by calculating correlation coefficients. A sensitivity analysis using a fixed-effects model was also performed to assess the consistency of results (*13*). The 5% significance level was used for alpha risk. Every estimate was given with its 95% CIs. In our study, the statistical software (RevMan 4.1 ; Update Software Ltd, Oxford, UK) freely provided by the Cochrane Collaboration was used.

Results

Literature review

Our electronic and manual searches identified 17 papers (*14-30*). All candidate studies were written in English. Six reports were excluded because they did not fulfil the inclusion criteria (*14-16,19*) or contained duplicate studies of the same patients (*17-18*). The remaining 11 studies (*20-30*), representing a total of 235 patients, were included in the meta-analysis. Three (27%) had controlled clinical design (CCT) (*20-21,24*), whereas the others (*22-23,25-30*) were cohort studies. There was 100% concordance between reviewers with respect to

Table 1. — **Characteristics of studies included in the meta-analysis and anti-HCV schedule**

Authors	Reference year	Anti-HCV therapy, duration	Anti-HCV therapy, medication	Anti-HCV therapy, dose
Misiani R. *et al*. (20)	1994	24 wks	Recombinant IFN-α2a, sc	1.5 MUI x3/wk (first wk)+ 3 MUI x3/wk (n=23 wks)
Dammacco F. *et al*. (21)	1995	48 wks	Natural IFN-α, IM	3 MUI x3/wk (n=18) 3 MUI x3/wk (n=18) + 16 mg daily CCS
Migliaresi S. *et al*. (22)	1995	12 mo	Recombinant IFN-α, sc or IM	3 MUI x3/wk
Casato M. *et al*. (23)	1997	12 mo	Recombinant IFN-α2a, sc or IM	3 MUI daily (6 mo)+ 3 MUI x3/wk (6 mo)
Adinolfi L. *et al*. (24)	1997	12 mo	Recombinant IFN-α2a, sc	3 MUI x3/wk
Mazzaro C. *et al*. (25)	1997	12 mo	Recombinant IFN-α2b, sc	3 MUI x3/wk
Cresta P. *et al*. (26)	1999	6 mo	Recombinant IFN-2α Recombinant IFN-α2b, IM	3 MUI x3/wk
Calleja S. *et al*. (27)	1999	12 mo	Recombinant IFN-α, sc	3 MUI x3/wk
Naarendorp M. *et al*. (28)	2001	21 (3-60) mo	Recombinant IFN-2α, sc	3 MUI x3/wk
Mazzaro C. *et al*. (29)	2002	12 mo	Leukocyte IFN, sc	3 MUI x3/wk
Joshi S. *et al*. (30)	2007	6 mo	Recombinant IFN-α2b, sc Pegylated IFN, sc	3 MUI x3/wk

Mo = months ; wks = weeks ; IM = intramuscular route ; sc = subcutaneous route.

Table 2. — **Characteristics of studies included in the meta-analysis**

Authors	Patients, n	Age, yrs	Study design	Country
Misiani R. *et al*.	27	62 (37-70)	RCT	Italy
Dammacco F. *et al*.	26	59.2 ± 7 56.3 ± 3	RCT, cross-over	Italy
Migliaresi S. *et al*.	9	48.2 (30-66)	Cohort, prospective	Italy
Casato M. *et al*.	22	56 (38-73)	Cohort, retrospective	Italy
Adinolfi L. *et al*.	25	54 (38-62)	CCT	Italy
Mazzaro C. *et al*.	42	54.8 ± 9.1	Cohort, retrospective	Italy
Cresta P. *et al*.	12	55.0 ± 10	Cohort, prospective	France
Calleja S. *et al*.	18	48 ± 12	Cohort, prospective	Spain
Naarendorp M. *et al*.	11	48 (39-68)	Cohort, prospective	US
Mazzaro C. *et al*.	28	51 ± 9	Cohort, prospective	Italy
Joshi S. *et al*.	15	55 ± 11.2	Cohort, retrospective	Canada

n = number ; CCT = controlled clinical trial ; RCT = randomized clinical trial.

final inclusion and exclusion of studies reviewed based on the predefined inclusion and exclusion criteria.

Patient characteristics

Shown in Tables 1-4 are some salient demographic and clinical characteristics of subjects enrolled in the included clinical trials. All the reports were published from 1994 to 2007 ; the majority (7/11 = 64%) were from southern Europe. The mean age of subject cohorts was between from 48.0 to 62.0 years of age. The gender distribution ranged from 45% to 75% female. The frequency of naïve patients was 100% in nine studies (*20-28*). Based on the data provided in Table 3-4 it appears that the majority of enrolled subjects were non-cirrhotic patients with HCV genotype 1.

