



# Understanding the Cryoglobulinemias

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## Abstract

**Purpose of the Review** Cryoglobulins are immunoglobulins with the ability to precipitate at temperatures  $<37^{\circ}\text{C}$ . They are related to hematological disorders, infections [especially hepatitis C virus (HCV)], and autoimmune diseases. In this article, the state of the art on Cryoglobulinemic Vasculitis (CV), in a helpful and schematic way, with a special focus on HCV related Mixed Cryoglobulinemia treatment are reviewed.

**Recent Findings** Direct – acting antivirals (DAA) against HCV have emerged as an important key in HCV treatment to related Cryoglobulinemic Vasculitis, and should be kept in mind as the initial treatment in non-severe manifestations. On the other hand, a recent consensus panel has published their recommendations for treatment in severe and life threatening manifestations of Mixed Cryoglobulinemias.

**Summary** HCV-Cryoglobulinemic vasculitis is the most frequent form of CV. There are new treatment options in HCV-CV with DAA, with an important number of patients achieving complete response and sustained virologic response (SVR). In cases of severe forms of CV, treatment with Rituximab and PLEX are options. The lack of data on maintenance therapy could impulse future studies in this setting.

**Keywords** HCV · Mixed Cryoglobulinemia · Type I Cryoglobulinemia · gC1qR · Direct-acting antivirals · Rituximab

## Introduction and Definitions

Cryoglobulins are immunoglobulins (Ig) that precipitate in vitro at cold temperature and dissolve at  $37^{\circ}\text{C}$ , while Cryoglobulinemia (CG) is defined as the presence of cryoglobulins in serum [1, 2]. It must be noted that CG is a laboratory finding and is not necessarily associated with any signs or disease symptoms. Cryoglobulinemic Vasculitis (CV) corresponds to the presence of clinical manifestations due to immune complex deposition along medium and small vessel walls [3]. The presence of symptoms without a positive test for serum cryoglobulins may occur because of an important deposi-

tion of the total pool of cryoprecipitable immunocomplexes in targeted vessels and due to false negative results owing to improper blood sampling or inadequate laboratory processes [4]. Due to the latter, cryoglobulin testing should be serially rechecked when CV is highly suspected. On the other hand, healthy people can have CG at low concentrations and infections can trigger the appearance of serum cryoglobulins temporarily without symptoms [3–5].

Since 1974, CG has usually been classified in three subgroups according to immunoglobulin composition (“Brouet’s classification”): Type I CG corresponds to one monoclonal isotype or subclass of Ig, typically IgM. It is always associated with hematological disorders like Multiple Myeloma (MM), Waldenstrom’s Macroglobulinemia (WM), Monoclonal Gammopathy of Undetermined Significance (MGUS), Chronic Lymphocytic Leukemia (CLL) and B Cell Non-Hodgkin Lymphoma (B-NHL). Type II and III CGs are typically composed of IgM and IgG, so they are called Mixed Cryoglobulinemias (MC). Characteristically the IgM component of MCs has rheumatoid factor activity so can form immune complexes. Type II CG is usually conformed of a combination of monoclonal IgM and polyclonal IgG while type III CG presents both polyclonal IgM and IgG [3, 6, 7••] (Fig. 1).

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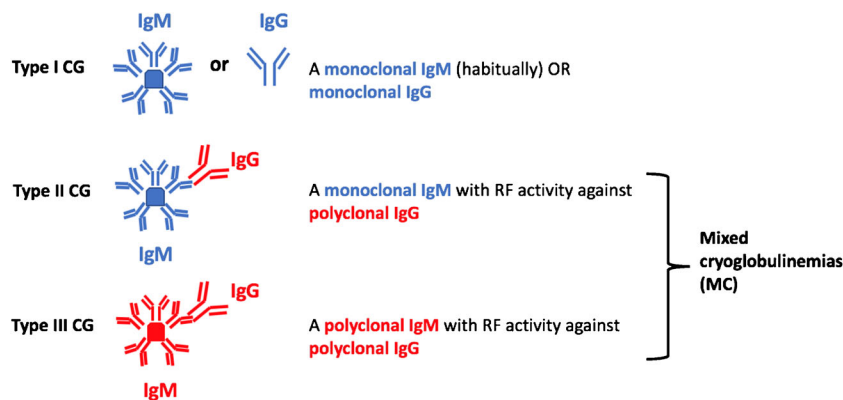
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**Fig. 1** Understanding the Cryoglobulinemias. CG: cryoglobulinemia, RF: Rheumatoid factor. Note that type I CGs are almost IgM, less frequently IgG and rarely IgA



