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New insights in cryoglobulinemic vasculitis

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ABSTRACT

Cryoglobulins are antibodies that precipitate at low temperatures and dissolve after rewarming. Cryoglobulinemia refers to the presence of circulating cryoglobulins and generally leads to a systemic inflammatory syndrome characterized by fatigue, arthralgia, purpura, ulcers, neuropathy and/or glomerulonephritis. The disease mainly involves small to medium-sized blood vessels and causes vasculitis due to cryoglobulin-containing immune complexes. Cryoglobulinemia is classified into three types (I, II and III) on the basis of immunoglobulin composition. Predisposing conditions include lymphoproliferative, autoimmune diseases and hepatitis C virus infection. The diagnosis of cryoglobulinemic syndrome is predominantly based on the presence of clinical features and laboratorial demonstration of serum cryoglobulins. The treatment strategy depends on the cause of cryoglobulinemia. For patients with chronic HCV infection, antiviral therapy is indicated. Immunosuppressive or immunomodulatory therapy, including steroids, plasmapheresis and cytotoxic agents, is reserved for organ-threatening manifestations. In this review, we discuss the main clinical presentations, diagnostic approach and treatment options.

1. Introduction

Cryoglobulins (CGs) are antibodies that precipitate in vitro at temperatures less than 37 °C and dissolve after rewarming. This definition discriminates CG from cryoproteins and cold agglutinins. Two types of cryoprecipitates are recognized: CG is present when proteins precipitate from serum and plasma, and cryofibrinogen when plasma, but not serum, forms a cryoprecipitate. CGs are either immunoglobulins (Igs) or a mixture of Igs and complement components [1].

Cryoglobulinemia refers to the presence of CG in serum, however many patients with cryoglobulinemia remain asymptomatic. When they present with symptoms, it is define as cryoglobulinemic syndrome or cryoglobulinemic vasculitis (CV) [1].

The pathological nature of CG was first suggested in 1933 when Wintrobe and Buell described the phenomenon of cryoprecipitation in a patient with signs and symptoms of hyperviscosity associated with multiple myeloma [2]. In 1947, Lerner introduced the term “cryoglobulins” which applied to precipitation of serum globulins [3]. Meltzer and colleagues reported in 1966 a typical clinical triad of purpura, arthralgia and weakness that may be associated with organic dysfunction and elevation of rheumatoid factor, defined as cryoglobulinemic disease [4].

1.1. Classification

Brouet's classification is the most widely used for recognizing three cryoglobulinemic subgroups based on Ig composition (Fig. 1). One of the advantages of this immunochemical classification is that it provides guidance for etiological diagnosis, clinical manifestations and treatment options. Cyoglobulins can be subdivided into three subgroups: type I contains an isolated monoclonal Ig; type II comprises IgG and an IgM rheumatoid factor (RF) of monoclonal origin, and type III comprises IgG and a polyclonal IgM RF [5].

Type I cryoglobulinemia is composed of a single type of monoclonal Ig, usually IgM or IgG. Between 10 and 15% of all cryoglobulinemia are type I. It is commonly found in patients with lymphoproliferative disorders (Waldenström's macroglobulinemia, multiple myeloma, non-Hodgkin's lymphoma, leukaemia chronic lymphocytic), even in its initial stages [Monoclonal gammopathy of undetermined significance (MGUS)]. Most cases of cryoglobulinemia type I behave symptomatically and symptoms are related to hyperviscosity, such as isolated Raynaud's phenomenon, acrocyanosis or gangrene [6–10].

Mixed cryoglobulinemia (MC), comprising both type II and type III cryoglobulinemia, is due to immunocomplexes composed of polyclonal IgG with monoclonal IgM (type II), or polyclonal IgM (type III) [11].

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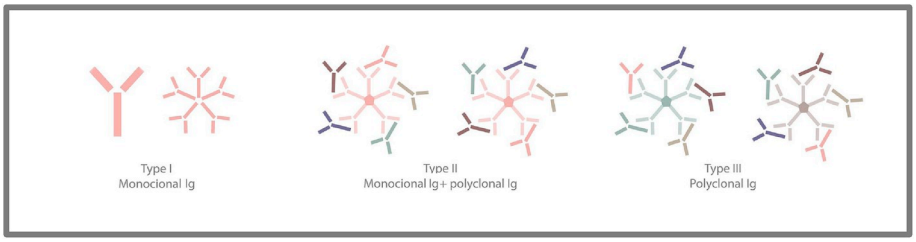

Fig. 1. Classification of cryoglobulinemia.

