Review

Treatment of HCV-related mixed cryoglobulinemia

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Abstract

Mixed cryoglobulinemia syndrome (MCs) is an immune-complex-mediated systemic vasculitis, also called cryoglobulinemic vasculitis, involving skin, joints, peripheral nerves, and several internal organs. Hepatitis C virus (HCV) is recognised as the etiologic agent for the majority of MCs patients (MC), as well as of number of autoimmune-lymphoproliferative and neoplastic disorders. In this context, HCV-related MCs represents an important model of virus-driven autoimmunity/cancer in humans. With regard the therapeutic strategies of MCs we can treat these patients at different levels by means of etiological (antivirals), pathogenetic/symptomatic drugs (mainly immunosuppressors, corticosteroids, plasmapheresis). In the majority of individuals, MCs shows a mild, slow-progressive clinical course needing only symptomatic treatments, generally low doses of corticosteroids. Considering the etiopathogenesis of the disease, the eradication of HCV infection should be considered the gold standard in the treatment of MCs. The use of combined peg-interferon-α/ribavirin and/or novel antiviral drugs may lead to HCV eradication in a significant percentage of cases with possible remission of MCs. On the other hand, the presence of rapidly progressive, diffuse vasculitis with multiple organ involvement may be successfully treated with aggressive immunosuppressive and anti-inflammatory therapies, mainly based on cyclophosphamide or rituximab, high dose corticosteroids, and plasmapheresis. Moreover, sequential/combined antiviral or immunosuppressive treatments could represent an useful therapeutic strategy particularly in MCs patients with major clinical manifestations. In clinical practice, the treatment of MCs should be tailored for the single patient according to the severity of clinical symptoms. In all cases, a careful clinical monitoring of the disease is mandatory, with particular attention to neoplastic complications, such as B-cell lymphoma. The present review focuses on the different therapeutic strategies in patients with MCs, including the treatment of cryoglobulinemic skin ulcers, which represents one of the most discouraging complications of the disease.

Introduction.

Cryoglobulinemia refers to a laboratory abnormality characterized by the presence in the serum of one or more immunoglobulins, which precipitate at temperatures below 37°C and re-dissolve on re-warming [1, 2]. It is usually classified into three subgroups according to Ig composition [3]: namely, type I cryoglobulins are single monoclonal immunoglobulins related to hematological disorders, often B-cell lymphoproliferation, and it is *per se* frequently asymptomatic. Mixed cryoglobulinemia type II (IgG + monoclonal IgM) or type III (IgG + polyclonal IgM) consists of polyclonal IgG with or without monoclonal IgM, respectively, which has rheumatoid factor activity [1-3]. Circulating mixed cryoglobulins are commonly detected in a number of infectious or systemic autoimmune/neoplastic disorders [2-5]. On the contrary, mixed cryoglobulinemia syndrome (MCs) is regarded as distinct disorder, which can be classified among systemic vasculitides, in the subgroup involving small-medium sized vessels; MCs and cryoglobulinemic vasculitis (CV) are synonyms [5].

The histological hallmark of the disease is the leukocytoclastic vasculitis secondary to the vascular deposition of circulating cryo- and non-cryoprecipitable immune-complexes and complement [5]. The immune-mediated vasculitic lesions are responsible for different MCs clinical features, including cutaneous and visceral organ involvement [1-3, 5].

The prevalence of MCs is geographically heterogeneous; the disease is more common in Southern Europe than in Northern Europe or Northern America [5, 6]. It is considered to be a rare disorder, however, there are no adequate epidemiological studies regarding its overall prevalence, up to date. MCs is characterized by a clinical polymorphism, thus patients with vasculitis are often referred to different specialty centres, and a correct diagnosis might be delayed or overlooked entirely; for the same reasons, the true prevalence of MCs might be underestimated [2, 5]. Overall, MCs is more common in women than in men (female to male ratio of 3:1), while the disease onset is particularly common in the fourth-fifth decades and in older people, and rarely seen in the young people. From a clinico-pathogenetic point of view MCs could represents a crossroad between classical rheumatic/autoimmune

diseases, such as rheumatoid arthritis and primary Sjögren's syndrome, and lymphoproliferative/neoplastic disorders [2, 5].

Etiopathogenesis of MCs.

A causative role of hepatitis C virus (HCV) in the large majority of patients has been definitely established on the basis of epidemiological, pathological, and laboratory studies [7-11]. The prevalence of serum anti-HCV antibodies and/or HCV RNA in patients with MCs varies from 70% to almost 100% among different patient populations [12-13]; this finding relegates the term ‘essential’ MCs to a few patients [14]. The clinical development of MCs is closely linked to the natural history of chronic HCV infection; generally, this immunological disorder represents a late complication of viral infection. The appearance of different MCs phenotypes may be the result of genetic and/or environmental cofactors, which remain largely unknown [4, 5].

The etiopathogenetic cascade of MC and other HCV-related disorders is summarized in figure 1 (left).

