The cryoglobulinaemias

Manuel Ramos-Casals, John H Stone, Maria C Cid, Xavier Bosch

Lancet 2012; 379: 348–60
Published Online
August 24, 2011
DOI:10.1016/S0140-6736(11)60242-0

Josep Font Laboratory of Autoimmune Diseases (M Ramos-Casals MD) **and Vasculitis Research Unit, Department of Autoimmune Diseases** (M C Cid MD), **and Department of Internal Medicine** (X Bosch MD), **Institut Clínic de Medicina I Dermatologia, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, University of Barcelona, Barcelona, Spain; and Rheumatology Unit, Massachusetts General Hospital, Boston, MA, USA** (J H Stone MD)

Correspondence to:
Dr Xavier Bosch, Department of Internal Medicine, Hospital Clinic, Villarroel 170, Barcelona 08036, Spain
xavbosch@clinic.ub.es

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[5] (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 F_c 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 oligomonoclonal B-cell population).[6] Other forms of cryoglobulinaemia have been reported.[7]

Causes

Infections

The discovery of the hepatitis C virus (HCV) in 1989[8] radically changed the focus of research from essential to HCV-related cryoglobulinaemia.[9,10] Ferri and colleagues[11] confirmed the detection of circulating HCV-RNA in nearly 90% of Italian patients with mixed cryoglobulinaemia, although later studies found wide geographical variations.[12–15] HCV is predominantly associated with type II cryoglobulinaemia. The hepatitis B virus is reported to be associated with mixed cryoglobulinaemia.[7,16] 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.

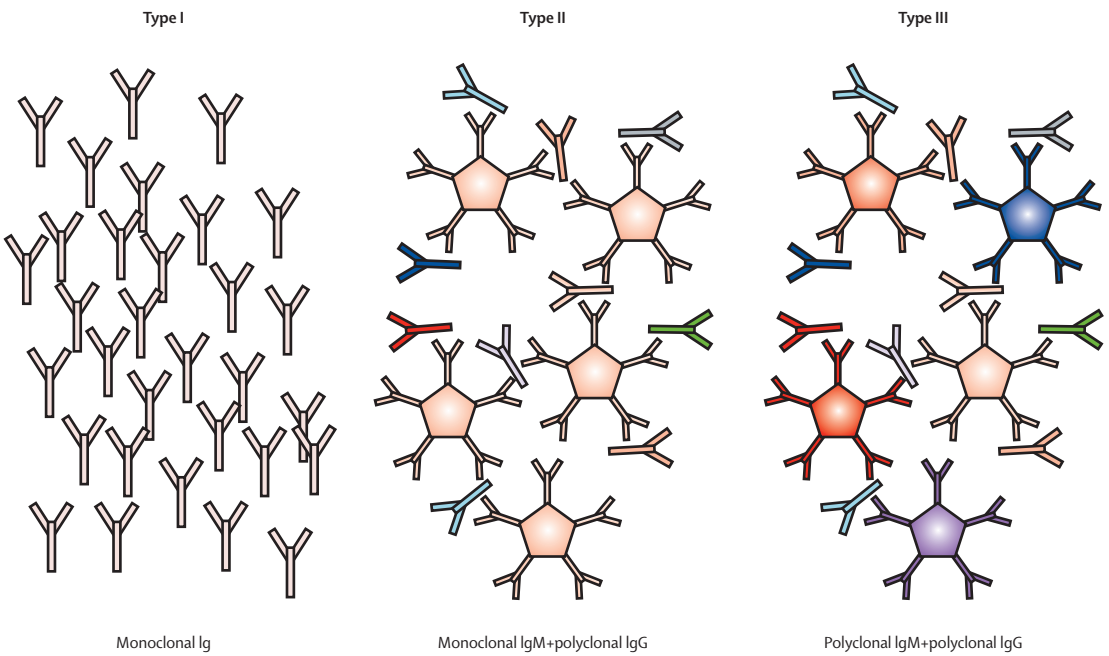

Figure 1: **Classification of cryoglobulinaemia[5]**

	Most frequent causes	Less frequent causes	Infrequent causes
Infections	Hepatitis C virus	HIV; Hepatitis B virus	*Streptococcus* spp; *Brucella* spp; *Coxiella* spp; *Klebsiella* spp; *Leishmania* spp; *Chlamydia* spp; *Mycobacterium tuberculosis;* leprosy; hepatitis A virus; cytomegalovirus; parvovirus B-19; chikungunya virus; Epstein-Barr virus; hantavirus; plasmodium; amoebaiasis; 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; intravesical BCG; moyamoya disease; endocarditis; chilblains

ANCA=antineutrophil cytoplasmic antibodies. *Associated with cryoglobulinaemic exacerbation.

Table: **Main causes associated with cryoglobulinaemia since 1990[23]**

In primary Sjögren's syndrome, cryoglobulinaemia is associated with extraglandular involvement, an enhanced risk of B-cell lymphoma, and poor survival.[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.[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.[23,28,29] Cryoglobulins can be detected in a wide range of other autoimmune diseases (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.[30] Mixed cryoglobulinaemias occur mainly in B-cell lymphomas.[31] Cryoglobulins can be detected in patients with solid cancers.[32]

Essential cryoglobulinaemia

Nearly 10% of cases of mixed cryoglobulinaemia are regarded as idiopathic or essential,[7] a percentage that rises to 25% in HCV-negative patients.[32] The possibility

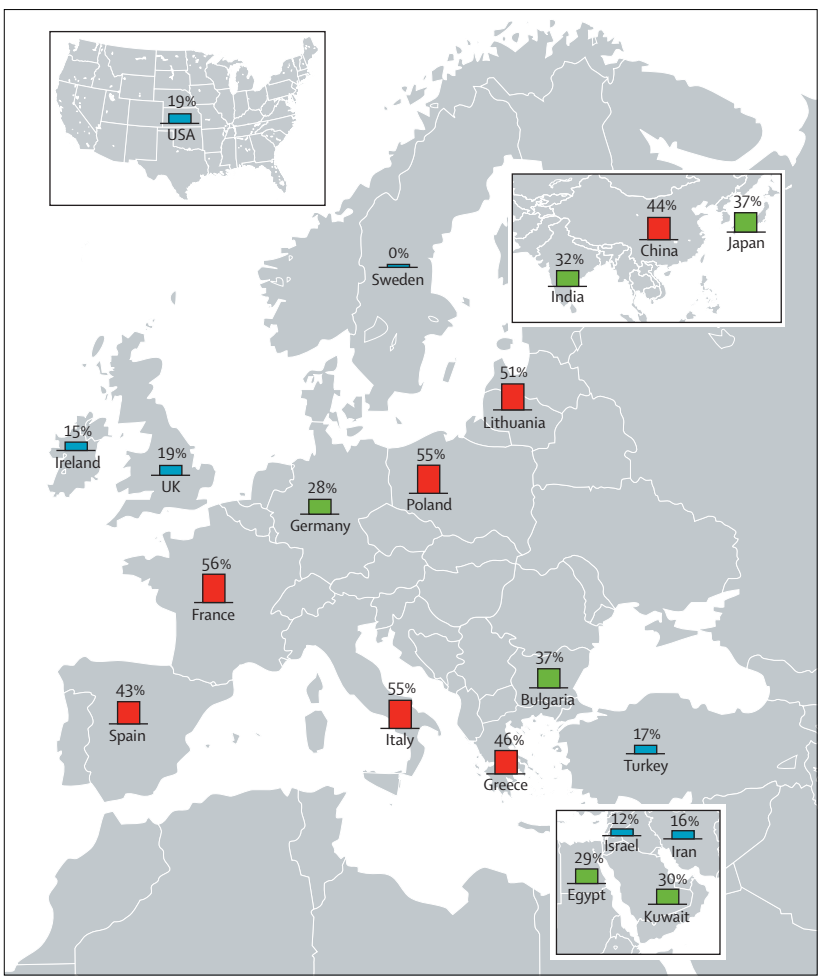

***Figure 2*: Prevalence of cryoglobulinaemia in patients with chronic HCV infection**
>40% in red, 20–40% in green, <20% in blue.

