REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

Cryoglobulinemia — One Name for Two Diseases

Patrice Cacoub, M.D., Matheus Vieira, M.D., and David Saadoun, M.D., Ph.D.

From Sorbonne Universités, Centre National de Références des Maladies Autoimmunes Systémiques Rares, Centre National de Références des Maladies Autoinflammatoires et de l'Amylose Inflammatoire, the Department of Inflammation, Immunopathology, and Biotherapy, Clinical Investigation Center in Biotherapy, INSERM 959, and Département de Médecine Interne et Immunologie Clinique, Groupe Hospitalier Pitié–Salpêtrière, Assistance Publique–Hôpitaux de Paris — all in Paris. Dr. Cacoub can be contacted at patrice.cacoub@aphp.fr or at Sorbonne Universités, AP-HP, Groupe Hospitalier Pitié–Salpêtrière, Département de Médecine Interne et Immunologie Clinique, F-75013, Paris, France.

N Engl J Med 2024;391:1426-39.
DOI: 10.1056/NEJMra2400092
Copyright © 2024 Massachusetts Medical Society.

CME

Cryoglobulinemia is a pathologic condition characterized by the precipitation of circulating immunoglobulins from human serum when cooled below 4°C; the immunoglobulins are reversibly soluble when reheated. These proteins were discovered by Wintrobe and Buell in 1933.[1] Lerner and Watson later identified these proteins as gamma globulins and introduced the term cryoglobulins (i.e., cold precipitable serum globulins).[2] Since the initial descriptions of clinical manifestations associated with the in vitro cryoprecipitation of immunoglobulins emerged in the late 1960s,[3] our understanding of cryoglobulinemia, which is characterized by circulating cryoglobulins in the serum, has undergone considerable change. The conditions associated with this unique biologic phenomenon were categorized into three groups of similar importance: lymphomas, Waldenström's macroglobulinemia, and the so-called essential forms, which occur in the absence of any known underlying disease. In 1974, Brouet and colleagues described a classification system, which is still used today, based on the isotype (or isotypes) of immunoglobulins constituting the cryoglobulinemia[4]: type I consists of a monoclonal immunoglobulin (IgM, IgG, or IgA), type II includes polyclonal IgG plus monoclonal IgM with rheumatoid-factor activity, and type III comprises polyclonal IgG, polyclonal IgM, or both. Both type II and type III are known as mixed cryoglobulinemias. Empirical therapeutic approaches have involved different combinations of glucocorticoids, conventional immunosuppressants, and plasma exchange and have yielded inconsistent outcomes and a poor prognosis.

The list of causes of cryoglobulinemia has expanded considerably over time, although causality has not been established in most cases.[5] A considerable shift occurred in the early 1990s with the discovery of the hepatitis C virus (HCV), which was rapidly acknowledged as the leading cause of what was previously termed "essential" mixed cryoglobulinemia.[6] The current list of causes has been narrowed down to a few hematologic disorders, systemic autoimmune diseases, and chronic infections (Table 1). The diagnostic, pathophysiological, and therapeutic advances made since the early 1990s have redefined the spectrum of cryoglobulinemias and led to the identification of two distinct conditions: type I cryoglobulinemia, which is characterized as a genuine hemostasis disorder accompanied by multiple thromboses of small and medium-sized vessels and sometimes by signs of vascular inflammation; and type II and III mixed cryoglobulinemias, which are considered to be a type of genuine inflammatory small-vessel vasculitis caused by complement-mediated immune-complex deposition. Despite the fact that recognition of cryoglobulinemia is better now than in the past, robust and comprehensive epidemiologic data are still scarce, and the disease is considered rare. A better understanding of the underlying mechanisms, however, led to the development of more targeted

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

KEY POINTS

CRYOGLOBULINEMIA

- The causes of cryoglobulinemias are currently restricted to a few hematologic disorders, systemic autoimmune diseases, and chronic infections.
- The cryoglobulinemia clinical syndrome comprises two major phenotypes.
- Type I cryoglobulinemia is a genuine hemostasis disorder that leads to mechanical obstruction of multiple small and medium-sized vessels (hyperviscosity, thrombosis, or both); patients with this condition sometimes show signs of vascular inflammation.
- Type II and III mixed cryoglobulinemias are characterized as autoimmune small-vessel vasculitis caused by complement-mediated immune-complex deposition.
- Whereas type I cryoglobulinemia typically arises from a hematologic cancer, mixed cryoglobulinemia is characterized by indolent B-cell lymphoproliferation that may eventually lead to overt lymphoma transformation.
- When pharmacologic therapy is indicated, the underlying disease must first be identified, and then the B-cell lineage clone can be targeted.

therapies (against either HCV or lymphocyte clones) that are changing the natural history of cryoglobulinemia. In view of their distinctive biologic properties — and therefore their different clinical and therapeutic implications — type I and mixed cryoglobulinemia constitute two distinct diseases categorized under the same name. This review delves into a detailed discussion and comparison of these conditions.

CRYOGLOBULINEMIA SYNDROME

CLINICAL CHARACTERISTICS

The clinical manifestations associated with cryoglobulinemia syndrome are highly diverse and can potentially affect virtually any organ; thus, the syndrome is categorized as a genuine systemic disease. However, not all manifestations are common to both types of cryoglobulinemia, and certain subtle differences can be helpful for clinicians in differentiating between type I cryoglobulinemia and mixed cryoglobulinemia. Data characterizing patients with type I cryoglobulinemia have been derived from retrospective cohorts that pooled cases involving IgG and IgM,[7-12] whereas data on patients with mixed cryoglobulinemia have come mainly from cases of HCV-related vasculitis[4,5,13-19]; both types are summarized in Figure 1.

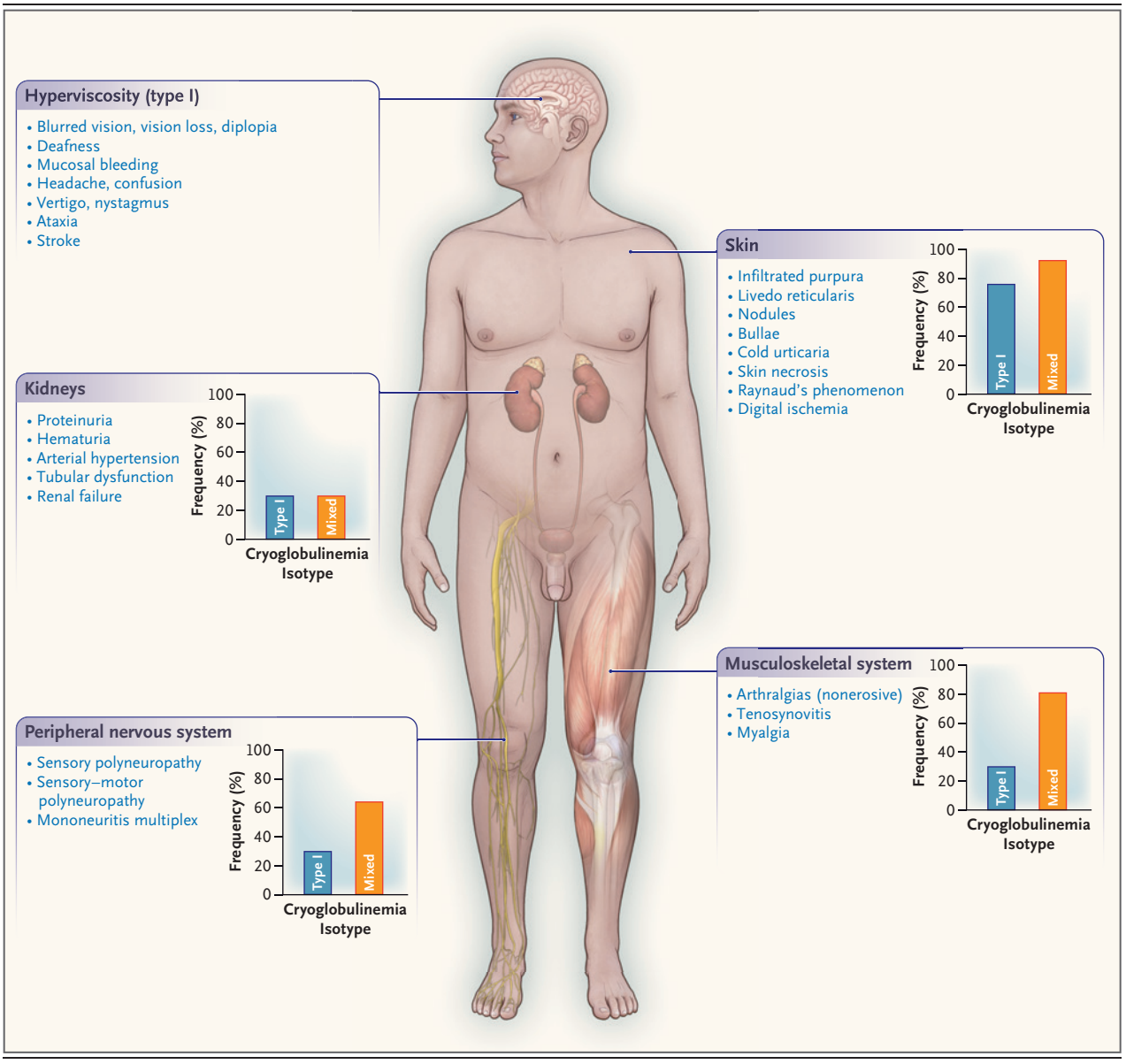
Skin involvement is present in most cases of mixed cryoglobulinemia and typically manifests as vascular purpura with lesions that are sometimes necrotic (Fig. 2A). In type I cryoglobulinemia, skin

