Learning objectives:

- Correctly classify/diagnose cryoglobulinaemia and mixed cryoglobulinaemia (MC).
- Use the correct technical procedures to detect and characterise cryoglobulin.
- Describe and explain the main mechanisms involved in the aetiopathogenesis of MC syndrome.
- Outline the epidemiology, prognosis and main clinical manifestations of MC syndrome (cryoglobulinaemic vasculitis).
- Make a differential diagnosis between MC syndrome and other autoimmune rheumatic disorders (Sjögren’s syndrome, rheumatoid arthritis, other systemic vasculitides).
- Define the main organ and systemic autoimmune disorders possibly triggered by hepatitis C virus (HCV) infection.
- List the possible neoplastic complications correlated with HCV infection.
- Describe and explain the pathogenetic mechanisms of HCV-related autoimmune and lymphoproliferative disorders.
- List the main targets of HCV-mixed cryoglobulinaemia therapy: clinical response (organ target manifestations), virological response (HCV RNA) and immunological response (cryoglobulinaemic, C4 serum level).
- Understand that antiviral therapy (Peg-interferon α plus ribavirin) is the cornerstone of HCV-mixed cryoglobulinaemia treatment.
- Recognise that HCV viral load correlates with clinical outcome.
- Know that B cell depleting therapy (rituximab) is an interesting additional therapeutic option.
- Explain the timing of action and the limitations of rituximab.
- Explain concerns about immunosuppressant agent use in HCV-mixed cryoglobulinaemia.
- Describe the use of steroids, immunosuppressant agents and plasmapheresis.
1. Cryoglobulinaemia

1.1 Definition

The presence in the serum of one (monoclonal cryoimmunoglobulinaemia) or more immunoglobulins (mixed cryoglobulinaemia, MC), which precipitate at temperatures below 37°C and redissolve on rewarming, is termed cryoglobulinaemia or cryoimmunoglobulinaemia and is an in vitro phenomenon (figure 1). Various hypotheses have been suggested to explain the cold precipitation of immunoglobulin(s): it can be secondary to the intrinsic characteristics of both mono- and polyclonal immunoglobulin (Ig) components or can be caused by interaction among single components of the cryoprecipitate. However, the intimate mechanism(s) of cryoprecipitation remains obscure.

1.2 Classification

Cryoglobulinaemia is usually classified into three subgroups according to immunoglobulin composition (table 1). Type I cryoglobulinaemia consists of only one isotype or subclass of immunoglobulin. Both type II and type III mixed cryoglobulins are immune complexes (IC) composed of polyclonal IgGs, autoantigens and mono- or polyclonal IgMs, respectively; the IgMs are the corresponding autoantibodies with rheumatoid factor (RF) activity. With more sensitive methodologies (i.e., immunoblotting or two-dimensional polyacrylamide gel electrophoresis), type II mixed cryoglobulins often show a microheterogeneous composition; in particular, oligoclonal IgM or a mixture of polyclonal and monoclonal IgM can be detected. This particular serological subset, termed type II–III MC, could represent an intermediate, evolutive state from type III to type II MC. Moreover, type II–III MC could fit together with the most recent molecular studies showing the presence of oligoclonal B lymphocyte proliferation in liver and bone marrow biopsy specimens from patients with MC. In two-thirds of patients with type II MC, a cross-idiotype WA monoclonal RF (first isolated from the serum of a patient with Waldenström’s macroglobulinaemia) has been demonstrated.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Composition</th>
<th>Pathological findings</th>
<th>Clinical associations</th>
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<tbody>
<tr>
<td>Type I cryoglobulinaemia</td>
<td>Monoclonal Ig, mainly IgG, or IgM, or IgA self-aggregation through Fc fragment of Ig</td>
<td>Tissue histological alterations of underlying disorder</td>
<td>Lymphoproliferative disorders: MM, WM, CLL, B cell NHL</td>
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<tr>
<td>Type II mixed cryoglobulinaemia</td>
<td>Monoclonal IgM (or IgG, or IgA) with RF activity (often cross-idiotype WA-mRF) and polyclonal Ig (mainly IgG)</td>
<td>Leukocytoclastic vasculitis B lymphocyte expansion with tissue infiltrates</td>
<td>Infections (mainly HCV), autoimmune/lymphoproliferative disorders, rarely ‘essential’</td>
</tr>
<tr>
<td>Type II–III mixed cryoglobulinaemia</td>
<td>Oligoclonal IgM RF or mixture of poly/m monoclonal IgM (often cross-idiotype WA-mRF)</td>
<td>Leukocytoclastic vasculitis B lymphocyte expansion with tissue infiltrates</td>
<td>Infections (mainly HCV), autoimmune/lymphoproliferative disorders, rarely ‘essential’</td>
</tr>
<tr>
<td>Type III mixed cryoglobulinaemia</td>
<td>Polyclonal mixed Ig (all isotypes) with RF activity of one polyclonal component (usually IgM)</td>
<td>Leukocytoclastic vasculitis B lymphocyte expansion with tissue infiltrates</td>
<td>Infections (mainly HCV), more often autoimmune disorders, rarely ‘essential’</td>
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CLL, chronic lymphocytic leukaemia; HCV, hepatitis C virus; Ig, immunoglobulin; MM, multiple myeloma; NHL, non-Hodgkin’s lymphoma; RF, rheumatoid factor; WM, Waldenström’s macroglobulinaemia.

Table 1 Classification and clinico-pathological characteristics of different cryoglobulinaemias
Type I cryoglobulinaemia is usually associated with well-known haematological disorders but is often itself asymptomatic. Similarly, circulating mixed cryoglobulins can be detected in a great number of infectious or systemic disorders; generally they represent an isolated laboratory finding without any clinical consequence. In contrast, ‘essential’ MC represents a distinct disorder, which is classified as one of the systemic vasculitides. Cryoglobulinaemic vasculitis (figure 2) is secondary to vascular deposition of circulating IC, mainly cryoglobulins and complement, with the possible contribution of both haemorheological and local factors. According to its clinical and histological features, MC is included in the subgroup of small-vessel systemic vasculitides, which also include cutaneous leukocytoclastic vasculitis and Henoch–Schönlein purpura (table 2).

Leukocytoclastic vasculitis is the histopathological hallmark of MC (figure 2). It may involve small and medium and may be responsible for multiple organ involvement. The term ‘cryoglobulinaemic vasculitis’ is often used as a synonym and better describes the typical histopathological alterations commonly detectable in the biopsy of cutaneous lesions.

2. Mixed cryoglobulinaemia (cryoglobulinaemic vasculitis)

2.1 Epidemiology

MC is considered to be a rare disorder, but no adequate epidemiological studies of its overall prevalence have been carried out. Numerous cohort studies of series of patients from different countries suggest that the prevalence of MC is geographically heterogeneous; the disease is more common in southern Europe than in northern Europe or Northern America. MC is characterised by clinical polymorphism – a single manifestation (skin vasculitis, hepatitis, nephritis, peripheral neuropathy, etc) is often the only apparent or clinically predominant feature – so that patients with MC
levels of circulating mixed cryoglobulins in over 50% of cases, while overt cryoglobulinaemic syndrome developed in about 5%. HCV infection is particularly diffuse worldwide; therefore, an increasing incidence of MC and of other HCV-related extrahepatic manifestations can be expected, especially in underdeveloped countries where HCV is prevalent in the general population.

In contrast, essential MC is generally seen in a significantly lower proportion of patients with MC, being quite rare in some geographical areas, such as southern Europe, where on the whole MC is prevalent (figure 4).

### 2.2 Aetiopathogenesis

Several clinico-epidemiological studies reported that chronic hepatitis was one of the most common symptoms...
Cryoglobulinemia and hepatitis C virus

Pathogenesis of MC. The prevalence of serum anti-HCV antibodies and/or HCV RNA in patients with MC ranged from 70% to almost 100% among different patient populations. Given the striking association between MC and HCV infection, the term ‘essential’ is now used to refer to a minority of patients with MC (in Italy <5%; figure 5). The clinical development of MC is closely linked to the natural history of chronic HCV infection. MC phenotypes are also the result of genetic and/or environmental cofactors, which remain largely unknown (figure 6). HCV has been recognised to be both a hepato- and lymphotropic virus, as suggested by the presence of active or latent viral replication in the peripheral lymphocytes of patients with type C hepatitis or MC. HCV is an RNA virus without reverse transcriptase activity; therefore the viral genome cannot integrate in the host genome. It is probable that HCV chronically stimulates the immune system through different viral proteins, such as core protein. Chronic stimulation of the lymphatic system exerted through viral epitopes, autoantigen production and/or a molecular mimicry mechanism has been suggested. In this respect, particularly interesting is the presence, in HCV-positive patients, of anti-GOR antibodies, which are cross-reactive autoantibodies directed both to the HCV core and a nuclear antigen called GOR. Another pathogenetic hypothesis suggested of MC; its prevalence progressively increases to over two-thirds of patients during the clinical course of the disease. This observation suggested a possible role for hepatotropic viruses in the pathogenesis of the disease and so a causative role for hepatitis B virus (HBV) has been sought since the 1970s. The same virus has been just correlated with another systemic vasculitis, namely, polyarteritis nodosa. However, HBV viraemia was rarely recorded, while anti-HBV antibodies varied among different groups of patients with MC. HBV may be a causative factor of MC in <5% of people (figure 5).

In 1989, the discovery of HCV as the major aetiological agent of non-A-non-B chronic hepatitis, was crucial for the aetio-pathogenetic studies of MC syndrome. A possible role of HCV infection in MC was proposed independently by two pioneering reports which showed a significantly higher prevalence of serum anti-HCV antibodies in these patients than in the general population. This hypothesis was confirmed in 1991, when the presence of HCV RNA was detected by polymerase chain reaction (PCR) in 86% of Italian patients with MC. Following this, an increasing number of studies including clinico-epidemiological observations, as well as both histopathological and virological investigations (HCV RNA detection by PCR and/or in situ hybridisation) established the important role of HCV in the pathogenesis of MC. The prevalence of serum anti-HCV antibodies and/or HCV RNA in patients with MC ranged from 70% to almost 100% among different patient populations. Given the striking association between MC and HCV infection, the term ‘essential’ is now used to refer to a minority of patients with MC (in Italy <5%; figure 5).

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that HCV, in association with very low-density lipoprotein (LDL), might induce a T-independent primordial B cell population, producing monoclonal immunoglobulin with WA idotype. In turn, HCV–very LDL complexes may trigger RF production as consequence of somatic mutations of WA clones; the possible evolution to B cell lymphomas might be the consequence of the accumulation of stochastic genetic aberrations. The chronic stimulation of B lymphocytes by HCV epitopes may cause expansion of some B cell subpopulations with favourable and/or dominant genetic characteristics. This hypothesis recalls the pathogenetic role of Helicobacter pylori in MALT lymphoma of the stomach, for which different evolutive phases are required.

The interaction between the HCV E2 envelope protein and CD81 molecule, a ubiquitous tetraspannin present on the surface of B cells, may represent another important pathogenetic factor in MC. The consequence of this interaction may be a strong and sustained polyclonal stimulation of the B cell compartment (figure 6). A following pathogenetic step of HCV-related autoimmune-lymphoproliferative disorders may be the t(14;18) translocation found in B cells of HCV-infected subjects. Even if not definitely confirmed, the t(14;18) translocation may lead to increased expression of Bcl-2 protein with consequent inhibition of apoptosis and abnormally prolonged B cell survival. Interestingly, a significantly high prevalence of t(14;18) translocation is found in patients with HCV-related MC (85% in type II MC), and also in patients with isolated hepatitis type C (about 37–38%). It can be hypothesised that during chronic HCV infection, several factors (including the interaction between the HCV E2 protein and CD81 molecule, the high viral variability and the persistent infection of both hepatic and lymphatic cells) may favour a sustained and strong B cell activation (figure 6). This latter may in turn favour the t(14;18) translocation and Bcl-2 overexpression; the consequent B lymphocyte expansion is responsible for autoantibody production, including the cryoglobulins. In addition, the prolonged B cell survival may represent a predisposing condition for further genetic aberrations, which may lead to frank B cell malignancy as a late complication of MC syndrome.

