The cryoglobulinaemias

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Cryoglobulins are immunoglobulins that precipitate in vitro at temperatures less than 37°C and produce organ damage through two main pathways: vascular sludging (hyperviscosity syndrome, mainly in type I cryoglobulinaemia) and immune-mediated mechanisms (principally vasculitis, in mixed cryoglobulinaemia). Cryoglobulinaemia is associated with many illnesses, which can be broadly grouped into infections, autoimmune disorders, and malignancies; the most common cause is infection with hepatitis C virus. Mixed cryoglobulinaemic syndrome is diagnosed when a patient has typical organ involvement (mainly skin, kidney, or peripheral nerve) and circulating cryoglobulins. Cutaneous purpura is the most common manifestation of cryoglobulinaemic vasculitis. The most frequently affected internal organs are the peripheral nerves, kidneys, and joints. The course varies widely and prognosis is influenced by both cryoglobulinaemic damage to vital organs and by comorbidities associated with underlying diseases. More than 90% of cases of cryoglobulinaemia have a known underlying cause; therefore treatment is focused on the cause of the disorder rather than merely symptomatic relief. Studies suggest that both combined or sequential antiviral therapies and targeted biological treatments might be more effective than monotherapy.

Definition and classification
Cryoglobulins are immunoglobulins that precipitate in vitro at temperatures less than 37°C and redissolve after rewarming. Cryoglobulinaemia refers to the presence of cryoglobulins in serum. However, the terms cryoglobulinaemic disease or cryoglobulinaemic vasculitis are used to describe patients with symptoms related to the presence of cryoglobulins. Many patients with cryoglobulinaemia remain asymptomatic.1

The pathological nature of cryoglobulins was postulated in 1933, in a patient with multiple myeloma.2 The term cryoglobulin was introduced in 1947.3 Cryoglobulinaemic disease was described in 1966 by Meltzer and colleagues,4 who reported 29 patients with cryoglobulins and a common clinical presentation (purpura, arthralgia, and weakness), accompanied by organ dysfunction and raised serum concentrations of rheumatoid factor.

The composition of cryoglobulins is heterogeneous. The most commonly used classification dates from 1974 (figure 1) but remains useful because of its consistency for the clinical manifestations of the three cryoglobulin subsets. Three basic types are recognised according to the clonality and type of immunoglobulins. Type I consists of monoclonal immunoglobulin, generally either IgM or IgG. Type II cryoglobulins are a mixture of monoclonal IgM and polyclonal IgG. The IgM component of type II cryoglobulins has rheumatoid factor activity (ie, these immunoglobulins bind to the F₃ portion of IgG). Type III cryoglobulins are a mixture of polyclonal IgM and IgG. Types II and III are referred to as mixed cryoglobulinaemias because they consist of both IgG and IgM components. Some investigators have suggested that type III cryoglobulinaemia is a transitional state that evolves to a type II cryoglobulin profile (ie, from a polyclonal to an oligomonomoclonal B-cell population).5 Other forms of cryoglobulinaemia have been reported.6

Causes
Infections
The discovery of the hepatitis C virus (HCV) in 1989 radicals changed the focus of research from essential to HCV-related cryoglobulinaemia.5,8,9 Ferri and colleagues10 confirmed the detection of circulating HCV-RNA in nearly 90% of Italian patients with mixed cryoglobulinaemia, although later studies found wide geographical variations.11-15 HCV is predominantly associated with type II cryoglobulinaemia. The hepatitis B virus is reported to be associated with mixed cryoglobulinaemia.16 In patients infected with HIV, the percentage with cryoglobulinaemia ranges from 7% to 17% but rises to between 35% and 64% in those co-infected with HCV.17-21 Highly-active antiretroviral therapy lowers the frequency of cryoglobulinaemia in HIV.22 Case reports have associated cryoglobulinaemic syndrome with a wide range of other infectious agents (table).

Autoimmune diseases
Patients with systemic autoimmune diseases can present with complications of mixed cryoglobulinaemia.

