HEPATITIS C VIRUS ANTIBODIES AMONG RISK GROUPS IN SPAIN

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Summary

The frequency of hepatitis C virus (HCV) infection in Spain was assessed by means of a recombinant-based immunoassay for serum anti-HCV antibodies. 836 serum samples were tested from 676 patients selected according to their risk of blood-borne viral infections and presence of liver disease. Among patients at high risk of infection (with or without liver disease) anti-HCV antibodies were found in 85% of prospectively followed patients with post-transfusion non-A, non-B hepatitis, 62% of patients with chronic hepatitis or cirrhosis and a history of blood transfusion, 70% of haemophiliacs receiving replacement therapy, 70% of intravenous drug abusers, and 20% of haemodialysis patients. Only 8% of homosexual men infected with human immunodeficiency virus and 6% of female contacts of drug abusers were positive. Among patients with liver disease and no history of parenteral exposure to blood, anti-HCV antibodies were detected in 38% with cryptogenic, alcoholic, or primary biliary cirrhosis and in 44% with chronic active hepatitis. Among healthy subjects without risk factors for hepatitis the overall prevalence of anti-HCV was 1.2%.

Introduction

More than 90% of transfusion-associated hepatitis cases worldwide are attributed to non-A, non-B hepatitis (NANBH).1-6 NANBH accounts for a substantial proportion of hepatitis cases among patients with frequent parenteral exposure to blood (eg, haemophiliacs), intravenous drug abusers,8 and haemodialysis patients9 and for more than 25% of cases of sporadic hepatitis without obvious percutaneous exposure.10,11 Researchers at the Chiron Corporation in California have lately isolated a blood-borne NANBH agent, designated hepatitis C virus (HCV).12 Virus isolation led to the development of a recombinant-based immunoassay for detection of specific anti-HCV antibodies. Initial evaluation of one such assay13 in serum samples from post-transfusion NANBH cases and implicated donors in the USA and in sera from Italian and Japanese patients with acute and chronic NANBH confirmed that HCV is the major causal agent of NANBH. To estimate the prevalence of HCV infection in Spain, we have studied 836 serum samples from three categories of patients—326 at high risk of viral hepatitis (group I);60 with biopsy-proven chronic liver disease but no apparent risk factor for viral hepatitis (group II); and 290 healthy subjects without liver disease and with no history of percutaneous exposure to blood (group III).

Subjects and Methods

Group I (High-risk Patients with or without Liver Disease)

Post-transfusion hepatitis and chronic NANBH patients.—54 patients with post-transfusion NANBH who had been enrolled in a prospective study between 1978 and 1984 were studied.6-4 54 samples (obtained at 1–2, 6–12, and 20–32 weeks post-transfusion) were assayed in 40 patients; 5–10 serial samples (obtained shortly after transfusion, during the acute phase, and at variable intervals during convalescence) in 10 patients; and 2 samples (1–2 and 20–25 weeks post-transfusion) in 4 patients. Samples were also tested from 8 symptom-free patients with biopsy-proven chronic active hepatitis (6 cases) or cirrhosis (2) who had a history of blood transfusion as the only known origin of their liver disease.

Haemophiliacs.—Serum samples had been obtained in 1985 from 97 patients aged 1 to 55 years (mean 20.1, SD 14) with coagulation disorders (73 haemophilia A; 7 haemophilia B; 11 von Willebrand disease; 6 other deficiencies) regularly attending the haemophilia unit at our centre. 12 patients had never been treated whereas the remaining 85 were receiving regular replacement therapy (61 factor VIII concentrate; 10 cryoprecipitate; 8 prothrombin complex; 6 fresh frozen plasma). 48 patients had antibodies against human
immunodeficiency virus (HIV) but none had symptoms of immunodeficiency when the sample was obtained.

*Intravenous drug abusers.*—Samples were obtained from 83 drug abusers (60 M, 23 F; mean age 26.5 years, range 18–35) with a mean duration of drug use of 6.1 (SD 3.5) years. All but 1 had been referred to the hospital because of HIV-seropositivity (6 patients had AIDS, 3 AIDS-related complex, and the rest were symptombarem). All but 3 had admitted frequent needle sharing. A history of hepatitis of unknown type was reported by 34 (41%).