The IgM component has RF activity and binds to the Fc portion of IgG. Of all cryoglobulinemias, 50–60% are from type II and 30–40% are type III. MC is associated with infections [especially Hepatitic C Virus (HCV)], autoimmune diseases [mainly Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE)] or neoplasms [12]. MC presents with the typical triad of purpura, arthralgias and myalgias, and may also have important visceral involvement. Complement levels are usually low, in particular C4 and C1q fractions, while C3 is usually normal. In general this classification system is useful, although the use of more sensitive methods of antibody detection (immunoblot, polyacrylamide gel electrophoresis) has allowed the identification of a new MC serological type, type II-III or biclonal, which is defined by the simultaneous presence of oligoclonal (ie, monoclonal and polyclonal) IgM and polyclonal IgG. This oligoclonal MC seems to be an intermediate stage in the evolution of MC type III towards MC type II, as with MGUS and multiple myeloma [13,14]. Nearly 10% of cases of MC are regarded as idiopathic or essential, a percentage that rises to 25% in HCV-negative patients.

1.2. Epidemiology

The prevalence of CV varies widely, being more frequent in southern Europe. This geographic distribution seems related to the endemic presence of HCV infection. On the whole, this disease is considered to be relatively rare, but its prevalence may be underestimated due to clinical polymorphisms and diagnostic methods. The prevalence of clinically significant cryoglobulinemia has been estimated at approximately 1 in 100,000, appears more commonly in patients aged 45–65 years, with a maximum incidence in women (sex ratio women to men, 2–3 to 1) [8]. Different prevalences of serum CG, ranging from 19% to 50%, have been reported in HCV-infected patients. The proportion of patients with a type I cryoglobulin varies substantially among case series, but accounts for approximately 5–25% of cases [15–19].

1.3. Etiopathogenesis

Although the etiology of cryoglobulinemia is not completely understood, three mechanisms are considered to be major pathogenic factors:

1. Chronic immune stimulation and/or lymphoproliferation, causing the production of high concentration of Igs that form CGs;
2. Increased immune complex formation;
3. Insufficient clearance of CGs or their immune complexes [5,20].

Type I CG is characterized by the presence of monoclonal CGs, whose production is due to the underlying lymphoproliferative disease. Exposure to cold provokes precipitation that leads to inflammatory vasculitis and vessel obstruction. On the other hand, in MC chronic inflammatory states (induced by viral infections or autoimmune diseases) lead to hyperactivation/hyperproliferation of B-cells, which in turn induce the production of CGs [21,22].

Ischemic lesions may be related to vascular obstruction by CG precipitates, notably in type I CG. MC causes true immune complex-mediated vasculitis. Why symptomatic vasculitis does not occur consistently is unclear. Recent studies have indicated that the development of lesions is dependent on the physicochemical properties of Igs, such as stereotactic properties and heavy-chain glycosylation. Depending on this, Igs vary in their propensity to form immune complexes, precipitate and induce an inflammatory response (via recruitment of complement and of macrophage Fc receptor) [1,23].

In chronic hepatitis C, HCV envelope glycoproteins E1 and E2 help the virus entering into the hepatocytes and lymphocytes, via CD81 cell receptor [1,24]. Chronic HCV infection induces persistent stimulation of intrahepatic and circulating B cells [24]. The expanded B-cell population is characterized by a distinctive repertoire, with predominance of certain clones, notably VH1-69, which produce an Ig with rheumatoid factor activity, thereby leading to the formation of a cryoglobulin [25,26]. Chronic antigen stimulation results in the gradual emergence of B-cell clones that produce polyclonal IgMs (type III cryoglobulin) initially, then oligoclonal IgMs (type II/III cryoglobulin), and finally monoclonal IgM (type II cryoglobulinemia) [1,27]. In patients with symptomatic cryoglobulinemia, the risk of developing lymphoma may be increased 35-fold compared to the general population [28].