Table 1. Main Causes of Cryoglobulinemia.*

Cause	Disorders
Hematologic disorders	Waldenström's macroglobulinemia Multiple myeloma Non-Hodgkin's lymphoma Chronic lymphocytic leukemia Monoclonal gammopathy of clinical significance
Systemic autoimmune diseases	Sjögren's syndrome Systemic lupus erythematosus Rheumatoid arthritis
Chronic viral infections	Hepatitis C Hepatitis B Human immunodeficiency virus
Others (very rare)	Viral infections (e.g., adenovirus, herpes viruses, Epstein–Barr virus, varicella–zoster virus, human T-cell leukemia virus type 1, influenza virus, parvovirus B19, rubella virus) Bacterial infections (e.g., brucella, infective endocarditis, Lyme disease, rickettsia, syphilis) Fungal infections (e.g., coccidioidomycosis) Parasitic infections (e.g., echinococcosis, leishmaniasis, malaria, schistosomiasis, toxoplasmosis, trypanosomiasis)

* Additional tests should be performed systematically according to the cryoglobulinemia type, clinical manifestation, and suspected underlying diagnosis. Diagnostic testing generally prescribed for type I cryoglobulinemia includes a complete blood count, protein electrophoresis, serum protein immunofixation, thoracic and abdominal computed tomographic scans, a positron-emission tomographic scan, and bone marrow biopsy. For type II and III mixed cryoglobulinemias, initial testing should include viral serologic tests (anti–hepatitis C virus [HCV] antibody, HCV RNA, hepatitis B surface antigen, anti–hepatitis B surface antibody, anti–hepatitis B core antibody, and anti–human immunodeficiency virus antibody); tests for antinuclear antibodies, antibodies against extractable nuclear antigens, and anti–double-stranded DNA antibodies; and salivary-gland biopsy, if applicable. In addition, although hematologic cancers are commonly associated with type I cryoglobulinemia, they may also cause type II, so tests specific to type II cryoglobulinemia should be considered during diagnostic testing.

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.



manifestations are highly dependent on ambient temperature, and cutaneous necrosis is much more marked than in mixed cryoglobulinemia. Joint and muscle involvement is present in most patients with mixed cryoglobulinemia and consists of inflammatory, bilateral, symmetric, and nondestructive arthralgias affecting large joints; synovitis is rare. The clinical manifestations of mixed cryoglobulinemia may resemble those of rheumatoid arthritis, particularly in the presence of rheumatoid factor. Many patients are treated accordingly, and when they present with systemic manifestations, patients often end up with a misdiagnosis of rheumatoid vasculitis. The absence of bone erosions and anti–citrullinated protein antibodies helps to distinguish between rheumatoid arthritis and cryoglobulinemia.[20] In patients with type I cryoglobulinemia, arthralgias occur less frequently, with painful swelling of the fingers and toes that is not necessarily of articular origin. In rare cases, small-fiber neuropathy might be the cause of widespread pain in patients with cryoglobulinemia.

The most common neurologic impairment in mixed cryoglobulinemia is sensory–motor polyneuropathy. Cerebral involvement is very rare,

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

Figure 1 (facing page). The Clinical Presentation of Cryoglobulinemia Syndrome.

Shown are the most frequent clinical manifestations occurring with cryoglobulinemia syndrome, along with the frequency of each category of clinical features according to cryoglobulinemia isotype. Skin involvement includes vascular purpura, which is typically gravity-dependent and which starts on the lower limbs and spreads to the upper limbs while avoiding the trunk and face. Recurrent flares may leave a trace of dermatitis with an ochre color. Skin involvement may also include livedo reticularis, subcutaneous nodules, bullae, vesicles, cold urticaria, and paresthesia in the fingers and toes, rather than true Raynaud's phenomenon. These features of skin involvement are closely linked to physical effort and orthostatism in type II and III mixed cryoglobulinemias, whereas the role of external cold is substantial in type I cryoglobulinemia. Skin manifestations seen in type I cryoglobulinemia mostly involve extremity hypoperfusion (e.g., acral necrosis or Raynaud's phenomenon), whereas mixed cryoglobulinemia manifests predominantly as inflammatory lesions (e.g., vasculitic purpura or subcutaneous nodules). Renal involvement is usually indistinguishable clinically between type I cryoglobulinemia and mixed cryoglobulinemia and may manifest as nephrotic-range proteinuria, arterial hypertension, hematuria, renal failure, or a combination of these features. Renal involvement in type I cryoglobulinemia may be related to the underlying hematologic condition (e.g., myeloma-related kidney disease with or without tubular dysfunction). The most common neurologic impairment in patients with mixed cryoglobulinemia is sensory–motor polyneuropathy, which starts in the feet and rarely extends above the knees; the hands may also be involved, although involvement usually does not affect the upper limbs above the wrists. Sensory symptoms are typically the initial feature and may last for several months or even years before the onset of motor-deficit symptoms. Less commonly, sudden onset of multiple mononeuropathies may mimic periarteritis nodosa. Cerebral involvement is very rare and may manifest as focal deficits or neurocognitive disorders. In type I cryoglobulinemia, the presence of monoclonal immunoglobulins in large quantities may lead to hyperviscosity symptoms, including mucosal bleeding and neurosensory signs. The broad spectrum of clinical manifestations encompasses blurred or diminished vision, diplopia, headache, confusion, deafness, vertigo, nystagmus, ataxia, stroke, or even coma.

manifesting as focal deficits or neurocognitive disorders. Neurologic symptoms associated with lymphoma infiltration or hyperviscosity syndrome may also contribute to the clinical manifestation of cryoglobulinemia. Hyperviscosity symptoms occur when monoclonal immunoglobulin is present in large amounts, which is characteristic of type I cryoglobulinemia, and can include mucosal bleeding and neurosensory signs.

Approximately one third of patients with mixed cryoglobulinemia have renal involvement, usually in the form of membranoproliferative glomerulonephritis. Successive relapses can lead to varying degrees of renal fibrosis, although end-stage renal failure is, fortunately, rare. Renal complications also occur in patients with type I cryoglobulinemia and may be related to the underlying hematologic cancer. General symptoms, such as fatigue and intermittent fever, are frequently associated with disease relapses. Other severe symptoms related to digestive, cardiac, pulmonary, or retinal vasculitis may occur, but they are rare.

The classification criteria for mixed cryoglobulinemic vasculitis include the most relevant aspects of the disease.[21] Even though the detection of cryoglobulinemia is a sine qua non condition for classifying vasculitis, these classification criteria might be useful in diagnosing cryoglobulinemia in patients without detected cryoglobulins in the serum.

DIAGNOSIS

The clinical manifestation of cryoglobulinemia syndrome should be confirmed. However, detection of cryoglobulins can be challenging because of the very strict sampling requirements.[22] In a large series, 9% of patients with cryoglobulinemia initially had false negative tests.[23] To prevent ex vivo cryoprecipitation, preanalytic conditions must be rigorous: the blood sample must be kept at 37°C until centrifugation at the laboratory. Cryoprecipitate manifests as a more or less thick border in the tube pellet, with its quantity measured in grams per liter or semiquantitatively (Fig. 2B). Immunophenotyping identifies the constituent immunoglobulin that determines the isotype (type I, II, or III in approximately 10%, 50%, and 40% of cases, respectively),[4,23] which then guides the etiologic assessment. The presence of cryofibrinogen, another cryoprotein, in plasma might be a potential confounder during diagnostic testing for type I cryoglobulinemia.[24] The biologic and pathologic profile of cryoglobulinemia is presented in Table 2.