The presence of oligoclonal IgM or a mixture of monoclonal and polyclonal IgM plus polyclonal IgG have been described as type II – III mixed CG and is considered a transition from type III to type II CG [7••, 8].

## Causes

The percentage of each type of cryoglobulin in total number of patients with CG and the most important causes related to CG are described in Table 1.

## Epidemiology

CV is a rare disease with an estimated prevalence of less than 5 cases per 100,000 patients in Europe and North America. Information on the prevalence in the rest of the world is scant, but the highest prevalence has been described in the Mediterranean basin [7••].

It is known that 80–90% of MC cases are caused by HCV infection and the presence of CG depends on how long the

individual has been infected (annual incidence of 3%) [11–14]. On the other hand, 40–60% of patients infected by HCV have Mixed Cryoglobulinemia while only 5% of these infected patients will develop CV [3, 11, 15]. Interestingly, for unknown reasons, patients infected with HCV from the Mediterranean basin have the highest prevalence of MC and this group of patients is more prone to be infected with HCV [3]. When comparing patients with HCV chronic infection, with and without Cryoglobulinemia, the former have higher incidence of cirrhosis and higher fibrosis scores [16, 17].

As previously described, other viruses have been related to the presence of MC. Findings of importance are that 7–17% of HIV patients have Cryoglobulins in their serum, while the coinfection of HIV/HCV increases the percentage to 35–64% [3, 18]. About 2% of CV is due to HBV infection [19].

MC has been observed in up to 25% of patients with Systemic Lupus Erythematosus and 10% of patients with Rheumatoid Arthritis [3, 20]. In patients with primary Sjogren's Syndrome (pSS), 5–20% may have type II CG [21]. The latter association is related with extra glandular involvement, development of systemic vasculitis and B cell lymphoma in pSS patients [22].

**Table 1** Main causes of Cryoglobulinemias

Type of CG	%	Main causes
Type I CG	10%	Hematological disorders: WM, MM, MGUS, CLL, B-NHL
Type II CG	65%	HCV
Type III CG	25%	(80–90% of MC)
		- AI diseases: Sjögren's Syndrome (mainly type II CG), SLE and RA
		- Non-HCV viruses: HBV, HIV/HCV coinfection, HIV alone, EBV, CMV
		- Other infectious causes: Bacteria or parasites, other viruses *
		- B- NHL (mainly type II CG)
		- Idiopathic or essential MC: 10% of cases

CG: cryoglobulinemia; AI: autoimmune; SLE: Systemic Lupus Erythematosus; RA: Rheumatoid Arthritis; WM: Waldenstrom's Macroglobulinemia; MM: Multiple Myeloma; MGUS: Monoclonal Gammopathy Of Undetermined Significance; CLL: Chronic Lymphocytic Leukemia; B-NHL: B Cell Non-Hodgkin Lymphoma; HCV: Hepatitis C Virus; HBV: Hepatitis B Virus; HIV: Human immunodeficiency Virus; EBV: Epstein Barr Virus; CMV: Cytomegalovirus; B-NHL: B non-Hodgkin lymphoma, MC: Mixed Cryoglobulinemia [3, 7••, 9, 10].

\*Other infectious causes of MC can be seen elsewhere [7••]. [3, 7••, 9, 10]

## Relationship between MC related to HCV and B - NHL

An Italian retrospective multicenter study of 1255 patients with symptomatic MC related to HCV, showed that these patients are 35 times more likely to develop B-NHL than people without MC, especially with low grade lymphomas [23, 24]. Some risk factors for the appearance of B-NHL in these patients were the presence of a symptomatic CG, more than 0.6 g/L of cryoglobulins in serum and low immunoglobulin levels [7••].