Of interest, HCV-driven lymphoproliferation may also explain the pathogenetic role of HCV infection in ‘idiopathic’ B cell lymphomas. This association was first described in unselected Italian patients with idiopathic B cell lymphomas and later confirmed by different epidemiological and laboratory studies, mainly in the same geographical areas as those in which HCV-associated MC is commonly found (figure 4).

Given its biological characteristics, HCV may be involved in a wide number of autoimmune and lymphoproliferative disorders. Figure 6 summarises the main causative factors – infectious, toxic, genetic and/or environmental – that are potentially involved in the pathogenesis of MC. These factors, alone or in combination, may trigger two multistep pathogenetic processes, not mutually exclusive, responsible
Cryoglobulinemia and hepatitis C virus

MC with or without overt clinical syndrome has been reported in patients with a great number of infectious agents, usually as anecdotal observations. A significant prevalence of MC has been found in patients with human immunodeficiency virus (HIV) infection, with and without HCV co-infection. HIV alone may exert a continuous antigenic stimulation of B lymphocytes; these latter may be responsible for type III MC production earlier in the course of HIV infection. In some patients the B cell disorder may evolve into monoclonal MC with a typical clinical syndrome. As observed for HCV infection, the prevalence of other virus-related MC is variable among patient series.

Figure 6 Aetiopathogenesis of mixed cryoglobulinaemia (MC) and other HCV-related disorders: the HCV syndrome. The figure summarises the putative mechanisms involved in the aetiopathogenic cascade of MC and other HCV-related disorders. This is probably a multifactorial and multistep process: the remote events include some infectious agents, mainly HCV, predisposing host factors and, possibly, unknown environmental/toxic triggers. Viral antigens (for example HCV core, envelope E2, NS3, NS4, NS5A proteins) may exert a chronic stimulus on the host immune system through specific lymphocyte receptors, such as CD81, which may interact with the viral E2. Predisposing host factors may include particular HLA alleles, and metabolic and hormonal conditions. The main consequence is a ‘benign’ B cell proliferation with a variety of autoantibodies produced, among which are rheumatoid factor (RF), and cryo- and non-cryoprecipitable immune complexes (IC). These serological alterations may be correlated with different organ- and non-organ-specific autoimmune disorders, including MC syndrome (or cryoglobulinaemic vasculitis). Moreover, the activation of Bcl2 proto-oncogene, responsible for prolonged B cell survival, may be a predisposing condition to other genetic aberrations, which may lead to frank B cell lymphomas and other malignancies. The appearance of malignant neoplasias can be seen in a small but significant percentage of patients, usually as a late complication. Both immunological and neoplastic disorders show a clinico-serological and pathological overlap. Often, autoimmune organ-specific manifestations may evolve to systemic conditions, such as MC, and less frequently to overt malignancies. Conversely, it is not uncommon that patients with malignancies develop one or more autoimmune manifestations. In this scenario, MC syndrome is at the junction between autoimmune and neoplastic disorders. HCV, hepatitis C virus; IC, immune complexes; sicca s., sicca syndrome; porphyria c.t., porphyria cutanea tarda; RF, rheumatoid factor.

for MC and other HCV-related disorders. The first process produces a ‘benign’ polyoligoclonal B lymphocyte proliferation responsible for organ- and non-organ-specific autoimmune disorders, including immune-complex-mediated cryoglobulinaemic vasculitis; the second is characterised by different oncogenetic alterations, which ultimately may lead to malignant complications. Comparable pathogenetic mechanisms could be also hypothesised for HCV-negative MC; this intriguing clinical subset might be correlated with other infectious agents or associated with some well-known autoimmune/rheumatic or lymphoproliferative disorders (figures 5 and 6).
from different geographical areas (figure 4). Moreover, a number of clinico-epidemiological studies showed a heterogeneous distribution of different HCV-related extrahepatic manifestations, including some autoimmune disorders such as primary Sjögren’s syndrome (pSS).

Cryoglobulinaemic syndrome may share a number of aetiopathogenetic events and clinical features with both autoimmune diseases such as autoimmune hepatitis (AIH), Sjögren’s syndrome and polyarthritis and B cell lymphomas. Therefore, a differential diagnosis should be carefully made in all patients with MC syndrome (figure 7; see also section 2.5); correct disease classification may decisively affect the overall clinico-therapeutic approach and prognosis.

### 2.3 Clinical manifestations

MC syndrome, or cryoglobulinaemic vasculitis, is characterised by a clinical triad of purpura, weakness and arthralgias, and by a variable combination of symptoms, including chronic hepatitis, membranoproliferative glomerulonephritis (MPGN), peripheral neuropathy, skin ulcers, diffuse vasculitis and, less frequently, lymphatic and hepatic malignancies. The clinical pattern of cryoglobulinaemic vasculitis is comparable in patients with type II or type III MC.

Table 3 gives the prevalence of MC manifestations in an Italian patient population referred to our university-based division of rheumatology. Patient recruitment at different specialist centres may influence the symptom composition of MC series; for example, patients with MC recruited at nephrology units naturally show a higher percentage of glomerulonephritis compared with those seen at rheumatology units (figure 3). This factor together with the different geographical origins of patient series reported in the literature may be responsible for the variable prevalence of individual MC symptoms.

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**Figure 7** Clinical overlap and differential diagnosis among some possible hepatitis C virus (HCV)-related rheumatic diseases. Mixed cryoglobulinaemia (MC) syndrome, primary Sjögren’s syndrome (SS) and rheumatoid arthritis (RA) show a clinico-pathological overlap, including a possible association with HCV infection. The following information may be useful for a correct differential classification/diagnosis: primary SS shows a typical histopathological pattern of salivary gland involvement and specific autoantibodies (anti-RoSSA/LaSSB), which are rarely found in patients with MC; conversely, cutaneous leukocytoclastic vasculitis, visceral organ involvement (glomerulonephritis, hepatitis), low C4 and HCV infection, are typically found in MC. Moreover, erosive symmetrical polyarthritis and serum anticyclic citrullinated peptide antibodies (anti-CCP) characterise classic RA. Finally, B cell non-Hodgkin’s lymphoma (B-NHL) may complicate these diseases, more frequently MC and primary SS. B-NHL may be suspected following careful clinico-serological monitoring and diagnosed by bone marrow/lymph node biopsies and total body CT scan. RF, rheumatoid factor.
At patient anamnesis, the presenting symptoms of MC vary greatly among cryoglobulinaemic patients; similarly, at the first examination, MC shows different clinico-serological patterns, varying from apparently isolated serum mixed cryoglobulins, in some cases associated with mild manifestations such as arthralgias and/or sporadic purpura, to severe cryoglobulinaemic syndrome with multiple organ involvement (figure 8). The disease shows a combination of serological findings (mixed cryoglobulins with RF activity and frequently low C4) and clinico-pathological features (purpura and leukocytoclastic vasculitis with multiple organ involvement). In some chronically HCV-infected subjects, asymptomatic serum mixed cryoglobulins can be found. This condition may precede the clinical onset of disease by years or decades. On the other hand, some patients show typical cryoglobulinaemic syndrome without serum cryoglobulins, the hallmark of the disease (figure 8). MC is characterised by large amount of cryoprecipitable IC, with the cryoglobulins representing a variable percentage of them among different patients as well as in the same patient during follow-up. Therefore, the absence of serum cryoglobulins may be a transient phenomenon owing to this variable percentage of cryoprecipitable IC; repeated cryoglobulin determinations are necessary for a correct diagnosis in these subjects.

The most common manifestations of MC are cutaneous lesions. Orthostatic purpura is generally intermittent, with the dimensions and diffusion of purpuric lesions varying greatly from sporadic isolated petechiae to severe vasculitic lesions, often complicated by torpid ulcers of the legs and malleolar areas (figure 2). In a significant proportion of patients, repeated episodes of purpura may lead to stable, often confluent, areas of ochraceous colouration on the legs (figure 2). Cutaneous manifestations, in particular orthostatic purpura and ulcers, are the direct consequence of vasculitic alterations with the possible contributions of various cofactors, in particular chronic venous insufficiency and physical stress, mainly prolonged standing and/or muggy weather. In addition, haemorheological disturbances due to high cryocrit levels may also be a contributing factor. In this respect, the purpuric outbreaks are often seen late in the afternoon when the highest cryocrit levels are generally found, often following prolonged standing.

Patients with MC frequently have arthralgias, while clear signs of arthritis (usually mild, non-erosive oligoarthritis) are less often seen.

Almost half of patients with MC complain of mild sicca syndrome, that is, xerostomia and xerophthalmia; however, only a few cases meet the current criteria for the classification of pSS (see section 2.5).

Peripheral neuropathy may frequently complicate the clinical course of MC, in most cases as mild sensory neuritis. The common symptoms are paraesthesias with painful and/or burning sensations in the lower limbs, often with nocturnal exacerbation. The patient’s quality of life may be
severely compromised because of the chronicity of these symptoms together with their poor response to treatment. In a minority of cases the peripheral neuropathy may be complicated by severe sensorimotor manifestations, which usually appear abruptly, often as asymmetric mononeuropathy. In some patients, peripheral neuropathy may complicate the interferon (IFN) α treatment, possibly in predisposed subjects, often during the first weeks of antiviral therapy. Central nervous system involvement, including dysarthria and hemiplegia, is rarely seen; in older patients, it is often difficult to distinguish these symptoms from the most common atherosclerotic vascular manifestations.

In over two-thirds of patients (table 3), overt chronic hepatitis, generally with a mild to moderate clinical course, can be seen at any time during the natural history of the disease. This manifestation, uncommon in other systemic vasculitides, is the direct consequence of HCV infection that represents the underlying disorder in MC. In our experience, chronic hepatitis may evolve to cirrhosis in one-quarter of patients, while only seven patients developed hepatocellular carcinoma. In a few cases, especially in patients with renal failure due to chronic glomerulonephritis, hepatorenal syndrome develops as a major life-threatening complication. On the whole, the clinical course and prognostic value of chronic hepatitis seem to be less severe than for classic type C hepatitis without MC syndrome; similarly, hepatocellular carcinoma less often complicates MC syndrome compared with the total population of HCV-positive subjects. These differences are quite intriguing, but difficult to fully explain. It may be that patients with MC characterised by a relatively low prevalence of HCV genotype 1b, along with a lower median consumption of alcohol, develop a rather benign clinical course of liver involvement.

MPGN type 1 is another important condition involving an organ, which may severely affect the prognosis and survival of patients with the disease. MC-related nephropathy is a typical IC-mediated glomerulonephritis, although other immunological mechanisms have also been hypothesised.

Widespread vasculitis involving medium to small arterioles, capillaries and venules with multiple organ involvement may develop in a small proportion of patients. This extremely severe complication may involve the skin, kidney, lungs, central nervous system and gastrointestinal tract. In rare cases, intestinal vasculitis may suddenly complicate the disease, often in patients with renal and/or liver disease; pain simulating an acute abdomen is the presenting symptom of intestinal vasculitis. A timely diagnosis and aggressive treatment are necessary for this life-threatening complication.