Pathogenesis may also depend on a poorly understood interaction between a polygenic host predisposition and environmental triggers. In a multicentric Genome Wide Association study, Zignego et al. have detected an association between two single-nucleotide polymorphisms (SNPs) on chromosome 6 and HCV induced MC: SNPs in intron of NOTCH4 gene and SNPs located between HLADRB1 and HLADQA1 segments of major histocompatibility complex [29].

1.4. Clinical features

Most people with CG remain asymptomatic; the percentage of patients who develop symptoms varies between 2 and 50% [30]. When symptoms appear there is a correlation with the type of CG set by Brouet. In Type I CGs stand out the symptoms of hematological disease and secondary to hyperviscosity and/or thrombosis. Thus, most frequent manifestations are the Raynaud's phenomenon, ischemic ulcers and distal gangrene, cold-induced urticaria, livedo reticularis, purpura, retinal hemorrhages, visual disturbances, headache and encephalopathy due to microcirculation involvement of the central nervous system, among others. Nonspecific symptoms such as arthralgia, fatigue, and myalgia, as well as cutaneous vasculitis and neuropathy, occur more frequently in MC. The triad of purpura, weakness and arthralgia, also known as Meltzer triad, is characteristic but only occurs in a minority of patients [21].

Recurrent palpable purpura is the most common and frequent manifestation (approximately 90% of patients). It typically consists in recurrent outbreaks lasting three to ten days of purpuric non-prurutic lesions palpable usually located in lower limbs and possibly extending to the abdomen. The characteristic histology is that of leukocytoclastic vasculitis. Ulcers may also appear accompanying the purpura, mainly located in the malleolus, which can be overinfected and even provoke osteomyelitis and sepsis [21,31]. Cold-induced symptoms such as Raynaud's phenomenon occur in 25% of patients overall, with higher

rates in patients who have type I cryoglobulinemia. Cold urticaria is a chronic systemic non-pruritic urticarial rash with plaques that remain unchanged for more than 24 h [1]. Porphyria cutanea tarda and lichen planus may occur in HCV infection. Raynaud's phenomenon, digital gangrene, livedo reticularis and acrocyanosis are more common in type I CG [31].

Joint manifestations (50–75%) often consist of non-migratory pain that predominantly involves the hands and knees in a bilateral and symmetric pattern; elbows and ankles are less often affected [1]. True arthritis is less common and does not cause joint destruction. If symmetric and erosive polyarthritis appears, a syndrome of overlap between CG and rheumatoid arthritis (RA). Spinal involvement is even rarer [32,33].

Neuropathy affects a variable percentage of MC cases (17–60%), in contrast to type I, but only 20% of patients with MC present clinically relevant peripheral neuropathy. It manifests with paresthesias or burning pain in legs and muscle weakness, which might worsen at night. Electromyographically, sensitive polyneuropathy can evolve to sensory-motor. Multiplex mononeuritis is also common, but cranial nerve involvement rarely occurs. In histological analysis, in addition to axonal degeneration, vascular alterations of chronic vasculopathy, ischemic occlusion or frank vasculitis can be seen. Involvement of central nervous system is rare and usually manifests as encephalopathic syndromes or ischemic stroke. Cases of transverse myelitis and medullary ischemia have been described [34,35].

Renal involvement occurs in 30–60% of CG patients (especially those with MC type II) [36]. It usually appears during or shortly after an outbreak of cutaneous vasculitis, and manifests with varying degrees of microhematuria, proteinuria, hypertension and/or renal failure [37]. Nephritic syndrome can be seen in 20% of cases, and occasionally nephrotic syndrome or isolated acute renal failure acute. Renal involvement causes significant morbidity and mortality. Membrano-proliferative glomerulonephritis is the most common histological pattern, with endocapillary proliferation with massive infiltration of monocytes/macrophages, amorphous subendothelial deposits and monocytes engulfing and degrading the deposits. Indirect immunofluorescence demonstrates immunoglobulins and C3 deposits. Electron microscopy shows characteristic deposits with in the macrophages. In hepatitis-C-related nephropathy, HCV core protein and immunoglobulin are homogeneously distributed along the capillary walls of the glomeruli and are a component of the immune complexes. Small vessel vasculitis has been reported in up to one third of cases. In CG type II (rarely in type I) pseudothrombus can be observed in capillaries [38–40].