Table 1 provides details on the study design, including dose and duration of IFN mono-therapy. The majority of studies (10/11 = 91%) regarded mono-therapy with conventional or recombinant IFN ; only a few patients (*n* = 5) received pegylated IFN (*30*).

Primary analysis

The summary (or pooled) estimate for SVR rate across the identified trials was 0.15 with a 95% CI of 0.08 ; 0.22 (random-effects model) (Fig. 1). Table 5 gives information on the summary estimate for SVR rate across various

Table 3. — **Characteristics of studies included in the meta-analysis**

Authors	Female, *n*	HCV genotype 1	Baseline purpura	Baseline kidney disease
Misiani R. *et al.*	23 (85%)	NA	16 (59%)	20 (74%)
Dammacco F. *et al.*	18 (56%)	NA	26 (81%)	15 (46%)
Migliaresi S. *et al.*	7 (78%)	NA	9 (100%)	4 (44%)
Casato M. *et al.*	13 (59%)	13 (59%)	22 (100%)	8 (36%)
Adinolfi L. *et al.*	15 (60%)	10 (40%)	15 (60%)	13 (52%)
Mazzaro C. *et al.*	27 (64%)	31 (73%)	41 (97%)	NA
Cresta P. *et al.*	7 (58%)	8 (67%)	4 (33%)	2 (17%)
Calleja S. *et al.*	11 (61%)	13 (72%)	8 (25%)	2 (11%)
Naarendorp M. *et al.*	5 (45%)	5 (45%)	8 (73%)	4 (36%)
Mazzaro C. *et al.*	20 (71%)	18 (64%)	NA	NA
Joshi S. *et al.*	8 (53%)	13 (87%)	12 (80%)	5 (33%)

Table 4. — **Characteristics of studies included in the meta-analysis**

Authors	Naïve pts, *n*	Baseline arthralgias	Baseline cirrhosis	Baseline peripheral neuropathy
Misiani R. *et al.*	100%	19 (70%)	NA	8 (30%)
Dammacco F. *et al.*	100%	23 (71%)	9 (28%)	9 (28%)
Migliaresi S. *et al.*	100%	5 (55%)	NA	9 (100%)
Casato M. *et al.*	100%	10 (45%)	1 (12%)	15 (68%)
Adinolfi L. *et al.*	100%	14 (56%)	8 (32%)	5 (20%)
Mazzaro C. *et al.*	100%	NA	7 (22%)	9 (21%)
Cresta P. *et al.*	100%	6 (50%)	3 (25%)	6 (50%)
Calleja S. *et al.*	100%	10 (55%)	0	3 (16%)
Naarendorp M. *et al.*	100%	6 (54%)	3 (27%)	7 (63%)
Mazzaro C. *et al.*	0	NA	3 (11%)	NA
Joshi S. *et al.*	80%	7 (47%)	5 (33%)	5 (33%)

