HEMOPHILIC PATIENTS ARE at high risk of acquiring viral hepatitis after repeated treatment with plasma-derived clotting factor products.1-3 With the implementation of procedures for inactivating viruses in plasma-derived products, a dramatic decrease in the transmission of hepatitis B and C viruses has been observed in this patient population.4,5 Virus inactivation procedures also have been successful in reducing the risk of transmitting the blood-borne hepatitis G virus (HGV).6,7 However, other blood-borne viruses, known and as yet unidentified, might escape the current virucidal procedures and establish infections in the recipients. For instance, an outbreak of hepatitis A virus (HAV) infection in plasma products and establishment infections in the recipients. For instance, an outbreak of hepatitis A virus (HAV) infection in plasma products and establish infections in the recipients. A total of 178 consecutive Italian hemophilic patients (mean age, 29 years) were included in this study. The only criterion used for selection was that all patients had been prospectively followed with annual liver function tests since their first treatment at the Center (mean follow-up of 16 years; range, 2 to 27 years). The epidemiological and clinical characteristics of these patients are summarized in Table 1. The 38 patients with mild hemophilia had been infused with concentrates because they did not respond to 1-deamino-8-D-arginine vasopressin (DDAVP). One hundred twenty-seven (71%) patients had been treated with unmodified concentrates until 1985 and with virus-inactivated concentrates thereafter; 33 (19%) patients were given only virus-inactivated concentrates, and 18 (10%) were given recombinant FVIII preparations exclusively. The virus inactivation method implemented by manufacturers from 1984 to 1987 was dry-heating at 60° to 68°C; this procedure was effective against the transmission of human immunodeficiency virus (HIV), but not of hepatitis C virus (HCV).8,9 Since 1987, more effective virucidal procedures, such as dry-heating at >80°C, vapor heating, solvent/detergent, and pasteurization were adopted. Although most patients received more than 1 virally-inactivated product, the solvent/detergent method was the most widely used, with at least two thirds of the patients having been treated with concentrates inactivated with this method. One hundred volunteer blood donors (73 men, 27 women; mean age, 36 years) from the hospital blood bank, all with persistently normal ALT and no serum hepatitis B surface antigen (HBsAg), anti-HCV, or anti-HIV were included as controls.

Virological and biochemical measurements in patient sera. Serum ALT activity was measured by an automated standardized colorimetric assay (normal value, ≤ 40 U/L). HBsAg and antibodies to HIV (anti-HIV) in sera were tested by immunoenzymatic assays (Abbott Laboratories, North Chicago, IL). Serum HGV-RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) and sequence-specific hybridization.

Study subjects. A total of 178 consecutive Italian hemophilic patients (mean age, 29 years; range, 2 to 73 years) treated at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milano, Italy, were included in this study. The only criterion used for selection was that all patients had been prospectively followed with annual liver function tests since their first treatment at the Center (mean follow-up of 16 years; range, 2 to 27 years). The epidemiological and clinical characteristics of these patients are summarized in Table 1. The 38 patients with mild hemophilia had been infused with concentrates because they did not respond to 1-deamino-8-D-arginine vasopressin (DDAVP). One hundred twenty-seven (71%) patients had been treated with unmodified concentrates until 1985 and with virus-inactivated concentrates thereafter; 33 (19%) patients were given only virus-inactivated concentrates, and 18 (10%) were given recombinant FVIII preparations exclusively. The virus inactivation method implemented by manufacturers from 1984 to 1987 was dry-heating at 60° to 68°C; this procedure was effective against the transmission of human immunodeficiency virus (HIV), but not of hepatitis C virus (HCV).8,9 Since 1987, more effective virucidal procedures, such as dry-heating at >80°C, vapor heating, solvent/detergent, and pasteurization were adopted. Although most patients received more than 1 virally-inactivated product, the solvent/detergent method was the most widely used, with at least two thirds of the patients having been treated with concentrates inactivated with this method. One hundred volunteer blood donors (73 men, 27 women; mean age, 36 years) from the hospital blood bank, all with persistently normal ALT and no serum hepatitis B surface antigen (HBsAg), anti-HCV, or anti-HIV were included as controls.

