Review

Hepatitis C-associated mixed cryoglobulinaemia: a crossroad between autoimmunity and lymphoproliferation

D. Saadoun1,2, D. A. Landau1,2, L. H. Calabrese3 and P. P. Cacoub1,2

Hepatitis C virus (HCV) infection is the second most common chronic viral infection in the world with a global prevalence of about 2%. Chronic HCV infection is commonly associated with a number of extrahepatic complications. Circulating mixed cryoglobulins (MCs) are detected in 40–60% of HCV-infected patients whereas overt cryoglobulinaemia vasculitis develops in only 5–10% of the cases. MC reflects the expansion of B cells producing a pathogenic IgM with rheumatoid factor (RF) activity. Because cryoglobulin-producing B cells in HCV are mostly monoclonal, HCV-associated MC can be viewed as a benign B cell lymphoproliferative condition. The disease expression of MC vasculitis is variable, ranging from mild clinical symptoms (purpura, arthralgia) to fulminant life-threatening complications (glomerulonephritis, widespread vasculitis). The overall risk of non-Hodgkin’s lymphoma in patients with HCV-MC is estimated to be 35 times higher than that in the general population. This review will focus on recent advances in our understanding of the clinical course, complications, pathophysiology and treatment of these immune-mediated disorders.

KEY WORDS: HCV infection, Mixed cryoglobulinaemia, Vasculitis, Non-Hodgkin’s lymphoma.

Introduction

Hepatitis C virus (HCV) infection is the second most common chronic viral infection in the world, with over 170 million cases worldwide and a global prevalence of about 2% [1]. HCV is an RNA virus belonging to the Flaviviridae family. HCV infection is characterized by a high rate of chronicity leading to chronic liver inflammation resulting in cirrhosis and/or hepatocellular carcinoma in 20%. HCV is uniquely associated not only with chronic hepatic inflammation but also an array of extrahepatic complications. In the majority of these associated extrahepatic manifestations the pathogenic mechanism appears to be immunologically driven, with features of autoimmunity in many.

Table 1 represents a partial list of immunological laboratory abnormalities and disorders that have been associated with HCV infection. In terms of the strength of association of these disorders, several have strong links based on epidemiological and clinical studies while for others the supporting data are limited or conflicting. Among these extrahepatic manifestations, cryoglobulinaemia and its clinical sequelae hold the strongest association. The history of cryoglobulinaemia as a disease dates back to the 1930s when Wintrobe and Buell [2] described its association in a single patient with multiple myeloma. The modern era of cryoglobulinaemia is dated to 1966 when Meltzer and colleagues [3] described nine patients with cryoglobulins but lacking any known disease process associated with such cryoproteins. Among these patients were eight women and one man with a characteristic clinical syndrome characterized by purpura, arthralgia and weakness. This seminal article on the disease recognized the importance of hepatic involvement but these patients had no form of infective hepatitis recognized at the time. Over the ensuing years several groups suggested hepatitis B as the etiological agent responsible for cryoglobulinaemia [4, 5].

TABLE 1. Extrahepatic immunological complications associated with chronic HCV infection

<table>
<thead>
<tr>
<th>Established associations</th>
<th>Possible associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed cryoglobulinaemia</td>
<td>B-cell non-Hodgkin’s lymphomas</td>
</tr>
<tr>
<td>Membranoproliferative glomerulonephritis</td>
<td>Arthralgia, myalgia</td>
</tr>
<tr>
<td>Porphyria cutanea tarda</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Sialadenitis</td>
<td>Polyarteritis nodosa</td>
</tr>
<tr>
<td>Autoantibody production (RF, ANA, SMA, ATG, ACL)</td>
<td>Autoimmune cytopenia</td>
</tr>
<tr>
<td></td>
<td>Autoimmune thyroiditis</td>
</tr>
</tbody>
</table>

Interferon-induced

Dysthyroidism
Psoriasis
Lichen planus
Sarcoidosis
Polyneuropathy
Cutaneous vasculitis

