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Hepatitis C virus-related vasculitis

Gonçalo Boleto, MD^{1,2}, Matheus Vieira, MD^{1,2}, David Saadoun, PhD^{1,2,3,4}, Patrice Cacoub MD, PhD^{1,2,3,4}

¹AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, F-75013, Paris, France;

²Centre de Référence des Maladies Auto-Immunes et Systémiques Rares, Centre de Référence des Maladies Auto-Inflammatoires et de l'Amylose, France

³Sorbonne Université, UPMC Univ Paris 06, UMR 7211, Inflammation-Immunopathology-Biotherapy Department (DHU i2B), F-75005, Paris, France;

⁴INSERM, UMR S 959, F-75013, Paris, France; CNRS, FRE3632, F-75005, Paris, France;

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Correspondence should be addressed to: Prof. Patrice Cacoub, AP-HP, Hôpital Pitié-Salpêtrière, Department of Internal Medicine and Clinical Immunology, 83 boulevard de l'hôpital. F-75013, Paris, France

Phone: + (33)(1) 42 17 80 09. Fax: + (33)(1) 42 17 80 33.

E Mail: patrice.cacoub@aphp.fr

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Abstract

Cryoglobulinemic vasculitis (CryoVas) is a small-to-medium vessel systemic vasculitis caused by the deposition of mixed cryoglobulins and immune complexes. Clinical spectrum of CryoVas ranges from mild symptoms to vasculitis involving multiple organs that may progress to more life-threatening illness. Hepatitis C virus (HCV) chronic infection is the most frequent condition to be assessed in patients with CryoVas. The mortality rate among patients with HCV-associated CryoVas is 3× that of the general population, with a 63% 10-year survival rate. The recent advent of interferon-free direct-acting antivirals (DAAs), which have the potential to induce sustained virological response rates greater than 95%, has dramatically changed the management of chronic HCV infection and HCV-related CryoVas. B-cell depleting strategies, mainly with rituximab, are the main therapeutic option in severe and refractory cases of HCV-associated CryoVas. Despite the progress in the last years on the management of chronic HCV infection, there are still unmet needs regarding therapeutic management of severe and refractory HCV-associated CryoVas.

Introduction

Cryoglobulins are abnormal immunoglobulins (Ig) which reversibly precipitate at low temperatures below 37°C. This definition distinguishes cryoglobulins from other cryoproteins and cold agglutinins. Cryoglobulinemia is defined as the persistent presence of cryoglobulins in serum. According to Brouet's classification [1], three subtypes of cryoglobulins exist based on immunoglobulin composition. Type I cryoglobulins comprise single monoclonal Ig [most commonly immunoglobulin M (IgM), rarely IgG or IgA]. Type II and type III cryoglobulins are classified as mixed cryoglobulins (MC) because they include two types of Ig (usually IgG and IgM). The presence of type I cryoglobulins is linked to a B cell lymphoproliferative disorder [2]. There is a vast number of conditions associated with MC: chronic hepatitis C virus (HCV) infection and other infections, autoimmune diseases [connective tissue diseases (CTD)], and B cell lymphoproliferative disorders [3]. When MC is found in the absence of an underlying condition, the syndrome is designated essential MC [4]. The elementary mechanism contributing to MC is aberrant autoantibody production by B cells and B cell proliferation [4]. MC can cause a systemic inflammatory syndrome called cryoglobulinemic vasculitis (CryoVas) that belongs to the group of systemic immune-complex-mediated small-to-medium vessel vasculitis according to Chapel Hill Consensus Conference reviewed in 2012 [5]. CryoVas patients most frequently present with purpura, arthralgia, and fatigue, or may progress to serious potentially life-threatening manifestations including neurologic, renal and cardiac involvement [6]. Infection with HCV is known to result in liver cirrhosis and hepatocellular carcinoma but it is also recognized as the virus most often associated with extrahepatic manifestations, including CryoVas [7]. Historically, the great majority (80-90%) of CryoVas cases were related to an HCV infection ; however, the recent development of all oral interferon (IFN)-free direct acting antiviral agents (DAA) has changed the landscape and incidence of CryoVas [8]. Viral clearance is associated with clinical improvement and therefore eradication of HCV should be a priority in the management of CryoVas [9]. Here, we review up-to-date data on HCV-related vasculitis.