## Pathophysiology and Disease Mechanisms

The continuum of production of cryoglobulins and the presence of clinical manifestations in Cryoglobulinemia may be summarized in three steps [3, 7••]: **(1)** In the context of lymphoproliferative disorders or a chronic stimulation of B lymphocytes (e.g. chronic infections or rheumatic diseases) there will be a monoclonal or polyclonal expansion of B cells (BC) in type I/II and III CG, respectively. **(2)** The subsequent synthesis of mono, oligo or polyclonal cryoglobulins. **(3)** Tissue injury due to Cryoglobulinemia; with vessel occlusion and hyper viscosity in type I CG and formation/deposit of immune complexes in type II/III CG.

The fact that cryoglobulins are cold insoluble is not fully understood [4]. The diminished presence of tyrosine in the molecule with a relative majority of hydrophobic amino acids; a smaller amount of galactose and sialic acid at Fc region of these immunoglobulins, and large IgM – IgG immune complexes in type II CG have been related to cold precipitation [4, 25, 26]. Because manifestations of CG are not exclusive to acral zones of the body (e.g. kidney compromise), other factors as pH, ionic strength, concentration of cryoglobulins in plasma or different local visceral concentration of chloride or calcium are also implicated in aggregation of cryoglobulins [4, 27–29].

## HCV infection Model

The most studied model corresponds to type II MC due to HCV infection. The E2 protein from envelope of HCV interacts with CD81, a transmembrane protein that is present in B lymphocytes (working as a complex with CD19 and CD21) and hepatocytes [30, 31]. Of consideration, the viral replication is seen in CD19 peripheral blood mononuclear cells (PBMC) but not in CD19 negative PBMC [32]. Once activated by HCV, the latter complex mentioned between CD81, CD19 and CD21 triggers a polyclonal expansion that apparently is not related to B cell receptor (BCR) stimulation [7••, 33]; also, an upregulation of B cell activator factor (BAFF/Blys) has been described [34, 35]. Importantly, the hallmark of type II MC is the presence of B cell clones that produce monoclonal IgM (the WA cross-reacting idio type) with rheumatoid factor activity against polyclonal IgG, which in turn binds to HCV core protein [3, 7••, 36–38].

These B cell clones are present as lymphoid infiltrates in the liver and other sites as bone marrow, spleen and peripheral blood [3, 7••]. Of note, beside cryoglobulins, HCV infection stimulates the production of various other antibodies that determine the presence of other autoimmune manifestations in the context of a “HCV syndrome” [39] (Table 2).

The immune dysregulation characterized by HCV infection and the presence of CG have been described as risk factors for lymphomagenesis [40–42]. In addition to chronic stimulation of B cells, other mechanisms related to HCV infection and the transformation from polyclonal to monoclonal BC are: (a) The presence of mutations in genes related to heavy and light immunoglobulin (Ig) chains [43]; (b) Overexpression of *BCL-2* oncogene due to augmented t(14:18) translocation and the overexpression of other oncogenes as *MYC* [7••, 44]; (c) The role of certain viral proteins (NS3 and core proteins) that cause alterations in DNA repair and (d) activation of different intracellular signaling pathways as JNK and ERK or transcription factors as NF-κB [42].

As discussed above, tissue injury in type I CG is derived from vessel occlusion with high concentrations of CGs. On the other hand, immune – complex (IC) deposition and subsequent complement activation in MC due to HCV infection is the most important mechanism. The ICs containing HCV core proteins also contain C1q protein which can bind to the receptor for the globular domain of C1q protein (gC1q-R) in the membrane of endothelial cells, a process that can be related to deposition and posterior vascular damage [38, 45]. Of note, the latter ICs that bind to gC1qR can promote activation of complement system (classical pathway) and deposition of C4, a fact that could explain the characteristically low C4 in these patients [46••]. Moreover, CGs escape from the erythrocyte transport system and the HCV infection interferes with the lysosomal activity of phagocytes so there is a diminished blood clearance of these ICs [47, 48].