RF, rheumatoid factor; ANA, antinuclear antibodies; SMA, anti-smooth muscle antibodies; ATG, antithyroglobulin antibodies; ACL, antithyroid peroxidase antibodies.
readily detectable in 40–60% of HCV-infected patients in most case series [10–13]. Cryoglobulins are immunochemically categorized into three types by the method of Brouet et al. [14]. Type I cryoglobulins are single monoclonal immunoglobulins usually found in haematological diseases. Type II consists of polyclonal IgG with monoclonal rheumatoid factor (RF) activity. Types III are comprised of polyclonal IgG and polyclonal RF. Types II and III are often referred to as mixed cryoglobulins (MCs) [14]. Beside the detection of serum cryoglobulin itself, other laboratory abnormalities may provide surrogate evidence of the presence of cryoglobulinaemia such as low C4, depressed total haemolytic complement levels, monoclonal proteinemia or RF activity. Most HCV-MC vasculitis patients demonstrate decreased levels of the early complement components C1, C4 and C2, whereas C3 levels fluctuate with the disease course. An RF activity is frequently observed, in up to 70% of the patients (Table 2). Electrophoreses and immuno-electrophoreses may show polyclonal hypergammaglobulinaemia or monoclonal Ig (mostly IgMk). Other immunological abnormalities in HCV-infected patients, whether MC-positive or -negative, include antinuclear (17–41%), anti-cardiolipin (20–27%), anti-smooth muscle cell (9–40%) and anti-thyroglobulin antibodies (8–13%) with important geographic differences in the prevalence [15–18]. There is usually no correlation between non-organ-specific autoantibodies and the main HCV-related features [18].

Main clinical features

Circulating MCs are frequently detected in HCV-infected patients (40–60%) whereas overt cryoglobulinaemia vasculitis develops in only 5–10% of the cases [10]. The most frequent target organs are skin, joints, nerves and kidney. The disease expression is variable, ranging from mild clinical symptoms (purpura, arthralgia) to fulminant life-threatening complications (glomerulonephritis, widespread vasculitis). The most common clinical and immunological manifestations of HCV-associated MC are shown in Table 2.

**Purpura.** Skin is the most frequently involved target organ and is the direct consequence of the small-size vessel vasculitis. The main sign is palpable purpura which is reported in 70–90% of the patients [19, 20]. It always begins at the lower limbs and may extend to the abdominal area and less frequently to the trunk and upper limbs. With petechial or papular, seldom necrotic aspect, this purpura can be compounded of erythematous maculae and dermal nodules. It persists 3–10 days with a residual brownish pigmentation. Skin biopsy shows a non-specific leucocytoclastic vasculitis involving small-size vessels with inflammatory infiltrates and, in some cases, fibrinoid necrosis of the arteriolar walls and endovascular thrombi. The association with other dermatological symptoms, like upper malleolar ulcers and urticarian vasculitis is not rare. Raynaud’s syndrome and acrocyanosis, which may evolve to digital ulcerations, can also occur.

**Arthralgia.** Arthralgia is reported in 40–80% of HCV-infected patients with MC [21, 22]. They are bilateral and symmetric, non-deforming and touched mainly great articular, knees and hands, more seldom elbows and ankles. Frank arthritis is rarely reported, being present in <10% of the patients [21, 22]. RF activity is found in 70–80% of the MC patients but is not correlated with the presence of articular disease as patients chronically infected with HCV in the absence of HCV-MC or RF may have prominent articular symptoms. There is no evidence of joint destruction, and antibodies to cyclic citrullinated peptide, which are highly specific of rheumatoid arthritis, are absent [23, 24].

**Neuropathy.** The prevalence of peripheral nervous system involvement varies in the literature and can be as high as 55% of the cases [25]. Neurological manifestations range from pure sensory axonopathy to mononeuritis multiplex. The most frequently described form is a distal sensory or sensory-motor polyneuropathy (PN). Bilateral, more often asymmetrical polyneuropathies represent 45–70% of the MC-PN and mononeuropathies multiplex 30–55% [26]. Motor deficit is inconsistent and mainly affects the lower limbs, appearing a few months to a few years after sensory symptoms. The tempo of the vasculitic neuropathy may be subacute, chronic or acute on chronic. In patients with distal polyneuropathy, nerve conduction studies are in keeping with a predominantly axonal process, mainly affecting sensory nerves. Neuropathological data show axonal degeneration, differential fasicular loss of axons, signs of demyelination and small-vessel vasculitis with mononuclear cell infiltrates in the perivascular area [25].

