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REVIEW ARTICLE

Clinical practice: hepatitis C virus infection, cryoglobulinemia and cryoglobulinemic vasculitis

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Abstract

Cryoglobulins are circulating immunoglobulins that reversibly precipitate at temperatures below 37 °C. Type-II cryoglobulins consist of monoclonal IgM/polyclonal IgG immune complexes (ICs), whereas in type-III cryoglobulins both IgM and IgG are polyclonal. The clinical condition resulting from the presence of cryoglobulins in the blood is called mixed cryoglobulinemia (MC), which can be asymptomatic or manifest as cryoglobulinemic vasculitis (CV). Type-I cryoglobulins, consisting of a single monoclonal isotype, are detected in patients with lymphoproliferative disorders. It is now established that > 90% of MCs are associated with HCV infection. Clinically, the spectrum of symptoms may range in severity from occasional purpuric eruptions to life-threatening features. In addition to the development of liver cirrhosis and hepatocellular carcinoma, the possible progression of HCV-positive CV patients to B-cell non-Hodgkin lymphoma (B-NHL) has been reported. The pathogenetic role played by HCV infection in the onset of B-NHL is suggested by regression of the latter following the achievement of a sustained virologic response (SVR). For several years, interferon-α alone or combined with ribavirin has been the standard of care. However, the rates of clinical, biochemical, and virologic responses have been low, and the occurrence of relapse frequent. The addition of rituximab has resulted in a higher rate of responses. With the advent of direct-acting antiviral agents, SVR has been achieved in ~95% of CV patients. However, in a minority of patients, despite SVR, CV may persist or reappear over variable lengths of time from the completion of therapy. The eventual appearance of B-NHL is also possible.

Keywords Cryoglobulinemia · Cryoglobulinemic vasculitis · Direct-acting antiviral agents · Hepatitis C virus · Non-Hodgkin lymphoma · Rheumatoid factor

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Abbreviations

BAFF	B-cell activating factor
CV	Cryoglobulinemic vasculitis
DAAs	Direct-acting antiviral agents
DLBCL	Diffuse large B-cell lymphoma
EMC	Essential mixed cryoglobulinemia
GCs	Glucocorticoids
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
ICs	Immune complexes
IFN-α	Interferon-α
MC	Mixed cryoglobulins or mixed cryoglobulinemia
MoAb	Monoclonal antibody
MZL	Marginal zone lymphoma
NHL	Non-Hodgkin's lymphoma
NVC	Nailfold videocapillaroscopy
pIFN-α	Pegylated interferon-α
RBV	Ribavirin
RF	Rheumatoid factor
RTX	Rituximab
SVR	Sustained virologic response

Historical context and definition

Cryoglobulinemia is a vasculitic disorder that mainly affects the small and, less frequently, medium-sized vessels. It is characterized by the occurrence of serum proteins that reversibly precipitate in vitro at temperatures < 37 °C and redissolve at body temperature. Initially described in 1933 by Wintrobe and Buell [1] in a patient with multiple myeloma, the phenomenon was later detected in association with other diseases. The proteins responsible for the occurrence of cryoglobulinemia are referred to as cryoglobulins [2]. Structural studies, clinical observations and, more recently, etiological and therapeutic advancements have remarkably improved our knowledge of this striking condition. Table 1 summarizes the milestones in the study of cryoglobulinemia and cryoglobulinemic vasculitis.

Immunochemical structure and classification

Three structural types of cryoglobulins have been identified [5]: (a) type-I or single cryoglobulins, consisting of a monoclonal immunoglobulin (Ig), usually IgM or IgG or, more rarely, IgA; (b) type-II mixed cryoglobulins (MC),

Table 1 Milestones in the study of cryoglobulinemia and cryoglobulinemic vasculitis (CV)

Years	Observations	References[a]
1933	A cold-precipitable protein is detected in the serum of a patient with multiple myeloma	[1]
1947	The same phenomenon is observed in various diseases and the terms "cryoglobulins" and "cryoglobulinemia" are coined	[2]
1962	Cryoglobulins are shown by chromatographic separation to be mixed, i.e., formed by two protein fractions of different molecular size (7S + 19S), the 19S fraction expressing rheumatoid factor (RF) activity	[3]
1966	The clinical picture and the structural heterogeneity of mixed cryoglobulinemia (MC) are carefully described and, in the ignorance of its etiology, MC is defined "essential"	[4]
1974	Cryoglobulins are classified into three main types according to their immunochemical structure	[5]
1987	Therapy with recombinant interferon-α (IFN-α) is shown to be clinically effective in patients diagnosed with "idiopathic" MC	[6]
1990…	The introduction of reagents for the detection of anti-hepatitis C virus (HCV) antibodies and the quantitation of HCV RNA allows to demonstrate that the large majority of patients with apparently "essential" MC are in fact HCV-infected	[7]
1991	IgMk RFs isolated from patients with MC are shown to display a major cross-reactive idiotype (CRI), designated WA	[8]
1994…	Progression to overt non-Hodgkin's lymphoma (NHL) is observed in a small percentage of HCV-positive MC patients, though with wide geographic variations	[9]
2002	Regression of splenic lymphoma with villous lymphocytes is reported after the successful treatment of HCV infection	[10]
2003…	Rituximab (RTX), a chimeric anti-CD20 monoclonal antibody, is found to be clinically effective in MC patients relapsing after, or refractory to, IFN-α therapy, alone or combined with Ribavirin (RBV). The rationale for the use of RTX was the demonstration that a B-cell clonal expansion involving RF-secreting cells is the biological hallmark of MC	[11]
2005…	An emerging, unforeseen and poorly clarified scenario is the observation of isolated CV patients in whom cryoglobulinemia and CV recur in spite of therapy-induced HCV eradication	[12]
2016…	The enlarged indication of a growing number of pan-genotypic direct-acting antiviral agents (DAAs) from patients with chronic HCV-positive hepatitis to those with CV is resulting in a high rate of clinical, virologic and immunological responses	[13]

[a]References are the presumed first papers dealing with each observation, but may not strictly reflect priority. Suspension points indicate that additional papers have subsequently addressed the same topic and are to a large extent mentioned throughout the present review

in which an IgM monoclonal component (mostly IgM kappa) with rheumatoid factor (RF) activity and predominantly bearing the WA cross-idiotype [14] is complexed with polyclonal IgG; (c) type-III MC, consisting again of IgM-IgG immune complexes (ICs) but the IgM and IgG fractions are polyclonal. Small amounts of serum cryoglobulins, asymptomatic and devoid of clinical consequences, are occasionally detected in several infectious, hematologic, and immunologic diseases and even in a few normal sera. However, the term cryoglobulinemic vasculitis (CV) refers to a well-defined illness whose clinical and pathological features are pathogenetically related to the occurrence of serum cryoglobulins.

Type-I cryoglobulins are detected in rare instances of clonal lymphoproliferative disorders such as Waldenström's macroglobulinemia, multiple myeloma, and B-cell non-Hodgkin's lymphoma (B-NHL), but the large majority of type-II and type-III MC are, as described below, associated with hepatitis C virus (HCV) infection. The present review, an updated extension of a previous report [15], is specifically aimed at providing a comprehensive overview of all the features of MC that are of clinical interest.

Epidemiology and etiology

Our cohort of cryoglobulinemic patients was retrospectively reviewed via a combination of electronic and paper chart review. The study was conducted in accordance with the International Council for Harmonisation Good Clinical Practice and was approved by the Institutional Review Board of the University of Bari, according to the current Bioethics and Safety Acts. All pictures are a selection from the archives of the authors and include biopsies coming from patients who provided written informed consent for utilizing their material in research.

In our previous report [16] based on 141 patients treated at our hospital, the mean (±SD) age at disease onset was 59±7.6 years (range 31–78 years), with a sex distribution of 96 females and 45 males (F/M ratio: 2.13). When the same evaluation was carried out at diagnosis on a total of 424 cryoglobulinemic patients whose data were collected from January 1991 to June 2018, the mean age at disease onset was 49±9 years (range 27–78 years) and the F/M ratio 3.3.

For many years, MC remained of undefined etiology and was therefore termed "essential" [4]. A turning point in the etiological definition of MC was the establishment of serological and biomolecular tests that allowed the detection of serum anti-HCV antibodies and then, later on, of HCV RNA. In 1990, in a short communication Pascual et al. [7] reported that many sera from patients with type-II MC tested positive for HCV. This first report was soon followed by many others that confirmed this unexpected association [17–22].

It is now established that CV is a chronic IC-mediated systemic vasculitis and the most typical extra-hepatic manifestation of HCV infection. Indeed, anti-HCV antibodies and variable levels of HCV RNA, with no viral genotypic prevalence, can be detected in > 90% of CV patients [16]. According to the 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides, which is based on the predominant type of vessels involved, CV is included in the subgroup "immune complex small vessel vasculitis," together with IgA vasculitis (Henoch-Schönlein) and hypocomplementemic urticarial vasculitis (anti-C1q vasculitis) [23]. Conversely, cryoglobulins are produced in roughly 25% of HCV-infected individuals, but the reasons why only 10–15% of them eventually develop CV are still unclear and may reflect the roles of genetic and/or environmental factors. [24]. The length of HCV infection seems directly related to the risk of developing CV [25].

As discussed later in this review, a few instances of MC have been found to be associated with HCV-negative connective tissue diseases or ascribed to other viral and pyogenic bacterial infections [26–28], including human immunodeficiency virus [29], hepatitis B virus [30], and hepatitis E virus [31]. The diagnosis of essential MC should therefore be restricted to the few patients in whom the etiology remains persistently undefined.

According to commonly accepted criteria, a disease is defined as rare when its prevalence in the general population is < 5/10,000. Although MC is in fact considered a rare disease, this is not strictly correct, as in southern European countries the prevalence of HCV infection is higher than in corresponding northern populations. An epidemiological study carried out in Origgio, a small town of < 8000 people not far from Milan, Italy, showed that the prevalence of HCV-related MC in the adult population was 8.5/10,000 and roughly 26/10,000 among residents ≥ 50 years of age [32].

In 2017, the International Task Force for Disease Eradication substantially revised its previously already high estimations, calculating that ~ 71 million (62–79 million) individuals worldwide were chronically infected with HCV, corresponding to a general prevalence of 1% (0.8–1.1%) but with obvious large geographic variations [33]. Assuming that cryoglobulins are present in 20–25% of HCV-positive patients and that CV develops in only 10% of them, then, globally, there are roughly 1,500,000 patients with clinically overt CV. In Italy, the Polaris Observatory estimated that, in 2017, out of a total number of 741,633 viremic HCV-infected people, only 325,365 had been actually diagnosed with HCV. From these data, a rough estimation of the total number of CV patients living in Italy is likely to be in the range of 6500–8000. CV remains, therefore, a rare disease

affecting (according to the European definition) fewer than 1 in 2000 people.

Pathogenetic mechanism

Although the pathogenesis of MC has been the focus of several studies, summarized in previous reviews [24, 34–39], the molecular mechanisms underlying this clinical condition are still poorly understood. By analogy with other autoimmune diseases, the role of B-lymphocyte stimulator, also referred to as B-cell activating factor (BAFF), a cytokine belonging to the tumor necrosis factor family of ligands, has been emphasized [37, 40, 41]. HCV infection is thought to be the early event leading to BAFF upregulation. In addition to overexpression in inflammatory cell-containing portal tracts in liver biopsy tissues [37], circulating levels of BAFF are significantly increased in CV patients [40, 41] and likely serve as an effective costimulatory mechanism sustaining B-cell clonal expansion.

A biological hallmark of CV patients is an antigen-driven, oligoclonal, non-neoplastic expansion of RF-synthesizing B-cells [42, 43]. As these cells carry mutations of IgV heavy and light chains, they are likely to be of germinal center or post-germinal center derivation. In addition, the expanded B-cells seem to originate from selected precursors endowed with anti-IgG specificity. These B-cell clones probably start expanding in the liver and then reach the circulation and other compartments. With few exceptions, the IgM monoclonal components with RF activity exhibit the WA (VH1-69) cross-reactive idiotype and are associated with both the light chain cross-idiotype 17-109 and the heavy chain cross-idiotype G6 [8].

The initial formation of ICs and their ability to immunize the host may result in the formation of T-cell-dependent, high-affinity RF, which can lead to larger, multi-molecular, cold-insoluble ICs. The immunochemical structure of these cryoprecipitating ICs consists of HCV nucleocapsid protein bound to IgG with specific anti-core reactivity, which in turn is linked to IgM directed to their Fc portion [44]. The reason(s) why anti-core IgG is recognized by RF is unknown, but it is conceivable that IgG molecules reactive with other antigens (E1 and E2 envelope glycoproteins and maybe even non-HCV antigens) are also present in the cryoprecipitates.

The deposition and binding of these ICs on the endothelial surface through the C1q receptor results in the generation of vasoactive peptides from the complement system, the recruitment of inflammatory cells, and, eventually, leukocytoclastic vasculitis (Fig. 1a–d). The inevitable consequence is impairment of the vessel lumen and ischemic injury of the tissues supplied by the damaged vessels.