The production of different organ-specific and systemic disorders is probably a multifactorial and multistep process: the trigger factors include some infectious agents, mainly HCV, host genetic predisposition, and possible unknown environmental/toxic causes. Some HCV antigens, i.e. HCV core, envelop E2, NS3, NS4, NS5A proteins, may represent a chronic stimulus to lymphocytes through specific receptors, such as CD81 that may interact with the viral E2 [15-17]. Predisposing factors may include particular HLA alleles [18], metabolic and hormonal status of infected individuals. The result is a 'benign' B-cell proliferation with a large autoantibody production, among which rheumatoid factor (RF), and cryo- and non-cryoprecipitable immune-complexes (IC) [19]. These immunological abnormalities may be correlated with different organ and non-organ-specific autoimmune disorders, including the systemic manifestations of MCs. On the other side, the activation of Bcl2 proto-oncogene, responsible for prolonged survival of B-lymphocytes, may be a precondition to other genetic aberrations, which ultimately may produce overt B-cell lymphomas and other malignant neoplasias [20, 21]. The appearance of different malignancies can be observed in a small but not negligible percentage of patients, usually late manifestation of chronic HCV

infection with/without MCs [20, 22-25]. Both immune-mediated and neoplastic diseases show a clinico-serological and pathological overlap. More frequently, autoimmune organ-specific manifestations may precede the development of systemic conditions, which in same individuals may be complicated by malignancies (Fig.1 left). From the other side, it is possible to observe the appearance of some autoimmune manifestations in patients with malignancies [23]. On the whole, the proposed 'HCV syndrome' encompasses this multiform complex of HCV-related clinical manifestations, among which the MCs represents a crossing road between autoimmune and neoplastic disorders [26].

Clinical features.

The MCs is clinically characterized by a triad -purpura, weakness, arthralgias-, present in the large majority of patients, and chronic hepatitis, membranoproliferative glomerulonephritis (MPGN), peripheral neuropathy, skin ulcers, diffuse vasculitis, and, less frequently, by lymphatic and hepatic malignancies [5; Fig. 2].

The presenting symptoms largely vary among patients with MCs. At the initial observation, patients show different clinico-serological patterns, ranging from apparently isolated serum mixed cryoglobulins to complete cryoglobulinemic syndrome (Fig. 2). For a correct clinical classification we can regard the MCs as a combination of serological findings (mixed cryoglobulins with RF activity and frequent low C4) and clinico-pathological features (purpura, leukocytoclastic vasculitis with multiple organ involvement) [27]. While, serum mixed cryoglobulins can be more frequently found in asymptomatic chronically HCV-infected individuals [5]. Figure 2 shows the main clinical features of MCs. Cutaneous manifestations represent the most frequent features of the MCs [2, 5]; the hallmark is the orthostatic, generally intermittent purpura, ranging from sporadic isolated petechiae to severe vasculitic lesions, while torpid ulcers of the legs and malleolar areas may affect a significant percentage of patients (Fig. 3).

MC patients may frequently develop a permanent ochreous coloration on the legs due to repeated episodes of purpura (Fig.3). Skin manifestations, in particular orthostatic purpura and ulcers, are the direct consequence of

vasculitic lesions with the possible contribution of chronic venous insufficiency, physical stress, including prolonged standing, and/or muggy weather. The majority of MCs patients suffers from arthralgias, while clear clinical signs of synovitis, usually mild, non-erosive oligoarthritis, are quite rare [28, 29, Sebastiani et al in the present issue]. Peripheral neuropathy may complicate the clinical course of MCs, frequently as mild sensory neuritis in about 80% of MCs patients [30, 31]; it is clinically characterized by paresthesias with painful and/or burning sensations in the lower limbs, often with nocturnal exacerbation. Therefore, the patients' quality of life is often compromised because of the chronicity of these symptoms along with their scarce sensibility to therapeutic attempts. In a minority of cases peripheral neuropathy may be complicated by severe sensory-motor manifestations; in some subjects it may complicate the alpha-interferon treatment [32]. Dysarthria and hemiplegia, consequence of central nervous system involvement, are rarely reported [5], while it is often difficult to distinguish these symptoms from the possible concomitant atherosclerotic manifestations. Moreover, mild-to-moderate chronic hepatitis can be observed at any time during the natural history of the disease as expression of the underlying HCV infection. Chronic hepatitis may evolve to cirrhosis, and less frequently may be complicated by hepatocellular carcinoma [23, 33]. With regards the overall prognostic value of hepatic manifestations, the liver involvement in patients with MCs seems to be less severe if compared to HCV-related chronic hepatitis alone [2, 5]. Xerostomia and xerophthalmia are present in almost half MCs patients; however, only a few cases meet the current criteria for the classification of primary Sjögren's syndrome [34, 35]. Raynaud phenomenon is reported in about 25% of patients, as showed in Fig. 2. Membranoproliferative glomerulonephritis (MPGN) type 1 is an important organ involvement, which may severely affect the prognosis and survival of the disease [36, 37]. MC-related MPGN is a typical immune-complex-mediated glomerulonephritis, although other immunological mechanisms have also been hypothesized. A widespread vasculitis involving medium-small sized arteries, capillaries and venules may develop in a small proportion of patients and may involve the skin, kidney, lungs, central nervous system, and gastrointestinal tract [2, 5, 38]. Interstitial lung involvement (mainly subclinical alveolitis) has been anecdotally observed in MCs as well as in

