Search for Intrafamilial Transmission of Hepatitis C Virus in Hemophilia Patients

By Stephan A. Brackmann, Andreas Gerritzen, Johannes Oldenburg, Hans-Hermann Brackmann, and Karl E. Schneweis

This study was performed to determine the risk of family members of anti-hepatitis C virus (HCV)–positive hemophilia patients (index patients) for infection with HCV compared with the risk of acquiring hepatitis B virus (HBV), human immunodeficiency virus (HIV), and hepatitis A virus (HAV) infection. All index patients (n = 141) were found to be positive by first and second generation anti-HCV enzyme immunoassays (EIAs). Among their household contacts (n = 228), 224 were negative and 1 positive by both assays.

Three contacts gave positive results in first generation anti-HCV EIA and negative results in second generation assay. This latter result was confirmed by further tests (neutralization test, synthetic peptides, and supplemental assay).

Percent positivity for anti-HBc was about the same in nonsexual household contacts and sexual partners (13 of 109 [12%] and 7 of 54 [13%], respectively). Percent prevalence of anti-HBc was higher in contacts of index patients with chronic hepatitis B than in those of index patients who had recovered from that disease (6 of 20 [30%] and 14 of 133 [10%], respectively; P < .05). The HBV infection rate of contacts participating in controlled self-treatment was not higher than that of controls (3 of 57 [5%] and 10 of 98 [10%, respectively). Of 44 sexual partners, 5 (11%) were found to be positive for anti-HIV. Prevalence of anti-HAV matched with the age-related distribution in the German population. These findings suggest that intrafamilial transmission of HCV to family members of hemophilia patients is uncommon. In contacts of hemophilia patients, the risk of acquiring HCV infection seems to be as high in household contacts as in sexual contacts. Participation in controlled self-treatment does not appear to be an additional risk for HCV and HBV infection. There is no doubt that sexual transmission of HCV is less common than that of HBV and HIV.

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PATIENTS AND METHODS

The index patients were 141 hemophiliacs observed at the Haemophilia Centre in Bonn. They had been positive for antibody to HCV on several occasions and were suffering from hemophilia A or B and from von Willebrand's disease. Over a period of 9 months (between October 1990 and June 1991), serum samples were obtained from their household contacts and stored at −40°C until serologic examination. A total of 294 family members took part in this study. In 66 cases, other sources of infection, such as blood transfusions and intravenous drug abuse, could not be ruled out. These subjects were excluded from the study. Among the remaining 228 individuals, there were 73 sexual partners (63 wives and 10 girlfriends) and 155 household contacts (60 fathets, 54 mothers, 7 nonhemophilic brothers, 17 sisters, and 17 offspring). Seventy-three contacts had taken part in the controlled self-treatment. At the time of blood specimen collection, the youngest contact was 1 year old and the oldest was aged 82. The mean age of the contacts was about 40 years.

The HAV, HBV, HCV, and HIV findings obtained from the index patients and available at this center were evaluated. The following commercial tests were used: anti-HAV IgG, anti-HAV IgM, HBsAg, anti-HBs, anti-HBe, anti-HBe from Abbott (Wiesbaden, Germany); first generation anti-HCV EIA and anti-HIV Western Blotting from Ortho (Neckargemünd, Germany); and anti-HIV EIA from Wellcome (Burgwedel, Germany). In addition, the serum samples of the index patients were stored at −40°C and subjected to a second generation anti-HCV test (Abbott) and the positive results in both anti-HCV EIAs were confirmed in a subset of the