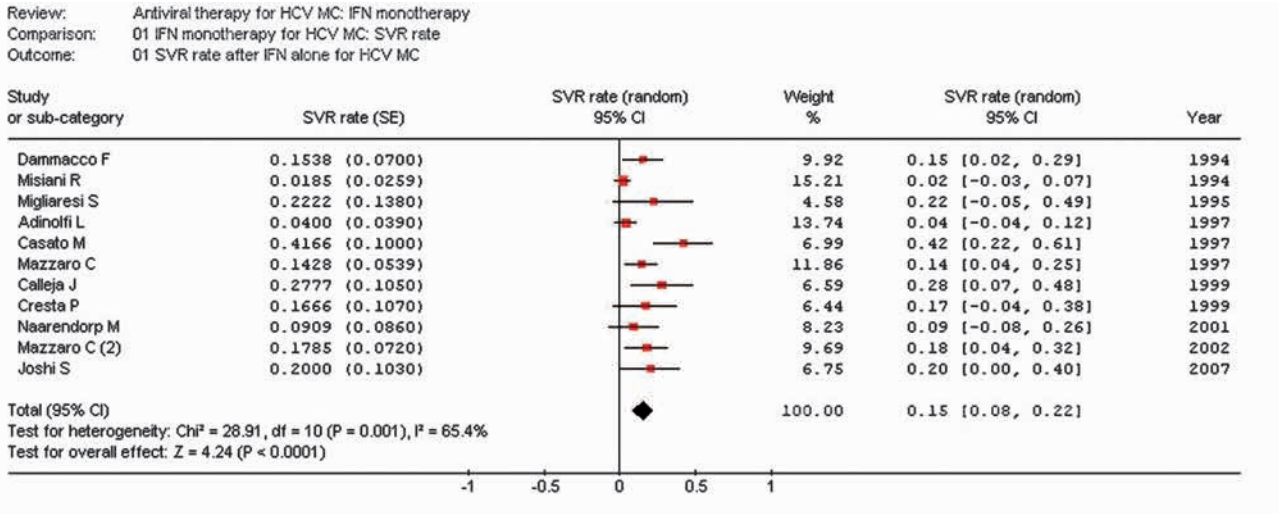

Fig. 1. — Summary estimate of SVR rate after antiviral therapy (IFN alone) for symptomatic HCV-MC (random-effects model)

Table 5. — **Stratified analysis : summary estimate for SVR in various subgroups of interest (random-effects model)**

	SVR estimate (95% Confidence Intervals)	Chi-squared test	I^2
All studies (n = 11)	0.15 (0.08 ; 0.22)	28.9 (P = 0.01)	65.4%
Cohort studies (n = 8)	0.19 (0.13 ; 0.26)	8.06 (P = 0.33)	13.2%
Studies published in 1990s (n = 8)	0.15 (0.06 ; 0.23)	25.7 (P = 0.006)	72.7%
Studies based on 12-month IFN (n = 7)	0.18 (0.10 ; 0.26)	16.95 (P = 0.02)	58.7%
Studies from Italy (n = 7)	0.14 (0.05 ; 0.22)	23.28 (P = 0.0007)	74.2%
CCTs (n = 3)	0.05 (-0.01 ; 0.10)	3.30 (P = 0.19)	39.4%
Recombinant IFN (n = 9)	0.12 (0.06 ; 0.19)	7.01 (P = 0.34)	48.2%

Table 6. — **Tolerance to antiviral therapy : drop-out rate among studies included in the meta-analysis (ITT analysis)**

Authors	Patients stopping IFN, n	Reasons for IFN withdrawal
Misiani R. *et al.*	2 (7%)	Atrial fibrillation (n = 1), depression (n = 1)
Dammacco F. *et al.*	0	
Migliaresi S. *et al.*	0	
Casato M. *et al.*	0	
Adinolfi L. *et al.*	1 (4%)	Depression
Mazzaro C. *et al.*	0	
Cresta P. *et al.*	0	
Calleja S. *et al.*	0	
Naarendorp M. *et al.*	3 (27%)	Depression (n = 1), anemia (n = 1), leukopenia (n = 1)
Mazzaro C. *et al.*	2 (7%)	Depression (n = 1), erythema (n = 1)
Joshi S. *et al.*	NA	

ITT : intention-to-treat ; NA = not available.

subgroups according to stratified analysis. As listed in Table 5, subgroup analysis did not meaningfully change these results- the SVR rate ranging between 0.05 (95% CI, -0.01 ; 0.10) and 0.19 (95% CI, 0.13 ; 0.26).

We found excellent agreement between viral and clinical response to combination antiviral therapy [weighted $K = 0.72 \pm 0.09$ (95% CI, 0.5-0.89)], by an analysis at individual level involving three studies giving information on it (n = 65 unique patients) (*23,29-30*).

The number of patients with symptomatic HCV-MC on IFN mono-therapy who interrupted the treatment due to side-effects was small (Table 6) ; many patients experienced minor side-effects during antiviral therapy such as influenza-like symptoms, insomnia, weight loss, or alopecia, but no permanent interruption of anti-HCV therapy was needed. The reasons for permanent IFN withdrawal are reported in Table 6.

Figure 2 shows the estimated SVR rate for each study ± two SEs, that is, the 95% CIs. The overall estimate of SVR rate was also reported.

