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## Prevalence of Hepatitis C Virus Antibody in a Cohort of Hemophilia Patients

By D.B. Brettler, H.J. Alter, J.L. Dienstag, A.D. Forsberg, and P.H. Levine

One hundred thirty-one patients followed at the New England Hemophilia Center (Worcester, MA) were tested for antibody to hepatitis C virus (HCV). All but two had used factor concentrate that had not undergone viral inactivation; two patients had used only cryoprecipitate. The overall prevalence of HCV antibody positivity was 76.3%. There was no significant difference in age or the amount of non-heat-treated factor concentrate used between the group that was HCV antibody positive and negative. There was also no significant difference between aminotransferase levels in the two groups. There was a positive association between HCV antibody and the presence of antibody to hepatitis B core antigen and antibody to

human immunodeficiency virus. A group of 31 patients were tested twice for HCV antibody at intervals of 35 to 71 months. In this subset, 25 were repeatedly seropositive, 4 were repeatedly seronegative, and 2 went from seropositive to seronegative. These data confirm the previous impression that non-A, non-B hepatitis is a major sequela to the use of pooled coagulation factor concentrates. HCV infection may account for most of the chronic liver disease observed in this population. Anti-HCV testing of plasma donors and improved methods of viral inactivation should prevent new cases from developing.

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**B**EFORE THE introduction of viral inactivated factor concentrates, infections with transfusion-transmitted viruses were widespread in the hemophilic population. Human immunodeficiency virus (HIV-1) infection entered the plasma supply in the middle to late 1970s, and by 1982 50% of hemophiliacs in the United States had become infected.<sup>1</sup> Although symptomatic infection with hepatitis B (HBV) virus is uncommon in this population, 85% to 90% of patients receiving factor before 1984 to 1985 are seropositive for antibody to hepatitis B surface antigen (anti-HBs)<sup>2</sup>; approximately 5% are hepatitis B virus carriers. Studies of multiple cohorts of hemophilic patients have shown that up to 90% of these patients also have elevated aminotransferase (ALT) levels.<sup>3,4</sup> Liver biopsies have demonstrated pathologic lesions ranging from chronic persistent hepatitis to chronic active hepatitis and cirrhosis.<sup>5,6</sup> It was believed that many patients with elevated ALT levels had non-A, non-B (NANB) hepatitis, although the diagnosis has been one of exclusion. Recently, the genome for an NANB hepatitis agent (hepatitis C virus, HCV) has been cloned, and an assay for detection of antibody to HCV has been developed and refined.<sup>7,8</sup> Thus, we studied a cohort of hemophilic patients to determine the prevalence of antibody to HCV and attempted to correlate it

with exposure to other transfusion transmitted viruses, particularly HIV-1.

### PATIENTS AND METHODS

**Patients.** A total population of 131 patients with hemophilia followed at the New England Hemophilia Center (Worcester, MA) were tested for the presence of antibody to HCV (anti-HCV). Of these patients 117 had hemophilia A, 12 had hemophilia B, 1 was an asymptomatic carrier, and 1 had von Willebrand disease. The median age of the patients studied was 25.8 years, with a range of 3 to 71.5 years. The median amount of nonviral inactivated factor concentrate used was 1,330 U/kg/y (range 42 to 8,906) for factor VIII concentrate and 696 U/kg/y (range 25 to 14,761) for prothrombin complex concentrate. The patients were seen at yearly intervals at which time liver biochemistry tests were performed, as were HBV serology, and beginning in 1985, HIV antibody.

**Methods.** Sixty-three stored serum samples drawn in 1983 to 1984 were assayed for anti-HCV with the radioimmunoassay (RIA) developed by Chiron Corp (Emeryville, CA).<sup>9</sup> An additional 99 stored samples, drawn in 1988 to 1989, were tested for anti-HCV by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's directions (Ortho HCV antibody ELISA, Raritan, NJ). The total of 162 samples represents 131 different patients; 31 patients were sampled on two different occasions. Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), and hepatitis B core antibody (anti-HBc) were measured by routine enzyme immunoassay (EIA) methods (Abbott Laboratories, North Chicago, IL). HIV antibody was measured using an ELISA test (Abbott Laboratories) and all positive samples were confirmed by Western blot. Liver biochemistry tests were done by routine methods.