Virological and biochemical measurements in patient sera. Serum ALT activity was measured by an automated standardized colorimetric assay (normal value, ≤ 40 U/L). HBsAg and antibodies to HIV (anti-HIV) in sera were tested by immunoenzymatic assays (Abbott Laboratories, North Chicago, IL). Serum HGV-RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) and sequence-specific hybridization.

MATERIALS AND METHODS

Study subjects. A total of 178 consecutive Italian hemophilic patients (mean age, 29 years; range, 2 to 73 years) treated at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milano, Italy, were included in this study. The only criterion used for selection was that all patients had been prospectively followed with annual liver function tests since their first treatment at the Center (mean follow-up of 16 years; range, 2 to 27 years). The epidemiological and clinical characteristics of these patients are summarized in Table 1. The 38 patients with mild hemophilia had been infused with concentrates because they did not respond to 1-deamino-8-D-arginine vasopressin (DDAVP). One hundred twenty-seven (71%) patients had been treated with unmodified concentrates until 1985 and with virus-inactivated concentrates thereafter; 33 (19%) patients were given only virus-inactivated concentrates, and 18 (10%) were given recombinant FVIII preparations exclusively. The virus inactivation method implemented by manufacturers from 1984 to 1987 was dry-heating at 60° to 68°C; this procedure was effective against the transmission of human immunodeficiency virus (HIV), but not of hepatitis C virus (HCV).8,9 Since 1987, more effective virucidal procedures, such as dry-heating at >80°C, vapor heating, solvent/detergent, and pasteurization were adopted. Although most patients received more than 1 virally-inactivated product, the solvent/detergent method was the most widely used, with at least two thirds of the patients having been treated with concentrates inactivated with this method. One hundred volunteer blood donors (73 men, 27 women; mean age, 36 years) from the hospital blood bank, all with persistently normal ALT and no serum hepatitis B surface antigen (HBsAg), anti-HCV, or anti-HIV were included as controls.

Virological and biochemical measurements in patient sera. Serum ALT activity was measured by an automated standardized colorimetric assay (normal value, ≤ 40 U/L). HBsAg and antibodies to HIV (anti-HIV) in sera were tested by immunoenzymatic assays (Abbott Laboratories, North Chicago, IL). Serum HGV-RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) and sequence-specific hybridization.

From Sentinel Biosciences, Inc. Palo Alto, CA; the Department of Internal Medicine, Blood Transfusion and Transplant Immunology Center, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital, University of Milan, Milan, Italy.

Submitted April 15, 1999; accepted August 9, 1999.

Supported by a grant from Istituto Superiore di Sanità (96/B/T24).

Address reprint requests to Massimo Colombo, MD, Professor and Chairman, Department of Internal Medicine, Via Pace 9, 20122 Milan, Italy; e-mail: mcolombo@imiucca.csi.unimi.it.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1999 by The American Society of Hematology.

0006-4971/99/9412-0011$3.00/0
The prevalence of TTV-DNA was higher in the 127 recipients of unmodified concentrates (78%) and in the 33 recipients of virus-inactivated products (67%) than in the 18 recipients of recombinant concentrates only (11%) (Table 2). To assess whether a correlation exist between treatment intensity and risk of acquiring TTV, TTV-DNA seroprevalences were related to the severity of hemophilia. TTV-DNA was present in 102 of 140 patients (73%) with severe or moderate hemophilia (FVIII 5% or less) compared with 21 of 38 (55%) with mild hemophilia ($P = .053$).