of occult HCV infection should be investigated in patients with cryoglobulinaemia who present with persistently abnormal liver function tests of unknown cause.[33] 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.[34] 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.[12] 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%.[15] Patients infected with HCV who have cryoglobulinaemia have a mean disease duration roughly twice that of patients without cryoglobulinaemia,[35] with an odds ratio for cirrhosis of 4·87.[36]

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.[5,7,37] 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.[10,11] In this context, HCV lymphotropism represents the first step of B-cell clonal expansion, irrespective of the presence or absence of cryoglobulinaemic disease.[38] 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.[39] This interaction is believed to trigger chronic B-cell stimulation.[40] 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.[41–43] B–cell clones produce monoclonal IgM that has a cross-reacting idiotype called WA, which binds immunoglobulins directed to anti-HCV core protein.[44] Nearly 10% of patients who are asymptomatic and HCV positive have circulating B cells that are positive for WA.[45] In-vivo and in-vitro cryoprecipitates from patients with HCV-related cryoglobulinaemia contain viral core proteins and RNA,[46–48] 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.[49] 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. 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.[7,16] 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,[54] and is very uncommon in patients with mixed cryoglobulinaemia (<3%).[16,55] 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 funduscopy 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).

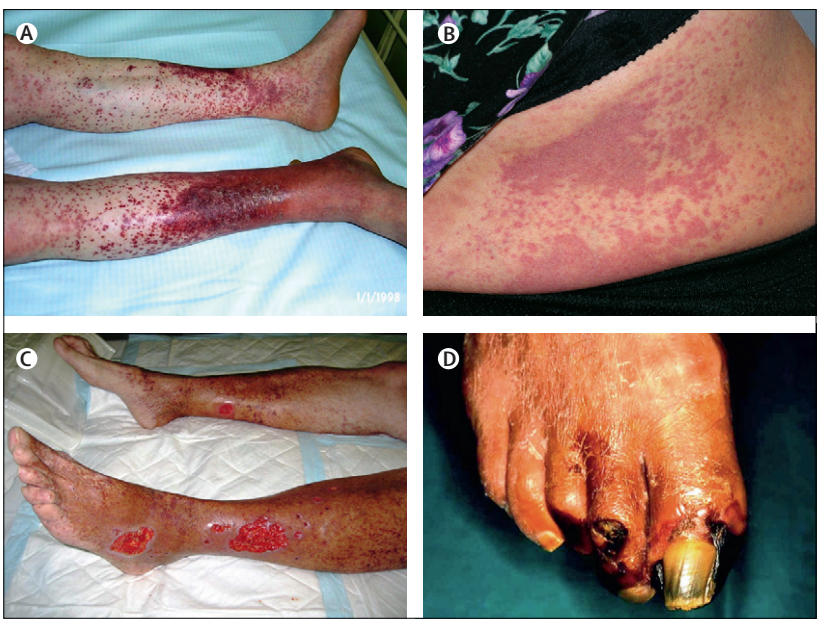

Figure 3: Cutaneous involvement in cryoglobulinaemia
(A) Purpura in legs, (B) atypical purpura, (C) cutaneous ulcers, (D) digital necrosis.

Cryoglobulinaemic vasculitis

Flares of cryoglobulinaemic vasculitis are often, but not always, accompanied by general symptoms such as fever, weakness, myalgia, and arthralgia. These symptoms, particularly when combined with purpura, strongly suggest vasculitis. Fevers of unknown origin can occur. Articular involvement consists mainly of joint pain in the hands, knees, and wrists, without clinical signs of inflammation (44–71%).[7,16,55,58] Arthritis is reported in fewer than 10% of patients.[59] Radiographs show no evidence of bone erosions and anticitrullinated antibodies are negative, by contrast with what is seen in rheumatoid arthritis.[59] Weakness and fatigue are reported by more than 50% of patients. Fibromyalgia and endocrine processes, such as hypothyroidism and diabetes, should be investigated because of their higher frequency in cryoglobulinaemic patients.[60–62]

Cutaneous purpura is probably the most characteristic manifestation of cryoglobulinaemic vasculitis (54–82%).[7,16,55,58,63] 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

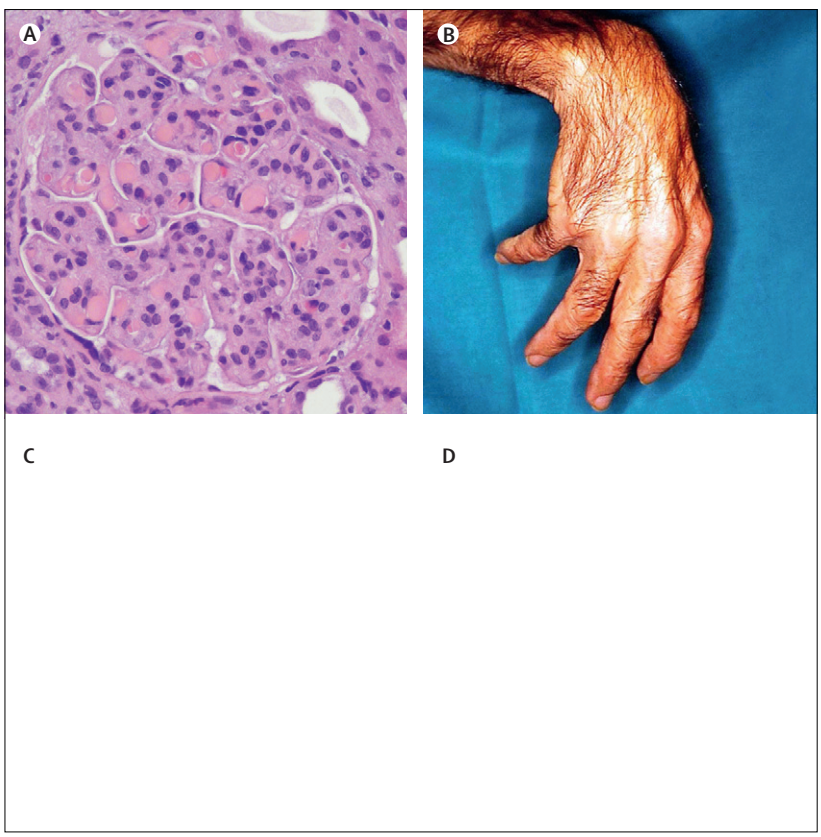

Figure 4: **Systemic cryoglobulinaemic vasculitis**
(A) Membranoproliferative glomerulonephritis associated with HCV-related type II cryoglobulinaemia. A glomerulus shows proliferative changes and subendothelial deposits within capillary loops (pink). These lesions resemble pseudothrombi. (B) Multineuritis (radial and cubital paralysis), (C) intestinal ischaemia (diffuse oedema of intestinal wall), (D) pulmonary haemorrhage.

