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CRYOGLOBULINEMIC VASCULITIS: FROM AETIOLOGY TO TREATMENT

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ABSTRACT – Cryoglobulinemic vasculitis refers to a vasculitic syndrome affecting predominantly small-sized vessels that develops because of cryoglobulin presence in the serum. Cryoglobulinemic vasculitis is considered a rare disorder which, however, could be underestimated given the clinical polymorphism of the syndrome and the difficulty in achieving a diagnosis. In addition, the detection of cryoglobulins is an extremely complex process that depends on the expertise of the laboratory technician and on the possible presence of interfering components. A breakthrough in its etiological definition was the development of serological and biomolecular tests that allowed anti-HCV antibodies to be detected in serum and so to identify that the 86% of patients with mixed cryoglobulinemia was positive for circulating HCV-RNA. The diagnosis is supported by clinical and laboratory finding, according to the current classification criteria. The management of the disease depends on the severity of the systemic involvement, but in all HCV positive cases the eradication with new direct antiviral agents is mandatory. The aim of this review is to collect the principal knowledge about this particular disease, and to introduce the new further knowledge about classification, epidemiology and therapy strategies.

KEYWORDS: Cryoglobulinemia, Vasculitis, Autoimmune diseases, Connective tissue diseases, Hepatitis.

INTRODUCTION

Cryoglobulinemia refers to the presence in the serum of proteins that reversibly precipitate in vitro at temperatures below 37°C and that redissolve again at body temperature. Cryoglobulinaemic vasculitis, on the other hand, refers to a vasculitic syndrome affecting predominantly small-sized vessels that develops because of cryoglobulin presence in the serum[1].

Wintrobe and Buell first described cryoglobulinemia in 1933 in a patient with multiple myeloma and more than thirty years later its mixed nature was identified[2], as it was characterised by cryoglobulins consisting of different molecular fractions[3]. The breakthrough came in 1989, with the discovery of the hepatitis C virus (HCV) when this chronic infection was correlated with mixed cryoglobulinemia cases, demonstrating that more than 80% of patients had HCV-RNA detected by PCR[4-6]. The later discovery of direct-acting antiviral agents (DAAs) reconfirmed the role of HCV in the pathogenesis of the disease and recorded an important relative increase in the number of clinical, virological, and immunological responses in patients with mixed cryoglobulinemia[7].

The aim of this review is to collect the principal knowledge about this particular disease and to introduce the new further knowledge about classification, epidemiology and therapy strategies.

CLASSIFICATION

Cryoglobulinemias are classically classified using a system designed by Brouet, depending on their composition. Cryoglobulinemia type 1 is characterised by the presence of only one immunoglobulin subclass, usually IgM or IgG and more rarely IgA; mixed cryoglobulinemia type 2 is characterised by the presence of a monoclonal IgM component (mainly IgM κ) with rheumatoid factor activity forming complexes with polyclonal IgG; mixed cryoglobulinemia type 3 is characterised by the presence of circulating immune complexes formed by both polyclonal IgG and IgM[8].

A probable intermediate form known as mixed cryoglobulinemia type 2-3 has also been described, and it is characterised by the presence of oligoclonal IgM or a mixture of monoclonal and polyclonal IgM in addition to polyclonal IgG; the latter could identify an intermediate developmental stage between mixed cryoglobulinemia type 2 and type 3[9].

Cases of particular mixed cryoglobulinemias have been described in literature, requiring a re-evaluation of this classification[10-12].

EPIDEMIOLOGY

Cryoglobulinaemic vasculitis is considered a rare disorder, with a prevalence of approximately 1:100000 population, which, however, could be underestimated given the clinical polymorphism of the syndrome and the difficulty in achieving a diagnosis[9].

A study conducted in 1993 on the population of Campogalliano – in the province of Modena (3800 inhabitants) – identified the presence of cryoglobulinaemic vasculitis in 1 subject per 2000 inhabitants, demonstrating that the prevalence changes depending on whether the disease is being sought or simply found (unpublished data). The case series is fragmented since patients are referred to different specialists depending on the primarily affected organ[13-15].