Epidemiology

CryoVas is considered a rare disease (<5 cases per 10,000 individuals in the western countries), although data on the prevalence and incidence of this condition in the general population are scarce [4]. As stated before, the main etiology of MC and

CryoVas is chronic HCV infection, accounting for > 80% of cases [10,11]. Chronic HCV infection is a common worldwide problem and is a major cause of cirrhosis, hepatocellular carcinoma and liver transplantation. The prevalence of HCV infection varies depending on the geographical region, being highly prevalent (>3.5%) in some parts of Asia, Middle East and Africa and less prevalent (<1%) in North America, western European and high-income Asia-Pacific countries [12]. MC is found in 40 to 60% of patients with chronic HCV infection whereas only 5%-15% will develop CryoVas [13,14]. Interestingly, patients with chronic HCV infection and MC seem to be at higher risk for severe liver illness with higher rates of cirrhosis and fibrosis scores than those without MC [15]. The presence of cryoglobulins seems to correlate with the duration of HCV infection [13]. A very recent study from our center showed that DAAs are dramatically changing the incidence of MC and CryoVas with HCV infection no longer being its main cause. Indeed, in our series, HCV represented only 36.4% of CryoVas cases whereas autoimmune diseases mainly systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS) appeared as the most common causes in 2018 [8]. We observed a steady decrease in the incidence of MC between 2011 and 2018 mainly due to a decrease in HCV-associated MC cases. This first signal should be interpreted with caution as the worldwide reported variation in the proportion of cases of MC and CryoVas that are attributable to HCV infection can be due to population selection bias and to the fact that clinical and laboratory assessment is not standardized and prone to false results.

Pathophysiology

The pathogenesis underlying the formation of cryoglobulins is only partially understood. It seems to depend on an interaction between predisposing host factors and environmental triggers leading to aberrant B cell lymphoproliferation [16]. Formation of cryoprecipitates appear to depend on several factors, including pH, ionic force, electrical charge and weak noncovalent interactions, cryoglobulin levels and temperature [17]. HCV has a tropism for B cells and their chronic stimulation results in polyclonal activation and expansion leading to production of an IgM with rheumatoid factor (RF) activity, and thereby to cryoglobulin formation [18]. Envelope glycoproteins E1 and E2 help HCV enter lymphocytes possibly via CD81 receptor [19]. Active HCV replication has been shown in CD19 positive B cells. HCV RNA and HCV core and non structural NS3 proteins can be detected in CD19 positive but not in CD19 negative peripheral mononuclear cells [20,21]. Persistent antigen presentation results in the

gradual emergence of B cell clones that produce initially polyclonal IgM (type III cryoglobulins), then oligoclonal IgM (type II/III cryoglobulins), and finally a monoclonal IgM (type II cryoglobulins) [22]. Furthermore, regulatory T cells (Tregs) have been shown to be reduced in HCV-associated CryoVas patients thus contributing to autoreactive B cell expansion and expression of autoimmune manifestations [23]. A previous study demonstrated a negative correlation between the number of IgM⁺ CD21⁻/_{low} memory B cells and Tregs and a positive correlation with the number of T follicular hepls (Tfh) cells and serum levels of cryoglobulins in patients with HCV-associated CryoVas. Therapy with DAAs is able to restore B-cell and Treg homeostasis [24]. Concerning predisposing host factors, patients with the HLA-DR11 antigen have an increased risk for CryoVas whereas those with HLA-DR7 have a decreased risk of cryoglobulin production [25]. Other predisposing factors include single nucleotide polymorphisms within an intronic region of NOTCH4 and between HLA-DRB1 and HLA-DQA1 [26]. Different expression profiles of microRNAs in the peripheral mononuclear cells with a high prevalence of homozygosity and a greater frequency of a particular allele of the B cell activating factor (BAFF) promoter were more frequent in patients with HCV-associated CryoVas [27,28]. The induction of B cell proliferation can result in B cell transformation and patients with HCV-associated CryoVas have a 35-fold increased risk of developing lymphoma as compared to the general population [29]. Patients with chronic HCV infection have clonal B cell populations that are predominantly IgM-producing memory B lymphocytes expressing hypermutated immunoglobulin genes. These immunoglobulin idiotypes and restricted gene sequence rearrangements are seen in both HCV-associated CryoVas and HCV-positive non-Hodgkin lymphoma (NHL) thus suggesting a shared pathogenesis [30–32]. The clinical symptoms of CryoVas are caused by the deposition of immune complexes in small-to-medium blood vessels resulting in endothelial injury. These circulating immune complexes are formed by HCV particles, anti-HCV polyclonal IgG and monoclonal IgM with RF activity. The presence of IgM in the circulating cryoprecipitates induces complement activation and consumption triggering immune responses and inflammation thus resulting in vasculitis [13,33,34].