Search strategy and selection criteria
We searched Medline and Embase (from January, 1990, to January, 2011). We used the search term “cryoglobulinemia” in combination with “epidemiology”, “diagnosis”, “virus”, “cancer”, “autoimmune”, “pathogenesis”, “hyperviscosity”, “vasculitis”, “prognosis”, and “therapy”. We focused on publications from the past 10 years but also included commonly referenced and highly regarded older publications. We searched the reference lists of articles identified by this search strategy and selected those publications we judged relevant. Review articles and book chapters are cited to provide readers with more detail than can be included in our Seminar. The date of the last search was Jan 25, 2011.
In primary Sjögren’s syndrome, cryoglobulinaemia is associated with extraglandular involvement, an enhanced risk of B-cell lymphoma, and poor survival.\textsuperscript{24–26} The prevalence of cryoglobulinaemia is five times higher in patients with both Sjögren’s syndrome and HCV infection compared with those not infected with HCV.\textsuperscript{27} Cryoglobulins are detected in nearly 10% of patients with systemic lupus erythematosus and rheumatoid arthritis, but cryocrit values are generally lower compared with those in patients with Sjögren’s syndrome, and the clinical manifestations of cryoglobulinaemic vasculitis are much less common.\textsuperscript{23,28,29} Cryoglobulins can be detected in a wide range of other autoimmune diseases (table).

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Most frequent causes} & \textbf{Less frequent causes} & \textbf{Infrequent causes} \\
\hline
Infections & Hepatitis C virus & Streptococcus spp; Brucella spp; Caxiella spp; Klebsiella spp; Leishmania spp; Chlamydia spp; Mycobacterium tuberculosis; leprosy; hepatitis A virus; cytomegalovirus; parvovirus B-19; chikungunya virus; Epstein-Barr virus; hantavirus; plasmodium; amoebiasis; toxoplasmosis \\
Autoimmune diseases & Sjögren’s syndrome & Systemic lupus erythematosus; Rheumatoid arthritis & Systemic sclerosis; antiphospholipid syndrome; inflammatory myopathies; adult-onset Still’s disease; polyarteritis nodosa; giant-cell arteritis; Takayasu’s arteritis; ANCA-associated vasculitis; autoimmune hepatitis \\
Cancer & B-cell lymphoma & Multiple myeloma & Hodgkin’s lymphoma; chronic lymphocytic leukaemia; chronic myeloid leukaemia; myelodysplasia; hepatocellular carcinoma; papillary thyroid cancer; lung adenocarcinoma; renal cell carcinoma; nasopharyngeal carcinoma \\
Other causes & Alcoholic cirrhosis & Co-trimoxazole;* interferon alfa;* cocaine;* intravenous radiographic contrast;* influenza vaccination; hepatitis B vaccination; intravenous BCG; moyamoya disease; endocarditis; chilblains \\
\hline
\multicolumn{3}{l}{ANCA=antineutrophil cytoplasmic antibodies. *Associated with cryoglobulinaemic exacerbation.}
\end{tabular}
\caption{Main causes associated with cryoglobulinaemia since 1990\textsuperscript{23}}
\end{table}

**Malignancy**

B-cell lymphoproliferative diseases are the major cause of cryoglobulinaemia associated with malignancy. Type I cryoglobulinaemia is reported predominantly in patients with Waldenström’s macroglobulinaemia, multiple myeloma, or chronic lymphocytic leukaemia.\textsuperscript{30} Mixed cryoglobulinaemias occur mainly in B-cell lymphomas.\textsuperscript{30} Cryoglobulins can be detected in patients with solid cancers.\textsuperscript{30}

**Essential cryoglobulinaemia**

Nearly 10% of cases of mixed cryoglobulinaemia are regarded as idiopathic or essential,\textsuperscript{7} a percentage that rises to 25% in HCV-negative patients.\textsuperscript{25} The possibility...
of occult HCV infection should be investigated in patients with cryoglobulinaemia who present with persistently abnormal liver function tests of unknown cause. Monoclonal gammopathies of unknown significance can have cryoglobulin activity and might account for an undetermined percentage of essential cryoglobulinaemias.

**Epidemiology**

The prevalence of cryoglobulinaemia remains unknown. Reasons include the careful laboratory technique needed to isolate and identify cryoglobulins and the absence of a standard clinical assessment of patients with possible cryoglobulinaemia. Even so, because HCV infects more than 170 million individuals worldwide, the number of patients at risk for the complications of mixed cryoglobulinaemia is substantial.

The prevalence of HCV infection in patients with mixed cryoglobulinaemia ranges from 30% to nearly 100%, with the highest prevalence in Mediterranean patients. Conversely, between 12% and 56% of HCV-infected patients have circulating cryoglobulins, again with the highest frequency in Mediterranean patients (figure 2). The reasons for this geographic variation are not clear. Genetic studies have identified few consistent risk factors. The risk of mixed cryoglobulinaemia has a well-described relation with the duration of HCV infection, with an annual incidence of cryoglobulinaemia of 3%. Patients infected with HCV who have cryoglobulinaemia have a mean disease duration roughly twice that of patients without cryoglobulinaemia, with an odds ratio for cirrhosis of 4.87.

