Characteristics and outcome of 49 patients with symptomatic cryoglobulinaemia


Departments of Internal Medicine, 1Biochemistry, 2Virology and 3Nephrology, Hôpital Avicenne, Assistance Publique-Hôpitaux de Paris, Université Paris-Nord, 125 rue de Stalingrad, 93009 Bobigny Cedex, France

Abstract

Objective. To describe a population of patients with symptomatic cryoglobulinaemia, comparing manifestations and outcome as a function of hepatitis C virus (HCV) status.

Patients and methods. A retrospective study on 179 patients who tested positive for cryoglobulins, seen between 1978 and 1998 in an internal medicine department.

Results. Among 179 cryoglobulin-positive patients, only 49 (18 men, 31 women; mean age 59.96 ± 12 yr) had clinical manifestations attributable to cryoglobulinaemia. Thirty-three had HCV infection, 20 had systemic autoimmune diseases, two had haematological diseases, one had human immunodeficiency virus and HCV co-infection, one had HCV and HBV co-infection and six had essential mixed cryoglobulinaemia. The clinical manifestations and cryoglobulin levels in HCV+ and HCV− patients did not differ significantly. Only arthralgias and elevated transaminases were significantly more frequent in HCV+ patients (P < 0.02 and <0.05, respectively). Five-year survival rates were comparable for HCV+ and HCV− patients. Eight patients died (six HCV+, two HCV−), with a median time between diagnosis and death of 38.7 months.

Conclusion. Clinical manifestations of cryoglobulinaemia, except arthralgias, were comparable for HCV+ and HCV− patients. When systemic manifestations are present, the prognosis is poor despite intensive or prolonged therapy.

KEY WORDS: Cryoglobulinaemia, HCV, Interferon alpha, Plasma exchange, Prognosis, Mortality.

Cryoglobulins are plasma proteins that precipitate at low temperatures and dissolve at higher temperatures. They were first described in 1933 by Wintrobe and Buell [1]. In 1962, Lospaluto et al. [2] showed that cryoglobulins contain more than one immunoglobulin and that there may be rheumatoid factor activity within the cryoprecipitate. Brouet et al.’s [3] classification recognized three types of cryoglobulin: type I is composed of monoclonal immunoglobulins, usually IgM; type II consists of a combination of polyclonal and monoclonal immunoglobulins, with the latter having rheumatoid factor activity; type III is characterized by a combination of polyclonal immunoglobulins. Types II and III are termed mixed cryoglobulins.

Cryoglobulinaemia is an immune complex-mediated systemic vasculitis involving mostly small, but sometimes larger vessels, and can be associated with a variety of clinical conditions including myeloma, macroglobulinaemia, chronic infection and inflammatory disorders. Type I cryoglobulins can be found in patients with lymphoproliferative disorders, such as Waldenström’s macroglobulinaemia or multiple myeloma, while mixed cryoglobulins are detected in patients with autoimmune disorders, lymphoproliferative disorders, or chronic infections. When no underlying disease is present, this is referred to as essential mixed cryoglobulinaemia (EMC).

In EMC, the possible role of hepatotropic viruses has been suggested by the high frequency of co-existing liver abnormalities [4]. Levo et al. [5] proposed hepatitis B virus (HBV) as a possible aetiology for EMC, but subsequent studies did not confirm the systematic responsibility or any relationship [6, 7]. Because liver involvement is frequent in EMC, the prevalence of anti-hepatitis C virus (HCV) antibodies and the correlation with clinical and serological parameters of EMC have been investigated [8–10]. A high prevalence of circulating anti-HCV antibodies and a very high HCV RNA concentration in the cryoprecipitate were
found, thereby suggesting a close relationship between EMC and chronic HCV infection [8–10]. The clinical manifestations of MC range from asymptomatic, mild vasculitis with palpable purpura, arthralgias and fatigue, to severe vasculitis with skin necrosis [11–13], involvement of the kidneys [14–16], peripheral nerves [17–20], central nervous system (CNS) [21–25], lungs [26], myocardium [3, 27, 28] and/or gastrointestinal (GI) tract [29]. The aim of this study was to describe the clinical and biological manifestations observed in patients with symptomatic cryoglobulinaemia, in light of the discovery of HCV infection.