Diagnosis and clinical presentation

The diagnosis of CryoVas relies on the detection of circulating cryoglobulins in combination with clinical presentation. Cryoglobulin detection should be performed in all HCV patients presenting with manifestations of CryoVas [4]. Due to the termal

instability of cryoglobulins which precipitate if the temperature falls below 37°C, cryoglobulin detection can be technically challenging. Clinicians should be aware that several precautions are important to avoid false-negative results i) blood samples must be kept at 37° during transport to the laboratory and during centrifugation to avoid premature precipitation ; ii) serum samples must be stored at 4°C for one week to ensure the detection of delayed cryoprecipitation ; iii) immunoelectrophoresis is performed after purification to identify the type of cryoglobulins ; iv) the cryoprecipitate is quantified using immunoblotting; and v) serum containing cryoglobulins should be tested for reversibility by rewarming at 37°C for 24 hours in order to avoid false positive results caused by cryofibrinogen or heparin precipitable proteins [4,35]. Usually, a concentration above 50mg/L is considered abnormal [35]. The levels of cryoglobulin do not usually correlate with the severity of the clinical presentation [36]. Complement abnormalities are frequently observed with decreased C4 levels and normal C3 levels. RF activity is also common in MC but is rarely observed in type I cryoglobulinemia [35]. MC can cause non necrotizing leucocytoclastic vasculitis involving small- to medium-sized vessels. The diagnosis of CryoVas is based on the detection of circulating cryoglobulins and the presence of the classic clinical triad of purpura, arthralgia and fatigue. Purpura affecting primarily the lower limbs and sometimes the abdomen is often the inaugural manifestation. By contrast with type I cryoglobulinemia, distal necrosis at the lower or upper limbs is less frequently observed in MC. Arthralgia is also one of the most frequent manifestations, often consisting of non migratory pain involving hands and knees in a bilateral and symmetric fashion ; arthritis is less common and non destructive. Some patients however can have more severe and life-threatening clinical course with renal, gastrointestinal, cardiac and/or central nervous system manifestations [6]. In patients with renal involvement proteinuria and microscopic hematuria are the main manifestations and hypertension and renal failure may develop. At the histological level, membranoproliferative glomerulonephritis with mesangial proliferation is the most common presentation. Extra-capillary proliferation, amorphous eosinophilic intraluminal thrombi and fibrinoid necrosis of the vessel walls are adverse prognostic features. Immunofluorescence shows subendothelial and intraluminal deposits of immunoglobulins identical to those present in the cryoprecipitate [37]. In patients with neurologic involvement, peripheral nervous system is usually the main target. Common inaugural clinical presentation includes limb neuropathic pain and paresthesia. Distal sensorymotor polyneuropathy at the lower

limbs can occur in up to two-thirds of patients and mononeuritis multiplex in one-third of patients. Motor deficit symptoms are less common and usually set in gradually. Vasculitis of the central nervous system (CNS) is rare but probably underestimated [38]. CNS vasculitis is characterized by a marked heterogeneity in clinical presentation including acute or subacute symptoms including headaches, seizures, cranial nerve palsies, and seldom cerebrovascular events. Since brain biopsy is rarely performed, presumptive diagnosis is based on magnetic resonance imaging, angiography or computed tomography findings [39]. Vasculitis affecting the heart is also rare but may progress to heart failure and is among the causes of death of patients with CryoVas. Microvascular disease with necrotizing vasculitis of the coronary arterioles is the main feature of cardiac involvement [40]. Other clinical manifestation include gastrointestinal involvement with abdominal pain and eventually bleeding and/or bowel perforation and pulmonary involvement with organizing pneumonia and alveolar hemorrhage which are extremely rare.

Patients with suspected HCV-CryoVas can present with symptoms that overlap with with a variety of different conditions, particularly Sjogren syndrome and B cell NHL [41]. A fair amount of patients with HCV-CryoVas may share several features observed in SS which complicates the diagnosis including sicca syndrome, arthralgia, purpura, RF activity as well as an increased risk of developing B cell lymphoma.

Management

In HCV-CryoVas, treatment should be initiated without delays and always include antiviral treatment [42]. A previous large cohort study of HCV patients found that viral clearance with interferon-based therapy dramatically reduced the risk of developing CryoVas. Indeed, patients who achieved sustained virological responses (SVR) were less likely to develop CryoVas (HR 0.61, 95% CI 0.39, 0.94; P=0.026), especially when compared with those who did not achieve SVR (HR 0.55, 95% CI 0.33, 0.90; P=0.017) [43]. The recent advent of oral interferon-free DAAs, which have the potential to induce SVR rates greater than 95%, has dramatically changed the management of chronic HCV infection and HCV-CryoVas [44,45]. Complete response of CryoVas manifestations are observed in 71%-95% of cases with an excellent safety profile as serious adverse events were reported in less than 8% [46–49]. In the first prospective, open-labelled VASCUVALDIC study, 74% of patients achieved SVR at week 12 and a complete clinical remission was observed in 87% of patients ; purpura, skin ulcers and arthralgia disappeared in all cases and renal involvement improved in four out of five cases [50].