Interstitial lung disease has been anecdotally observed in MC syndrome as well as in patients with isolated HCV infection. Almost invariably, lung involvement in MC is characterised by subclinical alveolitis, as demonstrated by bronchoalveolar lavage in an unselected patient series; this condition may predispose to pulmonary infectious complications and, in rare cases, may lead to clinically evident interstitial lung fibrosis.

The hyperviscosity syndrome due to high levels of serum cryoglobulins is another rare clinical manifestation of MC, even if haemorheological alterations may contribute to some clinical symptoms such as orthostatic purpura, skin ulcers and renal involvement.
Generally, there are no associations between the severity of clinical symptoms, such as glomerulonephritis, skin ulcers and/or diffuse vasculitis, and the serum levels of cryoglobulins and/or haemolytic complement. Low complement activity is almost invariably detected in MC. It is characterised by a typical pattern independently of disease activity; namely, low or undetectable C4 with normal or slightly reduced C3 serum levels (table 3). Moreover, in vitro consumption of complement can also be seen owing to the anticomplement activity of some cryoimmunoglobulins. In clinical practice, it is interesting to note sudden variations in C4, rising from very low to abnormally high levels, in some patients with MC developing a B cell lymphoma. The lack of correlation between circulating cryoglobulin levels and the severity/activity of MC manifestations might be explained by different hypotheses: the pathogenic role of other non-cryoprecipitable IC, their intrinsic ability to activate the complement and/or the in situ formation of IC, with a relative concentration of HCV virions.

Some endocrinological disorders are significantly more common in patients with MC than in the general population, including thyroid disorders, diabetes and gonadal dysfunction. The most common thyroid disorders are autoimmune thyroiditis, subclinical hypothyroidism and thyroid cancer; while hyperthyroidism is less common, it may appear as a reversible complication of IFN treatment. Moreover, a significantly increased incidence of diabetes mellitus type 2 has been found in HCV-positive patients with and without MC syndrome compared with the general population. Finally, HCV-positive men with or without cryoglobulinaemic vasculitis may develop erectile dysfunction, attributable to hormonal or neurovascular alterations, or both.

B cell lymphoma is the most common malignancy found, often as a late manifestation of MC syndrome. This complication may be related to peripheral B lymphocyte expansion and to lymphoid infiltrates found in the liver and bone marrow, which represent the pathological substrate of the disease. In particular, these infiltrates have been regarded by some authors as ‘early lymphomas’, since they are sustained by lymphoid components indistinguishable from those of B cell chronic lymphocytic leukaemia/small lymphocytic lymphoma (B-CLL) and immunocytoma. However, unlike frank malignant lymphomas, they tend to remain unmodified for years or even decades and are followed by overt lymphoid tumours in approximately 10% of cases. These characteristics justify the proposed term ‘monotypic lymphoproliferative disorder of undetermined significance (MLDUS)’. Interestingly enough, the incidence of type II MC-related MLDUS is highest in those geographical areas where about 30% of patients with ‘idiopathic’ B cell lymphomas also display HCV positivity and where an increased prevalence of HCV genotype 2a/c has been found in both MC and lymphomas (figure 4). Type II MC-associated MLDUS presents two main pathological patterns, namely, the B-CLL-like and the immunocytoma-like. In clinical practice, malignant B cell lymphomas are often seen in patients with MC with a mild clinical course, sometimes unexpectedly during a routine evaluation. It is possible to observe a sudden decrease or disappearance of serum cryoglobulins and RF, sometimes associated with abnormally high levels of C4 as the presenting symptom of complicating B cell malignancy.

Other neoplastic manifestations, for example, hepatocellular carcinoma or papillary thyroid cancer, are less often seen. Thus, MC can be regarded as a pre-neoplastic disorder; consequently, careful clinical monitoring is recommended, even in the presence of mild MC syndrome.

2.4 Diagnosis
To date there are no available diagnostic criteria for MC; in 1989 the Italian Group for the Study of Cryoglobulinaemias proposed preliminary criteria for MC classification, later revised by including clinico-pathological and virological findings. This classification is mainly based on the serological and clinical hallmarks of the disease, namely, circulating mixed cryoglobulins, low C4 and orthostatic skin purpura. Moreover, leukocytoclastic vasculitis, involving medium and, more often, small blood vessels (arterioles, capillaries and venules) is the typical pathological finding of affected tissues. It is easily detectable by a skin biopsy of recent vasculitic lesions (within the first 24–48 h).

More recently, preliminary classification criteria for cryoglobulinaemic vasculitis have been developed by a cooperative study using a standardised methodology. If formally validated in MC patients referred to experts from a larger number of countries, these criteria may be usefully employed in epidemiological and clinico-pathogenetic studies, as well as in therapeutic trials.

In all cases, cryoglobulin detection in the serum is necessary for a definite classification of MC syndrome (figure 1) and their characterisation as type II (IgG+IgM monoclonal) or type III (IgG+IgM polyclonal) mixed cryoglobulins (table 3). Unfortunately, there are no universally accepted methodologies for cryoglobulin measurement, but simple standardised indications are often sufficient for testing for
cryoglobulinaemia. Cryoglobulins are characterised by high thermal instability. For a correct evaluation of serum cryoglobulins, it is necessary to avoid false-negative results due to immunoglobulin cold precipitation which also occurs at room temperature. Blood sampling for cryoglobulin detection should be carried immediately after blood is drawn or blood should be rapidly transported to the laboratory using a thermostable device (37°C). In general, to avoid the possible loss of cryoglobulins, the first steps (blood sampling, clotting and serum separation by centrifugation) should always be carried out at 37°C. In contrast, isolated serum for cryoglobulin determination and characterisation should be managed at 4°C. The serum with cryoglobulins should be tested for reversibility of the cryoprecipitate by re-warming an aliquot at 37°C for 24 h. Cryocrit measurement is usually carried out in serum sample stored at 4°C for 7 days. The cryocrit corresponds to the percentage of packed cryoglobulins with reference to the total serum after centrifugation at 4°C (figure 9); it should be determined on blood samples without anticoagulation to avoid false-positive results due to cryofibrinogen or heparin-precipitable proteins. Without the above relatively simple precautions, not only will the quantities of cryoglobulins measured be incorrect but also the test may completely fail to detect cryoglobulins.

After isolating and washing the cryoprecipitate, the cryoglobulin components can be identified by immunoelectrophoresis or immunofixation. These analyses must be performed at 37°C to avoid precipitation and hence loss of the cryoglobulin during the procedures. More sophisticated methodologies, such as immunoblotting or two-dimensional polyacrylamide gel electrophoresis, may be used for laboratory investigations. Although the detection of serum cryoglobulins is fundamental for the diagnosis of MC, the levels of serum cryoglobulins usually do not correlate with the severity and prognosis of the disease. Very low levels of cryocrit, often difficult to quantify, can be associated with severe and/or active cryoglobulinaemic syndrome; in contrast, high cryocrit values may characterise a mild or asymptomatic disease course. In rare cases, very high cryocrit levels, possibly associated with a cryogel phenomenon, may be associated with classic hyperviscosity syndrome. A sudden decrease or disappearance of serum mixed cryoglobulins, with or without abnormally high levels of C4, should be regarded as alarming signal of complicating B cell malignancy.

Box 1 summarises the clinico-serological investigations at a patient’s first evaluation in order to classify the MC syndrome correctly and to identify possible overlapping disorders (see section 2.5, figure 7) or comorbidities, or both. The prevalence of these latter, in particular atherosclerosis, may be correlated with the disease duration, and with cumulative side effects of prolonged treatments. Diagnosis and monitoring of the major MC manifestations is essential for their timely treatment, especially for life-threatening liver, renal and/or neoplastic complications.

2.5 Differential diagnosis

Initially, the term ‘essential’ referred to MC as an autonomous disease once other well-known systemic, infectious or neoplastic disorders had been ruled out by a wide clinico-serological investigation. However, in some patients a definite diagnosis may be difficult because of the clinical polymorphism of the MC. Moreover, the association of MC with HCV infection may further complicate the differential diagnosis as there is frequent clinicopathological overlapping among different HCV-related disorders. Cryoglobulinaemic syndrome can result from the co-occurrence of
Cryoglobulinemia and hepatitis C virus

Ping MC/RA syndrome can be suspected. In these cases, the detection of serum anticyclic citrullinated peptide antibodies, markers of classic RA, may be a useful tool for the differential diagnosis.

Sicca syndrome may be suspected in about half of the patients with MC; however, current criteria for the classification of pSS are satisfied in only few cases. MC and pSS may share various symptoms: xerostomia and/or xerophthalmia, arthralgias, purpura, RF and serum cryoglobulins and possible complication with B cell lymphoma. However, a careful patient clinical assessment is usually sufficient for a correct diagnosis in the large majority of cases following consideration of some important findings: histopathological alterations of the salivary glands and the specific autoantibody pattern (anti-RoSSA/LaSSB) of pSS are rarely found in patients with MC; conversely, HCV infection, cutaneous leukocytoclastic vasculitis and visceral organ involvement (MPGN type 1, chronic hepatitis) are seldom recorded in pSS (figure 7). In view of the above considerations, it has been recently proposed that the presence of HCV infection itself should be considered an exclusion criterion for the diagnosis of pSS. In rare cases in which the differential

some autoimmune diseases (AIH, Sjögren’s syndrome, polyarthritis, glomerulonephritis, thyroiditis, type 2 diabetes, etc) and malignancies (B cell lymphomas, hepatocellular carcinoma). We can observe in the same patient a slow progression from mild HCV-associated hepatitis to various extrahepatic manifestations (arthralgias, sicca syndrome, Raynaud’s phenomenon, RF positivity, etc) and, ultimately, to overt MC syndrome with typical clinic-serological manifestations. In a minority of patients with MC, a malignancy may develop, generally after a long follow-up period. Therefore, a careful patient evaluation is necessary for a correct diagnosis of MC syndrome, particularly to differentiate it from other RF-positive, systemic rheumatic disorders such as rheumatoid arthritis (RA) and pSS (figure 7).

Arthralgias are one of the most common symptoms, while clear signs of synovitis are quite rare. Patients may develop mild, non-erosive oligoarthritis, often sensitive to low doses of corticosteroids with or without hydroxychloroquine. In contrast, rheumatoid-like polyarthritis is more common in patients with HCV-related hepatitis without MC syndrome. In patients with HCV-associated MC and symmetrical, erosive polyarthritis, the diagnosis of overlapping MC/RA syndrome can be suspected. In these cases, the detection of serum anticyclic citrullinated peptide antibodies, markers of classic RA, may be a useful tool for the differential diagnosis.