## Clinical Presentation

### Type I CG

The typical clinical picture of type I CG is related to vessel occlusion due to a characteristic ability for cryoprecipitation,

**Table 2** Some autoimmune manifestations of the “HCV syndrome”

Rheumatology	- Cryoglobulinemic vasculitis - Sicca syndrome
Endocrinology	- Autoimmune diabetes - Autoimmune thyroiditis
Hematology	- Hemolytic anemia - Thrombocytopenia - B- cell lymphoma
Pneumology	- Interstitial lung disease

but some vasculitis features can be seen too [9]. In this context, the presence of cold – induced symptoms (90–100%), purpura (80%), Raynaud phenomenon (25–40%) that sometimes can be complicated with digital gangrene; distal ulcers with necrosis (30–35%) and peripheral neuropathy (30–50%) are important findings. Less frequently, renal compromise, arthralgias and *livedo reticularis* are seen [49, 50]. Cryocrit is usually above 5% and can be higher than 50%. In the case of high cryocrit and therefore a high serum viscosity (> than 4.0 cP habitually), hyperviscosity syndrome (HS) could develop. Some features of HS are mental confusion and headache; visual loss or blurred vision; nasal bleeding and hypoacusia [3, 9].

### Type II & III CG (MC)

Due to RF activity and consequent immune complexes formation and activation of the complement system, the clinical manifestations of MC are related to small and medium vessel vasculitis [3, 9]. CV is characterized by a triad of purpura, weakness and arthralgias (“Meltzer’s triad”). Also, a variable combination of symptoms can be associated, including: skin ulcers, diffuse vasculitis, peripheral neuropathy, membranoproliferative glomerulonephritis (MPGN), chronic hepatitis, lymphatic and hepatic malignancies. Classic laboratory findings are low complement levels (specially C4) and positive RF. Cryocrit is lower than 5% in MC [9].

- **Cutaneous:** The most common manifestation of MC is purpura (90–98%) of the lower limbs which might be orthostatic and intermittent and is a key clue for diagnosis, often complicated with ulcers or hyperpigmentation at resolution [19]. Cutaneous ulcers and *livedo reticularis* traduce compromise of medium - sized vessels [3]. The typical histopathological pattern of CV is the presence of non-necrotizing leukocytoclastic vasculitis of small and medium vessels. Necrotizing vasculitis with fibrinoid necrosis can be seen in 20% [10].
- **Articular:** Frequently arthralgias (44–71%). Less often, a non-erosive oligoarthritis can be seen (10%) [3].
- **Sicca syndrome** (50%) [39].
- **Neurologic:** Neuropathies occur in 65% of HCV - related MC by clinical criteria and 80% by electrodiagnostic testing (ET); polyneuropathies are more common than mononeuritis multiplex [2, 51–53]. Most neuropathies are distal, slowly progressive, and sensorimotor (but sensory predominant). About 50% of neuropathies are painful, and approximately 25% of them are small fiber in character [54]. ET studies reveal an axonal, sensorimotor neuropathy and nerve biopsies reveal predominantly axonal alterations with mononuclear predominant perivascular inflammation in the epineurium, with definite vasculitis in 50% [55].

Central nervous system (CNS) involvement is rarely seen but may appear with dysarthria and hemiplegia [2].