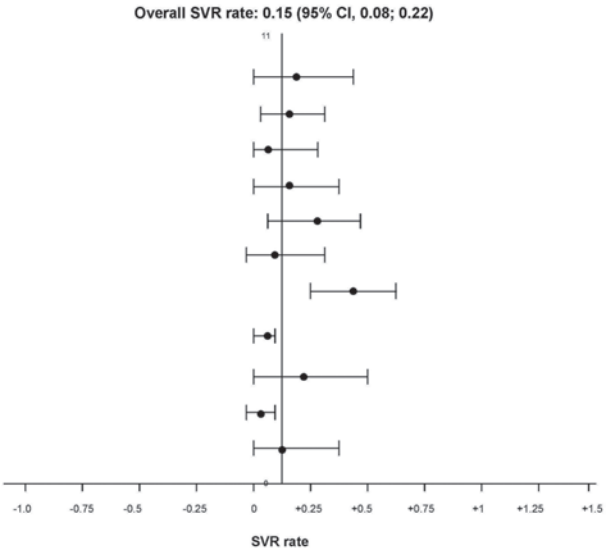

Fig. 2. — Estimate of SVR rate for each study and 95% CIs. The vertical line represents the pooled estimate of SVR rate.

Table 7. — **Relationship between the percentage of study patients achieving SVR and variables of interest in studies with available data. A '1' would mean complete correlation. A '0' would mean none. A negative number means that when one variable goes up, the other goes down. A positive number means they rise and fall together**

Independent Variable	Pearson's correlation coefficient	*P*
Publication year	0.11	0.36
Naïve patients (%)	-0.02	0.47
Age (years)	-0.210	0.26
Female (%)	-0.16	0.31
Genotype 1 (%)	0.34	0.22
Baseline cirrhosis (%)	-0.611	0.04
Baseline purpura (%)	0.259	0.23
Baseline arthralgias (%)	-0.593	0.04
Baseline neuropathy (%)	0.37	0.14
Baseline kidney disease (%)	-0.519	0.07
Patients discontinuing treatment (%)	-0.419	0.09
Anti-HCV duration	0.01	0.48

(Dependent variable : SVR rate).

Table 8. — **Relationship between the percentage of study patients achieving drop-outs (or reducing the dose of antiviral agents) and variables of interest in studies with available data. A '1' would mean complete correlation. A '0' would mean none. A negative number means that when one variable goes up, the other goes down. A positive number means they rise and fall together**

Independent Variable	Pearson's correlation coefficient	*P*
Publication year	-0.32	0.43
Naïve patients (%)	-0.81	0.4
Age (years)	0.312	0.2
Female (%)	-0.33	0.24
Genotype 1 (%)	-0.58	0.47
Baseline cirrhosis (%)	0.16	0.90
Baseline purpura (%)	-0.04	0.25
Baseline arthralgias (%)	0.18	0.13
Baseline neuropathy (%)	0.37	0.36
Baseline kidney disease (%)	0.188	0.22
Anti-HCV duration	0.677	0.51

(Dependent variable : Drop-out rate).

Heterogeneity analysis

As listed in Figure 1, there was significant heterogeneity (chi-square = 28.9, $P = 0.001$) in the primary analysis. According to the chi-squared tests (*P*-values) of our stratified analysis (Table 5), significant heterogeneity persisted in many subgroups of interest with regard to the primary points of interest (SVR). In other words, these studies may not be measuring the same underlying quantity from the same base population. The summary estimate may not be the best descriptive analysis, since they assume that all estimates from individual studies are from the same population. The sources of the between study heterogeneity are rather unclear. As listed in Table 7, we found a negative, significant association between cirrhosis, arthralgias and reported SVR ; baseline kidney involvement had negative influence on viral response but no statistical significance was reached. No relationship between drop-out rate and various clinical or background parameters was noted (Table 8).

Discussion

The use of interferon-α in MC started in 1987 (*20*), and at that time the aetiology of MC was unknown. Following evidence of a close link between hepatitis C virus and MC, antiviral therapy has been recommended according to various clinical guidelines (*7-8*) but the medical literature on this point is rather poor. Our pooled analysis of clinical studies has shown that antiviral therapy with standard or pegylated IFN alone is unsatisfactory as gives viral remission of the disease in a minority of patients only.

The number of long-term responders of our study is similar to that observed in the studies addressing pegylated IFN mono-therapy for chronic hepatitis C (*31*). However, it is difficult to compare the limited number of patients included in the current meta-analysis with the very large number of patients with chronic hepatitis C as patients with several medical conditions (such as thrombocytopenia, Raynaud phenomenon, neuropathy or kidney involvement) were excluded from the studies on chronic hepatitis C. Also, our patients were generally older than chronic hepatitis C patients without MC (*31-32*).