An open-label study evaluating the effectiveness and safety of a combination of sofosbuvir and daclatasvir in patients with HCV-CryoVas showed complete clinical remission in 90.2% of patients at week 24. Complete remission rate at 12 months was 90%, with an event free survival rate at 2 years of 100% [46]. Another study confirmed these data with very high rates of clinical and virological responses (90% and 100% respectively) in patients treated with sofosbuvir based IFN-free regimens [48]. In line with these data, Bonacci et al reported 71% and 94% of complete clinical and virological responses respectively. However, only 48% of cryoglobulin clearance was found in 35 patients with HCV-associated CryoVas treated with different DAAs [49]. Comarmond et al in a prospective study of 27 patients with HCV-associated CryoVas showed that DAA therapy restored disturbances in peripheral B and T cell homeostasis. DAA therapy increased numbers of Tregs, decreased percentages of IgM⁺CD21⁻/low memory B cells and decreased numbers of Tfh cells. Moreover, expression levels of B lymphocyte stimulator receptor 3 and programmed cell death 1 on B cells increased after DAA therapy [24]. Of note, 6-12 months after stopping DAAs, up to 60% of HCV-CryoVas patients still had detectable cryoglobulins and a subset of patients experienced CryoVas relapse and/or developed lymphoma despite successful eradication of the virus. These findings suggest that some patients continue to have B cell clonal expansion probably by an autonomous proliferative behaviour. Previous studies suggested that HCV particles remained trapped within cryoprecipitates even after SVR. A recent study did not confirm such findings as the authors did not find HCV RNA in the serum and/or cryoprecipitates in patients who had a SVR after DAA therapy [51].

Management of patients with HCV-CryoVas should be individualized according to the severity of CryoVas patients' clinical presentation. Patients with mild-to-moderate vasculitis and non-life-threatening symptoms (i.e. fatigue, arthralgia, arthritis, purpura, sensory neuropathy) should be treated with DAAs alone. In patients with severe HCV-CryoVas, immunosuppressant therapy always in combination with DAAs is useful in order to rapidly control vasculitis manifestations [52,53]. Intravenous methylprednisolone (0.5-1.0 g/day for 3-5 days) in combination with plasma exchanges (PLEX) (daily for 5 to 14 days sessions, followed by 3 sessions per week for two to three weeks; replacement fluid should always be warmed to prevent cryoprecipitation) should be used in patients with severe vasculitis manifestations such as severe kidney involvement, skin necrosis, gastrointestinal, progressive motor neuropathy and/or central nervous system involvement [42]. Rituximab, a chimeric monoclonal antibody

that binds to the CD20 antigen, a transmembrane protein selectively expressed on pre-B and mature lymphocytes, has demonstrated greater efficacy than conventional immunosuppressants in HCV-CryoVas. CD20-positive B cells are expanded and activated in CryoVas patients [54,55]. In a randomized controlled clinical trial of rituximab versus conventional therapy (azathioprine, cyclophosphamide, PLEX) in 59 patients with severe CryoVas, only rituximab showed to significantly reduce the Birmingham Vasculitis Activity Score (BVAS) from baseline up to month 24 [56]. Rituximab is thought to provide long-term remission by depleting CD19 positive-B cells, known to be HCV reservoirs [20]. The best rituximab dosing regimen is 375mg/m² given weekly for 4 consecutive weeks [57]. Tolerance is usually good. Nonetheless, clinicians should be aware of the possibility of rituximab-associated vasculitis flares due to the formation of immune complexes. A recent study identified 3.4% vasculitis flares after a median of eight days following rituximab infusion in patients with type II CryoVas. Patients with rituximab-associated CryoVas flare had more frequently renal involvement, higher cryoglobulin level (2.1 vs 0.4 g/l, p = 0.0004) and lower levels of C4 (0.02 vs 0.05g/L, p = 0.023). Vasculitis flares included acute kidney injury, purpura, gastrointestinal involvement and myocarditis. The 1-year survival rate was poorer in patients with vasculitis flare after rituximab [43% (95%CI 18-100) vs. 97% (95%CI 92-100), p < 0.001]. Interestingly, the authors observed the presence of rituximab, IgM, and IgG1-positive staining of endomembranous deposits and thrombi within kidney lesions [58]. In patients with risk factors of rituximab-induced vasculitis flare, lower doses of rituximab should be used (250 mg per infusion) [42].

