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## Chronic Hepatitis in Patients With Hemophilia A: Histologic Studies in Patients With Intermittently Abnormal Liver Function Tests

By Gilbert C. White II, Kenneth D. Zeitler, Henry R. Lesesne, Campbell W. McMillan, Martha S. Warren, Harold R. Roberts, and Philip M. Blatt

Recent studies in multiply transfused patients with hemophilia A and persistent liver function abnormalities have shown a high incidence of chronic active hepatitis. The purpose of the present study was to determine the severity of liver disease in multiply transfused patients with intermittent liver enzyme abnormalities. Fifteen patients with elevated enzymes on two or three out of four determinations at 6-mo intervals were studied. None had signs or symptoms of chronic liver disease. Thirteen had serologic evidence of prior exposure to the hepatitis B virus. Liver biopsy performed on these patients after replacement

therapy with factor VIII showed chronic persistent hepatitis or other mild forms of liver disease in 14 of the 15 patients. Patients with chronic persistent hepatitis had significantly higher mean liver enzymes at time of biopsy than patients with milder forms of hepatic inflammation, but there was no relationship between liver histology and hepatitis B serology or the amount of factor VIII used in the 6 mo preceding biopsy. These findings support the continued use of factor VIII concentrates in patients with hemophilia.

**T**HE REPEATED EXPOSURE to blood products places the individual with severe hemophilia at high risk to develop both acute and chronic posttransfusion liver disease. Several recent studies designed to document the incidence of liver dysfunction in hemophilia have shown that approximately 85% of patients with clinically severe hemophilia have serologic evidence of prior exposure to the hepatitis B antigen (HBsAg).<sup>1-6</sup> Approximately 10% demonstrate the presence of circulating HBsAg. The incidence of liver dysfunction is high. In the large study by the Cooperative Inhibitor Group, abnormal aminotransferase (AST or ALT) levels were present on at least one occasion in 76% of hemophiliacs followed at 6-mo intervals for up to 2 yr.<sup>6</sup> Of these patients, some demonstrated continuous enzyme elevations, but the majority had liver dysfunction that is best described as intermittent and mild.<sup>6,7</sup>

Four centers have reported the results of percutaneous liver biopsy in hemophiliacs with continuously abnormal liver function.<sup>8-11</sup> The results suggest an almost equal incidence of chronic persistent hepatitis and chronic active hepatitis, with histologic features of chronic active hepatitis that ranged from subacute hepatitis with bridging necrosis to active necrosis with fibrosis and cirrhosis. Similar histologic studies are not available in the larger group of patients with intermittent liver function abnormalities.

Since there appeared to be a high frequency of chronic active hepatitis in patients with continuously abnormal liver function tests, we have undertaken a prospective study to examine liver histology in hemophiliacs with intermittently abnormal liver function tests to determine the type and severity of liver disease in this portion of the hemophilic population.

### MATERIALS AND METHODS

#### Patient Population

Informed consent was obtained in accordance with the Helsinki Declaration and this study was reviewed and approved by the Committee on the Protection of Human Rights of the University of North Carolina School of Medicine.

Patients participating in the study were selected from the Comprehensive Hemophilia Diagnostic and Treatment Center at the University of North Carolina. All patients have liver function tests performed at 6-mo intervals during routine visits to the center. Of 195 patients with severe hemophilia enrolled in the Center, 152 had abnormal aminotransferase levels on one or more occasions. This group of patients was further divided into those who had been followed at least 1.5 yr (at least 4 determinations of aminotransferases at 6-mo intervals) and those followed less than 1.5 yr (less than 4 determinations). Patients in the former group were considered for the study.

Patients were considered eligible if abnormal aminotransferase levels were present on two or three of the most recent four determinations at 6-mo intervals. In many instances, this pattern of intermittent liver dysfunction had been present for many years. In those patients, eligibility for inclusion in the study was based on the most

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recent four determinations. Abnormal enzyme levels were considered to be any value above normal. Patients were excluded from the study if a factor VIII inhibitor was present or they were less than 3 yr of age.

Of the 60 patients who met criteria for inclusion into the study, 20 had a regularly scheduled clinic visit during the 6-mo course of the study and were approached to participate in the study. Five patients elected not to participate. The remaining 15 patients form the basis of this report.

Patients consenting to participate in the study were scheduled for admission to the Clinical Research Unit within 6 mo of their last enzyme determination. A factor VIII inhibitor assay was performed and checked the day prior to biopsy and liver enzymes, hepatitis serology [hepatitis B surface antigen (HBsAg), antibody to the hepatitis B surface antigen (anti-HBs), antibody to the hepatitis B core antigen (anti-HBc), and antibody to the hepatitis Be antigen (anti-HBe)] and blood group type were obtained the day before the biopsy. Patients were prepared for liver biopsy as described previously,<sup>7</sup> except that a fall-off study was not routinely performed. The biopsy was performed under coverage with factor VIII concentrates by continuous infusion (cryoprecipitate was used in one case) for a total of 60 hr after the biopsy, and the patients remained on bolus factor VIII therapy for an additional 12 hr at home. The average total in-patient use of factor VIII per patient for the biopsy procedure was 16,950 U.