HBsAg was detected in 6 (5%) recipients of unmodified concentrates only. The prevalence of HCV-RNA and anti-HIV was significantly greater in patients treated with unmodified concentrates (85% and 24%, respectively) than in the recipients of virus-inactivated (15% and 0) or recombinant concentrates (0 and 0). In contrast, HGV-RNA was rarely detected in the recipients of both unmodified concentrates and virus-inactivated or recombinant factors (8% < 3% and 0, respectively) (Table 2). TTV-DNA alone was detected in 27 patients (15%), HCV-RNA alone in 21 (12%), TTV-DNA + HCV-RNA in 36 (31%), more than 1 virus in 42 (24%), and no virus in 32 (18%) (Table 3).

Overall, 98 patients (55%) had either intermittently or persistently elevated serum ALT values. Only 2 of 27 (7%) patients exclusively infected by TTV had abnormal ALT compared with 43 of the 56 (77%) who were coinfected by TTV and HCV, and the 16 of 21 (76%) who circulated HCV-RNA alone (Table 3). The risk of acquiring TTV alone was 1.24 (95% CI, 0.27 to 5.79) for the recipients of unmodified concentrates and 0.09 (95% CI, 0.01 to 0.52) for the recipients of recombinant factors compared with patients with virus-inactivated concentrates. The corresponding figures for the risk of acquiring any other virus were 41.4 (95% CI, 9.3 to 204.5) and 0 (95% CI, 0 to 0.82), respectively (Table 4).

To assess whether exposure to TTV was affected by patients’ age, TTV-DNA prevalences were calculated in donors and patients after stratification by decades (Table 5). In donors, TTV-DNA rates were 19% in the 31 subjects aged 20 to 30 years, 16% in the 31 subjects aged 31 to 40 years, 28% in the 21 subjects aged 41 to 50 years, and 28% in the 17 subjects older than 50 years. In hemophiliacs, there was a clear cut increase of TTV-DNA prevalence in the group older than 10 years (76% vs 32%, $P < .001$). However, when the 28 patients younger than 10 years were stratified by treatment modalities, serum TTV-DNA was detected only in 2 of 16 (12%) who were exclusively treated by recombinant factors compared with 7 of 12 (58%) of those who had received recombinant and virus-inactivated factors ($P = .01$).
The TT virus was initially identified as a transfusion-transmissible agent present in a large number of patients with acute and chronic hepatitis of non-A to G etiology. This virus was also reported in severe hemophiliacs in Scotland, Japan, and France who were recipients of TTV-infected batches of clotting factor concentrates manufactured from large plasma pools. Sixty-nine percent of the hemophiliac patients evaluated in this study circulated TTV-DNA compared with 22% healthy blood donors. Unlike HBV, HCV, and HIV infections that prevailed in the recipients of unmodified concentrates, TTV was equally common in the recipients of unmodified concentrates and in those treated with virus-inactivated factors only (78% and 67%, respectively). The prevalence of TTV viremia in our Italian hemophiliacs, even in the recipients of virus-inactivated clotting concentrates, was higher than that reported for patients from other countries. A study in France reported a prevalence of TTV viremia in healthy blood donors. Unlike HBV, HCV, and HIV infections that prevailed in the recipients of unmodified concentrates, TTV was equally common in the recipients of unmodified concentrates and in those treated with virus-inactivated factors only (78% and 67%, respectively). The prevalence of TTV viremia in our Italian hemophiliacs, even in the recipients of virus-inactivated clotting concentrates, was higher than that reported for patients from other countries. A study in France reported a prevalence of TTV-DNA among recipients of virus-inactivated concentrates, whereas the studies in UK and Japan demonstrated lower rates of serum TTV-DNA among recipients of virus-inactivated products than in recipients of unmodified concentrates (5% and 43% v 27% and 78%, respectively). TTV is a nonlipid-enveloped virus and, therefore, it might be present in pooled coagulation factor-concentrates, virally-inactivated with solvent/detergent method. In vitro studies have shown that solvent/detergent is less effective than heating (pasteurization at 60°C for 10 hours) in inactivating TTV. As many as two thirds of our patients have been treated with solvent/detergent-inactivated products. Y et, the higher rate of TTV viremia observed in our patients is unlikely to be due exclusively to differences in efficiency of virus-inactivation procedures, because comparable prevalence of TTV was observed in patients who received clotting factors treated with other procedures. Both blood product-related and nonparenteral, community-acquired infections could contribute to the higher frequency of TTV in the Italian hemophiliacs, consistent with a higher prevalence of TTV in Italian blood donors compared with those in other western countries. A potential fecal transmission route of TTV has been suggested. In this study, TTV-DNA was detected in 22% healthy blood donors, despite the fact that this population was selected on the basis of being at low risk of blood-borne infections. High prevalence (>90%) of TTV-DNA also has been observed in multitransfused Italian thalassemic patients, however, the precise reasons for a higher carrier rate in Italy is presently unclear.