Pulmonary involvement occurs in less than 5% of patients and appears to be more common in MC. Patients present with mild-to-moderate dyspnea and dry cough. Rarely, acute alveolar hemorrhage, organizing pneumonia, pulmonary vasculitis and pleural effusions can be seen [1,41].

Other involvements by clinically significant vasculitis are rare in CV, with variable reported frequencies. Gastrointestinal disease can appear as hepatomegaly, alterations in liver function tests and recurrent abdominal pain due to mesenteric vasculitis [40]. Cardiac involvement is rare, but associated with increased mortality [1]. Ocular and oral dryness and/or bilateral parotid swelling can be present up to 30% and few met the criteria for SS [42].

1.5. Causes

Persistent stimulation of the immune system can be induced by hematological/lymphoproliferative disorders, chronic infections (HCV or other infections) or autoimmune diseases (SLE or SS).

Monoclonal cryoglobulinemia (type I) is predominantly associated with monoclonal gammopathies (Waldenström's macroglobulinemia, multiple myeloma or MGUS) or chronic lymphocytic leukaemia. MC (II and III) happens mainly in B-cell lymphomas [43]. CGs have also been described in patients with solid cancers [44].

After the discovery of HCV in 1989 it has been found that almost all MC cases, previously classified as essential, were related to chronic HCV infection. The prevalence of HCV infection in patients with MC ranges from 30% to 100% according to case series [45]. Between 12 and 56% of infected patients have CGs, although only 5–15% develop CV [41]. The reason is not clear why HCV induces MC in some patients, but not in others. HCV is predominantly associated with type II cryoglobulinemia.

Hepatitis B virus (HBV) is reported to be associated with MC [30]. In 1999, Dimitrakopoulos and colleges showed a high prevalence of CGs in a cohort of Human Immunodeficiency Virus (HIV)-1–infected patients representing all stages of HIV disease and all categories of HIV transmission. Active HIV replication results in persistent B-cell stimulation and hyperproduction of polyclonal Igs and polyclonal RFs, inducing the formation of type III CGs. The transformation from polyclonal to oligoclonal and, finally, to monoclonal RFs (type II mixed cryoglobulinemia) may be induced by HIV-1 as a direct or indirect consequence of the infection of peripheral blood mononuclear cells [46]. In patients infected with HIV cryoglobulinemia ranges from 7% to 17%, but rises to 35%–64% in those coinfected with HCV. Antiretroviral therapy lowers the frequency of cryoglobulinemia in HIV infection [42]. Case reports have associated CV with a wide range of other infectious agents.

The most frequently associated autoimmune disease is SS. This group of patients has a higher incidence of extraglandular manifestations, greater risk of B-cell lymphoma and death [30,47,48]. In 1986, Tzioufas and colleges shown that patients with SS had mixed monoclonal IgM Igs, whereas those observed in SLE and RA patients were mixed polyclonal. The presence of CGs in the sera of SS patients correlated with extraglandular disease, antibodies to Ro (SS-A), IgM RF and lower serum C4 levels [49]. The prevalence of cryoglobulinemia is five times higher in patients with both SS and HCV infection compared to those with HCV infection alone [50]. CGs are detected in patients with SLE and RA, but values are generally lower compared to those patients with SS, and clinical manifestations of CV are much less common [21].

Nearly 10% of cases of MC are regarded as idiopathic or essential [30], a percentage that rises to 25% in HCV-negative patients [44]. The possibility of occult HCV infection and monoclonal gammopathies should be investigated in patients with cryoglobulinemia of unknown cause.

1.6. Diagnosis

Diagnosis of CV is based on a combination of clinical, laboratory and histopathological data. For most patients, cryoglobulinemic disease is diagnosed by the presence of typical organ involvement (mainly skin, kidney or peripheral nerve) and circulating CGs. There are no standardized neither validated diagnostic nor classification criteria. The 2012 International Chapel Hill Consensus Conference on the Nomenclature of Vasculitides defined CV as a category of small vessel vasculitis, where immune deposits of CGs predominantly affect capillaries, venules and/or arterioles.