**Pathophysiology**

**Generation of cryoglobulins**

Cryoglobulins are generated by the clonal expansion of B cells, in the context of either lymphoproliferative disorders or persistent immune stimulation triggered by chronic infections or autoimmune diseases. Types I and II cryoglobulinaemias result from the monoclonal expansion of a clone that can be overtly malignant (multiple myeloma), smouldering (Waldenström’s macroglobulinaemia, plasmacytoid lymphoma), or indolent (as in monoclonal gammopathy of unknown significance). By contrast, B-cell expansion is polyclonal in type III cryoglobulinaemia.

HCV infection is the key model for studying aetiopathogenesis. In this context, HCV lymphotropism represents the first step of B-cell clonal expansion, irrespective of the presence or absence of cryoglobulinaemic disease. An HCV envelope protein, E2, interacts with the major extracellular loop of tetraspanin CD81, a signalling molecule expressed by both hepatocytes and by B and T lymphocytes. This interaction is believed to trigger chronic B-cell stimulation. B-cell clones can be found within the peripheral blood, bone marrow, and liver of patients with HCV infection, particularly those with type II cryoglobulinaemia. B-cell clones produce monoclonal IgM that has a cross-reacting idiotype called WA, which binds immunoglobulins directed to anti-HCV core protein.

Nearly 10% of patients who are asymptomatic and HCV positive have circulating B cells that are positive for WA. In-vivo and in-vitro cryoprecipitates from patients with HCV-related cryoglobulinaemia contain viral core proteins and RNA, suggesting that cryoglobulin formation results from the host immune response against chronic HCV infection.

**Precipitation of cryoglobulins**

Reversible precipitation on exposure to low temperatures permits laboratory detection of cryoglobulins, but the biochemical mechanisms of this process are not fully understood. However, temperature is probably not the only factor affecting the solubility of cryoglobulins. Some cryoglobulins precipitate in vivo at temperatures well above the 4°C at which they are precipitated in the laboratory.
laboratory. Thus cold exposure could be a contributing factor to clinical manifestations of cryoglobulinaemia in the distal extremities. Internal organ involvement is more difficult to explain on the basis of temperature changes alone because of the tight regulation of core body temperature. Protein solubility can depend on a range of factors, including primary structure and steric conformation which, in turn, depend on temperature, pH, and ionic strength.49 Scarcity of tyrosine residues, relative abundance of hydrophobic aminoacids, and reduced concentration of galactose and sialic acid in the glycosylated portion of the molecule can increase precipitation.49,50 In type II mixed cryoglobulinaemia, the formation of large, complement bound, IgM–IgG complexes is a major factor influencing cryoprecipitation.51

Pathogenesis of tissue injury

Two major mechanisms are at play to varying degrees across the different types of cryoglobulinaemia: cryoglobulin precipitation in the microcirculation, and immune-complex-mediated inflammation of blood vessels. Vascular occlusion is more frequent in type I cryoglobulinaemia, which is usually accompanied by high cryoglobulin concentrations, and can be associated with hyperviscosity syndrome and cold-induced acral necrosis. Immune-complex–mediated vasculitis is more frequent in mixed cryoglobulinaemias, particularly type II, in which the monoclonal IgM component generates large immune complexes with IgG and complement fractions, particularly C1q. C1q can bind to receptors on endothelial cells, facilitating immune complex deposition and subsequent vascular inflammation.52

Clinical manifestations

The percentage of patients with circulating cryoglobulins who develop symptoms varies from 2% to 50%.7 The most common presentation, the triad of purpura, arthralgia, and weakness, is reported in 80% of patients at disease onset.7 The development of cryoglobulinaemic symptoms is affected by age, underlying illness (such as HCV infection) and the characteristics of the cryoglobulins (type II subclass, high serum concentrations).7,53

Hyperviscosity syndrome

Hyperviscosity syndrome develops mainly in patients with type I cryoglobulinaemia associated with haematological neoplasia,26 and is very uncommon in patients with mixed cryoglobulinaemia (<3%).54 The key symptoms are neurological (headache, confusion), ocular (blurry vision, visual loss), and rhino-otological (epistaxis, hearing loss). Massive intratubular cryoprecipitation leading to rapidly progressive renal failure has been reported. The physical examination should include fundoscopy to exclude hyperviscosity-related retinal changes, especially haemorrhages. In patients in whom hyperviscosity syndrome is suspected, it is helpful to measure serum viscosity. Patients usually become symptomatic at viscosity measurements that exceed 4-0 centipoise,56 but some patients are symptomatic with lower viscosities.57 Symptomatic hyperviscosity requires urgent treatment (eg, plasma exchange).

