

A Multicenter Study of Viral Hepatitis in a United States Hemophilic Population

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Hemophilia A and B patients seen at nine US regional treatment centers were tested for serologic markers of hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis delta virus (HDV) during 1987 and 1988. Because human immunodeficiency virus (HIV) infection, a potentially confounding variable, was present in 53% of the group, the population was divided by HIV status for analysis purposes. In the HIV-positive group (N = 382), less than 1% had not been infected with HBV, HCV, or HDV, whereas 75% had evidence of infection with HBV and 98% with HCV. HBsAg, a marker of active HBV infection, was present in 12% of subjects; 96% of these were HCV positive. Anti-HDV was detected in 35 subjects (9.1%); all were anti-HBc positive. Ten of the 35 (29%) also were positive for IgM anti-HDV, indicating current infection. All 10 were HBsAg positive and 7 of the 9 tested were HDV RNA positive. Severe/moderate hemophilia B patients were more likely to have experienced an HBV infection and to be anti-HDV positive than were similar hemophilia A patients (22% v 8%, $P < .05$). In the HIV-negative group (N = 345), the subjects

were younger and had less severe hemophilia than the HIV-positive patients. No evidence of HBV, HCV, or HDV infection was found in 18%, whereas 33% had experienced HBV infection and 79% were anti-HCV positive. Within this group, 4% were HBsAg positive. All 13 subjects with anti-HDV (4% of the HIV-negative group) also possessed anti-HBc. One (7.7%) was IgM anti-HDV positive and the serum from another contained HDV RNA. Both of these individuals were HBsAg positive. As in the HIV-positive group, severe/moderate hemophilia B patients were more likely to be HBV and HDV positive than were hemophilia A patients (9% v 3%, $P < .05$). A prevalence study of viral hepatitis in a large US hemophilic population showed that active infection with HCV is common, occurring in 89% of all study patients regardless of HIV status. Evidence of active HBV infection was found in 8%; 19% of these were actively infected with HDV. HDV was more common in hemophilia B patients after controlling for disease severity.

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BECAUSE SEVERAL thousand donors are required for preparation of clotting factor concentrates, blood-borne infectious diseases, such as human immunodeficiency virus (HIV) infection and viral hepatitis, continue to be a serious health burden to hemophiliacs. Studies have shown that prevalence rates for hepatitis B and C are consistently greater than 60% in this population,¹⁻¹¹ and abnormal liver function tests are present in greater than 50%.^{1-3,12-18} Among hemophiliacs chronically infected with hepatitis B virus (HBV), cirrhosis has been predicted to develop in 10% to 20%.^{19,20} In addition, hepatitis C virus (HCV) infection is frequent in these patients.²¹ Hemophiliacs have been reported to be at increased risk for hepatocellular carcinoma²² and, in general,

liver disease is suspected to be responsible for 5% to 11% of the deaths that occur.

A third hepatitis agent, hepatitis delta virus (HDV), relies on HBV for replicative helper functions (although transplantation models suggest that a third mechanism exists in which HDV is capable of establishing latent, asymptomatic infections without apparent assistance from HBV)²³ and is transmitted through the same routes as HBV. HDV infection typically leads to a more severe clinical presentation than HBV infection alone,²⁴⁻²⁷ and patients with chronic HDV have an increased risk of cirrhosis and hepatocellular carcinoma.²⁸ HBV-infected recipients can provide rescue capabilities to HDV, apparently even when HBsAg is serologically undetectable in a chronic HBV carrier.²⁹ Thus, even though screening of blood donors for HBsAg is universal, concern remains about the potential transmission of HDV to hemophiliacs. Heat treatment of factor VIII and factor IX preparations was initiated in 1983 and 1985, respectively, but HDV is not inactivated completely by the dry heating process.³⁰ Thus, earlier "virus-attenuated" factor replacement products may still have been contaminated with infectious HDV. In addition, although anti-HBc screening was introduced into blood banks in 1986 and would have provided further protection against HDV transmission, this safety feature was not extended to donor blood used in the preparation of factor concentrate administered to hemophiliacs.