In Germany, there are about 2,500 hemophilia patients needing treatment. These patients have bleeding disorders due to inherited deficiencies of coagulation factors and therefore require replacement with clotting factor concentrates for the prevention and control of bleeding events. Before the introduction of virus-inactivated concentrates, such treatments were associated with a high risk for transmission of pathogens. Thus, a large number of hemophilia patients were infected with hepatitis B virus (HBV), the pathogen causing non-A, non-B hepatitis (hepatitis NANN), and the human immunodeficiency virus (HIV). While HBV and HIV were discovered in 1968 to 1970 and 1983, respectively, the pathogen responsible for the NANN hepatitis was unknown for a longer time. In 1989, the genome of a virus termed hepatitis C virus (HCV) was identified. HCV is related to the flaviviruses. After deriving proteins from fragments of its viral genome and using them as antigens in enzyme immunoassays (EIAs), HCV antibodies could be detected in the serum, providing evidence for an existing or previous infection with this virus. It became apparent that most hemophilia patients treated with nonvirus-inactivated products had contracted infection with HCV.

This study was undertaken to determine the prevalence of antibody to HCV, as compared with that of hepatitis A virus (HAV), HBV, and HIV antibody in the contacts of HCV antibody-positive hemophiliacs (index patients) and to analyze the distribution patterns.
index patients (n = 23) by second generation anti-HCV RIBA (Ortho, Raritan, NJ).

The serum samples of their contacts were tested for HAV IgG and HAV IgM (Abbott); for HBs-Ag, anti-HBs, and anti-HBe; and, where necessary, for HBe-Ag and anti-HBc (Abbott). EIA of the first (Ortho) and the second generation (Abbott) were used in the examinations for antibody to HCV. The first generation anti-HCV EIA is performed with a recombinant antigen from the NS3 and NS4 regions of the HCV genome (c22) are used. They and the second generation (Abbott) were used in the examinations for antibody to HCV. The first generation anti-HCV EIA is performed with a recombinant antigen from the NS3 and NS4 regions of the HCV genome (C100) expressed by fusion to superoxide dismutase (SOD) in Saccharomyces cerevisiae (SOD-ABCD). In the second generation anti-HCV EIA, a recombinant antigen from the NS3 region (33c) fused to parts of the C100 (“A” is missing) and an antigen from the core region of the HCV genome (c22) are used. They are expressed by fusion to the CMP-ketodoxozytoxenate synthetase (CKS) in Escherichia coli (CKS-33eBCD and CKS-c22). Furthermore, small amounts of the complete C100 (SOD-ABCD) are contained in that kit.

Contacts found positive for anti-HCV on several occasions or exhibiting limit values underwent an HCV neutralization test (Abbott). These samples were sent to the Abbott Reference Laboratory for further assays for antibody to HCV. In this neutralization test, the serum samples are incubated for 30 minutes with a neutralizing reagent and a diluent. The antigen in the reagent is a protein from a fragment of the HCV protein C100 and CKS (256 amino acids) expressed in E. coli. The diluent is free from antigen. Specific antibodies to the C100 antigen in the patient sera are inhibited by the neutralizing reagent. The reactivity of specific antibodies to C100 antigen in the first generation HCV EIA is reduced more than 50% by the neutralizing reagent as compared with the reactivity of the sample incubated with the diluent alone. In contrary, antibodies to SOD or yeast antigens giving false-positive results in the first generation anti-HCV EIA were not inhibited by the neutralizing reagent.

Additional assays developed by Abbott were based on a recombinant antigen from the NS3 region of the HCV genome (CKS-33c) and on synthetic peptides containing immunogenic epitopes from C100 and the core antigen.

Furthermore, a supplemental assay was performed. In this assay, antibody bound to HCV structural protein of the second generation anti-HCV EIA and antibody bound to HCV nonstructural protein can be determined separately.

Statistical analysis was performed by the $\chi^2$ independent test.

RESULTS

Prevalence of antibody to HCV in index patients. All 141 index patients gave positive reactions in the first and second generation anti-HCV EIAs. It may be assumed that these selected patients had been infected with HCV before 1984. Since spring of that year, virus-inactivated clotting factor concentrates have been used at this center for the treatment of hemophilia. Taking into account that none of the index patients has lost antibodies to HCV in 8 or more years, we assume that most of them are chronically infected.