Reports on well-documented central nervous system (CNS) involvement in patients with HCV-associated vasculitis are rare [25, 27–29], though it has been described as the initial extra hepatic manifestation of HCV infection. Clinically, transient ischaemic attacks, stroke, progressive reversible ischaemic neurologil deficits, lacunar infarctions or encephalopathic syndrome may occur [25, 27]. MRI findings of the brain have been consistent with ischaemia, showing either small lesions of the periventricular white matter and the cerebral trunk or extensive supra- and infratentorial white matter lesions suggesting cerebral vasculitis [25]. The presence of CNS vasculitis involving small- and medium-sized vessels has been documented in two patients [30, 31].

**Renal involvement.** Renal manifestations are reported in 20–35% of the MC patients [19, 32, 33]. The most frequent clinical and histological picture is that of acute or chronic type I membranoproliferative glomerulonephritis (MPGN) with sub-endothelial deposits [34]. It represents >70–80% of cryoglobulinaemic renal diseases and it is strongly associated with the type II cryoglobulinaemia with IgM RF [34–36]. The most frequent presentation (55%) is proteinuria with microscopic haematuria and a variable degree of renal insufficiency [37]. Acute nephritic (20%) or acute nephritic syndrome (25%) can also reveal cryoglobulinaemic renal involvement [35]. New onset arterial hypertension is seen in 80% of the cases. Early complement components (C1q, C4) are usually very low. Chronic uraemia may develop in 10–20% of the MC patients but only after prolonged follow-up of a decade or more [35]. Morphological features are characterized by important monocytes infiltrates with double contours of the basement membrane, large, eosinophilic and amorphous intra-luminal thrombi. In indirect immunofluorescence, intra-glomerular sub-endothelial deposits of IgG, IgM and complement components are observed. The electron microscopic features with sub-endothelial and intra-luminal deposits presenting a crystallloid aspect are pathognomonic. Vasculitis of small renal arteries or extra-capillary crescents are rarely observed [33–35, 38]. A number of

### Table 2. Main features in hepatitis C-associated MC

<table>
<thead>
<tr>
<th>Findings</th>
<th>Trejo et al. [150]</th>
<th>Ferri et al. [63]</th>
<th>Saadoun et al. [151]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male ratio</td>
<td>1.5</td>
<td>3.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>54</td>
<td>56</td>
<td>67</td>
</tr>
<tr>
<td>Purpura</td>
<td>67 (32)</td>
<td>187 (81)</td>
<td>75 (64)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>82 (40)</td>
<td>166 (72)</td>
<td>21 (18)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>35 (17)</td>
<td>134 (58)</td>
<td>51 (43)</td>
</tr>
<tr>
<td>Sclera syndrome</td>
<td>40 (19)</td>
<td>65 (29)</td>
<td>21 (18)</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>112 (56)</td>
<td>46 (20)</td>
<td>16 (14)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>23 (11)</td>
<td>79 (36)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Leg ulcers</td>
<td>9 (4)</td>
<td>25 (11)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>107 (52)</td>
<td>231 (100)</td>
<td>93 (79)</td>
</tr>
<tr>
<td>Type II cryoglobulin</td>
<td>–</td>
<td>143 (62)</td>
<td>100 (84)</td>
</tr>
<tr>
<td>Low C4 level</td>
<td>98 (56)</td>
<td>–</td>
<td>64 (54)</td>
</tr>
<tr>
<td>ANA</td>
<td>92 (52)</td>
<td>–</td>
<td>21 (18)</td>
</tr>
</tbody>
</table>

*Except where indicated otherwise, values are expressed as n (%). ANA, antinuclear antibodies.*
less common nephropathies have been reported, including mesangioproliferative or focal segmental glomerulonephritis.

**Liver involvement.** Clinical and biological evidence of liver disease is evidenced in 60–80% of the patients with essential MC whose liver biopsies showed chronic active hepatitis or cirrhosis [5, 39]. In asymptomatic HCV-infected patients, the significance of cryoglobulins in the development of cirrhosis has been the subject of speculation. A meta-analysis of 19 studies on a total of 2323 patients with chronic HCV infection found a 44% prevalence of cryoglobulinaemia and a highly significant association between cirrhosis and cryoglobulinaemia after adjustment for age, gender and estimated duration of disease [40]. In a recent study of 437 consecutive HCV-infected patients [41], advanced liver fibrosis and steatosis were found in 37 and 35% of MC patients, respectively. In multivariate analysis, MC increased by nearly 3-fold the risk of having advanced liver fibrosis and steatosis.