There have been limited studies assessing the role of HDV and other viral hepatitis infections in the hemophilic population.³¹⁻³⁵ Because of this, a prospective study was initiated in 1987 to assess the prevalence of HBV, HCV, and HDV in a large representative hemophilic population in the United States, and to relate these findings to various risk factors found in the study group, especially infection with HIV. Subsequent reports will examine liver disease as well as prospective follow-up data from this cohort.

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MATERIALS AND METHODS

Study Population (N = 727)

Subjects were enrolled at nine regional hemophilia treatment centers: The University of Texas Health Science Centers at Houston, San Antonio, and Dallas; Oklahoma Hemophilia Center; Hemophilia Center of Western Pennsylvania; Children's Hospital of Los Angeles; Great Lakes Hemophilia Foundation; N.E. Wisconsin Hemophilia Center; and The University of Wisconsin Center. The Eugene B. Casey Hepatitis Research Laboratory at Baylor College of Medicine served as the coordinating center. Human IRB approval was obtained at each center. Hemophiliacs were enrolled between May 1987 and October 1988. More than 80% with factor VIII or IX levels of less than 10% chose to participate. These patients were receiving or had received virtually all existing licensed and experimental clotting factor products. A study form was completed and blood specimens obtained from each participant.

Laboratory Studies

Blood samples were processed according to a standard protocol and frozen at -70°C . Once per month, samples were sent on dry ice to the coordinating center. Specimens were assayed for all HBV seromarkers as well as anti-HDV and anti-HCV. Commercial enzyme immunoassay (EIA) kits (Abbott Laboratories, North Chicago, IL) were used for most assays. Anti-HBs results were calculated in milli-international units per milliliter (mIU/mL)³⁶ using a radioimmunoassay (RIA) kit (Abbott Laboratories). A level of ≥ 1.5 mIU/mL was considered positive. All anti-HDV-reactive samples were tested for IgM anti-HDV (Hepatitis Branch, Centers for Disease Control [CDC], Atlanta, GA)³⁷ and HDV RNA (courtesy of Dr Margaret Gallagher, CDC, and Dr John Gerin, Georgetown University). Abbott EIA anti-HCV kits produced before licensure by the Federal Drug Administration (FDA) were used to assay for anti-HCV. Repeatedly reactive results were confirmed at Abbott by a supplemental blocking anti-HCV test using synthetic peptides sp42, sp117, and sp65 from the C100-3 region as antigens. In addition, all negative samples were retested by an improved, more sensitive anti-HCV assay that used epitopes from the nonstructural, envelope, and capsid regions of the genome (courtesy of Dr Mary Kuhns, Abbott Laboratories). HIV testing (EIA; confirmation by Western blot) had been performed previously at each hemophiliac treatment center.

Data Management and Statistics

Data entry was performed at the coordinating center. Testing for differences in proportions was accomplished using the χ^2 statistic or Fisher's Exact test. Differences in means were evaluated using the *t*-test or analysis of variance.

RESULTS

The majority of the 727 patients had hemophilia A (84%). Two-thirds (66%) of the subjects had severe hemophilic disease ($<1\%$ factor level) and 21% had moderate disease (1% to 5% factor level). Subjects with hemophilia A were more likely to be classified as severe (68%) than were those with hemophilia B (52%) ($P < .001$). The mean age for the entire group was 19 years (range, 0.75 to 71 years). HIV infection was present in 53%, whereas HCV was detected in 89%. Active HBV infection (HBsAg positive) was detected in 8%, and 19% of these were actively infected with HDV.

Because of the many confounding variables associated with viral hepatitis and HIV infection, the study was divided into two groups based on HIV status. One cohort consisted of

382 "HIV positive" hemophilic patients, whereas a second group was composed of 345 "HIV-negative" patients. The terms "anti-HIV positive" and "HIV positive" will be used interchangeably because all of these patients had either been confirmed positive by Western blot assay or had tested repeatedly positive by EIA over several years, both of which are consistent with active infection in this cohort. Study subjects who were negative for anti-HIV were considered to be "HIV negative".