In the event of strong clinical suspicion of cryoglobulinemia but difficulty in identifying

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society. Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only. No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

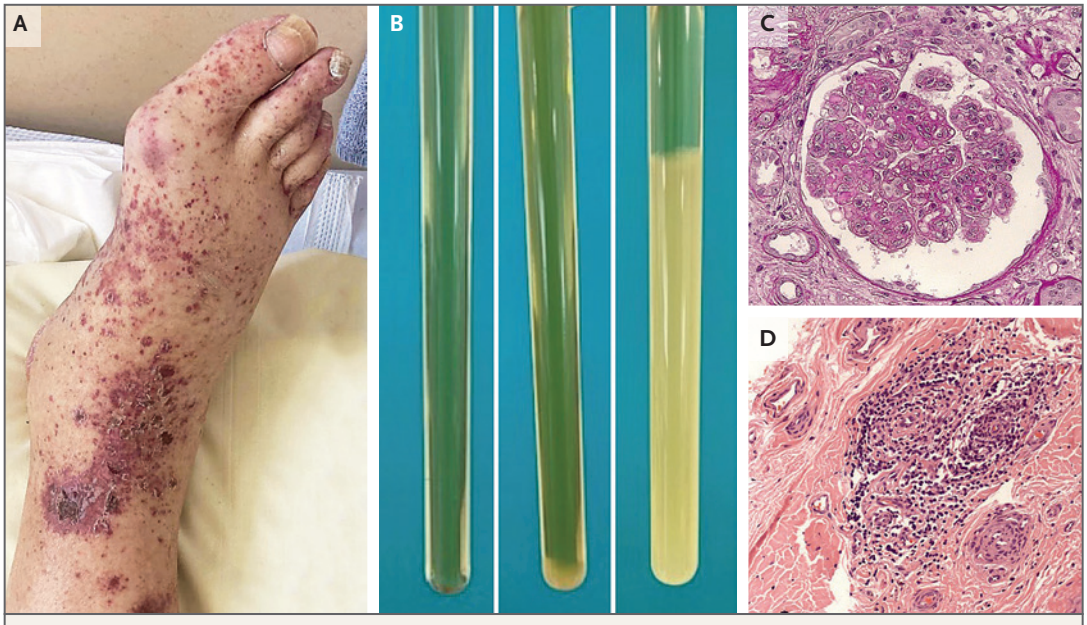


Figure 2. Diagnosis of Cryoglobulinemia.

The diagnosis of cryoglobulinemia is suspected on the basis of characteristic clinical features, such as vascular purpura of the lower limbs (Panel A). The diagnosis is confirmed through the detection of cold-induced precipitates on laboratory testing (Panel B). After centrifugation of blood at 37°C, serum is stored at 4°C for 7 days and then centrifuged at 4°C: tests might be negative (Panel B, left), positive with a low cryocrit (Panel B, middle), or positive with a very high cryocrit (cryoglobulin serum level) (Panel B, right). In cases in which a biologic diagnosis is not possible, targeted tissue biopsies may be helpful. Renal biopsy typically identifies lesions consistent with type I membranoproliferative glomerulonephritis, which is characterized by immunoglobulin deposition (the same isotype as cryoglobulinemia) and complement deposition (C3 and C1q) on immunofluorescence. Endocapillary proliferation, vasculitis, or small intrarenal artery thrombosis is common. Shown is a renal-biopsy specimen with an enlarged glomerulus with glomerular basement membrane duplication, mesangial hypercellularity, endocapillary hypercellularity, and positivity for pseudothrombi on periodic acid–Schiff staining (Panel C). Electron microscopy would show a double-contour pattern of the glomerular basement membrane. A peripheral-nerve biopsy may also be performed and would typically show a perivascular lymphocytic infiltrate adjacent to affected nerves (Panel D).

circulating cryoglobulins, their presence may be suspected on the basis of hypergammaglobulinemia (possibly with a monoclonal spike on serum protein immunofixation), complement consumption (low C4 and CH50 levels), and rheumatoid-factor activity.[25] In addition, the presence of serum cryoglobulins can interfere with automated biologic analyses, resulting in pseudoleukocytosis, pseudomacrocytosis, and even paradoxical hypogammaglobulinemia (due to significant immune-complex precipitation). Additional tests should be conducted on the basis of the underlying diagnostic workup (Table 1). In cases in which a biologic diagnosis remains elusive, a biopsy of targeted tissue (such as skin, kidney, or peripheral nerve) may provide conclusive evidence (Fig. 2C and 2D).[26-29]

NATURAL HISTORY

Much of our understanding of mixed cryoglobulinemia comes from its association with HCV. Among patients with HCV-related mixed cryoglobulinemia, 25 to 30% remain asymptomatic, 40 to 45% have predominantly mild cutaneous manifestations, 20 to 30% present with significant organ damage, 7 to 12% have progression to B-cell cancer, and 2 to 5% will have rapidly progressive and life-threatening vasculitis.[30] In practice, patients with type III cryoglobulinemia usually have milder clinical features than those with type II disease.[31] In a previous study, after the introduction of interferon-based anti-HCV therapies, which led to various sustained virologic responses, the 5-year overall survival among patients with mixed cryoglobulinemia was esti-

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

Table 2. Biologic and Pathologic Profile of Cryoglobulinemias.*

Characteristic	Type I Cryoglobulinemia	Type II Cryoglobulinemia	Type III Cryoglobulinemia
Range of cryoglobulin levels in serum — g/liter	1–30	0.5–2	0.05–0.5
Findings on serum protein electrophoresis	Monoclonal spike	Monoclonal spike and polyclonal elevation of gammaglobulins	Polyclonal elevation of gammaglobulins
Serum protein immunofixation	IgG (most frequent), IgM, IgA (least frequent)	Typically IgM kappa	None
Rheumatoid-factor activity	Very rare	Frequent	Variable
Low C4 level	Very rare	Frequent	Variable
Skin biopsy	Noninflammatory thrombotic lesions, with downstream infarction or hemorrhage	Leukocytoclastic vasculitis, hyaline thrombi	Leukocytoclastic vasculitis, hyaline thrombi
Peripheral-nerve biopsy	Pauci-inflammatory occlusive lesions with neuronal ischemia	Lymphocytic infiltrate around epineurial vessels, with axonal degeneration of affected nerves (vasa vasorum) Necrotizing vasculitis or demyelination might be present	Lymphocytic infiltrate around epineurial vessels, with axonal degeneration of affected nerves (vasa vasorum) Necrotizing vasculitis or demyelination might be present
Kidney biopsy	Thrombotic and hypocellular glomerular lesions Type I membranoproliferative glomerulonephritis may occur	Type I membranoproliferative glomerulonephritis, endocapillary proliferation, deposits of subendothelial or intraluminal immune complexes (or both) Electron microscopy: double-contour pattern of the glomerular basal membrane, microtubular immunoglobulin deposits Mesangial proliferative glomerulopathy, intraglomerular hyaline thrombi, and vasculitis with fibrinoid necrosis might be found	Type I membranoproliferative glomerulonephritis, endocapillary proliferation, deposits of subendothelial or intraluminal immune complexes (or both) Electron microscopy: double-contour pattern of the glomerular basal membrane, microtubular immunoglobulin deposits Mesangial proliferative glomerulopathy, intraglomerular hyaline thrombi, and vasculitis with fibrinoid necrosis might be found
Direct immunofluorescence	Monoclonal immunoglobulin, usually without complement deposition	Deposits of IgM, IgG, or C3 (or a combination of the three)	Deposits of IgM, IgG, or C3 (or a combination of the three)

* The cryocrit (i.e., the percentage of the total serum volume made up of cryoglobulins) is generally very high in type I, moderate in type II, and low in type III. Biopsy samples from patients with mixed cryoglobulinemia usually have lymphocytic infiltrates surrounding small vessels (arterioles, venules, or capillaries), with no infiltration of polymorphonuclear cells or macrophages and no true vascular wall destruction, which distinguishes mixed cryoglobulinemia from other systemic vasculitis conditions. Immunofluorescence may show arterial deposits of IgM, IgG, or C3 (or a combination of the three). Differentiating between type I and mixed cryoglobulinemia, and even differentiating between cryoglobulinemia and other conditions, can be challenging when interpreting renal biopsies because of potential overlap in the immunomorphologic characteristics of these diseases. Electron microscopy can be invaluable in this context. Tissues affected by type I cryoglobulinemia, rather than mixed cryoglobulinemia, have thrombotic vascular obstruction of small vessels with severe downstream ischemic findings and little-to-moderate cellular infiltrate.

mated at 75%. Bacterial infections and end-stage liver disease were the main causes of death, and liver fibrosis and severity of vasculitis (i.e., renal involvement) were the main prognostic factors.[15] The advent of therapy with direct-acting antiviral agents in the past decade, which has resulted in a sustained virologic response in more than 95% of patients and which has an excellent safety profile, has transformed the management and the prognosis of HCV-related mixed cryoglobulinemia. In a large prospective study that included 148 patients with HCV-related cryoglobulinemic vasculitis, only 2.8% died after a median follow-up of 15.3 months.[32] A prospective study involving an Italian cohort examined non–HCV-related cryoglobulinemic vasculitis (including hepatitis B virus [HBV]–related disease, underlying autoimmune diseases, and essential forms) and showed

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society. Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only. No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

that male patients with purpura, patients with type II cryoglobulinemia, and patients with underlying HBV had the worst outcomes.[31] The long-term prognosis of pure noninfectious mixed cryoglobulinemia remains to be determined.