*Homosexual men and female contacts of intravenous drug abusers.*—There were 26 homosexual men (96% anti-HIV positive) who had engaged in active and passive rectal intercourse and had a mean of 10.8 sexual partners/year. 10 patients had a history of hepatitis; 4 were chronic HBsAg carriers; and 15 were positive for anti-HBc. 10 of the 18 female partners of drug abusers were anti-HCV positive. In 5 cases the partner who abused drugs had never been treated, only 2 had anti-HCV antibodies when the sample was obtained.

*Haemodialysis patients.*—There were 42 patients on chronic haemodialysis who were seronegative for all HIV markers; 30 had received a mean of 26 weeks after transfusion (range 20–32). 5 of the 8 patients who remained seronegative the last tested sample had been obtained a mean of 26 weeks after transfusion (range 20–32).

*Group II (Low-risk Patients with Liver Disease)*

There were 34 patients (3 M, 31 F; aged 13–76 years, mean 54) with biopsy-proven chronic hepatitis (20) or active cirrhosis (14). 14 patients were symptombarem and had been studied because of persistently increased alanine aminotransferase (ALT) values, 4 had arthritis, 4 presented with symptoms of acute hepatitis, and 11 presented with jaundice, ascites, or variceal bleeding. 32 patients had antinuclear antibodies, in most cases together with other autoantibodies (anti-liver-membrane antigen, anti-smooth-muscle, or anti-gastric-parietal-cell). None had been transfused or recalled blood donor control sera.

3 patients with primary biliary cirrhosis, 8 with cryptogenic cirrhosis, and 15 men (mean age 61.5) with alcoholic cirrhosis were also included in this group. Most patients had been admitted to hospital because of ascites or variceal bleeding. None had a history of transfusion before the test sample was obtained.

*Group III (Low-risk Healthy Subjects)*

There were 49 unselected blood donors and 241 healthy pregnant women from whom blood samples had been taken at delivery as part of a vaccination programme of babies born to HBsAg carrier mothers. Of the pregnant women, 98 were healthy HBsAg carriers with repeatedly normal ALT values on several occasions for more than a year who had never received blood or had any known risk factor for hepatitis.

**Methods**

*Anti-HCV testing.*—All samples were shipped in dry ice to the assay laboratory and tested under code in duplicate. Anti-HCV was assayed with a microtiter radioimmunossay in which a recombinant HCV polypeptide obtained in yeast was used to capture specific viral antibodies, as previously described.12 Samples were considered positive when the counts per minute (cpm) were above the mean plus 3 SD cpm of 138 blood donor control sera (>3540 cpm).

*Statistical analysis.*—Statistical methods included Fisher's exact test to compute relative frequencies within groups and Student's t-test to evaluate the significance of differences among groups. All p values were two-sided.

**Results**

*Group I*

**Post-transfusion hepatitis and chronic NANBH patients.*—Unequivocal seroconversion was documented in 42 patients (78%). In 4 additional patients low-level anti-HCV antibodies were present from the first early post-transfusion sample, presumably owing to passive transfer of antibody from the donor. Thus, 85% of our post-transfusion NANBH cases became anti-HCV positive (table I). Of the 34 seroconverters from whom enough serial samples were available, anti-HCV was first detected during the acute phase of infection (6–8 weeks post-transfusion) in 13; between 20 and 26 weeks post-transfusion in 19; and at 38 and 52 weeks, respectively, in 2. In 6 of the 8 patients who remained seronegative the last tested sample had been obtained a mean of 26 weeks after transfusion (range 20–32).

5 of the 8 patients with chronic active hepatitis or cirrhosis and a history of blood transfusion were anti-HCV positive.

*Haemophiliacs.*—64% of patients in this group were anti-HCV positive (table I). 60 of the 85 treated patients (70%) were anti-HCV positive. By contrast, of the 12 who had never been treated, only 2 had anti-HCV antibodies (p < 0.05). 1 of the latter was a year-old infant whose mother's status was unknown. Among the treated patients, anti-HCV positivity was unrelated to age, type of haemophilia (71% haemophilia B, 68% haemophilia A, 58% von Willebrand disease, and 50% of patients with other deficiencies), type of replacement therapy (factor VIII 70%, cryoprecipitate 60%, prothrombin complex 87%, and fresh frozen plasma 50%) or serological status for HIV (anti-HIV positive 71%, anti-HIV negative 58%).

*Intravenous drug abusers.*—70% of patients were positive (table I). Overall, there was no difference between anti-HCV positive and negative addicts with respect to age (26.6 [SD 4.5] vs 25.8 [4.1], respectively), mean duration of drug abuse (6.4 [3.6] vs 5.5 [3.3]) and mean ALT levels (61.7 [53] vs 47.5 [38.4]).