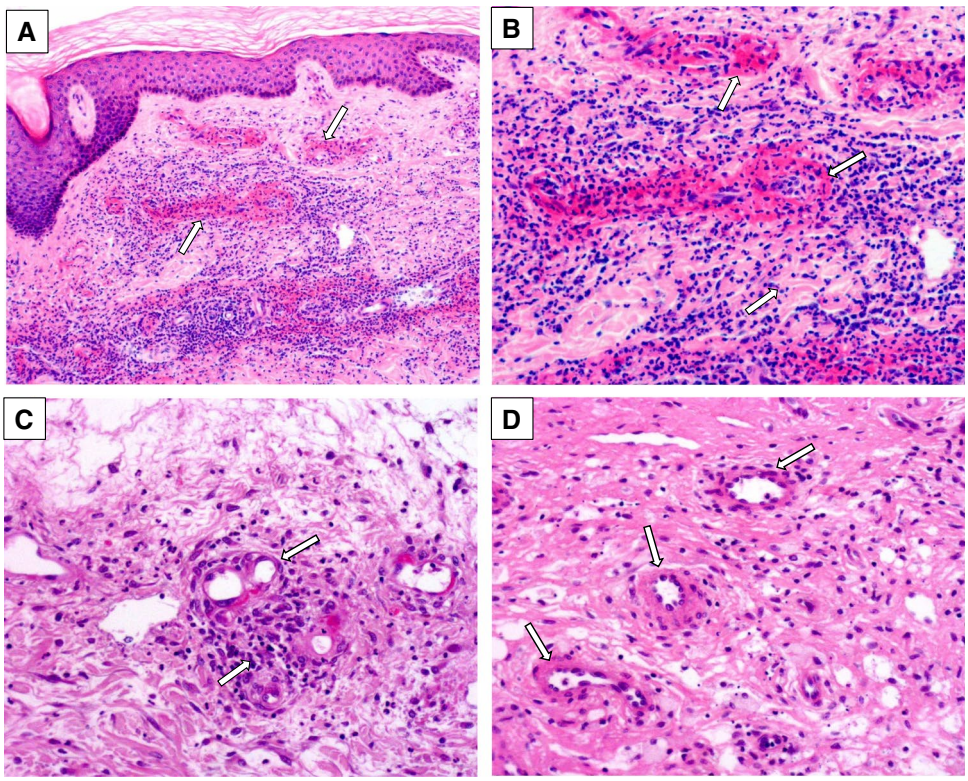

Fig. 1 Skin biopsy from a patient with cryoglobulinemic vasculitis (CV) showing typical features of leukocytoclastic vasculitis. Hematoxylin-eosin staining. **a** ×60: Epidermis is normally preserved, while at the derma level the vessel walls appear thickened by fibrin deposits (arrows). **b** ×120: Neutrophils with "nuclear dust" (leukocytoclasia) can be seen among bundles of connective tissue (arrows). **c** ×120, and **d** ×120: small and medium vessel walls appear covered by eosinophilic fibrinoid material scattered in the edematous derma, in which it is still possible to recognize nuclear dust with the characteristic phenomenon of leukocytoclasia (arrows)

Laboratory findings

The most common laboratory parameters that characterize CV are summarized in Table 2. Routine laboratory tests, including biochemical tests to assess kidney and liver function, are performed in all patients. Given that the large majority of CV patients are HCV-positive, determination of anti-HCV antibodies by enzyme-linked immunosorbent assay, measurement of HCV RNA levels by branched-chain DNA assay, and genotyping by INNO-LiPA are also mandatory. In CV patients who suffer frequent attacks of Raynaud's phenomenon (Fig. 2a), wide-field nailfold videocapillaroscopy (NVC) is performed as a noninvasive technique to ascertain microvascular involvement. NVC examination is, in fact, the most reliable technique to distinguish primary from secondary Raynaud's phenomenon. Abnormalities of distribution, a higher than normal number of shorter capillaries, and increased capillary tortuosity can be detected in 50–60% of CV patients (Fig. 2b, c). Ectasias, megacapillaries, and hemorrhage are of less frequent occurrence and are more typical of the scleroderma pattern [45]. Vascular function abnormalities are also revealed by digital photoplethysmography (Fig. 2d).

When the serum of CV patients is stored at + 4 °C, cryoglobulins form a whitish precipitate at the bottom of the tube, usually within 48–72 h but sometimes first appearing after one week (Fig. 2e, f). The amount of cold-induced serum precipitate varies widely from patient to patient and is quantified as a percentage of the whole serum. By analogy with the hematocrit, it is called the cryocrit, whose quantification is commonly related to clinical severity and can be used in the assessment of the response to treatment [38]. The isolated and purified cryoglobulins are then subjected to immunofixation, to establish their immunochemical structure as single or mixed cryoglobulins. The IgM monoclonal fraction of type-II MC shows (with rare exceptions) RF activity and, as mentioned above, bears the WA cross-idiotype [8].

The measurement of serum viscosity is advisable in all patients with type-I IgM cryoglobulins and in those with type-II cryoglobulins that also contain an IgM monoclonal component, especially when the clinical picture suggests hyperviscosity syndrome. Using the Ostwald method or red blood cell pipette viscosimetry, the results are expressed as the ratio of the time needed for a serum sample to pass through the tube relative to the time for a reference fluid (usually water). When automated methods are employed, the results are expressed in centipoise (cP) units of viscosity (normal values: 1.6–2.2 cP). Although serum IgM levels do not consistently correlate with the presence and degree of hyperviscosity syndrome, clinical features of the latter usually appear at a viscosity ≥ 4 cP.

Clinical symptoms

The clinical features of CV include a wide spectrum of symptoms ranging in severity from the occasional appearance of purpuric eruptions to life-threatening conditions. Figure 3 summarizes the spectrum of clinical

Table 2 Laboratory tests in the study of patients with single-type and mixed cryoglobulinemia (MC)

Laboratory tests	Expected results
Tests for anti-HCV antibodies and HCV RNA	At diagnosis, over 90% of patients with MC (but not of those with single-type cryoglobulins) are positive for anti-HCV antibodies and have variable levels of circulating HCV RNA. No genotype prevalence is usually found
Search of cryoglobulins by storing the patient's serum at + 4 °C from 2 days to one week	Detection of a cryoprecipitate that can redissolve by keeping the serum in a Wintrobe tube at + 37 °C for 1 to 3 h. The amount of the cryoprecipitate is expressed as percentage of the whole serum and is called cryocrit. The highest cryocrit levels are associated with type-I cryoglobulins
Immunofixation of the isolated and purified cryoglobulins	Immunochemical characterization of the cryoglobulins to establish if they are type I, or II or III
Tests for rheumatoid factor (RF) activity	Medium-to-high titers of RF activity are associated with type-II MC; lower titers with type III
Quantitative assessment of complement CH50, C3 and C4	Serum C4 levels are consistently and often remarkably reduced. C3 and CH50 are reduced to a lower extent in types-II and III MC. Variable results in type-I cryoglobulins
Ostwald viscosimetry or red blood cell pipette viscosimetry or automated methods	Serum viscosity can be remarkably increased in 8–10% of patients with type-I monoclonal IgM cryoglobulinemia. Hyperviscosity is a rare event in types-II and III MC
Nailfold videocapillaroscopy	Abnormalities of capillary distribution and morphology are detected in approximately half of the patients with Raynaud's phenomenon and any type of cryoglobulinemia

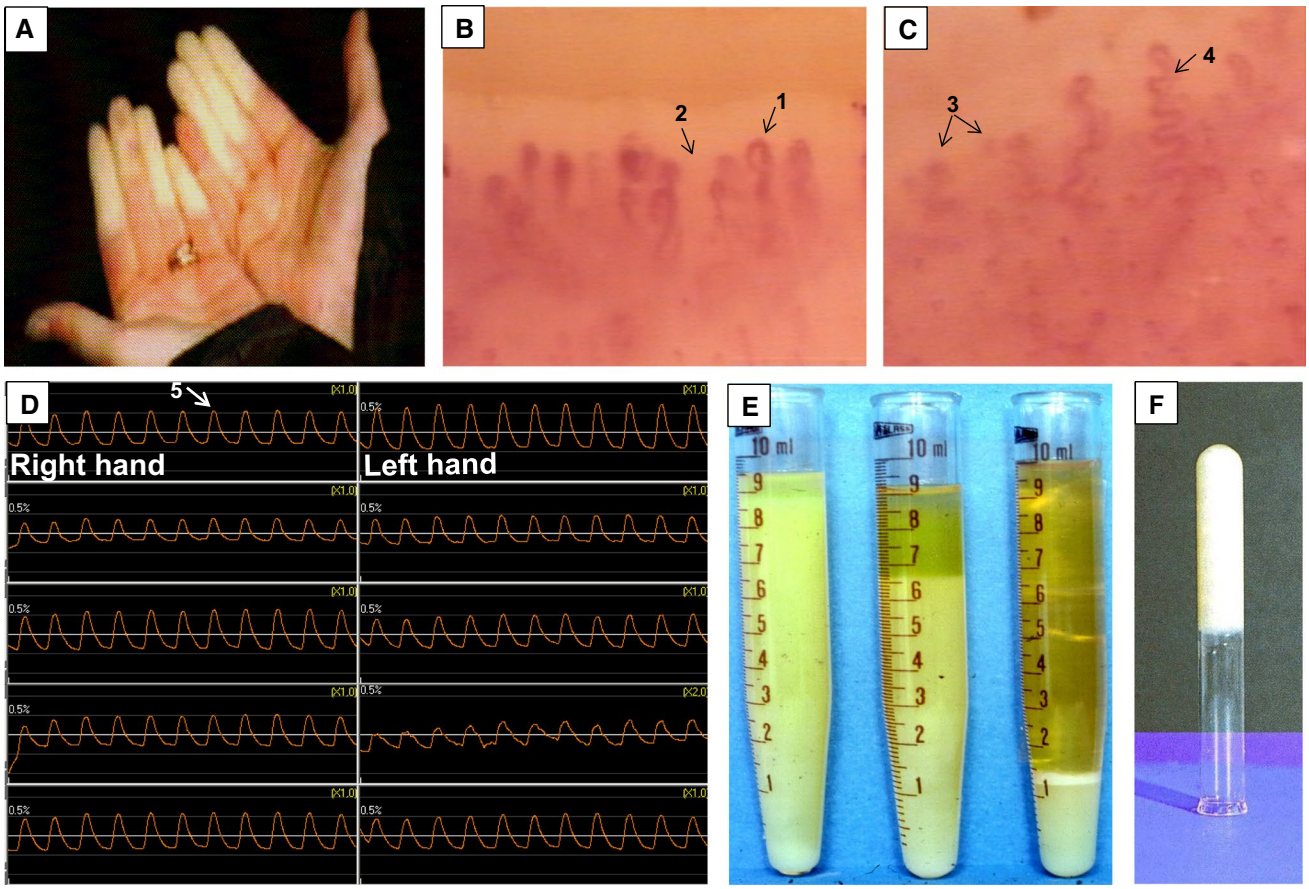

Fig. 2 **a** Severe Raynaud's phenomenon in a patient with CV, characterized by episodic vasospasm of the peripheral vessels in response to cold exposure, resulting in extremely pale appearance of the fingers. **b** and **c** Nailfold videocapillaroscopy showing abnormalities of variable severity in patients with CV. Notice irregular enlargement of the capillaries (arrow 1), disorganization of the normal capillary array, loss of capillaries (arrow 2), ramified capillaries (arrow 3), and capillary loop with typical 'ball' feature (arrow 4). **d** Digital photoplethysmography revealing alterations of the dicrotic wave (arrow 5) with disappearance of the pre-diastolic notch after exposure of both hands at 4 °C in a refrigerated box for 15 min. **e** Variable amounts of cold-induced serum cryoglobulins in 3 patients with HCV-related CV. **f** In another patient, the serum appears totally gelified, so that the tube can be turned upside down without the serum being poured out

symptomatology on the basis of our observations of a large cohort of 424 patients with HCV-related CV.

Although asthenia was recorded in only 48% of our patients (a figure that is largely underestimated for the incomplete collection of clinical data in the first years of enrollment), the triad purpura/arthralgia/asthenia is reported to occur in the large majority of patients and is thus considered a hallmark of CV [15, 38]. Many other features can be detected with variable frequency. Due to the recurrent bouts of palpable purpura, prevalently involving the legs, feet, and to a lesser extent the thighs (Fig. 4a–e), hemosiderin deposits gradually accumulate at the sites of purpuric eruptions. This results in a red-brownish hyperpigmentation of the lower limbs whose appearance in most patients is so typical that it alone is considered diagnostic of CV (Fig. 4f). Other common features are acrocyanosis, livedo reticularis, non-healing ulcers (Fig. 4g, h) in the lower extremities, and the pale appearance of the nose and auricles following cold exposure.

Approximately, half of CV patients will develop kidney involvement during the course of their disease, with a severity ranging from nephrotic syndrome and membranoproliferative glomerulonephritis to less frequent conditions of glomerulosclerosis and end-stage renal failure [38, 46] (Fig. 5). Proteinuria of variable degree, microscopic hematuria, granular and/or erythrocyte casts, increased serum creatinine levels, and arterial hypertension are also of relatively frequent occurrence.