Patients and methods

Cryoglobulinaemia
Venous blood was collected in a warm syringe and stored at 37°C until it clotted. After centrifugation at 37°C, the serum was collected and stored at 4°C for 1 week. After isolation and washing, the cryoprecipitate was quantified. The components of the cryoprecipitate were detected using an automated immunonephelometric assay and characterized using immunofixation electrophoresis (Sebia, Issy-les-Moulineaux, France). In our experience, the cryoglobulin concentration did not exceed 80 mg/l in 60 consecutive healthy blood donors. Therefore, only persistent cryoglobulin concentrations >80 mg/l were considered for the diagnosis of cryoglobulinaemia. Only 33 sera could be tested for baseline cryoglobulin concentration, as described above. For the 16 other patients, cryoglobulins had been previously determined in other centres (and measured as a percentage of cryoprecipitate, i.e. cryocrit); by the time they were assessed in our centre, all patients had started therapy and thus, the cryoglobulin concentrations were probably lower than at baseline.

Patients
Cryoglobulins were detected in the sera of 179 patients seen between 1978 and 1998 in our Department of Internal Medicine. Cryoglobulins were sought in patients presenting with polyarthralgias or myalgias when vasculitis or systemic diseases were suspected, or systematically tested when HCV infection was present. Patients with transient cryoglobulins (n = 8), <80 mg/l (n = 7), with HCV infection but no clinical manifestations (n = 61) or with a final diagnosis other than cryoglobulinaemia (n = 54) were not considered. The clinical characteristics of the 49 patients with clinical manifestations of cryoglobulinaemia are presented here.

HCV infection
Second- or third-generation HCV serological tests, ELISA-2, ELISA-3 (Ortho Diagnostic System, Raritan, NJ, USA) and RIBA-2 (Chiron/Ortho Diagnostic System, Emeryville, CA, USA) were used. HCV RNA was detected using reverse transcriptase–polymerase chain reaction (RT–PCR). Blood samples were collected and RNA was extracted as previously described [30].

Biochemical evaluation
Measurement of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, γ-glutamyltransferase (γ-GT) and gammaglobulin levels, a test for rheumatoid factor, measurements of serum complement components (C3 and C4) and total haemolytic complement activity (CH50) were performed using standard procedures. Haematuria and proteinuria were also assessed for each patient. Patients with clinical manifestations consistent with vasculitis or connective tissue disease were systematically tested for antinuclear autoantibodies and antineutrophil cytoplasm antibodies.

Therapeutic modalities
Only patients with symptomatic cryoglobulinaemia were treated. HCV-infected patients were treated with subcutaneous interferon alpha (IFNα) at the dose of 3 MU thrice weekly for 6 or 12 months, with or without plasma exchange (PE) scheduled as follows: 60 ml/kg bodyweight was removed and replaced with gelatin or human albumin three times/week for 2 weeks, two times/week for 2 weeks and one time/week for 3 weeks. No patients received ribavirin. PE was prescribed for glomerulonephritis with renal insufficiency, necrotic leg ulcers resistant to corticosteroids, recent-onset peripheral neuropathy, mononeuritis multiplex or CNS involvement. Corticosteroids (at the initial dose of 1 mg/kg/day, then tapered over several months) were given to patients presenting with severe visceral manifestations: glomerulonephritis with renal insufficiency, GI tract involvement, myocardial or CNS involvement or recent-onset mononeuropathy multiplex.

Treatment of HCV− patients and those for whom HCV was detected retrospectively several years after the onset of cryoglobulinaemia, consisted of corticosteroids with or without PE. Immunosuppressants (oral cyclophosphamide or azathioprine) were prescribed after relapse or failure of first-line therapy. IFNα (3 MU thrice weekly) was prescribed for patients refractory to immunosuppressants or when immunosuppressants were not tolerated or contraindicated.

Statistical analyses
Statistical analyses were performed using the χ² test and appropriate corrections when necessary for categorical variables. Quantitative values, expressed as mean ± standard deviation, were compared using Student’s t-test. Actuarial survival curves were established using the Kaplan–Meier method.

Results

Cryoglobulin characteristics
Cryoglobulin concentrations were determined for 33 patients (mean concentration: 2.002 ± 2.004 g/l)
and characterized in 43/49 patients (Table 1): type I in three patients (one IgM kappa, two IgG kappa; 7%); type II in 24 patients (20 IgM kappa, two IgM lambda, one IgG lambda, undetermined in one patient; 55.8%) type III in 16 patients (37.2%).

**Patient characteristics**

Our patient population consisted of 31 women and 18 men, with a mean age at diagnosis of 59.96 ± 12 yr (female/male ratio = 1.72).

**Presenting symptoms**

In this retrospective study, 34 patients had at least one cryoglobulinaemia-defining manifestation at first presentation. Palpable purpura (55.9%) was the most frequent presenting symptom, followed by arthralgias (29.4%), peripheral neuropathy (20.6%) and/or myalgias (8.8%), with renal disease (5.9%) and leg ulcers (5.9%) rarely present at onset.