*Haemodialysis patients.*—8 of the 42 tested patients were positive. Of the 30 patients who had received blood transfusions in the past, 7 were positive vs 1 of the 12 without desc
TABLE III—CHARACTERISTICS OF PATIENTS WITH AUTOIMMUNE LIVER DISEASE ACCORDING TO ANTI-HCV STATUS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Anti-HCV positive (n=15)</th>
<th>Anti-HCV negative (n=19)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>60.9 (41-76)</td>
<td>47.3 (13-76)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Symptoms at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Liver-related*</td>
<td>6</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ALT (SD)</td>
<td>53.8 (40.1)</td>
<td>128 (58)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean total immunoglobulin (SD range)</td>
<td>2.05 (1.1-3.4)</td>
<td>2.98 (1.8-4.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Autoantibodies (% positive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-nuclear antibodies</td>
<td>13</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>Other†</td>
<td>17</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>Liver histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
<td>9</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>Active cirrhosis</td>
<td>6</td>
<td>7</td>
<td>NS</td>
</tr>
</tbody>
</table>

*aScars, variceal bleeding, or symptoms of acute hepatitis.*
†Mean of three consecutive values per patient taken to obtain group mean ALT.
*Anti-liver-membrane antigen, anti-smooth-muscle, anti-gastra-parietal-cell, or anti-erytulin.
NS = not significant.

...a history of transfusion (difference not statistically significant).

Homosexual men and heterosexual contacts of intravenous drug abusers.—8% of homosexual men and 6% of female contacts of drug abusers had anti-HCV antibodies (table I). None of the 5 contacts of abusers known to be HCV infected had anti-HCV. Comparison of serum markers for HIV, hepatitis B virus (anti-HBc), and HCV in both groups is shown in table II.

Group II

Autoimmune chronic active hepatitis.—15 patients (44%) had circulating antibodies to HCV. Antibody-positive patients were significantly older and had lower mean ALT levels than those who were anti-HCV negative (table II).

Primary biliary cirrhosis, cryptogenic cirrhosis, and alcoholic cirrhosis.—Anti-HCV antibodies were detected in 1 of 3 patients with primary biliary cirrhosis, in 2 of 8 with cryptogenic cirrhosis, and in 7 of 15 with alcoholic cirrhosis. Overall frequency of anti-HCV in this group was 38%.

Group III

Antibodies to HCV were detected in 2 of 143 non-carrier pregnant women, 1 of 98 HBsAg carrier women, and none of 49 random blood donors. Overall frequency of anti-HCV in this group was 1.2%.

Discussion

Our results show that HCV accounts for most cases of post-transfusion hepatitis in Spain. Although seroconversion may occur during the acute phase of the infection (or, about a third of cases), in more than half of our patients anti-HCV antibodies were first detected 4-6 months after transfusion, and in some the antibody response was considerably later. This delayed response could explain why anti-HCV was not detected in all our post-transfusion cases. In 6 of the 8 seronegative cases the last tested sample had been obtained between 20 and 32 weeks after transfusion, so we might have underestimated seroconversion by almost 10%. In the remaining 2 patients there was a very long interval between the acute and convalescent samples tested (more than a year). Since anti-HCV antibodies may disappear with time (H. Alter, personal communication), as observed in 1 of our patients, transient seroconversion might have been missed because of infrequent sampling. The possibility that these seronegative patients did not have viral hepatitis or that their hepatitis was caused by a different agent, although unlikely, cannot be excluded.

The high frequency of anti-HCV antibodies among haemophiliacs and drug abusers was not unexpected. Among treated haemophiliacs seropositivity for anti-HCV was independent of age, type of haemophilia, type of replacement therapy, or serological status for HIV. Similarly, among intravenous drug abusers, presence of anti-HCV was independent of age, duration of drug abuse, absolute number of T-helper lymphocytes (data not shown), or clinical stage of HIV infection. However, within this group, mean ALT values of anti-HCV positive and negative patients (after excluding those with chronic HBV infection) did not differ significantly. 48% of patients in the anti-HCV negative group had abnormal ALT values in the absence of active HBV infection; liver biopsy was done in 5 patients in this group and histological examination showed chronic active hepatitis. This finding rises the possibility of chronic seronegative anti-HCV infection or chronic hepatitis by a different viral agent, although cryptic HBV infection or toxic hepatitis cannot be excluded.