Sicca syndrome may be suspected in about half of the patients with MC; however, current criteria for the classification of pSS are satisfied in only few cases. MC and pSS may share various symptoms: xerostomia and/or xerophthalmia, arthralgias, purpura, RF and serum cryoglobulins and possible complication with B cell lymphoma. However, a careful patient clinical assessment is usually sufficient for a correct diagnosis in the large majority of cases following consideration of some important findings: histopathological alterations of the salivary glands and the specific autoantibody pattern (anti-RoSSA/LaSSB) of pSS are rarely found in patients with MC; conversely, HCV infection, cutaneous leukocytoclastic vasculitis and visceral organ involvement (MPGN type 1, chronic hepatitis) are seldom recorded in pSS (figure 7). In view of the above considerations, it has been recently proposed that the presence of HCV infection itself should be considered an exclusion criterion for the diagnosis of pSS. In rare cases in which the differential

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Box 1  Clinico-diagnostic assessment of mixed cryoglobulinaemia (MC) syndrome

<table>
<thead>
<tr>
<th>Clinical and serological investigations at a patient’s first evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Past clinical history, physical examination</td>
</tr>
<tr>
<td>• Chest x-ray examination, ECG, abdominal ultrasonography (US), blood chemistry and urine analysis</td>
</tr>
<tr>
<td>• Cryoglobulin detection and characterisation (see table 1)</td>
</tr>
<tr>
<td>• Rheumatoid factor, C3 and C4, antinuclear antibodies (abs), antiretractable nuclear antigen abs, antineutrophil cytoplasmic abs, antisMOOTH muscle abs, antimitochondrial abs, antiliver/kidney microsome type 1 abs, other abs</td>
</tr>
<tr>
<td>• Virological markers: hepatitis C virus (genotyping), hepatitis B virus, others</td>
</tr>
<tr>
<td>• Evaluate possible comorbidities (cardiovascular, endocrine/metabolic, etc)</td>
</tr>
<tr>
<td>• MC classification (definite, essential, secondary)</td>
</tr>
</tbody>
</table>

**Diagnosis and monitoring of major MC complications**

- Chronic hepatitis, cirrhosis, hepatocellular carcinoma:
  - Monitoring (every 6–12 months) of alanine aminotransferase, alkaline phosphatase
  - Liver US (biopsy, CT scan)
- Glomerulonephritis:
  - Monitoring of urine analysis and serum creatinin (kidney US, biopsy)
- Peripheral neuropathy:
  - Clinical monitoring
  - EMG
- Skin ulcers:
  - Exclusion of vascular comorbidities (arteriovenous Doppler evaluation)
- Sicca syndrome:
  - Differential diagnosis with primary Sjögren’s syndrome (see section 2.5 and figure 7)
- Arthritis:
  - Differential diagnosis with rheumatoid arthritis (see section 2.5 and figure 7)
- Thyroid involvement:
  - Hormones
  - Autoantibodies
  - Neck US
  - Fine-needle aspiration
- B cell lymphoma:
  - Clinical monitoring
  - Bone marrow/lymph node biopsies
  - Total body CT scan

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Chapter 42

HCV-related extrahepatic disorders

HCV chronic infection is diffuse worldwide and may in a low but significant percentage of patients trigger a large number of extrahepatic manifestations. These include a variety of autoimmune and neoplastic disorders, among which MC is the prototypic HCV-associated extrahepatic disease (see section 2). It is a complex immunological disorder characterised by multiple organ involvement. Since MC syndrome, strongly associated with HCV infection, mimics other immune-mediated and neoplastic disorders, a possible role of HCV in these conditions has been also investigated. The spectrum of possible HCV-associated diseases includes a wide number of organ-specific and systemic diseases. Poly-oligoclonal B lymphocyte expansion seems to be the common underlying alteration in a significant percentage of HCV-infected patients, some of whom may develop a variable combination of both hepatic and extrahepatic manifestations; the term ‘HCV syndrome’ refers to this complex clinical condition.

HCV-related diseases can be grouped into three different categories according to the strength of association based on clinico-epidemiological, histopathological and molecular biology studies (figure 10).

3.1 Pathogenesis of HCV-related autoimmune and lymphoproliferative disorders

The main pathogenetic insights into HCV syndrome have been provided by studies of the biological peculiarities of this virus and its possible interaction with the host immune system. In addition, studies of HCV-associated MC provided important insights into the pathogenesis of other HCV-related disorders. HCV lymphotropism is an important step in the pathogenesis of virus-related immunological disorders. A number of epidemiological studies suggested a pathogenetic role for HCV in MC, a disorder characterised by ‘benign’ B lymphocyte expansion. Interestingly, the same immune-pathological alteration may also develop in a significant number of HCV-infected subjects, often in association with one or more serum autoantibodies or mixed cryoglobulins, or both.

Since HCV is a positive, single-stranded RNA virus without a DNA intermediate in its replicative cycle, viral genomic sequences cannot be integrated into the host genome. One possible hypothesis is that HCV infection exerts a chronic stimulus on the immune system, which facilitates clonal B lymphocyte expansion.

The t(14;18) translocation has been demonstrated in a significant percentage of peripheral blood lymphocytes from HCV-infected subjects, with consequent activation of the Bcl2 proto-oncogene (figure 6). In addition, identification of the HCV envelope protein E2, which can bind the CD81 molecule expressed on both hepatocytes and B lymphocytes, seems to be crucial for HCV-driven autoimmunity. CD81 is a cell-surface protein that, on B cells, is part of a complex with CD21, CD19 and Leu 13. This complex reduces the
Cryoglobulinemia and hepatitis C virus

may lead in predisposed subjects to overt malignant lymphoma (figure 6). The oncogenic potential of HCV has been confirmed in patients with hepatocellular carcinoma and in a significant percentage of B cell lymphomas. Of interest, the same virus could be also involved in other malignancies such as thyroid cancer.

There is great geographical heterogeneity in the prevalence of HCV-related immunological or neoplastic disorders (figure 4). This epidemiological observation contrasts with the homogeneous diffusion of HCV infection worldwide. In this respect, we can hypothesise that HCV itself might be insufficient to drive the different autoimmune-lymphoproliferative disorders seen in infected subjects. The involvement of particular HCV genotypes, environmental and/or host genetic cofactors (figure 6) may contribute to the pathogenesis of HCV syndrome. However, the actual pathogenetic relevance of the above cofactors still remains to be fully demonstrated.
It is well known that most HCV-infected subjects remain asymptomatic, some for a long time. In a small but significant percentage of patients the virus is responsible for both hepatic and extrahepatic disorders, usually as late manifestations (figure 6). These clinico-epidemiological observations suggest that HCV syndrome is the consequence of a multifactorial and multistep pathogenetic process. It is quite common to see in the same patient a progression from mild, often isolated manifestations, to systemic manifestations and, finally, to overt malignancies (figure 6).

3.2 HCV and rheumatic diseases

Various inflammatory rheumatic disorders are the most common extrahepatic manifestations of HCV syndrome. Among these, MC is at the junction between classic rheumatic diseases, such as RA and Sjögren's syndrome, and other autoimmune/lymphoproliferative disorders (figures 5 and 10).

Because of its clinical polymorphism, cryoglobulinaemic vasculitis may overlap with other rheumatic disorders such as Sjögren's syndrome and RA (figure 7); on the other hand, these rheumatic disorders may be occasionally associated with HCV infection (figure 7). The possible aetiopathogenetic role of HCV in pSS remains controversial. Patients with HCV-associated Sjögren's syndrome show a significantly low rate of anti-RoSSA/LaSSB (23%) together with a high prevalence of MC (50%), hypocomplementaemia (51%) and systemic vasculitic manifestations (58%) (figure 7). This particular condition cannot be classified as pSS; at least 50% of patients might be better classified as having cryoglobulinaemic vasculitis, which has important clinico-prognostic and therapeutic implications. The example of overlapping MC/Sjögren's syndrome suggests that in genetically predisposed subjects, HCV infection may trigger complex immune system alterations, which may produce variable phenotypes mimicking different well-known diseases, namely Sjögren's syndrome, RA, dermatomyositis, etc (figures 6, 7 and 10).

Chronic oligo-polyarthritis can be found in HCV-infected subjects, is often non-erosive and is barely progressive. In patients with HCV-related cryoglobulinaemic vasculitis, the joint involvement is generally characterised by mild oligoarthritis, while symmetrical rheumatoid-like polyarthritis may complicate IFN treatment in patients with type C hepatitis. Finally, given the relatively high prevalence of the two diseases, it is not uncommon to observe a simple association between classic RA and HCV infection (figure 11). Figure 7 summarises the main clinico-serological parameters for differential diagnosis between MC, pSS and RA in the setting of HCV syndrome.

Osteosclerosis is a rare condition described in adults with HCV infection; it can be defined as an acquired, painful skeletal disorder characterised by a marked increase in bone mass (figure 10). Osteosclerosis is clinically characterised by non-specific, often diffuse bony pain and tenderness over involved bones due to periosteal stretching, in the absence of joint swelling or motion limitation. The radiograph examination shows bony sclerosis and thickening of the long-bone cortices, mainly the diaphyseal cortices. Laboratory investigations frequently reveal abnormally increased markers of bone formation (alkaline phosphatase and bone-specific alkaline phosphatase, osteocalcin); these alterations were mirrored by a marked increase in bone mass (bone mineral density, BMD) and enhanced radionuclide uptake at scintigraphy (99mTc-MDP). Bone biopsy shows an increased number and thickness of trabeculae with a parallel reduction in bone marrow. Only a very small percentage of infected patients develop osteosclerosis compared to the widespread diffusion of HCV. The pathogenesis of this rare condition is still unknown; it has been suggested that HCV alone or in combination of other unknown agent(s) may infect and alter bone cells or their precursors in predisposed subjects. These alterations might be mediated by the production of bone growth factors, such as insulin-like growth factors or osteoprotegerin. The pathogenetic role of the latter factor seems to be relevant; an imbalance in the osteoprotegerin/RANKL system leading to a predominance of osteoprotegerin has been documented.

To date, only 16 cases of HCV-associated osteosclerosis have been reported in the literature. In some cases a partial or complete spontaneous remission of symptoms and/or bone sclerosis was observed during the follow-up period. Treatment with antiresorptive agents in some patients was ineffective, while symptomatic therapies may provide some benefit. The beneficial effect of HCV eradication observed in one patient is quite intriguing, but should be confirmed in larger numbers of patients.

The number of HCV-associated rheumatic diseases, as well as of other extrahepatic disorders, has grown during the last two decades (figure 10). In addition to the conditions described above, other rheumatic diseases have been associated with HCV infection, namely, myalgia, fibromyalgia, poly/dermatomyositis, polyarteritis nodosa, Behçet's syndrome, systemic lupus erythematosus and antiphospholipid syndrome.
In a large series of HCV-infected individuals, myalgia was mentioned by a significant number (15%) of patients. The pathogenesis of this symptom remains difficult to explain; the detection of viral genomic sequences within muscle fibres suggested a direct involvement of HCV in the pathogenesis of diffuse muscle pain. Fibromyalgia was also reported by some authors in a significant percentage of patients with chronic HCV infection, but other studies carried out in patients with typical clinical manifestations of fibromyalgia did not confirm this association. On the other hand, the differential diagnosis between fibromyalgia and muscle pain, frequently associated with weakness and arthralgias, may be very difficult in the setting of HCV-positive patients. Similarly to that proposed for sicca syndrome and pSS, some authors suggest considering HCV-associated myalgia and classic fibromyalgia as distinct entities.

The association of poly/dermatomyositis with HCV infection is described in numerous anecdotal observations, more often in patients with long-lasting viral infection or during IFNα treatment. Similarly, cases of vasculitis involving medium arteries suggesting the diagnosis of polyarteritis nodosa have been associated with HCV infection; moreover, HCV seropositivity has been reported in a significant percentage of patients with polyarteritis nodosa. This latter association is not surprising in light of the well-known relationship between polyarteritis nodosa and another hepatotropic virus, namely HBV. Polyarteritis nodosa may share numerous clinical symptoms with cryoglobulinaemic vasculitis; thus, patients with suspected HCV-associated polyarteritis nodosa should be correctly classified by means of wide clinico-serological and pathological investigation. As regards other possible HCV-associated disorders, namely Behçet’s syndrome, systemic lupus erythematosus and antiphospholipid syndrome, the data reported in the literature are generally anecdotal.