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)[7,16,55,63] 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.[1,16,51,55,58,63–68] 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.[67] More than 70% of patients present with hypertension and 40–60% have a raised serum creatinine concentration (>1·5 mg/dL) at diagnosis.[7,68] In a series of 105 patients with cryoglobulinaemic glomerulonephritis,[69] 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.[70,71]

Between 17% and 60% of cryoglobulinaemic patients present with peripheral neuropathy,[7,16,53,55] which can be the first sign of cryoglobulinaemia.[72] 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 polyneuropathy is more common than mononeuritis multiplex,[7,58,73] often disclose subclinical peripheral nerve dysfunction.[74] In a few patients, neuropathy can present as rapidly-progressive sensory-motor involvement with severe functional impairment.[75,76]

Less-common disease manifestations

2–6% of patients have gastrointestinal involvement.[7,55,77] 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.[77] Cryoglobulinaemic vasculitis has been identified within the gallbladder at cholecystectomy.

Pulmonary involvement occurs in less than 5% of patients.[7,16,78] In patients presenting with mild-to-moderate effort dyspnoea and dry cough, interstitial lung fibrosis should be suspected; bronchoalveolar lavage shows a predominance of macrophages, with slightly increased neutrophils or lymphocytes.[79] A few patients manifest with acute alveolar haemorrhage (haemoptysis, respiratory failure, and diffuse pulmonary infiltrates)[80] (figure 4D). Pleural effusions are rare.[58] CNS involvement, reported in up to 6% of patients, is often difficult to confirm.[7,58] 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.[80] 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.[80] Isolated cases of transverse myelitis (often recurrent) and ischaemic spinal-cord involvement have been reported.[81]

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.[80] There are reports of pericarditis or congestive heart failure complicating cryoglobulinaemia.[80,82]

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.[1]

Diagnosis

Cryoglobulin testing

There are no standardised or validated diagnostic or classification criteria for cryoglobulinaemic vasculitis.[83] 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.[84] 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.[85]

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),[86,87] and mixed polyclonal cryoglobulins often occur transiently during infection.[49] 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.[49] 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.[49,85]

Cryoglobulin concentration can be assayed indirectly by measurement of the total protein concentration within the cryoprecipitate and by estimation of the cryocrit.[49,75] Cryoglobulin concentration is usually more than 5 g/L in type I cryoglobulinaemia, but generally lower in types II and III.[49] 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 redissolved cryoprecipitate, 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[49,51,85] and can correlate with some clinical symptoms.[88]

***Panel 1:* Clinical, laboratory, and histopathological red flags advising cryoglobulin testing**

Regard cryoglobulinaemia as highly probable when at least two features of different subsets are present

Clinical findings

- Skin purpura in adults
- Cutaneous necrotic ulcers
- Glomerulonephritis
- Peripheral neuropathy
- Non-erosive arthritis
- Acral ischaemia
- Cold-induced acrocyanosis
- Raynaud's phenomenon

Laboratory abnormalities

- Monoclonal gammopathy, particularly of IgM isotype or with hyperviscosity
- Unexplained low concentrations of complement (especially C4)
- Unexplained high titres of rheumatoid factor
- Pseudothrombocytosis
- Formation of erythrocyte *rouleaux*

Histopathological findings

- Leukocytoclastic vasculitis in adults
- Membranoproliferative glomerulonephritis
- Hyaline thrombi in capillaries in context of glomerulonephritis or small-vessel vasculitis
- Endoneural vasculitis
- Unclassified systemic necrotising vasculitis involving small–medium-sized vessels

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, anti-citrullinated antibodies) is recommended, even in patients known to have HCV.[49]

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 endoneural microvessels. Hyaline thrombi are especially likely when the monoclonal component in type I and II cryoglobulinaemias is abundant.[67,89]

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.[51] Skin biopsies of purpuric lesions reveal

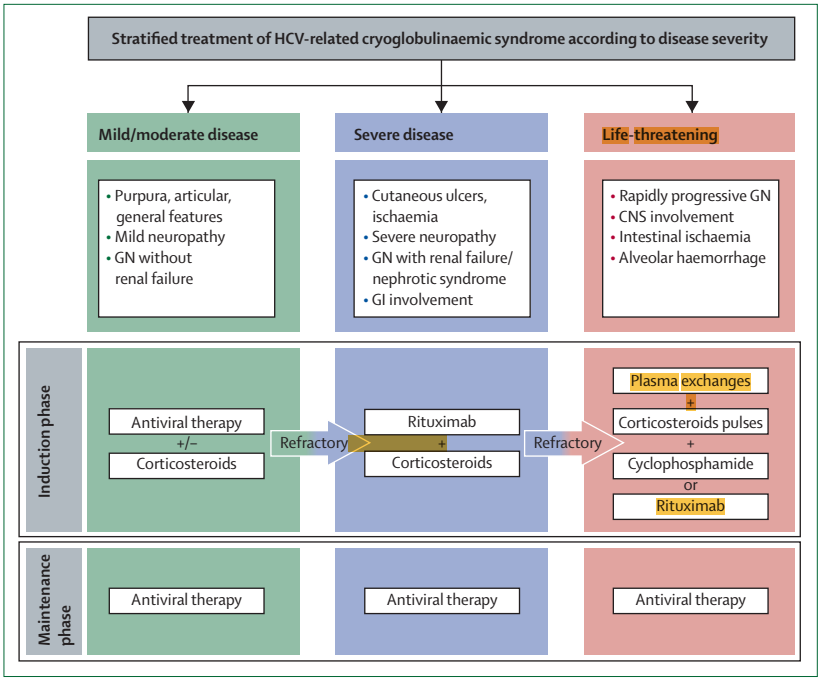

Figure 5: **Proposed therapeutic algorithm for patients with HCV-related cryoglobulinaemic syndrome according to disease severity**

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.[1,94,98,105,106] 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.

leukocytoclastic vasculitis of the capillaries and postcapillary venules. In cryoglobulinaemic glomerulonephritis, proliferative (highly cellular) glomerular infiltrates are recorded. Eosinophilic and periodic acid-Schiff test-positive subendothelial cryoprecipitate material might be detected.

Renal biopsy detects type I membranoproliferative glomerulonephritis in more than 70% of patients[16,58,67,68] (figure 4A). More specific histopathological findings are hyaline intraluminal thrombi that contain IgM, IgG, and C3 as endomembranous deposits and glomerular infiltration by monocytes. Glomerular crescents (10–20%), renal necrotising vasculitis (5–30%), and interstitial inflammation arise less often.[67,68] Renal biopsy discloses other histopathological patterns (focal, mesangioproliferative, or membranous glomerulonephritis) in smaller percentages of patients.[7,67] In these patients, the possible role of cryoglobulinaemia-associated processes (systemic lupus erythematosus, lymphoma, chronic viral diseases),[67,89,90] renal involvement associated with cardiovascular risk factors (hypertension, diabetes), and IgA nephropathy[90,91] should be investigated. Renal biopsy is recommended to confirm the diagnosis and assess the extent of involvement.