#### Liver Function Tests

HBsAg, anti-HBs, anti-HBc, and anti-HBe were determined using radioimmunoassay kits from Abbott Laboratories (North Chicago, Ill.). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured with an automated enzymatic assay (Smith, Kline Instruments, Plus-Chem Reagents, Sunnyvale, Calif.). Controls were measured with each run. Normal values for ALT and AST for this laboratory are less than 30 U/ml.

#### Liver Biopsy Interpretation

Biopsy specimens were fixed in paraformaldehyde and sections were stained with hematoxylin and eosin, periodic acid-Schiff reagent, trichrome for collagen and fibrin, silver impregnation for reticulin fibers, and Gormi's stain for iron. The histologic appearance was assessed at the time of the biopsy and blindly at the end of the study by one of the authors (H.R.L.) and by Dr. Joseph Grisham of the Department of Pathology. Biopsies were classified as chronic persistent hepatitis or chronic active hepatitis with or without cirrhosis, as defined by the Fogarty Diseases of the Liver and Biliary Tract International Committee.<sup>11</sup> The extent of fibrosis, if present, was estimated as mild, moderate, or severe.

## RESULTS

#### Comparison of Biopsy Group With Study Group

In order to extrapolate from the group chosen for biopsy to the whole group with intermittent liver dysfunction, the biopsied patients and the remainder of the group were compared with respect to age, use of factor VIII, and severity of liver dysfunction. Patients who were not biopsied had higher average ALT levels than patients who were biopsied, but AST levels, age, pattern of liver dysfunction, and exposure to factor VIII in the two groups were comparable (Table 1).

**Table 1. A Comparison of Liver Biopsy and Non-Liver Biopsy Patients With Intermittent Liver Dysfunction**

	Biopsy Patients*	Non-Biopsy Patients*
Number	15	45
Age (yr)†	22.9 ± 13.1	23.2 ± 14.9
Pattern of dysfunction‡	2.7/4	2.4/4
Factor VIII use/6 mo†	24,424 ± 42,445	27,091 ± 35,666
Cryoppt/concentrate	1/15	1/44
Aminotransferase levels§		
AST (U/ml)†	42.2 ± 23.0	44.6 ± 34.7
ALT (U/ml)†	60.1 ± 51.8	136.4 ± 991.0

\*Biopsy patients are hemophiliacs who underwent liver biopsy. Non-biopsy patients are hemophiliacs who qualified for the study but were not biopsied either because they were not seen for a regular clinic visit during the course of the study or they refused biopsy.

†Results are expressed as mean ± SD.

‡Patients had abnormal aminotransferase levels on either 2 or 3 of the most recent 4 determinations to qualify for the study. To arrive at a comparative score, the number of abnormal determinations per patient were averaged.

§Average aminotransferase levels were determined from the four determinations used to assess eligibility for the study.

#### Liver Dysfunction in Biopsy Group

None of the biopsied patients demonstrated clinical symptoms of chronic hepatitis and none had hepatomegaly, splenomegaly, or cutaneous stigmata of chronic liver disease. Table 2 shows the biochemical extent of liver dysfunction in the biopsied patients and the biopsy results. Four of 15 patients had enzyme abnormalities on 2 of the most recent 4 determinations, and 11 of 15 patients had abnormalities on 3 of the most recent 4 determinations. It should be emphasized that some patients (nos. 5, 6, 11, 12 and 14) had normal enzymes at the time of biopsy, while others (nos. 4, 13, and 15) had enzyme elevations that were more than twice normal. One of the 15 patients had a positive HBsAg and 12 patients had a positive anti-HBs or anti-HBc. Two patients, one (no. 2) exposed primarily to third-generation products that have been RIA-tested for HBsAg and the other (no. 9) exposed almost exclusively to cryoprecipitate, had no serologic evidence of prior exposure to hepatitis B virus.

On final biopsy interpretation, 11 of the 15 patients biopsied had classical chronic persistent hepatitis, 3 patients had mild chronic portal inflammation but did not have the changes of chronic persistent hepatitis, and 1 had chronic active hepatitis with mild fibrosis. None of the patients had histologic features of alcohol-related liver disease. Three patients (nos. 2, 4, and 12) were reclassified from mild focal chronic portal inflammation to chronic persistent hepatitis at the final biopsy interpretation. With either histologic clas-

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**Table 2. Liver Function and Histology in Severe Classical Hemophiliacs With Intermittent Liver Dysfunction**