Cutaneous purpura is probably the most characteristic manifestation of cryoglobulinaemic vasculitis (54–82%).7,26,55,63 The typical presentation consists of many small petechial lesions in the legs (figure 3A); bullous or vesicular lesions are uncommon. Purpura can extend to the abdominal region and, less frequently, to the upper limbs and thorax (figure 3B). Involvement at other sites is rare. Isolated purpura has a good prognosis, and patients usually recover spontaneously, often in less than 1 week, leaving discoloured skin

Figure 3: Cutaneous involvement in cryoglobulinaemia
(A) Purpura in legs, (B) atypical purpura, (C) cutaneous ulcers, (D) digital necrosis.
patches formed of haemosiderin deposits, although recurrent episodes are frequent. Patients should be questioned about exercise and depilation, which often trigger episodes of purpura. Other factors, including chronic venous insufficiency, protracted standing, and muggy weather should be considered.

The prognosis can be worsened by cutaneous ulcers caused by coalescence of vasculitic lesions (usually around the malleoli) or ischaemia in the distal regions (hands, feet, lips, ears, and nose) (figure 3C and 3D). Livedo reticularis and cutaneous ulcers usually indicate involvement of medium-sized vessels (ie, small arteries or arterioles). Cutaneous ulcers and digital necrosis signify a high risk of infection, sepsis, and death.

About 20% of patients with cryoglobulinaemia present with nephropathy at diagnosis and 30% have renal complications during the disease course. Renal features are indolent in nearly half the patients, with proteinuria, microscopic haematuria, red-blood-cell casts, and varying degrees of renal failure. Nephrotic (21%) or nephritic (14%) syndromes are less frequent. More than 70% of patients present with hypertension and 40–60% have a raised serum creatinine concentration (>1.5 mg/dL) at diagnosis. In a series of 105 patients with cryoglobulinaemic glomerulonephritis, 15 (14%) evolved to chronic renal failure after a mean follow-up of 6 years. Recurrence of cryoglobulinaemia in the renal allograft has been reported in patients receiving a kidney transplant.

Between 17% and 60% of cryoglobulinaemic patients present with peripheral neuropathy, which can be the first sign of cryoglobulinaemia. The main symptoms are paresthesiae, with painful or burning sensations, or both, in the lower limbs, which are often worse at night. These generally precede motor involvement (figure 4B). Electrodiagnostic studies, which show that polynuropathy is more common than mononeuritis multiplex, often disclose subclinical peripheral nerve dysfunction. In a few patients, neuropathy can present as rapidly-progressive sensory-motor involvement with severe functional impairment.

**Less-common disease manifestations**

2–6% of patients have gastrointestinal involvement. Intestinal ischaemia should be suspected in patients who present with acute abdominal pain and general malaise. Fever and bloody stools are reported by a third of patients (figure 4C). Some patients present with intestinal perforation and shock. Cryoglobulinaemic vasculitis involving the gastrointestinal tract can mimic cholecystitis, pancreatitis. Cryoglobulinaemic vasculitis has been identified within the gallbladder at cholecystectomy.

Pulmonary involvement occurs in less than 5% of patients. In patients presenting with mild-to-moderate exertional dyspnoea and dry cough, interstitial lung fibrosis should be suspected; bronchoalveolar lavage shows a predominance of macrophages, with slightly increased neutrophils or lymphocytes. A few patients manifest with acute alveolar haemorrhage (haemoptysis, respiratory failure, and diffuse pulmonary infiltrates) (figure 4D). Pleural effusions are rare. CNS involvement, reported in up to 6% of patients, is often difficult to confirm. The most common clinical presentation is stroke (motor or sensory deficits, aphasia, or dysarthria), although diffuse cerebral involvement manifesting as encephalopathy has also been described. MRI shows cerebral ischaemia in half of these patients and, less often, haemorrhagic lesions. A third of patients have diffuse small lesions that raise the spectre of cerebral vasculitis. Isolated cases of transverse myelitis (often recurrent) and ischaemic spinal-cord involvement have been reported.

Fewer than ten cases of myocardial vasculitis have been reported in cryoglobulinaemic patients. These patients can have myocardial infarctions in the absence of cardiovascular risk factors. There are reports of pericarditis or congestive heart failure complicating cryoglobulinaemia.