The Italian Group for the Study of Cryoglobulinemia (GISC) proposed a classification system, not a diagnostic one, in 2011, that was validated in 2014 for research and epidemiological purposes (Table 1). It includes three domains with sensitivity of 88.5% and specificity of 95.4% [51,52].

The diagnosis of cryoglobulinemia requires demonstration of CGs in serum. Appropriate sample collection and handling is essential. Blood should be collected in prewarmed syringes and tubes, transported, clotted and centrifuged at 37–40 °C, ensuring that temperature never falls below 37 °C. The serum should then be stored at 4 °C for up to 7 days. Precipitation of type I CGs usually occurs within hours. By contrast, MC, particularly type III, can take several days to precipitate (Table 2).

It is important to note that some healthy individuals have low

Table 1
Criteria fulfillment: at least two of the three items in a patient with cryoglobulinemia (detected at least two times during an interval of at least 12 weeks). Data from De Vita et al. Ann Rheum Dis. 2011.

CLASSIFICATION CRITERIA FOR CRYOGLOBULINEMIC VASCULITIS
ITEM 1: Subjective Symptoms
Positive answer to at least two of the following questions:
Do you remember one or more episodes of small red spots on your skin, particularly involving the lower limbs?
Have you ever had red spots on your lower extremities which leave a brownish color after their disappearance?
Has a doctor ever told you that you have viral hepatitis?
ITEM 2: Objective symptoms (present or past)
Presence of at least three of the following:
Constitutional symptoms (fatigue, fever, fibromyalgia)
Articular involvement (arthralgias, arthritis)
Vascular involvement (púrpura, skin ulcers, necrotising vasculitis, hyperviscosity syndrome, Raynaud' phenomenon)
Neurologic involvement (peripheral neuropathy, cranial nerve involvement, CNS involvement)
ITEM 3: Laboratory abnormalities
Presence at the time of the diagnosis of at least two of the following:
Reduced serum C4
Positive serum rheumatoid factor
Positive serum M component

Table 2
Best practice for cryoglobulin evaluation.

Essential rules for detection of cryoglobulins
•10–20 mL of blood are drawn into syringes and/or collection tubes that have been prewarmed to 37 °C without anticoagulants
•Sample should be delivered to the laboratory = / > 37 °C
•Allowed to clot at 37 °C – minimum 1 h
•Serum should be separated by centrifugation at 37 °C
•Aliquots of separated serum should be refrigerated (4 °C) to allow the precipitation of cryoglobulin for at least three (preferably seven) days. Inspect for a precipitate everyday
•If precipitate is present, re-solubilized at 37 °C
•Three washes with saline at 4 °C, centrifugation at 4 °C and re-solubilization at 37 °C for qualitative/quantitative analysis
•The simplest estimate of cryoglobulin concentration is the cryocrit, measured in a calibrated sedimentation tube
•Should analyze the proteins to classify the type of cryoglobulin
•Must be prepared to repeat the sample on multiple occasions to ensure that false-negative results do not occur when clinical suspicion remains high

concentrations of CGs and mixed polyclonal CGs often occur transiently during infection. On the other hand, a negative test for CGs does not exclude cryoglobulinemia, due to false-negative results caused by improper sample collection or inconsistent laboratory techniques. Moreover, CG concentrations can fluctuate (depending on their precipitation in target vessels/organs) and levels should be serially evaluated when there is a high degree of suspicion of CV. Immunofixation is the most accurate and easiest technique to determine CG isotype (IgG, IgA or IgM, kappa or lambda), monoclonality or polyclonality (Fig. 2) [1,49].

CG detection can be technically difficult due to the considerable thermal instability of CGs, which precipitate if the temperature of the blood sample falls below 37° before processing at the laboratory. Besides that, high concentrations of CGs may block analytical instruments that run at room temperature. CGs, which precipitate during serum centrifugation or storage, may escape detection. Electrophoresis of such samples may appear normal, because the monoclonal protein has been lost prior to electrophoresis. The presence of CG can cause falsely elevated cell counts in automated cell counters. Complement may be activated ex vivo and appear falsely low, hence complement measurements should be carried out on serum kept at 37 °C [1,40].

