The prevalence, clinical relevance, and risk factors of serum cryoglobulins in hemophilic patients with chronic hepatitis C virus (HCV) infection are unknown. We studied 135 consecutive hemophilic patients (median age, 31 years; range, 10 to 69 years) with chronic hepatitis C, exposed to the virus for 10 to 41 years. A total of 67 patients were infected with the human immunodeficiency virus (HIV), and 3 (2%) had signs of cirrhosis. Serum samples were tested for the presence of cryoglobulins, hepatitis B virus (HBV) markers, including HBV-DNA by hybridization assay, and antibody to HCV by enzyme immunoassay (EIA). Serum HCV-RNA was tested by polymerase chain reaction and typed with a hybridization technique. Samples were also tested for antitissue antibodies, immunoglobulins, rheumatoid factor, and C3 and C4 protein. Forty-two hemophiliacs (31%) circulated cryoglobulins (median levels, 166 mg/L; range, 66 to 480) predominantly type III (62% and 29% type II). None of the patients had clinical signs or symptoms of systemic vasculitis. Cryoglobulinemic patients had more often serum HCV-RNA (95% vs 80%, P < .05), rheumatoid factor (20% v 6%, P < .05), higher levels of IgG (2,354 ± 682 mg/dL v 1,928 ± 557 mg/dL, P < .0005) and IgM (323 ± 226 mg/dL v 244 ± 243 mg/dL, P < .05), and lower levels of serum C4 (19 ± 8 mg/dL v 24 ± 8 mg/dL, P < .05) than patients without cryoglobulins. The risk of producing cryoglobulins was greater for 114 patients circulating HCV-RNA than for 21 nonviremic patients (odds ratio [OR] = 4.9, 95% confidence interval [CI] = 1.1 to 22.0) and for the 31 patients with longer exposure to HCV (more than 26 years) than for the 24 patients with shorter (17 years or less) exposure (OR = 4.4 95% CI = 1.1 to 18.0). In conclusion a large number of multitransfused hemophiliacs with chronic HCV infection circulated serum cryoglobulins but none had clinical signs or symptoms of vasculitis. The risk of developing cryoglobulins parallels the duration of exposure to HCV.

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MATERIALS AND METHODS

Patients. From June 1995 to July 1996, we studied 135 consecutive anti-HCV antibody–positive hemophiliacs (134 men; 123 with hemophilia A and 12 with hemophilia B), who were regularly attending the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center (Milan, Italy) for their annual medical check-up. The duration of HCV infection was calculated assuming that the first infusion with blood, plasma, or non–virus-inactivated concentrates manufactured from large pools of plasma had transmitted the virus C. According to this criterion, 128 hemophiliacs have been infected with HCV for 10 to 41 years (median, 23 years). Their main epidemiological and clinical features are shown in Table 1. In each patient serum alanine aminotransferase (ALT) values had been measured every year since 1979 and were classified as persistently normal (<40 U/L), intermittently elevated, and persistently elevated. HCV genotype distribution was 1a in 39%, 1b in 35%, 1a plus 1b in 3%, 2b in 4%, 2a/c in 2%, 3 in 10%, 4 in 2%, and not performed in 5%. Sixty-seven hemophiliacs (50%) had serum antibody to human immunodeficiency virus (anti-HIV) for 11 to 14 years (median, 13 years); 35 (52%) had signs of disease progression, with CD4 cell counts less than 200/cmm and/or full-blown acquired immunodeficiency syndrome. These patients were receiving antiretroviral therapy. None of the patients had received treatment with interferon or steroids in the previous 12 months.

Study profile. Clinical history and physical examination, liver and kidney function, and routine chemistry tests were obtained in all patients. Special attention was given to the search for signs and symptoms of cirrhosis and for cryoglobulinemic syndrome. Cirrhosis was diagnosed clinically on the basis of laboratory signs of liver failure, ie, platelet count less than 150,000/mL, serum albumin less than 3.5 g/L, serum cholinesterase activity less than 4,500 U/L, endoscopic signs of portal hypertension (presence of esophageal varices and hypertensive gastropathy), and/or by abdominal ultrasound (irregular margins of the liver, dilated portal vein, and splenomegaly). All patients were also examined for signs and symptoms referable to serum cryoglobulins, ie, palpable purpura, active or healed skin ulcers, peripheral neuropathy, Raynaud’s phenomenon, sicca syndrome, or renal complications. Joint pain or damage were not considered among possible symptoms of cryoglobulinemia because hemophilic arthropathy may be a confounder.

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According to Brouet et al., 16 cryoglobulins were classified as type II (if Ig composition by immunoblotting according to Musset et al.15 was measured by reading the absorbance at 280 nm. Values less than 50 mg/L were considered negative. Washed cryoglobulins were precipitated from serum stored for up to 8 days at 4°C (0.1 g/L sodium azide). The precipitates were used as reference. The results were expressed as odds ratio and 95% confidence intervals (CI).