Cryoglobulinaemic neuropathy is characterised by vasculitis of the perineural and endoneural vessels, with various degrees of axonal degeneration and demyelination. Endoneural microvessel involvement is more common than in other systemic vasculitides. Endoneural capillaries are thickened and inflamed; extravasation of erythrocytes and macrophages (endoneural purpura) might be observed. Indirect immunofluorescence identifies deposits of immunoglobulins (of the same type as the serum cryoglobulins) and complement in skin, kidney, and nerve biopsies.[51,64,67,89,92,93]

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.[16] Patients with cryoglobulinaemic disease have a worse 10-year survival rate compared with the rate in the general population.[16,55] 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.[16,55,63,94,95]

Prognosis is influenced heavily by both cryoglobulinaemic damage to vital organs and by underlying diseases and comorbidities. Chronic HCV infection resulting in liver cirrhosis and cancer and haematological neoplasia are associated with a poor prognosis. Therapeutic interventions (glucocorticoids, immunosuppressive agents, plasma exchange) raise the risk of infection, especially in patients with chronic renal or liver failure. Patients with a combination of severe processes (chronic organ failure, infection, and vasculitis or neoplasia) are often seen and need an integrated, multidisciplinary approach.[96,97]

Life-threatening cryoglobulinaemia

Glomerulonephritis results in acute renal failure in 10% of patients[67] or can evolve progressively to chronic renal failure.[16,67,94] 10-year survival rates of glomerulonephritis range between 33% and 49%,[16,94] although a study reported in 2007 recorded nearly 80% survival after 10 years[67] 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.[67–69,94] Mortality rates associated with intestinal ischaemia and alveolar haemorrhage are very high (>80%),[94] although a 2010 study[77] suggested improved prognosis for gastrointestinal involvement.

Risk of neoplasia

Mixed cryoglobulinaemic syndrome is traditionally regarded as a crossroads between autoimmune disease and cancer.[98,99] 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.[93] B-cell lymphoma, the main neoplastic complication in patients with mixed cryoglobulinaemia,[16,100] has been reported in 5–22% of patients with mixed cryoglobulinaemia.[7,16,32,101,102] Hypogammaglobulinaemia can be a marker for impending lymphomagenesis.[102] Associated HCV infection confers a 20–30% increased risk of B-cell lymphoma.[103] 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.[31] Treatment usually includes standard chemotherapy regimens and rituximab.[32,101]

Liver cancer, the second most frequently diagnosed neoplasia in cryoglobulinaemic patients, is attributable to the close association with HCV.[7,16,104] Hepatocellular carcinoma occurs about a third as often as lymphoma in patients infected with HCV.

Treatment

Conventional immunosuppression

Treatment should be modulated according to the underlying aetiopathogenesis (hyperviscosity *vs* vasculitis) and the severity of clinical presentation (figure 5).[1,93,98,105,106] 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.[107] 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.[13,98,108]

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.

***Panel 2:* Key therapeutic points in patients with cryoglobulinaemia**

- Treat underlying cause of cryoglobulinaemia when possible
- Careful individual workup to assess how many organs are affected and severity of involvement is essential when planning therapeutic approaches
- Carefully assess intensity and duration of conventional immunosuppressive therapies, especially in patients infected with HCV
- Regard combination of pegylated interferon alfa and ribavirin as therapeutic cornerstone of HCV-related cryoglobulinaemic vasculitis
- Plasma exchange is valuable therapeutic option in life-threatening involvements, including hyperviscosity syndrome
- Off-label use of B-cell-depleting therapies should be reserved for severe refractory situations, and can improve therapeutic responses when combined with antiviral therapies

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.[109–111] Patients with HCV genotypes 2 and 3 respond most favourably to this regimen, with between 75% 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%).[112–114] The recommended regimen is shown in panel 3.[114] 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).

***Panel 3*: Recommended treatment regimen for combination interferon* and ribavirin† therapy[114]**

Therapeutic regimens

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

or

Pegylated interferon alfa-2b 1–5 µg/kg subcutaneously per week
+
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‡

- 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.

Interferon is currently contraindicated in recipients of kidney, heart, and lung allografts.[114]

Failure to achieve a virological response at weeks 12 and 24 is associated with a low probability of achieving a sustained virological response,[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.[115] Maintenance of antiviral therapies can be appropriate in patients with cryoglobulinaemia who seem to benefit.

Drug intolerance and dose reductions are common with both interferon alfa and ribavirin.[116] Influenza-like symptoms, cytopenia, depression, and autoimmune thyroiditis are common with interferon alfa. Exacerbation of vasculitic manifestations and delayed healing of necrotic ulcers have also been reported with interferon alfa.[117,118] Haemolytic anaemia is the major adverse effect associated with ribavirin. New generations of agents based on interferon alfa and ribavirin are likely to improve dosing schedules and lower the risk of adverse events.[119,120]

Biological therapies

The most-promising biological approach to cryoglobulinaemia used thus far is B-cell depletion with rituximab. Peripheral B-lymphocyte depletion should lead to a reduction in the B-cell clones that produce cryoglobulins. This idea needs further testing in mechanistic studies linked to clinical trials, but the results of small series are promising.[121–126] 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.[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.[122–124] Three studies reported in 2010 deserve careful scrutiny.[128–130] A prospective study[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/m² 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[129] was a prospective trial of 41 patients who were randomised to receive antiviral therapy alone or combined with rituximab (375 mg/m² 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.[130]

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)[131,132] should be made on an individual basis. Additionally, a substantial risk of complex formation between rituximab and IgM-κ with rheumatoid-factor activity has been reported,[133] especially in patients with high baseline concentrations of cryoglobulin.

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.[77,134] However, many aspects of the disease

remain unresolved.[1] 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[131] or type I[135,136] cryoglobulinaemia. The role of new antiviral agents in HCV-related cryoglobulinaemia, such as telaprevir,[137] 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.[138]

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[128,129] suggest greater benefit when rituximab is added to antiviral therapy, although some researchers support rituximab monotherapy.[139] Differences in study designs and therapeutic schedules do not allow definitive recommendations on the relative timing of antiviral and B-cell-depletion strategies. B-cell-activating factor of the family of tumour-necrosis-factor blocking agents[140] and Toll-like receptor agonists[141] might be promising therapies.

Cryoglobulinaemia is characterised by a wide range of causes, symptoms, and outcomes, with various aetiopathogenic 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, JHS, 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.

Acknowledgments

We thank David Buss for his technical assistance.