RF and complement (C3, C4, CH50) are part of the diagnostic workup. RF is usually present, often at high levels, in patients with type II MC. These serological abnormalities can lead to a misdiagnosis of rheumatoid vasculitis. Diminished complement levels may expose an ongoing consumption by CG-containing immune complexes. Type I CG typically produces few serological complement abnormalities; instead MC causes reduced serum levels of CH50, C1q, C2 and C4, particularly in patients with type II and III CG associated with collagen vascular disease. C3 levels are generally unaffected or slightly diminished [1,51]. Autoantibodies that should be included as part of the initial evaluation are: antinuclear antibodies (ANA), *anti*-dsDNA, *anti*-Sm, *anti*-Ro/SSa, anti-La/SSb, *anti*-RNP and antineutrophilic cytoplasmatic antibodies (ANCA) [40].

Serological studies, particularly for viral hepatitis, are always indicated during laboratory evaluation of a patient with MC. Hepatitis C has been reported in 60–90% of patients with mixed CG. Serological studies for other agents (HBV, HIV and Epstein-Barr Virus) have been inconsistently associated, but are worth considering during workup.

Acute phase reactants such as the erythrocyte sedimentation rate and C-reactive protein are usually elevated; mild to moderate hypergammaglobulinemia (IgM, IgA, and/or IgG) also typifies MC, with particularly elevated levels being more suggestive of type I cryoglobulinemia [22].

Histological evidence of vasculitis remains the gold standard for diagnosing CV. Biopsy is usually taken from affected organs, including skin, nerve and renal biopsies. Pathological features of CV differ from other types of vasculitis by: size of the involved vessels (small size vessels, i.e. arteries, capillaries, and venules), type of inflammatory infiltrate (lymphomonocytic rather than polymorphonuclear) and site of inflammation (mainly perivascular) [29,40]. Skin biopsy usually reveals leukocytoclastic vasculitis, which constitutes the hallmark histopathological feature, with an important finding that distinguishes it from other causes of leukocytoclastic vasculitis: infiltrating cells are T and mononuclear cells not neutrophils. Neural biopsies show endoneurial vasculitis with vessel wall destruction, patchy focal demyelination and axonal degeneration. Renal biopsies are usually consistent

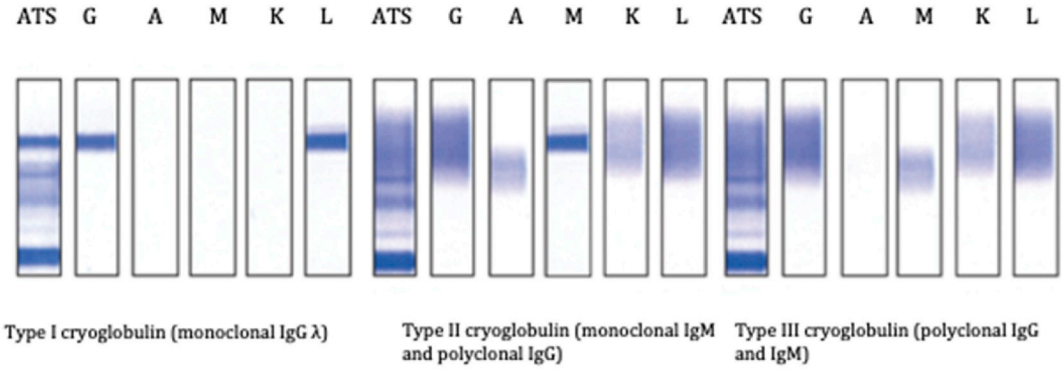

Fig. 2. Immunofixation of the cryoglubulin subtypes.

with membranoproliferative glomerulonephritis with subendothelial Igs and complement deposition. Electron microscopy reveals glomerular dense deposits with tubular, annular or fibrillar structures [29,34,40].

1.7. Treatment

The treatment of CV still presents a great challenge, given its complex etiopathogenesis, symptomatic diversity and occasionally life-threatening presentations. The therapeutic management of this syndrome must be individualized according to associated disease and severity. The natural history of CV is not predictable and depends on concomitant diseases and complications as well as response to treatment.