Prevalence of antibody to HCV in household contacts. A total of 228 household contacts of the index patients were subjected to the first and second generation anti-HCV EIAs. Two hundred twenty-four contacts were negative and 1 female was positive in both assays. Three gave positive reactions in the first generation assay on various occasions and negative reactions in the second generation assay (Table 1).

The woman found positive in both assays (3 to 5 times the limit value) was also definitely positive in the anti-HCV EIA neutralization test. These results were confirmed and specified by the Abbott Reference Laboratory. In this case, the antibodies were obviously directed against the nonstructural proteins of the HCV, because the serum gave positive reactions with synthetic peptides, which are equivalent to the nonstructural antigens of the first generation anti-HCV EIA (C100) and with the CKS-33c antigen. In the supplemental assay, the serum reacted with the antigen from the nonstructural region of the HCV genome.

Among the three contacts with different reactions in the first and second generation assays, two (father and wife) were found positive in the first generation assay (4 and 2 times the limit value, respectively) and definitely negative in the second generation assay, whereas one (girlfriend) showed a positive result near the limit value in the first generation assay and a negative result in the second generation assay. The father and the wife were found to be definitely negative in the HCV neutralization test, whereas a result near the limit value was once more obtained from the girlfriend. The three of them were clearly negative in the test using synthetic peptides and CKS-33c and in the supplemental assay.

On the basis of these results, one household contact was considered positive by the tests for HCV antibody and 227 were assessed as negative.

Prevalence of antibody to hepatitis B core antigen. The prevalence of antibody to hepatitis B core antigen was determined in the contacts of those index patients who were positive for anti-HBc. These tests were designed to serve as a guideline for the assessment of transmission of HBV from index patients infected with HBV (n = 98) to their contacts (54 sexual partners and 109 nonsexual household contacts). Twenty-one contacts were found to be positive for antibody to HBc. One of them, the father of an index patient, was apparently infected by his wife suffering from chronic aggressive hepatitis B. In all other cases, no sources of infection other than contact with an index patient positive for anti-HBc could be detected. Negative results were obtained in 142 cases. Nine of them had been vaccinated against HBV infection and were positive for antibody to HBs.

An attempt was made to determine the distribution pattern of the prevalence of anti-HBc among sexual and other household contacts. Subjects vaccinated against HBV infection and those with other sources of infection were excluded from this study. As shown in Table 2, 7 sexual contacts were positive for anti-HBc. Among household contacts, 13 were positive. Thus, almost as many household contacts as sexual contacts of the index patients were found to be positive for antibody to HBc (12% and 13%, respectively). Furthermore, we tried to find out whether the contacts of index patients suffering from chronic HBV infection (group 1, household contacts

<table>
<thead>
<tr>
<th>Index patients (n = 141)</th>
<th>First Generation</th>
<th>Second Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>141</td>
<td>None</td>
</tr>
<tr>
<td>Negative</td>
<td>224</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. First and Second Generation EIAs for Antibody to HCV in Index Patients and Their Household Contacts
Table 2. Anti-HBc Prevalence Among Sexual and Nonsexual Contacts of Anti-HBc–Positive Index Cases

<table>
<thead>
<tr>
<th>Category of Contact</th>
<th>Anti-HBc Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex partners of index case</td>
<td>N</td>
</tr>
<tr>
<td>Nonsexual household contact</td>
<td>109</td>
</tr>
<tr>
<td>Contacts of HBS Ag+ index case</td>
<td>20</td>
</tr>
<tr>
<td>Contacts of HBS Ag+/anti-HBc+ case</td>
<td>133</td>
</tr>
<tr>
<td>Contacts in home treatment</td>
<td>57</td>
</tr>
<tr>
<td>Contacts not in home treatment</td>
<td>98</td>
</tr>
</tbody>
</table>

* Not significant.
† Two sexual and 4 nonsexual contacts.
‡ Five sexual and 9 nonsexual contacts.