patients with isolated HCV infection [2, 39]. From a practical point of view, there is no relationship between the severity/activity of clinical symptoms, such as glomerulonephritis, skin ulcers, or diffuse vasculitis and the serum levels of cryoglobulins and/or hemolytic complement [5]. Some endocrinological disorders may be observed in MCs, mainly autoimmune thyroiditis with subclinical hypothyroidism, thyroid cancer, and an increased incidence of diabetes mellitus type 2 [40-43]. B-cell lymphoma is the most frequent neoplastic manifestation complicating MCs [24]. This malignancy may be related to the peripheral B-lymphocyte expansion and 'indolent' lymphoid infiltrates observed in the liver and bone marrow of MCs patients [25]. Other neoplastic manifestations, i.e. hepatocellular carcinoma or papillary thyroid cancer, are less frequently observed [33, 42]. In this light, the MCs can be regarded as a pre-neoplastic condition; consequently, careful clinical monitoring is recommendable, even in the presence of mild MCs. Low complement activity is almost invariably detectable in MCs, with the typical pattern of low/undetectable C4 and normal C3 serum levels, regardless the disease activity (Fig. 2). Of interest, a sudden increase in C4 to abnormally high levels can be observed in MCs patients complicated by B-cell lymphoma [5].

Treatment

MCs is characterized by mild, slow progressive clinical course, but in a significant number of patients with moderate/severe cutaneous and/or visceral organ involvement the therapeutical approach may result particularly challenging, considering the complex etiopathogenesis and clinical polymorphism of the disease. A correct strategy should deal with three concomitant factors; namely, HCV infection, autoimmune, and pre-neoplastic alterations (Fig. 1). Figure 1 shows the main steps of the etiopathogenetic process, i.e. viral infection, B-lymphocyte proliferation, and cryoglobulinemic vasculitis (Fig. 1); accordingly, we can treat MCs patients at different levels by means of etiologic, pathogenetic, and/or symptomatic therapies [2, 5, 26]. Considering the HCV as triggering and possibly perpetuating agent of the MCs through a chronic stimulus on the immune-system, an attempt at HCV eradication treatment should be done in all patients with HCV-associated MCs

[5, 44, 45; see also Zignego et al. in the present issue].

Initially, the treatment with Interferon alpha (IFNα) was associated with a relatively poor response and a high rate of disease relapses [46]. Combination therapy with IFNα plus ribavirin showed a greater antiviral efficacy with sustained virological response in a variable percentage of patients with chronic hepatitis C, as well as much better short- and long-term results in MCs patients than reported with IFNα monotherapy [46, 47]. However, the beneficial effect observed with IFNα and ribarivin is often transient and not rarely associated with important immune-mediated side effects such as peripheral sensory-motor neuropathy, thyroiditis, and rheumatoid-like polyarthritis [48]. It is possible that IFNα, both antiviral and immunomodulating agent, can trigger or exacerbate some symptoms in predisposed subjects, therefore IFNα therapy should be avoided at least in those patients with clinically evident peripheral neuropathy. The efficacy of antiviral therapy progressively increased during the time with the introduction of Peg-IFN and RBV, and ultimately it became very promising with the novel combination therapies, including interferon-free drugs [49; see also Zignego et al. in the present issue]. The long-term effects of HCV eradication needs to be deeply investigated; particularly the outcome of HCV-related immunological alterations, including cryoglobulinemia and its clinical manifestations. Although anecdotal or limited to small patients' series, preliminary observations suggest that the effects of HCV eradication on MCs remain quite unpredictable [50]. One of the possible factors could be the duration of the disease at the time of HCV eradication with self-perpetuating autoimmune mechanism underlying long-lasting MCs; therefore, it is possible to hypothesize a point of no return in the natural history of MCs. In the setting of HCV eradication, a recent study suggested that the presence of MCs may represent a negative prognostic factor of virological response, while the clearance of HCV may lead to persistent resolution or improvement of MCs [50]. The more recent use of triple therapy with Peg-IFNα, ribavirin, and a specifically targeted antiviral agent so called DAAs (i.e. Boceprevir, Telaprevir, Sofosbuvir) led to improve sustained virological response rates in patients infected with HCV genotype 1 [51]. Boceprevir-based therapy was reported as safe and effective in cryoglobulinemic patients [50; see also Zignego et al in the present issue], while the use of sofosbovir produced still

contrasting effects [52].

Further studies are needed to confirm and clarify the activity of these IFN-free antiviral combination in larger series of MCs patients.