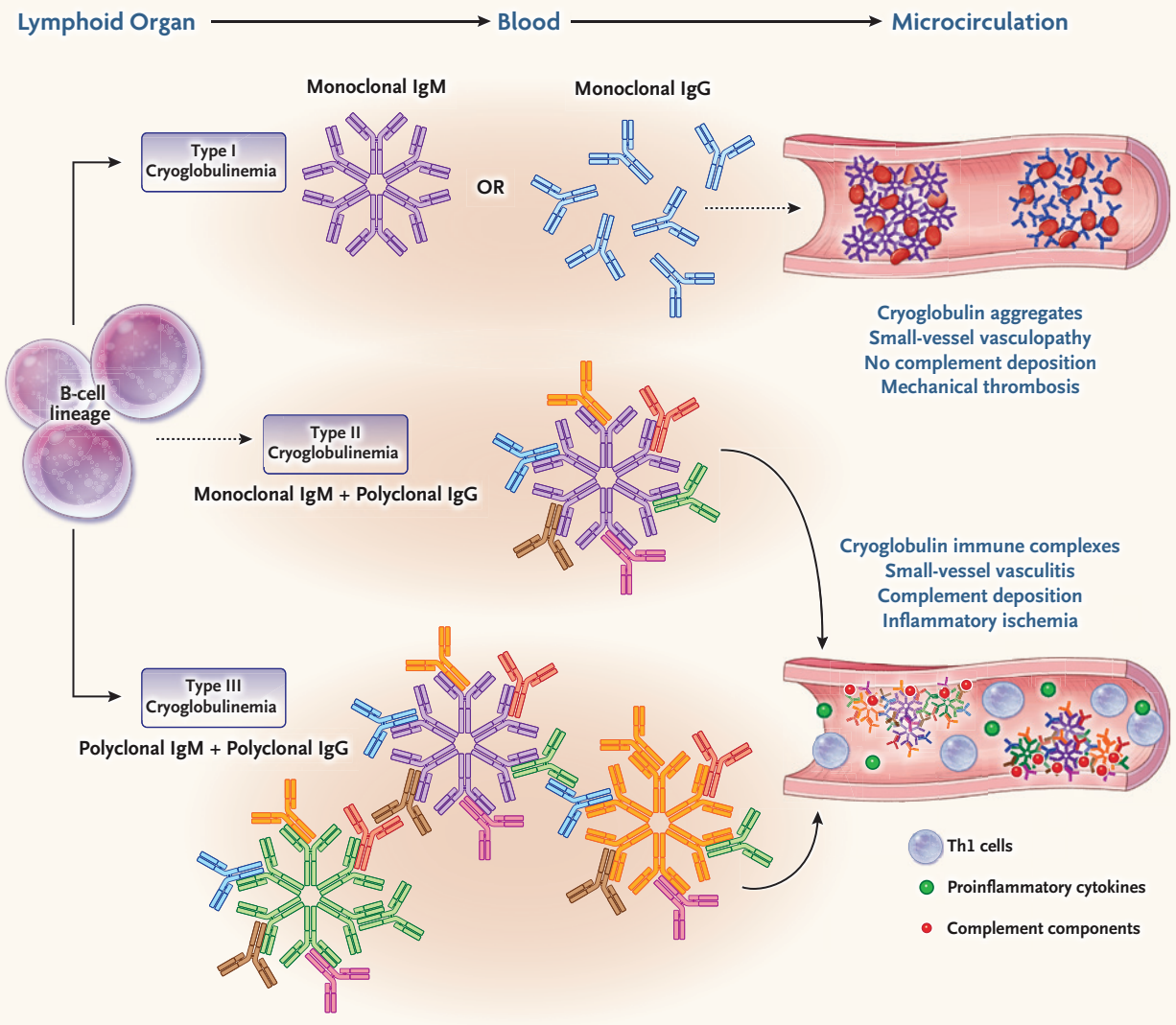


Figure 3. Mechanisms of Cryoglobulinemia.

Cryoglobulinemia has two distinct underlying mechanisms: type I cryoglobulinemia evolves as a vasculopathy affecting small and medium-sized arteries, whereas type II and III mixed cryoglobulinemias manifest as a true autoimmune vasculitis affecting small-to-medium-sized vessels. In both types, lymphocytes from the B-cell lineage (such as memory B cells or plasma cells) produce cryoglobulins in a monoclonal or polyclonal manner. Type I cryoglobulins are monoclonal IgM or IgG produced in large amounts by plasma cells, forming tightly compacted macromolecular nets that physically trap cells within blood vessels, a process known as *rouleaux* formation. Because type I cryoglobulins rarely have rheumatoid-factor activity, complement-mediated inflammatory vasculitis is infrequent. Instead, the primary mechanism in type I cryoglobulinemia involves mechanical vascular obstruction by cold-induced aggregates within the microcirculation, which leads to microthromboses of small vessels. Mixed cryoglobulins consist exclusively of polyclonal immunoglobulins (type III) or a combination of polyclonal immunoglobulins with monoclonal IgM (type II). This IgM typically has rheumatoid-factor activity, which activates complement within immune complexes, ultimately leading to tissue inflammation and injury. Another key mechanism in mixed cryoglobulinemia is the expansion and tissue infiltration of lymphocyte type 1 helper T cells (Th1) and effector T cells, which produce proinflammatory cytokines such as tumor necrosis factor α and interferon-γ, at the expense of regulatory T cells.

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

In a large cohort study involving patients with type I cryoglobulinemia, the estimated survival without clinical relapse, treatment complications, or death was 26% at 5 years.[7] Independent factors associated with poor event-free survival included the IgG isotype and kidney involvement.

PATHOPHYSIOLOGY

CAUSES OF CRYOGLOBULINEMIA: AN EVOLVING CONCEPT

More than 55 years after the initial description by Meltzer et al.,[3] a number of well-deciphered pathophysiological mechanisms now allow us to delineate, within the overarching term "cryoglobulinemia," two distinct diseases: type I cryoglobulinemia, a hematologic process involving thromboses in small and medium-sized arteries; and type II and III mixed cryoglobulinemias, an autoimmune vasculitis. The general mechanisms involved in cryoglobulinemia are outlined in Figure 3.

Throughout the history of research into cryoglobulinemia, the essential forms of mixed cryoglobulinemia have become increasingly rare. The demographic profile of patients is largely dependent on the underlying condition. In addition, direct antiviral therapies against HCV have brought about a considerable shift in the epidemiologic profile of mixed cryoglobulinemia. Although autoimmune diseases (such as Sjögren's syndrome and systemic lupus erythematosus) accounted for approximately 10 to 20% of type II and III cryoglobulinemias in older series, since 2015 they have represented more than 50% of cases, emerging as the leading cause of mixed cryoglobulinemia.[33] With the declining global prevalence of HCV infection, the overall incidence of mixed cryoglobulinemia has decreased, with a younger median age and a higher proportion of female patients being more prevalent.[33]

Type I cryoglobulinemia typically arises from the expansion of a single clone within pathologic blood cells, which can be indolent, smoldering, or malignant. In a more recent cohort of patients with type I cryoglobulinemia, the distribution of underlying hematologic conditions was as follows: monoclonal gammopathies of clinical significance in 31% of patients, Waldenström's disease in 27%, non-Hodgkin's lymphoma in 20%, multiple myeloma in 15%, and chronic lymphocytic leukemia in 7%.[7]

MECHANISMS OF MIXED CRYOGLOBULINEMIC VASCULITIS

As both a hepatotropic and lymphotropic virus, HCV is capable of inducing a range of B-cell lymphoproliferative disorders, from isolated polyclonal hypergammaglobulinemia with detectable cryoglobulins in the serum to cryoglobulinemic vasculitis and, ultimately, B-cell cancer. This spectrum is substantiated by robust data and has been extensively reviewed elsewhere.[34]