Table 3. Prevalence of Antibody to HIV, HBc, and HCV in Sexual Partners of Index Patients

<table>
<thead>
<tr>
<th>Anti-HIV EIA and WB (n = 44)</th>
<th>Anti-HBc EIA (n = 54)</th>
<th>Anti-HCV First Generation EIA (n = 73)</th>
<th>Anti-HCV Second Generation EIA (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5 (11%)</td>
<td>7 (13%)*</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Negative</td>
<td>39 (89%)</td>
<td>44 (81%)*</td>
<td>70 (96%)</td>
</tr>
</tbody>
</table>

Abbreviation: WB, Western blotting.
* The total number of positive and negative cases is not equal to 100% because 3 immunized sexual partners were excluded.

of index patients who had been positive for HBs antigen for more than 1 year) showed a higher incidence of positivity for antibody to HBc than those of index patients who had been affected with acute hepatitis B sometime in the past (group 2, household contacts of index patients negative for HBs antigen and positive for antibody to HBc). The percentage of positivity for HBc antibody was actually higher in group 1 than in group 2 (30% and 10%, respectively). This difference is significant (Table 2).

Prevalence of antibody to HBc in contacts involved or not involved in the controlled self-treatment of the index patients. Of the 228 contacts, 73 (30%) have provided home care to an index patient (controlled self-treatment). Nonsexual household contacts were divided in two groups and tested for antibody to HBc. Group A consisted of 57 subjects taking part in the controlled self-treatment of an index patient. Group B contained 98 contacts not participating in such treatment.

In group A, 3 individuals (5%) gave positive reactions to HBc antibody. In group B, positive results were obtained in 10 cases (10%). Although the contacts in group A had been exposed to a greater risk for infection, the infection rate was not higher in group A than in group B (Table 2).

Relationship between the prevalence of antibody to HCV and to HBV and HIV in sexual contacts of index patients. Of the 141 index patients, 79 (56%) were positive for antibody to HIV, as judged by the EIA and by Western blotting. Forty-four sexual contacts of the HIV-positive index patients were evaluated. Five (11%) were found to be positive for antibody to HIV by the EIA and by Western blotting, and 39 were negative. It is of interest to note that none of the positive sexual contacts had evidence of HCV or HIV infection, although all 5 of the index patients concerned gave positive reactions in the first and second generation assays for HCV antibody and in the serologic tests for HBV. Three index patients were found positive for antibody to HBs and HBc and two had been positive for more than 6 years for HBs antigen and HBe antibody.

The prevalence of antibody to HIV, HBV, and HCV in the sexual contacts of the index patients is summarized in Table 3. The percentages of sexual partners giving positive reactions in the tests for HIV and HBe antibodies were almost the same (5 of 44 [11%] and 7 of 54 [13%]), whereas that of sexual contacts with positive reactions to HCV antibody was much lower (1 of 73).

Prevalence of antibody to HAV in contacts. As seen in Table 4, the prevalence of HAV antibodies increases with age. Such antibodies were detected in 109 of 228 contacts (48%). Positive results were obtained from 1 contact in the first year of life, from 14 of 65 contacts (21%) between 1 and 30 years of age, from 78 of 142 contacts (55%) between 30 and 60 years, and from 16 of 20 contacts (80%) over 60.

DISCUSSION

Comparison of the results from the anti-HCV EIA of the first and the second generation. Of 228 contacts of 141 hemophilia patients who were positive for antibody to HCV, 4 (1.7%) gave positive reactions in the first generation EIA for HCV antibody but only 1 of them (0.4%) was found positive in the second generation assay. The prevalence of antibodies determined in the first generation assay is somewhat higher than that obtained from blood donors in Germany (0.48%,13 0.42%14). On the other hand, Polivka and Laufer13 searched for antibodies to C100 antigen in sera from 42 household contacts of subjects who were positive for HCV antibody, without obtaining positive results. In a study performed by Everhart et al.,16 62 household contacts of patients suffering from hepatitis NANB were also found to be negative. Contrarily, Ideo et al17 encountered 7 contacts (8%) with positivity for antibody to C100 in a slightly larger population (n = 88). A similar prevalence (8%) was observed by Kiyosawa et al18 in 196 household contacts of patients with chronic hepatitis NANB. It is noteworthy that, in these studies, almost the same percentages of positive results were seen in sexual and other household contacts of the index patients. By contrast, in the study of Riestra et al.,19 5 of 71 sexual partners (7%) and only 2 of 149 nonsexual household contacts (1.3%) were found to be positive for antibody to C100.