HIV-Positive Group

Demographics. The mean age of the 382 HIV-infected subjects was 22 years (range, 1.9 to 62 years). Ninety percent were diagnosed with hemophilia A. Severe to moderate hemophilia was present in 93%.

Hepatitis serology. The distribution of seromarkers for a past or present infection with HBV (HBsAg and/or anti-HBc), HCV (anti-HCV positive), and HDV (anti-HDV positive) among HIV-positive hemophiliacs is shown in Table 1. Only three subjects ($<1\%$) had no serologic evidence of infection with these viruses, whereas 75% had experienced infection with HBV and 98% infection with HCV. Sixty-five percent had serologic evidence of infection with both HBV and HCV.

HIV-positive patients were classified into one of five serologic categories depending on their HBV marker status (Fig 1A). One group consisted of 18 patients (5%) with no seromarkers of HBV, whereas 78 subjects (20%) expressed anti-HBs without concurrent HBsAg or anti-HBc. A third group of 20 hemophiliacs (5%) was positive for anti-HBc, but negative for other HBV markers. Greater than half of the HIV-positive patients (58%) had evidence of HBV immunity (anti-HBc and anti-HBs positive/HBsAg negative). A total of 46 patients (12%) were positive for HBsAg and anti-HBc, indicating active HBV infection. One of these subjects also was positive for IgM anti-HBc. A subsequent specimen showed that he cleared his HBV infection with the development of anti-HBs. The remaining 45 subjects were presumed to be chronic carriers of HBV.

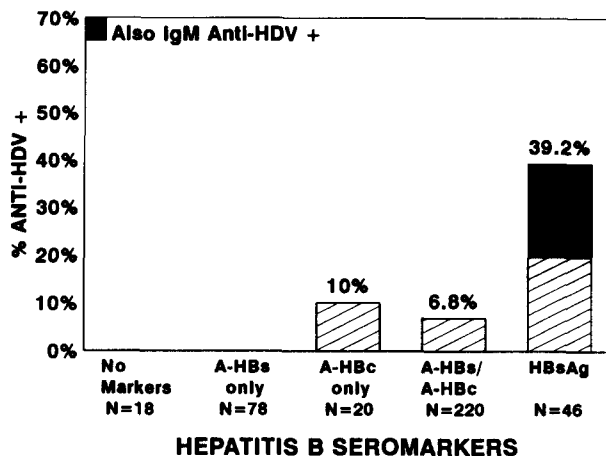
Thirty-five subjects (9.2% of the HIV-infected group) were positive for anti-HDV, of which 18 (51%) were HBsAg positive. Significantly, serologic evidence of HDV infection occurred only in subjects who were currently or previously infected with HBV. The mean age of the anti-HDV-positive

Table 1. Viral Hepatitis Serology in Relation to HIV Status

Hepatitis Serology	HIV-Positive (N = 382)		HIV-Negative (N = 345)	
	N	%	N	%
None	3	0.8	68	20
HBV only	1	0.3	4	1
HBV and HDV	2	0.5	0	0
HCV only	93	24	157	46
HBV and HCV	250	65	103	30
HBV, HCV, and HDV	33	9	13	4

Abbreviations: None, no markers or anti-HBs positive only; HBV, anti-HBc positive; HCV, anti-HCV positive; HDV, anti-HDV positive.