Chronic stimulation of the viral antigen plays a central role in HCV-related lymphoproliferation. The binding of the E2 protein of HCV to the CD19–CD21–CD81 membrane complex in B cells reduces the activation and proliferation threshold of HCV and induces biased immunoglobulin somatic hypermutation (in the gene encoding IgH VH1-69), resulting in hypergammaglobulinemia.[35-37] Cryoglobulins are subsequently produced in a polyclonal manner (type III cryoglobulinemia), and if viral stimulation persists, a monoclonal component is added (type II cryoglobulinemia), marking a progression toward autonomous lymphoproliferation. IgM molecules with rheumatoid-factor activity are able to form immune complexes with IgG and complement fractions, leading to tissue inflammation and injury.[38]

B cells undergo clonal expansion, assuming an atypical memory phenotype (CD21–CD27+IgM+ T-bet+). The decreased expression of CD21 (i.e., complement receptor CR2) renders the B cells functionally anergic to generic stimuli and less prone to clearance from circulation.[37,39] However, atypical memory B cells respond to Toll-like receptor 9 stimulation and induce a lymphocyte type 1 helper T-cell (Th1) response, at the expense of regulatory T cells.[40,41] Autoantigen-driven clonal expansion and exhaustion of selected rheumatoid-factor–producing B cells occurs in HCV-related cryoglobulinemia, essential forms of cryoglobulinemia, and cryoglobulinemias resulting from primary Sjögren's syndrome, which suggests common pathogenetic mechanisms throughout mixed cryoglobulinemias.[42] B-lymphocyte stimulator plays a critical role in the survival of B cells, and its overexpression induces the expansion of B-cell clones, possibly contributing to the progression of lymphoproliferation seen in cryoglobulinemia related to both HCV and Sjögren's syndrome.[43-45]

Over time, the initial HCV-driven B-cell proliferation gradually becomes independent of the

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society. Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only. No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

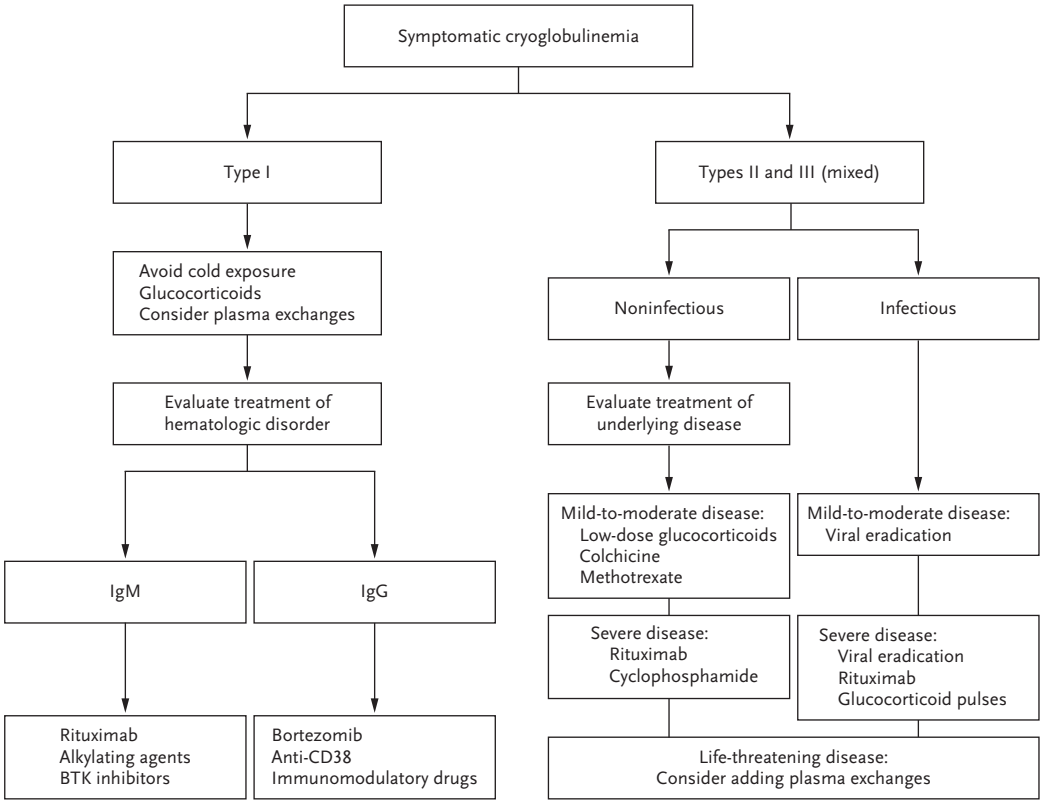


Figure 4. Treatment Algorithm for Cryoglobulinemias.

Patients presenting with symptomatic cryoglobulinemia should be assessed to determine treatment, with particular consideration given to the underlying causes. For every patient with type I cryoglobulinemia, avoiding cold exposure is an essential measure, and compression socks might be enough for patients presenting only with purpura. Glucocorticoids and plasma exchanges are usually considered among the first-line strategies for patients with a pharmacologic indication. Close collaboration with hematologists is imperative, and in the absence of an indication for treating the underlying hemotologic condition, the treatment for type I cryoglobulinemia should be determined on the basis of the disease isotype. Rituximab (a first-generation anti-CD20 agent), alkylating agents, and Bruton's tyrosine kinase (BTK) inhibitors are preferred in the management of IgM-mediated disease, whereas bortezomib (a proteasome inhibitor), anti-CD38 agents (e.g., isatuximab and daratumumab), and immunomodulatory drugs (e.g., lenalidomide and thalidomide) have been used for IgG-mediated disease. For type II and III mixed cryoglobulinemias, therapeutic regimens are chosen on the basis of the severity of vasculitis and involve eradication of the viral trigger when the cause is infectious. In hepatitis C virus–related mixed cryoglobulinemia, direct antiviral agents are usually sufficient to achieve a sustained virologic response and vasculitis remission in mild-to-moderate disease. Short-term, low-dose glucocortoid treatment (i.e., ≤0.5 mg per kilogram of body weight per day) might eventually be prescribed concomitantly, although most patients recover with a glucocorticoid-free regimen. For severe forms of disease, direct-acting antiviral agents should be combined with rituximab (375 mg per square meter of body-surface area on days 1, 8, 15, and 22). In life-threatening circumstances (e.g., glomerulonephritis with impaired renal function, motor deficit, multiple mononeuropathy, extensive skin necrosis, or digestive, cardiac, or pulmonary involvement), direct-acting antiviral agents should be combined with high-dose glucocorticoids (i.e., 0.5–1.0 g of methylprednisolone for 3 consecutive days, followed by tapering doses of oral glucocorticoids) and plasma exchanges. In rare forms complicated by a hematologic cancer, combinations of rituximab, fludarabine, and cyclophosphamide have been used successfully in expert centers. For patients who do not have a response to direct-acting antiviral agents or who have a contraindication to them, rituximab can be used alone for induction, followed by a 500-mg infusion every 6 to 9 months as maintenance treatment. Noninfectious mixed cryoglobulinemia can be managed further with the use of the arsenal of treatments available for the underlying cause (e.g., colchicine or methotrexate).

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

virus. Antigen-insensitive lymphoproliferation may lead to overt lymphoma transformation, thus completing the continuum. Cryoglobulinemic vasculitis is by far the most significant risk factor for B-cell cancer in patients with chronic HCV infection, conferring a risk that is 35 times as high in these patients as in the general population.[46] The most frequent HCV-related hematologic cancers in this context are marginal-zone lymphoma, lymphoplasmacytic lymphoma, and high-grade diffuse large B-cell lymphoma.[34]

MECHANISMS OF TYPE I CRYOGLOBULINEMIA

Type I cryoglobulinemic vasculopathy has more similarities to plasma-cell–associated disorders than to autoimmune diseases. When not associated with an overt lymphoproliferative disorder, type I cryoglobulinemia is considered a monoclonal gammopathy of clinical significance.[47] Monoclonal IgG cryoglobulins have finely structured morphologic features and have been shown to form highly structured macromolecular nets capable of physically entrapping cells, a process known as *rouleaux* formation within blood vessels.[48]

In general, B-cell clones have a genetic and molecular signature similar to that of type I cryoglobulinemia, but monoclonal gammopathies and overt hematologic cancers form two ends of a continuum in terms of the number of somatic mutations.[49,50] Because type I cryoglobulins have rheumatoid-factor activity only in rare cases,[23] complement-mediated inflammatory vasculitis typically occurs less often with type I cryoglobulinemia than with mixed cryoglobulinemia. A mouse model of cryoglobulinemic renal disease highlighted the major role of immune-complex deposition in glomerular capillaries rather than complement-dependent and Fc receptor–dependent nephrotoxic mechanisms.[51] Above all, mechanical obstruction of microcirculation caused by microthromboses is the primary mechanism underlying type I cryoglobulinemia.[48]

THERAPEUTIC APPROACHES

In the past two decades, therapeutic strategies for cryoglobulinemia have become increasingly targeted because of advancements in our understanding of the underlying mechanisms of the disease. Treatment of mixed cryoglobulinemia often involves direct-acting antiviral agents against HCV, with or without rituximab (an anti-CD20 monoclonal antibody), and much less frequently involves the use of glucocorticoids. For type I cryoglobulinemia, treatment regimens may include glucocorticoids, plasma exchange, and immunosuppressants. Plasmapheresis is a therapeutic option in the context of IgM disease because 80% of IgM antibodies remain in the circulation. The treatment approach for cryoglobulinemias is shown in Figure 4.