- **Renal:** Renal involvement (RI) has been reported in 18–40% of patients with CV and these patients have higher mortality [19, 56–58]. The occurrence of RI follows the diagnosis of Cryoglobulinemia after 2.6–4 years and occurs until 41 years after diagnosis [59, 60]. It is mainly characterized by urinary abnormalities: proteinuria is seen in 88–100% of cases and hematuria in almost all patients. Elevation of plasma creatinine is described in 47–63% of patients with RI [59, 60]. Kidney biopsy must be performed for confirmation of RI in CV: the histopathological features observed are characterized by a glomerular monocyte infiltration with double contours of the basement membrane and hyaline intraluminal thrombi, similar to a type I membranoproliferative glomerulonephritis (MPGN) [61]. Immunofluorescence analysis defines the RI as an immune complex-mediated MPGN, showing intra-glomerular sub-endothelial deposits of Ig identical to those of the cryoprecipitate, and complement components. Renal necrotizing vasculitis and extra- capillary crescents are rarely observed [61, 62]. Some predictive factors of RI in CV are the presence of Type II CG, a high cryocrit, an IgG kappa monoclonal component and diabetes [63].
- **Liver / Gastrointestinal (GI):** Due to HCV infection, over 70% of patients develop chronic hepatitis. This has a mild to moderate clinical course, but may evolve to cirrhosis in 25% of patients. Around 3% of patients develop hepatocellular carcinoma [39]. GI tract compromise has been observed in 2–6% of patients. Some manifestations are abdominal pain, GI bleeding and perforation due to intestinal ischemia [3, 64].
- **Hyper viscosity Syndrome:** Rare manifestation, < 3% in MC [65].
- **Respiratory system (RS):** The compromise of the RS accounts for less than 5%. The presence of interstitial lung disease, alveolar hemorrhage due to vasculitis and pleural effusion have been described [3].
- **Diffuse vasculitis:** Severe and unusual complication of the disease. Involves medium to small vessels with multiple organ involvement (skin, lungs, kidneys, gastrointestinal and CNS) [2].
- **Endocrinological disorders:** More frequent than in patients without CV. Thyroid disorders (including cancer); diabetes mellitus type 2 and erectile dysfunction can be seen [66–68].
- **Cardiovascular disease:** Reports of myocarditis, myocardial infarction, heart failure and pericarditis have been published [69, 70].



Some manifestations are considered as life – threatening (LT) events (GI ischemia, RI, alveolar hemorrhage and CNS compromise) (Table 3). In a Spanish series of 209 patients diagnosed with CG between 1991 and 1999, 14% of them developed LT manifestations of CG. Of these, 59% presented LT events as the initial manifestation of the disease and 66% died. Interestingly, intestinal ischemia and pulmonary hemorrhage had 100% of mortality. With regard to etiology, 76% had associated chronic viral infection (HCV and HIV) and 31% of patients had autoimmune diseases. Patients with LT Cryoglobulinemic Vasculitis had a higher frequency of fever, type II cryoglobulins, low C3 levels and a higher mean value of cryocrit than patients with milder forms of CV [69, 71].

## Diagnosis

Diagnosis is based on history, clinical manifestations of the disease and the presence of cryoglobulins in serum. It is necessary to rule out differential diagnosis as Rheumatoid Arthritis, primary Sjögren’s syndrome, B cell neoplasms and MPGN. Also, the viral status of the patient must be explored (HCV, HBV, HIV) [7••, 39].

For the detection of cryoglobulins, 10 to 20 mL of blood is drawn into collection tubes, prewarmed to 37 °C without anticoagulants. After clotting at 37 °C for 30 to 60 min, the serum is separated by centrifugation at 37 °C, placed in a graduated (Wintrobe) tube and refrigerated (4 °C) to allow the precipitation of cryoglobulin for 3–7 days. After that, if positive, a precipitate should appear at the bottom of the tube. If the precipitate is truly cryoglobulins, it will re-dissolve when the tube is heated at 37 °C. In this process, pre-analytical conditions are probably the most important. If the temperature of the pre-analytical conditions is not controlled to prevent cooling, cryoglobulins may already precipitate during this stage and give false negative results [72].

The next step for laboratories is to determine a cryocrit, which is a measure of the packed (centrifuged) volume of

the precipitate as a percentage of the original serum volume at 4 °C. The cryocrit in individuals without CG is close to zero and a cryocrit over 0.5 to 1% or cryoglobulin concentration above 2 to 5 mg/dL is considered clinically significant [59]. It should be kept in mind that there is no strict relationship between the cryoglobulin concentration and the severity of the clinical manifestations [56]. Posteriorly, the precipitate can be used for typing and quantification of the cryoprecipitate [72]. Typing of the cryoglobulin is normally performed by immunofixation or immune-electrophoresis. Typing enables identification of the cryoprecipitate as immunoglobulin, and allows to make clinical associations and prevent possible complications for the three types of cryoglobulins [73].