Hopefully, a vaccine-based therapy [53] with recombinant HCV proteins in HCV-infected individuals could be able to prevent the progression of viral infection and possibly to interrupt the virus-driven autoimmune disease.

Immunosuppression with rituximab or cyclophosphamide represents the pathogenetic treatment of patients with MCs (Fig. 1 right and 4). These treatments include also steroids, low-antigen-content (LAC) diet, and plasma exchange [2, 26, 46, 54, 55].

The immunosuppressive agents are typically reserved for patients with severe disease manifestations such as MPGN, sensory-motor neuropathy and life-threatening complications. In these patients high dose corticosteroids and immunosuppressors has been used for the control of severe vasculitis lesions, alone or as sequential/combined therapy, while awaiting the generally slow response to antiviral treatments [46, 54-56].

Low dose corticosteroids may help to control minor intermittent inflammatory symptoms such as sporadic purpura, arthralgia, weakness, mild peripheral sensory neuropathy [2, 13, 26].

Plasmapheresis represents a pathogenetic/symptomatic therapy by removing circulating immune-complexes, mainly cryoglobulins, complement, and other pro-inflammatory agents; it is particularly effective for rapidly progressive glomerulonephritis, sensory-motor neuropathy, diffuse vasculitis, and/or severe skin ulcers [2, 57]. Both traditional plasma exchange and double-filtration plasma exchange, in combination with steroids and immunosuppressors may be able to markedly reduce the circulating immune-complex levels with rapid symptom amelioration [2]. Immunosuppressors (rituximab or cyclophosphamide) may reinforce the beneficial effect of plasma exchange and also prevent the possible rebound phenomenon during the tapering of aphaeretic sessions [38, 46].

LAC-diet can improve the clearance of circulating immune-complexes by restoring the activity of the reticule-endothelial system, overloaded by large amounts of circulating cryoglobulins [58]. This particular dietetic treatment is able to reduce the input of alimentary macromolecules crossing the mucosal barrier of the gut; some foods, particularly dairy products and eggs, present a

potential antigenic activity, and consequently might be involved in the pathogenesis of some immune-mediated diseases. The reduction of the alimentary input of macromolecules directed to mononuclear phagocyte system may improve its function in those diseases characterized by abnormal endogenous production of immune-complexes responsible for organ damage, i.e. mixed cryoglobulinemia and other immune-complex mediated diseases. In patients with clinically mild MCs symptoms LAC-diet has been usefully employed with low dosage of steroids (6- methyl-prednisolone 2–4 mg/day), often sufficient to improve mild MCs manifestations (arthralgias, sporadic purpura) [2, 54].

Anti-CD20 monoclonal antibody (rituximab, RTX) represents the first-line immunosuppressive/immunomodulating treatment of HCV-related MCs during the last years considering its efficacy and safety [49, 55, 56, 59]. In the first reports, the main indication for RTX was the failure or intolerance to other treatments, and/or associated lymphoma [60, 61].

RTX may be usefully employed alone or in combination/sequence with Peg-IFNα-ribavirin; in MCs patients, combined antiviral (boceprevir) and RTX treatment may be followed by complete remission of MCs manifestations and sustained virological response (see also Zignego et al. in this issue).

Tailoring specific treatment strategies

In clinical practice, the treatment of MCs should be tailored for the single patient according to the severity of clinical symptoms (Fig. 4). Clinically asymptomatic patients usually do not need any treatment, even in the presence of high levels of cryocrit. However, antiviral therapy might represent the etiological treatment also for patients with mild MCs manifestations, with the purpose to avoid the progression of the immunological disorder as well as of the most severe hepatic manifestations. Patients with mild or moderate disease (i.e. arthralgia, purpura, and/or mild sensory polyneuropathy) may be treated with low dose corticosteroids and/or LAC-diet able to control these minor inflammatory manifestations. On the contrary, sequential/combined antiviral and immunosuppressive therapy should be considered in patients with moderate-severe symptoms; an attempt with antivirals might be firstly used in subjects with active chronic hepatitis, with high risk to develop cirrhosis and/or hepatocellular carcinoma. Finally, patients with severe vasculitic

manifestations, MPGN, sensory-motor neuropathy, and/or widespread vasculitis must be timely treated with high doses of steroids, plasma exchange, and/or cyclophosphamide or RTX. For the above manifestations the therapeutic strategy should be tailored on the single patient according to the variable combination of clinical symptoms and possible comorbidities (fig. 4, right). For MCs patients presenting with the fulminant clinical variants, including peripheral necrosis of extremities, rapidly progressive glomerulonephritis, abdominal, cardiac, pulmonary and/or central nervous system vasculitic involvement and/or the rare hyperviscosity syndrome, plasmapheresis may lead to immediate beneficial effects. In general, the apheresis should be followed by immunosuppression, in order to avoid post-apheretic rebound of symptoms. In all cases, a careful clinical monitoring of MCs patients is mandatory, with particular attention to metabolic**,** autoimmune, and neoplastic complications [62-66].