**Clinical manifestations from onset to the end of follow-up (Table 2)**

**Cutaneous and vasomotor symptoms.** Forty (81.6%) patients had skin involvement. Palpable purpura was the most common manifestation, usually beginning in the legs. Skin biopsies of purpuric lesions confirmed the diagnosis of vasculitis in 22/27 patients (81.5%). Leg ulcers developed in 20 patients (40.8%). Raynaud’s phenomenon was noted in 17 (34.7%), livedo in three (6.1%), urticaria in two (4.1%) and subcutaneous nodules in one (2%).

**Rheumatological manifestations.** Arthralgias were present in 25 patients (51%) and diffuse myalgias were present in 10 (20.4%).

**Neurological disorders.** Peripheral neuropathy was present in 27 patients (55.1%) and was documented by electromyography in 25 patients: 14 symmetrical distal sensory polyneuropathies and 11 mononeuritis multiplex. Nerve biopsies from 15 patients showed lymphocytic vasculitis involving epineurial small vessels in 14 patients (93.3%) and was normal in one patient. CNS involvement was suspected in three patients: one had vertigo; another cerebral ischaemia with multifocal infarcts on magnetic resonance imaging; the third patient had dysarthria, aphasia and confusion which regressed dramatically after intensive PE.

**Renal disease.** Renal manifestations were present in 12 patients (24.5%). Renal insufficiency, defined as creatininaemia >140 μmol/l, was present in five patients, microscopic haematuria was found in 9/12 patients. Proteinuria (>1 g/day) was present in 10/12 patients (83%), seven (58%) of whom had nephrotic syndrome. Renal biopsies performed in 10 patients revealed membranous proliferative glomerulonephritis in all of them; vasculitis was seen in two patients. Six of these 12 patients had type II IgM kappa, three had type III, and for three, cryoglobulins were not typed. Eight patients were HCV+ and four were HCV−.

**GI involvement.** Episodes of diffuse abdominal pain, one of them with GI bleeding, were observed in six patients (12.2%). None necessitated laparotomy. Coeliomesenteric angiography, performed in two patients, did not show microaneurysms; distal opacification was slow and late, consistent with diffuse distal mesenteric ischaemia.

**Cardiac manifestations.** During the course of the disease, four patients (8.2%) (mean age 63.3 yr) had myocardial infarctions; only one of them had a risk factor for coronary disease (hypertension). Mitral insufficiency was diagnosed in one patient and pericarditis in another.

**Respiratory tract diseases.** Pleural effusions were observed in two patients; one had a nephrotic syndrome and the other suffered from pulmonary fibrosis associated with progressive systemic sclerosis.

**Sicca syndrome.** Sicca syndrome, defined as dryness of the mouth and/or the eyes, was found in 17 patients (34.7%), but none of them had anti-SSA antibodies. Four salivary gland biopsies could be reviewed and showed diffuse, non-nodular, lymphocytic infiltrates, without ductal atrophy; vasculitis was seen in one patient.

**Miscellaneous.** Asthenia, weight loss and/or fever were observed in 18 patients (36.7%). Mild depression was noted in six patients (12.2%), two during the first

---

**Table 1. Main characteristics of and differences between the cryoglobulins detected in 49 symptomatic patients, according to HCV status**

<table>
<thead>
<tr>
<th>Cryoglobin types</th>
<th>HCV+</th>
<th>HCV-</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV+</td>
<td>33</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>HCV-</td>
<td>14</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*HCV serum was not performed for two patients. NT, not typed.

**Table 2. Clinical manifestations of MC in symptomatic patients according to HCV status**

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>HCV+ (n = 33)</th>
<th>HCV- (n = 14)</th>
<th>All patients (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpura</td>
<td>21 (63.6%)</td>
<td>11 (78.6%)</td>
<td>32 (67.3%)</td>
</tr>
<tr>
<td>Leg ulcers</td>
<td>14 (42.4%)</td>
<td>6 (42.9%)</td>
<td>20 (40.8%)</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>26 (78.8%)</td>
<td>12 (85.7%)</td>
<td>40 (81.6%)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>8 (24.2%)</td>
<td>7 (50%)</td>
<td>15 (34.7%)</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>20 (60.6%)</td>
<td>3 (21.4%)</td>
<td>23 (48.9%)</td>
</tr>
<tr>
<td>Myalgias</td>
<td>7 (21.2%)</td>
<td>1 (7.1%)</td>
<td>8 (16.3%)</td>
</tr>
<tr>
<td>Sicca syndrome</td>
<td>10 (30.3%)</td>
<td>6 (42.9%)</td>
<td>16 (32.6%)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>7 (21.2%)</td>
<td>4 (28.6%)</td>
<td>11 (22.4%)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>18 (54.5%)</td>
<td>8 (57.1%)</td>
<td>26 (53.1%)</td>
</tr>
<tr>
<td>CNS involvement</td>
<td>3 (9.1%)</td>
<td>0</td>
<td>3 (6.1%)</td>
</tr>
<tr>
<td>GI involvement</td>
<td>5 (15.2%)</td>
<td>1 (7.1%)</td>
<td>6 (12.2%)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>3 (9.1%)</td>
<td>1 (7.1%)</td>
<td>4 (8.2%)</td>
</tr>
</tbody>
</table>