The possible involvement of the central and peripheral nervous systems in HCV-associated CV has been addressed in several reports. An example of the neurological abnormalities that can be detected in isolated instances of CV is shown in Fig. 6. As stroke is a rare event, for the sake of brevity we will restrict our discussion to motor-sensory

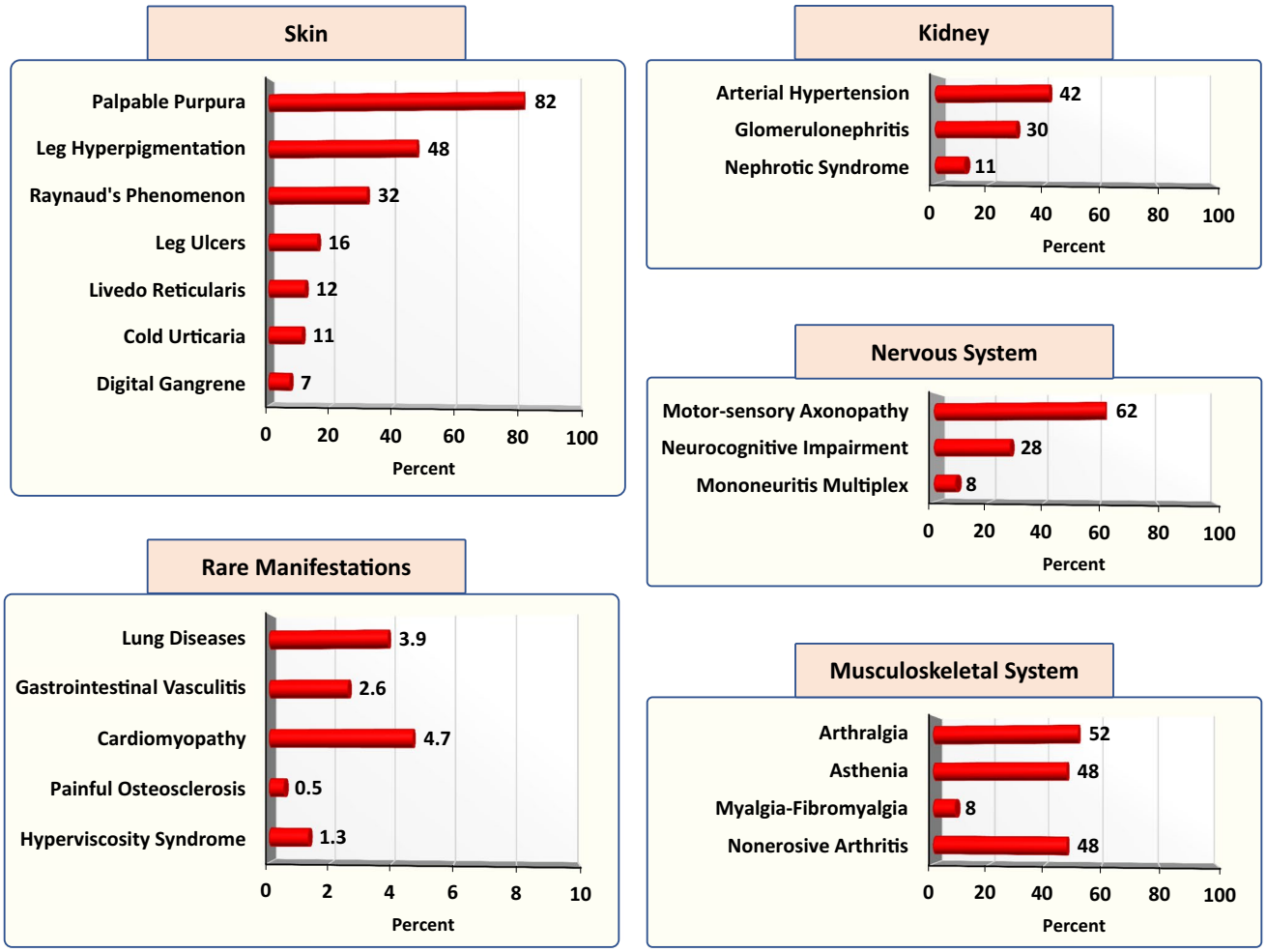

Fig. 3 The spectrum of clinical features observed at diagnosis in 424 patients with HCV-related CV, collected in the period from January 1991 to June 2018

axonopathy, which occurs in ~60% of CV patients [44]. The symptoms include a burning sensation in the legs associated with diffuse paresthesias as well as neurocognitive and neuropsychiatric disorders [47, 48]. In one study, a deficiency in one or more of the ten cognitive domains examined was detected in 89% of the patients tested, with attention (70%), executive functions (44%), visual construction (37%), and visual spatial functions (33%) as the domains most commonly involved [47]. Among the signs and symptoms of neuropsychological dysfunction, impairment in executive function, sustained attention, working memory, verbal learning and verbal recall have been reported in patients with chronic HCV infection. These alterations occur independently of HCV genotype and stage of liver disease, and are not associated with abnormalities on conventional brain magnetic resonance imaging [48]. Sexual dysfunction, emotional distress, and a higher risk of depression, anxiety, somatization, and insecurity as well as fatigue and sleep disorders have also been noted [48].

Additional, albeit less common manifestations of CV should also be mentioned. Pulmonary involvement (< 5%) may consist of exertional dyspnea, dry or productive cough, hemorrhagic alveolitis, and interstitial lung fibrosis, with or without pleural effusions. Isolated instances of bronchiolitis obliterans organizing pneumonia have been described as well [49]. These respiratory symptoms are likely related to the development of vasculitis involving the small arteries, capillaries, and venules [35, 38]. Gastrointestinal vasculitis involving the small and medium-sized vessels may result in intestinal ischemia, with subjective signs such as abdominal pain and bloody stools that can mimic an acute abdomen [50]. Acute pancreatitis or cholecystitis may be mistakenly diagnosed in these patients [38]. CV-induced heart failure, reversible dilated cardiomyopathy, and hypertrophic cardiomyopathy, probably reflecting myocardial vessel disease, have been additionally reported [51–53].

Finally, mention should be made of the rare, singular occurrence of painful osteosclerosis, characterized by a

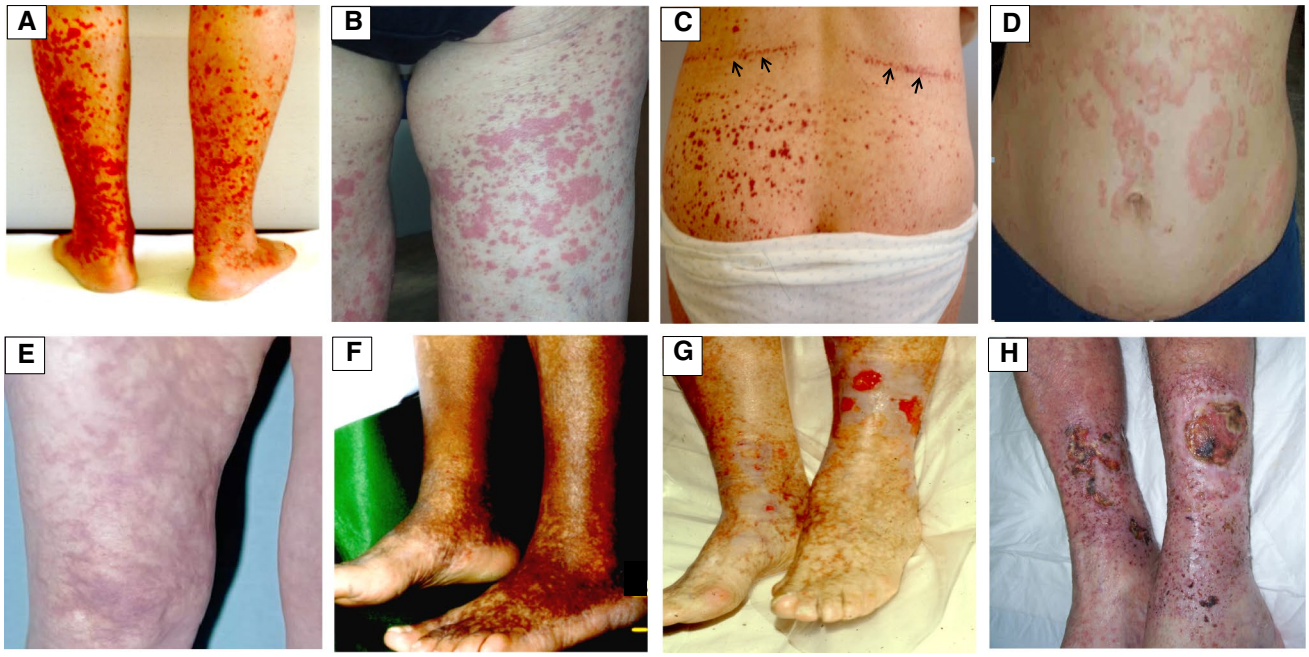

Fig. 4 **a** Crops of palpable purpura, the clinical hallmark of CV, are present on the lower extremities, possibly as a consequence of venous stasis and environmental exposure that can enhance cryoglobulin precipitation. **b** Confluent purpuric eruptions extensively involving the thighs. **c** Buttocks can be involved less frequently, and pressure related to the elastic band of the panties is able to induce the appearance of linear purpura (arrows). **d** Urticarial vasculitis is characterized by recurrent urticarial wheals that tend to persist for more than 24 h and induce a burning rather than itching sensation. **e** Sometimes the skin lesions of urticarial vasculitis may resemble the ring-like mottling of livedo reticularis. **f** The deposition of hemosiderin at sites repeatedly affected with purpuric eruptions over the years may result in the local appearance of a brownish, irreversible pigmentation that can by itself raise the suspicion of CV. **g**, **h** Torpid, long-lasting ulcers may appear distally on the legs, are usually painful and bilateral, and may require double-filtration plasmapheresis in addition to the etiological treatment for their healing

highly increased bone mass [54] that, by stretching the richly innervated periosteum, causes bone pain. These patients typically complain of pain affecting several bone districts, whereas the joints are spared and motion is not affected. Thickening and sclerosis of the diaphyseal cortical bone can be detected on plain radiographs [55, 56], and serum levels of bone alkaline phosphatase activity are remarkably increased. The onset of HCV-associated osteosclerosis has been attributed to an imbalance of the osteoprotegerin/receptor activator of nuclear factor-kB ligand [57, 58]. We have found only two CV patients with osteosclerosis, who were incompletely studied because rapidly lost to follow-up, but no clues are so far available as to the potential role of CV/MC in the pathogenesis of osteosclerosis.

The clinical features of hyperviscosity syndrome can be recognized in 10–15% of patients with type-I cryoglobulinemia but they are rare in those with type-II and even rarer in those with type-III cryoglobulinemia. Patients usually describe a variable combination of symptoms that includes blurred vision, recurrent epistaxis, headaches, tinnitus, vertigo, and dizziness. In a few patients with severe hyperviscosity syndrome, confusion, ataxia, and heart failure may be present. Funduscopic examination reveals vessel tortuosity, marked retinal venous engorgement with a "sausage-like" appearance of the affected vessels, and, in some cases, retinal hemorrhage [38, 59, 60].

Among the extra-hepatic manifestations of HCV infection, thyroid disorders (including autoimmune thyroiditis, hypothyroidism, and papillary thyroid cancer) as well as type-2 diabetes mellitus have been described with relative frequency in patients with or without CV. The appearance of the different clinical phenotypes has been considered a multifactorial and multistep process that implies the contribution by genetic and environmental factors [61, 62]. The immunopathogenesis of these associations has been ascribed to HCV infection of thyrocytes or pancreatic beta cells, resulting in the upregulation of CXCL10 secretion by the same cells and the recruitment of Th1 lymphocytes. In genetically predisposed patients, a Th1 response is associated with the increased production of interferon (IFN)-γ and tumor necrosis factor-α. These cytokines stimulate CXCL10 secretion by target cells, thereby perpetuating the immune cascade and thus the dysfunction of thyrocytes and/or beta cells, leading to thyroid autoimmunity and type-2 diabetes, respectively [61, 62].

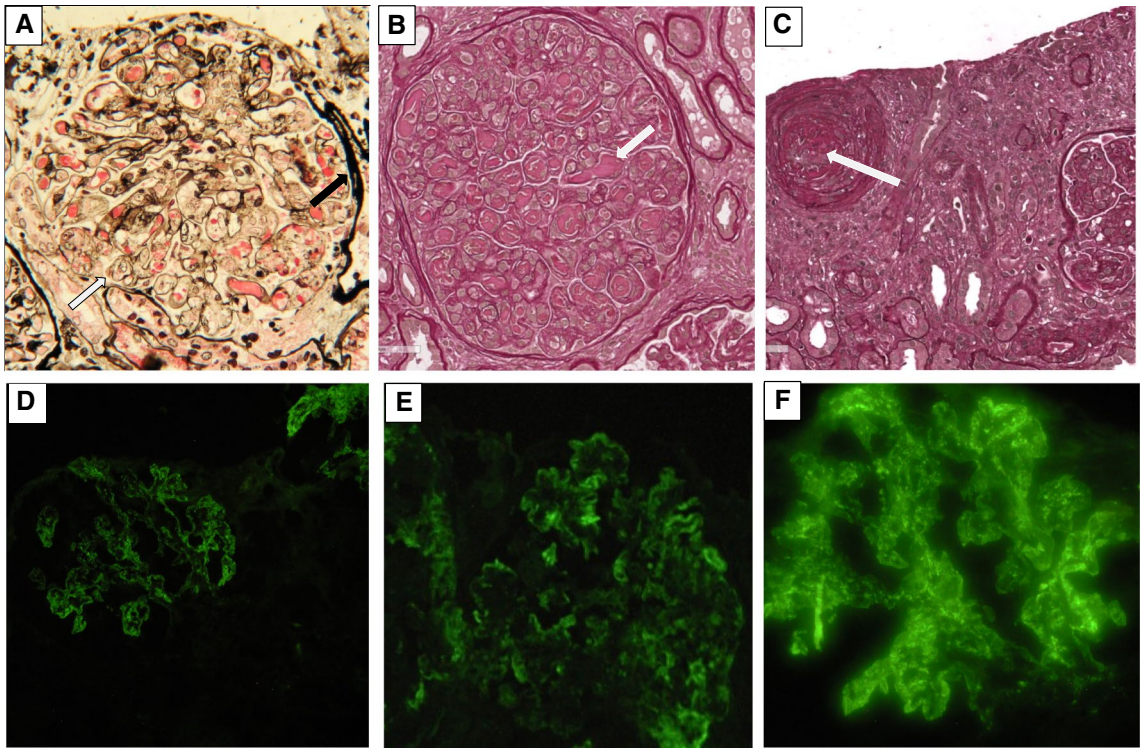

Fig. 5 Kidney biopsy from a patient with cryoglobulinemic glomerulonephritis. **a** A glomerulus showing mesangial and endocapillary proliferation with "double contours" (black arrow) of the glomerular basement membranes and segmental cellular interposition (membrano-proliferative pattern) (white arrow). Several thrombi are present within capillary lumens (Jones Silver stain, ×400). **b** PAS staining showing strong periodic acid positivity within the thrombi revealing the endoluminal presence of cryoglobulins ("pseudothrombi") (arrow) (PAS staining, ×400). **c** A small artery showing accumulation of periodic acid positive material in the capillary lumen and within the vessel wall (arrow), accompanied by lymphomonocytic infiltrate (PAS staining, ×400). From D to F: immunofluorescence staining of renal biopsy samples from patients with cryoglobulinemic glomerulonephritis. **d** Subendothelial IgG deposition along glomerular basement membranes and within mesangial areas with a coarse granular pattern (anti-IgG, ×200). **e** Subendothelial IgM deposition along glomerular basement membranes and within mesangial areas with a coarse granular pattern (anti-IgM, ×200). **f** Subendothelial C3 deposition along glomerular basement membranes and within mesangial areas with a coarse granular pattern (anti-C3, ×400)