A. HIV POSITIVE



B. HIV NEGATIVE

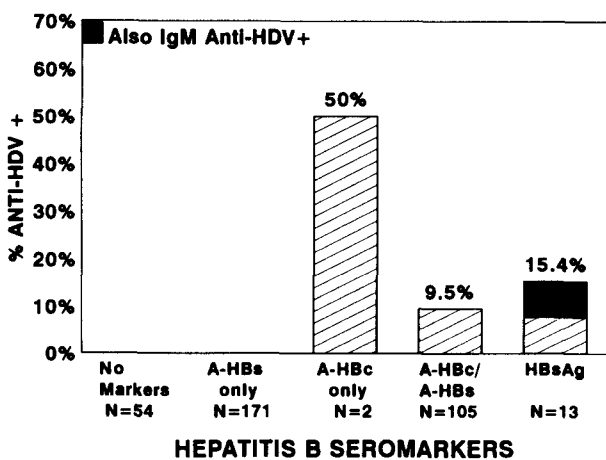


Fig 1. HDV infection rates among hemophiliacs by HIV and hepatitis B seromarker status.

hemophiliacs was 22 years (range, 8 to 49 years), identical to the group as a whole. HDV infection was distributed differently between individuals with hemophilia A and hemophilia B (Fig 2). Among 357 HIV-positive patients with moderate or severe hemophilic disease, 8.4% of the 321 hemophilia A patients were positive for anti-HDV compared with 22% of the 36 hemophilia B patients ($P < .05$). Ten of the 35 anti-HDV-positive subjects (29%) also were positive for IgM anti-HDV, a marker of active HDV disease.^{38,39} All 10 IgM anti-HDV-positive samples came from patients who were expressing HBsAg. As expected, those individuals who were positive for IgM anti-HDV antibody had a higher total anti-HDV concentration than was present in the serum of the IgM anti-HDV-negative group (mean cutoff-to-sample optical density [OD] ratios of 83 v 11; $P < .01$). Of the 35 HIV-positive/anti-HDV-positive patients, 34 were tested for the presence of HDV RNA by slot blot hybridization. Seven of

the nine (78%) IgM anti-HDV-positive sera tested were HDV RNA positive. In contrast, none of the remaining 25 anti-HDV-positive specimens, negative for IgM anti-HDV, tested positive for HDV RNA ($P < .001$).

HCV infection was highly prevalent in the HIV-infected population (98% anti-HCV positive). Thus, no differences in anti-HCV prevalence rates were found based on hemophilia A or B diagnosis or on hemophilia disease severity or found by HBV serogroups. In the three subjects less than 5 years of age, two were already anti-HCV positive and this rate increased to 98% in those between 5 and 10 years of age.

Relationship of HBV immunization status to hepatitis B serology. Only 11% of 371 HIV-positive hemophiliacs indicated that they had been immunized. Anti-HBs (\pm anti-HBc) was detected in 84% of those who indicated that they had received one or more doses of vaccine. However, among the serogroup positive for anti-HBs alone, only 32% indicated that they had received one or more doses of HBsAg vaccine. These subjects were significantly younger than those who did not report a history of immunization (9 years v 24 years; $P < .001$). However, there was no significant difference between the geometric mean anti-HBs levels of the two groups. It is noteworthy that 3 of 13 (23%) hemophiliacs with no identifiable HBV markers had received two or more inoculations of the HBsAg vaccine.

HIV-Negative Group

Demographics. In our study, 345 hemophiliacs repeatedly tested anti-HIV negative. The average age of this group was 16 years (range, 0.75 to 71 years). Patients with hemophilia A accounted for 77% of this study group, while severe to moderate disease was present in 78%.

Hepatitis serology. Table 1 shows the prevalence rates for antibodies to HBV, HDV, and HCV among HIV-negative individuals. One-fifth of the group was free of viral hepatitis markers that would indicate past or current infection, whereas 35% had experienced an HBV infection and 80% an HCV

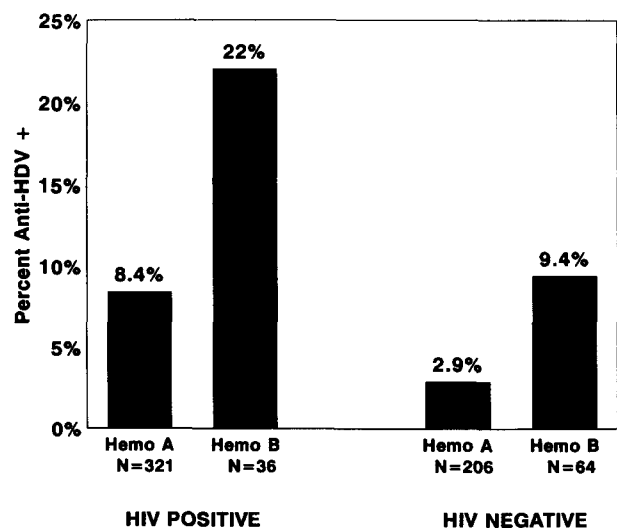


Fig 2. HDV infection rates by hemophiliac diagnosis.