A small proportion of cryoglobulinaemic patients present with multiorgan disease (skin, kidneys, lungs, CNS, and gastrointestinal tract) involving the small-medium-sized arteries, capillaries, and venules.
Diagnosis

Cryoglobulin testing

There are no standardised or validated diagnostic or classification criteria for cryoglobulinaemic vasculitis. Diagnosis is based on clinical, laboratory, and histopathological data. For most patients, cryoglobulinaemic disease is diagnosed by the presence of typical organ involvement (mainly skin, kidney or peripheral nerve) and circulating cryoglobulins.

The diagnosis of cryoglobulinaemia requires demonstration of the presence of cryoglobulins in serum (panel 1). Appropriate sample collection and handling is crucial. Blood should be collected in prewarmed syringes and tubes, transported, clotted, and centrifuged at 37–40°C, ensuring that the temperature never falls below 37°C. The serum should then be stored at 4°C for up to 7 days. Precipitation of type I cryoglobulins usually occurs within hours. By contrast, mixed cryoglobulins, particularly type III, can need days to precipitate.

Expert laboratory interpretation that considers the patient in the appropriate clinical context is essential. Some healthy individuals have low concentrations of cryoglobulins (<0.06 g/L) and mixed polyclonal cryoglobulins often occur transiently during infection. On the other hand, a negative test for cryoglobulins does not exclude cryoglobulinaemia because of the possibility of false-negative results caused by improper sample collection or inconsistent laboratory techniques. Moreover, cryoglobulin concentrations can fluctuate, depending on their in-vivo precipitation in target vessels. Cryoglobulin should be assayed serially when there is a high degree of suspicion of disease.

Cryoglobulin concentration can be assayed indirectly by measurement of the total protein concentration within the cryoprecipitate and by estimation of the cryocrit. Cryoglobulin concentration is usually more than 5 g/L in type I cryoglobulinaemia, but generally lower in types II and III. Quantification of the cryocrit is important because the amount of serum cryoprotein can correlate with the severity of symptoms and is useful in monitoring the response to treatment, particularly in patients with hyperviscosity syndromes. Immunofixation of the cryocrit, if feasible, allows identification of the type of cryoglobulin. If the cryoprecipitate is too small, the type of cryoglobulin can be estimated indirectly by serum immunofixation. The monoclonal component detected usually corresponds to the existing monoclonal cryoglobulin.

Laboratory tests

Laboratory tests are a key instrument for evaluating functional results of visceral involvement and causal factors. Biochemical tests for renal and liver involvement are mandatory. Low complement levels (particularly C4) and raised titres of serum rheumatoid factor are usually observed in mixed cryoglobulinaemias and can correlate with some clinical symptoms.

Cryoglobulin detection demands thorough evaluation to identify potential underlying diseases. HCV testing is mandatory (antibodies and serum HCV-RNA detection) in patients with mixed cryoglobulinaemia. Testing for other viruses (hepatitis B virus, HIV) and autoimmune diseases (antinuclear, anti-DNA, anti-Ro/La, anticitrullinated antibodies) is recommended, even in patients known to have HCV.

Histopathological findings

Histopathological examination is usually appropriate to confirm the diagnosis in an affected organ. Precipitated cryoglobulins appear in vivo as hyaline thrombi that occlude small blood vessels, including the glomerular tuft and endoneurial microvessels. Hyaline thrombi are especially likely when the monoclonal component in type I and II cryoglobulinaemias is abundant.

Vasculitis, the typical pathological lesion of mixed cryoglobulinaemia, consists of mixed inflammatory infiltrates involving small and, less often, medium sized vessels. Fibrinoid necrosis can occur. The most commonly affected organs are the skin, kidneys, and peripheral nerves. Skin biopsies of purpuric lesions reveal...
Corticosteroids should be used at minimum dose and period necessary; short courses and therapeutic regimen of rituximab is unknown. Timing between rituximab and antiviral therapy administration is not clear. There are no available data on immunosuppressive maintenance therapy; similar regimens and doses to those used in other autoimmune diseases (lupus, vasculitis) are suggested. Best dose and therapeutic regimen of rituximab is unknown. Timing between rituximab and antiviral therapy administration (simultaneous, or 1–4 weeks after starting rituximab, or after 4–6 months) is not clear.

