Recommendations for the management of mixed cryoglobulinemia syndrome in hepatitis C virus-infected patients.

Recommendations for the management of mixed cryoglobulinemia syndrome in hepatitis C virus-infected patients

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Abstract
Objective: The objective of this review was to define a core set of recommendations for the treatment of HCVassociated mixed cryoglobulinemia syndrome (MCS) by combining current evidence from clinical trials and expert opinion. Methods: Expert physicians involved in studying and treating patients with MCS formulated statements after discussing the published data. Their attitudes to treatment approaches (particularly those insufficiently supported by published data) were collected before the consensus conference by means of a questionnaire, and were considered when formulating the statements. Results: An attempt at viral eradication using pegylated interferon plus ribavirin should be considered the first-line therapeutic option in patients with mild–moderate HCV-related MCS. Prolonged treatment (up to 72 weeks) may be considered in the case of virological non-responders showing clinical and laboratory improvements. Rituximab (RTX) should be considered in patients with severe vasculitis and/or skin ulcers, peripheral neuropathy or glomerulonephritis. High-dose pulsed glucocorticoid (GC) therapy is useful in severe conditions and, when necessary, can be considered in combination with RTX; on the contrary, the majority of conference participants discouraged the chronic use of low–medium GC doses. Apheresis remains the elective treatment for severe, life-threatening hyper-viscosity syndrome; its use should be limited to patients who do not respond to (or who are ineligible for) other treatments, and emergency situations. Cyclophosphamide can be considered in combination with apheresis, but the data supporting its use are scarce. Despite the limited available data, colchicine is used by many of the conference participants, particularly in patients with mild–moderate MCS refractory to other therapies. Careful monitoring of the side effects of each drug, and its effects on HCV replication and liver function tests is essential. A low-antigencontent diet can be considered as supportive treatment in all symptomatic MCS patients. Although there are no data from controlled trials, controlling pain should always be attempted by tailoring the treatment to individual patients on the basis of the guidelines used in other vasculitides. Conclusion: Although there are few controlled randomised trials of MCS treatment, increasing knowledge of its pathogenesis is opening up new frontiers. The recommendations provided may be useful as provisional guidelines for the management of MCS.

Introduction

Mixed cryoglobulins (MCs) are immune complexes that typically consist of an IgM rheumatoid factor and immunoglobulins (most frequently IgG) that can precipitate at temperatures below 37 °C [1]. Associated with acute and chronic infections, lymphoproliferative disorders, and autoimmune diseases, they often have no real pathological significance but, in some cases, they may be responsible for serious and debilitating vasculitis syndromes with organ damage and a sometimes fatal outcome [2]. The mixed cryoglobulinemia syndrome (MCS) or symptomatic mixed cryoglobulinemia is defined by the Meltzer and Franklin triad of purpura, fatigue and arthralgia [3], and can be classified as one of the immune complex-mediated systemic vasculitides involving small-sized vessels. Since the discovery of hepatitis C virus (HCV), it has been shown that the vast majority of MCs previously defined as 'essential' or idiopathic can be attributed to it [4–7]. Circulating cryoglobulins are frequently detected in HCV-positive patients, but only a minority of patients with chronic HCV infections (usually women aged more than 50 years) develop frank MCS [8]. However, although it is generally considered to be rare, MCS is actually not uncommon, especially in southern European countries where the prevalence of HCV infection is high among the elderly. Various attempts at defining a clinical classification have been made since the 1990s and the GISC (Italian Group for the Study

of Cryoglobulinemia) has recently completed a large international cooperative study aimed at establishing classification criteria on the basis of standard methods [9]. Managing MCS means dealing with multiple and often very different clinical patterns, activity and severity, and should have the aim of preventing irreversible organ damage, reducing pain and improving the patients' quality of life. However, the treatment is still largely empirical. Any rational therapeutic approach should have three main objectives [10]: to eradicate HCV; to limit or suppress B lymphocyte proliferation; and to contain and symptomatically treat the vasculitis and reduce the damage caused by circulating immune complexes. Each of these therapeutic targets requires the use of different classes of drugs or specific procedures, but there are still very few data available from randomised controlled studies. Furthermore, the involvement of different organs means that MCS may be diagnosed by specialists in different fields, who tend to focus on the therapeutic approaches that are more in line with their own experience. For this reason, the GISC promoted a consensus conference to discuss currently used therapies and the published evidence concerning their efficacy. The aim of this conference was to define a core set of practical treatment recommendations by combining clinical trial data and expert opinion.