All these investigators used the first generation EIA for HCV antibody. This assay seems to be not sufficiently sensitive, because in 8 of 265 patients, Inaba et al.20 for example, observed seroconversion for C100 antibody after blood...
transfusions that were negative for C100. The development of anti-HCV after infection with this virus was often delayed. In some cases, these antibodies could not be detected a few years after convalescence. On the other hand, a number of investigators reported false-positive reactions, which are thought to be due to antibodies to SOD, yeast antigens, or to the presence of rheumatoid factor, above all in patients with autoimmune hepatitis. Moreover, the limit values of the first generation assay for HCV antibody may have been set too low.

Recently, a new generation of HCV assays permits surveillance of the prevalence of antibodies to HCV in index patients and their household contacts. The sensitivity of this assay was improved by using another two HCV antigens. False-positive reactions should be avoided by antigen fusion to CKS rather than to SOD and by expression in E. coli rather than in yeast.

In this study, the higher specificity of this assay was noted in the examination of contacts. Of 4 subjects who had been found positive for antibody to C100, only 1 gave a positive reaction in the second generation assay. These findings were confirmed by the results of the neutralization assay and of the tests performed in the Abbott Reference Laboratory.

The higher sensitivity of the second generation assay for HCV antibody, which was documented, for example, by Aach et al., did not lead to a larger number of positive results in the present investigation. It is not probable that this was due to delayed development of antibodies observed in acute HCV infection or to the loss of the antibodies late after acute HCV infection.

Unlike their contacts, the index patients gave positive reactions to the first and the second generation assays for HCV antibody. Thus, the observation has been confirmed that the first generation assay gives few false-positive results in subjects likely to have been infected with HCV.

Transmission of HBV to household contacts. There was no evidence of HCV transmission to household contacts. This might be due to the generally low infectivity titer of HCV in the blood. By contrast, transmission of the highly viremic HBV by household contact seems to be possible. The raised prevalence of antibody to HBe in contacts of index patients with chronic hepatitis B leads to this conclusion. Furthermore, there was no substantial difference between sexual partners and household contacts with regard to the distribution pattern of antibodies to HBe. On the other hand, Koff et al. observed that the prevalence of antibody to hepatitis B core antigen was three times greater in sexual partners than in other persons within households of nonhemophilic patients with hepatitis B. This discrepancy cannot be accounted for by a larger number of patients with chronic hepatitis B in the group of nonsexual contacts than in the group of sexual partners. As shown in Table 2, chronic hepatitis was equally distributed among the groups. Obviously these data provide evidence that household contacts of hemophiliacs found positive for antibody to HBe are at a greater risk of contracting HBV infection than the family members of a nonhemophilic patient suffering from hepatitis B infection or having a history of such infection. The higher incidence of bleedings (eg, from the gums) in hemophilia patients and the associated contamination of towels, cutlery, toys, and similar objects might account for this finding.

Transmission of HCV and HBV by home treatment. Exposure to clotting factor concentrates and injection needles that may be contaminated as well as the risk of incurring injuries during the care of index patients in home treatments would appear to increase the danger of infection of household contacts who are virtually at the same risk as health care workers in hospitals. Hofmann and Kunz detected antibodies to C100 in 2 of 46 physicians (4.3%) and in 4 of 123 nurses (3.3%). Kiyosawa et al. observed a seroconversion for C100 antibody in 3 of 110 subjects (2.7%) sustaining needle injuries in the care of a patient found positive for anti-C100. With regard to HBV infections of physicians and nursing staff, Janzen et al. reported positive results to HBs antigen or to anti-HBs obtained from 37 of 203 physicians (18.2%) and 96 of 469 nurses (20.5%). Thus, the prevalence of hepatitis B markers appears to be significantly higher in health care workers than in controls.