*HCV serum was not performed for two patients. Only arthralgias were significantly more frequent in HCV+ patients (P = 0.025).
3 months of IFNz therapy, while the other four did not receive IFNz. Two men had orchitis (11.1%). Vasculitis was found in two unusual biopsy sites: a liver biopsy in the first, and a collateral arteriole of the temporal artery biopsy in the second. Anti-thyroid autoantibodies were negative in all 16 patients tested; dysthyroidism was not found in any of them.

**Associated diseases** (Table 3)

**Infectious diseases.** Anti-HCV antibodies were present in 33 (67.3%) patients (23 women, 10 men), absent in 14 and not tested in two. HCV RNA was sought in 25 HCV+ patients and was present in all cases.

One HCV+ patient was co-infected with human immunodeficiency virus (HIV). Serum hepatitis B surface antigen was detected in two patients, one of whom was co-infected with HCV and the other was positive for anti-hepatitis Be antibodies and negative for HBV DNA.

**Haemato logical diseases.** Two patients (two men, mean age 54 yr) had monoclonal gammopathies (one IgG kappa and one IgM kappa) of unknown significance.

**Systemic diseases.** Twenty patients (15 women, five men, mean age 59.3 ± 10.8 yr) had systemic disease as detailed in Table 3. The female/male sex ratio was 3. Seventeen had sicca syndrome (10 with HCV infection), one had progressive systemic sclerosis (and HCV infection), one had primary biliary cirrhosis and one had co-existing polyarteritis nodosa (HCV and HBV co-infection).

Only six patients (three women, three men; mean age 71.4 ± 15.3 yr) had no associated or underlying disease.

**Laboratory findings** (Table 4)

Rheumatoid factor was sought in 34/49 patients and detected in 16 (47.1%). Low C4 levels were noted in 36/43 (83.7%) patients and CH50 was low in 14/25 (56%) patients. Elevated serum ALT was observed in 21/49 (42.8%) patients, elevated serum AST was observed in 19/49 (38.8%) patients. Transaminases were significantly higher in the HCV+ patients than in the HCV− patients (57.6 vs 14.3%, P < 0.05). Polyclonal hypergammaglobulinaemia was found in 10/49 (20.4%), hypogammaglobulinaemia was found in 12/49 (24.5%).

**Histological findings** (Table 5)

One hundred biopsies from 39 patients were reviewed, and an attempt was made to classify the vasculitic lesions according to vessel diameter (Table 5). Venules, capillaries and arterioles were considered to be small vessels; arteries with a diameter >200 μm were considered to be medium sized.

Forty-nine biopsies (49%) showed evidence of vasculitis: 43 (87.8%) involved small vessels, six (12.2%) involved medium-sized vessels, five of them co-existing with small vessel vasculitis. In seven biopsies, the inflammatory infiltrate was perivascular and did not invade the vessel wall.

Vascular infiltrates were composed as follows: predominately neutrophils (15.2%), predominantly mononuclear cells (45.4%), association of the two (39.4%). Immunofluorescence assays for immunoglobulins and C3 deposits were negative for 22%, and slightly positive