Methods

For practical reasons, the GISC board organising the conference focused on what is known about the drugs used to treat MCS. The discussion was also strictly limited to the treatment of HCV-related MCS. The Consensus Committee included physicians working in various medical fields (internal medicine, rheumatology, haematology, nephrology, hepatology, infectious diseases, and neurology) who were involved in caring for patients with MCS. The therapeutic schedules currently used by each GISC centre were collected by means of a questionnaire and submitted for preliminary evaluation by the GISC Scientific Board. The questionnaire asked which of the following drugs or treatment procedures were used to treat MCS: interferons ± ribavirin; pegylated interferons± ribavirin; anti-CD20; glucocorticoids; immunosuppressors, including cyclophosphamide, azatioprine, methotrexate, and cyclosporine; colchicine; NSAIDs and other drugs used in pain control; apheretic procedures; and a lowantigen-content diet. These were then divided into three groups to be discussed at the conference: 1) antiviral (interferon-based) therapies; 2) biological (anti-CD20-based) therapies; and 3) other therapeutic approaches. A panel of four members (two senior clinicians plus two junior clinicians as bibliographic reviewers) was formed for each group and, after carefully reviewing the literature and questionnaires (see below), each panel formulated preliminary statements to be discussed at the consensus conference by the full Consensus Committee. The literature review was carried out in phases based on the Cochrane systematic review guidelines [11]. The results of the review and the experts' discussions were then translated into epidemiological terms using Sackett's patients, intervention, comparison and outcome (PICO) methods [12]. Specific key words and MeSh terms were selected in MEDLINE and variously combined to search for papers concerning the treatment of patients aged more than 18 years with HCV-related MCS available in MEDLINE, EMBASE and Cochrane Central. The search was restricted to papers written in English, French and Italian. The selected papers included randomised controlled trials (RCTs), observational studies (prospective and retrospective cohort and case– control studies), and case series of at least three patients. Given the scarcity of data concerning some treatments, the Scientific Committee also included single case reports that provided information about otherwise neglected issues. The publication details, patient characteristics, dosage, therapeutic strategy and relevant outcomes were extracted from all of the included articles using standard forms. Levels of evidence (Oxford, May 2001, http://www.cebm.net) of each study were assessed and classified from level 1a (a systematic review of RCTs) to level 4 (case series), with level 5 being used for expert opinions without any explicit critical appraisal. The strength of the experts' recommendations was classified from A (consistent level 1 studies) to D (inconsistent or inconclusive studies at any level). The statements formulated on these bases were then discussed by the panel of experts together, and the recommendations were reformulated by combining the best available evidence from the literature with the experts' opinion. A second level of agreement was provided for each recommendation by the committee

members who had acknowledged competence in the field of each recommendation. Subsequently published additional data were taken into account when preparing the final report, which was resubmitted to the expert panel for a final on-line discussion.

Results

Antiviral therapy

Since the discovery of the association between HCV and MCS, many studies have assessed the efficacy of antiviral therapy [13]. Interestingly, because of its antiproliferative and immunomodulatory effects, interferon-α (IFN) was successfully used to treat MC even before the identification of HCV [14,15]. The antiviral treatment of MCS essentially followed the evolution of chronic hepatitis C treatment, but the studies are difficult to compare because of the heterogeneity of treatment regimens, patient selection, response evaluations, and follow-up [16]. Furthermore, they all had poor statistical power because of their small sample sizes. The first studies of the effects of IFN monotherapy showed the remission of symptoms in the majority of patients, but this benefit was often transient and relapses were very frequent after treatment discontinuation [17–34]. However, the presence of cryoglobulinemia did not affect the response to IFN in patients with chronic HCV infection [34–36]. It is also worth noting that the regression of peripheral blood and bone marrow monoclonal B lymphocytes was observed in the MCS patients who cleared HCV as a result of IFN therapy [37]. In comparison with IFN monotherapy, IFN plus ribavirin (RBV) improved viral eradication and the cure of symptoms [38–44]. However, only the patients who cleared the virus achieved a complete and sustained clinical response [38,39,41–43]. Once again, the presence of cryoglobulins did not affect the response to antiviral treatment [39] and it was confirmed that antiviral therapy can induce the disappearance of circulating B cell clones bearing the t(14;18) translocation [45–47]. Over the last ten years, the development of pegylated IFN (Peg-IFN) α-2a and α-2b, which have prolonged bioavailability and greater antiviral efficacy than standard IFN, has opened up new opportunities for the treatment of HCV-related chronic hepatitis and MCS [48–50]. Peg-IFN combined with ribavirin is now the standard of care for HCV treatment and leads to 41–54% sustained viral responses (SVRs) in the case of genotype 1, and approximately 80% in the case of genotypes 2 and 3. SVR is defined as undetectable HCV viremia six months after the completion of antiviral therapy [51]. There are only two pilot studies of the treatment of MCS with PegIFN+RBV [52,53], although additional data can be gathered from a few other studies in which both standard IFN and Peg-IFN were used [54,55]. Mazzaro et al. [52] studied 18 consecutive MCS patients treated with Peg-IFN α-2b (1 μg/kg/week) and RBV (1000 mg/day) for 48 weeks, after which 15 patients (83%) had undetectable HCV RNA levels, and most of the patients showed a clinical improvement. At the end of the 6- month follow-up period, only eight patients (44%) were sustained clinical and virological responders, and cryoglobulins had disappeared in six cases (33%). One major weakness of this study was the use of a Peg-IFN dose that was lower than that usually recommended in HCV therapeutic guidelines. Cacoub et al. [53] studied nine consecutive MCS patients (78% with HCV genotype 1) who received Peg-IFN α-2b (1.5 μg/kg/week) and RBV (800–1200 mg/day) for a mean of 13.5 months (range of 10–26). After a mean follow-up of 18.6 months (range of 6–33) following the discontinuation of antiviral therapy, seven patients (78%) showed a SVR and were complete clinical responders, and one a partial virological and a complete clinical response. The treatment was found to be safe and well tolerated in both of these studies, which demonstrated that in HCV-associated MCS combined Peg-IFN+RBV therapy leads to a SVR rate similar to that of HCV-infected patients without MCS, and strongly suggested this combination as the first-line treatment for MCS patients. However, few patients occasionally show the persistence of cryoglobulins or symptoms even after the clearance of HCV RNA [56,57]. Furthermore, antiviral treatment is sometimes associated with major immune-mediated adverse events, such as peripheral sensorymotor neuropathy, thyroiditis, rheumatoid-like polyarthritis, and other vasculitic manifestations [8,17,58–63]. Because of its immunomodulatory properties, IFN may precipitate or exacerbate some pre-existing and often subclinical disorders. Unfortunately, there are no parameters for predicting these complications [8], and so antiviral therapy should be very carefully administered to patients with mixed cryoglobulinemiarelated peripheral