1.7.1. Type I cryoglobulinemia

Treatment is reserved for symptomatic disease and is directed against the underlying disorder [53]. In patients with myeloma, treatment options follow general recommendations, and include drugs such as corticosteroids, bortezomib, thalidomide, lenalidomide or alkylating agents. Autologous stem cell transplant may be an option. In patients with Waldenström's macroglobulinemia -associated cryoglobulinemia, the use of bortezomib as primary therapy is recommended. Ibrutinib is emerging as an option for WM, but there is no data available for WM-associated cryoglobulinemia [1,53]. IgG MGUS is treated with myeloma drugs that target plasma cells, as it results from plasma cell proliferation; instead rituximab is generally preferred in IgM MGUS, which derives from lymphoplasmacytic proliferation. Plasma exchange therapy is reserved for severe renal involvement, extensive leg necrosis or to prevent IgM flare in patients treated with rituximab (who have IgM above 4 g/dL). Exposure to low temperatures induces CG formation and should be prevented. Foot and leg care is important to prevent wound complications [1,53–55].

1.8. Noninfectious mixed cryoglobulinemia

Given the rarity of non-HCV MC, it is challenging to provide evidence-based treatment recommendations [52]. In patients with overt hematologic malignancy, treat malignancy and modify treatment according to disease extent and organ dysfunction. Patients with severe or life-threatening manifestations need urgent intervention to suppress immune complex formation. This is accomplished with immunosuppressive therapy (IS), which is used in other systemic vasculitides (high-dose corticosteroids, cyclophosphamide, rituximab and/or plasmapheresis). IS aims to halt end-organ damage, and once such goal has been achieved, the dose should be tapered to the lowest possible and discontinued, if possible [53,56,57]. Successful use of mycophenolate mofetil was shown in a patient with severe CV related to systemic sclerosis and secondary SS, refractory to previous treatment with glucocorticoids and cyclophosphamide. Inhibitors of tumor necrosis factor alpha are not recommended for the treatment of CV [58]. Rituximab, as a B-cell depletion agent, constitutes a major tool in the current paradigm of immunosuppressive approach. The largest dataset on the use of rituximab in noninfectious MC comes from a Cryovac multicenter survey that included 242 patients. The use of rituximab in combination with corticosteroids achieved the greatest benefit in terms of clinical, renal and immunologic responses (ie, > 50% decrease in baseline CG levels and/or a > 50% increase in serum C4). This combination was more efficacious than corticosteroids alone or corticosteroids in combination with an alkylating agent [56,57]. In life or organ-threatening events, cyclophosphamide can be used in conjunction with pulse corticosteroids (intravenous methylprednisolone 500–1000 mg for three days followed by prednisone taper). A response to plasma exchange in MC is seen in 70%–80% of patients, and plasma exchange is a rational therapeutic option when severe disease manifestations (membranoproliferative glomerulonephritis, leg ulcers) are present or in the case of life-threatening events such as pulmonary hemorrhage or intestinal vasculitis. The exchange solution should be warmed to body temperature to avoid CG precipitation [53].

Controversial reports exist regarding the effect of the infusion of gammaglobulins in patients with type II cryoglobulinemia. Potential causes of poor outcome include changes in cryoprecipitability or increases in serum viscosity [59].

1.9. HCV-related CV

The therapeutic principles of infection associated MC follow those used in noninfectious MC. Because HCV-CV activity usually correlates with viremia, treatment should be focused on targeting this causal agent and in severe cases are usually offered IS followed by antiviral therapy. The choice of antiviral treatment should be made according to existing guidelines, because the presence of cryoglobulinemia does not determine the choice of antiviral therapy [53,60].

Direct-acting antiviral (DAA) agents are more effective than pegylated interferon-ribavirin combination, are given orally and for a shorter duration with a better safety profile. Their development has radically transformed the management of HCV-related CV. The highest clinical response rate was demonstrated in a prospective study by Saadoun and colleges, in which all patients (n = 41) achieved sustained virologic response and a complete (90%) or partial (10%) clinical response after 12 or 24 weeks of sofosbuvir and daclatasvir [61]. Although clinical improvement can occur even without achieving HCV RNA clearance, patients who achieved sustained virologic response were more likely to have improvement in their disease manifestations. Virologic relapses after an initial response were usually accompanied by relapsing vasculitis [53].