TYPE II AND III MIXED CRYOGLOBULINEMIAS

For cases associated with HCV, eradication of the virus is imperative. Although B-cell lymphoproliferation is predominantly driven by chronic viral stimuli, a sustained virologic response should suffice to abolish it. Direct-acting antiviral agents alone have been shown to reduce the activity of cryoglobulinemic vasculitis[52-54] and to restore the balance between increased atypical memory B cells and pathogenic T lymphocytes and decreased regulatory T cells.[55] The persistent presence of cryoglobulins in serum may serve as a surrogate marker of indolent lymphoproliferation.[34] A very large international cohort study showed that up to 12.6% of patients have vasculitis relapse in the long term despite an adequate sustained virologic response after therapy with a direct-acting antiviral agent,[56] similar to the percentages observed in a multicenter Italian study.[57] In addition to persistent cryoglobulins, combined monoclonal B-cell lymphocytosis, t(14;18) translocation, and abnormal free light-chain kappa-to-lambda ratios have recently been suggested as predictors of clinical response after a sustained virologic response.[58] Antigen-insensitive lymphoproliferation and uncontrolled B-cell proliferation may progress to overt lymphoma that warrants specific clone-targeted treatment.[34]

Overall, therapeutic regimens are determined by the severity of the vasculitis[32,52,59-62] and include eradication of viral triggers in cases with infectious causes. In patients with mild-to-moderate disease (e.g., arthralgias, myalgias, nonnecrotic purpura, or purely sensory neuropathy), direct-acting antiviral therapy alone is usually sufficient to achieve a sustained virologic response and remission of vasculitis. In patients

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society. Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only. No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

with severe forms (e.g., glomerulonephritis with impaired renal function, motor deficits, multiple mononeuropathy, extensive skin necrosis, or digestive, cardiac, or pulmonary involvement), direct-acting antiviral therapy should be combined with rituximab (375 mg per square meter of body-surface area on days 1, 8, 15, and 22 of treatment). For life-threatening manifestations, plasma exchanges and glucocorticoid pulses can be added.[62] In patients who have not had a response to direct-acting antiviral agents or who have contraindications to these agents, rituximab can be used alone as induction treatment, followed by a 500-mg infusion every 6 to 9 months as maintenance treatment.

Several clinical trials and observational studies showed good efficacy and a favorable safety profile of rituximab in patients with severe mixed cryoglobulinemia.[19,63-68] However, physicians should be aware of a particular adverse event that seems to be very specific to cryoglobulinemia: the occurrence of a drug-induced vasculitis flare, similar to serum sickness, between days 2 and 9 after rituximab infusion, with associated mortality exceeding 50%.[69] Among the risk factors for this severe complication are increased cryoglobulin levels, increased disease severity, and two 1000-mg doses of rituximab administered 2 weeks apart. For this reason, we recommend four weekly infusions of 375 mg as the induction regimen, along with standard premedication with methylprednisolone, paracetamol, and chlorphenamine before each infusion.[69] If clinical relapse occurs after discontinuation of rituximab, retreatment with rituximab alone seems to be an effective and safe strategy in the long term.[70]

Low-dose interleukin-2 is emerging as a potential treatment in distinct scenarios involving autoimmune disease and could serve as an alternative for refractory cases. It was investigated in a phase 1–2a, prospective, open-label study in which it was shown to have a favorable safety profile and led to clinical improvement in 8 of 10 patients, in addition to restoration of the balance between regulatory and effector T cells.[71]

For the rare cases of cryoglobulinemic vasculitis due to HBV infection or human immunodeficiency virus infection, targeting the chronic viral stimulation should also be a primary objective. Although the general therapeutic approach used in cases of HBV replication is similar to that used for HCV infection, rituximab should be used with caution in cases of HBV replication.[72,73] For noninfectious mixed cryoglobulinemia, treatment is tailored to the pathophysiological cause of the underlying disease, which is generally an autoimmune disease with excessive B-cell activity (e.g., systemic lupus erythematosus or Sjögren's syndrome).[74] Glucocorticoids, with or without methotrexate, are generally sufficient for cutaneous–articular forms, whereas colchicine may be prescribed for isolated cutaneous vasculitis. In severe cases of mixed cryoglobulinemia, rituximab is recommended, particularly in the presence of renal or neurologic involvement or for refractory cutaneous–articular forms. Cyclophosphamide is used in refractory cases, and plasma exchanges can be used in cases involving severe or life-threatening manifestations.

TYPE I CRYOGLOBULINEMIA

In patients with an indication for systemic treatment (usually because of skin ulceration), targeting the plasma cell clone or lymphoplasmacytic cell clone is crucial, because either will eventually lead to other severe hematologic manifestations. Given the difficulty in conducting prospective clinical trials in this very rare context, little progress has been made in recent years. The prognosis of type I cryoglobulinemia has not improved over time, with 5-year overall survival ranging from 77 to 83% in studies involving patients recruited between 1983 and 2018.[7-9,11] Treating the underlying monoclonal disorder in patients with type I cryoglobulinemia results in the clearance of serum cryoglobulins in only half of patients, despite a decrease in symptoms.[8]

Currently, no standard of care or international guidelines have been established for the treatment of type I cryoglobulinemia, and recommendations are derived primarily from expert opinion.[75] The therapeutic options available are quite diverse because current strategies are mainly based on specific drugs that address the underlying hematologic condition.[7-11] In cases in which a clear hematologic indication is lacking, the choice of treatment for type I cryoglobulinemia is typically guided by the monoclonal immunoglobulin isotype. For the IgM isotype, rituximab, sometimes combined with bendamustine or cyclophosphamide, may be used. For the IgG or IgA isotype, treatment combinations may include

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

bortezomib, anti-CD38 agents (e.g., daratumumab or isatuximab), or other drugs that target plasma cells. Plasma exchanges are commonly used.[47] Glucocorticoids and immunosuppressants may also be added in varying degrees as part of the treatment regimen. However, some patients presenting only with purpura might be treated with nonpharmacologic measures, such as compression socks. Avoiding cold exposure is important for every patient presenting with type I cryoglobulinemia.

FUTURE PERSPECTIVES

Although treatment with rituximab leads to a satisfactory clinical response in patients with mixed cryoglobulinemia, it may not effectively restore defective early B-cell tolerance checkpoints.[76] Elevated serum concentrations of B-lymphocyte stimulator in patients with mixed cryoglobulinemia are associated with increased B-cell proliferation, serum cryoglobulin levels, and vasculitis activity and therefore may contribute to relapse.[77,78] In light of retrospective observations in patients with mixed cryoglobulinemia who had disease that was refractory to rituximab alone,[79] ongoing clinical trials involving patients with noninfectious mixed cryoglobulinemia are evaluating the sequential therapeutic combination of rituximab and belimumab (an anti–B-lymphocyte stimulator monoclonal antibody; ClinicalTrials.gov number, NCT04629144), as well as investigating the efficacy and safety of the next-generation anti-CD20 agent obinutuzumab. In patients with type I cryoglobulinemia, the use of alkylating agents and bortezomib is associated with several side effects.[11] Despite their importance in the underlying pathophysiologic mechanisms of the disease, autoreactive plasma cells have not been systematically targeted in type I cryoglobulinemia. Targeting CD38 has been successful in treating refractory plasma-cell disorders such as multiple myeloma or AL amyloidosis.[80,81] Inhibition of CD38 in patients with type I cryoglobulinemia is promising, and an ongoing phase 2, open-label, pilot study assessing the efficacy and the safety of isatuximab (an anti-CD38 monoclonal antibody; ClinicalTrials.gov number, NCT05114109) may provide further insights into the treatment of these patients. Finally, other promising methods — for example, therapy involving chimeric antigen receptor technology or bispecific antibodies — are expanding rapidly in the management of immune-mediated and hematologic conditions and could represent new options in the treatment of cryoglobulinemia.