neuropathy or active skin ulcers. Moreover, some patients may have major contraindications to IFN and/or RBV, such as advanced age, uncompensated cirrhosis, major uncontrolled depressive illness, significant coronary heart disease, untreated thyroid disease [51], which makes antiviral treatment inadvisable. Finally, the slow and uncertain response to antiviral therapy means that particularly severe and rapidly progressive MCS complications (including nephrotic or acute nephritic syndrome, extensive cutaneous ulcers, widespread vasculitis, and hyperviscosity syndrome) require prompter and more aggressive treatment; in such settings, antiviral therapy may be used after or concomitantly with more rapid, immunosuppressive regimens [8,16,64–66]. In clinical practise, the treatment of MCS should therefore be tailored to the individual patient on the basis of the progression and severity of its clinical manifestations [8,64,65]. RCTs with adequately sized populations and an appropriate follow-up are needed to clarify whether higher virological or clinical response rates can be obtained using different antiviral schedules, such as longer treatment duration in virological non-responders showing a clinical and laboratory improvement (up to 48 weeks in the case of HCV genotypes 2 or 3, and 72 weeks in the case of HCV genotypes 1 or 4). On the basis of their answers to the questionnaire, almost all of the conference participants use Peg-IFN+ RBV in HCV-related MCS, although infectious disease specialists, gastroenterologists and hepatologists are more likely to use antiviral therapy than rheumatologists. Nevertheless, all of the experts agreed to extend the current standard of care for chronic HCV infection to MCS. Given the risks associated with progressive MCS, many of the participants also believe that MCS patients older than age limits established by the international guidelines can receive IFN-based treatment in the absence of other major contraindications.

Statements

• HCV RNA suppression is associated with an improvement or the disappearance of the clinical and laboratory manifestations of HCVrelated mixed cryoglobulinemia syndrome (MCS). The achievement of a sustained virological response (SVR) leads to a complete recovery from all signs and symptoms of disease in the majority of patients (3b C).

• In patients with HCV infection, the presence of MCS does not substantially affect the rate of SVRs to combined pegylated interferon and ribavirin therapy (4 C).

• An attempt at viral eradication should be considered a first-line therapeutic option in patients with mild–moderate HCV-related MCS in the absence of any major contraindication (4 C).

• The current guidelines for the treatment of chronic hepatitis C should also be followed in the case of patients with HCV-related MCS (1b A).

• An extended duration of treatment (up to 48 weeks for HCV genotypes 2 or 3 and 72 weeks for HCV genotypes 1 or 4) may be considered in the case of virological non-responders who show clinical and laboratory improvements in MCS (5 D).

• The possible onset or worsening of some vasculitic manifestations (e.g. peripheral neuropathy, skin ulcers, etc.) should be carefully evaluated before starting treatment (5 D).