**Other manifestations.** Other organs may more rarely be involved. Abdominal pains are reported and gastrointestinal bleeding secondary to mesenteric vasculitis has been described [22, 42]. Lungs can be involved more frequently without clinical symptoms but some patients may present moderate effort dyspnoea, dry cough, interstitial lung fibrosis, pleural effusions or haemoptysis, which can be consequent of intra-alveolar haemorrhages [43, 44]. Cardiac involvement including mitral valvular damage, coronary vasculitis complicated by myocardial infarction, pericarditis or congestive cardiac failure have all been described [45]. HCV-MC can also rarely manifest as chronic fever of unknown origin.

**Sicca syndrome and Sjögren’s syndrome.** Other autoimmune conditions associated with HCV have also been reported [46–48]. Studies have suggested an epidemiological association between Sjögren’s syndrome and HCV infection [49, 50]. HCV seropositivity was found in 156 (12%) of the 1309 reported SS patients tested for HCV infection [50]. Symptomatic xerophthalmia and xerostomia affects 10–53% of HCV-infected patients [49, 51], although this association was not demonstrated in a large cohort of US veterans [52]. However, this latter study only included males, a bias that greatly underestimated the prevalence of sicca syndrome [52]. HCV treatment was not demonstrated as effective for sicca syndrome by Cacoub et al. [53] whereas Doffoel-Hantz et al. [54] found improvement of sicca features in one-third of the SS patients treated with anti-HCV therapy. The main differential aspect between primary and HCV-related Sjögren’s syndrome is the immunological pattern, with a predominance of MC-related markers (low C4, RF and MC) in HCV-related Sjögren’s syndrome [46]. A sicca syndrome has been reported in 20–40% of MC patients, however, those meeting most definitions of definite Sjögren’s syndrome are less commonly encountered. Some authors have distinguished the sicca syndrome of HCV infection from Sjögren’s syndrome based on several differences, including absence of anti-SSA and anti-SSB antibodies, pericapillary and non pericapillary, lymphocytic infiltrate, and lack of glandular canals damages [49]. However, others studies showed that HCV-related sialadenitis was indistinguishable from those patients with primary Sjögren’s syndrome [15, 55]. In a large series of HCV-related SS patients, the prevalence of anti-SSA and anti-SSB antibodies was nearly 20% [55]. The mechanisms through which HCV may induce sialadenitis include its sialotropism [51]. In rodent models, the expression of the HCV E1 and E2 glycoproteins by transgenic mice is associated with the development of sialadenitis [56].

**Natural history of cryoglobulinaemic vasculitis**

During chronic HCV infection, the immunochemical properties of serum cryoglobulins may fluctuate. The detection of type II mixed cryoglobulins is more stable over time than type III and oligoclonal MC [57]. The oligoclonal type appears to be an intermediate stage in the course of type III changing to type II MC. Circumstances predisposing HCV-infected patients to develop cryoglobulinaemic vasculitis remains unclear. Interaction between HCV and lymphocytes directly modulates B- and T-cell function [58] and result in polyclonal activation and expansion of B cell producing IgM with RF activity. CD4+CD25+ regulatory T cell, which have been shown to control autoimmunity, are significantly reduced in HCV-MC vasculitis patients [59]. This defect in immune regulation may account for the expansion of peripheral autoreactive B cell that drive MC vasculitis. Furthermore, HLA type II polymorphism may predispose to HCV-MC. HLA-DR11 is associated with MC vasculitis whereas HLA-DR7 appears to protect from the production of type II MC [60]. In contrast, specific virological factors have not yet been identified. Specific changes in HCV envelope sequence distribution are unlikely to be directly involved in the establishment of pathological B-cell monoclonal proliferation [61]. Cryoglobulinaemic vasculitis is usually associated with advanced age, longer duration of HCV infection, type II MC, a higher MC serum level and clonal B-cell expansions in both the blood and liver [57, 62]. The natural history and prognosis of MC vasculitis are variable and highly dependent on renal involvement or on the extent of vasculitis lesions. The worse prognostic factors are an age older than 60 yrs at diagnosis and renal involvement [63]. The overall 5 yrs survival after the diagnosis of vasculitis range from 90% to 50% in case of renal involvement [37]. In historic series by Meltzer et al. [3] and Gorevic et al. [30], renal involvement was the main cause of death. Even in the absence of significant renal failure, increased mortality from liver involvement, cardiovascular disease, infection and lymphoma has been reported [63]. In a recent retrospective Italian study of 231 patients, 79 of 97 deaths were linked to vasculitis (46%, of whom one-third due to renal involvement), cancer or haemopathy (23%), or liver disease (13%) [63]. Life-threatening MC complications are observed in up to 10% of the patients with almost two-thirds of death [64]. Intestinal ischaemia, pulmonary haemorrhage, high cryocrit levels and type II MC are associated with severe prognosis [64].