Our analysis shows that tolerance to IFN mono-therapy was satisfactory. The drop-out rate was low (3.4%), and many patients experienced minor symptoms which did not require permanent interruption of antiviral agents. In some individuals, dose reduction or transient interruption of antiviral agents was needed and the disappearance of symptoms was obtained in all. These events occurred despite the older age and the more common organ involvement in our study group than other HCV-infected populations.

As listed in Table 7, we identified some predictive factors of response to anti-HCV therapy ; baseline co-morbidities such as cirrhosis, or arthralgias played a significant and negative impact on the viral response to IFN mono-therapy. The role of baseline kidney involvement on anti-HCV therapy did not reach statistical significance ($P = 0.07$) according to our heterogeneity analysis ; it is well known that CKD is a major cause of morbidity and mortality among patients with HCV-MC (*33-34*).

We found an excellent association between clinical and viral response, as emphasized by kappa statistics ($K = 0.72 \pm 0.09$). In other words, most patients reaching sustained virological response obtained clinical remission of symptomatic HCV-MC ; this confirming the pivotal role of HCV infection in the pathogenesis of MC. We identified a subgroup of patients ($n = 7$) who obtained persistent HCV RNA clearance with incomplete remission of MC signs or symptoms- the reasons are unclear. It has been cited the persistence of HCV replication below the sensitivity of the available assays for HCV RNA testing in serum ; alternatively, HCV partially interferes with lymphocyte B clone activity. The latter hypothesis is in keeping with the most important view on the pathogenesis of symptomatic HCV-associated MC-HCV is the initial step followed by pathogenic events downstream the triggering infection where auto-antigens, infections, cell deregulation can sustain autoimmunity abnormalities and B-cell expansion (*35*).

The results of our review call for an improvement of anti-HCV therapy in symptomatic HCV-MC. The frequent kidney involvement in patients with symptomatic HCV-MC has not supported the use of ribavirin. Physicians have been reluctant to use ribavirin in CKD patients given the fear of the drug-related side-effects, particularly haemolytic anemia, that can be exacerbated in the presence of end stage renal disease. However, some authors have provided evidence on the successful ribavirin use in patients with low glomerular filtration rate and recent clinical guidelines suggest a cautious use of ribavirin in kidney failure patients. According to these, patients with glomerular filtration rate < 60 ml per min per 1.72 m^2 were recommended to receive ribavirin only after the adoption of some procedures such as low ribavirin dose, weekly monitoring of haemoglobin levels, and erythropoietin use to correct the anaemia induced by ribavirin (*8*). Some studies on combination antiviral therapy of symptomatic HCV-MC have been recently made (*36-37*). Novel evidence has been recently accumulated on rituximab, an anti-CD20 monoclonal antibody targeting B cell clones, for the treatment of HCV-MC with severe organ involvement and/or without an early viral response (*38-39*). Encouraging results have been given and the sequential rituximab/antiviral treatment has been introduced for symptomatic HCV-MC (*40*) ; however, safety concerns on rituximab use exist, particularly among solid organ transplant recipients (*41*).

The current meta-analysis is subject to several limitations, an examination of which may inform the design and conduct of future studies on this topic. First, the number of patients available for our analysis was not very large ($n = 235$). Secondly, we have made a meta-analysis of observational studies but it is well known that a meta-analysis of randomized clinical trials is provided with better accuracy and reliability (*42*). Finally, the quality of the reports included in this systematic review was not high and there is increasing evidence showing that the quality of studies affect outcome estimates (*42*). The minimal changes on the effect size (pooled estimate of SVR) obtained with stratified analysis, and the absence of heterogeneity in some subgroup analyses strengthen our conclusions ; also, none of the studies we included was published as an interim report, and it is well known that the information published in preliminary format (i.e., abstract) can give greater treatment effect (*43*).

The rate of viral response after IFN mono-therapy observed in our meta-analysis is similar to recent data showing that around 10-20% of HIV positive (or negative) subjects with acute HCV clear hepatitis C virus spontaneously (*44-45*) ; however, our study regarded individuals with symptomatic and chronic HCV-associated mixed cryoglobulinemia and no information is available in these patients (*46*).

In conclusion, this meta-analysis shows that antiviral therapy with standard or pegylated IFN alone is safe but effective only in a few patients with symptomatic HCV-MC. Clinical trials based on combined antiviral therapy and/or novel immunosuppressive agents for symptomatic HCV-MC are under way.

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