Mixed cryoglobulinemia without any type of vasculitic manifestation has been found in a significant proportion of patients with chronic infection or inflammation: 15 to 20% of patients with HIV infection, 15 to 25% of patients with connective tissue disease, 25% of patients with HCV infection and in more than 64% of patients with both HIV and HCV co-infection[16-20]. In this regard, it is more prevalent in southern Europe compared to northern Europe and North America, but the incidence is expected to increase in underdeveloped countries where HIV and HCV are more common than in the general population[21].

Following an analysis on a cohort of 250 patients with mixed cryoglobulinemia from the Rheumatology Department of the Policlinico of Modena, the mean age (±DS) at onset is 54±13 years, the mean duration (±DS) of the disease is 12±10 years and the female-to-male ratio is 3:1[22].

AETIOLOGY

For many years, mixed cryoglobulinemia was considered an accessory syndrome to chronic hepatitis, so much so that there were suggestions about the involvement of hepatotropic viruses in its pathogenesis. However, hepatitis B virus (HBV) was shown to be the causative factor in less than 5% of individuals, so the remaining cases were defined as “essential”[23-26].

A breakthrough in its etiological definition was the development of serological and biomolecular tests that allowed anti-HCV[4] antibodies to be detected in serum. This made it possible to realise both that the etiological agent of non-A-non-B hepatitis was the hepatitis C virus (HCV)[6] and that mixed cryoglobulinemia could be correlated with chronic HCV infection: 86% of patients with mixed cryoglobulinemia had HCV-RNA detected by PCR[5]. In contrast, a patient with chronic HCV infection develops mixed cryoglobulinemia in 25% of cases, which only manifests as cryoglobulinaemic vasculitis in 10-15% of cases; the reason for this remains unclear but may reflect the role played by genetic and/or environmental factors[1].

Considering patients with non-HCV-related mixed cryoglobulinemia, we can divide them into two major categories according to the presumed aetiology: patients with other types of infections and patients with other pathological conditions without demonstrable infections.

In the first group of patients, many infections have been reported, although rare, and include hepatitis B virus (HBV), hepatitis A virus (HAV), Leishmania donovani, Brucella melitensis, Cytomegalovirus (CMV), parvovirus B19, Epstein-Barr virus (EBV), human immunodeficiency virus (HIV), Mycobacterium leprae, Ascaris lumbricoides, Candida albicans as well as pyogenic infections such as streptococcal endocarditis[27,28]. According to a French study, it appears that patients with non-HCV-related mixed cryoglobulinemia are younger and more frequently male (M:F 3:1)[28].

The second group of patients can be further divided into two categories: patients with connective tissue diseases, including Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis; and patients with non-Hodgkin's lymphoma or, more rarely, with tumours of other histotype. Finally, for those patients in whom the aetiology is persistently undefined, the diagnosis that is still made today is of ''essential'' mixed cryoglobulinemia[1,29].

CLINICAL DIAGNOSIS AND CLASSIFICATION CRITERIA

An Italian group studying cryoglobulinemia (GISC) proposed the first classification criteria for mixed cryoglobulinemia in 1989. The criteria currently used are those introduced in 2011 by Professor Salvatore De Vita's study group (Table 1)[30]. The sensitivity and specificity of these classification criteria are 89.9% and 93.5%, respectively[31].

Table 1. Classification criteria of cryoglobulinaemic vasculitis[30].

Satisfied if at least two of three items (questionnaire, clinical, laboratory) are positive.
The patient must be positive for serum cryoglobulins at least 2 determinations at major or equal 12-week interval.

1. Questionnaire item: at least two out of the following
   - 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 colour after their disappearance?
   - Has a doctor told you that you have viral hepatitis?