However, an increased incidence of HCV and HBV infection of household contacts involved in home treatments was not observed in this investigation. None of these persons was found positive by the first and second generation enzyme immunoassays for antibody to HCV and the prevalence of antibodies to hepatitis B core antigen was even lower than in subjects not participating in such treatments. It seems reasonable to assume that the health consciousness of family members who had been instructed how to conduct home treatment was helpful to reduce the risk of infection.

Sexual transmission of HCV compared with HBV and HIV. Evidence of sexual transmission of HCV was provided in some investigations. According to Alter et al., contact with more than two heterosexual partners within 6 months and a history of hepatitis of these partners constitute a significant risk of acquiring hepatitis NANB. Using the anti-HCV test of the first generation, Esteban et al., Pachucki et al., and Tor et al. examined heterosexual partners of subjects found positive for HCV (intravenous drug abuse) and obtained positive results in 1 of 18, 1 of 25, and 16 of 143 sexual contacts, respectively. These findings are in line with the prevalence of antibody to C100 (first generation test) in the sexual partners of our index patients (positivity in 3 of 73 cases) and with the results achieved by Riestra et al., who found 5 of 71 sexual contacts to be positive for anti-C100. Esteban et al. and Tor et al. studied their patients additionally for antibody to HBV and HIV. These investigators observed a higher prevalence of antibody to HBe and HIV than to HCV in heterosexual partners of their index patients.

### Table 4. Prevalence of Antibody to HAV in Household Contacts

<table>
<thead>
<tr>
<th>Age</th>
<th>Negative for HAV</th>
<th>Positive for HAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 1 yr</td>
<td>—</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>1 to 30 yr</td>
<td>51/65 (79)</td>
<td>14/66 (21)</td>
</tr>
<tr>
<td>30 to 60 yr</td>
<td>64/142 (45)</td>
<td>78/142 (55)</td>
</tr>
<tr>
<td>Over 60 yr</td>
<td>4/20 (20)</td>
<td>16/20 (80)</td>
</tr>
<tr>
<td>Total</td>
<td>119/228 (52)</td>
<td>109/228 (48)</td>
</tr>
</tbody>
</table>

Percentages are in parentheses.
In the present study as well, far fewer sexual partners were positive to anti-HCV than to anti-HBc or anti-HIV. Based on the results achieved by the second generation EIA for antibody to HCV it would appear that the HCV transmission rate associated with heterosexual activity was overestimated due to false-positive reactions in the first generation assays. Although it has to be considered that HCV could be transmitted more frequently from female to male spouses as compared with male to female spouses, sexual transmission of HCV seems to be less important than that of HBV or HIV and seems to occur only in a few exceptional cases.

Eyster et al. observed that transmission of HCV between sexual partners was more liable to occur when hemophiliac patients were infected with both HIV and HCV. This could not be definitely confirmed in the present investigation, because only 1 of the sexual partners of 44 index patients found positive for HIV and HCV, gave a positive reaction to HCV antibody, whereas all sexual contacts of 29 index patients having only antibodies to HCV were found to be negative for anti-HCV.

Prevalence of HAV antibodies in family members. The age-related prevalence of antibody to HAV in the contacts of the index patients agrees with the distribution in the total German population, described in 1979 by Frösner et al. Because there are many sources of infection other than household contact, no further analysis was made.

The data in this study suggest that sexual and nonsexual contact with HCV infected hemophiliac patients within households and the care of such patients by controlled self-treatment are not associated with an increased risk for HCV infection. More research is needed to assess the extent and the causes of nonparenteral transmission of HCV.

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