### Table 3. Systemic manifestations and infections associated with symptomatic cryoglobulinaemia in 49 patients

<table>
<thead>
<tr>
<th>HCV status</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV+</td>
<td>33</td>
</tr>
<tr>
<td>HCV alone</td>
<td>20</td>
</tr>
<tr>
<td>HCV and sicca syndrome</td>
<td>10</td>
</tr>
<tr>
<td>HCV + HBV co-infection (and HBV PAN)</td>
<td>1</td>
</tr>
<tr>
<td>HCV + HIV co-infection</td>
<td>1</td>
</tr>
<tr>
<td>HCV and PSS</td>
<td>1</td>
</tr>
<tr>
<td>HCV</td>
<td>14</td>
</tr>
<tr>
<td>Sicca syndrome</td>
<td>6</td>
</tr>
<tr>
<td>EMC</td>
<td>5</td>
</tr>
<tr>
<td>Monoclonal gammopathies</td>
<td>2</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>1</td>
</tr>
<tr>
<td>HCV undetermined</td>
<td>2</td>
</tr>
<tr>
<td>Sicca syndrome</td>
<td>4</td>
</tr>
<tr>
<td>EMC</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HCV status</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV+</td>
<td>33</td>
</tr>
<tr>
<td>HCV alone</td>
<td>20</td>
</tr>
<tr>
<td>HCV and sicca syndrome</td>
<td>10</td>
</tr>
<tr>
<td>HCV + HBV co-infection (and HBV PAN)</td>
<td>1</td>
</tr>
<tr>
<td>HCV + HIV co-infection</td>
<td>1</td>
</tr>
<tr>
<td>HCV and PSS</td>
<td>1</td>
</tr>
<tr>
<td>HCV</td>
<td>14</td>
</tr>
<tr>
<td>Sicca syndrome</td>
<td>6</td>
</tr>
<tr>
<td>EMC</td>
<td>5</td>
</tr>
<tr>
<td>Monoclonal gammopathies</td>
<td>2</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>1</td>
</tr>
<tr>
<td>HCV undetermined</td>
<td>2</td>
</tr>
<tr>
<td>Sicca syndrome</td>
<td>4</td>
</tr>
<tr>
<td>EMC</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 4. Biochemical and immunological data for 49 patients according to the presence of HCV infection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCV+ (n = 33)</th>
<th>HCV− (n = 14)</th>
<th>All patients (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated transaminases</td>
<td>19 (57.6%)a</td>
<td>2 (14.3%)a</td>
<td>42.8%</td>
</tr>
<tr>
<td>Hypermangamaglobulinaemia</td>
<td>7 (21.2%)</td>
<td>3 (21.4%)</td>
<td>20.4%</td>
</tr>
<tr>
<td>Rheumatoid factord</td>
<td>12/18 (75%)</td>
<td>4 (28.6%)</td>
<td>16/34 (47.1%)</td>
</tr>
<tr>
<td>Low C4 leveld</td>
<td>23/27 (85%)</td>
<td>12 (85.6%)</td>
<td>36/43 (83.7%)</td>
</tr>
<tr>
<td>Cryoglobulin level (g/l)</td>
<td>1.911 ± 1.628</td>
<td>2.230 ± 2.865</td>
<td>2.004 ± 2.004</td>
</tr>
<tr>
<td>Type I cryoglobulinsd</td>
<td>0b</td>
<td>2 (14.3%)b</td>
<td>2/43 (4.7%)</td>
</tr>
<tr>
<td>Mixed cryoglobulins (types II and III)d</td>
<td>27/27 (100%)c</td>
<td>11 (78.6%)</td>
<td>41/43 (95.3%)</td>
</tr>
</tbody>
</table>

aP = 0.02.
bP < 0.05.
cThe cryoglobulin type was not available for six patients.
dThese parameters were not available for some patients.
Table 5. Histological findings in 100 biopsies from 39 patients with symptomatic cryoglobulinaemia

<table>
<thead>
<tr>
<th>Biopsy sites</th>
<th>Presence of vasculitis</th>
<th>Small vessels</th>
<th>Medium-sized vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>27</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Liver</td>
<td>22</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Neuromuscular</td>
<td>15</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Muscle</td>
<td>10</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Kidneys</td>
<td>10</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>9</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sinus</td>
<td>1</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Temporal artery</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Colon</td>
<td>1</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

*Co-existence with small-vessel vasculitis.

for C3 and IgM in 78%. Proof of vasculitis was most often obtained in skin (primarily palpable purpura: 81.5%), neuromuscular (especially epineurial vessels: 14/15 or 93.3%), and muscle (5/10 or 50%) biopsies.

Patients with HCV infection (Table 2)

Thirty-three patients (67.3%) had HCV infection. Their mean age was 58.8 ± 10.3 yr vs 61.5 ± 14 yr for the HCV− patients (NS). The female/male sex ratio was 0.43. The route of contamination was known for 14: intravenous drug use (n = 3) or blood transfusion (n = 11). The mean time between contamination and diagnosis of cryoglobulinaemia was 13.8 ± 8 yr. Biochemical data are reported in Table 4. No significant difference was found between the mean cryoglobulin concentrations of the HCV+ patients (1.911 ± 1.628 g/l) and those of the HCV− patients (2.230 ± 2.865 g/l). AST and ALT were significantly higher in the HCV+ patients (P = 0.05; see Table 4). Only arthralgias were significantly more frequent in HCV+ patients (P = 0.025; see Table 2).

Treatment and outcome

Therapeutic modalities. First-line therapy for HCV-infected patients consisted of IFNα (23 patients), with corticosteroids (22 patients) or PE (11 patients), according to the severity of the clinical manifestations. Immunosuppressants (cyclophosphamide for four patients and azathioprine for one patient) were prescribed after failure of first-line therapy.