**Statistical methods.** The hepatitis C positive and negative groups were compared with the Mann-Whitney test, and associations were evaluated with the Fisher exact test.

### RESULTS

The prevalence of HCV antibody in this hemophilic cohort was 76.3% (100 of 131), including 93 of 118 (74%) patients with hemophilia A and 8 of 12 (67%) patients hemophilia B. As can be seen in Table 1, there was no significant difference in either the median age or the median amount of non-heat-treated factor concentrate consumed between those that were seropositive and seronegative for HCV antibody. There was also no significant difference in ALT levels between the anti-HCV positive and anti-HCV negative groups. Of the 31 patients that were anti-HCV negative, 19 (61%) had an

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**Table 1. A Comparison Between HCV Antibody Positive and Negative Hemophilic Patients**

	HCV Antibody Positive (n = 100)	HCV Antibody Negative (n = 31)
Median age (y)	27.3 (3.6-71.5)	23.2 (5.3-62.0)
Median factor usage (U/kg/y)	1,119 (125-4,271)	1,433 (92-8,906)
Median ALT levels (IU/mL)	69 (26-329)	85 (14-621)
No. (%) of patients with ALT elevation	73 (73%)	19 (61%)

elevated ALT at the time the HCV antibody test was obtained or intermittently within the 2 years before the sample was drawn. Conversely, in the seropositive group, 27 (27%) patients had normal ALT levels at the time of testing and on yearly testing in the preceding 2 years.

As can be seen in Table 2, there was a positive association between the presence of anti-HBc and anti-HCV. The correlation occurred only when the patients were seropositive for both anti-HBs and anti-HBc, and not when they were anti-HBc positive alone. There was also a positive correlation between the presence of anti-HIV and anti-HCV ( $P < .025$ ). There was no correlation between antibody to HCV and the presence of either HBsAg or anti-HBs.

**Repeat testing.** A group of 31 patients had HCV serology performed on two different occasions separated by 35 to 71 months. This subgroup did not differ from the group as a whole. The age ranged from 3.6 to 60.2 years (median 26.9). The majority had hemophilia A (30) and one had hemophilia B. All had used clotting factor concentrate that was not viral-inactivated. In this group, 25 were repeatedly seropositive, 4 were repeatedly seronegative and 2 appeared to lose antibody (being positive when first tested and negative on repeat testing). Of the 25 that were repeatedly seropositive, 24 had persistent or intermittently elevated ALT; one seropositive patient had persistently normal ALT although he had infused regularly with noninactivated clotting factor concentrate. Of the four patients that were seronegative on repeat testing, two had abnormal ALT and two had persistently normal ALT. One patient remained seronegative and had persistent normal ALT despite multiple infusions of factor concentrate, including products prepared before 1984. In the second seronegative patient with normal ALT on repeat testing, a high titer factor VIII inhibitor was present and the patient infused infrequently with prothrombin complex concentrates.

Both patients that appeared to lose anti-HCV maintained seropositivity for HIV over the same time period. One patient remains asymptomatic from HIV infection and the second patient has undergone a splenectomy secondary to HIV-induced thrombocytopenia.

## DISCUSSION

In this study, we use the anti-HCV assay to analyze a hemophilia cohort in which 81.6% of patients had chronic or intermittently elevated ALT. The anti-HCV test has been shown to be a reliable indicator of NANB hepatitis infection.<sup>9</sup> Anti-HCV was detected in 76% of patients, confirming the high prevalence of HCV infection in this population and supporting the etiologic role of this virus in the development of chronic liver disease in the hemophilic. Similar high anti-HCV prevalences had been reported.<sup>10-13</sup> In this study, we found that the prevalence of antibodies to HBV and HCV was approximately equal.