The treatment of MCs associated with malignant lymphoproliferation, mainly B-cell NHL, is a priority than of the underlying hematological disorder.

Treatment of cryoglobulinemic skin ulcers

The updated review of the literature revealed the presence of skin ulcers in around a quarter of MCs patients [5]. These are often non-healing cutaneous lesions, possibly complicated by local infection and gangrene; moreover, they may severely affect the patients' quality of life and the overall prognosis [67]. Therefore, the treatment of cryoglobulinemic skin ulcers is particularly challenging in the clinical practice. These lesions are prevalent in the lower limbs, where other pathogenetic co-factors may be associated - namely, venous insufficiency and/or arteriosclerotic alterations, which are not rare in older patients with comorbidities and frequently treated with steroids [67]. Figure 5 summarizes the therapeutic strategy for cryoglobulinemic skin ulcers, including both systemic (immunosuppressors, corticosteroids, and/or plasma exchange) and local treatments [67]. Among systemic treatments, the anti-CD20 monoclonal antibody rituximab represents one of the most effective and frequently employed therapy [55, 56, 59]; while, available data focusing on local therapeutic approach are generally limited to anecdotal observations

[67]. Local treatments consisted of sharp or surgical debridement, as well as interactive dressing according to the condition of wound bed, perilesional skin, and the possible presence of infection, usually detected in the majority of patient with skin ulcers. Analgesic treatment is mandatory for background pain due to skin ulcers, as well as for procedural pain; this latter is critical for an effective local skin ulcers management. The majority of cryoglobulinemic skin ulcers heal at a variable time interval according to the severity of the single lesion; only a low percentage of patients with very severe, non-healing skin ulcers need amputation [67]. Overall, a trained team of operators with specific experience might be involved for an integrated therapeutic approach of these very challenging manifestations.

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Legend to the Figures

Fig. 1 Left: etiopathogenetic cascade of MCs. It is a multifactorial and multistep process, including HCV infection, predisposing host factors, and unknown environmental/toxic triggers. The main consequence is a 'benign' B-cell proliferation with a variety of autoantibody production, among which rheumatoid factor (RF), and cryo- and non-cryoprecipitable immune-complexes (IC), which may be correlated to various autoimmune disorders, including the MCs (or cryoglobulinemic vasculitis). Moreover, the activation of Bcl2 proto-oncogene, followed by other genetic aberrations, may lead to frank B-cell lymphomas and other malignancies. Both immunological and neoplastic disorders show a clinico-serological and pathological overlap. In several cases, autoimmune organ-specific manifestations may evolve to systemic conditions, and less frequently to malignancies. Conversely, it is not rare that patients with malignancies may develop one or more autoimmune manifestations. In this context, cryoglobulinemic vasculitis represents a crossing road between autoimmune and neoplastic disorders. While the overall symptom complex triggered by viral infection may be termed 'HCV syndrome'.