In a previous prospective study carried out on 184 HCV-positive patients, we determined that renal failure, neurologic involvement, and B-cell NHLs (but not hepatocellular carcinoma [HCC]) were significantly more frequent in patients with than without CV [16]. On the basis of the experience gained from relatively large cohorts of CV patients, the following conditions should be considered potentially life-threatening: (a) infectious complications favored by the administration of glucocorticoids (GCs) and immunosuppressive drugs; (b) membrano-proliferative glomerulonephritis with renal failure; (c) cerebral ischemia or cerebral hemorrhage; (d) gastrointestinal hemorrhage, intestinal ischemia, or acute pancreatitis; (e) myocardial infarction from coronary involvement; and (f) respiratory failure [16, 35, 38].

In a cohort of 231 CV patients, the 10-year cumulative survival rate, computed using the Kaplan-Meier method, was 56.3%, compared with 93.4% in the sex- and age-matched general Italian population [63], and 63% in another large cohort of French patients [64]. According to our own experience, after 15 years of follow-up of our CV patients, the cumulative survival rate was 70.2%, with the survival duration significantly better in CV patients receiving antiviral therapy and achieving a sustained virologic response (SVR) than in those who were not treated [16]. During the same 15-year follow-up, the mortality rate was 29.7%. Death was mainly related to serious infections enhanced by concomitant immunosuppressive agents but also to end-stage liver disease and renal insufficiency. Heart and respiratory failure were less frequent causes [16]. There are, as yet, no data on the effects of the recently introduced direct-acting antiviral agents (DAAs) on the long-term survival of HCV-positive CV patients, but the use of these drugs is expected to greatly improve the overall survival of CV patients. In a French study, the death rate decreased from 24.4% in the pre-DAA era to 14.8% in the DAA era [65].

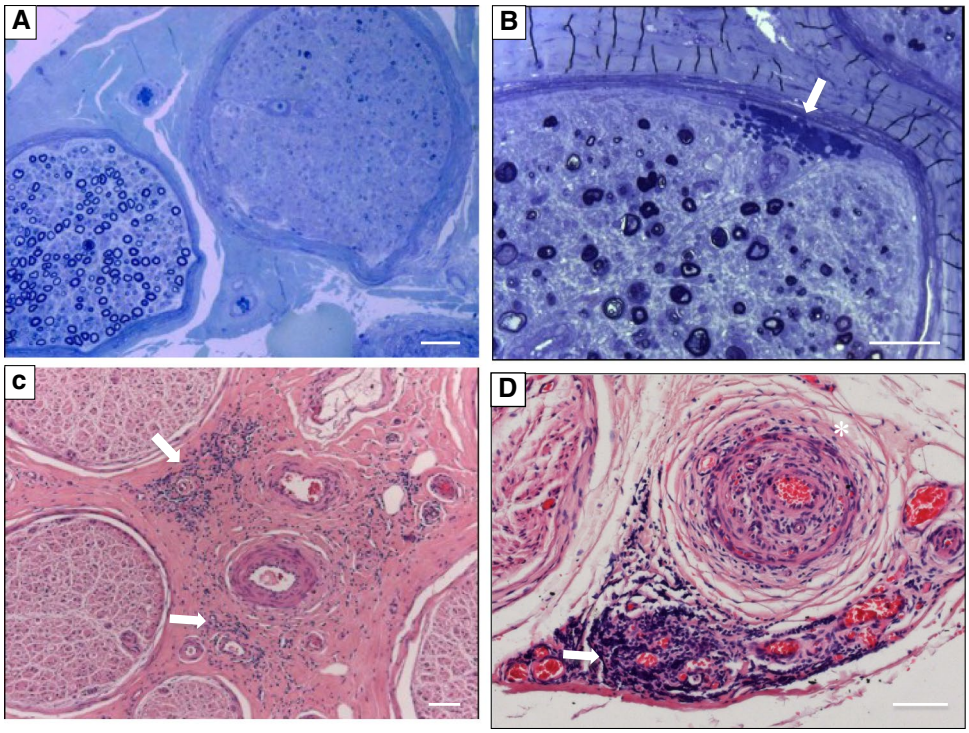

Fig. 6 Neuropathological changes in sural nerves of patients with HCV-related mixed cryoglobulinemia and neuropathy. **a** Plastic semi-thin sections stained with toluidine blue show unequal depletion of myelinated nerve fibers in two nerve fascicles, representing a pathological clue of vasculitic neuropathy. **b** Ongoing wallerian-type degeneration involving most of myelinated fibers and perivascular endoneurial purpura with red cells extravasation (arrow) in a nerve fascicle. **c** Paraffin-embedded nerve sections stained with hematoxylin and eosin showing extensive epineurial mononuclear cell infiltrates (arrows) with preferential involvement of small vessels. **d** Epineurial artery with luminal occlusion, recanalization and neovascularization, consistent with inactive vasculitis (asterix), and massive epineurial infiltrates with diffuse small-sized vessels involvement. Bar = 50 µm

Cryoglobulinemia and the risk of progression to liver cirrhosis, HCC, or NHL

Since the large majority of CV patients are HCV-positive, the natural history of the pathology induced by this hepatotropic virus includes a possible progression to liver cirrhosis and HCC. Among our HCV-positive patients, the 15-year cumulative probability of developing cirrhosis, diagnosed by a variable combination of clinical features, liver histology, elastography, contrast-enhanced ultrasonography, and computed axial tomography, was 15.4% in those with and 26.7% in those without MC ($p < 0.005$). The corresponding rates for the occurrence of neoplastic progression to HCC were 10.6% and 20.3% ($p = 0.001$) [16]. Why progression to liver cirrhosis and HCC is slower in HCV-infected patients with MC than in those lacking circulating cryoglobulins is unclear, given that the mean levels of circulating HCV RNA, viral genotypes, tumor stage, frequency of HCC nodules, and treatment with IFN-α-based regimens are comparable in the two groups of patients. The explanation may perhaps lie in the fact that these sequelae are more common in male HCV patients, while CV/MC affects more females (3.3-fold excess).

A striking and interesting observation is the reported association between HCV infection, in patients with or without CV, and B-NHL (Table 3). However, a meta-analysis of epidemiologic studies reported wide geographic variations [75], with the prevalence of the association between HCV infection and B-NHL reflecting differences in the prevalence of HCV infection. Thus, while several studies have documented progression from infection to NHL in 5% of patients in Italy [76] and 5.8% in Spain [66], the rates in northern Europe and North America are significantly lower [67–69, 77] and thus suggest a role for genetic and/or environmental factors in this process.

The most common NHL histotypes include low-grade marginal zone lymphoma (MZL) of splenic origin and diffuse large B-cell lymphoma (DLBCL). On the contrary, based on the odds ratio and 95% CI shown in Table 3, there is no significant association with follicular lymphoma [68, 70] (Table 3). In addition, two subgroups of DLBCL have been identified: DLBCLs of new onset and aggressive DLBCLs that are likely derived from the transformation of

Table 3 Risk for progression of HCV-positive cryoglobulinemic vasculitis (CV) to B-cell non-Hodgkin lymphoma (B-NHL): epidemiologic, histological and clinical features

HCV-positive B-NHLs	Description	References
Epidemiology	Increased risk in geographic areas with elevated prevalence of HCV infection. Approximately, 8 to 10% of CV patients eventually develop a lymphoproliferative disorder, whose risk has been calculated to be 35 times higher than in the control population. Odds ratio (OR) 2.4; 95% confidence interval (CI) 2.0–3.0. Conversely, compared with controls, the prevalence of HCV infection is significantly higher in NHL patients. OR 1.6; 95% CI 1.3–1.9	[66–71]
Histological subtypes of most frequent occurrence	(A) Low-grade marginal zone lymphoma (MZL), in particular of splenic origin OR 2.47; 95% CI 1.44–4.23 (B) Diffuse large B-cell lymphoma (DLBCL) (a) of newly onset (b) from transformation of MZL OR 2.24; 95% CI 1.68–2.99 (C) Lymphoplasmacytic lymphoma OR 2.57; 95% CI 1.14–5.79 (D) Follicular lymphoma OR 1.02; 95% CI 0.65–1.60	[68, 71]
Peculiar clinical features	(A) B-NHL is likely preceded by chronic hyperplastic lymphadenopathy (B) It occurs after 15 or more years of chronic HCV infection (C) Extranodal sites, including spleen and salivary glands, are involved with frequency higher than in the HCV-negative counterpart (D) The International Prognostic Index and the subsequently proposed "HCV Prognostic Score" as well as serum LDH are usually high in the DLBCL subtype (E) Indolent B-NHLs, especially of MZL subtype, are responsive to IFN-α-based and DAA-based antiviral therapy in 70 to 80% of the patients	[10, 71–74]

MZLs. The latter phenomenon occurs more frequently in HCV-positive than in HCV-negative patients [78].

At least two observations strongly argue for an important role of chronic HCV infection in NHL development: First, in HCV-infected patients with splenic B-NHL characterized by villous lymphocytes, the achievement of SVR following treatment with IFN-α2b, with or without RBV, was associated with a regression of the lymphoma, whereas none of the HCV-negative NHL patients in that study had a response to IFN therapy [10]. These results were later confirmed in larger cohorts of patients treated with IFN or DAAs [71, 79]. Second, in a retrospective study from Japan, the rate of NHL development in a cohort of untreated patients steadily increased, reaching 2.6% in the 15th year of observation, whereas in treated patients who had achieved a SVR the rate at the same time point was 0% [80]. These observations suggest a preventive effect of SVR against the onset of NHL.

The pathogenetic mechanisms underlying this relationship are still poorly defined (Fig. 7). Among the possible pathways of transformation, each acting alone or variably combined with the others, microenvironment-driven lymphomagenesis, resulting from the persistent stimulation of lymphocyte receptors by HCV antigens, is of particular relevance. As emphasized in our previous review [15], the key pathogenetic event of this pathway is oligoclonal B-cell expansion, resulting in the potentially pre-lymphomatous condition seen in CV patients. HCV-E2 and NS3 proteins are likely the chronic inciting stimuli that induce this benign proliferation. Through a multistep process, occurring in a subgroup of CV patients, sequential genetic translocations result in the activation of proto-oncogenes and, consequently, a subversion of T-cell regulatory functions [81]. The patient's genetic background should therefore be considered a predisposing factor in the onset of the lymphomatous process. Progression from an indolent lymphoproliferation to lymphomagenesis would then occur as the end-point of a multi-stage process in genetically prone patients.

A second theory involves HCV replication in B-cells, in which oncogenic transformation is mediated by intracellular viral proteins. Although direct oncogenic effects exerted by HCV replication in B-cells is an intriguing hypothesis, HCV RNA negative strands, the viral replicative intermediates that indicate active replication of HCV in human lymphocytes in vivo, have been detected in some studies [82] but not in others [83, 84] nor in our own analyses of HCV-NHL tissue [85].