infection (versus 75% and 98% for the HIV-positive group, respectively). Serologic evidence for both HBV and HCV infection was observed in 34% of the group (versus 75% of the HIV-positive group). However, 46% of the subjects (versus 24%) had been exposed to HCV only.

Figure 1B illustrates the various hepatitis B seromarker patterns detected in this group. Nearly one-sixth (16%) of the HIV-negative individuals were hepatitis B marker negative, whereas an additional 50% expressed anti-HBs only. Thus, two-thirds of the HIV-negative group had no serologic evidence for a past or current infection with HBV (versus only 25% of the HIV-positive group). Thirty percent of the HIV-negative group was HBV immune and only 4% was positive for HBsAg and anti-HBc. None of this latter group was IgM anti-HBc positive. Only two patients were anti-HBc positive without other seromarkers of HBV infection. Among severe hemophiliac patients, 41% had evidence of current or previous HBV infection, whereas only 27% of those with mild disease had such evidence.

Thirteen HIV-negative individuals (3.8%) were anti-HDV positive. These patients were significantly older than those without markers of HDV infection (26 years ν 16 years; $P \leq .01$). As observed in the HIV-infected group of patients, HDV infection was not detected in the absence of an anti-HBc response, and anti-HDV was distributed differently between the 206 HIV-negative hemophilia A and 64 hemophilia B patients with severe to moderate disease (Fig 2). Specifically, hemophilia B patients were significantly more likely to be anti-HDV positive (9.4% ν 2.9%; $P < .05$). Only one of the anti-HDV-positive patients had detectable IgM anti-HDV, and he was HBsAg positive. After HDV RNA testing of the 13 anti-HDV-positive samples, the one IgM anti-HDV-positive serum and an additional HBsAg/anti-HDV-positive sample (IgM anti-HDV negative) contained detectable HDV RNA. Thus, both HDV infections that occurred in the HBsAg group appeared to be active.

In contrast to the HIV-positive subjects, HCV infection was not evenly distributed across the five HBV serogroups. Slightly less than half of the group with no markers of HBV exposure expressed HCV seromarkers compared with 92% of those with past or current HBV infection ($P < .001$). Hemophilia A patients were significantly more likely to be seropositive for HCV than were hemophilia B patients (83% ν 65%; $P < .001$). In addition, severe hemophilia A patients were more often infected with HCV (85%) than were those with moderate (78%) or mild (59%) factor level deficiencies ($P < .001$). This trend continued among hemophilia B patients (severe, 68%; moderate, 50%; mild, 36%; $P < .001$).

Relationship of HBV immunization status to hepatitis B serology. Fifty-three percent of 335 HIV-negative subjects indicated they had received one or more inoculations of HBsAg vaccine, a level of participation that was significantly greater than the 11% reported by the HIV-positive group ($P < .001$). Anti-HBs (\pm anti-HBc) was detected in 90% of this group. However, in contradistinction to the HIV-positive group, 87% of the subjects who were anti-HBs positive only indicated that they had received one or more doses of vaccine. Compared with the nonimmunized group expressing anti-HBs only, these vaccinated hemophiliac patients, like the

HIV-positive subjects, were significantly younger (8 years ν 28 years; $P < .001$), but their geometric mean level of anti-HBs was much higher (839 mIU/mL ν 102 mIU/mL; $P < .001$). Among 54 HBV seromarker-negative individuals, 19% stated that they had received two or more inoculations of the HBsAg vaccine, a frequency similar to that seen in the HIV-positive group.