2. Clinical item: at least three out of the following four (present or past)

• Constitutional symptoms	Fatigue
	Low grade fever (37-37,9°C, >10 days, no cause)
	Fever (>38°C, no cause)
	Fibromyalgia
• Articular involvement	Arthralgias
	Arthritis
• Vascular involvement	Purpura
	Skin ulcers
	Necrotizing vasculitis
	Raynaud's phenomenon
	Hyperviscosity syndrome
• Neurologic involvement	Peripheral neuropathy
	Cranial nerve involvement
	Vasculitic CNS involvement

3. Laboratory item: at least two out of the following three (present)
   - Reduced serum C4
   - Positive serum rheumatoid factor
   - Positive serum M component

LABORATORY DETERMINATION

Due to the thermal instability of cryoglobulins, to avoid false results due to their precipitation at room temperature, the first step in the laboratory determination of cryoglobulins, consisting of all procedures including blood sampling, coagulation, and centrifugation, must be performed at a temperature of +37°C. The blood sample must be placed immediately in a thermostat or, when not possible, kept at +37°C until arrival in the laboratory. The second step involves storing the patient's serum at a temperature of +4°C for one week.

The cryocrit parameter is expressed in percentage (%) and it is measured by reading the amount of cryoprecipitate on a graduated test tube; this amount will then be compared to the amount of serum of the sample that has been analysed after centrifugation. The cryoprecipitate is subsequently analysed by either electrophoresis or immunofixation to identify the cryoglobulin components. In order to obtain a reliable result, it is important to perform the analysis on blood samples that do not contain anticoagulant, thereby avoiding false positives caused by the presence of cryofibrinogen and/or other heparin-precipitable proteins[9,32,33]. Several studies report

the coexistence of cryoglobulins and cryofibrinogen, in varying proportions within test specimens, depending primarily on the type of specimen and the type of underlying disease to which the cryoglobulinemia and/or cryofibrinogenemia are secondary[34-39]. Cryofibrinogen syndrome is a condition that presents the clinical spectrum typical of cryoproteinemias; therefore, a patient presenting with indicative signs and symptoms should also be tested for cryofibrinogen remembering that its determination is in the plasma and not in the serum[40-43].

The detection of cryoglobulins is an extremely complex process with a diagnostic accuracy that is subject to user-dependent variability and to the possible presence of interfering components in the serum[40-43].

PATHOGENESIS

If the cryoglobulinemia is HCV-related, the structure of these immune complexes consists of an HCV nucleocapsid protein molecule bound to an IgG with specific anti-core reactivity, which is itself bound to an IgM directed to the Fc portion of the same IgG[44]. However, this combination of typed immunoglobulins is not exclusive, but only prevalent, and there are many possible combinations[12]. The reason for this binding is not known, but it is possible that the precipitate may also contain IgG directed against other antigens, which would also justify the existence of cryoprecipitable immune complexes even in the absence of chronic HCV infection. The deposition and binding of these immune complexes on the endothelial surface via the receptor for C1q causes the release of vasoactive peptides of the complement system, the recruitment of inflammatory cells and, finally, leukocytoclastic vasculitis. The inevitable consequence is an alteration of the vessel line and ischaemic damage to the tissues affected by the vascular lesion[1].

The hypothesised molecular mechanisms are not yet fully understood, but similarly to other autoimmune diseases, the role of B-cell activating factor (BAFF), a cytokine belonging to the tumour necrosis factor (TNF) ligand family, has been emphasised[45,46]. HCV can cause a chronic stimulation of the immune system through several viral proteins, in particular the core protein is able to provoke the formation of anti-GOR antibodies directed against both the GOR nuclear antigen and the HCV core antigen[47]. In addition, HCV infection appears to be the early event that sustains the up regulation of BAFF, leading both to its overexpression at the level of inflammatory cells contained in portal tracts evaluated on liver biopsies[48], and to an increase in circulating levels of BAFF especially in patients with cryoglobulinaemic vasculitis, probably supporting the clonal expansion of B cells.

The biological feature of patients with cryoglobulinaemic vasculitis is the oligoclonal, non-neoplastic, antigen-driven expansion of rheumatoid factor-synthesizing B cells derived from the germinal or post-germinal centre[49,50]. These clones also appear to originate from precursors with specific anti-IgG activity and probably begin to expand in the liver and then reach the circulation and other compartments[1].