A "hit and run"-mechanism, in which mutations in tumor suppressor genes are induced by the presence of a transiently intracellular virus, has also been proposed. Accordingly, the transforming B-cell damage is not associated with viral replication inside tumor cells. Rather, in this scenario, HCV induces a mutator phenotype through alterations in proto-oncogenes and tumor suppressor genes, leading to the neoplastic transformation of B-cells, even if the virus

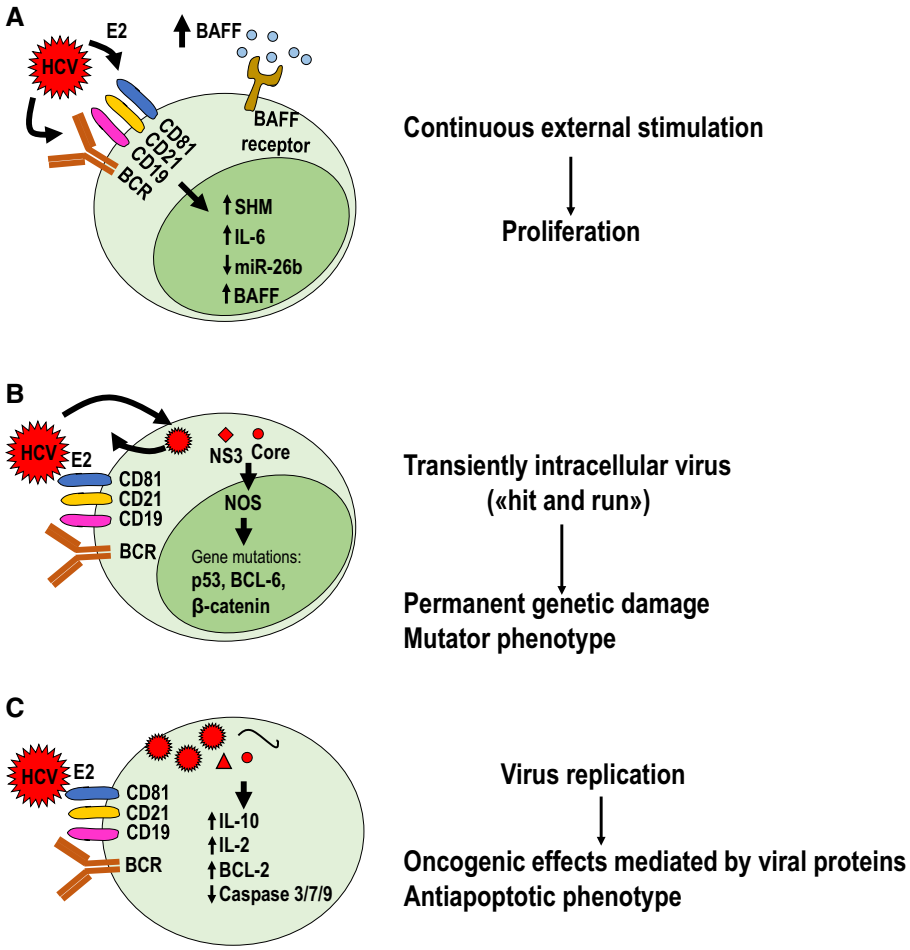

Fig. 7 Putative mechanisms of HCV-related lymphomagenesis. **a** Chronic stimulation of lymphocyte receptors (CD19, CD21, CD81) and B-cell receptor (BCR) by viral antigens and consecutive B-cell proliferation. Binding of HCV envelope glycoprotein E2 to CD81 facilitates the assembly of the CD81/CD19/CD21 costimulatory complex with lowering of the B-cell activation threshold, and induce somatic hypermutation (SHM) of the immunoglobulin gene locus. Evidence of upregulation of oncogenic signals (IL-6 and BAFF) and downregulation of tumor suppressive signals (miR26b). **b** "Hit and run"-mechanism. A transient intracellular presence of HCV core protein and non-structural protein 3 (NS3) induces a mutator phenotype by causing production of nitric oxide synthase (NOS), DNA damage, and subsequent mutations in tumor suppressor genes (p53, BCL-6 and β-catenin). **c** HCV replication within B-cell. The expression of intracellular viral proteins results in an anti-apoptotic phenotype by overexpression of IL-10, IL-2, and BCL-2 and downregulation of caspases 3, 7 and 9

is no longer detectable in the cells [81, 86]. This model, however, conflicts with the finding that B-cells from chronic HCV carriers do not display mutations in the cancer-related genes TP53, CTNNB1, and BCL6 [87] and with the complete regression of HCV-associated lymphomas after antiviral therapy [74].

Front-line therapy and response assessment

Given the heterogeneity and variable severity of cryoglobulin-related illnesses, the extent of treatment will not be the same in all patients. For many years, due to a lack of knowledge regarding its etiology, clinically active CV was treated empirically with GCs and cytotoxic drugs, including chlorambucil, azathioprine, and cyclophosphamide, with no or only partial and transient clinical efficacy. The effectiveness of an antiviral agent such as IFN-α was first recognized empirically [6, 88], with the rational basis of this response then becoming apparent when the large majority of CV patients were shown to be HCV-positive [17–20]. IFN-α, administered alone or with GCs, was shown to induce clinical, biochemical, and virologic responses in 40–60% of patients [89, 90], although a high relapse rate was found to occur within 6 months from the completion of treatment [91].

Addition of the nucleoside antimetabolite ribavirin (RBV) to pIFN-α led to a higher rate of complete clinical response

and SVR. This combination thus became the standard of care, although the daily dose of RBV had to be reduced in patients with renal impairment. Moreover, several host and viral factors, such as obesity, advanced age, alcohol consumption, high viral load, and difficult-to-treat viral genotypes (1 or 4), were found to be associated with a poor response to treatment or no response at all [39, 92].

A significant therapeutic advancement followed two key observations: (a) CV involves an HCV-driven, oligoclonal, non-neoplastic expansion of intrahepatic B-cells that spontaneously synthesize RFs and display the cross-reactive idiotype WA [42, 43]; (b) CD20-positive cells are expanded and activated in CV patients [93, 94]. A rational therapeutic approach therefore relied on the deletion of B-cell clonalities and could be realized with the development of rituximab (RTX), a chimeric monoclonal antibody specifically directed against the CD20 antigen expressed on pre-B and mature B lymphocytes.

In the first of two pioneering studies [11], RTX was administered to HCV-positive CV patients who had become refractory to conventional antiviral therapy or had relapsed after therapy. A major improvement in clinical signs, a significant reduction in RF activity, disappearance or remarkable reduction of cryoglobulins, and an increase in mean serum levels of C3 and C4 were achieved in 80% of the treated patients, with the response maintained throughout one year of follow-up in 75%. In the second study [95], published consecutively with the first one, 12 HCV-positive CV patients, 3 of whom also had low-grade NHL, were given RTX together with medium-to-low doses of GCs. Significant improvements in both the clinical symptoms and the cryoglobulin level were observed in the majority of these patients, including the subset with NHL. In addition, a complete response was achieved in a patient with glomerulonephritis of recent onset.

From these and subsequent studies, summarized in a comprehensive review [96], the following important points can be noted: First, RTX is an effective and safe biologic agent, with acceptable side effects, that should be considered for the treatment of CV patients who have become resistant to, or relapsed after, conventional antiviral therapy. Second, in the large majority of responding patients, a conversion of B-cell populations in the bone marrow, liver, and peripheral blood from oligoclonal to polyclonal can be shown by clonal analysis [97–99].

A question repeatedly raised in the IFN + RBV era was whether RTX should be given to CV patients from the very beginning or restricted to second-line therapy. With the advent of the DAAs era, the answer has been thoroughly reconsidered. Although the use of first-generation NS3/4A protease inhibitors, such as telaprevir or boceprevir, was short-lived because of their high rate of side effects [100], the subsequent introduction of newer-generation, highly effective, well-tolerated DAAs marked a milestone in the standard of care of chronically HCV-infected patients. The arsenal of IFN-free, all-oral, pan-genotypic drugs is steadily expanding. The response rates (SVR) obtained with these drugs in both easily treated and difficult-to-treat patients have been exceptional, often exceeding 95% when they are given once daily as single or co-formulated drugs in first-line treatment for 8 or 12 weeks.

DAAs, given alone or in variable combinations, are included in the recommendations of the European Association for the Study of the Liver (EASL) and the American Association of the Study of Liver Disease (AASLD) for the treatment of chronically HCV-infected patients [101, 102]. Therapies resulting in SVR are associated with a 74% reduction in mortality of any cause, an 85% decrease in the onset of HCC, and a 93% decline in liver-related mortality and the need for liver transplantation [103].

Impressive positive therapeutic results have been obtained with the administration of DAAs in patients with MC (and thus, by definition, asymptomatic) and in those with active CV. With the deliberate omission of single case reports, Table 4 summarizes the results of the major studies of cryoglobulinemic patients treated with variable combinations of DAAs. Based on the experience derived from those studies, the following points should be emphasized: (a) Unless a different approach is necessitated by a rapidly progressive and life-threatening condition, DAAs should be considered as the front-line, etiologic therapy for patients with HCV-positive MC, with or without CV; (b) approximately 95% of treatment-naïve and treatment-experienced (relapsed or resistant to pIFN ± RBV) patients will likely achieve SVR/12; (c) complete clinical and immunological responses can be expected in 40–95% of patients, with limitations of improvement possibly conditioned by factors such as coexistent NHL, cirrhosis, renal disease, duration of HCV infection, greater severity and a more advanced stage of CV; (d) the safety and tolerability of the large majority of DAAs are very good, with only a few cases of a moderate increase in serum bilirubin and transaminase levels and rare reports of photosensitivity and drug–drug interactions.

Contraindications are limited to simeprevir and paritaprevir in patients with liver cirrhosis Child-Pugh B or C, and to sofosbuvir in those with renal failure and an estimated glomerular filtration rate < 30 ml/min [104]. Detailed recommendations for the use of DAAs, the levels of evidence and of agreement, and the strength of the recommendations were submitted in a recent report by the International Study Group of Extrahepatic Manifestations Related to HCV [105].

Given the high rate of SVR, achieved by nearly all CV patients treated with last-generation DAAs, the role of non-etiologic agents is under critical consideration. RTX, either at a dose of 375 mg/m^2 once a week for 4 weeks, as described above, or at a lower, reportedly equally effective

Table 4 Efficacy of IFN-free, all-oral direct-acting antiviral agents (DAAs) in the therapy of HCV-related mixed cryoglobulinemia: an overview of results in chronologic order of publication

Number of patients (M/F)	% of genotype-1 (1a + 1b)	Therapeutic regimen (% of treated patients)	SVR/12[a] %	Immunologic response[b] %	Clinical response[c] %	References
12 (7/5)	58.3	Sofosbuvir/simeprevir (67) Sofosbuvir/RBV (33)	83	89	50	[13]
24 (13/11)	50	Sofosbuvir/RBV (100) [plus RTX in 7 patients]	74	Not clearly defined	87.5	[111]
44 (16/28)	52	Sofosbuvir/RBV (41) Sofosbuvir/simeprevir (27) [±RBV] Sofosbuvir/daclatasvir (9) [[±RBV]] Sofosbuvir/ ledipasvir (23) [±RBV] [plus a reduced dose of RTX in 2 patients]	100	95[d]	95[d]	[112]
7 (4/3)	42.8	Paritaprevir/ ritonavir/ombitasvir/dasabusvir (29) Sofosbuvir/RBV (29) Sofosbuvir/daclatasvir (28) Sofosbuvir/simeprevir (14)	100	57	29	[113]
64 (24/40)	95.3	Paritaprevir/ ritonavir/ombitasvir/dasabusvir (31) Sofosbuvir/ledipasvir (28) Sofosbuvir/simeprevir (6) Simeprevir/daclatasvir (6) Sofosbuvir/daclatasvir (5) Grazoprevir/elbasvir (5) Faldaprevir/daleobuvir (3) [+RBV in 70% of the whole cohort] [pIFN + DAAs] (16)	93.7	48	71	[114]
22 (8/14)	63.6	Sofosbuvir/RBV (64) Paritaprevir/ ritonavir/ombitasvir/dasabusvir (14) [± RBV] Sofosbuvir/ledipasvir (18) Sofosbuvir/daclatasvir (4)	100	82	73	[115]
83[e] (51/32)	83	Sofosbuvir/RBV (14) Sofosbuvir/simeprevir (26) Sofosbuvir/ledipasvir (50) [± RBV] Paritaprevir/ ritonavir/ombitasvir/dasabusvir (10)	92.4	47.1	38.8	[116]
41 (19/22)	60.9	Sofosbuvir/daclatasvir (100)	100	50	90.2	[117]

[a]Undetectable HCV RNA levels 12 weeks after therapy completion

[b]Complete immunologic response was defined as no detection of circulating cryoglobulins and normalized levels of complement C4 and rheumatoid factor; partial immunologic response as > 50% reduction of cryocrit and rheumatoid factor titer, and improved levels of C4

[c]Complete clinical response was defined as regression of clinical manifestations of vasculitis; partial clinical response as improvement in some but not all organs involved at baseline

[d]Partial vasculitis response and roughly 50% reduction of cryocrit was observed in 2 patients with NHL

[e]66 patients received pIFN-free and 17 patients pIFN-containing regimens. The therapeutic regimen of the second group is not mentioned because it does not fall within the aim of the present table

dose of 250 mg/m^2 given twice one week apart [106], should be limited to the following situations: (a) the few CV patients who are resistant to or who relapsed after DAA administration; (b) with or without chemotherapy, patients who develop B-NHL and are unresponsive to DAAs alone; (c) as initial treatment in patients with rapidly progressive or life-threatening CV, with or without additional measures such as GCs, cyclophosphamide and plasmapheresis and followed by etiologic treatment with DAAs once the hyperactive, dangerous phase has been successfully addressed; (d) patients in whom, despite successful HCV treatment after DAA administration, CV persists or relapses and the oligoclonal B-cell populations from the peripheral blood, bone marrow, and liver compartments are not converted to polyclonal populations.