Biological therapy

Rituximab (RTX) and infliximab are the only two biological agents that have been tested in MCS so far. RTX has led to encouraging results in open studies and single case reports [67–93], whereas the initial results obtained using infliximab were contrasting and did not support its further use in MCS [94–96]. RTX is a monoclonal antibody against the CD20 antigen, which is selectively expressed on B cells. CD20-positive cells are expanded and activated in MCS, may harbour and present viral antigens, and play a crucial pathogenetic role in cryoglobulin production. The rationale underlying RTX treatment is to intervene downstream of the triggering disease mechanism more selectively than when the conventional immunosuppressive treatments are used [97]. It has been reported that RTX can improve or cure various clinical manifestations of MCS, including fatigue, skin manifestations (purpura and skin ulcers), arthralgias and arthritis, glomerulonephritis (GN) (in about 90% of cases), peripheral neuropathy (in about 75% of cases) and hyper-viscosity syndrome [67–93]. It has also been reported that it can also be effective in some

life-threatening cases of gastrointestinal vasculitis [74,77]. GN responds to RTX within 1–6 months, more frequently within the first three months. Skin ulcers usually improve within three months, but complete healing requires a longer time [67,68,70]. Both sensitive and motor neuropathy improve within 1– 5 months, with a stable or improved electromyography picture [67,68,70–72,89]. At the time of the final review of our statements (October 2010), there were more than 150 published cases of MCS treated with RTX, most of which came from uncontrolled studies or single case reports. A recent multicentre RCT involving MCS patients who had failed or were not eligible for antiviral therapy compared RTX monotherapy (at the dose recommended in rheumatoid arthritis, i.e., 1 g every two weeks for a total of two infusions, with or without low-dose steroids) with the best conventional immunosuppressive treatment (corticosteroids, cyclophosphamide, azathioprine or plasma exchange, as chosen by the clinician) and the standard dose of RTX (375 mg/m2 in four weekly infusions) used in most published MCS cases. The preliminary results of the trial supported the superiority of RTX [98].

B cell depletion has occurred in the vast majority of MCS patients receiving RTX. It has also been reported that RTX decreases serum cryoglobulin and rheumatoid factor levels (although their disappearance is less frequent), and increases C4 levels. The activity of RTX is also supported by the restoration of some MCS-related immune abnormalities [99], and the disappearance of bone marrow B cell clonal expansion [100,101]. The duration of the response to RTX is difficult to define because most of the studies lack long-term follow-up data. However, while bearing in mind this limitation, it is worth noting that short-term relapses (within 3–4 months of RTX discontinuation) have been observed in a minority of patients, and long-term responses (more than one year) have been the most frequent outcome. In some cases, more intense induction regimens or maintenance regimens have been attempted [69,71,73,74] Retreatment with RTX after a disease relapse has proved to be effective in most cases [67,70,71,74,78,79]. Maintenance RTX therapy has rarely been described in patients with MCS, but may be considered in those with severe nephritis or abdominal vasculitis [71,73,74]. Interestingly, RTX has a steroid-sparing effect in patients with MCS [67], some of whom (including cases with active nephritis) could be treated without steroids ab initio or with only short glucocorticosteroids courses [71,98]. Some authors suggest that naïve patients with serious clinical manifestations may receive RTX before antiviral therapy, which can be introduced later after the efficacy and safety of RTX have been assessed [97]. This advice is based on the observation of continuing MCS-related symptoms in patients with persistently negative serum HCV RNA findings [57,102], which suggests that an autoimmune process can become independent of viral triggering and play a predominant role in the pathogenesis of the disease. Short-term reactions to RTX infusions do not seem to be any more frequent in MCS than in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or Sjogren's syndrome (SS). Serum sickness was never observed in most studies and appeared to be rarely reported (about 1% in pooling data), even with high RTX doses [80,98]. A French Group, however, reported a higher incidence of serum sickness (about 10%), though stating that RTX is overall well tolerated in MCS [103]. Therefore, patients should be carefully monitored, in particular when high cryocrit levels are present. Pre-medication with 100 mg of methylprednisolone, anti-histamine drugs and paracetamol may reduce the risk of such reactions [104]. In patients with a history of heart failure or arrhythmia, consideration can be given to the administration of half a dose per day on two consecutive days and/ or to prolonging the administration of each infusion. RTX can increase HCV viral load, generally without significant liver impairment or serum albumin decrease [67,68,70,75]. At the time of submission of this paper, there were no data supporting a substantial risk of liver toxicity directly caused by RTX or HCV reactivation, although there is a lack of long-term follow-up data. Moreover, RTX has been given to MCS patients with liver cirrhosis and led to an improvement in MCS symptoms and liver function despite a transient increase in serum HCV RNA [86,93]. RTX may induce the severe reactivation of hepatitis B virus (HBV) infection and so, regardless of the presence of HBV DNA, should only be used in HBsAg-positive patients when strictly needed, and in combination with antiviral therapy. The same policy should be adopted in the case of potential occult HBV carriers (HBsAgnegative/anti-HBc-positive patients) [105]. Severe infections (lethal disseminated cryptococcosis and severe bacterial pneumonia) have been reported after RTX administration in two severely