Since a pathogenetic link with HCV cannot be totally excluded, the therapeutic approach in these patients is difficult.

Figure 11 Proposed flow chart for the classification of patients with chronic arthritis and hepatitis C virus (HCV) infection. Patients referred for either symmetrical polyarthritis or mono-oligoarthritis should undergo careful clinical and laboratory investigation, including virological evaluation. On the basis of different immunological tests, namely, rheumatoid factor (RF), anticyclic citrullinated peptides antibodies (anti-CCP), antinuclear antibodies (ANA), cryoglobulins and complement, it is possible to correctly classify anti-HCV-positive patients into two main groups: (a) those with simple association RA+HCV infection, and (b) those with HCV-related arthritis. This latter condition may include patients with arthritis in the setting of chronic HCV infection and variable degree of liver involvement and patients with HCV-related cryoglobulinaemic syndrome. A possible therapeutic approach to different clinical subsets includes: for HCV-related arthritis, sequential treatment with antiviral agents (interferon (IFN) α+ribavirin), and immunomodulating treatments (steroids, hydroxychloroquine (HCQ) and other disease-modifying antirheumatic drugs, rituximab) can be usefully employed, with some limitation for IFNα – see text). In patients with concomitant HCV infection and arthritis, the use of tumour necrosis factor α blocking agents (anti-TNFα) seems to be useful and safe, alone or in combination with ciclosporin A (CyA).
As regards HCV-associated MC, patients with concomitant HCV infection and autoimmune systemic disorders, such as poly/dermatomyositis, polyarteritis nodosa and systemic lupus erythematosus, may be usefully treated with immunosuppressors (cyclophosphamide, rituximab, etc) with some important precautions and limitations due to viral infection. In all instances, a preliminary clinical evaluation of liver involvement and viral replication is necessary before any therapeutic decisions are made. The latter may be based on standard immunosuppressive treatments, possibly integrated into sequential/combined antiviral treatment.

### 3.3 Other HCV-related autoimmune disorders

A variety of organ- and non-organ-specific, immune-mediated diseases can be correlated with HCV infection. Cutaneous manifestations are often seen in HCV-infected subjects. Among these, porphyria cutanea tarda (PCT) is one of the most investigated. It is the commonest type of porphyria and is characterised by reduced activity of uroporphyrinogen decarboxylase, an enzyme involved in the haem biosynthetic pathway, and by frequent chronic liver disease. Since uroporphyrinogen decarboxylase deficiency is an essential condition, but not sufficient, for definite classification/diagnosis of PCT, various triggering factors, including viral infection, should be taken into account. A role of HCV infection has been investigated in several studies worldwide, which have reported a variable percentage of associations. The pathogenesis of HCV-related PCT is particularly intriguing: both metabolic factors – in particular, altered genes involved in iron metabolism – and a cross-reactivity of host versus HCV antigens have been proposed. Generally, HCV-positive patients without PCT do not show significant alteration in porphyrin metabolism; therefore, it may be supposed that a genetically driven reactivity is decisive, while HCV may exert an indirect role, possibly as a triggering factor.

HCV-related lichen planus is another important association, in particular, oral lichen planus, which has a variable geographical prevalence. Moreover, several mucocutaneous manifestations are variably reported in HCV-infected subjects, generally based on limited or anecdotal observations. HCV-positive patients may develop acute episodes or chronic manifestations of well-known skin diseases; these symptoms are an expression of immune-mediated cutaneous injury, triggered by HCV antigens and often amplified by IFN treatment. In many cases these cutaneous manifestations, often with a contribution from peripheral nerve alterations, severely affect patients’ quality of life. Peripheral neuropathy is a common complication of HCV infection, mainly in cryoglobulinaemic vasculitis, while central nervous system involvement is less common. Peripheral neuropathy is more often seen in patients with overt MC syndrome. Vascular manifestations, including central nervous system involvement, may represent late comorbidities of HCV syndrome, particularly in patients with more severe extrahepatic manifestations and receiving long-term steroid treatment. Some cardiovascular manifestations have been reported during HCV infection, mainly in patient series from eastern Asian countries; if confirmed by further studies, they may support the role of genetic and/or environmental cofactors in the pathogenesis of HCV-related diseases.

An intriguing, still controversial, aspect is the possible aetiological role of HCV in autoimmune hepatitis. Patients with autoimmune hepatitis may present serum mixed cryoglobulins, HCV infection and extrahepatic manifestations such as thyroiditis, sicca syndrome and arthritis; conversely, patients with HCV infection show one or more non-organ-specific autoantibodies and different organ involvements. In this respect, the antigenic target specificity of HCV-related autoantibodies shows only quantitative differences compared with those associated with ‘primary’ autoimmune hepatitis. Again, the heterogeneous geographical distribution of HCV-associated autoimmune hepatitis suggests a possible involvement of various pathogenetic cofactors; among these HCV might trigger a peculiar AIH clinico-serological subset which is prevalent in specific geographical areas (figure 4).

Glomerular and tubulointerstitial renal involvement in both native and transplanted kidneys may be associated with HCV infection. HCV-related glomerulonephritis may include various histopathological types: MPGN with and without MC and, less frequently; membranous nephropathy, fibrillar and immunotactoid glomerulonephritis, rapidly progressive glomerulonephritis and exudative-proliferative glomerulonephritis. Cryoglobulinaemic glomerulonephritis – namely, type 1 MPGN – is more commonly found, while ‘primary’ MPGN represents less than one-third of HCV-associated MPGN. This latter disease has been described mainly in the USA and Japan. However, the real prevalence of MPGN without detectable cryoglobulinaemia is difficult to assess; it may represent a subclinical form of MC, possibly owing to difficulties and/or inadequate methods of detecting serum cryoglobulins. The possible methodological biases have been examined in section 2.4.

In some patients, MPGN is the presenting symptom of MC syndrome that may develop later in the course of the
Cryoglobulinemia and hepatitis C virus

Thyroid involvement is perhaps the most common and thoroughly investigated endocrine alteration in HCV-positive patients. The prevalence of abnormally high levels of antithyroid antibodies found in these patients varies markedly, ranging from 2% to 48%, with a heterogeneous geographical distribution (figure 4). These discrepancies may be correlated with variable genetic predisposition and environmental cofactors, such as iodine intake or diffusion of other infectious agents. Moreover, subclinical hypothyroidism has been found in 2–9% of patients with chronic hepatitis C, while miscellaneous thyroid alterations and raised serum thyroid autoantibodies are generally higher in chronic hepatitis C than in hepatitis B or D. More recently, the prevalence of thyroid involvement was investigated in a large series of patients with chronic hepatitis C and compared with that in control groups from the general population from regions with different iodine intake, as well as that in patients with chronic hepatitis B. Autoimmune thyroid involvement and hypothyroidism were significantly more common in patients with chronic hepatitis C than in the comparison groups, whereas the prevalence of hyperthyroidism was similar. Comparable findings were also found in another study focusing on the thyroid abnormalities of HCV-positive patients with MC.

Recent studies have focused on the possible pathogenetic mechanisms responsible for HCV-related thyroid disorders. HCV thyroid infection may act by upregulating CXCL10 gene expression and secretion in thyrocytes (as previously shown in human hepatocytes) recruiting Th1 lymphocytes, which secrete IFNγ and tumour necrosis factor α (TNFα). In turn, these cytokines may induce CXCL10 secretion by thyrocytes, thus perpetuating the immune cascade. The consequence may be the appearance of thyroid autoimmune disorders in genetically predisposed subjects (figure 12). This hypothesis has been recently confirmed by a study that evaluated CXCL10 serum levels in HCV-positive patients with MC, in the presence or absence of autoimmune thyroid involvement. Chronic immune-mediated inflammatory thyroid lesions may be responsible for the papillary thyroid disease. Renal involvement is one of the most harmful complications of HCV-associated MC syndrome, and may severely affect the patient’s clinical outcome. It is the consequence of cryoprecipitable and non-cryoprecipitable IC deposition in the glomeruli. However, the exact role of HCV in the aetiology of glomerulonephritis is not universally accepted. The presence of HCV particles in IC seems to support an indirect involvement of this virus in the pathogenesis of glomerulonephritis.

Figure 12 Possible aetipathogenetic mechanisms of hepatitis C virus (HCV)-related thyroid disorders and diabetes type 2. HCV thyroid infection may act by upregulating CXCL10 gene expression and secretion in thyrocytes (as previously shown in human hepatocytes) recruiting Th1 lymphocytes, which secrete interferon γ (IFNγ) and tumour necrosis factor α (TNFα). In turn, these cytokines may induce CXCL10 secretion by thyrocytes, thus perpetuating the immune cascade. The consequence may be the appearance of thyroid autoimmune disorders in genetically predisposed subjects.
cancer found in a significant percentage of HCV-infected subjects compared with controls.

Analogous pathogenetic mechanisms can be involved as consequence of HCV infection of pancreatic β cells responsible for the upregulation of CXCL10 gene expression and secretion. The recruited Th1 lymphocytes, which secrete IFNγ and TNFα, amplify CXCL10 secretion by β cells, thus perpetuating the immune cascade. The final result is the appearance of β cell dysfunction, with the probable contribution of genetic predisposition.

The abnormalities in thyroid function should be included among the complications of HCV syndrome. These patients should be periodically screened for thyroid involvement to identify those needing treatment and to diagnose the rare but harmful neoplastic complication at an early stage.

Type 2 diabetes may be another important manifestation of HCV syndrome, regardless of the presence and severity of liver damage.

Initially, clinic-based studies found an excess of type 2 diabetes in non-cirrhotic HCV-positive patients compared with patients with chronic hepatitis of other origin; however, this was not confirmed by another large study. An Italian case–control study evaluated 564 non-cirrhotic HCV-positive patients compared with 302 control subjects without a history of alcohol abuse, drug addiction or positivity for markers of viral hepatitis and 82 non-cirrhotic HBV-positive patients. A significantly high prevalence of type 2 diabetes was recorded in non-cirrhotic HCV-positive patients compared with control subjects (12.6% vs 4.9% and 7%, respectively; p=0.008). Interestingly, the prevalence of type 2 diabetes in non-cirrhotic HBV-positive patients (7%) was within the range of the reported age-adjusted prevalence rates for the Italian population (4%).

A comparison between clinical phenotype of hepatic and diabetic patients showed that non-cirrhotic HCV-positive type 2 diabetes was characterised by slightly older age, higher body mass index (BMI), elevated serum triglycerides, raised blood pressure levels and lower high-density lipoprotein cholesterol concentrations. Moreover, type 2 diabetic non-cirrhotic HCV-positive patients had a significantly lower BMI than type 2 diabetic control subjects and a slightly but significantly (p<0.05) higher BMI than non-diabetic non-cirrhotic HCV-positive patients. Type 2 diabetes itself is characterised by older age, overweight, dyslipidaemia and higher blood pressure levels, the so-called ‘metabolic syndrome’ phenotype. In contrast, non-diabetic non-cirrhotic HCV-positive patients were lean and had low LDL cholesterol levels. Low LDL cholesterol levels have been correlated with HCV-induced hypo-β-lipoproteinaemia due to a binding competition between the virus and hepatic LDL receptor.