During IFNα therapy, cutaneous manifestations of the HCV+ patients regressed, but recurred when IFNα was stopped. Sensory peripheral neuropathy stabilized but did not regress, even when IFNα was combined with corticosteroids and/or PE. Nevertheless, no exacerbation of peripheral neuropathy was seen. Prolonged virus eradication could not be obtained in any of the patients.

First-line therapy for HCV− patients and patients for whom HCV was detected retrospectively and sometimes several years after the onset of the disease, consisted of corticosteroids (11 patients) with or without PE (five patients). Immunosuppressants (cyclophosphamide for five patients) or IFNα (three patients) were prescribed after relapse or failure of first-line therapy.

Twenty-nine patients received more than one treatment regimen during the course of the disease. Two patients (one HCV+ and one HCV−) did not receive any treatment because the flare was mild and regressed spontaneously.

Eighteen patients (13 HCV+, five HCV−) underwent PE, with (n = 12) or without (n = 6) low-dose corticosteroids and with (n = 11) or without (n = 7) IFNα. Most of them had recent-onset peripheral neuropathy or recurrent leg ulcers. All of them had dramatic regression of ulcers, while peripheral neuropathy responded poorly. Four patients with recurrent and painful leg ulcers were successfully treated with PE over several years. Pertinently, comparison of the long-term survival rates between patients who were treated with PE and those who were not showed a slight but significant difference in favour of those without PE (Fig. 1A). The explanation is probably complex and is discussed below.

Outcome. For 25/35 (71.4%) patients, clinical remission could be obtained but clinical manifestations recurred in all of them when treatment was stopped, and maintenance therapy with IFNα, low-dose corticosteroids and/or PE was necessary. For 14 patients, the long-term response to therapy was not well documented or was unknown.

Eight (16.3%) patients (six HCV+, two HCV−) died during follow-up, and the 5-yr survival curves did not differ significantly as a function of HCV status (Fig. 1B) or cyclophosphamide administration (Fig. 1C). The mean age at the time of death was 66.2 yr, and the mean time between diagnosis and death was 38.7 ± 36.7 months. The causes of death were: systemic infection in two (both with septic shock on indwelling catheters while receiving long-term corticosteroids, in addition to PE for one), major clinical deterioration in two patients (the first was being treated with long-term corticosteroids and had previously received cyclophosphamide; the second was treated with corticosteroids and IFNα and had been undergoing intensive PE for 2 weeks), stroke complicated by aspiration pneumonia in two patients (the first was on low-dose corticosteroids and PE for chronic and painful leg ulcers, the second received corticosteroids and IFNα), GI bleeding in one (untreated HCV-related cirrhosis, 2 yr after the transient efficacy of IFNα alone against purpura and peripheral neuropathy) and HCV-related hepatocarcinoma in a patient on corticosteroids 2 months after the end of PE which had successfully treated mononeuritis multiplex.

Discussion

Clinical manifestations

Cryoglobulinaemia, a systemic vasculitis involving primarily small vessels, can be associated with lymphoproliferative malignancies, autoimmune diseases, or
Symptomatic cryoglobulinaemia

Fig. 1. Kaplan–Meier 5-yr survival curves according to (A) PE, (B) HCV status, (C) cyclophosphamide (CYC) therapy.
cryoglobulins are found in more than half of HCV+ cases formerly diagnosed as EMC with HCV, this virus has been considered to be the cause of Sjögren’s syndrome.  

A | B | C | D | E
---|---|---|---|---
10 | 86 | 40 | 63 | 49
48.9 | NR | 49.6 | 45.5 | 60
9/1 | NR | 25/15 | 32/31 | 31/18
Mixed | All | Mixed | All |
100 | 55 | 100 | 34 | 67.3
30 | 5 | 30 | NR | 40.8
10 | 50 | 25 | NR | 34.7
100 | 35 | 72.5 | 17 | 51
40 | 21 | 55 | NR | 24.5
20 | NR | 45 | 11 | NR
10 | 15 | 20 | 41 | 55.1
20 | 0 | 30 | NR | 8.2
20 | 2 | 20 | NR | 12.2
90 | 0 | 70 | 52 (HCV+) | 67.3 (HCV+)
20 | 7 | 15 | NR | 34.7
20 | 2 | NR | NR | 6.1
60 | 0 | 17.5 | NR | NR

NR, not reported.

chronic bacterial, fungal or viral infections [3, 28], whereas transient and asymptomatic cryoglobulins can be detected during acute infectious diseases and even in healthy blood donors [31]. Since the discovery of HCV, this virus has been considered to be the cause of cases formerly diagnosed as EMC [8–10, 31, 32].