**HCV and B cell non-Hodgkin’s lymphoma (NHL)**

**Clinical aspects**

The lymphomagenic role of HCV is still a matter of debate. In HCV-infected patients, lymphoproliferative processes may evolve out of MC type II [65], or independently in patients without MC [66]. In long-term follow-up studies, only 4–6% of the patients with HCV-type II cryoglobulinaemia develop frank B-cell malignancies [67, 68], although in a recent multicentre Italian study, the estimated risk for lymphoproliferative disease was found to be 35-fold in patients with MC compared with the general population [69]. Epidemiological studies on the prevalence of HCV infection in B-cell NHL patients have yielded conflicting results [70–72]. Data from several countries indicate that the prevalence of HCV infection among patients with B-cell NHL ranges from 9 to 37%, values that are significantly higher than those found in patients with other haematological disorders, or in the general public [70, 73–78]. In contrast, several reports have not found an increased prevalence of HCV infection among patients with B-cell NHL [79, 80], suggesting a geographical influence that may signify that other factors are involved [72, 81, 82]. Meta-analysis studies have also investigated this association. In a meta-analysis published in 2003,
Gisbert et al. [83] have shown that the prevalence of HCV infection in patients with B-cell NHL is 15%. An additional meta-analysis, published in 2004 by Matsuo et al. [84], estimated the odds ratio (OR) for NHL for HCV seropositive relative to seronegative persons at 5.7 [95% confidence interval (CI) 4.09–7.96]. This meta-analysis also included a comparison of studies performed in endemic vs non-endemic countries which showed similar results for both. Additionally, studies that have utilized blood donors as a control group had higher OR compared with studies that have chosen different control groups (OR ≈ 8.43 and 4.65, respectively). Certain histological subtypes of lymphomas may be more strongly associated with HCV infection. Generally, low-grade lymphomas are more frequently associated with HCV than high-grade lymphomas [82]. In most studies, marginal zone B-cell lymphoma (MZL), lymphoplasmacytic lymphoma/immunocytoma and diffuse large B-cell lymphoma are the more frequent histological subtypes reported in HCV-infected patients [66, 74, 85–87]. A large case series published by Trejo et al. [88] has also demonstrated a frequent extra-nodal localization of NHL in HCV-infected patients and in particular, the liver and the major salivary gland were significantly overrepresented. An association between HCV infection and other B-cell lymphoproliferative processes, such as chronic lymphocytic leukaemia and multiple myeloma, has been suggested but was not demonstrated in a consistent manner, and thus remains debatable [82].