An immune-mediated mechanism for HCV-associated diabetes has been postulated and a similar pathogenesis might be involved in the diabetes of HCV-positive patients with MC. This hypothesis is strengthened by the finding that autoimmune phenomena in patients with type 2 diabetes are more common than previously thought. Similarly to the above-mentioned pathogenetic mechanisms involved in HCV-related thyroid disorders, we can hypothesise that HCV infection of β cells may act by upregulating CXCL10 gene expression and secretion. The recruited Th1 lymphocytes, which secrete IFNγ and TNFα, amplify CXCL10 secretion by β cells, thus perpetuating the immune cascade. The final result is the appearance of β cell dysfunction, possibly in genetically predisposed subjects (figure 12).

Finally, sex hormone alterations have been observed in HCV-positive MC. Erectile dysfunction has been anecdotally reported in patients with HCV-related chronic hepatitis undergoing treatment with IFNα.

To investigate the possible role of HCV infection in gonadal dysfunction, 207 male patients with HCV infection

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**Summary points**

- Types II and III mixed cryoglobulinaemia (MC), or cryoglobulinemic vasculitis, are classified among the small-vessel systemic vasculitides. The disease is a combination of serological findings (mixed cryoglobulins with rheumatoid factor (RF) activity and frequent low C4) and clinico-pathological features (purpura and leukocytoclastic vasculitis with multiple organ involvement).
- There are no diagnostic criteria for MC. The disease can be correctly classified on the basis of typical orthostatic purpura with the histological pattern of leukocytoclastic vasculitis on skin samples taken within the first 24–48 h, detection of serum mixed (IgG-IgM) cryoglobulins, low complement C4 and positive RF.
- MC may be associated with other well-defined immunological, infectious or neoplastic disorders; when isolated, it is a distinct disease, the so-called ‘essential’ MC. Following the discovery of a striking association between MC and hepatitis C virus (HCV) infection, the term ‘essential’ is now used to
Summary points—Cont’d

- The main clinical features of cryoglobulinaemic vasculitis are the typical triad of purpura, arthralgias and weakness. Liver and renal involvement, peripheral neuropathy, skin ulcers and the possible development of malignancies, mainly B cell lymphomas, generally as a late complication, may also be seen.
- Liver and/or renal involvement, as well as neoplastic complications, may severely affect the overall prognosis of MC. Such patients, more often women, aged 50–60 years at the time of diagnosis, have a worse prognosis than the general population.
- HCV is both a hepatotropic and a lymphotropic virus; it may exert a chronic stimulus on the immune system with both T and B lymphocyte alterations; “benign” B cell lymphoproliferation is responsible for altered autoantibody production, mainly of RF and cryoglobulins. In addition to cryoglobulinaemic vasculitis, HCV may trigger different immune-mediated extrahepatic disorders (thyroiditis, diabetes type 2, polyarthritis, glomerulonephritis, porphyria cutanea tarda, sicca syndrome, etc), as well as some malignancies, mainly B cell lymphomas.
- The large geographical heterogeneity in the prevalence of HCV-related extrahepatic manifestations suggests that HCV itself might be insufficient to trigger and maintain these different autoimmune-lymphoproliferative disorders. A variable combination of HCV with other unknown environmental and/or host genetic cofactors may lead to the different clinical phenotypes that characterise HCV syndrome.
- The main targets in the treatment of HCV-related cryoglobulinaemic vasculitis are the clinical (improvement of organ target manifestations), virological (clearance of HCV RNA) and immunological (serum cryoglobulin and C4 levels).

(102 with cryoglobulinaemic vasculitis) were compared with 207 age-matched men, randomly selected from a series of 2010 subjects from the Italian general population previously investigated for erectile dysfunction. Exclusion criteria were patients aged >55 years, IFNα treatment during the past 12 months, and the presence of diabetes, renal failure, hypothyroidism, and cardiovascular and psychiatric disorders. Erectile dysfunction was significantly more common in HCV-infected subjects than in control subjects (p<0.001).

Sex hormone determinations showed that total and free testosterone plasma levels were abnormally reduced in HCV-positive patients with erectile dysfunction. Neither erectile dysfunction nor low levels of total and free testosterone were related to the severity of liver damage. These sex hormone alterations along with the possible contribution of peripheral neuropathy may be responsible for erectile dysfunction, which should be confirmed by further investigations. The above findings further support the role of the host hormonal environment in the pathogenesis of HCV-driven autoimmune disorders. We can hypothesise that reduced endogenous immunosuppressive activity due to low levels of adrenal-gonadal androgens may amplify the autoreactive lymphocyte proliferation triggered by HCV infection.

3.4 HCV and malignancies

HCV infection is the major aetiological factor in hepatocellular carcinoma. The oncogenic role of the virus in hepatocellular carcinoma has been definitely established.

Since 1993, a possible role of this virus in the pathogenesis of malignant B cell neoplasias has also been suggested. This hypothesis was initially suspected because of the strikingly association between HCV and MC, a condition that may be complicated by B cell lymphomas, and was further reinforced by the demonstration of HCV lymphotropism. In 1994, a surprisingly high prevalence of HCV infection in unselected Italian patients with B cell non-Hodgkin’s lymphoma (B-NHL) was first reported. After this initial observation, an increasing number of epidemiological and laboratory investigations in patient series from different countries, as well as in animal models, confirmed the aetiopathogenetic role of HCV in a significant percentage of patients with B-NHL (figure 6). As for HCV-related MC, this association has a variable geographical distribution. The HCV-induced B cell malignancies have two main variants: the lymphomas complicating HCV-positive MC and isolated HCV-positive B-NHL. B-NHL complicating MC syndrome is discussed in section 3.3.

A significantly high prevalence of thyroid cancer complicating HCV-related hepatitis and HCV-related MC compared with controls was first noted in 1999. These data were subsequently confirmed in a case–control study, which reported a high prevalence of HCV in patients undergoing surgery for papillary thyroid cancer. Overall, HCV infection was associated with a high risk for liver cancer, multiple myeloma, B-NHL and thyroid cancer (figure 6). Furthermore, a high prevalence of thyroid cancer in subjects with a
history of transfusion and/or liver disease indirectly supports the role of HCV in this malignancy. A review of the literature shows discordant results, possibly owing to important epidemiological and methodological bias. The results of a recent study on a large number of HCV-infected patients seem to confirm the high prevalence of thyroid papillary cancer, excluding the influence of some possible biases such as gender, age and iodine intake.

In our studies, features of thyroid autoimmunity were more commonly found in patients developing thyroid papillary cancer irrespective of whether they had type C hepatitis or HCV-related MC syndrome. This observation suggests that thyroid autoimmunity may be a predisposing condition for this malignancy. Although a possible association between thyroid cancer and HCV infection is suggested by the above clinico-epidemiological studies, this needs to be verified by further investigations.

3.5 HCV syndrome

The strength of association as well as the pathogenetic role of HCV varies greatly among different diseases and for each disease among series of patients from different countries (figures 4 and 10). Often, each disease itself represents a clinical syndrome which may comprise different clinicoserological subsets. These latter are the resulting phenotypes of a variable combination of different – genetic, environmental, infectious – pathogenetic cofactors. In this scenario, HCV infection, with the contribution of other pathogenetic agents, may produce distinct autoimmune or neoplastic disease subsets. The complex of HCV-related disorders is a continuum, as suggested by the clinical history of some patients, which may display the entire spectrum. It is not uncommon to see HCV-infected subjects with mild, often limited manifestations, which may progress, generally during a long follow-up period, to more severe systemic manifestations, including malignancies (figure 6).

In general, HCV syndrome is a multifaceted, clinico-pathological condition (figure 6); the challenge for future investigations is to better elucidate the exact boundaries of this syndrome and the actual pathogenetic role of the virus in different conditions.

4. Treatment of MC vasculitis

Treatment of HCV-associated MC vasculitis may target either the viral trigger (HCV) or the downstream B cell arm of autoimmunity. Aggressive antiviral therapy with Peg-IFNα and ribavirin should be considered as induction therapy for HCV-MC with mild to moderate disease severity and activity. In patients presenting with severe disease (ie, 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. Combination therapy with rituximab and Peg-IFNα plus ribavirin appears logical as it may target both the viral trigger (HCV) and cryoglobulin producing B cells. This review will focus on recent advances in our understanding of the treatment of HCV-MC vasculitis.

4.1 Pre-HCV era

Prior to the discovery of HCV infection, patients with HCV-MC were treated in similar fashion to other forms of systemic vasculitis based on data derived from small uncontrolled studies. In severe systemic disease, patients were treated aggressively with high-dose corticosteroids, cyclophosphamide and plasmapheresis. The outcome of this therapy was generally unfavourable and associated with a high mortality rate. In those who did respond, there was frequently untoward toxicity and high rate of relapse. Interestingly and in the absence of evidence of a viral aetiology, the empiric use of IFNα, as an antiproliferative agent, was thought to be effective treatment for cryoglobulinaemic vasculitis.
4.3 Antiviral agents

4.3.1 INFα

Treatment of HCV-related cryoglobulinaemic vasculitis with IFNα was associated with a relatively poor response and a high relapse rate, especially in severe cases. IFN monotherapy was effective in 50–100% of patients with purpuric skin lesions, but did not clearly demonstrate efficacy on neural or renal involvement. Clinical improvement in HCV-related vasculitis correlated with virological response, that is, a negative or significant decrease in serum HCV RNA level. A virological response at the end of treatment has been reported in 15–60% of patients receiving IFN monotherapy, at a dosage of between 2 and 3 MIU thrice weekly for 6–12 months. However, follow-up, when long enough, showed that most of the responders developed virological and clinical relapses following IFN withdrawal. Such results are quite similar to those reported in patients without extrahepatic manifestations, where a 12-month course of IFN monotherapy leads to a sustained virological response in only 15–20% of patients. These results were probably due to the mechanisms of action of IFN on viral turnover. Combination therapy with IFN plus ribavirin is much more efficacious, with a sustained virological response in 35–80% of patients with chronic hepatitis C depending on their pretreatment characteristics. Improved rates of sustained response can be achieved when daily ribavirin doses exceed 10.6 mg/kg.

4.3.2 INFα plus ribavirin

The early attempts to control HCV-MC with standard thrice weekly IFNα was not surprisingly associated with a relatively poor response and a high relapse rate, especially in severe cases. Combination therapy with IFN plus ribavirin seems to provide much better short- and long-term results in patients with HCV-related vasculitis than historically reported with IFN monotherapy. In three recent uncontrolled studies, combination therapy with IFN and ribavirin demonstrated enhanced efficacy on the main HCV-related vasculitic manifestations (cutaneous, 100%; renal, 50%; and neural, 25–75%). In a small series, Zuckerman et al reported after a short duration of treatment and follow-up that only 2/9 patients had a virological response. A clinical response was noted in all patients with skin involvement, but in only half of the patients with nerve or renal involvement. In a study of 27 patients with chronic hepatitis C complicated by systemic vasculitis, patients received IFN over 20±14 months, associated with ribavirin for 14±12 months. After a mean follow-up of 57 months, 25/27 patients (93%) were alive and being followed as outpatients, while two patients had died secondary to cirrhosis. Most patients (75%) with a negative viraemia at the end of follow-up were complete clinical responders for their vasculitis. In complete clinical responders, HCV RNA was significantly more often undetectable or HCV viral load lower and genotype 1 less frequent. Age, gender, clinical vasculitic involvement, mean duration or total cumulative dose of IFN or ribavirin, and use of steroids or plasmapheresis did not differ significantly according to clinical response.

In rare cases of HCV-related vasculitis, complete clinical responders had viral clearance long after clinical remission. In addition, some patients (three patients reported by Casato et al, and one by Cacoub et al) may remain in clinical remission despite the persistence of viraemia. Although these recent studies were retrospective and uncontrolled, they suggest that treatment with IFN plus ribavirin can achieve a complete clinical response in most patients with HCV-related systemic vasculitis. Complete clinical response usually, but not always, correlates with the virological response.