Double-filtration plasmapheresis merits a brief mention. Its most obvious applications include the removal of both the cryoprecipitating proteins and the viral particles in CV patients with hyperviscosity syndrome. Strikingly positive results have been reported [107, 108], although multicenter, randomized studies comparing plasma exchange with either placebo or immunosuppressive therapy are not available [107]. An additional application is the treatment of CV patients with inveterate, indolent leg ulcers, in whom the

procedure may result in progressive scarring and eventual complete wound healing [24].

DAAs and the risk of HCC

The success associated with DAAs has met with worldwide enthusiasm, given that, compared with the previous pIFN-α + RBV standard of care, the achievement of viral clearance and SVR in > 95% of chronically HCV-infected patients, in a shorter time frame and with an improved side-effect profile, has represented a major breakthrough. However, sobering news came from the Barcelona Clinic Liver Cancer Group [109]. In their study of 103 patients with HCV infection and a prior history of treated HCC who achieved SVR after DAA treatment, HCV clearance was accompanied by *de novo* or recurrent HCC in a large percentage of patients (27.6%). Several mechanisms behind tumor relapse, that are not discussed here because they are outside the aim of the present review, have been hypothesized [110].

The need for caution should be noted by the entire hepatology community, including those clinicians treating CV patients, given that the large majority of these patients are in fact HCV-positive and the administration of DAAs has now replaced conventional pIFN-α + RBV therapy. An overview of the clinical and molecular data on the long-term role of DAAs can be found in recent reviews [118–121]. In a large, prospective, population-based study of almost 4000 HCV-positive patients treated with DAAs and followed-up for > 500 days, the risk of new-onset HCC during the first year was not higher than in untreated patients and it declined thereafter. It was therefore suggested that the early appearance of HCC reflects preexisting, microscopic, and at the time of study enrollment undetectable tumors [122].

Until further randomized and controlled studies resolve this critical issue, ongoing HCC surveillance should be conducted in all patients with established cirrhosis, with or without CV, as well as in those already treated for HCC.

Non-HCV-related CV

In a minority of patients with CV, HCV infection cannot be demonstrated. In the following, we refer to patients with the usual, largely prevalent HCV-positive CV as type-A and those who persistently test HCV-negative as type-B. The prevalence of the latter in a relatively early Italian study was 15.9% [123], but according to our more recent experience and that of a French group it is 6.7% and 5.5%, respectively [124]. The etiology of type-B CV is multifaceted and may be attributable to other infectious agents reportedly associated with MC, albeit rarely, including hepatitis B virus, *Leishmania donovani*, *Brucella melitensis*, cytomegalovirus, parvovirus B19, Epstein–Barr virus, human immunodeficiency virus, hepatitis A virus, *Mycobacterium leprae*, *Ascaris lumbricoides*, and *Candida albicans*. Streptococcus endocarditis and other pyogenic infections have also been implicated [124, 125].

In addition to this small subset of cases of non-HCV-related CV of infectious origin, the following type-B conditions should also be mentioned: (a) MC associated with connective tissue diseases such as Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis; (b) MC occurring in patients with NHL or, more rarely, tumors of different histotypes; (c) MC not ascribable to any concomitant or underlying factors and consequently defined as "essential." The disease features in patients with "essential" MC are roughly the same as those in patients with the highly prevalent HCV-positive form.

Two major reports addressed non-infectious CV. In the French multicenter CryoVas survey [126] of a large cohort of 242 patients, baseline manifestations, such as purpura, peripheral neuropathy, arthralgia or arthritis, glomerulonephritis, cutaneous ulcers, and cutaneous necrosis, were mostly similar to those commonly occurring in type-A patients. The underlying diagnoses were connective tissue diseases, B-NHL, and "essential" MC (Fig. 8). In the prospective observational study of 160 type-B patients (plus 15 HBsAg-positive patients not considered in this context of non-infectious CV) evaluated by the Italian Group for the Study of Cryoglobulinaemias, the following associated conditions were diagnosed: primary Sjögren's syndrome, systemic lupus erythematosus, other autoimmune disorders, lymphoproliferative diseases, solid tumors, and essential CV [125] (Fig. 8).

The clinical, prognostic, and therapeutic features of type-B patients reflect those of the underlying major diagnosis. Nonetheless, arthro-myalgias, purpuric eruptions, and renal and peripheral nerve involvement can be detected in highly variable combinations but usually at a lower frequency than in type-A patients [124]. In addition, the isolated cryoglobulins are monoclonal IgMK-polyclonal IgG or polyclonal IgM-polyclonal IgG populations, with a ratio of roughly 1:1, and serum levels of complement fraction C4 are significantly decreased in approximately 50% of patients. The characteristics of renal involvement in HCV-unrelated CV were the subject of a recent review [127].

The therapeutic management of type-B patients is largely dependent on the underlying disease. When endocarditis or another pyogenic infection is diagnosed or a specific infectious agent has been identified, properly selected antibacterial, antiviral, antiparasitic or antifungal agents should be administered. In patients with a connective tissue disease, GCs and immunosuppressive drugs are the first-line therapy, with the addition of biological agents (for example, belimumab in systemic lupus erythematosus or infliximab in

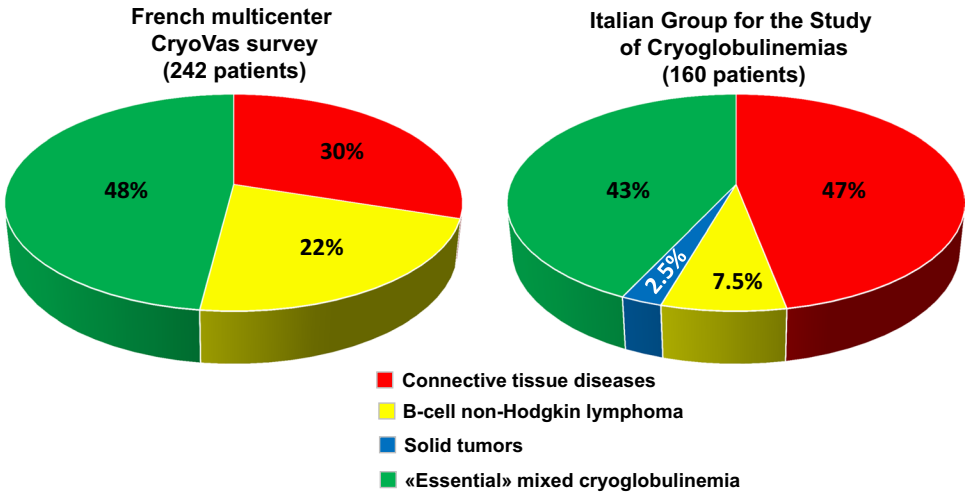

Fig. 8 Clinical diagnoses in two large cohorts of non-infectious CV [125, 126]

rheumatoid arthritis) as second-line therapy in patients with refractory or relapsing disease. Finally, NHL patients usually receive GCs and rituximab as first-line therapy, replaced by a suitable chemotherapy regimen in refractory or relapsing cases. Given the heterogeneity of the underlying diseases in type-B patients, the outcome is correspondingly variable, ranging from sustained remission in those in whom the infection is eradicated to death in those with endocarditis or NHL refractory to treatment.

The emerging challenges of CV

A largely unforeseen situation occurred during the pIFN-α era, namely a dissociation in a few patients successfully treated for CV between the achievement of SVR and the persistence or reappearance of CV or the persistence or relapse of both circulating cryoglobulins and RF activity [12]. When tested by transcription-mediated amplification assays for HCV RNA, the serum samples and cryoprecipitates of these patients were consistently negative, and HCV RNA replication could not be detected in peripheral blood mononuclear cells [128]. In some patients, this condition was eventually followed by the appearance of B-NHL [128].

With the advent of the new DAA regimens capable of inducing a remarkably higher rate of SVR, a similar phenomenon has been reported more frequently [15, 113, 114, 129]. In the most recent studies, while SVR was associated with clinical improvement in the majority of patients with CV, a mere reduction or, less commonly, no change in the levels of circulating cryoglobulins was detected in over one-third of the patients 12 weeks after therapy completion. A longer follow-up will provide much-needed information on the persistence of viral clearance and the clinical response [114–116]. In two patients with CV and NHL, the clinical response of the vasculitis was only partial and the cryocrit decreased by roughly 50%, but in neither patient was there a significant decrease in monoclonal B-cell lymphocytosis [112]. In another study, tumor progression occurred in a patient with small lymphocytic NHL despite viral clearance [115]. A possible explanation for the persistence of cryoglobulins despite viral load clearance is more advanced liver damage and thus a decreased ability to clear ICs [130]. In addition, DAAs are devoid of the immunomodulatory properties of pIFN-α, are unlikely to interfere with the pathogenesis of CV, and thus cannot be expected to impact the immune-mediated injury underlying the tissue targets [129].

As discussed earlier in this review, the immunochemical structure of cryoprecipitating ICs consists of HCV nucleocapsid protein bound to IgG with specific anti-core reactivity and linked to IgM directed to their Fc portion [44]. Thus, the virus is presumed to be the inciting agent that triggers a specific IgG antibody response and then the synthesis of a monoclonal IgM component with RF activity. However, the formation of circulating ICs despite viral clearance suggests that virus-independent ICs of different immunochemical structures are formed in those anti-HCV-positive patients who have achieved SVR after antiviral therapy but who continue to exhibit the clinical and immunologic features of CV.

The persistence and/or relapse of CV point to cryoglobulinemia as part of a dynamic process in which virus-dependent activation of the immune system results in the clonal expansions of B-cells and the production of RF molecules. The continued synthesis of cryoproteins in patients achieving SVR but with partial immunologic and clinical responses implies that HCV is not directly involved in the cryoprecipitation process. Rather, HCV can be considered as an independent variable capable of activating the immune system and generating the clonal expansion of B-cells, but these clonotypes then become viral-autonomous, remarkably stable, and often refractory to RTX. As we discussed in a previous paper, clonal expansion in these patients probably

occurs in an environment that favors immortalization of the cells and which may therefore be a predisposing factor for transforming events [97]. The persistent expansion of monoclonal B-cells should thus be considered as a major predictor of, on the one hand, a poor or no response, and, on the other, of potential progression to B-NHL.

No general therapeutic indications can be defined for patients with SVR but persistent or relapsed CV. Low-to-medium doses of GCs, possibly in combination with cyclophosphamide, should be considered. In resistant cases, since some patients have RTX-refractory disease, the use of a different humanized CD20 monoclonal antibody, such as ofatumumab, which targets an epitope distinct from the one recognized by RTX, may be a feasible alternative [131, 132]. Patients diagnosed with overt B-NHL will require a suitable chemotherapeutic regimen [133].

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Compliance with ethical standards

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References

1. Wintrobe MM, Buell MV. Hyperproteinemia associated with multiple myeloma. Bull Johns Hopkins Hosp. 1933;52:156–65.
2. Lerner AB, Watson CJ. Studies of cryoglobulins. I. Unusual purpura associated with the presence of a high concentration of cryoglobulin (cold precipitable serum globulin). Am J Med Sci. 1947;214:410–5.
3. LoSpalluto J, Dorward B, Miller W, Ziff M. Cryoglobulinemia based on interaction between a gamma macroglobulin and 7S gamma globulin. Am J Med. 1962;32:142–7.
4. Meltzer M, Franklin EC. Cryoglobulinemia—a study of twenty-nine patients. I. IgG and IgM cryoglobulins and factors affecting cryoprecipitability. Am J Med. 1966;40:828–36.
5. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. Am J Med. 1974;57:775–88.
6. Bonomo L, Casato M, Afeltra A, Caccavo D. Treatment of idiopathic mixed cryoglobulinemia with alpha interferon. Am J Med. 1987;83(4):726–30.
7. Pascual M, Perrin L, Giostra E, Schifferli JA. Hepatitis C virus in patients with cryoglobulinemia type II. J Infect Dis. 1990;162:569–70.
8. Gorevic PD, Frangione B. Mixed cryoglobulinemia cross-reactive idiotypes (CRI): implications for the relationship of MC to rheumatic and lymphoproliferative diseases. Semin Hematol. 1991;28:79–94.
9. Ferri C, Monti M, La Civita L, Careccia G, Mazzaro C, Longombardo G, et al. Hepatitis C virus infection in non-Hodgkin's B-cell lymphoma complicating mixed cryoglobulinemia. Eur J Clin Invest. 1994;24:781–4.
10. Hermine O, Lefrère F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med. 2002;347:89–94.
11. Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. Blood. 2003;101:3818–26.
12. Levine JW, Gota C, Fessler BJ, Calabrese LH, Cooper SM. Persistent cryoglobulinemic vasculitis following successful treatment of hepatitis C virus. J Rheumatol. 2005;32:1164–7.
13. Sise ME, Bloom AK, Wisocky J, Lin MV, Gustafson JL, Lundquist AL, et al. Treatment of hepatitis C virus-associated mixed cryoglobulinemia with direct-acting antiviral agents. Hepatology. 2016;63:408–17.
14. Abel G, Zhang QX, Agnello V. Hepatitis C virus infection in type II mixed cryoglobulinemia. Arthritis Rheum. 1993;36:1341–9.
15. Dammacco F, Racanelli V, Russi S, Sansonno D. The expanding spectrum of HCV-related cryoglobulinemic vasculitis: a narrative review. Clin Exp Med. 2016;16:233–42.
16. Lauletta G, Russi S, Conteduca V, Sansonno L, Dammacco F, Sansonno D. Impact of cryoglobulinemic syndrome on the outcome of chronic hepatitis C virus infection: a 15-year prospective study. Medicine (Baltimore). 2013;92:245–56.
17. Ferri C, Greco F, Longombardo G, Palla P, Moretti A, Marzo E, et al. Antibodies to hepatitis C virus in patients with mixed cryoglobulinemia. Arthritis Rheum. 1991;34:1606–10.
18. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med. 1992;327:1490–5.
19. Dammacco F, Sansonno D. Antibodies to hepatitis C virus in essential mixed cryoglobulinaemia. Clin Exp Immunol. 1992;87:352–6.
20. Misiani R, Bellavita P, Fenili D, Borelli G, Marchesi D, Massazza M, et al. Hepatitis C virus infection in patients with essential mixed cryoglobulinemia. Ann Intern Med. 1992;117:573–7.
21. Dammacco F, Sansonno D, Cornacchiulo V, Mennuni C, Carbone R, Lauletta G, et al. Hepatitis C virus infection and mixed cryoglobulinemia: a striking association. Int J Clin Lab Res. 1993;23:45–9.
22. Cacoub P, Fabiani FL, Musset L, Perrin M, Frangeul L, Leger JM, et al. Mixed cryoglobulinemia and hepatitis C virus. Am J Med. 1994;96:124–32.
23. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum. 2013;65:1–11.
24. Dammacco F, Sansonno D. Therapy for hepatitis C virus-related cryoglobulinemic vasculitis. N Engl J Med. 2013;369:1035–45.
25. Lunel F, Musset L, Cacoub P, Frangeul L, Cresta P, Perrin M, et al. Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. Gastroenterology. 1994;106:1291–300.
26. Mascia MT, Ferrari D, Campioli D, Sandri G, Mussini C, Ferri C. Non HCV-related mixed cryoglobulinemia. Dig Liver Dis. 2007;39(Suppl 1):S61–4.
27. Terrier B, Marie I, Lacraz A, Belenotti P, Bonnet F, Chiche L, et al. Non HCV-related infectious cryoglobulinemia vasculitis: results from the French nationwide CryoVas survey and systematic review of the literature. J Autoimmun. 2015;65:74–81.
28. Galli M, Oreni L, Saccardo F, et al. HCV-unrelated cryoglobulinaemic vasculitis: the results of a prospective observational

