

# Cryoglobulinemia

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## Abstract

The term "cryoglobulinemia" refers to the presence in the serum of one or more immunoglobulins (Ig), which precipitate below 37°C and redissolve on re-warming. Cryoglobulinemia is usually classified into three subgroups: **Type I, simple cryoglobulinemia** composed of one monoclonal Ig is often associated with hematological diseases; it is frequently asymptomatic per se; **Type II, and type III mixed cryoglobulinemia (MC)** consisting of circulating immune-complexes composed by polyclonal IgGs, as autoantigens, and mono- (type II) or polyclonal (type III) IgMs, as corresponding autoantibodies. MC may be secondary to numerous infectious or immunological disorders; when isolated, MC may represent a distinct disease, the so-called "essential" MC. Given the striking association with hepatitis C virus (HCV) infection (>90%), the term "essential" is now referred to a minority of MC patients (<10%). HCV may infect the lymphoid tissues; consequently it may trigger a mono-polyclonal B-lymphocyte proliferation with different autoantibody production, including the cryoglobulins. MC syndrome is a systemic vasculitis secondary to the deposition of circulating immune-complex and complement in small-sized vessels. Clinically, it is characterized by variable organ involvement: purpura, skin ulcers, hepatitis, glomerulonephritis, peripheral neuropathy, and/or widespread vasculitis. Some patients may develop a malignancy, usually as late complication; namely, B-cell non-Hodgkin lymphoma (10 %), hepatocellular carcinoma (<5%), or thyroid cancer (<1%). The first-line treatment of MC should be directed to HCV eradication by interferon and ribavirin; however, this treatment is often unable to eradicate the virus and it may be complicated by important side effects (neuropathy, thyroiditis, etc.). Pathogenetic treatments (plasmapheresis, immunosuppressors, and/or corticosteroids) should be tailored for single patient according to the activity and severity of clinical manifestations. A careful patient monitoring is recommended for a timely diagnosis and treatment of life-threatening MC complications.

## Keywords

Cryoglobulinemia, mixed cryoglobulinemia, cryoglobulinemic vasculitis, hepatitis C virus, lymphoma

## Disease name and synonyms

Cryoglobulinemia, mixed cryoglobulinemia, cryoglobulinemic vasculitis

The term cryoglobulinemia refers to the presence in the serum of one (monoclonal cryoimmunoglobulinemia) or more

immunoglobulins (mixed cryoglobulinemia), which precipitate at temperatures below 37°C and redissolve on re-warming (1,2). This is an in vitro phenomenon; the actual mechanism(s) of cryoprecipitation remains obscure; it could be secondary to intrinsic characteristics of both

mono- and polyclonal immunoglobulin (Ig) components, it can be caused as well by the interaction among single components of the cryoprecipitate (3).

Cryoglobulinemia is usually classified into three subgroups (4) according to Ig composition (Table 1).

**Table 1. Classification of Cryoglobulins**

Classification of Cryoglobulins	Composition of cryoprecipitates
Type I cryoglobulinemia	simple or single-component (Ig) cryoglobulinemia
Type II mixed cryoglobulinemia	polyclonal IgGs + monoclonal IgM
Type III mixed cryoglobulinemia	polyclonal IgGs + polyclonal IgMs

Type I cryoglobulinemia is composed of only one isotype or subclass of immunoglobulin. Both type II and type III mixed cryoglobulinemia (MC) are immune complexes composed of polyclonal IgGs, the autoantigens, and mono- or polyclonal IgMs, respectively; the IgMs are the corresponding autoantibodies with rheumatoid factor (RF) activity (3-6).

Type I or monoclonal cryoglobulinemia is almost invariably associated with a well-known hematological disorder and it is frequently asymptomatic per se. Similarly, circulating mixed cryoglobulins are commonly detected in a great number of infectious or systemic disorders (1-6). On the contrary, "essential" mixed cryoglobulinemia (MC) represents a distinct disorder (3-6); it can be classified among systemic vasculitides (3-10). The histopathological hallmark of MC is a leukocytoclastic vasculitis involving small- and medium-sized vessels and responsible for multiple organ involvement. A frequent synonym of the disease is the term "cryoglobulinemic vasculitis" which better focus on the typical histopathological alterations responsible for MC cutaneous and visceral organ involvement (3, 10).