immunocompromised renal transplant recipients with type III cyoglobulin-related graft dysfunction [73,106]. On the contrary, no life-threatening infections have been reported in patients with typical HCV-relatedMCS. Progressivemultifocal leukoencefalopathy has been never reported in MCS after rituximab therapy. However, it must be remembered that the published reports have rarely provided longterm follow-up data. The conference participants concluded that special care should be given to the prevention and management of infections, particularly in patients previously treated with immunosuppressants or steroids, or those with low serum immunoglobulin levels. De novo hypo-gammaglobulinemia [76], panniculitis [67], neutropenia [67,71,75,101] and retinal vascular occlusion [67,68] have been rarely reported as side effects of RTX treatment for MCS. The administration of aspirin has been proposed in the case of patients at cardiovascular risk [67] or with GN [69]. Direct experience of the use of RTX for patients with MCS was reported by about 70% of the conference participants. It has been hypothesised that the combination of antiviral and RTX therapy has a synergistic effect. Retrospective data and case reports have been published by French and Italian Authors [65,66,75,97]. Two very recent studies have compared combined therapy with antiviral treatment alone. A prospective, non-randomised cohort study of 93 patients found that combined therapy reduced the time to clinical remission (5.4± 4 versus 8.4± 4.7 months; P= 0.004), improved renal response rates (but not those of other organic manifestations), and led to higher rates of cryoglobulin clearance and clonal VH1-69+ B cell suppression than Peg-IFN+ RBV alone [107]. The authors concluded that the combined therapy is well tolerated and more effective than antiviral therapy alone. Similar results were achieved by the second study, which involved 37 patients [108]. However, none of these studies was designed to define the best first-line treatment in MCS and the advantages of combined therapy versus RTX monotherapy have never been investigated up to now in randomised trials. Some of the experts participating in the conference prefer the sequential treatment option (starting with RTX or antiviral therapy depending on the condition of the patient), and others favoured combination therapy ab initio. Nevertheless, all agreed that further investigations are required.

Statements

• Rituximab (RTX) is the only biological therapy that has proved to be beneficial in MCS, and should be considered when treating patients with severe clinical manifestations such as glomerulonephritis, skin ulcers or peripheral neuropathy (3 C).
• In the same clinical situations, RTX should be preferred over other more conventional treatments such as glucocorticoids, immunosuppressants or apheresis (3 C).
• In the same clinical situations, RTX may significantly reduce glucocorticoids administration (3 C).
• The monitoring of RTX infusion reactions should follow the guidelines used in the other clinical situations in which the drug is used (5 C).
• Patients receiving RTX should be carefully monitored for infectious complications, especially those with severe immunodepression (5 C).
• HCV viral load and liver function should be carefully monitored in patients receiving RTX, and antiviral prophylaxis should be given to HBV carriers (5 C) statements.
• Rituximab may be used in combination with antivirals in some cases of MCS (4 B).

Glucocorticoids

Glucocorticoids (GCs) are widely used to treat systemic vasculitis and, in the critical manifestation of MCS, have been prescribed at high doses (1–10 mg/kg) or as pulse therapy. Data from small case series support the effectiveness of high-dose pulse therapy in controlling disease flares [109–112]. The answers to our questionnaire indicated that high-dose pulse therapy is used by 94.7% of GISC centres, in the majority of cases for one single cycle and to treat a critical condition (renal, neurological or hyperviscosity syndromes). The long-term administration of low–medium GC doses (0.1– 0.5 mg/kg/day) is widely used in clinical practise, but the results of small controlled studies are conflicting [111–114]. Moreover, no studies have evaluated the effectiveness of long-term GC administration, although it is well known that the side effects of

long-lasting steroid therapy can be very serious and irreversible. All of the GISC centres reported the use of low (0.1–0.5 mg/kg/day) or intermediate GC doses (0.5–2 mg/kg/day) to control vasculitis symptoms or pain in MCS patients: only occasionally and for short courses in 32%, and with treatment limited to a few weeks in a further 18%, but 36% have prescribed chronic treatments for more than one year. GCs are frequently used in combination with other drugs [109– 116], and a RCT comparing INF with combined IFN and steroid treatment found that the combination led to better results [111]. The consensus panel concluded that high-dose GC pulse therapy is useful during MCS flares but, although there is considerable clinical experience of the use of low steroid doses, there are few data from controlled studies and therapeutic efficacy is controversial. Moreover, the side effects of long-lasting steroid therapy can be serious and irreversible, and careful patient monitoring is recommended to prevent them.