In our patients, the prevalence of HCV-RNA and anti-HIV was significantly greater in patients treated with unmodified concentrates (85%) than in recipients of virus-inactivated (15%) or recombinant concentrates (0%). Anti-HIV and HBsAg were only detected in recipients of unmodified concentrates, 24% and 5%, respectively. Hepatitis G viral RNA, however, was rarely detected (0% to 8%) in hemophiliacs, regardless of the source of clotting factors. Interestingly, the risk of acquiring TTV alone was greater for the recipients of unmodified concentrates than for the recipients of recombinant factors when compared with patients who received virus-inactivated concentrates, 24% and 5%, respectively. The corresponding figures for the risk of acquiring any HCV, HBV, HGV, or HIV were 41.4 (95% CI, 9.3 to 204.5) and 0 (95% CI, 0 to 0.82), respectively.

In previous studies, the potential of TTV causing hepatitis was implicated by the association of TT viremia with ALT elevation. In this study, biochemical signs of liver disease were present in half the patients, but only in 7% of those exclusively infected by TTV. The possibility of underestimating TTV-related liver damage due to the imperfect diagnostic accuracy of serum ALT patterns, in patients with transfusion-related hepatitis, cannot be ruled out, as we learned from HCV-infected patients. Taken together, however, results in this study do not suggest a causal effect of TTV on chronic liver disease of unknown etiology in this small population. There is also no evidence of the virus affecting the degree of liver damage when present as coinfection with HBV or HCV. Nevertheless, association of TTV with transient elevation of ALT and its potential for
causing hepatitis has been reported in transfusion recipients and immunocompromised organ-transplant patients.\textsuperscript{17} A better understanding of the pathogenicity of TTV and the use of recombinant clotting factor should reduce any further hepatic complications in hemophilic patients.

**ACKNOWLEDGMENT**

The authors thank Dr R. Bohenzky and the sequencing-informatics team at Sentinel Biosciences, Inc for their expert technical support; Professor J.P. Allain and Dr H. Lee for their helpful discussion and encouragement, and Dr A. Russo for statistical analysis.

**REFERENCES**


**Table 5. Prevalence of TTV in Hemophiliacs and Blood Donors**

<table>
<thead>
<tr>
<th>Age</th>
<th>Positive for TTV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donors</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>20-30</td>
</tr>
<tr>
<td>31</td>
<td>31-40</td>
</tr>
<tr>
<td>21</td>
<td>41-50</td>
</tr>
<tr>
<td>17</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Hemophiliacs</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>150</td>
<td>&gt; 10</td>
</tr>
</tbody>
</table>
TT Virus Is Present in a High Frequency of Italian Hemophilic Patients Transfused With Plasma-Derived Clotting Factor Concentrates

Benjamin P. Chen, Maria Grazia Rumi, Massimo Colombo, Yu-Huei Lin, Latha Ramaswamy, Jac Luna, Jen-Kuei Liu, Daniele Prati and Pier Mannuccio Mannucci