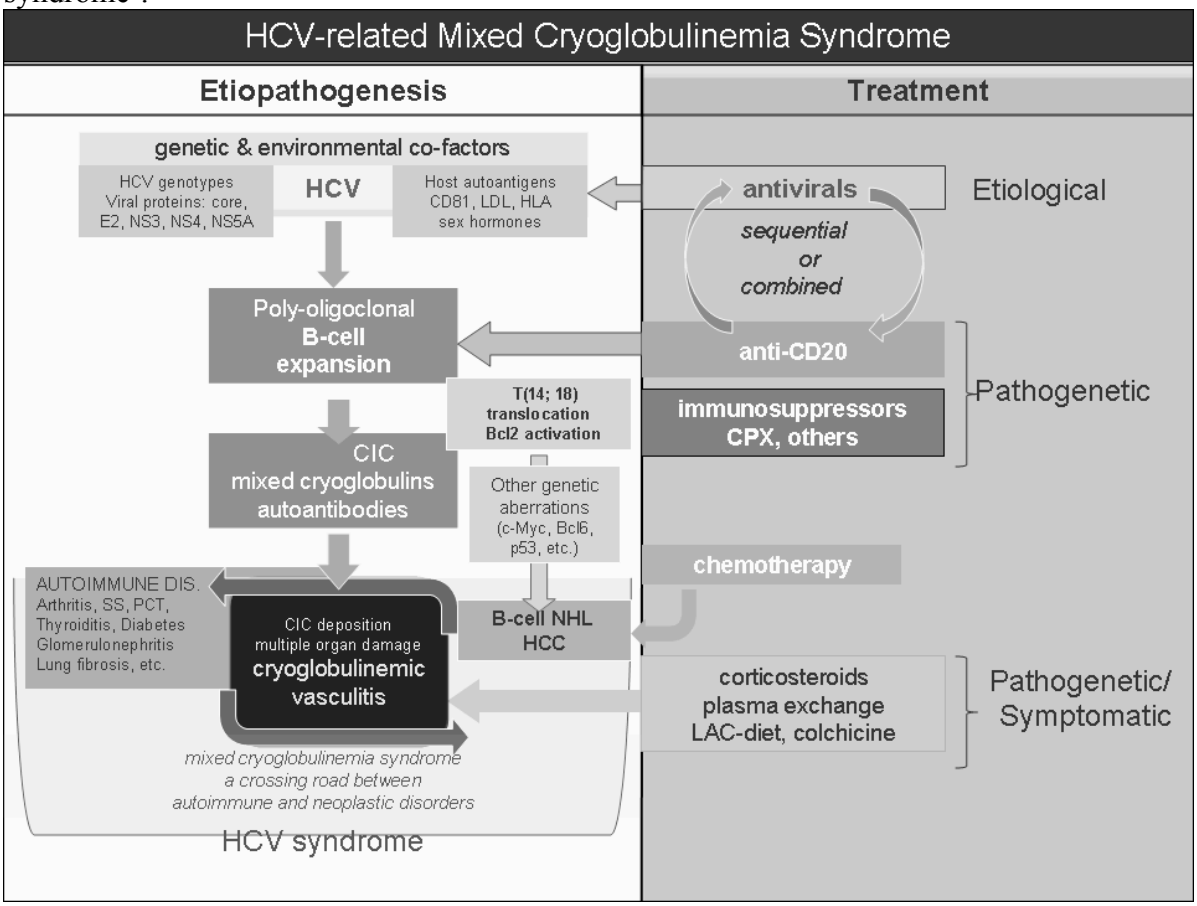

Fig. 2. Clinico-serological features of MCs.

HCV: hepatitis C virus; HBV: hepatitis B virus; ANA: antinuclear antibodies: ENA: anti-extractable nuclear antigen antibodies; ASMA: anti-smooth muscle antibodies AMA: antimitochondrial antibodies; B-NHL: B-cell non-Hodgkin's lymphoma

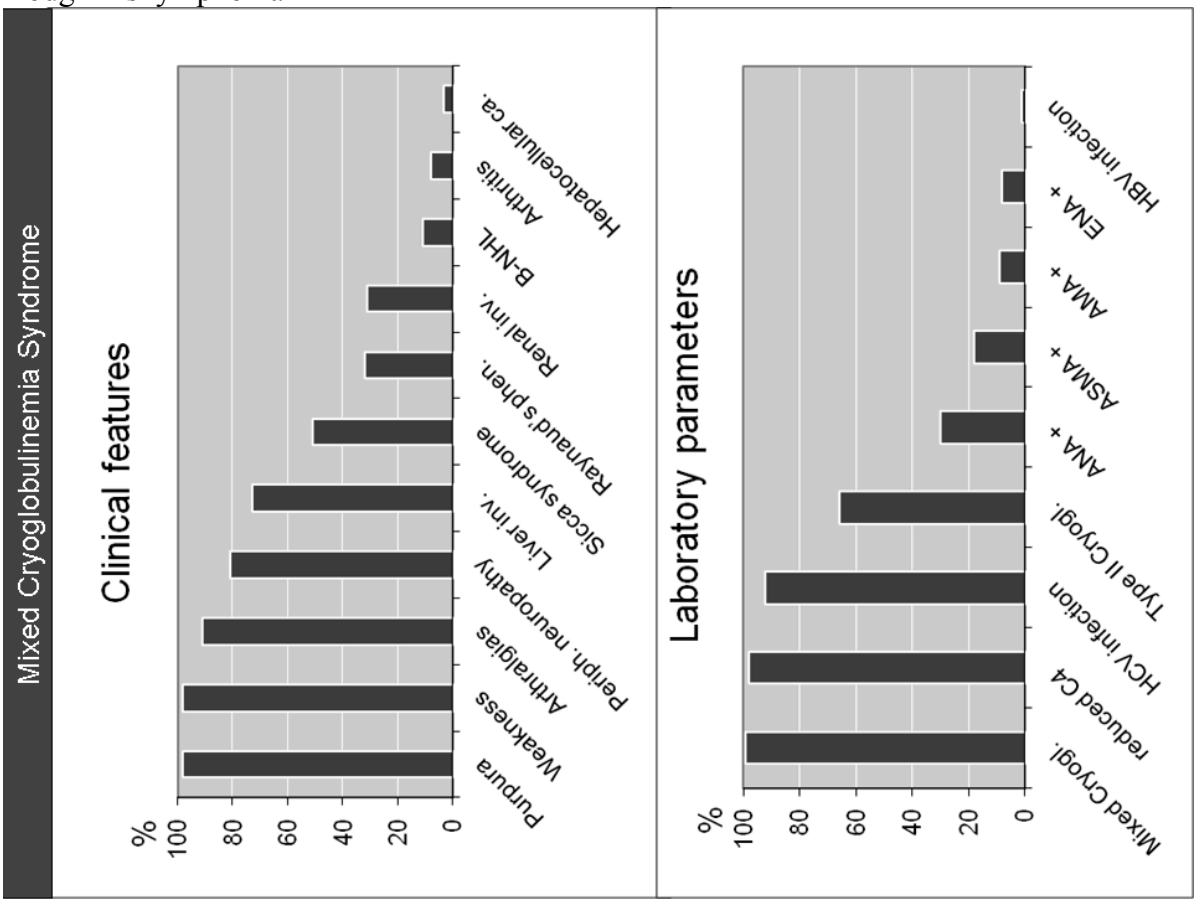

Fig. 3. Cutaneous manifestations of MCs.