The 20% seropositivity among haemodialysis patients without a history of HBV exposure seems to be almost exclusively related to blood transfusion (7 of the 8 who were anti-HCV positive had received blood). Although homosexual men have a high risk of HBV infection, the frequency of anti-HCV among HIV-infected homosexuals in our study was low—58% of the homosexuals tested were anti-HBc positive whereas only 2 (8%) had anti-HCV antibodies. The similarly low frequency (6%) of anti-HCV among female contacts of intravenous drug abusers (5 of whom were known to be anti-HCV positive) seems to indicate that HCV is not readily transmitted by sexual contact.

Perhaps the most important finding of our study is the high prevalence of anti-HCV among patients in group II. 30% of patients with chronic active hepatitis or cirrhosis of unknown or alcoholic origin and 44% of those with autoimmune chronic active hepatitis had anti-HCV in the absence of exposure to blood. Since the finding of anti-HCV seems to be associated with chronic infection, HCV may be important in the pathogenesis of almost half of the patients whose liver disease is currently attributed to non-viral causes. For patients with chronic active hepatitis and markers of autoimmunity who are infected with HCV, it remains to be established whether autoimmune manifestations are induced by viral infection or whether the association is coincidental. In any event, the high frequency of infection among these patients should influence management: HCV testing should now become mandatory in the routine evaluation of these patients. The seroprevalence of anti-HCV among healthy subjects in our study does not differ significantly from that reported in preliminary estimates from the USA.13

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ANTI-HEPATITIS C ANTIBODIES AND NON-A, NON-B POST-TRANSFUSION HEPATITIS IN THE NETHERLANDS

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Summary
In a prospective study carried out in the Netherlands (1984-86) to establish the incidence of post-transfusion hepatitis non-A, non-B (PTH-NANB) in patients undergoing open heart surgery, 393 patients received 5315 blood product transfusions. PTH-NANB developed in 9 patients (index cases); stored serum samples from these patients and from 9 control patients, matched for age, sex, and number of blood product transfusions, as well as serum samples of all implicated blood products, were selected retrospectively. Sera were tested under code with a radioimmunoassay for the detection of antibodies to hepatitis C virus (anti-HCV). PTH-NANB patients received 151 blood product transfusions and control patients 140. 4 of 9 PTH-NANB patients (3/5 chronic, 1/4 acute resolved hepatitis) and 0/9 controls seroconverted. 7 of the transfusions given to PTH-NANB patients but none of those given to control patients were anti-HCV positive. Among the donors implicated in transmission of PTH-NANB there was a strong correlation between raised alanine aminotransferase levels and the presence of anti-HCV antibodies.

Introduction
Researchers at the Chiron Corporation in the USA have lately isolated a cDNA clone from a parenterally transmitted non-A, non-B (NANB)-hepatitis viral genome. This virus has been named hepatitis C (HCV), and appears to be a lipid-enveloped, single-stranded RNA virus.

Materials and Methods
Serum samples from the 9 PTH-NANB patients in the 1984-86 study (5 chronic, 4 acute resolved; index cases) and from 9 retrospectively matched control patients (matched for age, gender, and number of transfusions), obtained before, and 3 and 6 months after transfusion, were tested for anti-HCV antibodies. Serum samples from all blood products implicated in the index cases (n=151), and from blood products that had been given to the matched controls (n=140), were similarly tested. Information about ALT values and anti-HBc antibodies was obtained from the original study. ALT levels were measured by a spectrophotometric test at 20°C, and anti-HBc was tested with a radioimmunoassay (Abbott, Chicago, USA). Radioimmunoassay for anti-HCV antibodies was done on coded serum samples at the Chiron Corporation, Emeryville, California. Briefly, wells of microtitre plates were coated with 0.1 µg of purified C100-3 HCV antigen before incubation for 1 h at 37°C with 100 µl of diluted (1 in 100) serum. Wells were washed, and bound antibody was detected by incubation for 1 h at 37°C with 100 µl of 125I-labelled sheep anti-human immunoglobulin. Values above the mean of uninfected controls plus 3 SD were considered positive. Statistical methods included z2, one-tailed Fisher's exact test, and Bartolomew's trend test.

Results
The results of anti-HCV testing are shown in table 1. Of the 9 PTH-NANB patients seroconverted to none of the 9 controls (p<0.05). Anti-HCV seroconversion was observed 6-7 weeks after onset of hepatitis in 3 of 5 patients with...