**Figure 5: Proposed therapeutic algorithm for patients with HCV-related cryoglobulinaemic syndrome according to disease severity**

GN=glomerulonephritis, GI=gastrointestinal. Limitations: there is little evidence for treatment of cryoglobulinaemic features (available therapeutic data relies mainly on uncontrolled studies and case series). There is no standardised classification of disease severity. Recommended strategies are based on personal experience and expert opinions. Corticosteroids should be used at minimum dose and period necessary; short courses and low doses (<30 mg/day) can be used. There are no available data on immunosuppressive maintenance therapy; similar regimens and doses to those used in other autoimmune diseases (lupus, vasculitis) are suggested. Best dose and therapeutic regimen of rituximab is unknown. Timing between rituximab and antiviral therapy administration (simultaneous, or 1-4 weeks after starting rituximab, or after 4-6 months) is not clear.

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**Stratified treatment of HCV-related cryoglobulinaemic syndrome according to disease severity**

- **Mild/moderate disease**
  - • Purpura, articular, general features
  - • Mild neuropathy
  - • GN without renal failure

- **Severe disease**
  - • Cutaneous ulcers, ischaemia
  - • Severe neuropathy
  - • GN with renal failure/nephrotic syndrome
  - • Gl involvement

- **Life-threatening**
  - • Rapidly progressive GN
  - • CNS involvement
  - • Intestinal ischaemia
  - • Alveolar haemorrhage

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**Outcome**

The evolution of cryoglobulinaemic disease varies widely. Roughly half of patients have chronic disease with no involvement of vital organs. A third of patients have moderate-to-severe disease, with chronic renal failure or cirrhosis, and nearly 15% present with sudden life-threatening disease. Patients with cryoglobulinaemic disease have a worse 10-year survival rate compared with the rate in the general population. Risk factors for poor outcomes include male sex, age more than 60 years, glomerulonephritis, gastrointestinal or pulmonary involvement, chronic HCV infection and type II cryoglobulinaemia.

**Life-threatening cryoglobulinaemia**

Glomerulonephritis results in acute renal failure in 10% of patients or can evolve progressively to chronic renal failure. 10-year survival rates of glomerulonephritis range between 33% and 45%, although a study reported in 2007 recorded nearly 80% survival after 10 years resulting from improved therapeutic management. The outlook is poor in men and patients with HCV infection, high cryocrit values, low C3 values, and raised creatinine at diagnosis. Mortality rates associated with intestinal ischaemia and alveolar haemorrhage are very high (>80%), although a 2010 study suggested improved prognosis for gastrointestinal involvement.

**Risk of neoplasia**

Mixed cryoglobulinaemic syndrome is traditionally regarded as a crossroads between autoimmune disease and cancer. Expansion of the peripheral B-lymphocyte pool and lymphoid infiltrates within the liver and bone marrow, characteristic of overt B-cell lymphoproliferative
processes, are often seen in patients with mixed cryoglobulinaemia.⁶⁻⁷ B-cell lymphoma, the main neoplastic complication in patients with mixed cryoglobulinaemia,⁶⁻⁷ has been reported in 5–22% of patients with mixed cryoglobulinaemia.⁶⁻⁷,9⁻¹²,¹³,¹⁄₂¹,¹²¹,¹²² Hypogammaglobulinaemia can be a marker for impending lymphomagenesis.¹²¹ Associated HCV infection confers a 20–30% increased risk of B-cell lymphoma.¹⁰¹ B-cell lymphomas usually occur within 10 years of diagnosis of cryoglobulinaemia. The most common types are diffuse, large, nodal marginal zone, and lymphoplasmacytoid B-cell lymphomas.¹¹ Treatment usually includes standard chemotherapy regimens and rituximab.¹²,¹³¹

Liver cancer, the second most frequently diagnosed neoplasia in cryoglobulinaemic patients, is attributable to the close association with HCV.⁷,¹⁄₂¹,¹²¹ Hepatocellular carcinoma occurs about a third as often as lymphoma in patients infected with HCV.

**Treatment**

**Conventional immunosuppression**

Treat should be modulated according to the underlying aetiopathogenesis (hyperviscosity vs vasculitis) and the severity of clinical presentation (figure 5).¹⁻³¹,¹⁄₅,¹⁹,¹⁰⁸,¹⁰⁹,¹¹⁶ There are three broad strategies in the treatment of cryoglobulinaemia: conventional immunosuppression, antiviral treatments, and biological therapies (panel 2).

