High Rates of Hepatitis G Virus Infection in Multitransfused Patients With Hemophilia

By Francesca De Filippi, M. Colombo, Maria Grazia Rumi, F. Tradati, D. Prati, A. Zanella, and P.M. Mannucci

The parallel measurement of serum antibodies to the hepatitis G virus (anti-HGV) and of viremia (HGV-RNA) should improve our understanding of HGV transmission by coagulation factor concentrates. The aim of this study was to assess the relationship between HGV, the type of concentrate infused, and liver disease in multitransfused hemophiliacs. To this end, anti-HGV and HGV-RNA were evaluated by an enzyme-linked immunosorbent assay and a nested-polymerase chain reaction assay in patients treated lifelong with nonvirus-inactivated plasma-derived concentrates (n = 128), virus-inactivated concentrates (n = 33), or recombinant factors (n = 7), and in 200 regular blood donors. The prevalence of serum HGV-RNA and anti-HGV was higher in the recipients of nonvirus-inactivated factors than in blood donors (HGV-RNA: 9% vs 1.5%, P = .002; anti-HGV: 32% vs 5%, P < .0001). In the recipients of virus-inactivated concentrates the prevalences of these markers were similar to those in blood donors (HGV-RNA: 3% vs 1.5%; anti-HGV: 15% vs 5%). The prevalence of either marker in the recipients of nonvirus-inactivated concentrates was higher than in the recipients of virus-inactivated factors (39% vs 18%, P = .04). The former group had serum hepatitis C virus (HCV) RNA or anti-HCV more frequently than the latter group (HCV-RNA: 86% vs 15%, P < .0001; anti-HCV: 96% vs 18%, P < .0001). Serum alanine aminotransferase was persistently high in 83 (81%) patients with HCV-RNA alone, in 8 (89%) with HCV/HGV coinfection, and in none of the three patients with HGV-RNA only. Thus, HGV infection in hemophiliacs is more common than previous studies of HGV-RNA prevalence have suggested, but it resolved in most cases and caused chronic viremia only in a small number of patients, without biochemical evidence of persistent liver damage.

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T HE GREAT MAJORIT Y of hemophilic patients treated with nonvirus-inactivated clotting factor concentrates prepared from pooled human plasma have chronic hepatitis as a consequence of infection with the blood-borne hepatitis C virus (HCV).1,2 The recently discovered hepatitis G virus (HGV), a flavivirus with a similar genome organization to HCV, is present in the serum of approximately 2% of volunteer blood donors and has been associated with transfusion-related and community-acquired cases of acute and chronic hepatitis.3,4 In recipients of nonvirus-inactivated clotting factor concentrates, relatively low rates (14%) of HGV infection were found with the polymerase chain reaction (PCR) for HGV-RNA despite frequent contamination of concentrate batches with HGV-RNA.5 These findings, while indicating that HGV is transmitted by pooled plasma products, suggest that HGV viremia may be time-limited, perhaps as a result of the activation of immunity against HGV.

Recently, an enzyme-linked immunosorbent assay (ELISA) detected IgG antibodies against the recombinant HGV envelope protein E2 (anti-HGV) in 9% of healthy donors and up to 41% of patients with parenteral risks, including those recovering from posttransfusion acute hepatitis.6

MATERIALS AND METHODS

Patients. Between September 1995 and June 1996, fresh serum samples were obtained from 168 consecutive hemophiliacs (mean age 29 years; range 2 to 71 years) attending the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, University of Milan; and the Blood Transfusion and Transplant Immunology Center, Istituto Ricovero Cura Carateure Scientifico Maggiore Hospital, Milan, Italy. Seven patients were treated exclusively with recombinant factor concentrates (HGV-RNA: 3% vs 1.5%; anti-HGV: 15% vs 5%). The prevalence of either marker in the recipients of nonvirus-inactivated concentrates was higher than in the recipients of virus-inactivated factors (39% vs 18%, P = .04). The former group had serum hepatitis C virus (HCV) RNA or anti-HCV more frequently than the latter group (HCV-RNA: 86% vs 15%, P < .0001; anti-HCV: 96% vs 18%, P < .0001). Serum alanine aminotransferase was persistently high in 83 (81%) patients with HCV-RNA alone, in 8 (89%) with HCV/HGV coinfection, and in none of the three patients with HGV-RNA only. Thus, HGV infection in hemophiliacs is more common than previous studies of HGV-RNA prevalence have suggested, but it resolved in most cases and caused chronic viremia only in a small number of patients, without biochemical evidence of persistent liver damage.

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sequences of the sense and antisense primers used for the first round of the nested PCR were, respectively: 5'-dGGCCAAAAGGTGGT-3' and 5'-dGTGGGCGTCGTTTGCCCAGG-3'. PCR was done with Taq DNA Polymerase for 35 cycles, consisting of denaturation at 96°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The second round of PCR was performed for 25 cycles (consisting of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute) with nested primers: sense (5'-dITTGGTAGCCACTATAGGGG-3') and antisense (5'-dGTTAGGACCAACACTCGTGG-3'). PCR products of the expected size (140 bp) were resolved on 3% agarose gel. To avoid PCR product carry-over, all recommended precautions were observed and appropriate controls were used.

Anti-HGV ELISA. An ELISA was used for the qualitative determination of IgG antibodies to the HGV E2-antigen. The E2-transmembrane protein was bound onto streptavidin-coated microtiter plates, which were incubated with the diluted specimen, and the antibodies directed against E2-protein were detected using an anti-human IgG-peroxidase conjugate and 2,2' azino di-[3 ethylbenzothiazoline sulfonate (6)] (ABST) as peroxidase substrate. Extinction was measured at 405 nm (Anti-HGenv, Boehringer Mannheim, Mannheim, Germany).