Another hypothesized mechanism involving HCV is the interaction of the E2 protein with the CD81 receptor on the B-cell surface[51] with consequent sustained polyclonal stimulation of the B-compartment. This results in stochastic[52] genetic aberrations, including translocation [14:18]; the latter becomes highly prevalent in patients with type II mixed cryoglobulinemia, ranging from 37-38% in individuals with hepatitis C to 85% in patients with HCV-related mixed cryoglobulinemia. Higher survival may result, as a late complication of the disease, in the genesis of a frank malignant lymphoma due to accumulation of additional genetic mutations[47,53-55].

CLINICAL MANIFESTATIONS

Cryoglobulinaemic vasculitis is clinically characterised by a triad of symptoms, the Meltzer-Franklin triad, consisting of purpura, asthenia, and arthralgia[22]. Although this triad occurs in the majority of patients, the reality is that the symptom spectrum of cryoglobulinaemic vasculitis is much broader and

includes several symptoms with an extremely variable frequency[1]. In the case of non-HCV-related mixed cryoglobulinemia, the symptom picture is almost similar, although the clinical scenario mainly reflects the main diagnosis, especially in the case of mixed cryoglobulinemia occurring in conjunction with connective tissue disease[28].

It is important to note that there are also patients with mixed cryoglobulinemia who are totally asymptomatic and patients with frank cryoglobulinaemic vasculitis without detectable cryoglobulins in the serum. The latter case is thought to be related to the fact that the precipitability of immune complexes may depend on several factors, so that during laboratory determination, despite the presence of cryoglobulins, precipitation does not occur. Alternatively, pre-analytical and post-analytical difficulties related to the laboratory methods may alter the results. In these cases, if the physician has a strong suspicion of cryoglobulinaemic syndrome, it is very important to perform multiple, time-series determinations in order to better characterise the patient's serological picture, also considering the fact that the presence of cryofibrinogen may cause clinical pictures similar to cryoglobulinaemic syndrome[9,39,43,56-58]. The reason why some patients with mixed cryoglobulinemia have no clinical manifestations is due to the fact that environmental and/or genetic factors that are not fully known are certainly involved[44-47].

The most frequent skin manifestation is palpable purpura in the lower limbs, usually orthostatic and intermittent. As a consequence of repeated purpura, haemosiderin accumulates in the subcutaneous layer, giving the skin a brownish hyperpigmentation, a finding that is considered diagnostic even in the absence of active purpura[1,30]. The extent and magnitude of the lesions may also depend on the presence of co-factors such as chronic venous insufficiency, physical stress, prolonged sitting, and humid hot weather[22].

Raynaud's phenomenon is another relatively frequent cutaneous manifestation, which is associated, in 50-60% of cases, with a videocapillaroscopic pattern characterised by an increase in the number of capillaries and an increase in their tortuosity[1].

Xerostomia and xerophthalmia occur in approximately 50% of patients, but only half of these manage to reach the diagnosis of Sjögren's syndrome[15,54,59]. It is worth mentioning that this type of manifestation is classically related to chronic HCV infection[60].

Other skin manifestations of note are skin ulcers, which are difficult to heal, livedo reticularis, cold urticaria and digital gangrene[1].

The two most frequent musculoskeletal manifestations are asthenia and arthralgia. The patient's clinical picture may also include a pattern of non-erosive arthritis, usually oligoarticular, mild and often migrating. Myalgia or frank fibromyalgia has been identified in a smaller proportion of patients[1,22].

Approximately 60% of patients with cryoglobulinaemic vasculitis present with an axonal sensory-motor neuropathy that causes paraesthesia and burning sensations, especially in the lower limbs, with a typical stocking distribution, but also in the upper limbs with a glove distribution[44]. Neurocognitive and neuropsychiatric alterations were also noted: 86% of the patients examined had a deficit in one or more of the ten cognitive domains[61]. In addition, sexual dysfunction, emotional distress, an increased risk of depression, anxiety, somatisation, and insecurity, as well as fatigue and sleep disturbances were noted[62].

Approximately half of patients develop renal involvement during the disease, ranging from a nephrotic syndrome and membranous-proliferative glomerulonephritis to less frequent manifestations such as glomerulosclerosis and terminal renal failure[63,64]. As a consequence of the renal damage, arterial hypertension, proteinuria of varying degrees, microhaematuria, granular and/or erythrocytic cylinders and increased creatinine levels may be recorded[1]. Renal damage, when manifest, is a factor that may worsen the prognosis of the disease[22].