#### **Etiology of Mixed Cryoglobulinemia**

Since the first description of MC syndrome, chronic hepatitis has been reported as frequent manifestation appearing during the clinical course of the disease (3-6). Therefore, a possible role for hepatotropic viruses in the pathogenesis of the disease has long been suggested (3, 11). A role for hepatitis B virus (HBV) has been firstly investigated; however, HBV viremia is rarely recorded, while anti-HBV antibodies largely varied among different MC patient populations (3). It can be estimated that HBV can represent a causative factor of MC in

less than 5% of individuals. Following the discovery of hepatitis C virus (HCV) as the major etiologic agent of non-A-non-B chronic hepatitis (12), a great number of clinico-epidemiological, histopathological, and virological studies (HCV RNA detection by polymerase chain reaction - PCR- and/or in situ hybridization -ISH) have been definitely established the relevant role for HCV in the pathogenesis of MC (3, 8-10, 15-19). The prevalence of serum anti-HCV antibodies and/or HCV RNA in MC patients ranged from 70% to almost 100% among different patient populations (3, 20).

#### **Etiopathogenesis of MC and its overlapping with other diseases**

Because of the frequent association between MC and HCV, the behavior of MC is closely linked to the natural history of HCV chronic infection (3). However, the MC is also the result of concomitant genetic and/or environmental factors, which remain largely unknown. HCV has been recognized to be both an hepato- and lymphotropic virus, as suggested by the presence of active or latent viral replication in the peripheral lymphocytes of patients with type C hepatitis or MC (21-22). Moreover, Bcl-2 recombination in B-lymphocytes may be observed in a significant percentage of HCV-infected individuals, particularly in patients with HCV-related MC (23, 24). The activation of this anti-apoptotic protooncogene may be responsible for B-lymphocyte expansion and consequent autoantibody production, including the cryoglobulins. Due to its biological characteristics HCV may be involved in a wide number of autoimmune and lymphoproliferative disorders (3, 9, 18, 20, 25-31).

Besides MC syndrome, other important HCV-associated disorders are porphyria cutanea tarda (PCT), autoimmune hepatitis, membranoproliferative glomerulonephritis, B-cell lymphomas, and other malignancies (3, 9, 18, 20, 25-31). Thus a frequent clinico-pathological overlapping may exist between MC and other HCV-related disorders (3, 25).

#### **Diagnosis criteria / classification**

There are no available diagnostic criteria for MC; in 1989 the Italian Group for the Study of Cryoglobulinaemias has proposed preliminary criteria for MC classification (32). A revised version of these criteria (Table 2), including clinico-pathological and virological findings, has been recently proposed (3). Circulating mixed cryoglobulins, low C4, and orthostatic skin purpura are the hallmarks of the disease; moreover, leukocytoclastic vasculitis, involving medium- and, more often, small-sized blood vessels (arterioles, capillaries, and venules) is responsible for MC tissue injury (3-6, 9-10).

**Table 2. Proposed criteria for the classification of mixed cryoglobulinaemia patients.**

criteria	serological	pathological	clinical
major	- mixed cryoglobulins - low C4	- Leukocytoclastic vasculitis	- purpura
minor	- rheumatoid factor + - HCV + - HBV +	- clonal B-cell infiltrates (liver and/or bone marrow)	- chronic hepatitis - MPGN - peripheral neuropathy - skin ulcers

**Definite mixed cryoglobulinemia syndrome:**

- a. serum mixed cryoglobulins (± low C4) + purpura + leukocytoclastic vasculitis
- b. serum mixed cryoglobulins (± low C4)+ 2 minor clinical symptoms  
+ 2 minor serological/pathological findings

**Essential or secondary mixed cryoglobulinemia:**

absence or presence of well-known disorders (infectious, immunological or neoplastic)

HCV+ or HBV+: markers of hepatitis C virus or hepatitis B virus infection (anti-HCV ± HCV RNA; HBV DNA or HBsAg); MPGN: membranoproliferative glomerulonephritis.

Cryoglobulinemic vasculitis is secondary to vascular deposition of circulating immune-complexes, mainly cryoglobulins, and complement, with the possible contribution of both hemorheological and local factors (3, 33). Due to its clinical and histological features, MC is classified among systemic vasculitides, in the subgroup of small vessel vasculitides, which also includes cutaneous leukocytoclastic vasculitis and Schonlein-Henoch purpura (3, 10).