CONCLUSION

More than a half century after their initial description, mixed and type I cryoglobulinemias should be regarded as two distinct entities, each characterized by unique underlying mechanisms, therapeutic approaches, and prognoses. Pathophysiological and therapeutic advances in HCV-related cryoglobulinemia over the past three decades have been considerable and have led to substantial improvements in patient care. The increased emphasis on underlying autoimmune diseases has underscored the existing knowledge gap in this domain. The range of underlying hematologic conditions in patients with type I cryoglobulinemia highlights the challenges inherent in studying this condition. Future efforts should concentrate on translational research and multicenter, randomized trials focusing on noninfectious mixed cryoglobulinemia and type I cryoglobulinemia, with the ultimate goal of improving prognosis.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Isabelle Brocheriou, Pascale Ghillani-Dalbin, and Thierry Maisonobe, for providing images related to the main manifestations of cryoglobulin, and the following collaborators, who have been involved in many of our scientific works about cryoglobulinemia: Chloe Comarmond, Anne Claire Desbois, Damien Sene, Zoubida Tazi Mezalec, and Benjamin Terrier.

REFERENCES

1. Wintrobe MM, Buell MV. Hyperproteinemia associated with multiple myeloma: with report of a case in which an extraordinary hyperproteinemia was associated with thrombosis of the retinal veins and symptoms suggesting Raynaud's disease. Bull Hopkins Hosp 1933; 52:156-64 (https://babel.hathitrust.org/cgi/pt?id=uc1.b4330364&seq=594).

2. Lerner AB, Watson CJ. Studies of cryoglobulins; unusual purpura associated with the presence of a high concentration of cryoglobulin (cold precipitable serum globulin). Am J Med Sci 1947;214: 410-5.

3. Meltzer M, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia — a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. Am J Med 1966;40:837-56.

4. 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.

5. Trejo O, Ramos-Casals M, García-Carrasco M, et al. Cryoglobulinemia: study of etiol0ogic factors and clinical

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society. Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only. No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

and immunologic features in 443 patients from a single center. Medicine (Baltimore) 2001;80:252-62.
6. Cacoub P, Saadoun D. Extrahepatic manifestations of chronic HCV infection. N Engl J Med 2021;384:1038-52.
7. Ghembaza A, Boleto G, Bommelaer M, et al. Prognosis and long-term outcomes in type I cryoglobulinemia: a multicenter study of 168 patients. Am J Hematol 2023;98:1080-6.
8. Sidana S, Rajkumar SV, Dispenzieri A, et al. Clinical presentation and outcomes of patients with type 1 monoclonal cryoglobulinemia. Am J Hematol 2017;92:668-73.
9. Néel A, Perrin F, Decaux O, et al. Long-term outcome of monoclonal (type 1) cryoglobulinemia. Am J Hematol 2014;89:156-61.
10. Zhang L-L, Cao X-X, Shen K-N, et al. Clinical characteristics and treatment outcome of type I cryoglobulinemia in Chinese patients: a single-center study of 45 patients. Ann Hematol 2020;99:1735-40.
11. Harel S, Mohr M, Jahn I, et al. Clinicobiological characteristics and treatment of type I monoclonal cryoglobulinaemia: a study of 64 cases. Br J Haematol 2015;168:671-8.
12. Terrier B, Karras A, Kahn J-E, et al. The spectrum of type I cryoglobulinemia vasculitis: new insights based on 64 cases. Medicine (Baltimore) 2013;92:61-8.
13. Ferri C, Sebastiani M, Giuggioli D, et al. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. Semin Arthritis Rheum 2004;33:355-74.
14. Terrier B, Carrat F, Krastinova E, et al. Prognostic factors of survival in patients with non-infectious mixed cryoglobulinaemia vasculitis: data from 242 cases included in the CryoVas survey. Ann Rheum Dis 2013;72:374-80.
15. Terrier B, Semoun O, Saadoun D, Sène D, Resche-Rigon M, Cacoub P. Prognostic factors in patients with hepatitis C virus infection and systemic vasculitis. Arthritis Rheum 2011;63:1748-57.
16. Retamozo S, Díaz-Lagares C, Bosch X, et al. Life-threatening cryoglobulinemic patients with hepatitis C: clinical description and outcome of 279 patients. Medicine (Baltimore) 2013;92:273-84.
17. Monti G, Galli M, Invernizzi F, et al. Cryoglobulinaemias: a multi-centre study of the early clinical and laboratory manifestations of primary and secondary disease. GISC. Italian Group for the Study of Cryoglobulinaemias. QJM 1995;88:115-26.
18. Cohen SJ, Pittelkow MR, Su WP. Cutaneous manifestations of cryoglobulinemia: clinical and histopathologic study of seventy-two patients. J Am Acad Dermatol 1991;25:21-7.
19. Roccatello D, Fornasieri A, Giachino O, et al. Multicenter study on hepatitis C virus-related cryoglobulinemic glomerulonephritis. Am J Kidney Dis 2007;49:69-82.
20. 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 cryoglobulinemia. Arthritis Rheum 2004;50:2305-8.
21. De Vita S, Soldano F, Isola M, et al. Preliminary classification criteria for the cryoglobulinaemic vasculitis. Ann Rheum Dis 2011;70:1183-90.
22. Musset L, Diemert MC, Taibi F, et al. Characterization of cryoglobulins by immunoblotting. Clin Chem 1992;38:798-802.
23. Kolopp-Sarda MN, Nombel A, Miossec P. Cryoglobulins today: detection and immunologic characteristics of 1,675 positive samples from 13,439 patients obtained over six years. Arthritis Rheumatol 2019;71:1904-12.
24. Saadoun D, Elalamy I, Ghillani-Dalbin P, Sene D, Delluc A, Cacoub P. Cryofibrinogenemia: new insights into clinical and pathogenic features. Am J Med 2009;122:1128-35.
25. Stoyanov A, Toong C, Kong Y, Chen R, Urriola N. Serum protein electrophoresis and rheumatoid factor analysis is an effective screening strategy for cryoglobulinaemia. Pathology 2023;55:391-6.
26. Gorevic PD, Kassab HJ, Levo Y, et al. Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients. Am J Med 1980;69:287-308.
27. Leung N, Bridoux F, Batuman V, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. Nat Rev Nephrol 2019;15:45-59.
28. Bonetti B, Invernizzi F, Rizzuto N, et al. T-cell-mediated epineurial vasculitis and humoral-mediated microangiopathy in cryoglobulinemic neuropathy. J Neuroimmunol 1997;73:145-54.
29. Ojemakinde K, Turbat-Herrera EA, Zeng X, Gu X, Herrera GA. The many faces of cryoglobulinemic nephropathy: a clinico-pathologic study of 47 cases with emphasis on the value of electron microscopy. Ultrastruct Pathol 2014;38:367-76.
30. Dammacco F, Sansonno D. Therapy for hepatitis C virus-related cryoglobulinemic vasculitis. N Engl J Med 2013;369:1035-45.
31. Galli M, Oreni L, Saccardo F, et al. HCV-unrelated cryoglobulinaemic vasculitis: the results of a prospective observational study by the Italian Group for the Study of Cryoglobulinaemias (GISC). Clin Exp Rheumatol 2017;35:Suppl 103:67-76.
32. Cacoub P, Si Ahmed SN, Ferfar Y, et al. Long-term efficacy of interferon-free antiviral treatment regimens in patients with hepatitis C virus-associated cryoglobulinemia vasculitis. Clin Gastroenterol Hepatol 2019;17:518-26.
33. Boleto G, Ghillani-Dalbin P, Musset L, et al. Cryoglobulinemia after the era of chronic hepatitis C infection. Semin Arthritis Rheum 2020;50:695-700.
34. Cacoub P, Comarmond C, Vieira M, Régnier P, Saadoun D. HCV-related lymphoproliferative disorders in the direct-acting antiviral era: from mixed cryoglobulinaemia to B-cell lymphoma. J Hepatol 2022;76:174-85.
35. 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 U S A 2005;102:18544-9.
36. Machida K, Cheng K-TH, Pavio N, Sung VM-H, Lai MMC. Hepatitis C virus E2-CD81 interaction induces hypermutation of the immunoglobulin gene in B cells. J Virol 2005;79:8079-89.
37. Charles ED, Green RM, Marukian S, et al. Clonal expansion of immunoglobulin M+CD27+ B cells in HCV-associated mixed cryoglobulinemia. Blood 2008;111:1344-56.
38. 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.
39. Isnardi I, Ng Y-S, Menard L, et al. Complement receptor 2/CD21- human naive B cells contain mostly autoreactive unresponsive clones. Blood 2010;115:5026-36.
40. Comarmond C, Lorin V, Marques C, et al. TLR9 signalling in HCV-associated atypical memory B cells triggers Th1 and rheumatoid factor autoantibody responses. J Hepatol 2019;71:908-19.
41. Boyer O, Saadoun D, Abriol J, et al. CD4+CD25+ regulatory T-cell deficiency in patients with hepatitis C-mixed cryoglobulinemia vasculitis. Blood 2004;103:3428-30.
42. Del Padre M, Minafò YA, Marrapodi R, et al. Rheumatoid factor-producing CD21low anergic clonal B-cells in essential mixed cryoglobulinaemia: a model for autoantigen-driven pathogenesis of infectious and non-infectious cryoglobulinaemias. Clin Exp Rheumatol 2020;38:Suppl 124:139-47.
43. Fabris M, Quartuccio L, Sacco S, et al. B-Lymphocyte stimulator (BLyS) up-regulation in mixed cryoglobulinaemia syndrome and hepatitis-C virus infection. Rheumatology (Oxford) 2007;46:37-43.
44. Quartuccio L, Salvin S, Fabris M, et al. BLyS upregulation in Sjogren's syndrome associated with lymphoproliferative disorders, higher ESSDAI score and B-cell clonal expansion in the salivary glands. Rheumatology (Oxford) 2013;52:276-81.
45. Glauzy S, Boccitto M, Bannock JM, et al. Accumulation of antigen-driven lymphoproliferations in complement receptor 2/CD21$^{-/low}$ B cells from patients with

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.