different skin manifestations of cryoglobulinemic vasculitis:

a) orthostatic purpura secondary to necrotizing leukocytoclastic vasculitis of the skin;
b) symmetrical hyperpigmentation of the skin on the legs after repeated episodes of purpura; both orthostatic purpura and these permanent ochraceous lesions represent the typical skin manifestations of CV;
c-d) more severe vasculitic manifestations showing multiple skin ulcers with the presence of central necrotic tissue and slough (adherent fibrous material derived from proteins, fibrin and fibrinogen) in the wound bed. The presence of devitalized tissue acts as a physical barrier to epidermal cell migration and healing process; the borders of skin ulcers are irregular and scarcely reactive.

e) distal gangrene of the second toe of the right foot

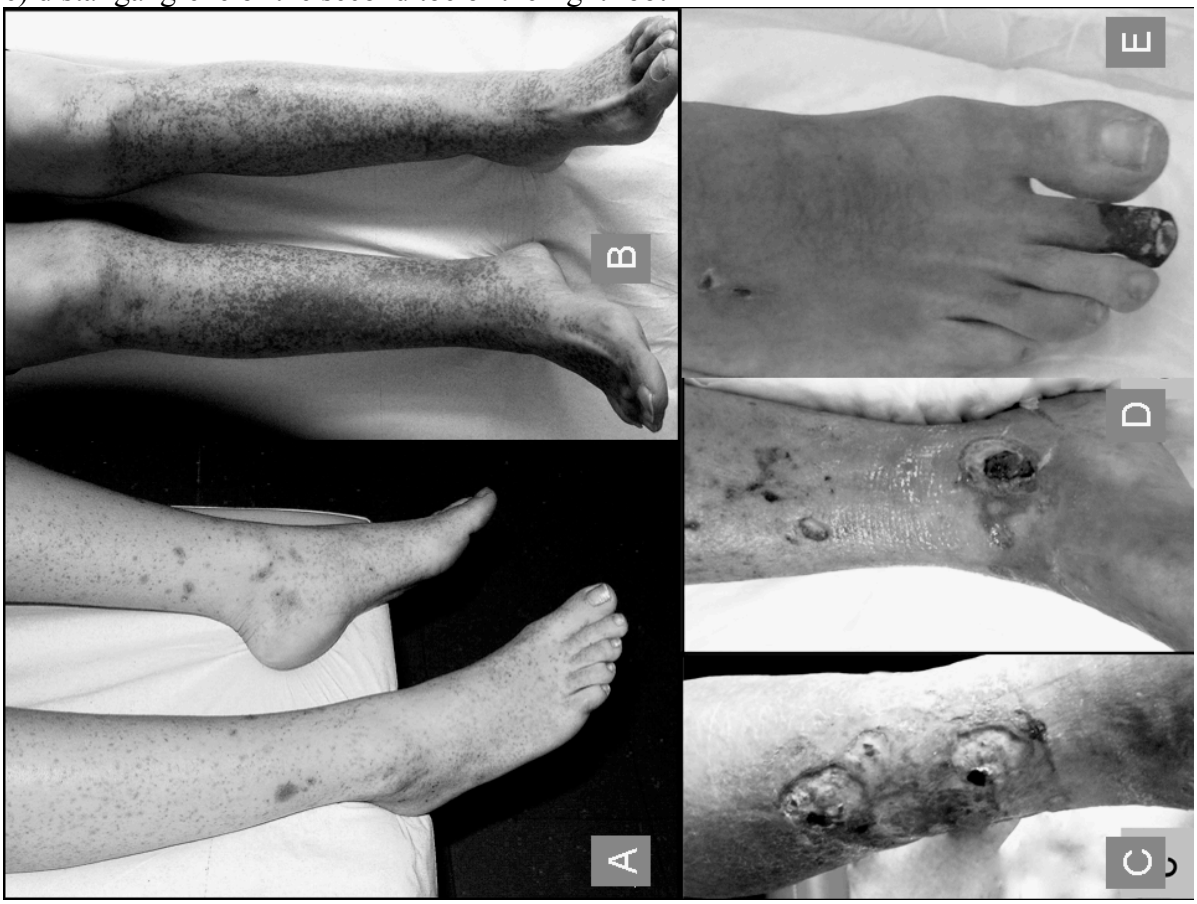

Fig. 4. The treatment of mixed cryoglobulinemic syndrome (MCs) may be modulated according to the clinical status of individual patients (see text).
CPX, cyclophosphamide; CS, corticosteroid; LAC, low antigen-content; RTX, rituximab.