**Pathogenesis**

Direct lymphocyte transformation by a given microbial agent account for lymphotropic transforming viruses such as Epstein–Barr virus, human herpesvirus 8 and human T lymphotropic virus 1 that directly infect a subset of lymphoid cells in which they express viral oncogenes. The mechanisms by which HCV leads to the development of B-cell lymphoma remain to be elucidated. HCV may also exert a direct oncogenic activity on B cells. There is evidence at least in some experimental conditions for a direct effect of some HCV proteins, such as HCV core and NS3 on cellular proliferation and viability [89, 90]. HCV is capable of infecting B lymphocytes through LDL receptors or CD81 [91, 92]. HCV is a positive, single-strand RNA virus but without a DNA intermediate in its replicative cycle ruling out the possibility of an insertional oncogenesis. Although HCV can infect B cells in vitro, only one case of B-cell lymphoma associated with direct infection of B cells by HCV has been described so far [93]. Recently, active replication of HCV was demonstrated in cells (mostly CD20 B cells) of peri-hepatic lymph nodes of cirrhotic patients [94]. Alternatively, HCV can exert its oncogenic potential indirectly by interacting with the host immune system [95]. HCV could exert a chronic stimulus on the immune system, leading to the selection of abnormal clones, in a scenario reminiscent of the role of Helicobacter pylori in gastric mucosal-associated lymphoid tissue (MALT) lymphoma [96]. In MC patients, the BCR was demonstrated to bind with HCV-E2 and NS3 [97, 98]. Clonal B-cell expansion has been demonstrated in liver, blood and bone marrow of patients with chronic HCV infection in the absence of overt B-cell malignancy [62, 99]. The B-cell repertoire in cryoglobulinaemic vasculitis is highly restricted. B cell clones in HCV-associated MC and lymphomas often use the VH1-69 gene and V kappa27 segment which is also used by anti-E2 antibodies elicited by HCV [100, 101]. Sequencing of these Ig variable regions has also revealed that they are the product of somatic hypermutation [102, 103]. Data support the hypothesis that MC and the subsequent evolution to NHL are antigen-driven processes possibly sustained by HCV [104]. The increased percentage of extra-nodal marginal cell lymphomas in HCV infection and the report of a more than 10-fold greater risk for developing B-NHL of the liver and salivary gland than the risk of lymphomas at other sites is consistent with the hypothesis that B-NHL arise selectively from marginal zone B cell [105]. Antigenic stimulation of spleen marginal zone B cells by persisting HCV antigens and particularly the E2 viral protein might be involved in the pathogenesis [97, 106]. In this line, enhanced mutations of immunoglobulin heavy chain and proto-oncogenes (p53, Bcl2 and β-catenin) have been demonstrated in HCV-associated lymphoma [107]. A higher rate of bcl-2 over-expression was reported in HCV patients with MC compared with HCV-infected patients without MC, with a further increase in patients with MC type II and NHL [108, 109]. Although HCV-associated bcl-2 over-expression in the mRF producing B cells (i.e. in cryoglobulins producing B-cell clones) remains to be convincingly demonstrated [110], the strongest argument for a role of HCV infection in NHL is provided by interventional studies (Table 3). A recent systematic review [111] summarized the response rate of lymphoproliferative disorders to anti-viral treatment in 65 HCV-infected patients, in a total of 16 different reports [93, 109, 112–125]. Complete remission was achieved in 75% (95% CI: 64–84%) of the cases. Additional studies published after the systematic review supported these results [126, 127].

**Therapy for cryoglobulinaemic vasculitis**

With the discovery of HCV as the aetiological agent for most cases of mixed cryoglobulinaemia, new opportunities and

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**Table 3. Efficacy of antiviral treatment in B-cell Non-Hodgkin's Lymphoma associated with Hepatitis C infection**

<table>
<thead>
<tr>
<th>Histological subtype</th>
<th>n</th>
<th>Antiviral treatment</th>
<th>SVR (%)</th>
<th>Haematological response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CR</td>
<td>PR</td>
</tr>
<tr>
<td>SLVL [122, 125, 126]</td>
<td>20</td>
<td>IFN (n=6)</td>
<td>6 (75%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN+RIBA (n=11)</td>
<td>7 (87.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PegIFN+RIBA (n=1)</td>
<td>8 (72.7)</td>
<td></td>
</tr>
<tr>
<td>MZL [125, 127, 152, 153]</td>
<td>18</td>
<td>IFN (n=1)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN+RIBA (n=4)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PegIFN+RIBA (n=13)</td>
<td>4(57.1)</td>
<td>1(25)</td>
</tr>
<tr>
<td>Immunocytoma [112, 121, 127]</td>
<td>10</td>
<td>IFN (n=7)</td>
<td>2 (69%)</td>
<td>1(33.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PegIFN+RIBA (n=3)</td>
<td>2(69%)</td>
<td>1(33.3)</td>
</tr>
<tr>
<td>Folicular [127]</td>
<td>1</td>
<td>IFN (n=1)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td>Large B cell [154]</td>
<td>1</td>
<td>IFN (n=1)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td>Mantle cell [93]</td>
<td>1</td>
<td>IFN (n=1)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoctic [142]</td>
<td>1</td>
<td>IFN (n=1)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td>Multiple myeloma [124]</td>
<td>1</td>
<td>IFN (n=1)</td>
<td>1(100)</td>
<td>0</td>
</tr>
<tr>
<td>MALT [116]</td>
<td>1</td>
<td>IFN (n=1)</td>
<td>1(100)</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Including one patient with stable disease, bResults for one patient not available.