4.3.3 Peg-IFNα plus ribavirin

The treatment of HCV infection (ie, in the absence of HCV-MC) has progressed dramatically over the past 15 years with now the standard of pegylated IFNα and ribavirin therapy leading to sustained virological clearance in nearly 60% of patients.

Mazzaro et al have reported on the results of 18 consecutive HCV-MC patients treated with Peg-IFNα-2b (1.0 μg/kg each week, subcutaneously) plus ribavirin (1000 mg daily) for 12 months. At the end of treatment HCV RNA had become undetectable in 15 (83%) patients and most patients had improved clinically. At the end of follow-up, only eight (44%) patients were still sustained clinical and virological responders and cryoglobulin disappeared in six (33%) cases. One major weakness of this study was the lower Peg-IFN dosage used in comparison with that usually recommended in HCV therapeutic guidelines.

We reported the results of a monocentric study with 72 consecutive HCV-MC patients who received treatment with IFNα-2b (n=32) (3 MIU thrice weekly) or Peg-IFNα-2b (n=40) (1.5 μg/kg/week), both combined with oral ribavirin (600–1200 mg/day) for at least 6 months. Following antiviral therapy, purpura resolved in 86% of cases, arthralgia in 80%, peripheral neuropathy in 68% and renal involvement...
in 41%. A significant decrease in proteinuria was observed in sustained virological responders, whereas no significant change in serum creatinine level was seen. Cryoglobulin disappeared in 25 (37.8%) cases. Peg-IFNα 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 IFNα plus ribavirin, regardless of HCV genotype and viral load. Subgroup analysis of the 40 HCV-MC patients treated with Peg-IFNα 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. Compared with standard IFNα-2b/ribavirin, there was a shorter duration of anti-HCV therapy (13.2 vs 18.3 months), less frequent use of corticosteroids (35% vs 47%) and a lower rate of death (5% vs 18.7%) with Peg-IFNα-2b/ribavirin. In multivariate analysis, an early virological response (ie, at month 3 and later) (odds ratio (OR) 3.53; 95% CI 1.18 to 10.59) was independently associated with a complete clinical response in MC. A glomerular filtration rate lower than 70 ml/min (OR 0.18; 95% CI 0.05 to 0.67) was negatively associated with a complete clinical response in MC. Although, HCV-MC patients with HCV genotype 1 and/or previous therapy failure displayed a lower clinical response rate, these factors were not independently associated with a poor clinical response in multivariate analysis. Epidemiological features, HCV viral load, transaminases or liver damage did not influence the clinical outcome in this study. Reappearance of HCV RNA was observed in eight (11.1%) patients with a median time after discontinuing therapy of 2 months (range 1–3 months). Six of the patients experienced a relapse in MC vasculitis. Eight deaths were noted due to cardiovascular disease (n=3), hepatocarcinoma (n=2), liver failure (n=2) and sepsis (n=1). In 39/70 (56%) patients, side effects included fatigue (47.2%), fever (37.5%), anaemia (33.3%), myalgia (25%), neutropenia (20%), depression (15.2%), thrombocytopenia (5%), pruritus (4.1%) and alopecia (2.7%). When compared with IFNα-2b/ribavirin, patients who received Peg-IFNα-2b/ribavirin had a similar rate of adverse events (53.1% vs 55%, respectively). No therapy interruptions were needed. A dose reduction of antiviral therapy was required in 11 patients.

4.3.4 New antiviral agents

Triple therapy with Peg-INF, ribavirin and a specifically targeted antiviral agent such as a protease inhibitor (boceprevir or telaprevir) will soon be available to improve sustained virological response in patients infected with HCV genotype 1 and a clinical response of vasculitis. In an open-label trial, we are currently evaluating the efficacy of 800 mg of boceprevir or 750 mg of telaprevir (thrice daily), an NS3 protease inhibitor, in combination with Peg-IFNα-2a (180 μg) or 2b (1.5 μg/kg) and ribavirin (800–1400 mg/day) in 19 HCV-MC patients with genotype 1. Seventeen of the 19 (89.5%) have cleared the virus and are in clinical remission from the vasculitis. The two remaining patients were considered virological non-responders (ie, persistent HCV RNA) and partial clinical responders. Tolerance seems satisfactory, although asthenia was frequent and four patients developed bacterial infections (pneumonia, n=2; kidney, n=2).

4.3.5 Treatment of HCV-related vasculitis relapses

Clinical relapses in HCV-related vasculitis are usually associated with relapsing HCV viraemia. A prolonged duration of 18–24 months of treatment might show higher efficacy of IFN-based therapy to avoid such relapses, particularly in cases with peripheral nerve or renal involvement. Although there is still controversy, the occurrence of B-NHL might be higher in MC patients compared with the general population, even in patients who have cleared HCV RNA following antiviral therapy.

We have recently reported on eight patients who presented with cryoglobulinaemic vasculitis due to chronic active HCV infection with no evidence of underlying malignant disease. After successful treatment of the HCV infection, the patients were sustained virological responders as they remained persistently HCV RNA negative. However, cryoglobulinaemia-related symptoms later reappeared, although with no HCV infection relapse (as demonstrated by numerous negative tests for HCV viraemia), but malignant B-NHL was found in two cases. Therefore clinicians should be aware of the possibility of malignant lymphoma when HCV positive patients experience a relapse in cryoglobulinaemia vasculitis without HCV virological relapse.

The recurrence of vasculitis-related symptoms after withdrawal of antiviral therapies with virological relapse (HCV RNA repositivation) can be treated with another course of combination antiviral therapy with a good response. Vasculitis relapses usually present the same vasculitis manifestations as were noted at presentation, being either cryoglobulinaemic or polyarteritis nodosa-type. Despite the successes with combination antiviral treatment,
Cryoglobulinemia and hepatitis C virus

Figure 13 Therapeutic interventions according to the aetiopathogenetic process of cryoglobulinaemic vasculitis. mAb, monoclonal antibody.

Figure 14 Therapeutic strategies in patients with hepatitis C virus (HCV) infection and different hepatic and extrahepatic manifestations. The treatment of mixed cryoglobulinaemia (MC) vasculitis should be decided according to the severity of clinical manifestations. In case of contraindication to antivirals, use rituximab alone. Optimal antiviral treatment should probably soon include Peg-interferon-alpha (Peg-IFNα), ribavirin (RBV) and protease inhibitor. B-NHL, B cell non-Hodgkin’s lymphoma.
HCV-related vasculitis remains a severe disease. Most series reporting on the effects of treatment reported a death rate of 8–15% after a sufficient follow-up period. Usually, death occurs in non-responders after a prolonged course of vasculitis, and it is often attributed to vasculitis, sepsis, liver disease or haemopathy. Careful monitoring for adverse effects is mandatory since some manifestations of HCV-related vasculitis, such as peripheral neuropathy or skin ulcers, may worsen under IFN therapy. In most cases, however, IFN treatment can be reinitiated without further problems. Tolerance of ribavirin is also reasonable with the exception of haemolytic anaemia which requires dosage reduction in some patients, and renal insufficiency which requires dose adjustment.

We recently reported on the long-term follow-up of 32 HCV-MC patients treated with rituximab±Peg-IFNα-ribavirin. After a mean follow-up of 23 months, seven patients (22%) experienced a clinical relapse. Six patients were re-treated with rituximab and all these patients had a complete clinical response, 50% had a complete immunological response, and 50% had a partial immunological response. Rituximab was well tolerated overall.

4.4 Immunosuppressive agents

Immunosuppressive agents are typically reserved for patients with severe disease manifestations such as MPGN, severe neuropathy and life-threatening complications. Traditionally a combination of corticosteroids and immunosuppressants such as cyclophosphamide and azathioprine has 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 to or intolerant of these immunosuppressive drugs. 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. Despite this aggressive approach, long-lasting remission of renal disease was achieved in only 14% of cases, and the 10-year survival rate was only 49%.

Corticosteroids, used alone or in addition to IFNα, did not improve HCV-related vasculitic manifestations in two controlled studies. In one randomised trial, methylprednisolone (MP) alone given for 1 year was associated with clinical response in 16.7% of patients, compared with 53.3% and 52.9% of patients receiving IFNα or IFNα plus MP, respectively. Low dose corticosteroids may help to control minor intermittent inflammatory signs such as arthralgia, but are not successful in cases of major organ involvement (ie, 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 usually needs to be accompanied by plasma exchange in order to avoid the rebound increase in cryoglobulinaemia that is commonly seen after discontinuation of apheresis. When used in combination with anti-HCV treatment, plasmapheresis did not modify the virological response if IFNα was given after each plasma exchange session.

4.5 Rituximab

Recently, several groups have reported on the efficacy of anti-CD20 monoclonal antibody (rituximab) in patients with HCV-MC vasculitis. 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 is an interesting therapy in HCV-MC patients as it targets B cells which are responsible for the cryoglobulin production, IC deposition and, finally, MC vasculitis lesions. Overall, 13 reports were identified which described a total of 57 cases which were analysed in detail for the present study. There were two large uncontrolled series of 20 and 15 patients, and two smaller series of six and five patients each. All other publications were case reports describing one or two patients.

Patients had a cryoglobulinaemia vasculitis secondary to chronic active HCV infection in 75.4% of cases or an essential MC in 24.6%. The main clinical manifestations of cryoglobulinaemia vasculitis were skin involvement (84.2%), arthralgia (61.4%), peripheral neuropathy (54.4%) and glomerulonephritis (31.6%). The main indication for rituximab therapy was no response to previous other treatments (ie, mostly IFNα monotherapy and/or steroids) (n=50), intolerance to previous treatments (n=3), associated lymphoma (n=2) or first line therapy for cryoglobulinaemic vasculitis (n=2). Most patients (48/57) received 4 weekly consecutive intravenous infusions of 375 mg/m² of rituximab. In other cases (9/57), six to eight infusions of rituximab were given. The mean follow-up after rituximab infusions lasted 9.7 months (range 3–24 months). Rituximab infusions proved effective for the main vasculitis signs, with a complete clinical response in 24/33 (73%) patients for skin involvement, 16/30 (53%) for arthralgia, 9/25 (36%) for neuropathy and 9/13 (70%) for glomerulonephritis.
However, cryoglobulaemic vasculitis relapse was noted in 13/36 (36.1%) patients within few days to 19 months (mean 6.7 months) after the last rituximab infusion. Eight out of 13 relapsers showed complete remission after a second course of rituximab infusion. There was no significant difference in the efficacy of rituximab therapy whether patients presented with HCV-induced or essential cryoglobulinaemia vasculitis. B cell depletion was achieved in most patients and did not influence the clinical outcome. B cell reconstitution began from 6 months. A relatively small number of side effects were reported. During the short-term follow-up they included bradycardia (n=3), hypotension (n=2), infection (n=3 renal transplanted patients), mild transaminases elevation (n=3), retinal arterial thrombosis (n=1), panniculitis of elbows and knees (n=1) and serum sickness (n=1). Two deaths were reported: one 12 months after rituximab infusion in an HCV-infected patient with renal insufficiency, and the other 2 months after rituximab infusion in a renal transplanted HCV-negative patient due to Candida neoformans meningoencephalitis. During the long-term follow-up (>12 months), two cases of lymphoma and one case of breast cancer were noted.