Since HCV infection is involved in most cases of mixed cryoglobulinemia, clear chronic hepatitis, generally of mild to moderate degree, often coexists. Chronic hepatitis becomes life-threatening, especially when it is combined with renal involvement; generally, its progression is less severe than that of the general HCV-positive population and it is also less frequently associated with the development of hepatocellular carcinoma (HCC)[53,59].

Patients with HCV-related mixed cryoglobulinemia with or without vasculitis, amongst extra-hepatic manifestations, present relatively frequently with thyroid disorders and type 2 diabetes mellitus. HCV infection of thyrocytes and pancreatic β-cells results in up-regulation of CXCR10 secretion and recruitment of Th1 lymphocytes that, in genetically predisposed individuals, respond with increased production of interferon γ and TNFα. In turn, they support and stimulate CXCL10 secretion by the target cells; this mechanism self-perpetuates a vicious cycle that ultimately leads to the onset of autoimmune thyroiditis and type 2 diabetes mellitus[27,65].

The involvement of the lungs in the disease is probably due to the development of vasculitic phenomena in the small vessels of the lungs and can manifest itself in various ways such as dyspnoea on exertion, dry or productive cough, haemorrhagic alveolitis, interstitial lung fibrosis with or without pleural effusion, and even cases of cryptogenic interstitial pneumonia have been described[1,66-68].

Gastrointestinal vasculitis is manifested by the involvement of small and medium vessels and causes abdominal pain and blood-streaked stools, resembling an acute abdomen, or frank patterns of intestinal perforation; these patients are often misdiagnosed as having acute pancreatitis and cholecystitis[66,69].

Hyperviscosity syndrome is a manifestation that mainly affects individuals with type 1 cryoglobulinemia, rather than type 2 and type 3[1]. The symptoms of hyperviscosity syndrome are characterised by blurred vision, recurrent nosebleeds, headache, tinnitus, dizziness, and vertigo. If the condition becomes severe, patients may experience confusion, ataxia, and heart failure[66,70,71].

Cardiovascular involvement and osteosclerosis have been occasionally reported[1].

MALIGNANCIES

The most frequently occurring malignancies in patients with mixed cryoglobulinemia are hepatocellular carcinoma (HCC) and non-Hodgkin's lymphoma, because the vast majority of patients have chronic HCV infection[1,22].

Interestingly, it appears that patients with mixed cryoglobulinemia are almost twice less likely to develop HCC and liver cirrhosis compared to the population without mixed cryoglobulinemia. The reason for this correlation is not fully known, but it can be speculated that it is due to the fact that HCV-related sequelae are more frequent in men, whereas mixed cryoglobulinemia is more frequent in women[1,72].

The known association between chronic HCV infection and B-cell non-Hodgkin's lymphoma is, however, subject to a wide geographical variability[73]. This difference probably reflects a possible role played by genetic and/or environmental factors, as the rate of progression to non-Hodgkin's lymphoma in Italy is 5%[74], in Spain 5.8%[75], while in Northern Europe and North America it is significantly lower[76-79]. The overall risk of aggressive non-Hodgkin's lymphoma in Italy is about 12 times higher than in the general population[76]. The most frequent histotypes of non-Hodgkin's lymphoma are splenic marginal low-grade lymphoma and diffuse large B-cell lymphoma[78,80]. There are two subtypes of the latter: the first is of primary origin while the second is an aggressive lymphoma that probably derives from the transformation of a marginal lymphoma. The transformation of a marginal lymphoma into aggressive diffuse large B-cell lymphoma is a phenomenon that occurs more frequently in chronically HCV-infected individuals than in non-infected individuals[81].