Sjögren's syndrome. Arthritis Rheumatol 2018;70:298-307.
46. 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;165:101-5.
47. Khwaja J, D'Sa S, Minnema MC, Kersten MJ, Wechalekar A, Vos JM. IgM monoclonal gammopathies of clinical significance: diagnosis and management. Haematologica 2022;107:2037-50.
48. Podell DN, Packman CH, Maniloff J, Abraham GN. Characterization of monoclonal IgG cryoglobulins: fine-structural and morphological analysis. Blood 1987; 69:677-81.
49. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. N Engl J Med 2012; 367:826-33.
50. Varettoni M, Zibellini S, Defrancesco I, et al. Pattern of somatic mutations in patients with Waldenström macroglobulinemia or IgM monoclonal gammopathy of undetermined significance. Haematologica 2017;102:2077-85.
51. Strait RT, Posgai MT, Mahler A, et al. IgG1 protects against renal disease in a mouse model of cryoglobulinaemia. Nature 2015;517:501-4.
52. Gragnani L, Visentini M, Fognani E, et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. Hepatology 2016;64:1473-82.
53. Bonacci M, Lens S, Londoño M-C, et al. Virologic, clinical, and immune response outcomes of patients with hepatitis C virus-associated cryoglobulinemia treated with direct-acting antivirals. Clin Gastroenterol Hepatol 2017;15(4):575-583.e1.
54. Saadoun D, Pol S, Ferfar Y, et al. Efficacy and safety of sofosbuvir plus daclatasvir for treatment of HCV-associated cryoglobulinemia vasculitis. Gastroenterology 2017;153(1):49-52.e5.
55. Comarmond C, Garrido M, Pol S, et al. Direct-acting antiviral therapy restores immune tolerance to patients with hepatitis C virus-induced cryoglobulinemia vasculitis. Gastroenterology 2017;152(8): 2052-2062.e2.
56. Fayed A, Hegazy MT, Biard L, et al. Relapse of hepatitis C virus cryoglobulinemic vasculitis after sustained viral response after interferon-free direct-acting antivirals. Am J Gastroenterol 2022;117: 627-36.
57. Kondili LA, Monti M, Quaranta MG, et al. A prospective study of direct-acting antiviral effectiveness and relapse risk in HCV cryoglobulinemic vasculitis by the Italian PITER cohort. Hepatology 2022; 76:220-32.
58. Gragnani L, Lorini S, Marri S, et al. Hematological and genetic markers in the rational approach to patients with HCV sustained virological response with or without persisting cryoglobulinemic vasculitis. Hepatology 2021;74:1164-73.
59. Sise ME, Bloom AK, Wisocky J, et al. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. Hepatology 2016;63:408-17.
60. Emery JS, Kuczynski M, La D, et al. Efficacy and safety of direct acting antivirals for the treatment of mixed cryoglobulinemia. Am J Gastroenterol 2017; 112:1298-308.
61. Bonacci M, Lens S, Mariño Z, et al. Long-term outcomes of patients with HCV-associated cryoglobulinemic vasculitis after virologic cure. Gastroenterology 2018;155(2):311-315.e6.
62. Ramos-Casals M, Zignego AL, Ferri C, et al. Evidence-based recommendations on the management of extrahepatic manifestations of chronic hepatitis C virus infection. J Hepatol 2017;66:1282-99.
63. De Vita S, Quartuccio L, Isola M, et al. A randomized controlled trial of rituximab for the treatment of severe cryoglobulinemic vasculitis. Arthritis Rheum 2012;64:843-53.
64. Sneller MC, Hu Z, Langford CA. A randomized controlled trial of rituximab following failure of antiviral therapy for hepatitis C virus-associated cryoglobulinemic vasculitis. Arthritis Rheum 2012;64: 835-42.
65. Terrier B, Saadoun D, Sène D, et al. Efficacy and tolerability of rituximab with or without PEGylated interferon alfa-2b plus ribavirin in severe hepatitis C virus-related vasculitis: a long-term followup study of thirty-two patients. Arthritis Rheum 2009;60:2531-40.
66. Dammacco F, Tucci FA, Lauletta G, et al. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. Blood 2010;116: 343-53.
67. 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;45:842-6.
68. Roccatello D, Sciascia S, Baldovino S, et al. Improved (4 plus 2) rituximab protocol for severe cases of mixed cryoglobulinemia: a 6-year observational study. Am J Nephrol 2016;43:251-60.
69. Desbois AC, Biard L, Sène D, et al. Rituximab-associated vasculitis flare: incidence, predictors, and outcome. J Rheumatol 2020;47:896-902.
70. Quartuccio L, Zuliani F, Corazza L, et al. Retreatment regimen of rituximab monotherapy given at the relapse of severe HCV-related cryoglobulinemic vasculitis: long-term follow up data of a randomized controlled multicentre study. J Autoimmun 2015;63:88-93.
71. Saadoun D, Rosenzwajg M, Joly F, et al. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. N Engl J Med 2011;365:2067-77.
72. Mazzaro C, Dal Maso L, Gragnani L, et al. Hepatitis B virus-related cryoglobulinemic vasculitis: review of the literature and long-term follow-up analysis of 18 patients treated with nucleos(t)ide analogues from the Italian Study Group of Cryoglobulinemia (GISC). Viruses 2021; 13:1032.
73. Droz N, Gilardin L, Cacoub P, et al. Kinetic profiles and management of hepatitis B virus reactivation in patients with immune-mediated inflammatory diseases. Arthritis Care Res (Hoboken) 2013;65: 1504-14.
74. Terrier B, Krastinova E, Marie I, et al. Management of noninfectious mixed cryoglobulinemia vasculitis: data from 242 cases included in the CryoVas survey. Blood 2012;119:5996-6004.
75. Muchtar E, Magen H, Gertz MA. How I treat cryoglobulinemia. Blood 2017;129: 289-98.
76. Chamberlain N, Massad C, Oe T, Cantaert T, Herold KC, Meffre E. Rituximab does not reset defective early B cell tolerance checkpoints. J Clin Invest 2016;126: 282-7.
77. Landau DA, Rosenzwajg M, Saadoun D, Klatzmann D, Cacoub P. The B lymphocyte stimulator receptor-ligand system in hepatitis C virus-induced B cell clonal disorders. Ann Rheum Dis 2009; 68:337-44.
78. Sène D, Limal N, Ghillani-Dalbin P, Saadoun D, Piette J-C, Cacoub P. Hepatitis C virus-associated B-cell proliferation — the role of serum B lymphocyte stimulator (BLyS/BAFF). Rheumatology (Oxford) 2007; 46:65-9.
79. Pouchelon C, Visentini M, Emmi G, et al. Management of nonviral mixed cryoglobulinemia vasculitis refractory to rituximab: data from a European collaborative study and review of the literature. Autoimmun Rev 2022;21:103034.
80. Kaufman GP, Schrier SL, Lafayette RA, Arai S, Witteles RM, Liedtke M. Daratumumab yields rapid and deep hematologic responses in patients with heavily pretreated AL amyloidosis. Blood 2017; 130:900-2.
81. Attal M, Richardson PG, Rajkumar SV, et al. Isatuximab plus pomalidomide and low-dose dexamethasone versus pomalidomide and low-dose dexamethasone in patients with relapsed and refractory multiple myeloma (ICARIA-MM): a randomised, multicentre, open-label, phase 3 study. Lancet 2019;394:2096-107.
Copyright © 2024 Massachusetts Medical Society.

The New England Journal of Medicine is produced by NEJM Group, a division of the Massachusetts Medical Society.
Downloaded from nejm.org at Chinese Academy of Medical Sciences (CAMS) on October 22, 2024. For personal use only.
No other uses without permission. Copyright © 2024 Massachusetts Medical Society. All rights reserved.