References

1 Ferri C. Mixed cryoglobulinaemia. *Orphanet J Rare Dis* 2008; **3:** 25.
2 Wintrobe MM, Buell MV. Hyperproteinemia associated with multiple myeloma. *Bull Johns Hopkins Hosp* 1933; **52:** 156–65.
3 Lerner AB, Watson CJ. Studies of cryoglobulins I: unusual purpura associated with the presence of a high concentration of cryoglobulin (cold precipitable serum globulin). *Am J Med Sci* 1947; **214:** 410–15.
4 Meltzer M, Franklin EC. Cryoglobulinaemia: a study of 29 patients. I: IgG and IgM cryoglobulins and factors effecting cryoprecipitability. *Am J Med* 1966; **40:** 828–36.
5 Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins: a report of 86 cases. *Am J Med* 1974; **57:** 775–88.
6 Tissot JD, Schifferli JA, Hochstrasser DF, et al. Two-dimensional polyacrylamide gel electrophoresis analysis of cryoglobulins and identification of an IgM-associated peptide. *J Immunol Meth* 1994; **173:** 63–75.
7 Trejo O, Ramos-Casals M, García-Carrasco M, et al. Cryoglobulinaemia: study of etiologic factors and clinical and immunologic features in 443 patients from a single center. *Medicine (Baltimore)* 2001; **80:** 252–62.
8 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood borne non-A, non-B viral hepatitis genome. *Science* 1989; **244:** 359–62.
9 Bambara LM, Biasi D, Caramaschi P, Carletto A, Pacor ML. Cryoglobulinaemia and hepatitis C virus (HCV) infection. *Clin Exp Rheumatol* 1991; **9:** 96–97.
10 Ferri C, Greco F, Longombardo G, et al. Antibodies to hepatitis C virus in patients with mixed cryoglobulinaemia. *Arthritis Rheum* 1991; **34:** 1606–10.
11 Ferri C, Greco F, Longombardo G, et al. Association between hepatitis C virus and mixed cryoglobulinemia. *Clin Exp Rheumatol* 1991; **9:** 621–24.
12 Sansonno D, Carbone A, De Re V, Dammacco F. Hepatitis C virus infection, cryoglobulinaemia, and beyond. *Rheumatology (Oxford)* 2007; **46:** 572–78.
13 Tedeschi A, Baratè C, Minola E, Morra E. Cryoglobulinaemia. *Blood Rev* 2007; **21:** 183–200.
14 Persico M, De Marino FA, Di Giacomo Russo G, et al. Prevalence and incidence of cryoglobulins in hepatitis C virus-related chronic hepatitis patients: a prospective study. *Am J Gastroenterol* 2003; **98:** 884–8.
15 Adinolfi LE, Utili R, Attanasio V, et al. Epidemiology, clinical spectrum and prognostic value of mixed cryoglobulinaemia in hepatitis C virus patients: a prospective study. *Ital J Gastroenterol* 1996; **28:** 1–9.
16 Ferri C, Sebastiani M, Giuggioli D, et al. Mixed cryoglobulinaemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin Arthritis Rheum* 2004; **33:** 355–74.
17 Cohen P, Roulot D, Ferrière F, et al. Prevalence of cryoglobulins and hepatitis C virus infection in HIV-infected patients. *Clin Exp Rheumatol* 1997; **15:** 523–27.
18 Dimitrakopoulos AN, Kordossis T, Hatzakis A, Moutsopoulos HM. Mixed cryoglobulinaemia in HIV-1 infection: the role of HIV-1. *Ann Intern Med* 1999; **130:** 226–30.
19 Fabris P, Tositti G, Giordani MT, et al. Prevalence and clinical significance of circulating cryoglobulins in HIV-positive patients with and without co-infection with hepatitis C virus. *J Med Virol* 2003; **69:** 339–43.
20 Ramos-Casals M, Forns X, Brito-Zerón P, et al. Cryoglobulinaemia associated with hepatitis C virus: influence of HCV genotypes, HCV-RNA viraemia and HIV coinfection. *J Viral Hepat* 2007; **14:** 736–42.
21 Bonnet F, Pineau JJ, Taupin JL, et al. Prevalence of cryoglobulinaemia and serological markers of autoimmunity in human immunodeficiency virus infected individuals: a cross-sectional study of 97 patients. *J Rheumatol* 2003; **30:** 2005–10.
22 Kosmas N, Kontos A, Panayiotakopoulos G, Dimitrakopoulos A, Kordossis T. Decreased prevalence of mixed cryoglobulinaemia in the HAART era among HIV-positive, HCV-negative patients. *J Med Virol* 2006; **78:** 1257–61.
23 Ramos-Casals M, Trejo O, García-Carrasco M, Cervera R, Font J. Mixed cryoglobulinaemia: new concepts. *Lupus* 2000; **9:** 83–91.
24 Tzioufas AG, Boumba DS, Skopouli FN, Moutsopoulos HM. Mixed monoclonal cryoglobulinaemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjogren's syndrome. *Arthritis Rheum* 1996; **39:** 767–72.
25 Brito-Zerón P, Ramos-Casals M, Bove A, Sentis J, Font J. Predicting adverse outcomes in primary Sjögren's syndrome: identification of prognostic factors. *Rheumatology (Oxford)* 2007; **46:** 1359–62.
26 Baimpa E, Dahabreh IJ, Voulgarelis M, Moutsopoulos HM. Hematologic manifestations and predictors of lymphoma development in primary Sjögren syndrome: clinical and pathophysiologic aspects. *Medicine (Baltimore)* 2009; **88:** 284–93.
27 Ramos-Casals M, Loustaud-Ratti V, De Vita S, et al, for the SS-HCV Study Group. Sjögren syndrome associated with hepatitis C virus: a multicenter analysis of 137 cases. *Medicine (Baltimore)* 2005; **84:** 81–89.
28 García-Carrasco M, Ramos-Casals M, Cervera R, et al. Cryoglobulinaemia in systemic lupus erythematosus: prevalence and clinical characteristics in a series of 122 patients. *Semin Arthritis Rheum* 2001; **30:** 366–73.

29 Perlemuter G, Cacoub P, Sbaï A, et al. Hepatitis C virus infection in systemic lupus erythematosus: a case-control study. *J Rheumatol* 2003; **30:** 1473–78.

30 Dammacco F, Lauletta G, Montrone M, Sansonno D. Mixed cryoglobulinaemia: a model of virus-related disease in internal medicine. *Dig Liver Dis* 2007; **39** (suppl 1)**:** S8–12.

31 Trejo O, Ramos-Casals M, López-Guillermo A, et al. Hematologic malignancies in patients with cryoglobulinaemia: association with autoimmune and chronic viral diseases. *Semin Arthritis Rheum* 2003; **33:** 19–28.

32 Saadoun D, Sellam J, Ghillani-Dalbin P, Crecel R, Piette JC, Cacoub P. Increased risks of lymphoma and death among patients with non-hepatitis C virus-related mixed cryoglobulinaemia. *Arch Intern Med* 2006; **166:** 2101–08.

33 Casato M, Lilli D, Donato G, et al. Occult hepatitis C virus infection in type II mixed cryoglobulinaemia. *J Viral Hepat* 2003; **10:** 455–59.

34 Trendelenburg M, Schifferli JA. Cryoglobulins are not essential. *Ann Rheum Dis* 1998; **57:** 3–5.

35 Lunel F, Musset L, Cacoub P et al. Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. *Gastroenterology* 1994; **106:** 1291–300.

36 Kayali Z, Bochwold VE, Zimmermann B, Schmidt WN. Hepatitis C, cryoglobulinemia, and cirrhosis: A meta-analysis. *Hepatology* 2002; **36:** 978–85.

37 Ramos-Casals M, De Vita S, Tzioufas AG. Hepatitis C virus, Sjögren's syndrome and B-cell lymphoma: linking infection, autoimmunity and cancer. *Autoimmun Rev* 2005; **4:** 8–15.

38 Ferri C, Antonelli A, Mascia MT, et al. HCV-related autoimmune and neoplastic disorders: the HCV syndrome. *Dig Liver Dis* 2007; **39** (suppl 1)**:** S13–21.