Not all patients with transaminase elevation and presumed NANB hepatitis were seropositive for anti-HCV. There are several possible explanations for this: (1) ALT elevations may have been medication-related or due to other nonviral causes; (2) anti-HCV antibody may have been previously present, but become undetectable with time; (3) the anti-HCV response may have been diminished by coexistent HIV infection as has been demonstrated in HBV infection<sup>14</sup>; (4) the NANB hepatitis could be related to an additional NANB hepatitis agent. A second agent has been suggested by both clinical and chimpanzee transmission studies and such a possibility can now be explored using the same approaches that elucidated HCV; and (5) the sensitivity of the first generation assay may be too low to detect all cases of HCV infection. It is probable that some anti-HCV negative cases will be shown to be HCV-related when newer serologic techniques and in situ hybridization studies are applied.

We found no correlation between elevation of ALT and HCV seropositivity. Of the 31 HCV seronegative patients, 19 (61%) had elevated ALT, and of the 100 seropositive patients, 27 (27%) had consistently normal ALT over a period of 6 to 7 years. However, there was a definite correlation between anti-HCV and the presence of HIV antibody and anti-HBc. Before procedures to inactivate viruses in the production of factor concentrates, these concentrates, made from donor pools as large as 20,000, were uniformly contaminated with HCV, HIV, and HBV. Thus, the evidence for exposure to each of these agents in this cohort is not unexpected. The concurrence of hepatitis markers in populations at high risk for blood-borne and sexually transmitted virus infections was reflected in studies that showed a correlation between donor anti-HBc and recipient NANB hepatitis,<sup>15</sup> and in blood bank policy deci-

**Table 2. Comparison of Viral Serology Between HCV Positive and Negative Hemophilia Patients**

	HCV Antibody Positive (n = 100) (%)	HCV Antibody Negative (n = 31) (%)
<b>HBV Serology</b>		
HBs Ag positive	12/98 (12.2)	2/31 (6.5)
Anti-HBsAb positive	81/93 (82.7)	26/31 (83.9)
Anti-HBc positive, total	64/73 (87.7)	14/23 (60.9)*
Anti-HBc positive, anti-HBs positive	55/73 (75.3)	12/23 (52.2)*
Anti-HBc positive alone	9/73 (12.9)	2/23 (8.7)
<b>HIV serology</b>		
Anti-HIV positive	69/80 (86.3)	19/26 (73.1)†

\* $P < .01$ .† $P < .025$ .

sions to use the anti-HBc assay as a surrogate index for NANB hepatitis virus carriers.

It appears that as with HIV antibody, the presence of anti-HCV generally denotes chronic infection.<sup>9</sup> Hence, a large segment of the hemophilia population is chronically infected with an agent that induces cirrhosis, and that more recently has been associated with hepatocellular carcinoma (HCC).<sup>16</sup> A preliminary multicenter surveillance study is now underway to ascertain the incidence of HCC in the hemophilic population. The interval to the clinical presentation of cirrhosis and HCC has been found to be prolonged.<sup>17</sup> Because many hemophilic patients have been infected since the early 1970s when factor concentrate was first widely used, the incidence of severe, overt liver disease may soon increase in this population.

It is hoped that with licensure of the anti-HCV assay, both

plasma and plasma-derived factor concentrates can be made safer by better screening and more effective viral inactivation. Although earlier inactivation procedures failed to eradicate the risk of clotting factor-associated NANB hepatitis transmission,<sup>18</sup> newer generation factor VIII concentrates using virucidal methods such as pasteurization or exposure to solvent/detergents have been shown to have a high probability of freedom from HCV contamination.<sup>19,20</sup> Recombinant factor concentrates now in widespread clinical trials should eliminate totally the burden of human transfusion-transmitted viruses.<sup>21,22</sup>

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