The management of rituximab-refractory CryoVas remains a major challenge in HCV patients. Refractory disease can be defined as the absence of CryoVas clinical response within 4-6 weeks after induction therapy, or less than 50% improvement after 12 weeks. The possibility of the presence of underlying malignant lymphoma when patients develop a relapse of CryoVas despite SVR should always be ruled out. Low-grade lymphoproliferation after HCV eradication might explain persistent circulating cryoglobulins and vasculitic symptoms in some individuals, especially those with previous long lasting infection. A cohort study found that the effect of SVR against CryoVas and other extrahepatic manifestations was lessened as the time to initiation of HCV therapy increased, thus emphasizing the importance of early treatment of HCV infection [59] Some alternative approaches targeting B cells such as the use of

ofatumumab (a potent anti-CD20 monoclonal antibody) or belimumab (an anti-B-cell activator antibody) have been proposed [60]. Modulation of Tregs also represents a promising approach in this setting. Saadoun et al showed in a previous open-label study that administration of low-dose interleukin-2 (IL-2) significantly reduced cryoglobulin levels and improved vasculitic symptoms in 8 out of 10 HCV-CryoVas patients. Low-dose IL-2 significantly increased numbers of Tregs and decreased the percentage of marginal-zone B cells [61]. A proposed treatment algorithm for HCV-associated CryoVas is shown in **Figure 1**.

Prognosis

HCV-CryoVas is associated with significant morbidity and mortality. The worst prognostic factors identified in previous reports were age >60 years and renal involvement, followed by the severity of the underlying liver disease, and the presence of cardiovascular disease, infection and lymphoma [62,63]. A previous study on 151 consecutive patients with HCV-CryoVas before DAA era identified age >65 years (HR 4.55), severe hepatic fibrosis (METAVIR Score \geq 3) (HR 5.31), heart (HR 4.2), central nervous system (HR 2.74) and renal involvement (HR 1.91) as the main adverse prognostic factors. Overall, 1-year, 3-year, 5-year and 10-year survival rates were 96%, 86%, 75% and 63%, respectively. IFN-based antiviral therapy was associated with decreased mortality rate (HR 0.34), whereas treatment with immunosuppressive therapy including steroids was associated with increased mortality rate after adjustment for the severity of vasculitis (HR 4.05) [64].

Conclusion

HCV-CryoVas is a unique condition with complex pathogenesis involving antigen-driven lymphoproliferation and formation of circulating cryoglobulins. CryoVas clinical presentation ranges from mild disease (i.e. arthralgia, purpura, sensory neuropathy) to more severe form with renal, gastrointestinal, cardiac, progressive motor neuropathy and/or central nervous system involvement. The advent of DAAs has changed the landscape of HCV-CryoVas management. High rates of viral clearance are commonly accompanied by improvements in HCV-CryoVas clinical symptoms and biological abnormalities. Rituximab always in combination with DAAs can now be considered standard of care treatment of moderate-to-severe CryoVas cases. Management of rituximab-refractory HCV-CryoVas still remains a major challenge.

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Legends

Figure 1. Proposed algorithm for the management of HCV-associated cryoglobulinemic vasculitis.

HCV, hepatitis C virus; CNS, central nervous system; GC, glucocorticoids; PLEX, plasma exchanges; RTX, rituximab.

HCV-associated
cryoglobulinemic vasculitis

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graph TD; A[HCV-associated cryoglobulinemic vasculitis] --> B[Mild/moderate<br/>(arthralgia, purpura, sensory neuropathy)]; A --> C[Severe<br/>(renal disease, skin necrosis, progressive motor neuropathy, CNS, digestive, or heart involvement)]; B --> D[Direct acting antiviral therapy +/- low dose GC (<0.5 mg/kg/d)]; C --> E[IV methylprednisolone, PLEX, RTX plus optimized direct acting antiviral therapy];
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Mild/moderate
(arthralgia, purpura, sensory neuropathy)

Direct acting antiviral therapy +/-
low dose GC (<0.5 mg/kg/d)

Severe
(renal disease, skin necrosis,
progressive motor neuropathy, CNS,
digestive, or heart involvement)

IV methylprednisolone, PLEX, RTX
plus optimized direct acting
antiviral therapy