39 Pileri P, Uematsu Y, Campagnoli S, et al. Binding of hepatitis C virus to CD81. *Science* 1998; **282:** 938–41.

40 Rosa D, Saletti G, De Gregorio E, et al. Activation of naïve B lymphocytes via CD81, a pathogenetic mechanism for hepatitis C virus-associated B lymphocyte disorders. *Proc Natl Acad Sci USA* 2005; **102:** 18544–49.

41 Sansonno D, Lauletta G, De Re V et al. Intrahepatic B cell clonal expansions and extrahepatic manifestations of chronic HCV infection. *Eur J Immunol* 2004; **34:** 126–36.

42 Quartuccio L, Fabris M, Salvin S, et al. Bone marrow B-cell clonal expansion in type II mixed cryoglobulinaemia: association with nephritis. *Rheumatology (Oxford)* 2007; **46:** 1657–61.

43 Charles ED, Green RM, Marukian S, et al. Clonal expansion of immunoglobulin M+CD27+ B cells in HCV-associated mixed cryoglobulinaemia. *Blood* 2008; **111:** 1344–56.

44 Chen PP, Fong S, Goni F, et al. Cross-reacting idiotypes on cryoprecipitating rheumatoid factor. *Semin Immunopathol* 1988; **10:** 35–55.

45 Knight GB, Gao L, Gragnani L, et al. Detection of WA B cells in hepatitis C virus infection: a potential prognostic marker for cryoglobulinemic vasculitis and B cell malignancies. *Arthritis Rheum* 2010; **62:** 2152–59.

46 Sansonno D, Cornacchiulo V, Iacobelli AR, Di Stefano R, Lospalluti M, Dammacco F. Localization of hepatitis C virus antigens in liver and skin tissues of chronic hepatitis C virus-infected patients with mixed cryoglobulinaemia. *Hepatology* 1995; **21:** 305–12.

47 Sansonno D, Gesualdo L, Manno C, Schena FP, Dammacco F. Hepatitis C virus-related proteins in kidney tissue from hepatitis C virus-infected patients with cryoglobulinaemic membranoproliferative glomerulonephritis. *Hepatology* 1997; **25:** 1237–44.

48 Sansonno D, Lauletta G, Nisi L, et al. Non-enveloped HCV core protein as constitutive antigen of cold-precipitable immune complexes in type II mixed cryoglobulinaemia. *Clin Exp Immunol* 2003; **133:** 275–82.

49 Sargur R, White P, Egner W. Cryoglobulin evaluation: best practice? *Ann Clin Biochem* 2010; **47:** 8–16.

50 Mizuochi T, Pastore Y, Shikata K, et al. Role of galactosylation in the renal pathogenicity of murine immunoglobulin G3 monoclonal cryoglobulins. *Blood* 2001; **97:** 3537–43.

51 Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. *Lancet Infect Dis* 2005; **5:** 227–36.

52 Sansonno D, Tucci FA, Ghebrehiwet B, et al. Role of the receptor for the globular domain of C1q protein in the pathogenesis of hepatitis C virus-related cryoglobulin vascular damage. *J Immunol* 2009; **183:** 6013–20.

53 Sene D, Ghillani-Dalbin P, Thibault V, et al. Longterm course of mixed cryoglobulinaemia in patients infected with hepatitis C virus. *J Rheumatol* 2004; **31:** 2199–206.

54 Della Rossa A, Tavoni A, Bombardieri S. Hyperviscosity syndrome in cryoglobulinaemia: clinical aspects and therapeutic considerations. *Semin Thromb Hemost* 2003; **29:** 473–77.

55 Della Rossa A, Tavoni A, D'Ascanio A, et al. Mortality rate and outcome factors in mixed cryoglobulinaemia: the impact of hepatitis C virus. *Scand J Rheumatol* 2010; **39:** 167–70.

56 Treon SP. How I treat Waldenström macroglobulinemia. *Blood* 2009; **114:** 2375–85.

57 Menke MN, Treon SP. Hyperviscosity syndrome. In: Sekeres MA, Kalaycio ME, Bolwell BJ, eds. Clinical malignant hematology. New York: McGraw-Hill, 2007: 937–41.

58 Rieu V, Cohen P, André MH, et al. Characteristics and outcome of 49 patients with symptomatic cryoglobulinaemia. *Rheumatology (Oxford)* 2002; **41:** 290–300.

59 Wener MH, Hutchinson K, Morishima C, Gretch DR. Absence of antibodies to cyclic citrullinated peptide in sera of patients with hepatitis C virus infection and cryoglobulinaemia. *Arthritis Rheum* 2004; **50:** 2305–08.

60 Poynard T, Cacoub P, Ratziu V, et al, for the Multivirc group. Fatigue in patients with chronic hepatitis C. *J Viral Hepat* 2002; **9:** 295–303.

61 Antonelli A, Ferri C, Fallahi P, et al. Thyroid involvement in patients with overt HCV-related mixed cryoglobulinaemia. *QJM* 2004; **97:** 499–506.

62 Antonelli A, Ferri C, Fallahi P, et al. Type 2 diabetes in hepatitis C-related mixed cryoglobulinaemia patients. *Rheumatology (Oxford)* 2004; **43:** 238–40.

63 Bryce AH, Kyle RA, Dispenzieri A, Gertz MA. Natural history and therapy of 66 patients with mixed cryoglobulinaemia. *Am J Hematol* 2006; **81:** 511–18.

64 Matignon M, Cacoub P, Colombat M, et al. Clinical and morphologic spectrum of renal involvement in patients with mixed cryoglobulinaemia without evidence of hepatitis C virus infection. *Medicine (Baltimore)* 2009; **88:** 341–48.

65 Alpers CE, Smith KD. Cryoglobulinaemia and renal disease. *Curr Opin Nephrol Hypertens* 2008; **17:** 243–49.

66 Pioltelli P, Maldifassi P, Vacca A, et al. GISC protocol experience in the treatment of essential mixed cryoglobulinaemia. *Clin Exp Rheumatol* 1995; **13** (suppl)**:** S187–90.

67 Roccatello D, Fornasieri A, Giachino O, et al. Multicenter study on hepatitis C virus related cryoglobulinaemic glomerulonephritis. *Am J Kidney Dis* 2007; **49:** 69–82.

68 Beddhu S, Bastacky S, Johnson JP. The clinical and morphologic spectrum of renal cryoglobulinaemia. *Medicine (Baltimore)* 2002; **81:** 398–409.

69 Tarantino A, Campise M, Banfi G, et al. Long-term predictors of survival in essential mixed cryoglobulinaemic glomerulonephritis. *Kidney Int* 1995; **47:** 618–23.

70 Basse G, Ribes D, Kamar N, Esposito L, Rostaing L. Life-threatening infections following rituximab therapy in renal transplant patients with mixed cryoglobulinemia. *Clin Nephrol* 2006; **66:** 395–96.

71 Hiesse C, Bastuji-Garin S, Santelli G, et al. Recurrent essential mixed cryoglobulinemia in renal allografts. Report of two cases and review of the literature. *Am J Nephrol* 1989; **9:** 150–54.

72 Gemignani F, Melli G, Inglese C, Marbini A. Cryoglobulinaemia is a frequent cause of peripheral neuropathy in undiagnosed referral patients. *J Peripher Nerv Syst* 2002; **7:** 59–64.