SLVL, splenic lymphoma with villous lymphocytes; MZL, marginal zone lymphoma; INF, interferon; RIBA, Ribavirin; PEG INF, pegylated interferon; SVR, sustained viral response; CR, complete remission; PR, partial remission; NR, no response; NA, not available.
problems for crafting therapy of HCV-MC have emerged. A new and major concern was the potential adverse effects that immunosuppressive therapy with glucocorticoids and cytotoxic drugs could have on an underlying chronic viral infection. Alternatively, the discovery of HCV provided the opportunity to control HCV-MC with antiviral therapy based on the belief that the underlying infection was driving immune complex formation and resultant vasculitis. Unfortunately, the initial efforts to treat HCV with weekly interferon were associated with low rate of virological cure. Furthermore, the cornerstone of HCV antiviral therapy has been and continues to be interferon which has the potential to exacerbate autoimmune disease states [128]. Though rare, interferon has been documented to induce vasculitis in some patients previously without it and to exacerbate certain complications such as ulcer healing or neuropathy thus confounding the use of interferon-based therapies as sole treatment of HCV-MC [129]. While optimum therapy is still unclear a series of recent studies suggest a role for both antiviral as well as limited immunosuppressive therapy in the treatment of HCV-MC depending on the activity and severity of the underlying vasculitis and the status of the underlying infection. Figure 1 represents the therapeutic options in patients with HCV-MC.

**Antiviral agents**

The treatment of HCV infection (i.e. in the absence of HCV-MC) has progressed dramatically over the past 15 years with now the standard of pegylated interferon-α and ribavirin therapy leading to sustained virological clearance in nearly two-thirds of the patients. The early attempts to control HCV-MC with standard thrice weekly IFN-α was not surprisingly associated with a relatively poor response and a high relapse rate, especially in severe cases [130]. IFN-α monotherapy was effective in 50–100% of the patients with purpuric skin lesions, but did not clearly demonstrate efficacy on neurological or renal involvement. However, when follow-up was sufficient, most of the responders developed virological and clinical relapses following IFN-α withdrawal [130–132]. Combination therapy with standard IFN-α plus ribavirin has provided much better short- and long-term results in patients with HCV-related vasculitis than historically reported with IFN-α. In three uncontrolled studies [133–135], combination therapy with standard IFN-α and ribavirin demonstrates enhanced efficacy on main HCV-related vasculitic manifestations (cutaneous, 100%; renal, 50% and neural, 25–75%). Two studies reported a loss of proteinuria and haematuria in sustained viral responders treated by IFN-α.
HCV-related MC

plus ribavirin [136, 137]. We recently reported in 72 consecutive HCV-MC patients that Peg-IFN-α plus ribavirin achieved a higher rate of complete clinical (67.5 vs 56.2%), virological (62.5 vs 53.1%) and immunological response (57.5 vs 31.2%) as compared with standard IFN-α plus ribavirin, regardless of HCV genotype and viral load [138]. In multivariate analysis, an early virological response (OR, 3.53; 95% CI 1.18–10.59) was independently associated with a complete clinical response of MC. A glomerular filtration rate <70 ml/min (OR 0.18; 95% CI 0.05–0.67) was negatively associated with a complete clinical response of MC [138]. Although, HCV-MC patients with HCV genotype 1 and/or previous failure to therapy displayed a lower clinical response rate, these factors were not independently associated with a poor clinical response in multivariate analysis [138]. Epidemiological features, HCV viral load, transaminases or liver damage did not influence the clinical outcome in this study [138]. The sub-group analysis of the 40 HCV-MC patients treated with Peg-IFN-α plus ribavirin showed a complete recovery of skin involvement in 21/24 (87.5%), arthralgia in 18/22 (81.8%), peripheral neuropathy in 20/27 (74%) and nephropathy in 5/10 (50%) cases, respectively [138].

Immunosuppressive agents

Immunosuppressive agents are typically reserved for patients with severe disease manifestations such as membranoproliferative glomerulonephritis, severe neuropathy and life-threatening complication. Traditionally a combination of corticosteroids and immunosuppressants such as cyclophosphamide and azathioprine have been used for the control of severe vasculitis lesions while awaiting the generally slow response to antiviral treatments. Mycophenolate mofetil may represent an alternative therapeutic option in patients refractory or intolerant to these immunosuppressive drugs [139]. In a large retrospective study of 105 patients with renal disease associated with cryoglobulinaemia vasculitis, 80% were administered corticosteroids and/or cytotoxic agents, while 67% underwent plasmapheresis [37]. Despite this aggressive approach, long-lasting remission of the renal disease was achieved in only 14% of the cases, and the 10-yr survival rate was only 49%.