study by the Italian Group for the Study of Cryoglobulinaemias (GISC). Clin Exp Rheumatol. 2017;35(Suppl 103):67–76.
29. Kosmas N, Kontos A, Panayiotakopoulos G, Dimitrakopoulos A, Kordossis T. Decreased prevalence of mixed cryoglobulinemia in the HAART era among HIV-positive, HCV-negative patients. J Med Virol. 2006;78:1257–61.
30. Mazzaro C, Dal Maso L, Urraro T, Mauro E, Castelnovo L, Casarin P, et al. Hepatitis B virus related cryoglobulinemic vasculitis: a multicentre open label study from the Gruppo Italiano di Studio delle Crioglobulinemie—GISC. Dig Liver Dis. 2016;48:780–4.
31. Bazerbachi F, Leise MD, Watt KD, Murad MH, Prokop LJ, Haffar S. Systematic review of mixed cryoglobulinemia associated with hepatitis E virus infection: association or causation? Gastroenterol Rep (Oxf). 2017;5:178–84.
32. Monti G, Saccardo F, Castelnovo L, Novati P, Sollima S, Riva A, et al. Prevalence of mixed cryoglobulinaemia syndrome and circulating cryoglobulins in a population-based survey: the Origgio study. Autoimmun Rev. 2014;13:609–14.
33. Meeting of the International Task Force for Disease Eradication, June 2017. Wkly Epidemiol Rec. 2017;92:537–56.
34. Agnello V. The etiology and pathophysiology of mixed cryoglobulinemia secondary to hepatitis C virus infection. Springer Semin Immunopathol. 1997;19:111–29.
35. Lamprecht P, Gause A, Gross WL. Cryoglobulinemic vasculitis. Arthritis Rheum. 1999;42:2507–16.
36. Ferri C, Mascia MT. Cryoglobulinemic vasculitis. Curr Opin Rheumatol. 2006;18:54–63.
37. Sansonno D, Carbone A, De Re V, Dammacco F. Hepatitis C virus infection, cryoglobulinaemia, and beyond. Rheumatology (Oxford). 2007;46:572–8.
38. Cacoub P, Saadoun D. Hepatitis C virus infection induced vasculitis. Clin Rev Allergy Immunol. 2008;35:30–9.
39. Ramos-Casals M, Stone JH, Cid MC, Bosch X. The cryoglobulinaemias. Lancet. 2012;379:348–60.
40. Fabris M, Quartuccio L, Sacco S, De Marchi G, Pozzato G, Mazzaro C, Ferraccioli G, Migone TS, De Vita S. B-Lymphocyte stimulator (BLyS) up-regulation in mixed cryoglobulinaemia syndrome and hepatitis-C virus infection. Rheumatology (Oxford). 2007;46:37–43.
41. Lake-Bakaar G, Jacobson I, Talal A. B cell activating factor (BAFF) in the natural history of chronic hepatitis C virus liver disease and mixed cryoglobulinaemia. Clin Exp Immunol. 2012;170:231–7.
42. Sansonno D, De Vita S, Iacobelli AR, Cornacchiulo V, Boiocchi M, Dammacco F. Clonal analysis of intrahepatic B cells from HCV-infected patients with and without mixed cryoglobulinemia. J Immunol. 1998;160:3594–601.
43. De Vita S, De Re V, Gasparotto D, Ballarè M, Pivetta B, Ferraccioli G, et al. Oligoclonal non-neoplastic B cell expansion is the key feature of type II mixed cryoglobulinemia: clinical and molecular findings do not support a bone marrow pathologic diagnosis of indolent B cell lymphoma. Arthritis Rheum. 2000;43:94–102.
44. Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. Lancet Infect Dis. 2005;5:227–36.
45. Cutolo M, Pizzorni C, Secchi ME, Sulli A. Capillaroscopy. Best Pract Res Clin Rheumatol. 2008;22:1093–108.
46. Roccatello D, Fornasieri A, Giachino O, Rossi D, Beltrame A, Banfi G, et al. Multicenter study on hepatitis C virus-related cryoglobulinemic glomerulonephritis. Am J Kidney Dis. 2007;49:69–82.
47. Casato M, Saadoun D, Marchetti A, Limal N, Picq C, Pantano P, et al. Central nervous system involvement in hepatitis C virus cryoglobulinemia vasculitis: a multicenter case-control study using magnetic resonance imaging and neuropsychological tests. J Rheumatol. 2005;32:484–8.
48. Monaco S, Mariotto S, Ferrari S, Calabrese M, Zanusso G, Gajofatto A, Sansonno D, Dammacco F. Hepatitis C virus-associated neurocognitive and neuropsychiatric disorders: advances in 2015. World J Gastroenterol. 2015;21:11974–83.
49. Zackrison LH, Katz P. Bronchiolitis obliterans organizing pneumonia associated with essential mixed cryoglobulinemia. Arthritis Rheum. 1993;36:1627–30.
50. Terrier B, Saadoun D, Sène D, Scerra S, Musset L, Cacoub P. Presentation and outcome of gastrointestinal involvement in hepatitis C virus-related systemic vasculitis: a case-control study from a single-centre cohort of 163 patients. Gut. 2010;59:1709–15.
51. Culclasure TF, Dorogy ME, Enzenauer RJ. Cryoglobulinemia: a reversible cause of dilated cardiomyopathy. Am Heart J. 1996;131:1044–6.
52. Karras A, Potier L, Reboux AH, Coldea N, Perdrix L, Jacquot C, et al. Cryoglobulin-induced cardiomyopathy. J Am Coll Cardiol. 2010;55:e13.
53. Terrier B, Karras A, Cluzel P, Collet JP, Sène D, Saadoun D, et al. Presentation and prognosis of cardiac involvement in hepatitis C virus-related vasculitis. Am J Cardiol. 2013;111:265–72.
54. Sebastiani M, Giuggioli D, Colaci M, Fallahi P, Gragnani L, Antonelli A, et al. HCV-related rheumatic manifestations and therapeutic strategies. Curr Drug Targets. 2017;18:803–10.
55. Lormeau C, Falgarone G, Roulot D, Boissier MC. Rheumatologic manifestations of chronic hepatitis C infection. Joint Bone Spine. 2006;73:633–8.
56. Ferri C, Sebastiani M, Antonelli A, Colaci M, Manfredi A, Giuggioli D. Current treatment of hepatitis C-associated rheumatic diseases. Arthritis Res Ther. 2012;14:215.
57. Fiore CE, Riccobene S, Mangiafico R, Santoro F, Pennisi P. Hepatitis C-associated osteosclerosis (HCAO): report of a new case with involvement of the OPG/RANKL system. Osteoporos Int. 2005;16:2180–4.
58. Manganelli P, Giuliani N, Fietta P, Mancini C, Lazzaretti M, Pollini A, et al. OPG/RANKL system imbalance in a case of hepatitis C-associated osteosclerosis: the pathogenetic key? Clin Rheumatol. 2005;24:296–300.
59. Della Rossa A, Tavoni A, Bombardieri S. Hyperviscosity syndrome in cryoglobulinemia: clinical aspects and therapeutic considerations. Semin Thromb Hemost. 2003;29:473–7.
60. Stone MJ, Bogen SA. Evidence-based focused review of management of hyperviscosity syndrome. Blood. 2012;119:2205–8.
61. Antonelli A, Ferri C, Ferrari SM, Colaci M, Fallahi P. Immunopathogenesis of HCV-related endocrine manifestations in chronic hepatitis and mixed cryoglobulinemia. Autoimmun Rev. 2008;8:18–23.
62. Ferri C, Colaci M, Fallahi P, Ferrari SM, Antonelli A, Giuggioli D. Thyroid involvement in hepatitis C virus-infected patients with/without mixed cryoglobulinemia. Front Endocrinol (Lausanne). 2017;8:159.
63. Ferri C, Sebastiani M, Giuggioli D, Cazzato M, Longombardo G, Antonelli A, et al. Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. Semin Arthritis Rheum. 2004;33(6):355–74.
64. Landau DA, Scerra S, Sene D, Resche-Rigon M, Saadoun D, Cacoub P. Causes and predictive factors of mortality in a cohort of patients with hepatitis C virus-related cryoglobulinemic vasculitis treated with antiviral therapy. J Rheumatol. 2010;37:615–21.
65. Cacoub P, Vautier M, Desbois AC, Lafuma A, Saadoun D. Effectiveness and cost of hepatitis C virus cryoglobulinaemia vasculitis treatment: from interferon-based to direct-acting antivirals era. Liver Int. 2017;37:1805–13.