IS is indispensable in patients with severe vasculitis (severe kidney dysfunction, skin necrosis, involvement of the gastrointestinal tract or central nervous system). Classically, glucocorticoids, cyclophosphamide or azathioprine have been used by analogy to other systemic vasculitides, but there are no clinical trials that demonstrate their effectiveness in MC. In very severe cases (pulmonary hemorrhage or progressive renal failure) or if there is hyperviscosity syndrome, plasmapheresis is usually performed [60]. Rituximab has demonstrated greater efficacy than conventional IS or placebo. In earlier studies, adding rituximab to pegylated interferon and ribavirin shortened time to clinical remission and increased renal response and CG clearance rates [62]. In recent studies, a small proportion of patients with MC received rituximab in conjunction with DAA treatment. These patients had progressive forms of MC, and no significant differences were found in virologic responses to DAA treatment alone compared to patients who received additional rituximab. Rituximab is currently considered the best biological agent option for patients with MC and its use should consider both benefits and risks. It is still debatable whether it should be administered concomitantly with DAAs or sequentially [60]. Another potentially useful agent is belimumab, a monoclonal antibody against the B-lymphocyte stimulator (BLyS). It was shown that different types of B-cell activating factor (BAFF) might contribute to production of CG in chronic HCV infection [58].

Studies on therapeutic approaches in non-HCV infectious cryoglobulinemia are limited, given the rarity of this disease. Anti-infective therapies (antiviral or antibacterial) are more likely to achieve a sustained response, even in the absence of IS. The use of IS alone resulted in a poor response to therapy. In refractory cases, use of targeted anti-infective agents in combination with IS may overcome refractoriness. Rituximab (as well as other forms of IS) should be given only to patients who are receiving concomitant *anti*-HBV and/or *anti*-HIV therapies [53].

1.10. Prognosis

CV remains a challenging disease to manage due to severe organ-

specific involvements and occasionally life-threatening presentations. The most common causes of death in CV are infection, HCV-related end-stage liver disease, cardiovascular disease and more rarely lymphoma or neoplasms. A recent study on noninfectious mixed CV found poorer outcomes in patients with pulmonary involvement, gastrointestinal involvement, creatinine clearance below 60 mL/min and age older than 65 years. Survival rates after 1, 2, 5, and 10 years were 91%, 89%, 79%, and 65%, respectively [1,63,64].

The natural history of CV is not predictable and strongly depends on concomitant diseases and complications as well as response to treatment. Death usually occurs after a prolonged course of vasculitis, often lasting years. Morbidity due to CV may also be important. Careful monitoring of life-threatening complications (mainly nephropathy, widespread vasculitis and B cell lymphoma or other malignancies) should be carried out in all patients with CV [1,40,62,63].

2. Conclusion

A new era in the epidemiology and treatment of HCV-related CV has begun with broader treatment coverage of HCV using very effective and safe DAA. In the last decade, vaccination against HBV infection was also associated with a significant decrease in the incidence of polyarteritis nodosa. We can expect that a wider treatment of HCV infection may lead to a similar reduction in the prevalence of HCV-associated CV, at least in countries that can afford the high cost of antiviral therapy [65].

Nevertheless many aspects of the disease remain unsolved. Elucidation of the mechanisms and predisposing factors involved in the development of cryoglobulinemia remains a research question. Identification of a trigger can revolutionize treatment, it has occurred 20 years ago with HCV. While some studies support the use of rituximab for non-HCV-related CV, the optimal treatment regimen for this type of vasculitis remains unclear. Further studies are needed to identify rescue treatments for rituximab-refractory and/or intolerant patients. Some emerging alternative approaches include abatacept (a soluble fusion protein that consists of an extracellular domain of human cytotoxic T lymphocyte-associated antigen 4 linked to the modified Fc portion of human IgG1) or tocilizumab (a humanized monoclonal antibody against IL-6 receptor). Clarification of such aspects can lead to improvement in the prognosis of this heterogeneous and challenging disease.

Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.102313.

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