DISCUSSION

Hemophiliacs have many risk factors that are associated with hepatitis infections, including age; disease severity; the use of factor concentrate versus cryoprecipitate or designated donor replacement therapy, type, and amount of concentrate used; and the virucidal treatment procedures that the concentrate has undergone. A multicenter study population of 727 hemophiliac patients was assembled to determine the 1987 to 1988 period prevalence of viral hepatitis. The data presented here form the baseline for a prospective study of the incidence of HBV, HCV, and HDV in this group. Basic demographic analysis shows that our study group is similar to the hemophiliac population in the United States in type of factor deficiency, severity, sex, and age distribution.⁴⁰

An important confounding variable in this investigation is HIV infection, which can alter immune function and affect a patient's antibody status and the progression of liver disease. Because of these potential uncertainties, it was decided to divide the hemophilia population into HIV-positive and -negative groups at the start of these analyses. As expected, the HIV-positive group was older and more likely to be classified as having severe hemophilia. While 74% of HIV-positive subjects had serologic evidence of HBV and HCV infection, only 34% of the HIV-negative subjects were similarly infected. Correspondingly, more HIV-negative patients were likely to have a single infection with HCV. These differences are presumably related to three factors, all of which are more likely to affect younger (ie, HIV-negative) patients: (1) hepatitis B vaccination, (2) recent HBsAg and ALT screening of blood products used in the manufacture of factor concentrate, and (3) use of virus-attenuated products as well as blood donor self-exclusion criteria initiated after the advent of acquired immunodeficiency syndrome (AIDS) and the introduction of new tests for hepatitis.

Patients were classified into hepatitis B groups depending on the pattern of their HBV markers, representing different outcomes with HBV. The percentage of patients who had no serologic evidence of exposure to HBV was relatively low in the HIV-positive group (25%), but reached a level of 65% in the HIV-negative group. How these hemophiliacs avoided exposure is of some interest. One might expect that these patients were mild hemophiliacs who rarely needed factor replacement therapy. However, this was not the case, as 61% of the 18 HIV-positive hemophiliacs and 26% of the 54 HIV-negative hemophiliacs with no HBV seromarkers had factor levels less than 1%. An alternative explanation is that these patients were young children who had only received virus-attenuated or HBV-screened clotting factor. However, the average age of the subjects in this subset was 17 years for the HIV-positive group and 20 years for the HIV-negative group, similar to the remaining population. Vaccination followed

by undetectable but potentially protective immune responses might be another consideration, but only 3 of 13 (23%) HIV-positive and 10 of 54 (19%) HIV-negative patients with no hepatitis B markers admitted to previous vaccination. Thus, the lack of HBV infection in this group may simply be due to a fortuitous set of circumstances whereby they were not exposed to HBV-contaminated lots of factor concentrates, may have been infected in the past with HBV but lost all markers of this infection, were genetically resistant to infection, and/or were protected by previous immunization. In addition, the presence of anti-HBs in the donor pool may have further reduced the possibility of infection. The high prevalence of HCV in the HIV-positive (97%) and -negative (67%) groups without HBV markers is presumably due to lack of screening tests for this virus, the higher proportion of carriers of HCV, and a lack of neutralizing antibody among blood donors.

Of the 22 individuals in the anti-HBc-only serogroup, 20 were HIV positive. This serologic pattern may represent a nonspecific (false-positive) anti-HBc reaction, an active infection in which HBsAg remains undetectable by conventional assays, a superinfection with HCV that interferes with HBV expression⁴¹ or a selective loss of anti-HBs over time,³⁶ especially when HIV infection is present. Nonspecific reactions are generally characterized by EIA OD readings near the cutoff value.⁴² In this study, 21 of these 22 subjects had OD levels that were more than two times greater than the cutoff value. This would suggest that nonspecificity is less likely to account for this serologic pattern. While HDV infection is also known to depress HBsAg levels, none of these 22 patients were actively infected with HDV, although 96% had an HCV infection.