However, the incidence of indolent non-Hodgkin's lymphoma could be overestimated if one did not consider the existence of monoclonal lymphoproliferative disorders of undetermined significance (MLDUS), clinical pictures between a simple polyclonal lymphoproliferation and a low-grade lymphoma. Following the multistep model of carcinogenesis, this model explains the need for the accumulation of additional mutations and chromosomal translocations, such as those of p53 or RB genes, for a lymphoproliferative process to assume neoplastic characteristics. This is referred to as 'undetermined significance', analogous to MGUS (monoclonal gammopathy of undetermined significance), as they may not progress to lymphoma. Chronic antigenic stimulation caused by chronic infections induces a polyclonal proliferation of B cells, which over time leads to the selection of a clone that has gained a proliferative advantage, but not enough to constitute a frank lymphomatous disease[82]. In fact, bone marrow and liver infiltrates do not tend to evolve[83]. It was, therefore, concluded that type II mixed cryoglobulinemia is not a malignant haematological disorder, although the anatomopathological findings are very similar to those of indolent B-cell lymphomas[49].

Throughout the studies, two observations have been made that emphasise the role of chronic HCV infection in the genesis of B-cell non-Hodgkin's lymphoma. In a study by Hermine et al[84] in 2002 on patients with B-cell non-Hodgkin's lymphoma of marginal hair-cell histotype with a chronic HCV infection, if a sustained virologic response was achieved with an interferon α2b-based treatment, with or without ribavirin, regression of the lymphoma was also observed; the same consequence was not achieved in HCV-negative patients. The same results were later confirmed with larger cohorts of patients treated with interferon and direct-acting antiviral agents (DAAs)[85,86]. In a retrospective study in 2007, Kawamura et al[87] found that a cohort of chronically HCV-infected, untreated patients experienced a steady increase in the rate of development of non-Hodgkin's lymphoma to 2.6% in the fifteenth year of observation, whereas patients who achieved a sustained virologic response had the same rate of development of 0% at the same time of observation. Based on these observations, it can be assumed that sustained virological response plays a preventive role in the development of non-Hodgkin's lymphoma.

In a study by Michaud et al[37] patients with coexisting cryoglobulinemia and cryofibrinogenemia had an increased risk of malignancy compared to those with cryofibrinogenemia alone.

The association between mixed non-HCV-related cryoglobulinemia and solid tumours is extremely weak and may be a coincidence. It is, therefore, not necessary to systematically screen a patient with apparently essential cryoglobulinemia for solid tumours[88].

MANAGEMENT

Mild manifestations of the disease

All patients should be on a low antigen diet to enhance the ability of the reticuloendothelial system to eliminate circulating cryoglobulins[89].

In cases of mild disease, therefore, characterised by fatigue, arthralgia, arthritis, purpura and skin manifestations or sensory neuropathy, symptomatic therapy may be combined with aetiological therapy depending on the type of manifestations.

Skin purpura and non-necrotising skin manifestations, arthralgia or asthenia can be treated with colchicine at a dose of 1 mg daily[90,91]. In cases of arthritis, it is possible to combine hydroxychloroquine[92] and, as in the case of inflammatory arthritis, non-steroidal anti-inflammatory drugs (NSAIDs); prednisone can be used at a low-intermediate dose of 0.1-0.5 mg/kg per day in cases of impaired renal function or insufficient control; its discontinuous or chronic use is discouraged as the evidence is conflicting[89].

Pain management related to sensory neuropathy can be treated with tricyclic antidepressants, gabapentin, pregabalin, local anaesthetics or, as a second-line treatment, opioid analgesics[93].

In HCV-related mixed cryoglobulinemia, aetiological therapy should be initiated at an early stage and should always include antiviral treatment both because the use of direct-acting antiviral agents (DAAs) has an efficacy rate greater than 95% and because sustained antiviral response (SVR) prevents the development of cryoglobulinaemic vasculitis[94-96].

For all patients with mixed cryoglobulinemia related to an infection not related to HCV, appropriate eradication treatment should be administered, if available, for the infection in question, and patients usually achieve remission, sometimes with the addition of corticosteroids, plasmapheresis or immunosuppressants[28].

Moderate-severe manifestations of the disease

In HCV-related cryoglobulinaemic vasculitis, if severe manifestations are also involved (renal failure and/or nephritic or nephrotic syndrome, interstitial pulmonary fibrosis, low-to-medium grade coronary artery disease, severe recurrent abdominal pain, skin ulcers, progressive motor neuropathy and severe liver damage without liver failure), DAAs should be combined with a regimen of Rituximab (RTX), an anti-CD20 monoclonal antibody, at a dose of 375 mg/m^2 per week for 4 weeks. This leads to a marked reduction in the Birmingham Vasculitis Activity Score (BVAS) at 24 months[89,97].