When cryoglobulins are systematically tested, mixed cryoglobulins are found in more than half of HCV+ patients; but in the large majority of them, they remain at low concentrations and are clinically asymptomatic [31, 33]. The long-term significance of asymptomatic cryoglobulinaemia in HCV+ patients remains to be elucidated.

The present study set out to describe the characteristics, aetiology and outcome of patients with cryoglobulinaemia observed in a single department of internal medicine.

Clinical and biological manifestations of cryoglobulinaemia were described before the discovery of HCV [3, 28, 34] (see Table 6). The frequency of cutaneous manifestations in our patients was comparable with those reported in the literature, and no difference was observed with regard to HCV infection [3, 11–13, 28].

It is pertinent that four of our patients had long histories of recurrent and painful chronic leg ulcers requiring numerous hospitalizations; their general condition progressively deteriorated over several years, leading to death, even though three of them never developed extracutaneous involvement. These deaths might have resulted from the cumulative toxicity of long-term low-dose corticosteroids. In our experience, chronic leg ulcers never healed with IFNα alone; repeated PE was the sole effective therapy.

Peripheral neuropathy was more frequent in our patients (55.1%) than previously published frequencies, which vary from 8.3 to 53% [3, 18–20, 28, 31, 35]. Our patients presented with two distinct forms of neurological involvement: progressive and chronic distal sensory polyneuropathy involving the legs, or acute mononeuritis multiplex. Epineural vasculitis was found in 14/15 nerve biopsies.

One of our three patients with CNS manifestations recovered dramatically from encephalopathy after intravenous administration of corticosteroids and one PE. We postulate that encephalopathy could have been the consequence of a rapid rise in the cryoglobulin concentration, as it was >15 g/l. Reversible acute or subacute encephalopathy has been described [21]. In another patient, magnetic resonance imaging revealed multifocal T2-weighted hypersignals. Stroke-like syndromes have been attributed to ischaemia or haemorrhage [22, 23, 25]. In our experience, the frequency of renal involvement (24.5%) was comparable with that observed in other series [3, 28]; only one patient required chronic dialysis. In the Italian experience [16], renal insufficiency usually occurred late in the course of the disease in <10% of the patients.

Myocardial involvement is rare. In our study, four patients (mean age 63.3 yr) suffered myocardial infarctions, three of them did not have any coronary risk factor; one underwent a coronarography that showed occlusion of the right coronary artery. Gorevic et al. [28] reported that 12 patients required management for congestive heart failure, two for pericarditis and four for myocardial infarctions; among the nine post-mortem examinations, coronary vasculitis was found in two patients, and a subendothelial infarct was found in one.

As observed in our patients, GI involvement is common (<20%) and is thought to be due to intestinal vasculitis.

In contrast to HBV-related polyarteritis nodosa [36], only two of our HCV+ patients had orchitis, neither was co-infected with HBV.
All of our patients experienced more than one flare; only purpuric rashes regressed spontaneously within a few weeks, while painful leg ulcers necessitated continuing care or intensive and prolonged therapies, such as PE.

Comparison of HCV+ and HCV− patients

Few studies have compared HCV+ and HCV− cryoglobulinemic patients. In our experience, the mean age and sex ratio of HCV+ and HCV− patients did not differ significantly. Unlike Cacoub et al. [31], we did not find higher cryoglobulin concentrations in our HCV+ patients (see Table 4). In two French series [31, 35] of 105 and 63 patients, hepatic and cutaneous involvement were more frequent, and cryoglobulin levels higher in HCV+ patients. Buezo et al. [37] also found a high frequency of skin manifestations in HCV+ patients. In our study, only arthralgias (P < 0.02) and elevated serum transaminase levels (P < 0.05) were significantly more frequent in HCV+ patients. These findings are not surprising as these patients had chronic HCV infections. Others have suggested a role for HCV in leucocytoclastic vasculitis independently of cryoglobulins and liver involvement [38, 39]. The direct role of HCV, suggested by Agnello and Abel [40], who detected HCV RNA in biopsy specimens from six patients with cutaneous vasculitis, was not confirmed by other authors [41].

Peripheral neuropathy was present in more than half of our patients, regardless of HCV status. It was recently reported that HCV RNA was detected in nerve biopsies from five HCV+ patients. Using in situ RT–PCR, HCV RNA was localized in the epineurium, suggesting a major role for HCV in patients with neuropathies and HCV-related mixed cryoglobulinaemia (MC) [42]. But Khella et al. [43] suggested indirect damage of the nerves by cryoglobulins or inflammatory cells because they did not find HCV particles in their study on nerve biopsies.