Statements

• High-dose or pulsed glucocorticoid (GC) therapy plays a substantial role in the management of critical patients with renal or neurological complications or serious vasculitic manifestations (4 C).
• The use of low–intermediate GC doses (0.1–0.5 mg/kg/day) has proved ineffective (1b A), but it has been reported that they improve the results of IFN therapy (1b A).
• In the opinion of some experts, short courses (weeks) of low– intermediate GC doses might be considered to control vasculitic flares in patients who do not respond or who are refractory to other treatments (5 D).
• Chronic treatment with low GC doses should be avoided whenever possible and in any case carefully monitored. Alternative therapies (colchicine, a low-antigen-content diet) should be considered for the maintenance treatment of MCS (5 D).

Apheresis

Many different apheretic procedures have been used to treat various clinical situations associated with MCS but, in the absence of controlled trials and large cohort studies, the available data comes only from case reports [109,117–132]. Most of these date back to before the association of HCV and MCS was recognised, and apheretic methods used were significantly different. A number of observations support the role of apheresis in improving acute renal disease [118–120] and in treating neuritis [115,118] and ulcers [109,132]. Furthermore, it remains the first-choice treatment for cryoglobulinemic hyperviscosity syndrome despite the lack of RCT data, which are obviously difficult to obtain in this rare and dramatic condition. The use of combined apheresis and immunosuppressants is supported by some clinical reports [124–129], but the majority of conference participants suggested great caution in using these drugs, which should be avoided in the case of HBV-infected patients. There is some evidence that apheresis synchronised with the intravenous administration of high-dose immunoglobulins can be used to treat ulcers and MC-related peripheral neuropathy, but this may also have a considerable immunosuppressive effect [132]. Apheresis is used in 84% of GISC centres, the majority of them (87%) using it in combination with cytotoxic drugs; 53% of the centres accept treatments lasting for more than three months. Almost all of the centres reserve its use to the treatment of critical complications (renal or neurological impairment, or hyper-viscosity syndrome). Plasma exchange and double filtration are considered the best apheretic approaches [119,132], with many experts preferring plasma exchange in the case of life-threatening complications. There was no agreement among the experts concerning treatment intensity, duration or frequency.

Statements

• Apheresis (usually combined with other treatments) can be used in the case of severe, life-threatening cryoglobulinemic manifestations (4 C), and is the treatment of choice for hyper-viscosity syndrome (5 B).
• Apheresis can be used to treat severe cryoglobulinemic manifestations when other therapies have failed or cannot be used (4 C).

• Apheresis should be used very cautiously in patients with severe HCV liver disease (5 D), and the evolution of HCV or HBV infection should be carefully monitored after apheresis, particular when it has been combined with immunosuppressants (5 D).

Cyclophosphamide

Cyclophosphamide (CTX) is the cytotoxic drug that is most frequently used in MCS patients [121–129] but, as it is usually used in combination with apheresis or other drugs, it is often impossible to distinguish its specific effects. Moreover, most of the available data was collected before it was discovered that HCV is a major determinant of MCS and the risk of using CTX in HCV-infected patients is poorly defined. The rationale underlying the use of CTX to treat MCS seems to be that of obtaining temporary immunosuppression after acute apheretic treatment, but there are no data from large cohort studies or RCTs to support this. Our systematic search of the literature revealed only small case series [121–129] in which CTX has been mainly considered in the case of membrano-proliferative GN or severe polyneuropathy. The use of an intravenous CTX bolus is believed to be safer than oral administration as it reduces the cumulative dose [133]. Almost all of the GISC centres use CTX in combination with apheresis. HCV infection and liver function should be carefully monitored after the administration of CTX. The use of other immunosuppressants such as cyclosporine, azathioprine, and methotrexate to treat MCS was only anecdotal and could not be evaluated because of the lack of data.

Statements

• Despite some observations of improvements in renal function and purpura after cyclophosphamide (CTX) treatment, the use of CTX alone to treat MCS is not recommended. However, its combined use with apheresis can be considered in the case of serious MCS-related conditions, when other therapeutic approaches fail or are contraindicated (4 C).
• CTX increases plasma HCV RNA levels (4 C) and the effects of CTX on liver function should be strictly monitored.