73 Nemni R, Sanvito L, Quattrini A, Santuccio G, Camerlingo M, Canal N. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. *J Neurol Neurosurg Psychiatry* 2003; **74:** 1267–71.

74 Santoro L, Manganelli F, Briani C, et al; HCV Peripheral Nerve Study Group. Prevalence and characteristics of peripheral neuropathy in hepatitis C virus population. *J Neurol Neurosurg Psychiatry* 2006; **77:** 626–29.

75 Taieb G, Maisonobe T, Musset L, Cacoub P, Léger JM, Bouche P. Cryoglobulinaemic peripheral neuropathy in hepatitis C virus infection: Clinical and anatomical correlations of 22 cases. *Rev Neurol (Paris)* 2010; **166:** 509–14.

76 Vidaller A, Cid MC. Tetraparesis and paresthesias in the lower extremities in a 58-year-old man. *Med Clin (Barc)* 1999; **112:** 470–76 (in Spanish).

77 Terrier B, Saadoun D, Sène D, Scerra S, Musset L, Cacoub P. Presentation and outcome of gastrointestinal involvement in hepatitis C virus-related systemic vasculitis: a case-control study from a single-centre cohort of 163 patients. *Gut* 2010; **59:** 1709–15.

78 Amital H, Rubinow A, Naparstek Y. Alveolar hemorrhage in cryoglobulinaemia—an indicator of poor prognosis. *Clin Exp Rheumatol* 2005; **23:** 616–20.

79 Ferri C, La Civita L, Fazzi P, et al. Interstitial lung fibrosis and rheumatic disorders in patients with hepatitis C virus infection. *Br J Rheumatol* 1997; **36:** 360–65.

80 Retamozo S, Diaz-Lagares C, Bosch X, De Vita S, Ramos-Casals M. Life-threatening cryoglobulinemia. In: Khamashta MA, Ramos-Casals M, eds. Autoimmune diseases: acute and complex situations London: Springer-Verlag, 2011: 133–62.

81 Morgello S. The nervous system and hepatitis C virus. *Semin Liver Dis* 2005; **25:** 118–21.

82 Safadi R, Ilan Y, Ashur Y, Shouval D. Hepatitis C-associated cryoglobulinaemia presenting with pericardial effusion. *Am J Gastroenterol* 1997; **92:** 710–12.

83 Basu N, Watts R, Bajema I, et al. EULAR points to consider in the development of classification and diagnostic criteria in systemic vasculitis. *Ann Rheum Dis* 2010; **69:** 1744–50.

84 Bakker AJ, Slomp J, de Vries T, et al. Adequate sampling in cryoglobulinaemia: recommended warmly. *Clin Chem Lab Med* 2003; **41:** 85–89.

85 Vermeersch P, Gijbels K, Mariën G, et al. A critical appraisal of current practice in the detection, analysis, and reporting of cryoglobulins. *Clin Chem* 2008; **54:** 39–43.

86 Maire MA, Mittey M, Lambert PH. The presence of cryoprecipitable immunoglobulins in normal human sera may reflect specific molecular interactions. *Autoimmunity* 1989; **2:** 155–64.

87 Shihabi ZK. Analysis and general classification of serum cryoglobulins by capillary zone electrophoresis. *Electrophoresis* 1996; **17:** 1607–12.

88 Vitali C, Ferri C, Nasti P, et al. Hypercomplementaemia as a marker of the evolution from benign to malignant B cell proliferation in patients with type II mixed cryoglobulinaemia. *Br J Rheumatol* 1994; **33:** 791–92.

89 D'Amico G. Renal involvement in hepatitis C infection: cryoglobulinaemic glomerulonephritis. *Kidney Int* 1998; **54:** 650–71.

90 Gopalani A, Ahuja TS. Prevalence of glomerulopathies in autopsies of patients infected with the hepatitis C virus. *Am J Med Sci* 2001; **322:** 57–60.

91 Sumida K, Ubara Y, Hoshino J, et al. Hepatitis C virus-related kidney disease: various histological patterns. *Clin Nephrol* 2010; **74:** 446–56.

92 Vallat JM, Leboutet MJ, Loubet A. Cryoglobulinaemic neuropathy: ultrastructural study of the nerve capillaries. *Acta Neuropathol* 1981; **7:** 252–54.

93 Vallat JM, Desproges-Gotteron R, Leboutet MJ, Loubet A, Gualde N, Treves R. Cryoglobulinaemic neuropathy: a pathological study. *Ann Neurol* 1980; **8:** 179–85.

94 Ramos-Casals M, Robles A, Brito-Zeron P, et al. Life-threatening cryoglobulinaemia: clinical and immunological characterizsation of 29 cases. *Semin Arthritis Rheum* 2006; **36:** 189–96.

95 Monti G, Saccardo F, Pioltelli P, Rinaldi G. The natural history of cryoglobulinaemia: symptoms at onset and during follow-up: a report by the Italian Group for the Study of Cryoglobulinaemias (GISC). *Clin Exp Rheumatol* 1995; **13** (suppl)**:** S129–33.

96 Monti G, Saccardo F. Emergency in cryoglobulinaemic syndrome: what to do? *Dig Liver Dis.* 2007; **39** (suppl 1): S112–15.

97 Ramos-Casals M, Trejo O, García-Carrasco M, et al. Triple association between hepatitis C virus infection, systemic autoimmune diseases, and B cell lymphoma. *J Rheumatol* 2004; **31:** 495–99.

98 Saadoun D, Landau DA, Calabrese LH, Cacoub PP. Hepatitis C-associated mixed cryoglobulinaemia: a crossroad between autoimmunity and lymphoproliferation. *Rheumatology (Oxford)* 2007; **46:** 1234–42.

99 La Civita L, Zignego AL, Monti M, Longombardo G, Pasero G, Ferri C. Mixed cryoglobulinaemia as a possible preneoplastic disorder. *Arthritis Rheum* 1995; **38:** 1859–60.

100 McColl MD, Singer IO, Tait RC, McNeil IR, Cumming RL, Hogg RB. The role of hepatitis C virus in the aetiology of non-Hodgkins lymphoma—a regional association? *Leuk Lymphoma* 1997; **26:** 127–30.

101 Monti G, Pioltelli P, Saccardo F, et al. Incidence and characteristics of non-Hodgkin lymphomas in a multicenter case file of patients with hepatitis C virus-related symptomatic mixed cryoglobulinemias. *Arch Intern Med* 2005; **165:** 101–05.

102 Geri G, Terrier B, Semoun O, et al. Surrogate markers of B cell non-Hodgkin's lymphoma in patients with hepatitis C virus-related cryoglobulinaemia vasculitis. *Ann Rheum Dis* 2010; **69:** 2177–80.

103 Giordano TP, Henderson L, Landgren O, et al. Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. *JAMA* 2007; **297:** 2010–17.

104 Saccardo E, Novati P, Sironi D, Castelnovo L, Rinaldi G, Monti G. Causes of death in symptomatic cryoglobulinaemia: 30 years of observation in a Department of Internal Medicine. *Dig Liver Dis* 2007; **39** (suppl 1)**:** S52–54.