Statistical analysis. The prevalence of serum markers of HGV, HCV, and HIV and their relation to other variables were analyzed by the chi-squared test or Fisher's exact test. AID Blood 0022 / 5h43$$$421 10-30-97 01:29:42 blda WBS: Blood

**RESULTS**

**HGV.** Table 2 shows the results of HGV markers in hemophilic patients and blood donors. The prevalence of serum HGV-RNA and anti-HGV was higher in the recipients of unmodified concentrates than in blood donors (HGV-RNA: 9% v 1.5%, P = .002; anti-HGV: 32% v 5%, P < .0001). On the other hand, the corresponding prevalence in the recipients of virus-inactivated concentrates was similar to those in donors (HGV-RNA: 3% v 1.5%; anti-HGV: 15% v 5%). None of the 7 patients treated exclusively with recombinant factor had HGV-RNA or anti-HGV. Only two patients, who had received nonvirus-inactivated concentrates, circulated both HGV-RNA and anti-HGV (Table 2). The prevalence of either marker in the 128 recipients of nonvirus-inactivated concentrates was higher than in the 33 recipients of virus-inactivated factors (39% v 18%, P = .04).

**Other viruses.** HCV-RNA and anti-HCV were more frequent in the recipients of nonvirus-inactivated concentrates than in those given virus-inactivated concentrates (HCV-RNA: 86% v 15%, P < .0001; anti-HCV: 98% v 18%, P < .0001; Table 2). Both markers were absent in patients treated exclusively with recombinant factors. Anti-HIV was detected in 30 recipients (23%) of noninactivated concentrates but in none of those given virus-inactivated or recombinant factors (Table 2). In the recipients of noninactivated concentrates, exposure to HGV was less frequent than exposure to HCV (32% anti-HGV v 98% anti-HCV, P < .0001) and as frequent as exposure to HIV (32% anti-HGV v 23% anti-HIV, P = .08). Nine (7%) recipients of unmodified concentrates concurrently circulated HGV-RNA and HCV-RNA (Table 3).

**Liver disease.** Serum ALT was persistently high in 83 patients with HCV-RNA alone, in eight with HCV/HGV coinfection, but in none with HGV-RNA only (Table 3).

**DISCUSSION**

The first goal of this study was to evaluate the prevalence of HGV infection in hemophiliacs treated with large-pool coagulation factor concentrates by using a new serological method that measures antibodies to this virus. Previous findings of a relatively low prevalence of HGV viremia (6% to 14%) in recipients of nonvirus-inactivated clotting factor concentrates, despite frequent contamination of HGV-RNA sequences in batches, 6,11 led us to surmise that HGV is not efficiently transmitted with concentrates or that, unlike HCV, it does not establish long-term viremia in the majority of infected patients. Our findings of low rates (9%) of viremia but a higher prevalence (32%) of anti-HGV in the recipients of nonvirus-inactivated concentrates favor the latter interpretation. Because anti-HGV may mark recovery from infection, 7 perhaps these findings indicate that HGV was transmitted with concentrates but that most patients recovered from infection. Because the rates of anti-HGV seroconversion in high-risk patients increase with time, 7 perhaps currently viremic hemophiliacs are those who have been more recently infected with HGV. It must be recognized, however, that the biological and clinical significance of anti-HGV is not fully understood. The anti-HGV assay used in this study seems to be specific for the envelope protein E2 of HGV, because it does not detect cross-reacting anti-HCV E2 antibodies. However, the probability of cross-reaction with other related viruses cannot be ruled out. Except in two patients, serum
Table 2. Prevalence of Serum Markers of HGV, HCV, and HIV in Blood Donors and Hemophiliacs Treated With Different Types of Concentrates

<table>
<thead>
<tr>
<th>Recipients of Concentrates</th>
<th>Blood Donors* (N = 200)</th>
<th>Unmodified² (n = 128)</th>
<th>Inactivated³ (n = 33)</th>
<th>Recombinant (n = 7)</th>
<th>All (n = 168)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNA</td>
<td>3 (1.5%)</td>
<td>11 (9%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>12 (7%)</td>
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<tr>
<td>Antibody</td>
<td>10 (5%)</td>
<td>41 (32%)</td>
<td>5 (15%)</td>
<td>0</td>
<td>46 (27%)</td>
</tr>
<tr>
<td>Either</td>
<td>13 (6.5%)</td>
<td>50 (39%)</td>
<td>6 (18%)</td>
<td>0</td>
<td>56 (33%)</td>
</tr>
<tr>
<td>HCV</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>RNA</td>
<td>0</td>
<td>107 (86%)</td>
<td>5 (15%)</td>
<td>0</td>
<td>112 (67%)</td>
</tr>
<tr>
<td>Antibody</td>
<td>0</td>
<td>125 (98%)</td>
<td>6 (18%)</td>
<td>0</td>
<td>131 (78%)</td>
</tr>
<tr>
<td>Either</td>
<td>0</td>
<td>125 (98%)</td>
<td>6 (18%)</td>
<td>0</td>
<td>131 (78%)</td>
</tr>
<tr>
<td>HBsAg</td>
<td>0</td>
<td>7 (5%)</td>
<td>0</td>
<td>0</td>
<td>7 (4%)</td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>0</td>
<td>30 (23%)</td>
<td>0</td>
<td>0</td>
<td>30 (18%)</td>
</tr>
</tbody>
</table>

* versus ²: HGV-RNA P < .002, HCV RNA P < .0001, anti-HGV P < .0001, anti-HCV P < .0001, anti-HIV P < .0001.

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