The immunosuppressive approaches used in cryoglobulinaemic vasculitis, based on high-dose glucocorticoids and cyclophosphamide, were derived mainly from strategies used in other systemic vasculitides before it was understood that most cases result from HCV infection. No clinical trial has studied these agents in cryoglobulinaemic vasculitis. However, they remain essential to control severe disease quickly and can help to alter the course of disease if used shrewdly as a bridge to antiviral or biological agents. In patients with severe cryoglobulinaemic vasculitis, one goal of therapy should be to discontinue conventional immunosuppressive agents within 2–3 months, once the major end-organ effects have been controlled.

Patients with moderate to severe manifestations of cryoglobulinaemic vasculitis can be treated with glucocorticoids to control inflammation rapidly.¹¹⁰ Regimens of methylprednisolone (0·5–1·0 g/day) for 3 days followed by prednisone (1·0 mg/kg daily; not to exceed 80 mg/day) are appropriate in the setting of skin ulceration, sensorimotor neuropathy, glomerulonephritis, and other severe vasculitic manifestations. When disease control is achieved, glucocorticoids should be tapered to the minimum dose necessary to suppress activity and discontinued altogether if possible.

Cyclophosphamide is used in the most severe cases of cryoglobulinaemic vasculitis, either daily (oral, 2 mg/kg daily) or intermittent (intravenous, 750 mg/m² monthly) regimens. Azathioprine (2 mg/kg daily) and mycophenolate mofetil (1 g twice daily) are often used instead of cyclophosphamide or after cyclophosphamide for remission maintenance, although neither has been assessed in clinical trials for this aim.¹¹¹,¹¹₈,¹₁₈

Immunosuppression requires close monitoring of patient’s blood counts and other variables. Patients given glucocorticoids and cyclophosphamide should receive prophylaxis for *Pneumocystis* pneumonia and surveillance for the development of other opportunistic infections.

**Apheresis**

Both plasma exchange and plasmapheresis remove cryoglobulins from the circulation, thereby interrupting the immune-complex-mediated pathogenesis of cryoglobulinaemic vasculitis. These interventions are useful in patients with immediately life-threatening disease and for those with hyperviscosity syndrome. However, apheresis does not treat the underlying disease and can lead to rebound in which cryoglobulin production increases after the cessation of apheresis. Cyclophosphamide for up to 6 weeks might be needed to prevent this rebound.

**Antiviral treatments**

The most robust antiviral approach to the treatment of HCV-related cryoglobulinaemic vasculitis involves the use of both pegylated interferon alfa and ribavirin.¹¹⁰⁻¹¹¹ Patients with HCV genotypes 2 and 3 respond most favourably to this regimen, with between 73% and 90% achieving sustained virological responses at 24 weeks, whereas those with genotypes 1 and 4 have a lower likelihood of achieving sustained virological responses (45–52%).¹¹³⁻¹¹⁵ The recommended regimen is shown in panel 3.¹¹⁶ The dose of interferon should be carefully adjusted in cirrhotic patients, whereas the dose of ribavirin should be adjusted in patients with renal failure (glomerular filtration rate <60 mL/min per m²).
Interferon is currently contraindicated in recipients of kidney, heart, and lung allografts.\textsuperscript{114}

Failure to achieve a virological response at weeks 12 and 24 is associated with a low probability of achieving a sustained virological response,\textsuperscript{111} and the guidelines of the American Association for the Study of Liver Diseases recommend discontinuation of antiviral therapy in this setting. However, the effectiveness of antiviral therapy in patients with cryoglobulinaemic vasculitis should be assessed not only according to the virological response, but also by the full effect of other clinical and immunological responses achieved.\textsuperscript{115} Maintenance of antiviral therapies can be appropriate in patients with cryoglobulinaemic patients with advanced HCV-related liver disease, including improvement in cirrhosis.\textsuperscript{110}

While awaiting the results of randomised trials, some notes of caution about the off-label use of rituximab should be highlighted. A reasonable assessment of the benefits and risks (especially severe infections)\textsuperscript{111,112} should be made on an individual basis. Additionally, a substantial risk of complex formation between rituximab and IgM-\kappa with rheumatoid-factor activity has been reported,\textsuperscript{113} especially in patients with high baseline concentrations of cryoglobulins. This idea needs further testing in mechanistic studies linked to clinical trials, but the results of small series are promising.\textsuperscript{121–128} These studies suggest that disease relapses are associated with the absence of virological control and the recovery of peripheral B cells. In patients receiving conventional therapy, a third had a relapse of vasculitis despite sustained virological response.\textsuperscript{127} This finding suggests that B-cell proliferation can become independent of HCV over time and that a targeted B-cell approach combined with pegylated interferon alfa and ribavirin might be successful in deleting both virus-dependent and virus-independent clones.