**Differential diagnosis**

The so-called "essential" MC was first described by Meltzer *et al.* in 1966 (5); originally, this term was referred to autonomous disease when other well known systemic, infectious or neoplastic disorders have been ruled out by means of a wide clinico-serological work-up.

Given the striking association between MC and hepatitis C virus (HCV) infection (>90%), the term "essential" is now referred to a minority of MC patients (<10%) (3). Moreover, HCV is the triggering factor of a variety of disorders (3, 9, 18, 20, 25-31). In this scenario MC can represent a crossroads between some autoimmune disorders (autoimmune hepatitis, sicca syndrome, glomerulonephritis, thyroiditis, etc.) and malignancies (B-cell lymphomas, hepatocellular carcinoma) (3, 9, 18, 20, 25-31). Therefore, a careful patient evaluation is necessary for a correct diagnosis of MC syndrome. It is not rare in clinical practice to observe in the same patient a slow progression from mild HCV-associated hepatitis to various extrahepatic manifestations (arthralgias, sicca syndrome, Raynaud's phenomenon, rheumatoid

factor positivity, etc.), and ultimately to overt MC syndrome with typical clinico-serological manifestations. In only a minority of MC patients a malignancy may develop, generally after a long lasting follow up period (3, 20, 31, 34-36).

For these reasons a clinical monitoring of MC patients is recommended for a timely diagnosis of life-threatening MC complications, mainly the nephropathy, widespread vasculitis, and B-cell lymphoma or other malignancies.

**Frequency**

The prevalence of MC presents great geographic heterogeneity; the disease is more common in Southern Europe than in Northern Europe or Northern America (3-9). The disease is considered to be a relatively rare disorder; however, as yet there are no adequate epidemiological studies regarding its overall prevalence. Given its clinical polymorphism, a single manifestation (skin vasculitis, hepatitis, nephritis, peripheral neuropathy, etc.) is often the only apparent or clinically predominant feature, so that MC patients are often referred to different specialties. A correct diagnosis might thus be delayed or overlooked entirely; consequently, the actual prevalence of MC is probably underestimated.

For the same reasons, the clinical pattern of the MC syndrome may vary largely among patients series referred to different tertiary care facilities (3, 6, 8, 19, 37).

It has been estimated that low levels of circulating mixed cryoglobulins can be detected in over 50% of HCV-infected individuals, while overt cryoglobulinemic syndrome develops in about 5% (3, 28-29). Because of the wide diffusion of HCV infection worldwide, a growing incidence of HCV-related MC can be expected, especially in underdeveloped countries where HCV in the general population is quite prevalent (3, 38).

**Clinical description**

MC syndrome is characterized clinically by a triad -purpura, weakness, arthralgias- and by a series of pathological conditions, including chronic hepatitis, membranoproliferative glomerulonephritis (MPGN), peripheral neuropathy, skin ulcers, diffuse vasculitis, and less frequently by lymphatic and hepatic malignancies (3-10, 19, 25-27, 30-31, 34-37). The clinical pattern of cryoglobulinemic vasculitis is comparable in patients with type II or type III MC (3). The prevalence of MC manifestations reported in Table 3 regards an Italian patient population referred to a rheumatology-immunology division; a variable patient recruitment at different specialist centers together with racial differences among patients

series is often responsible for some contrasting data present in the literature (3-10, 19). At the initial observation, MC can present with different clinico-serological patterns, varying from isolated serum mixed cryoglobulins, probably expression of early stage of the disease, to complete cryoglobulinemic syndrome. This is 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). Moreover, incomplete MC, i.e. mixed cryoglobulinemia with one or two typical MC features (arthralgias, peripheral paresthesias, mild hepatitis, RF-seropositivity, low C4, etc.) is frequently found, mainly in rheumatic outpatient clinic. In these subjects overt MC syndrome can develop during long-term follow-up. On the other hand, we may see some patients with typical cryoglobulinemic syndrome, but without serum cryoglobulins, the hallmark of the disease. This is generally a transient phenomenon due to the wide variability of cryoprecipitable immune-complex levels (39). Repeated cryoglobulin determinations are necessary for a correct diagnosis in these subjects;