In the total study population, 60 participants (8%) were actively infected with HBV. However, these rates were significantly different between the HIV-positive (12%) and -negative groups (4%), a function of the similarity of modes of transmission of HBV and HIV. When evaluating only those subjects with HBV markers of infection, eg, anti-HBc-positive individuals, the prevalence of HBsAg was found to be 16% of 289 HIV/HBV-infected hemophiliacs versus 12% of 119 HBV-infected subjects who were anti-HIV negative ($P = .36$). While HIV infection is known to increase the risk of becoming a carrier after acute HBV infection,⁴³⁻⁴⁵ this only occurs when HIV infection exists before the HBV infection. The reverse situation is more likely to have occurred in most of the HBV/HIV-infected hemophiliacs in our study, thus leading to the similar ratio of HBsAg carriage seen among the anti-HBc-positive individuals.

Forty-eight subjects (6.6%) were anti-HDV positive and all had HBV seromarkers of infection (anti-HBc positive). This attests to the inherent problem of delta hepatitis in the hemophilic population regardless of whether the patients are HIV infected or not. Despite the higher frequency of hepatitis B in HIV patients, the data suggest that HBV and HDV infections frequently occur as coinfections in hemophiliacs who subsequently resolve both infections. Eleven of 20 (55%) HBsAg carriers who were anti-HDV positive also had IgM anti-HDV and 8 of 10 tested were positive for HDV RNA, presumably indicating active infection. Although numbers

are small, rates of active infection with HDV among HBsAg carriers were similar in the HIV-positive and -negative groups. This suggests that HIV infection, whether acquired before or after HBV and HDV infection, does not influence the outcome of HDV infection. The young age of two anti-HDV-positive patients (8 and 9 years) indicates that screening of donors for HBV, which began in 1974, has not eliminated the risk of contracting delta hepatitis, at least by 1987. However, concerns expressed about the ability of HBV immune patients to provide rescue capabilities to HDV appear to be unfounded as all cases of active HDV infection occurred only in HBsAg-positive patients.

None of the 71 patients with no viral markers (or anti-HBs only) reported a history of clinical hepatitis compared with 21% of the 656 subjects who experienced an infection with one or more of the three viruses ($P < .001$). Rates of clinical expression of disease were not significantly different among the HIV-positive or -negative group.

Because a greater percentage of hemophilia A patients were classified as having severe factor level deficiency, hemodiagnosis was stratified by disease severity. Among the HIV-positive subjects, there was no significant difference in hepatitis B or C infection rates after stratification. However, when those patients negative for HIV were stratified by disease severity and hemodiagnosis, it was found that severe hemophilia B patients, but not moderate or mild, were more likely to have experienced hepatitis B infection than were severe hemophilia A patients (57% v 37%; $P < .05$). Because all factor level groups studied showed a higher incidence of hepatitis B infection among the hemophilia B patients, it is probably the case that this was the only group showing statistical significance due to its larger number of hemophilia B patients ($n = 39$). An important observation is that HDV markers also were most prevalent in type B hemophilia after controlling for disease severity ($P < .02$). This occurred despite the fact that vaccination rates were equal among the hemophilia diagnosis groups. These data provide evidence of differences in safety between factor VIII and factor IX preparations and suggest that factor IX preparations have been contaminated by HBV, HCV, and HDV more heavily and/or for a longer period of time than factor VIII preparations. The lag in technology applications for virus-attenuation of factor IX or differences in the manufacturing techniques that may result in higher levels of HBV/HDV contamination of prothrombin complex concentrates are potentially responsible for this observation. These factor IX materials have not been tested for infectivity in previously untreated persons, lending credibility to this hypothesis.

In conclusion, a large scale study of HBV, HCV, and HDV seromarkers in hemophiliacs has confirmed that these diseases continue to be prevalent in this population. Ongoing longitudinal studies should provide additional information regarding incidence rates of HBV, HCV, and HDV, the clinical course of hepatitis in hemophiliacs, and the impact of currently used factor replacement products.

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