Substantial clinical experience in the off-label use of rituximab in patients with HCV-related cryoglobulinaemia has been accumulated.\textsuperscript{122–124} Three studies reported in 2010 deserve careful scrutiny.\textsuperscript{128–130} A prospective study\textsuperscript{128} compared the outcomes of 55 patients given antiviral therapy (pegylated interferon alfa and ribavirin for 48 weeks) to those of 38 patients given a sequential regimen of rituximab (intravenous administration of four infusions at 375 mg/m\textsuperscript{2} per week or two fortnightly infusions of 1000 mg), followed 1 month later by antiviral therapy. The rituximab-treated patients had a shorter mean time to clinical remission (5.4 months vs 8.4 months, respectively), better renal response rates (81% vs 40%), and higher rates of cryoglobulin clearance (68% vs 44%) compared with those assigned to antiviral therapy. Treatment discontinuations were similar in the two groups (13% vs 9%).

The second study\textsuperscript{131} was a prospective trial of 41 patients who were randomised to receive antiviral therapy alone or combined with rituximab (375 mg/m\textsuperscript{2} once a week for 1 month, plus two 5-monthly infusions during the 48-week course of antiviral treatment). A higher rate of complete response was reported in patients given the combined regimen (54% vs 33%). A third study, limited by small sample size, reported excellent tolerance of rituximab in cryoglobulinaemic patients with advanced HCV-related liver disease, including improvement in cirrhosis.\textsuperscript{130}

Future perspectives

A new era in our understanding of and therapeutic approach to cryoglobulinaemia began 20 years ago with the discovery of the HCV. The progressive introduction of antiviral therapies, with eradication of HCV currently regarded as the therapeutic gold standard, has improved survival rates.\textsuperscript{77,134} However, many aspects of the disease

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**Panel 3: Recommended treatment regimen for combination interferon\textsuperscript{*} and ribavirin\textsuperscript{†} therapy\textsuperscript{114}

**Therapeutic regimens**

**Pegylated interferon alfa-2a**
- 180 μg subcutaneously per week
- or
- 1000 mg orally per day (bodyweight <75 kg)
- 1200 mg orally per day (bodyweight >75 kg)

**Ribavirin**
- 800 mg orally per day (bodyweight ≤65 kg)
- 1000 mg orally per day (bodyweight 65–85 kg)
- 1200 mg orally per day (bodyweight 85–105 kg)
- 1400 mg orally per day (bodyweight >105 kg)

**Recommended duration of antiviral courses\textsuperscript{‡}**
- Genotypes 1 and 4: 48 weeks
- Genotypes 2 and 3: 24 weeks

\*Use with extreme caution in cirrhotic patients. \†Dose adjustment in patients with glomerular filtration rate <60 mL/min. \‡Duration also determined by evaluation of early virological response and clinical and immunological responses.
remain unresolved. The causes of essential cryoglobulinaemia are not known. Disease management remains difficult in many patients, because many do not respond or are intolerant to antiviral therapies. Few data are available on the treatment of patients with essential or type cryoglobulinaemia. The role of new antiviral agents in HCV-related cryoglobulinaemia, such as telaprevir, remains to be defined. Similarly, international efforts are needed to develop a set of criteria for a homogeneous classification of patients in future epidemiological and therapeutic studies.

The new century has seen the emergence of B-cell-targeted therapies, whose use is currently limited by the absence of specific licensing. Two studies reported in 2010 suggest greater benefit when rituximab is added to antiviral therapy, although some researchers support rituximab monotherapy. Differences in study designs and therapeutic schedules do not allow definitive recommendations on the relative timing of antiviral designs and therapeutic schedules do not allow definitive support rituximab monotherapy.

Cryoglobulinaemia is characterised by a wide range of causes, symptoms, and outcomes, with various aetio-pathogenic pathways involved in organ damage. This complex scenario results in equally complex management considerations. The present emphasis on approaching the different causes in a simultaneous or closely sequenced manner could produce improved therapeutic results for patients with this challenging disorder.

Contributors
All authors contributed to the literature search and writing of the Seminar. MR-C, HJS, MCC contributed to the figures.

Conflicts of interest
MCC has received consultancy fees, research grants and speaker’s fees from Centocor; and speaker’s fees from Bristol-Myers Squibb and Roche. JS has received consultancy fees from Genentech. XB and MR-C declare that they have no conflicts of interest.

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References