### RESULTS

Forty-five patients (31%) circulated cryoglobulins, with concentrations ranging between 66 and 480 mg/L (median, 166 mg/L). Cryoglobulins were type III in 26 patients (62%) and type II in 12 (29%). In 4 patients (9%) cryoglobulins were not typed. Although in patients with type-II cryoglobulin there was a tendency for less severe liver disease (as expressed by the prevalence of persistently elevated ALT), differences were not statistically significant (35% vs 50% in type III). Age, severity of hemophilia, pattern of serum ALT, prevalence of cirrhosis, antitissue antibodies, anti-HCV, HBsAg, HBV-DNA, anti-HIV, and HIV disease progression were similar in patients with or without serum cryoglobulins (Table 1 and 2). There were no differences in HCV genotype distribution between cryoglobulinemic and noncryoglobulinemic patients (data not shown). Conversely, prevalence of serum HCV-RNA (95% vs 80%, P < .05) and of rheumatoid factor (20% vs 6%, P < .05), mean serum levels of IgG (2,354 ± 682 mg/dL vs 1,928 ± 557 mg/dL, P < .0005), and IgM (323 ± 226 mg/dL vs 244 ± 243 mg/dL, P < .05) were higher in patients with cryoglobulins than in those without. Instead, the mean serum levels of C4 were lower in the cryoglobulinemic patients (18 ± 8 mg/dL vs 24 ± 8 mg/dL, P < .05; Table 2). None of the patients had or has had signs of palpable purpura, healed or active skin ulcers, peripheral neuropathy, kidney failure, sicca syndrome, or Raynaud phenomenon. In patients with serum HCV-RNA the risk of developing serum cryoglobulins was 4.9 (95% CI, 1.1 to 22) times that for nonviremic patients and it remained significantly higher after adjusting for HIV infection (Table 3). Compared with patients

### Table 1. Epidemiological and Clinical Features of the 135 Hemophilic Patients With Chronic Hepatitis C According to the Presence or Absence of Serum Cryoglobulins

<table>
<thead>
<tr>
<th>Features</th>
<th>All Patients n = 135</th>
<th>Cryoglobulins (+) n = 42</th>
<th>Cryoglobulins (−) n = 93</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>31 (10-69)</td>
<td>32 (14-69)</td>
<td>31 (10-66)</td>
<td>NS</td>
</tr>
<tr>
<td>Mild/moderate</td>
<td>31 (10-69)</td>
<td>32 (14-69)</td>
<td>31 (10-66)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ALT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistently normal</td>
<td>14 (10%)</td>
<td>2 (4%)</td>
<td>12 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td>Intermittently high</td>
<td>69 (51%)</td>
<td>20 (48%)</td>
<td>49 (53%)</td>
<td>NS</td>
</tr>
<tr>
<td>Persistently high</td>
<td>52 (39%)</td>
<td>20 (48%)</td>
<td>32 (34%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>3 (2%)</td>
<td>0</td>
<td>3 (2%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: NS, not significant; ALT, alanine aminotransferase.

### Table 2. Virological and Immunological Features of the 135 Hemophilic Patients With Chronic Hepatitis C According to the Presence or Absence of Serum Cryoglobulins

<table>
<thead>
<tr>
<th>Serum Markers</th>
<th>All Patients n = 135</th>
<th>Cryoglobulins (+) n = 42</th>
<th>Cryoglobulins (−) n = 93</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HCV</td>
<td>135 (100%)</td>
<td>42 (100%)</td>
<td>93 (100%)</td>
<td>NS</td>
</tr>
<tr>
<td>HCV-RNA</td>
<td>114 (84%)</td>
<td>40 (95%)</td>
<td>74 (80%)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>HBsAg</td>
<td>9 (7%)</td>
<td>7 (17%)</td>
<td>2 (2%)</td>
<td>NS</td>
</tr>
<tr>
<td>HBV-DNA</td>
<td>14 (10%)</td>
<td>2 (5%)</td>
<td>12 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>67 (50%)</td>
<td>24 (57%)</td>
<td>43 (46%)</td>
<td>NS</td>
</tr>
<tr>
<td>Antitissue antibodies (titer ≥ 1:40)</td>
<td>31/123 (25%)</td>
<td>8/38 (21%)</td>
<td>23/85 (27%)</td>
<td>NS</td>
</tr>
<tr>
<td>Rheumatoid factor &gt; 60 U/mL</td>
<td>13/129 (10%)</td>
<td>8/40 (20%)</td>
<td>5/69 (6%)</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>IgG (mg/dL)</td>
<td>2,066 ± 630</td>
<td>2,354 ± 682</td>
<td>1,928 ± 557</td>
<td>&lt;.0005</td>
</tr>
<tr>
<td>IgM (mg/dL)</td>
<td>269 ± 240</td>
<td>323 ± 226</td>
<td>244 ± 243</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>C4 (mg/dL)</td>
<td>22 ± 8</td>
<td>19 ± 8</td>
<td>24 ± 8</td>
<td>&lt;.05</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