## Treatment

The treatment of cryoglobulinemic manifestations depends on the type of CG, the etiology and its severity. It should be noted that cold and long standing avoidance must be encouraged in all patients [9].

In type I CG the basic principle of treatment will be the management of the underlying hematologic disorder [9, 10]. As discussed before, CLL, MM, WM, MGUS or B-NHL are the most common malignancies associated with type I CG [7••, 9]. The use of steroids plus Rituximab (RTX), Bortezomib, Lenalidomide or other agents as Cyclophosphamide have been described as therapeutic options in a large American series of 102 patients with type I CG and WM guidelines [49, 74]. In the case of hyper viscosity symptoms, plasma exchange (PLEX) should be performed together with the agents exposed above [9]. Of note, it must be considered that RTX has been associated with hyper viscosity syndrome due to IgM flare in WM patients, so preemptive plasmapheresis before RTX should be considered in the case of a basal IgM of >4 g/dl [9, 75].

The treatment of Cryoglobulinemic vasculitis (CV) in the context of MC must be divided into infectious or non-infectious causes. The next step is to classify CV manifestations as non-severe, severe or life threatening (Table 3) [9]. In the case of infectious CV due to HCV, non-severe symptoms can be treated with direct antiviral agents (DAA) alone. DAA has emerged as a “game changer” in the treatment of HCV virus, with a sustained viral response (SVR) at 12 weeks of 97–100% and less toxic effects than interferon- based schemes. At long term follow up, SVR is associated with a negative detection of HCV RNA and morbimortality associated with HCV [76, 77]. The larger study of CV due to HCV infection is an international, prospective and open - label trial (VASCUVALDIC 3) of 148 patients with HCV associated CV, all of them received a free interferon regimen with DAA for 12 or 24 weeks. At 12 weeks after DAA were stopped, 72% of patients had a complete clinical response (defined as improvement of every organ affected at baseline without a

**Table 3** Severity of manifestations in Cryoglobulinemia Vasculitis

Non severe	<ul style="list-style-type: none"> <li>- Articular symptoms</li> <li>- Purpura or livedo reticularis (non-necrotizing lesions)</li> <li>- Constitutional symptoms</li> <li>- Non-severe peripheral neuropathy</li> </ul>
Severe	<ul style="list-style-type: none"> <li>- Cutaneous ulcers</li> <li>- Peripheral neuropathy</li> <li>- Glomerulonephritis</li> </ul>
Life threatening	<ul style="list-style-type: none"> <li>- Central nervous system compromise</li> <li>- Gastrointestinal ischemia</li> <li>- Rapidly progressive glomerulonephritis</li> <li>- Alveolar hemorrhage</li> </ul>

clinical relapse), 22.6% had a partial response and 97.2% had a sustained virologic response (SVR). Cryoglobulinemia disappeared in 53% of patients. Interestingly, a total of 29.1% of patients had a severe form of vasculitis and 14.3% required Steroids, Rituximab or PLEX. Baseline factors independently associated with poor response were peripheral neuropathy and a severe form of CV [78••].

Severe manifestations require the use of glucocorticoids (GC) and immunosuppressants (IS), and DAA can be considered once the patient is stable. Initial use of high dose steroids is needed to quickly subjugate vasculitic damage [78••]. In light of evidence, Rituximab is preferred instead of other immunosuppressive agents in this setting (Table 4) [51, 79, 80•, 81]. Moreover, it seems that RTX at a dose of 250 mg/m2

given two times separated by one week is equally effective as 325 mg/m2 per week for 4 weeks in CV treatment, and the use of repeated low doses is also a safe and efficacious strategy in case of relapsing disease [82, 83].