105 Ferri C, Mascia MT. Cryoglobulinemic vasculitis. *Curr Opin Rheumatol* 2006; **18:** 54–63.

106 Saadoun D, Delluc A, Piette JC, Cacoub P. Treatment of hepatitis C-associated mixed cryoglobulinemia vasculitis. *Curr Opin Rheumatol* 2008; **20:** 23–28.

107 Vassilopoulos D, Calabrese LH. Hepatitis C virus infection and vasculitis. Implications of antiviral and immunosuppressive therapies. *Arthritis Rheum* 2002; **46:** 585–97.

108 Ramos-Casals M, Font J. Mycophenolate mofetil in patients with hepatitis C virus infection. *Lupus* 2005; **14** (suppl)**:** S64–72.

109 Feld JJ, Hoofnagle JH. Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature* 2005; **436:** 967–72.

110 Cacoub P, Saadoun D, Limal N, Sene D, Lidove O, Piette JC. PEGylated interferon alfa-2b and ribavirin treatment in patients with hepatitis C virus-related systemic vasculitis. *Arthritis Rheum* 2005; **52:** 911–15.

111 Saadoun D, Resche-Rigon M, Thibault V, Piette JC, Cacoub P. Antiviral therapy for hepatitis C virus associated mixed cryoglobulinemia vasculitis: a long-term follow-up study. *Arthritis Rheum* 2006; **54:** 3696–706.

112 Fried MW, Shiffman ML, Reddy KR et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347:** 975–82.

113 Hadziyannis SJ, Sette H Jr., Morgan TR, et al, for the PEGASYS International Study Group. Peginterferon-alpha 2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140:** 346–55.

114 Ghany MG, Strader DB, Thomas DL, Seeff LB, for the American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49:** 1335–74.

115 Joshi S, Kuczynski M, Heathcote EJ. Symptomatic and virological response to antiviral therapy in hepatitis C associated with extrahepatic complications of cryoglobulimia. *Dig Dis Sci* 2007; **52:** 2410–17.

116 Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003; **124:** 1711–19.

117 Gordon AC, Edgar JD, Finch RG. Acute exacerbation of vasculitis during interferon-alpha therapy for hepatitis C-associated cryoglobulinaemia. *J Infect* 1998; **36:** 229–30.

118 Cid MC, Hernández-Rodríguez J, Robert J, et al. Interferon-alpha may exacerbate cryoblobulinemia-related ischemic manifestations: an adverse effect potentially related to its anti-angiogenic activity. *Arthritis Rheum* 1999; **42:** 1051–55.

119 Nelson DR, Rustgi V, Balan V, et al. Safety and antiviral activity of albinterferon alfa-2b in prior interferon non-responders with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2009; **7:** 212–18.

120 Gish RG, Arora S, Rajender Reddy K, et al. Virological response and safety outcomes in therapy-naïve patients treated for chronic hepatitis C with taribavirin or ribavirin in combination with pegylated interferon alfa-2a: a randomizsed, phase 2 study. *J Hepatol* 2007; **47:** 51–59.

121 Quartuccio L, Soardo G, Romano G, et al. Rituximab treatment for glomerulonephritis in HCV-associated mixed cryoglobulinaemia: efficacy and safety in the absence of steroids. *Rheumatology (Oxford)* 2006; **45:** 842–46.

122 Lamprecht P, Lerin-Lozano C, Merz H, et al. Rituximab induces remission in refractory HCV-associated mixed cryoglobulinemic vasculitis. *Ann Rheum Dis* 2003; **62:** 1230–33.

123 Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. *Blood* 2003; **101:** 3818–26.

124 Zaja F, De Vita S, Mazzaro C, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. *Blood* 2003; **101:** 3827–34.

125 Saadoun D, Rosenzwaig M, Landau D, Piette JC, Klatzmann D, Cacoub P. Restoration of peripheral immune homeostasis after rituximab in mixed cryoglobulinemia vasculitis. *Blood* 2008; **111:** 5334–41.

126 Roccatello D, Baldovino S, Rossi D, et al. Long-term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. *Nephrol Dial Transplant* 2004; **19:** 3054–61.

127 Landau DA, Saadoun D, Halfon P, et al. Relapse of hepatitis C virus-associated mixed cryoglobulinemia vasculitis in patients with sustained viral response. *Arthritis Rheum* 2008; **58:** 604–11.

128 Saadoun D, Resche-Rigon M, Sene D, et al. Rituximab plus PEG-interferon alpha/ribavirin compared to PEG-interferon alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. *Blood* 2010; **116:** 326–34.

129 Dammacco F, Tucci FA, Lauletta G, et al. Pegylated interferon-alfa, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. *Blood* 2010; **116:** 343–53.

130 Petrarca A, Rigacci L, Caini P, et al. Safety and efficacy of rituximab in patients with hepatitis C virus-related mixed cryoglobulinemia and severe liver disease. *Blood* 2010; **116:** 335–42.

131 Terrier B, Launay D, Kaplanski G, et al. Safety and efficacy of rituximab in nonviral cryoglobulinemia vasculitis: data from the French Autoimmunity and Rituximab registry. *Arthritis Care Res (Hoboken)* 2010; **62:** 1787–95.

132 Gea-Banacloche JC. Rituximab-associated infections. *Semin Hematol* 2010; **47:** 187–98.

133 Sene D, Ghillani-Dalbin P, Amoura Z, Musset L, Cacoub P. Rituximab may form a complex with IgM-kappa mixed cryoglobulin and induce severe systemic reactions in patients with hepatitis C virus-induced vasculitis. *Arthritis Rheum* 2009; **60:** 3848–55.

134 Landau DA, Scerra S, Sene D, Resche-Rigon M, Saadoun D, Cacoub P. Causes and predictive factors of mortality in a cohort of patients with hepatitis C virus-related cryoglobulinemic vasculitis treated with antiviral therapy. *J Rheumatol* 2010; **37:** 615–21.

135 Spizzo G, Mitterer M, Gunsilius E. Bortezomib for the treatment of refractory type-1 cryoglobulinaemia. *Br J Haematol* 2010; **150:** 235–37.

136 Talamo G, Claxton D, Tricot G, Fink L, Zangari M. Response to bortezomib in refractory type I cryoglobulinemia. *Am J Hematol* 2008; **83:** 883–84.

137 Hezode C, Forestier N, Dusheiko G, et al, for the PROVE2 Study Team. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360:** 1839–50.

138 De Vita S, Soldano F, Isola M, et al. Preliminary classification criteria for the cryoglobulinemic syndrome. *Ann Rheum Dis* 2011; **70:** 1183–90.

139 De Vita S, Quartuccio L. Rituximab monotherapy, rather than rituximab plus antiviral drugs, for initial treatment of severe hepatitis C virus-associated mixed cryoglobulinemia syndrome: comment on the article by Terrier et al. *Arthritis Rheum* 2010; **62:** 908–09.

140 Sène D, Limal N, Ghillani-Dalbin P, Saadoun D, Piette JC, Cacoub P. Hepatitis C virus-associated B-cell proliferation—the role of serum B lymphocyte stimulator (BLyS/BAFF). *Rheumatology (Oxford)* 2007; **46:** 65–69.

141 McHutchinson J, Bacon BR, Gordon SC, et al. Phase 1B, randomized, double-blind, dose-escalation trial of CPG 10101 in patients with chronic hepatitis C virus. *Hepatology* 2007; **46:** 1317–18.