However, in case of life-threatening manifestations (acute kidney damage with oligo-anuria and rapidly progressive renal failure, diffuse alveolar haemorrhage, acute cardiovascular or cerebrovascular events, ischaemic colitis, infected skin ulcers complicated by sepsis and critical liver failure), since Rituximab has a latent effect, the first line of treatment is plasmapheresis, normally associated with high doses of glucocorticoids, performed in an intensive care setting. This involves 3 to 8 sessions for a total of 1-1.5L of plasma, replaced with albumin or fresh plasma in the case of alveolar haemorrhage, to restore pro-coagulant factors[98].

It is of paramount importance to mention the possibility of a flare-up of Rituximab-associated vasculitis, a clinical entity occurring in 3.4% of patients at a median of 8 days after administration. It is most frequently associated with renal involvement, high cryoglobulin levels and increased C4 use. Clinically it causes renal damage, purpura, gastrointestinal involvement, and myocarditis, with a 1-year survival rate of 43%. In patients with an increased risk of RTX-induced relapse, lower doses should be administered (250 mg/m^2 at each infusion)[99].

Patients resistant to RTX treatment are those who do not respond at 4-6 weeks after induction or who have less than 50% improvement after 12 weeks. In this case it is always important to exclude the presence of an underlying lymphoma[100]. A study by Michaud et al[37] states that if cryofibrinogenemia exists in association with cryoglobulinemia, then a more intensive treatment for vasculitis is required compared to those with cryofibrinogenemia alone.

Treatment alternatives include Ofatumumab (anti-CD20 monoclonal antibody), Belimumab (anti-BLYS/BAFF monoclonal antibody) or the administration of interleukin 2[101,102].

It is important to remember that despite SVR, only two-thirds of patients have clearance of cryoglobulins in the serum[103] and that RTX therapy significantly reduces serum cryoglobulin levels[104].

In non-HCV-related but still infection-related cryoglobulinaemic vasculitis, in cases of refractory or relapsing disease, often related to chronic HBV infection, remission can therefore be achieved by adding an RTX-based regimen to the treatment[28].

Considering patients with non-infectious, thus essential, mixed cryoglobulinemia related to lymphoproliferative diseases or connective tissue diseases, in patients with severe disease manifestations, a regimen based on Rituximab and corticosteroids has been shown to be superior in inducing remission compared to a regimen based on immunosuppressants and corticosteroids. However, a particular sub-

set of patients (GFR<60 mL/min, older age, high corticosteroid doses), has been shown to have a higher rate of infections secondary to therapy in the Rituximab+corticosteroid regimen, therefore, in general, it is important to consider rapid corticosteroid tapering to decrease the risk of severe infections[105].

PROGNOSIS

The prognosis of non-HCV-related cryoglobulinaemic vasculitis is related to the underlying disease and, therefore, highly variable[1].

For HCV-related cryoglobulinaemic vasculitis, the negative prognosis factors are age greater than 60 years and renal involvement, in addition to the severity of the underlying liver disease, the presence of cardiovascular involvement, infections, and the presence of lymphoma[53,106,107].

In addition, it would appear that the co-existence of cryofibrinogen generates a more severe clinical phenotype of cryoglobulinaemic vasculitis[37].

CONCLUSIONS

The cryoglobulinemic vasculitis, despite is considered a rare disorder, is a disease characterized by an extremely wide clinical spectrum and necessitate, therefore, to a high index of suspicion. It is fundamental to distinguish an isolated laboratory definition, as a transient finding without a confirm in the following control or an asymptomatic state, from a real syndromic state. On the other way, it is important to consider the presence of a variability on the laboratory process of analysis, often characterized by a high rate of false negative. These considerations are significant to meet the necessity to treat an underestimated condition that could become a potentially lethal disorder.

Conflict of interests:
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors report no conflicts of interest for this work.

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