Colchicine

The rationale underlying the use of colchicine to treat MCS is based on the drug's activity in reducing Ig secretion [134,135]. A controlled, retrospective study of 17 patients treated with colchicine 1 mg/day for 6–48 months showed that it had favourable effects on purpura, weakness, leg ulcers and MCS-related laboratory abnormalities. Mild to substantial gastrointestinal side effects may occur during the first days/weeks of therapy, and prolonged treatment can cause haematological abnormalities [134]. Colchicine is prescribed for symptomatic patients in 56% of GISC centres, 44% of which currently prescribe treatments lasting 2–3 years, stopping only when side effects appear or the patients enter stable remission. In the experts' opinion, colchicine seems to be safe and effective in controlling the symptoms of patients with mild MCS and, in such cases, can also be considered as a means of sparing steroid therapy when other treatments can not be administered.

Statements

• Purpura and pain can be improved by administering colchicine 1 mg/day for at least six months (4 C).
• Long-term treatment maymaintain the effect and reduce glucocorticoid consumption (3b B).

Low-antigen-content diet

A low-antigen-content (LAC) diet can improve phagocyte activity and modify the composition of immune complexes. MCS patients who strictly follow such a diet experience a significant reduction in symptoms within 4–8 weeks [136,137]. A chronic LAC diet has a steroid sparing effect, reduces purpura and pain, is not expensive, and does not cause adverse effects; [138] however, some patients are not completely compliant or are unable to change their eating habits [116]. A LAC diet is currently used in 74% of GISC

centres, 57% of which limit its use to symptomatic patients, 78% prescribe it for as long as possible (without any pre-defined limit), and 43% continue its prescription during MCS flares.

Statements

• A low-antigen-content (LAC) diet improves MCS symptoms and laboratory abnormalities when strictly followed for 4–8 weeks (1b A).

• A LAC diet can be considered as supportive treatment in all symptomatic MC patients (3b B).

Analgesic and non-steroidal anti-inflammatory drugs

Pain is one of the major symptoms of MCS, and often severely limits the patients' quality of life. Interventions aimed at controlling pain are frequently necessary, even when patients are undergoing a LAC diet or receiving antiviral therapy or colchicine. No published data are available concerning the use of analgesics or non-steroidal antiinflammatory drugs (NSAIDs) in patients with MCS. Among the potentially useful drugs controlling pain, the participating centres currently use gabapentin or pregabalin (87% of cases), acetaminophen (62%), opioids (31%), NSAIDs (31%), amitriptyline (25%), and benzodiazepines (12%), with the treatment for each patient being tailored on the basis of the physician's approach. The experts participating in the conference agreed to suggest acetaminophen as the first-choice analgesic. Combined analgesic treatments seem to be necessary in patients with severe pain associated with peripheral neuropathy.

Statements

• Interventions aimed at controlling any pain in MCS patients should be attempted even during the administration of ‘etiological’ treatments (5 C).

• In the absence of controlled studies, the management of pain in MCS patients should be individually tailored and based on the drugs that have proved to be effective in controlling pain due to other vasculitides and neuropathies (5 C.)

The consensus recommendations are summarised in Table 1.

Discussion

The complexity of the etiopathogenesis of MCS and its polymorphic clinical manifestations make its treatment particularly challenging. Our Consensus Conference specifically focused on current strategies for treating MSC ‘as a whole’, leaving discussion of the management of its particular manifestations, such as skin ulcers, peripheral neuropathy, and GN to future projects. The relatively rare HCV-negative MCS and the monoclonal cryoglobulinemias (type I cryoglobulinemias in the immunochemical classification of Brouet et al.) [139] were also excluded as they presumably have a different pathogenesis from that of HCV-related MCS. The main limitation of the conference (but also the most important reason for holding it) was the scarcity of data from controlled trials of MCS treatment. However, the available data concerning the pathogenesis of the syndrome could be used as a reference to make some treatment suggestions when there were few or no clinical data. It was widely agreed that, regardless of the severity of MCS, an attempt to eradicate HCV should be made whenever possible because suppressing viral replication may limit or (in the most favourable cases) arrest the immunopathogenic process triggered by HCV [37,45–47,52–54]. Although the current guidelines for the treatment of chronic hepatitis C do not contraindicate antiviral treatment in patients with normal ALT and/or serum cryoglobulin levels, the percentage of MCS patients receiving antiviral treatment remains relatively low, regardless of the virus genotype, and depending on the specialisation of the caring centre. The lack of studies assessing the long-term results and side effects of antiviral treatment for MSC, or the best therapeutic schedule, may reduce the chances of its use, particularly in patients with mild–moderate liver disease. In clinical practise, the treatment of HCV-related MCS should be tailored to each individual patient on the basis of the progression and severity of the clinical manifestations. RCTs with an adequate statistical power and appropriate follow-up are required to clarify