**Table 3. Demographic, clinico-serological and virological features of 200 MC patients**

Age, mean ± SD years (range)*	54±12 (29-74)
female/male ratio	3
Disease duration, mean ± SD years (range)	11±6 (1-36)
Purpura	95%
Weakness	94%
Arthralgias	85%
Arthritis (non-erosive)	9%
Raynaud's phenomenon	35%
Sicca syndrome	35%
Peripheral neuropathy	45%
Renal involvement**	31%
Liver involvement	71%
B-cell non-Hodgkin's lymphoma	8.5%
Hepatocellular carcinoma	2.5 %
Cryocrit, mean ± SD %	3.8±7.4
Type II/type III mixed cryoglobulins	2/1
C3, mean ± SD mg/dl (normal 60-130)	86±33
C4, mean ± SD mg/dl (normal 20-55)	11±14
Antinuclear antibodies	25%
Antimitochondrial antibodies	11%
Anti-smooth muscle antibodies	22%
Anti-extractable nuclear antigen antibodies	8%
Anti-HCV antibodies	92%
HCV RNA	86%
Anti-HBV antibodies	40%
HBsAg	3%

\*at the disease onset; \*\*invariably membrano-proliferative glomerulonephritis;

### Diagnostic methods

The main diagnostic parameter of MC is the presence of serum mixed (IgG-IgM) cryoglobulins. Unfortunately, there are no universally accepted methodologies for cryoglobulin measurements; however, simple standardized indications are often sufficient for testing cryoglobulinemia (3, 9, 38). Due to their thermal instability, the measurement of cryoglobulin level in the blood should be performed immediately in the same place where the blood is sampled. For a correct evaluation of serum cryoglobulins it is necessary to avoid false-negative results due to Ig cold precipitation also at room temperature: the first steps (blood sampling, clotting, and serum separation by centrifugation) should be always carried out at 37°C and the cryocrit determination and cryoglobulin characterization at 4°C (after 7 days). Moreover, cryocrit determinations (percentage of packed cryoglobulins referred to total serum after centrifugation at +4°C) should be done on blood samples without anticoagulation to avoid false-positive results due to cryofibrinogen. Without the above relatively simple precautions, not only will the quantities of cryoglobulins measured be incorrect, but the test may completely fail to even detect cryoglobulins. While the detection of serum cryoglobulins is fundamental for the diagnosis of MC, the levels of serum cryoglobulins usually do not correlate with the severity and prognosis of the disease (3). Very low levels of cryocrit, often difficult to quantify, can be associated with severe, active cryoglobulinemic syndrome; on the contrary, high levels of serum cryoglobulins may characterize an oligo- or asymptomatic disease course. In these subjects, particularly in the presence of a cryogel phenomenon, rheological alterations due to blood hyperviscosity can be observed (33, 38). Finally, a sudden decrease or disappearance of serum mixed cryoglobulins, sometimes associated with abnormally high levels of C4, can be the presenting manifestation of complicating B-cell malignancy (40).

The analysis of cryoprecipitates is generally carried out by means of immunoelectrophoresis or immunofixation. Using more sensitive methodologies, i.e. immunoblotting or two-dimensional polyacrylamide gel electrophoresis, type II MC shows a microheterogeneous composition; in particular, oligoclonal IgM or a mixture of polyclonal and monoclonal IgM can be detected (3, 37, 41). This particular serological subset, termed type II-III MC, could represent an intermediate, evolutive state from type III to type II.

## Management

Clinical course, treatment, and prognosis of type I cryoglobulinemia largely depend on the underlying disorder, varying from benign monoclonal gammopathy of undetermined significance to malignant B-cell neoplasias. In some individuals, type I cryoglobulinemia may be responsible for hyperviscosity syndrome; in these instances a short plasma exchange course is often able to resolve this complication (3). On the contrary, both type II and type III MC are often responsible for a more or less severe clinical syndrome with multiple organ involvement (Table 3). Due to its complex etiopathogenesis and clinical polymorphism, the treatment of MC is particularly challenging. For a correct therapeutic approach we must deal with three important factors; namely, the HCV infection, the presence of autoimmune disorder, and the possible neoplastic complications (3). Following the etiopathogenesis process leading from HCV infection to cryoglobulinemic vasculitis we can treat the disease at different levels by means of different –etiologic, pathogenesis, symptomatic- therapies (Table 4). Since HCV represents the triggering factor of the disease as well as it may exert a chronic stimulus on the immune-system (3, 9, 10, 16, 21-25, 35-36), an attempt at HCV eradication by alpha-interferon treatment should be done in all cases of HCV-associated MC (3, 10, 42-46). However, the beneficial effect observed with this drug is often transient and not rarely associated with important immune-mediated side-effects such as peripheral sensory-motor neuropathy, thyroiditis, and rheumatoid-like polyarthritides (47-49). Probably, in predisposed subjects alpha-interferon, both antiviral and immunomodulating agent, can trigger or exacerbate some pre-existing, often subclinical, symptoms. Unfortunately, there are no available parameters for predicting this harmful complication; thus, alpha-interferon therapy should be avoided at least in those patients with clinically evident peripheral neuropathy. On the whole, the usefulness of alpha-interferon treatment in MC patients is limited by the low rate of responders and frequent side effects. The association of alpha-interferon and ribavirin might achieve the eradication of HCV infection in a rather significant number of treated subjects, as recently demonstrated in patients with type C chronic hepatitis (50-52). Controlled clinical trials are necessary to evaluate the usefulness of such combination therapy in HCV-associated MC. Hopefully, with the rapid growth of molecular biology a vaccine against HCV might be available in the near future. A vaccine-based therapy (54) with recombinant HCV proteins in HCV-infected individuals could be able to prevent the progression of viral infection and