*Cryoglobulins (+) vs Cryoglobulins (−).
†Data are mean ± standard deviation.
with 10 to 17 years exposure to HCV, the risk of developing cryoglobulins was 2.4 (95% CI, 0.6 to 10.8) for patients with 18 to 21 years exposure to HCV, 3.7 (95% CI, 1.0 to 14.4) for those with 22 to 26 years exposure, and 4.4 (95% CI, 1.1 to 18.0) for those with longer than 26 years exposure. A similar trend was observed after adjusting for HIV infection. The odds ratio of developing serum cryoglobulins in anti-HIV–positive patients was 1.6 (95% CI, 0.8 to 3.2) compared with anti-HIV–seronegative patients (Table 3). Serum levels of cryoglobulins and type of cryoglobulin distribution were similar in anti-HIV–positive and –negative patients (data not shown). Anti-HIV–positive patients had higher serum levels of IgG (2,406 ± 608 mg/dL v 1,720 ± 435 mg/dL, P < .001) and IgM (295 ± 208 mg/dL v 243 ± 267 mg/dL, P < .05) than anti-HIV–seronegative patients.

**DISCUSSION**

This is the first study evaluating the prevalence, clinical relevance, and risk factors of serum cryoglobulins in multitransfused hemophiliac patients with chronic HCV infection. Approximately one third of the patients circulated serum cryoglobulins, which were predominantly type III with polyclonal rheumatoid factor. Further confirming the strict association between HCV replication and appearance of serum cryoglobulins, serum HCV-RNA was more often detected in patients with cryoglobulins than in those without (95% v 80%, P < .05). Interestingly, despite the relatively high serum levels of circulating cryoglobulins, none of the patients had clinical signs or symptoms of systemic vasculitic disorders. Three previous studies in nonhemophilic patients with HCV-related chronic hepatitis or cirrhosis found similar rates (35% to 37%) of serum cryoglobulins.2,4 Another study reported a 54% rate in patients with chronic hepatitis C.1 These studies showed a tendency for higher rates to occur in women, older patients, and those with long-lasting HCV-related infection or cirrhosis. Perhaps the fact that all except one of our hemophilic patients were men and had a median age of 31 years, and that only few of them had clinical signs of cirrhosis, would account for the relatively low rate (31%) of serum cryoglobulins that we observed. Studies in nonhemophilic patients suggested an association between serum cryoglobulins and genotype 2a/c or 1b of HCV.9,10 We could not assess whether any HCV genotype was pathogenetically relevant as to cryoglobulinemia, because most of our patients were chronically infected with the coagulation-concentrate-related genotype 1 of HCV.18-20 The low rates of genotype 2a/c could be another factor contributing to the relatively low rates of serum cryoglobulin found in these patients. Finally, it must be pointed out that this study was not prospective and was based on the evaluation of one serum sample only. It is possible that cryoglobulins may have appeared and disappeared over time in hemophilic patients with hepatitis C.

The most important finding of this study was the positive correlation existing between duration of hepatitis C and risk of producing serum cryoglobulins. The existence of such a correlation is difficult to show in nonhemophilic patients, because most of them have community-acquired hepatitis in which the starting point of infection is difficult to assess. Another important finding was the absence of clinical signs of systemic vasculitis in hemophiliacs with cryoglobulins. These data contrast with those obtained in nonhemophilic patients, showing signs and symptoms of vasculitis in 10% to 50% of patients.22,23 Perhaps such factors as the young age of our patients and the male sex, which seem to influence negatively the production of serum cryoglobulins, are also influencing the risk of vasculitis. Preliminary data indicate that patients infected with HCV later in life are at higher risk of cirrhosis and liver cancer than those infected earlier.22-24 Assuming that this holds true also for the risk of developing serum cryoglobin-related sequelae, hemophiliacs could be at low risk of vasculitis because they were predominantly infected by HCV early in life at the time of the first infusion with blood products.

Similar prevalences and amounts of serum cryoglobulins were found in patients with serum anti-HIV and those without, indicating that there is no obvious association between HIV infection and cryoglobulins. However, association studies in HIV-infected hemophilic patients should be interpreted with caution. HIV/HCV-coinfected hemophiliacs could have more serious HCV-related sequelae than HIV-uninfected patients, but this is difficult to show by retrospective studies because of the shortened survival of patients with serum anti-HIV.25,26

In conclusion, a significant number of hemophilic patients with chronic hepatitis C circulated cryoglobulins, but they had no clinical sign of vasculitis. Because the risk of developing serum cryoglobulins seems to increase with the duration of exposure to HCV, a prospective study is necessary to establish whether or not in these patients serum cryoglobulinemia will remain asymptomatic or become a clinically relevant complication of HCV infection.

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High Prevalence of Serum Cryoglobulins in Multitransfused Hemophilic Patients With Chronic Hepatitis C

E. Santagostino, M. Colombo, D. Cultraro, M. Muça-Perja, A. Gringeri and P.M. Mannucci