whether better response rates can be obtained by extending treatment duration in virological non-responders who show clinical and laboratory improvements after a standard course of Peg-IFN+RBV. Given the complete absence of data concerning the use of HCV polymerase or protease inhibitors in patients with MCS, no recommendations could be included in this paper. It is conceivable that trials of these drugs in MCS patients will be proposed in the near future. RTX treatment is proposed for patients with severe MCS, i.e. with active GN, skin ulcers, or worsening/refractory peripheral neuropathy [67–93]. A number of important papers on the use of this drug have been very recently published [66,88–93,108], but the clinical criteria for its use in MCS patients, as well as its optimal positioning in relation to antiviral therapy, need to be better defined. Preliminary data from the first longterm, multicentre, RCT comparing RTX monotherapy with conventional immunosuppressive treatment encouraged the use of RTX in patients not responding to or ineligible for antiviral therapy [98]. In our experts' opinion, high-dose glucocorticoid therapy can be administered together with RTX, but should be tapered and discontinued as soon as possible. The steroid-sparing effect of RTX has been highlighted [98] and is a very important question that deserves further investigation. There is a rationale for combining RTX and antiviral therapy, but the advantages must be thoroughly investigated. Many other questions concerning the use of RTX in MCS patients remain open, including its long-term effects on liver function and immune response, response duration, re-treatment, and possible maintenance strategies, particularly in the case of severe, life-threatening manifestations. There was general agreement concerning the use of high-dose and pulsed GC therapy during MCS flares, when the severity of the clinical picture requires emergency intervention. On the contrary, the benefit of the chronic administration of low–medium doses was one of the most debated issues: the effectiveness of this treatment is questioned, and there is some evidence from controlled studies against its use. Moreover, the age of many patients, the frequent presence of co-morbidities, concomitant chronic HCV infection, and the risk of other infections are all significant caveats. However, as shown by the responses to the questionnaire, this therapeutic approach has been and is still widely used in GISC centres, particularly in the case of patients with mild–moderate MCS, and when IFN or RTX therapy are considered too hazardous. Nevertheless, the majority of the participating experts thought that the long-term administration of low–medium GC doses should be discouraged for its side effects. Colchicine may be an alternative for patients who have failed on, or could not be treated with antivirals or RTX. However, although it is quite widely use in GISC centres, most of the available data are anecdotal and little is known about the long-term side effects. Apheresis (with or without CTX) should be restricted to lifethreatening situations in which the other therapeutic approaches have failed or could not be used. A LAC diet is safe and inexpensive, and can be considered in all cases of MCS. Its only limitation is patient compliance, which may be insufficient. Finally, the conference strongly recommended the need to manage pain, which often greatly affects the quality of life of MCS patients. Unfortunately, this aspect has not yet been considered in any controlled trial. In conclusion, recent findings concerning the pathogenesis of MCS and the current availability of new drugs have significantly increased the possibility of treating MCS, and there is a possibility that other new drugs might improve treatment further in the near future. However, there is still a lack of RCT, and so physicians must still tailor individual treatments and make choices that are not always based on solid data. In the meantime, we hope that the recommendations made in this paper will help to support medical decision making.

Take-home messages

• Mixed cryoglobulins (MCs) are immune complexes that can precipitate at temperatures below 37 °C associated with acute and chronic infections, lymphoproliferative disorders, and autoimmune diseases.
• The treatment is still largely empirical, but the rational therapeutic approach should have three main objectives - to eradicate HCV; to limit or suppress B lymphocyte proliferation; and to contain and symptomatically treat the vasculitis and reduce the damage caused by circulating immune complexes.
• The GISC board organised the conference that is focused on what is known about the drugs used to treat MCS, and formulated the statements and the recommendations by combining the best available evidence from the literature with the experts' opinion.

• In clinical practise, the treatment of HCV-related MCS should be tailored to each individual patient on the basis of the progression and severity of the clinical manifestations.
• All of the conference participants use pegylated interferon + ribavirin in HCV-related MCS, although infectious disease specialists, gastroenterologists and hepatologists are more likely to use antiviral therapy than rheumatologists. Nevertheless, all of the experts agreed to extend the current standard of care for chronic HCV infection to MCS.
• Rituximab treatment is proposed for patients with severe MCS, i.e. with active glomerulonephritis, skin ulcers, or worsening/ refractory peripheral neuropathy.
• There is a rationale for combining rituximab and antiviral therapy, but the advantages must be thoroughly investigated.
• Long-term administration of low–medium corticosteroid doses should be discouraged for its side effects.
• Colchicine may be an alternative for patients who have failed on, or could not be treated with antivirals or rituximab.
• Apheresis (with or without cyclophosphamide) should be restricted to life-threatening situations in which the other therapeutic approaches have failed or could not be used.
• A low-antigen-content diet is safe and inexpensive, and can be considered in all cases of MCS.
• To manage pain, which often greatly affects the quality of life of MCS patients, is strongly recommended.

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