possibly to interrupt the self-perpetuating autoimmune mechanism underlying the MC. In rare cases of non-HCV-associated MC the immunosuppressive treatment, mainly cyclophosphamide, is still the first-line intervention. For HCV-associated MC immunomodulating/immunosuppressive treatments should be considered, especially in patients who have failed to respond to alpha-interferon. These treatments include steroids, low-antigen-content (LAC) diet, plasma exchange, and immunosuppressors (3, 54-57). In particular, both traditional plasma exchange and double-filtration plasma exchange are able to markedly reduce the levels of circulating immune-complex, especially the cryoglobulins (100-101). Oral cyclophosphamide (50-100 mg/day for 2-6 weeks) during the tapering of aphaeretic sessions can reinforce the beneficial effect of plasma exchange; moreover, it can prevent the rebound phenomenon that may be observed after the aphaeresis discontinuation. Plasma exchange is particularly useful in severe MC complications such as active membranoproliferative glomerulonephritis (Table 4). LAC-diet is a particular dietetic treatment that can improve the clearance circulating immune-complexes by restoring the activity of the reticulo-endothelial system, overloaded by large amounts of circulating cryoglobulins (54, 57). LAC-diet and/or low dosage of steroids may be sufficient to improve mild manifestations of the MC (Table 4). Patients with mild-moderate symptoms, such as palpable purpura, are particularly sensitive to the smallest variations of daily steroid dosage (1-2 mg). In clinical practice, MC treatment should be tailored for the single patient according to the severity of clinical symptoms. Therefore, patients with severe vasculitic manifestations must be promptly treated with high doses of steroids and/or plasma exchange and/or cyclophosphamide, while clinically asymptomatic patients usually do not need any treatment, even in the presence of high levels of cryocrit. In all cases a careful clinical monitoring of the disease is mandatory, with particular attention to neoplastic complications.

**Table 4. Treatment of HCV-associated Mixed Cryoglobulinaemia**

State of patient	Proposed treatments	
Asymptomatic	none	Attempt at HCV eradication
Mild manifestations purpura, weakness arthralgias, arthritis, peripheral sensory neuropathy	LAC-diet, low dosage of steroids other symptomatics	Attempt at HCV eradication
Severe manifestations nephropathy, skin ulcers, sensory-motor neuropathy, widespread vasculitis, active hepatitis	steroids, plasma exchange, cyclophosphamide, alpha-interferon + ribavirine	Attempt at HCV eradication
Cancer B-cell NHL, HCC	chemotherapy, surgery	Attempt at HCV eradication

NHL: non-Hodgkin's lymphoma, HCC: hepatocellular carcinoma.

### Unresolved questions

After the first description of MC syndrome as distinct disorder in 1966 (5), the discovery of the association between HCV and MC in the large majority of patients (3, 17) represents a decisive contribute for a better comprehension of the pathogenesis mechanisms responsible for the disease, and consequently for adequate therapeutic strategy.

However, the following questions remain still to be answered:

1. The exact role of HCV in the pathogenesis of the disease; HCV could be only the triggering factor of the MC or it could also contribute to the self-perpetuating mechanism of the disease
2. The role of antiviral treatment that could positively affect the natural history of the disease
3. The possible role of new/alternative therapies

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