66. Gisbert JP, García-Buey L, Pajares JM, Moreno-Otero R. Prevalence of hepatitis C virus infection in B-cell non-Hodgkin's lymphoma: systematic review and meta-analysis. Gastroenterology. 2003;125:1723–32.
67. Monti G, Pioltelli P, Saccardo F, Campanini M, Candela M, Cavallero G, et al. Incidence and characteristics of non-Hodgkin lymphomas in a multicenter case file of patients with hepatitis C virus-related symptomatic mixed cryoglobulinemias. Arch Intern Med. 2005;165:101–5.
68. de Sanjose S, Benavente Y, Vajdic CM, Engels EA, Morton LM, Bracci PM, et al. Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. Clin Gastroenterol Hepatol. 2008;6:451–8.
69. Zignego AL, Gragnani L, Piluso A, Sebastiani M, Giuggioli D, Fallahi P, et al. Virus-driven autoimmunity and lymphoproliferation: the example of HCV infection. Expert Rev Clin Immunol. 2015;11:15–31.
70. Iqbal T, Mahale P, Turturro F, Kyvernitakis A, Torres HA. Prevalence and association of hepatitis C virus infection with different types of lymphoma. Int J Cancer. 2016;138:1035–7.
71. Couronné L, Bachy E, Roulland S, Nadel B, Davi F, Armand M, et al. From hepatitis C virus infection to B-cell lymphoma. Ann Oncol. 2018;29:92–100.
72. Sansonno D, De Vita S, Cornacchiulo V, Carbone A, Boiocchi M, Dammacco F. Detection and distribution of hepatitis C virus-related proteins in lymph nodes of patients with type II mixed cryoglobulinemia and neoplastic or non-neoplastic lymphoproliferation. Blood. 1996;88:4638–45.
73. Merli M, Visco C, Spina M, Luminari S, Ferretti VV, Gotti M, et al. Outcome prediction of diffuse large B-cell lymphomas associated with hepatitis C virus infection: a study on behalf of the Fondazione Italiana Linfomi. Haematologica. 2014;99:489–96.
74. Peveling-Oberhag J, Arcaini L, Bankov K, Zeuzem S, Herrmann E. The anti-lymphoma activity of antiviral therapy in HCV-associated B-cell non-Hodgkin lymphomas: a meta-analysis. J Viral Hepat. 2016;23:536–44.
75. Dal Maso L, Franceschi S. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. Cancer Epidemiol Biomarkers Prev. 2006;15:2078–85.
76. Mele A, Pulsoni A, Bianco E, Musto P, Szklo A, Sanpaolo MG, et al. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an Italian multicenter case-control study. Blood. 2003;102:996–9.
77. Paydas S. Hepatitis C virus and lymphoma. Crit Rev Oncol Hematol. 2015;93:246–56.
78. Besson C, Canioni D, Lepage E, Pol S, Morel P, Lederlin P, Groupe d'Etude des Lymphomes de l'Adulte Programs, et al. Characteristics and outcome of diffuse large B-cell lymphoma in hepatitis C virus-positive patients in LNH 93 and LNH 98 Groupe d'Etude des Lymphomes de l'Adulte programs. J Clin Oncol. 2006;24:953–60.
79. Arcaini L, Besson C, Frigeni M, Fontaine H, Goldaniga M, Casato M, et al. Interferon-free antiviral treatment in B-cell lymphoproliferative disorders associated with hepatitis C virus infection. Blood. 2016;128:2527–32.
80. Kawamura Y, Ikeda K, Arase Y, Yatsuji H, Sezaki H, Hosaka T, et al. Viral elimination reduces incidence of malignant lymphoma in patients with hepatitis C. Am J Med. 2007;120:1034–41.
81. Peveling-Oberhag J, Arcaini L, Hansmann ML, Zeuzem S. Hepatitis C-associated B-cell non-Hodgkin lymphomas. Epidemiology, molecular signature and clinical management. J Hepatol. 2013;59:169–77.
82. Lerat H, Berby F, Trabaud MA, Vidalin O, Major M, Trepo C, et al. Specific detection of hepatitis C virus minus strand RNA in hematopoietic cells. J Clin Invest. 1996;97:845–51.
83. Lanford RE, Chavez D, Chisari FV, Sureau C. Lack of detection of negative strand hepatitis C virus RNA in peripheral blood mononuclear cells and other extrahepatic tissues by the highly strand-specific rTth reverse transcriptase PCR. J Virol. 1995;69:8079–83.
84. Ito M, Murakami K, Suzuki T, Mochida K, Suzuki M, Ikebuchi K, et al. Enhanced expression of lymphomagenesis-related genes in peripheral blood B cells of chronic hepatitis C patients. Clin Immunol. 2010;135:459–65.
85. Sansonno D, Lotesoriere C, Cornacchiulo V, Fanelli M, Gatti P, Iodice G, et al. Hepatitis C virus infection involves CD34(+) hematopoietic progenitor cells in hepatitis C virus chronic carriers. Blood. 1998;92:3328–37.
86. Machida K, Cheng KT, Sung VM, Shimodaira S, Lindsay KL, Levine AM, et al. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. Proc Natl Acad Sci U S A. 2004;101:4262–7.
87. Tucci FA, Broering R, Johansson P, Schlaak JF, Kuppers R. B cells in chronically hepatitis C virus-infected individuals lack a virus-induced mutation signature in the TP53, CTNNB1, and BCL6 genes. J Virol. 2013;87:2956–62.
88. Casato M, Laganà B, Antonelli G, Dianzani F, Bonomo L. Long-term results of therapy with interferon-alpha for type II essential mixed cryoglobulinemia. Blood. 1991;78:3142–7.
89. Ferri C, Marzo E, Longombardo G, Lombardini F, La Civita L, Vanacore R, et al. Interferon-alpha in mixed cryoglobulinemia patients: a randomized, crossover-controlled trial. Blood. 1993;81:1132–6.
90. Dammacco F, Sansonno D, Han JH, Shyamala V, Cornacchiulo V, Iacobelli AR, et al. Natural interferon-alpha versus its combination with 6-methyl-prednisolone in the therapy of type II mixed cryoglobulinemia: a long-term, randomized, controlled study. Blood. 1994;84:3336–43.
91. Misiani R, Bellavita P, Fenili D, Vicari O, Marchesi D, Sironi PL, et al. Interferon alfa-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med. 1994;330:751–6.
92. Asselah T, Estrabaud E, Bieche I, Lapalus M, De Muynck S, Vidaud M, et al. Hepatitis C: viral and host factors associated with non-response to pegylated interferon plus ribavirin. Liver Int. 2010;30:1259–69.
93. Vallat L, Benhamou Y, Gutierrez M, Ghillani P, Hercher C, Thibault V, et al. Clonal B cell populations in the blood and liver of patients with chronic hepatitis C virus infection. Arthritis Rheum. 2004;50:3668–78.
94. Charles ED, Brunetti C, Marukian S, Ritola KD, Talal AH, Marks K, et al. Clonal B cells in patients with hepatitis C virus-associated mixed cryoglobulinemia contain an expanded anergic CD21low B-cell subset. Blood. 2011;117:5425–37.
95. Zaja F, De Vita S, Mazzaro C, Sacco S, Damiani D, De Marchi G, et al. Efficacy and safety of rituximab in type II mixed cryoglobulinemia. Blood. 2003;101:3827–34.
96. Ferri C, Cacoub P, Mazzaro C, Roccatello D, Scaini P, Sebastiani M, et al. Treatment with rituximab in patients with mixed cryoglobulinemia syndrome: results of multicenter cohort study and review of the literature. Autoimmun Rev. 2011;11:48–55.
97. Dammacco F, Tucci FA, Lauletta G, Gatti P, De Re V, Conteduca V, et al. Pegylated interferon-alpha, ribavirin, and rituximab combined therapy of hepatitis C virus-related mixed cryoglobulinemia: a long-term study. Blood. 2010;116:343–53.
98. Saadoun D, Resche Rigon M, Sene D, Terrier B, Karras A, Perard L, et al. Rituximab plus Peg-interferon-alpha/ribavirin compared with Peg-interferon-alpha/ribavirin in hepatitis C-related mixed cryoglobulinemia. Blood. 2010;116:326–34.
99. Sneller MC, Hu Z, Langford CA. A randomized controlled trial of rituximab following failure of antiviral therapy for hepatitis C

virus-associated cryoglobulinemic vasculitis. Arthritis Rheum. 2012;64:835–42.
100. Saadoun D, Resche Rigon M, Pol S, Thibault V, Blanc F, Pialoux G, et al. PegIFNα/ribavirin/protease inhibitor combination in severe hepatitis C virus-associated mixed cryoglobulinemia vasculitis. J Hepatol. 2015;62:24–30.
101. European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2016. J Hepatol. 2017;66:153–94.
102. American Association for the Study of Liver Disease, Infectious Diseases Society of America. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. 2017 (http://www.hcvguidelines.org).
103. van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, et al. Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. JAMA. 2012;308:2584–93.
104. Zignego AL, Ramos-Casals M, Ferri C, Saadoun D, Arcaini L, Roccatello D, ISG-EHCV, et al. International therapeutic guidelines for patients with HCV-related extrahepatic disorders. A multidisciplinary expert statement. Autoimmun Rev. 2017;16:523–41.
105. Ramos-Casals M, Zignego AL, Ferri C, Brito-Zerón P, Retamozo S, Casato M, International Study Group of Extrahepatic Manifestations related to HCV (ISG-EHCV), et al. Evidence-based recommendations on the management of extrahepatic manifestations of chronic hepatitis C virus infection. J Hepatol. 2017;66:1282–99.
106. Visentini M, Ludovisi S, Petrarca A, Pulvirenti F, Zaramella M, Monti M, et al. A phase II, single-arm multicenter study of low-dose rituximab for refractory mixed cryoglobulinemia secondary to hepatitis C virus infection. Autoimmun Rev. 2011;10:714–9.
107. Rockx MA, Clark WF. Plasma exchange for treating cryoglobulinemia: a descriptive analysis. Transfus Apher Sci. 2010;42:247–51.
108. Olson N, Yerrabothala S, Dunbar N. Successful use of cryocrit for monitoring response to therapeutic plasma exchange in type 1 cryoglobulinemia. J Clin Apher. 2016;31:403–4.
109. Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, et al. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol. 2016;65:719–26.
110. Grandhe S, Frenette CT. Occurrence and recurrence of hepatocellular carcinoma after successful direct-acting antiviral therapy for patients with chronic hepatitis C virus infection. Gastroenterol Hepatol (N Y). 2017;13:421–5.
111. Saadoun D, Thibault V, Si Ahmed SN, Alric L, Mallet M, Guillaud C, et al. Sofosbuvir plus ribavirin for hepatitis C virus-associated cryoglobulinaemia vasculitis: VASCUVALDIC study. Ann Rheum Dis. 2016;75:1777–82.
112. Gragnani L, Visentini M, Fognani E, Urraro T, De Santis A, Petraccia L, et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinemia. Hepatology. 2016;64:1473–82.
113. Sollima S, Milazzo L, Peri AM, Torre A, Antinori S, Galli M. Persistent mixed cryoglobulinaemia vasculitis despite hepatitis C virus eradication after interferon-free antiviral therapy. Rheumatology (Oxford). 2016;55:2084–5.
114. Bonacci M, Lens S, Londoño MC, Mariño Z, Cid MC, Ramos-Casals M, et al. Virologic, clinical, and immune response outcomes of patients with hepatitis C virus-associated cryoglobulinemia treated with direct-acting antivirals. Clin Gastroenterol Hepatol. 2017;15:575–83.
115. Lauletta G, Russi S, Pavone F, Vacca A, Dammacco F. Direct-acting antiviral agents in the therapy of hepatitis C virus-related mixed cryoglobulinaemia: a single-centre experience. Arthritis Res Ther. 2017;19:74.
116. Emery JS, Kuczynski M, La D, Almarzooqi S, Kowgier M, Shah H, et al. Efficacy and safety of direct acting antivirals for the treatment of mixed cryoglobulinemia. Am J Gastroenterol. 2017;112:1298–308.
117. Saadoun D, Pol S, Ferfar Y, Alric L, Hezode C, Si Ahmed SN, et al. Efficacy and safety of sofosbuvir plus daclatasvir for treatment of HCV-associated cryoglobulinemia vasculitis. Gastroenterology. 2017;153:49–52.
118. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, et al. Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol. 2016;65:727–33.
119. Butt AS, Sharif F, Abid S. Impact of direct acting antivirals on occurrence and recurrence of hepatocellular carcinoma: biologically plausible or an epiphenomenon? World J Hepatol. 2018;10:267–76.
120. Roche B, Coilly A, Duclos-Vallee JC, Samuel D. The impact of treatment of hepatitis C with DAAs on the occurrence of HCC. Liver Int. 2018;38(Suppl 1):139–45.
121. Huang AC, Mehta N, Dodge JL, Yao FY, Terrault NA. Direct-acting antivirals do not increase the risk of hepatocellular carcinoma recurrence after local-regional therapy or liver transplant waitlist dropout. Hepatology. 2018. https://doi.org/10.1002/hep.29855 **[Epub ahead of print]**.
122. Romano A, Angeli P, Piovesan S, Noventa F, Anastassopoulos G, Chemello L, et al. Newly diagnosed hepatocellular carcinoma in patients with advanced hepatitis C treated with DAAs: a prospective population study. J Hepatol. 2018. https://doi.org/10.1016/j.jhep.2018.03.009 **[Epub ahead of print]**.
123. Mascia MT, Ferrari D, Campioli D, Sandri G, Mussini C, Ferri C. Non HCV-related mixed cryoglobulinemia. Dig Liver Dis. 2007;39(Suppl 1):S61–4.
124. Terrier B, Marie I, Lacraz A, Belenotti P, Bonnet F, Chiche L, et al. Non HCV-related infectious cryoglobulinemia vasculitis: results from the French nationwide CryoVas survey and systematic review of the literature. J Autoimmun. 2015;65:74–81.
125. Galli M, Oreni L, Saccardo F, Castelnovo L, Filippini D, Marson P, et al. HCV-unrelated cryoglobulinaemic vasculitis: the results of a prospective observational study by the Italian Group for the Study of Cryoglobulinaemias (GISC). Clin Exp Rheumatol. 2017;35(Suppl 103):67–76.
126. Terrier B, Krastinova E, Marie I, Launay D, Lacraz A, Belenotti P, et al. Management of noninfectious mixed cryoglobulinemia vasculitis: data from 242 cases included in the CryoVas survey. Blood. 2012;119:5996–6004.
127. Spatola L, Generali E, Angelini C, Badalamenti S, Selmi C. HCV-negative mixed cryoglobulinemia and kidney involvement: in-depth review on physiopathological and histological bases. Clin Exp. Med 2018 (accepted for publication).
128. Landau DA, Saadoun D, Halfon P, Martinot-Peignoux M, Marcellin P, Fois E, et al. Relapse of hepatitis C virus-associated mixed cryoglobulinemia vasculitis in patients with sustained viral response. Arthritis Rheum. 2008;58:604–11.
129. Roccatello D, Sciascia S, Rossi D, Solfietti L, Fenoglio R, Menegatti E, et al. The challenge of treating hepatitis C virus-associated cryoglobulinemic vasculitis in the era of anti-CD20 monoclonal antibodies and direct antiviral agents. Oncotarget. 2017;8:41764–77.
130. Cornella SL, Stine JG, Kelly V, Caldwell SH, Shah NL. Persistence of mixed cryoglobulinemia despite cure of hepatitis C with new oral antiviral therapy including direct-acting antiviral sofosbuvir: a case series. Postgrad Med. 2015;127:413–7.

131. Castillo JJ, Ghobrial IM, Treon SP. Biology, prognosis, and therapy of Waldenström Macroglobulinemia. Cancer Treat Res. 2015;165:177–95.
132. Chen LY, Shah R, Cwynarski K, Lambert J, McNamara C, Mohamedbhai SG, et al. Ofatumumab is a feasible alternative anti-CD20 therapy in patients intolerant of rituximab. Br J Haematol. 2018. https://doi.org/10.1111/bjh.15110 **[Epub ahead of print]**.
133. Merli M, Frigeni M, Gotti M, Grossi P, Bruno R, Passamonti F, et al. Direct-acting antivirals during or after immunochemotherapy in hepatitis C virus-positive diffuse large B-cell lymphomas. Hepatology. 2017;66:1341–3.