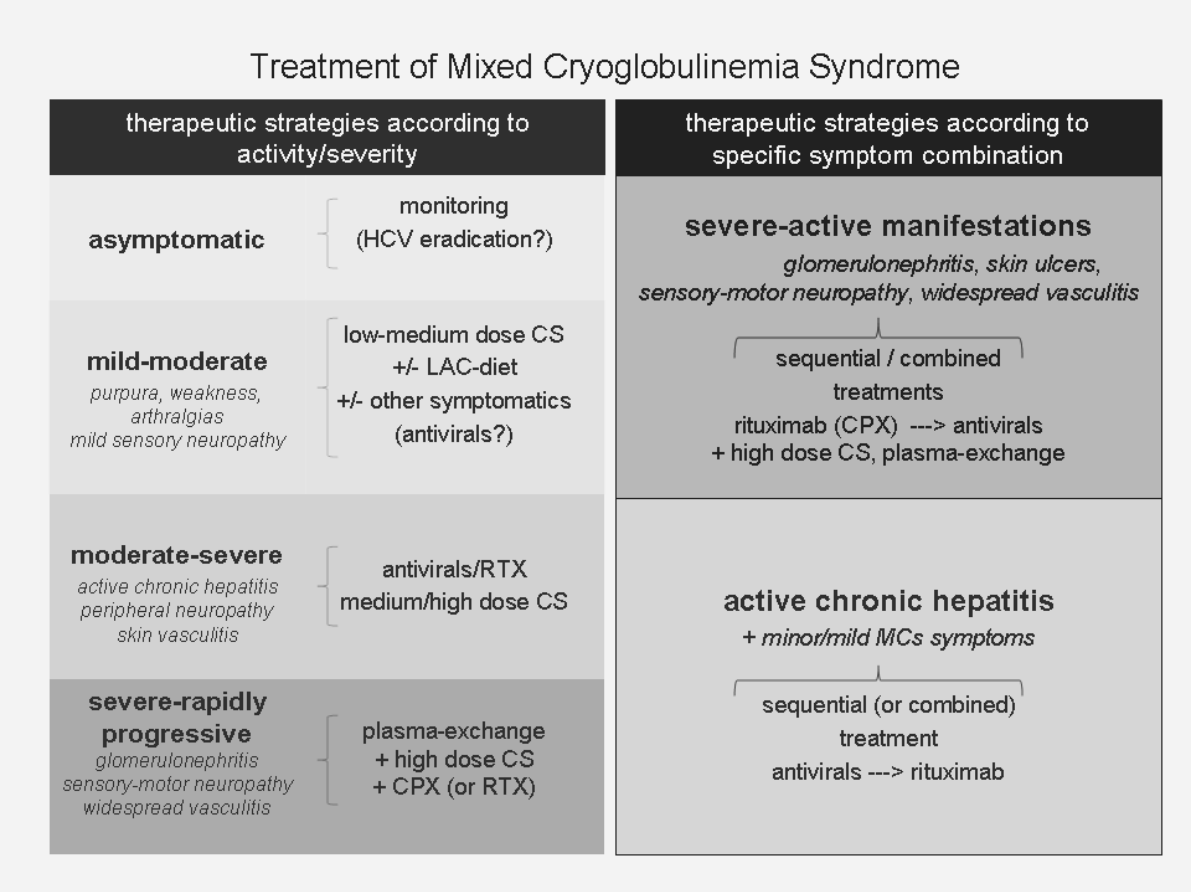

Fig. 5. Therapeutic strategy of cryoglobulinemic cutaneous ulcers should be based on both systemic and local treatments (see also text).

After clinical work-up considering the entire MCs, including possible comorbidities (venous insuffi ciency and/or arteriosclerotic alterations, diabetes, and so on), and careful examination of the ulcer characteristics, the systemic treatment may be based on etiological, pathogenetic, and/ or symptomatic therapies. More aggressive combined treatments (immunosuppressors, steroids, and plasmapheresis) may be necessary in the presence of very severe, non-healing skin ulcers. Long-term administration of analgesics is often necessary to improve the patient's chronic pain and compliance with local treatment, which should be carried out at a wound care clinic. Wound bed preparation, with particular regard to the prevention and treatment of infections, is crucial for the healing of cryoglobulinemic skin ulcers.

Therapeutic Strategies of Cryoglobulinemic Skin Ulcers (SU)

Systemic treatment

according to activity/severity of MCs and SU

Sequential/ combined

- Antivirals & Pathogenetic therapies
  - immunosuppressors
  - steroids
  - plasmapheresis

Symptomatics:

- vasoactive drugs
- low dosage steroids
- analgesics

Local treatment

Wound bed preparation:

- remove necrotic tissue
- prevent/treat infections
- moisture balance
- stimulate epithelial advancement
- procedural pain treatment

Growth factors:

- platelet gel

Autologous skin grafting