PLEX is recommended in life threatening CV (also with CS and IS) in a recent published consensus, on the basis of retrospective studies and only one small randomized trial, that used immunoadsorption apheresis plus immunosuppressive therapy versus the latter alone (Table 4) [69, 80•, 84, 85•]. It should be noted that RTX must be administered after PLEX because the latter removes large quantities of the drug (about half of the dose if PLEX is applied in the first three days after infusion) [86]. Hepatitis B virus (HBV) is a relevant non HCV infectious cause, with frequent relapsing episodes [87]. Albeit

**Table 4** Randomized controlled trials of Rituximab and plasmapheresis in Mixed Cryoglobulinemia

Author	Patients	Intervention	Comparison	Primary outcome
Dammacco 2010 [81]	Total: 37 patients All patients had MC with “Meltzer triad”; chronic hepatitis confirmed by biopsy and HCV (+)	22 patients Peg – IFN <sup>a</sup> for 48 w + RBN <sup>a</sup> for 48 w + RTX: 375 mg/m2 once a week for 1 month, then two 5-monthly infusions	15 patients Peg – IFN + RBN (same doses as IG)	<b>Complete response<sup>b</sup>:</b> 54.5% IG v/s 33.3% CG ( $P < .05$ ) at 1 year
De Vita 2012 [51]	Total: 57 patients. Patients with severe active CV and type II MC, related or not to HCV infection. HCV (+) patients (93%) had received Peg – IFN and RBN and failed, had poor tolerance to them or were contraindicated	28 patients RTX <sup>c</sup> : 1 g on days 0 and 14	29 patients Non – RTX <sup>d</sup> : - CS (58.6%)- CYC (13.8%) or AZA (10.3%)- PA (17.2%)	<b>Survival of treatment<sup>e</sup>:</b> 64.3% IG v/s 3.5% CG ( $P < 0.0001$ ) at 12 months
Sneller 2012 [79]	Total: 24 patients. Patients with CV related to HCV infection (cutaneous vasculitis, PN or GN) in whom treatment with RBN and IFN <sup>a</sup> failed or was not tolerated	12 patients RTX <sup>f</sup> : 375 mg/m2 once a week for 1 month	12 patients Non – RTX <sup>g</sup> : CYC, MTX or PLEX	<b>Remission at study month 6<sup>h</sup>:</b> 83% IG v/s 8% CG ( $P < 0.001$ )
Stefanutti 2009 [84]	Total: 17 patients. MC related to HCV infection with at least 1 severe complication <sup>i</sup>	9 patients IA: between 24 and 35 times + IT <sup>j</sup> : Peg – IFN, CYC, CsA, RBN or MP for 12 w	8 patients IT: Peg – IFN, CYC, CsA, RBN or MP for 12 w (same doses as IG)	<b>CSc at 0, 12 and 24 weeks<sup>k</sup></b> CSc at w 12 v/s CSc IG at week 24 ( $P < 0.001$ ) CSc IG v/s CSc CG at week 24 ( $P = 0.03$ )

MC: mixed cryoglobulinemia; HCV: hepatitis C virus; Peg – IFN: pegylated interferon- $\alpha$ ; RBN: ribavirin; RTX: rituximab; IG: intervention group; CG: comparison group; CV: cryoglobulinemic vasculitis; CS: corticosteroids; CYC: cyclophosphamide; AZA: azathioprine; PA: plasmapheresis; PN: peripheral neuropathy; GN: glomerulonephritis; IFN<sup>a</sup>: interferon alpha; MTX: methotrexate; PLEX: plasma exchange IA: immunoadsorption apheresis; IT: Immunosuppressive therapy; CsA: cyclosporine; MP: melphalan; CSc: Clinical score

<sup>a</sup> Doses: Pegylated interferon 180  $\mu$ g or 1.5  $\mu$ g/kg weekly, Ribavirin 1 or 1.2 g daily. <sup>b</sup> Complete response (CR) was defined as remarkable improvement or absence of clinical manifestations, absence of cryoglobulins, clearance of HCV RNA and clearance of B cell clonalities from blood