Corticosteroids, used alone or in addition to IFN-α, did not favourably affect the response of HCV-related vasculitic manifestations in two controlled studies [131, 132]. In one randomized trial, methylprednisolone (MP) alone given for 1 yr was associated with clinical response in 22% of the patients, compared with 66 and 71% in patients receiving IFN-α or IFN-α plus MP, respectively [132]. Low-dose corticosteroids may help to control minor intermittent inflammatory signs such arthralgia, but do not succeed in cases of major organ involvement (i.e. neurological, renal), or in the long-term control of vasculitis.

Plasmapheresis offers the theoretical advantage of removing the pathogenic cryoglobulins from the circulation of patients with HCV-MC vasculitis. Immunosuppressive therapy is usually associated with plasma exchange in order to avoid the rebound increase in cryoglobulinaemia that is commonly seen after discontinuation of apheresis [35]. When used in combination with anti-HCV treatment, plasmapheresis did not modify the virological response if IFN-α was given after each plasma exchange session [140].

Recently, several groups have reported on the efficacy of anti-CD20 monoclonal antibody (rituximab) in patients with HCV-MC vasculitis [141–148]. Such an approach involves the use of monoclonal antibodies directed to CD20 antigen, a transmembrane protein expressed on pre-B lymphocytes and mature lymphocytes. Rituximab proved effective on skin vasculitis manifestations, subjective symptoms of peripheral neuropathy, arthralgia and low-grade B-cell lymphoma. The pooled data of the eight rituximab studies including 43 HCV-MC patients showed a complete recovery of skin involvement in 24/33 (73%), arthralgia in 16/30 (53%), peripheral neuropathy in 9/25 (36%) and nephropathy in 9/13 (70%) cases, respectively [141–148]. Most clinical responders also had a decrease in serum cryoglobulin levels and an increase in C4 serum levels, though not to undetectable or normal levels. However, one potential concern regarding the use of such therapy is that it has a propensity to worsen HCV viraemia [141], which may lead patients to develop more severe HCV-induced liver lesions and/or cryoglobulinaemic relapses in ensuing years [147, 148]. These studies did not allow conclusions to be drawn concerning the efficacy of anti-CD20 monoclonal antibody on peripheral neuropathy and nephropathy [141, 148, 149]. The absence of efficacy on HCV viral clearance and furthermore, the potential increase in HCV viral load stresses the need for combined antiviral therapy to block the HCV infection trigger.

Therapeutic guidelines

In general, it appears logical that aggressive antiviral therapy with Peg-IFN and weight-based ribavirin be considered as induction

![Fig. 2. Therapeutic strategies in patients with HCV-associated MC vasculitis. CNS, central nervous system.](http://rheumatology.oxfordjournals.org/)
therapy for HCV-MC with mild-to-moderate disease severity and activity (i.e. without rapidly progressive nephritis, motor neuropathy or other life-threatening complications) (Figure 2). The duration of therapy has not yet been rigorously determined but treatment courses longer than those estimated merely based on genotype alone appear more likely to be effective [135]. The current treatment duration in HCV-MC is 12 months for all HCV genotypes with the Peg-IFN and ribavirin combination [138]. With this strategy patients with mild or moderate disease (i.e. arthralgia, purpura, sensory-motor polyneuropathy and/or isolated proteinuria) may be able to be managed without immunosuppressive therapy. In patients with more severe disease (i.e. worsening of renal function, mononeuritis multiplex, extensive skin disease including ulcers and distal necrosis), an induction phase of immunosuppression is often necessary while awaiting the generally slow response to antiviral treatments (Figure 2). Combination therapy with rituximab and Peg-IFN-α plus ribavirin appears logical and may target both the viral trigger (HCV) and the downstream B-cell arm of autoimmunity. Biologic therapy with B cell directed therapy is promising in the treatment of HCV-MC but many questions exist regarding the appropriate position of this strategy in treatment. In this setting, as with the use of rituximab in the treatment of other autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus, the duration of effect appears finite with response durations typically lasting 6–12 months and its combination with antiviral drugs is necessary. The continued efficacy and safety of repeated therapy in HCV-MC needs further investigation. For patients presenting with the most fulminant presentations including peripheral necrosis of extremities, rapidly progressive nephritis, digestive, pulmonary and or central nervous system involvement and or signs and symptoms of hyperviscosity, apheresis can have immediate beneficial effects but must be combined with immuno-suppression (cytotoxic agents, steroids) to avoid post-apheresis rebound of MC. Antiviral therapy with Peg-IFN-α and ribavirin combination should be differed after the critical phase (Figure 2).

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