No difference was found for renal involvement in terms of histological findings and mean creatininemia between our HCV+ and HCV− patients.

Therapy

Treatment of MC with or without HCV infection is difficult and prospective or controlled trials are rare [44–58]. Therapeutic strategies have been proposed according to the severity of the clinical manifestations. When visceral involvement is present, corticosteroids and/or immunosuppressants and PE have been prescribed [3, 28, 46, 59]. But regardless of the associated diseases, or combinations of treatments, relapses are frequent and long-term remission is rare [28, 32, 44, 50, 51, 53, 55].

Although some authors reported promising results with PE, indications of PE in MC remain controversial [45–48, 57, 58]. As already published, prompt regression of skin and joint manifestations was obtained, whereas neurological improvement was slower [45]. The beneficial effect was observed after the first 2–3 weeks of treatment. Other authors also found PE to be effective against recurrent or refractory leg ulcers [56], and was performed every 10–14 days in some patients as a maintenance therapy [60]. The high mortality rate of our patients who had been treated with PE cannot be explained by a single factor: these patients had long histories of recurrent disease and all had received prolonged corticosteroids with or without immunosuppressants. The causes of death were different, but two deaths resulted from iatrogenic infectious complications on indwelling catheters, whereas another followed the progressive deterioration of the patient’s general condition, even though no specific involvement of the heart, CNS, lungs or acute renal failure was present. In our opinion, patients with recurrent, chronic and painful leg ulcers undergo slow general deterioration and are at high risk of infectious complications.

In our study, eight (16.3%) patients died. Most of them had a long history of cryoglobulinaemia with corticosteroids and/or immunosuppressive therapy. Only one of them (who was <50 yr old) developed renal involvement, but it was not the cause of death. The others were over 60 yr old, without renal involvement. It should be noted that, although heart disease was not the leading cause of death, three of our four patients with myocardial infarction died during therapy with prolonged PE and oral corticosteroids. Specific myocardial involvement may be underdiagnosed, as it is not a rare finding on autopsy [28, 34] and could be a poor prognostic factor for patients with cryoglobulinaemia, especially when prolonged immunosuppressive therapy is still needed. Some authors found that renal disease was associated with a poor prognosis [28, 34], whereas Cordonnier et al. [61] reported that death was caused by extrarenal complications, e.g. infections, cardiovascular disease, CNS involvement or haematological malignancies. Tarantino et al. [62] reported the outcomes of 105 patients with EMC and renal disease: 42 died of cardiovascular disease, liver disease or infection; 15 developed chronic renal failure; two achieved complete remission; 15 experienced recurrence of only renal manifestations; 31 were alive with recurrent or persistent manifestations. Independent risk factors for death or dialysis were age >50 yr, purpura, splenomegaly, cryocrit = 10%, plasma C3 level <54 mg/dl and serum creatinine >140 μmol/l.

None of our patients has developed lymphoma; one HCV+ patient infection had isolated lymphoid infiltrates on a bone marrow biopsy, but the search for clonality was negative. This absence of lymphoma is strikingly different from the Italian and North American experiences [63–67]; in those studies, the risk of developing lymphoma rose from 13% at the time of MC diagnosis in a prospective study [67] to 35% after 10 yr of follow-up [63]. This discrepancy has not been correlated to HCV genotypes and remains unexplained.

In this retrospective study, the role of the different regimens is difficult to assess as some patients received immunosuppressive therapy before the discovery of
HCV. Furthermore, our department is a tertiary care centre, and many patients had received various treatments, including corticosteroids or immunosuppressants. It is known that long-term eradication of HCV with IFNα is more difficult to obtain when the duration of HCV infection is longer and the HCV load is higher, as it is in immunocompromised patients [68]. Whether prolonged exposure to immunosuppressants can influence the course of HCV-related MC remains unknown. In our experience, short-term control of vasculitic manifestations was obtained with corticosteroids, but most patients relapsed when therapy was tapered or stopped.

In light of the central role of HCV, it seems logical to state that therapeutic strategies for HCV-related MC must include HCV eradication. In a controlled study on patients with HCV-related MC, the disappearance of HCV RNA from serum during IFNα therapy correlated with clinical and biological improvement, but was not predictive of prolonged virological and clinical remissions [53]. Preliminary results with ribavirin alone [69] or combined with IFNα [58, 70] are encouraging. In a French study [69], five patients who did not tolerate IFNα were treated with ribavirin alone for 10–36 months; they all benefited from significant clinical improvement but no virological response was obtained. Large-scale trials are needed to confirm those preliminary data.

References

67. Rasul I, Shepherd FA, Kamei-Reid S, Krajden M, Pantalony D, Heathcote EJ. Detection of occult low-