<sup>c</sup> Corticosteroids were allowed as concomitant treatment

<sup>d</sup> Patients received only one of the treatment options exposed depending on physician’s choice. The CS group received 1 mg/kg of prednisone or equivalent with or without pulse steroids. The CYC/AZA (1–2 mg/kg/ day) and PA (at least 2 procedures per week in the first month) groups received their treatment with or without steroids. In case of response, any of the treatments could be suspended at the end of month 6 after randomization

<sup>e</sup> Proportion of patients who continued taking their initial therapy

<sup>f</sup> Patients were allowed to continue any immunosuppressive medication they were receiving during randomization

<sup>g</sup> Patients used the same therapy they were using at randomization

<sup>h</sup> Remission was defined as a Birmingham Vasculitis Activity Score (BVAS) of 0

<sup>i</sup> The following were considered severe complications of mixed cryoglobulinemia: peripheral neuropathy, Sjögren’s syndrome, rash, nephritis, lower extremity vasculitis and skin ulcer with/without necrosis. All patients received immunosuppressive therapy 12 weeks before randomization. <sup>j</sup> Doses: IFN 3 MU 3 times/week, cyclophosphamide 7.5 to 15 mg/kg (IV), CSA 2 to 5 mg/kg/d, ribavirin 10 to 15 mg/kg/d, or melphalan 0.1 to 0.2 mg/kg/d

<sup>k</sup> A clinical score that includes cryocrit, renal function, neurologic system, skin, vascular system, and joints related signs and symptoms

the principles of treatment for HBV are the same as HCV infection, antivirals always should be used as first line therapy besides the use of IS and CS in severe and life threatening cases. The previous recommendation is based on the potential risk of flare or reactivation of HBV and HIV with RTX [7••, 9, 88, 89]. Indeed, the use of RTX in active hepatitis flare has been associated with fatal outcomes [90].

In the case of non-infectious CV, the aim of treatment for non-severe manifestations is the management of the underlying disease. When CV is due to essential MC (without apparent cause) low dose CS could be an option [9]. Severe manifestations require the use of IS, specially RTX instead of cyclophosphamide (CYC) according to a large retrospective study of 242 patients from the French multicenter *CryoVas* survey that showed a better response (but also a greater number of severe infections and similar death rates) with the combination of RTX plus CS versus CYC plus CS or CS alone [91]. The use of PLEX is also encouraged in severe or life threatening cases with warming of the exchange solution [92].

## Prognosis

Overall, the survival rates at 10 years are similar for HCV or non-infectious related CV (75% and 79%, respectively). The leading cause of death in CV corresponds to infections (nearly 50% in non-infectious CV and 35% in HCV CV) while other described causes are liver disease (HCV CV), cardiovascular manifestations and organ damage because of vasculitis [58, 93, 94]. The prognosis of patients with symptomatic Cryoglobulinemia is diverse and depends on the organ or system compromised and the underlying disease that caused it. In general, presence of an hematologic neoplasm is an important adverse prognostic factor [10]. The main prognostic baseline factors in HCV related CV are severe liver fibrosis, a high Five-factors score and involvement of heart, kidneys and central nervous system; in non-infectious CV, prognostic factors are: age > 65 years and involvement of lungs, kidneys or gastrointestinal tract [58, 93]. Of interest is that renal compromise could be worst in related HCV than in essential CG [62].

## Conclusions

Cryoglobulinemia is a rare disease that acts like a syndrome even within its spectrum of different diseases and in many cases requires a different approach and treatment. Its real prevalence is undetermined mostly due to difficulties in the laboratory processes needed for diagnosis.

However, there are new treatment options for HCV-CV with DAA, with a significant number of patients achieving

complete response and SVR; but Rituximab and PLEX are still prescribed in cases that develop severe forms of Vasculitis. The lack of data on maintenance therapy could impulse future studies in this context.

In the setting of Essential Cryoglobulinemia, there is an absence of new treatment options and more research on this subgroup of patients is needed.

## Compliance with Ethical Standards

**Conflict of Interest** No potential conflicts of interest relevant to this article were reported.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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