One potential concern regarding the use of such therapy is its propensity to worsen HCV viraemia. In this setting, rituximab cannot be seen as a curative treatment as long as the viral starter antigen of the vasculitis (ie, HCV) remains. Indeed, despite a dramatic reduction in the number of circulating B cells and deletion of B cell clones, Sansonno et al observed the appearance of different clones in MC patients responding to rituximab, demonstrating that selected antigens may be recognised as part of a limited host response to a virus capable of undergoing spontaneous long-term mutations. As a first examination of the immunological effects of rituximab in MC vasculitis, we recently evaluated both B and T cell subsets in patients at baseline, in the setting of selective B cell depletion by anti-CD20 monoclonal antibodies, and during the B cell recovery phase. B cell depletion (<1% of the total peripheral blood lymphocytes) was achieved in 67% (14/21) of the MC vasculitis group regardless of the clinical response. The percentage of CD19+ cells (12.7±2.2%) dropped to less than 1% after the fourth infusion. Recovery of B cell count began at 6–9 months. Compared with healthy and HCV controls, pretreatment abnormalities in MC patients included a decreased percentage of naive B cells (p<0.05) and CD4+CD25+FoxP3+ regulatory T cells (p=0.02) with an increase in memory B cells (p=0.03) and plasmablasts (p<0.05). These abnormalities were reverted at 12 months after rituximab. Clonal VH1–69+ B cells dramatically decreased following treatment (32±6% vs 8±2%; p=0.01). Complete responders to rituximab exhibited an increase in regulatory T cells (p<0.01) accompanied by a decrease in CD8 T cell activation (p<0.01) and decreased production of interleukin 12 (IL-12; p=0.02) and IFNγ (p=0.01).

A recent pilot study reported the efficacy of a lower dosage of rituximab (two infusions of 250 mg/m² instead of 4 weekly infusion of 375 mg/m²). Six consecutive patients with MC were treated. All patients had severe or life-threatening disease manifestations, including necrotising skin ulcers, renal disease, hyperviscosity or intestinal vasculitis. Four of five evaluable patients (excluding one early death) had >80% decrease in cryocrit and remission of vasculitis at the end of a 22–55-week (median 40-week) follow-up. The non-responder failed to respond to additional rituximab treatment. Based on their prior pilot study, the authors designed a phase II single-arm two-stage study to evaluate the efficacy of a lower dosage of rituximab, 250 mg/m² given twice, for refractory MC. They have recently reported the preliminary results in the first 27 patients enrolled. The overall response rate in 24 evaluable patients was 79%, and the mean time to relapse was 6.5 months, similar to the 6.7 months reported in studies with high-dose rituximab. Side effects were comparable to those seen in patients treated with a high dose. An increase in HCV viral load, reported in some high-dose studies, was not observed in our patients. In conclusion, low-dose rituximab may provide a more cost-effective and possibly safer alternative for treating refractory HCV-associated MC.

We recently reported six cases of HCV-MC vasculitis in patients who experienced systemic drug reactions after rituximab infusion. Four patients developed a severe flare of MC vasculitis 1 or 2 days after rituximab infusion. Two patients developed serum sickness syndrome 7 and 9 days after the first 1000 mg rituximab infusion. Compared with patients without drug reactions, those with drug reactions had higher mixed cryoglobulin levels and lower C4 levels and more of them received the 1000 mg high-dose rituximab protocol (50% vs 6.25%; p=0.046). In vitro immunochemical assays showed that rituximab formed a complex with the cryoprecipitating IgM kappa that had RF activity. Moreover, the in vitro addition of rituximab to serum containing an RF-positive IgM kappa type II mixed cryoglobulin was associated with accelerated cryoprecipitation. As such, rituximab should be administered with caution in MC vasculitis, with use of the 375 mg/m² protocol and plasma...
Exchanges prior to rituximab infusion in patients with high baseline levels of mixed cryoglobulin.

In a recent prospective study, 19 HCV-positive patients with MC and advanced liver disease, who were excluded from antiviral therapy, were treated with rituximab and followed for 6 months. MC symptoms included purpura, arthralgias, weakness, sensory-motor polyneuropathy, nephropathy and leg ulcers. Liver cirrhosis was observed in 15 of 19 patients, with ascitic decompensation in six cases. A consistent improvement in MC syndrome was evident at the end of treatment and the end of follow-up. Variable modifications in both mean viral titres and alanine aminotransferase values were observed. Improvement in liver protidiosynthetic activity and ascites degree was observed at the end of treatment and the end of follow-up, especially in more advanced cases. This study shows the effectiveness and safety of rituximab in MC syndrome with advanced liver disease.

4.6 Rituximab plus Peg-IFNα and ribavirin

Based on the limitations of each therapy (ie, antiviral and rituximab), and the 30% of MC patients who continue to have active disease while receiving anti-CD20 monoclonal antibody or antiviral therapy, the combination of rituximab with Peg-IFNα-ribavirin appears logical. We reported on 16 consecutive HCV-MC patients being treated with rituximab (375 mg/m² intravenously each week for 4 weeks) combined with Peg-IFNα-2b (1.5 μg/kg/week, subcutaneously) plus ribavirin (600–1200 mg/day orally) for 12 months. All patients had severe active disease resistant to previous combination therapy with standard or Peg-IFNα-ribavirin. Fifteen patients (93.7%) showed clinical improvement, 10 of whom (62.5%) were complete responders. Clinical improvement was observed after a mean duration of 6±4.1 months. HCV RNA and serum cryoglobulin became undetectable in 11 (68.7%) and 10 (62.5%) patients, respectively. Peripheral blood B cell depletion was achieved in all patients with reconstitution starting at the end of antiviral therapy. Compared with clinically complete responders, the partial or non-responders had a 3.6-fold longer duration of vasculitis prior to therapy and a lower rate of early virological response. Treatment was well tolerated with no infectious complications. Flare-up of psoriasis and worsening of peripheral neuropathy occurred in one patient each. After a mean follow-up of 19.4±3.6 months, one death occurred due to liver failure. Two patients (12.5%) experienced clinical relapse associated with simultaneous reappearance of HCV RNA and cryoglobulin and an increase in the number of B cells.

We recently extended our experience to 38 HCV-MC patients who received a combination of rituximab (375 mg/m²) once a week for 1 month followed by Peg-IFNα (2a: 180 μg, or 2b: 1.5 μg/kg) weekly plus ribavirin (600–1200 mg) daily for 48 weeks. They were compared to 55 HCV-MC patients treated with Peg-IFNα/ribavirin with the same modalities. Compared with Peg-IFNα/ribavirin, patients treated with rituximab plus Peg-IFNα/ribavirin had a shorter time to clinical remission (5.4±4 vs 8.4±4.7 months; p=0.004), better renal response rates (80.9% vs 40% complete response; p=0.040) and higher rates of cryoglobulin clearance (68.4% vs 43.6%; p=0.001) and clonal VH1–69+ B cell suppression (p<0.01). Treatment was well tolerated with 11% of discontinuations due to antiviral therapy. Taken together, rituximab combined with Peg-IFNα/ribavirin is well tolerated and more effective than Peg-IFNα/ribavirin in HCV-MC. Rituximab synergises the immunological effect of antiviral therapy.

Dammacco et al recently reported on 22 patients with HCV-related MC who received Peg-IFNα (2a: 180 μg, or 2b: 1.5 μg/kg) weekly plus ribavirin (1000 or 1200 mg) daily for 48 weeks, and rituximab (375 mg/m²) once a week for 1 month followed by two 5-monthly infusions (termed PIRR). Fifteen additional patients received Peg-IFNα/ribavirin with the same modalities as the PIRR schedule. Complete response was achieved in 54.5% (12/22) and in 33.3% (5/15) of the patients who received PIRR and Peg-IFNα/ribavirin, respectively (p<0.05). Clearance of HCV RNA and conversion of B cell populations from oligoclonal to polyclonal in liver, bone marrow and peripheral blood was maintained for up to 3 years in 10 of 12 (83.3%) and in two of five (40%) patients receiving PIRR and Peg-IFNα/ribavirin, respectively (p<0.01). Cryoproteins in 22.7% (5/22) of patients treated with PIRR and in 33.3% (5/15) treated with Peg-IFNα/ribavirin persisted despite sustained HCV RNA clearance. No response occurred in the remaining five patients in both groups. PIRR therapy is well tolerated and more effective than the Peg-IFNα/ribavirin combination in HCV-related MC. Its effect may last for more than 3 years.

4.6.1 Therapeutic guidelines

Aggressive antiviral therapy with Peg-IFNα (1.5 μg/kg/week, subcutaneously) and ribavirin (1000–1200 mg daily) should be considered as induction therapy for HCV-MC with mild to moderate disease severity and activity...
(ie, without rapidly progressive nephritis, motor neuropathy or other life-threatening complications). The duration of therapy has not yet been rigorously determined but current treatment duration in HCV-MC is 48 weeks for all HCV genotypes. Extending therapy with Peg-INFα/ribavirin to 72 weeks decreases the probability of relapse in patients with slow virological response (ie, undetectable HCV RNA level or ≥2-log decrease at week 12). If they can be maintained on extended-duration therapy, sustained virological response rates also may improve. With this strategy, patients with mild or moderate disease (ie, arthralgia, purpura, sensory-motor polyneuropathy and/or isolated proteinuria) may be able to be managed without immunosuppressive agents. In patients presenting with severe disease (ie, worsening of renal function, mononeuritis multiplex, or extensive skin disease including ulcers and distal necrosis), an immunosuppression induction phase is often necessary while awaiting the generally slow response to antiviral treatments. Combination therapy with rituximab plus Peg-IFNα and ribavirin appears logical as it may target both the viral trigger (HCV) and the downstream B cell arm of autoimmunity. Biological therapy with B cell directed therapy is promising for the treatment of HCV-MC but many questions remain 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 RA or systemic lupus erythematosus, the duration of effect appears finite, with response durations typically lasting 6–12 months, so it should be combined with antiviral drugs. The 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 immunosuppression (cytotoxic agents, steroids) to avoid MC rebound after apheresis. Antiviral therapy with Peg-IFNα and ribavirin combination should be postponed until after the critical phase.

### Summary points

- Treatment of HCV-mixed cryoglobulinaemia (MC) may target either the viral trigger (HCV) or the downstream B cell arm of autoimmunity.
- Antiviral therapy with Peg-interferon (IFN) α and ribavirin should be considered as induction therapy for HCV-MC with mild to moderate disease severity and activity.
- A new antiviral combination (Peg-IFNx, ribavirin and a protease inhibitor) seems very promising in HCV-MC.
- In patients presenting with severe disease (ie, worsening of renal function, mononeuritis multiplex, or extensive skin disease including ulcers and distal necrosis), an immunosuppression induction phase is often necessary while awaiting the generally slow response to antiviral treatments.
- Combination therapy with rituximab and optimal antiviral treatment appears logical as it may target both the viral trigger (HCV) and cryoglobulin producing B cells.
- An early virological response to antiviral therapy is correlated with a complete clinical response in MC.
- Clinical relapses in HCV-related vasculitis are usually associated with relapsing HCV viraemia.
- Recovery of B cell count began 6–9 months after rituximab therapy.
- Low-dose corticosteroids may help to control minor intermittent inflammatory signs such arthralgia, but are not successful in cases of major organ involvement (ie, neurological, renal) in the long-term control of vasculitis.
- Careful monitoring for adverse effects is mandatory since some manifestations of HCV-related vasculitis, such as peripheral neuropathy or skin ulcers, may worsen under IFN therapy.
- Clinicians should be aware of the possibility of malignant lymphoma when HCV-positive patients experience a relapse in cryoglobulinaemia vasculitis without HCV virological relapse.

### Key references

**Complete list of references available at http://**