Patient	Age (yr)	Pattern of Liver Dysfunction*	ALT/AST at Biopsy†	HBsAg/Anti-HBs/anti-HBc/Anti-HBe	Liver Histology
1	6	N-A-N-A	34/39	+/- / -	Chronic persistent hepatitis
2	7	N-A-N-A	24/35	-/- / -	Chronic persistent hepatitis
3	9	A-A-A-N	31/38	-/+ / +	Chronic persistent hepatitis
4	9	A-A-N-A	204/113	-/- / +	Chronic persistent hepatitis
5	10	A-N-N-A	18/23	-/+ / +	Chronic persistent hepatitis
6	11	A-N-A-A	19/26	-/+ / +	Mild focal chronic portal inflammation
7	18	A-A-N-A	85/25	-/+ / -	Chronic persistent hepatitis
8	23	A-A-A-N	25/19	-/+ / +	Mild focal chronic portal inflammation
9	30	N-N-A-A	93/36	-/- / -	Chronic persistent hepatitis
10	32	N-A-A-A	60/20	-/+ / +	Chronic persistent hepatitis
11	33	A-A-N-A	15/13	-/+ / +	Mild focal chronic portal inflammation
12	34	A-A-A-N	24/29	-/+ / +	Chronic persistent hepatitis
13	35	A-N-A-A	245/95	-/+ / -	Chronic persistent hepatitis
14	40	A-A-N-A	21/21	-/+ / +	Chronic persistent hepatitis
15	47	A-N-A-A	144/87	-/+ / +	Chronic active hepatitis, mild fibrosis

\*The pattern of liver dysfunction is the pattern of liver enzymes at 6-mo intervals. A, one or both enzymes abnormal. N, both enzymes normal. Thus, A-A-A-N indicates that on the first, second, and third clinic visits, one or both enzymes were abnormal, on the fourth visit, both were normal.

†Normal values for ALT and AST are less than 30 U/ml.

sification, there was no apparent relationship between liver histology and age or hepatitis B serology (Table 3). Patients with histologic evidence of chronic persistent hepatitis had higher enzyme levels at the time of biopsy and received more factor VIII in the 6 mo preceding biopsy than patients with focal chronic portal inflammation, but these differences were not statistically significant.

#### DISCUSSION

The high frequency of liver function abnormalities in patients with hemophilia coupled with biopsy evidence of chronic active hepatitis and the documentation of progression to cirrhosis<sup>8-11,13</sup> have led some authors to question current policies of replacement

with concentrates of factor VIII for some patients.<sup>14</sup> While some patients, primarily those with moderately severe and severe enzyme elevations, will have histologic evidence of chronic active hepatitis and/or cirrhosis,<sup>8-11,13</sup> the results of the present study indicate that a larger proportion of patients will have milder degrees of enzyme abnormalities and predominantly chronic persistent hepatitis or milder forms of liver disease. Thus, for many transfusion-requiring hemophiliacs, the frequent exposure to factor VIII concentrates is not accompanied by the development of the more severe forms of chronic liver disease.

Patients with chronic persistent hepatitis received more factor VIII in the 6-mo period preceding liver biopsy and had significantly higher enzyme levels at the time of biopsy than patients with nonspecific inflammation of the liver. The difference in factor VIII use did not achieve statistical significance because of the small sample size, but there was a trend suggesting that there might be a relationship between factor VIII use and the histologic appearance of the liver. Such a relationship might have important clinical implications and should be evaluated in an appropriately designed study.

The development of chronic forms of liver disease may begin very early in patients whose hemophilia requires transfusion. Patients 1-5 had all developed the classic histologic features of chronic persistent hepatitis by the age of 10 and, although we did not observe more aggressive forms of liver disease in our

**Table 3. A Comparison of Patients With Different Histologic Forms of Liver Disease**

	Focal Chronic Portal Inflammation	Chronic Persistent Hepatitis
Number	3	11
Age (yr)*	22.3 ± 11.0	20.9 ± 13.3
Aminotransferase levels		
AST (U/ml)*	19.3 ± 6.5	43.1 ± 31.1†
ALT (U/ml)*	19.6 ± 5.0	76.2 ± 78.2‡
Factor VIII/6 mo*	15,453 ± 10,520	25,818 ± 34,740§

\*All results are expressed as mean ± SD.

†One-sided  $p = 0.03$  using Mann-Whitney test.

‡One-sided  $p = 0.04$  using Mann-Whitney test.

§One-sided  $p = 0.23$  using Mann-Whitney test.

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younger patients with intermittent enzyme abnormalities, Mannucci and coworkers have described chronic active hepatitis in 3 patients below the age of 10 yr with continuous liver enzyme abnormalities.<sup>11</sup>

The etiolog(ies) of the liver disease in the population that was biopsied in this study is uncertain. Two patients, nos. 2 and 9, were negative for hepatitis B markers and may be presumed to have chronic liver disease from non-A, non-B viruses. The remainder of the patients were positive for hepatitis B markers but probably have been exposed to multiple hepatitis viruses. Histologically, the two patients negative for hepatitis B markers had chronic persistent hepatitis, but their histology was not discernably different from that of patients with chronic persistent hepatitis and positive hepatitis B markers. None of the patients had histologic features of alcohol-induced liver disease.

In summary, a prospective study of liver biopsy has been undertaken in a group of hemophiliacs with intermittent and mild liver enzyme abnormalities. The results show primarily chronic persistent hepatitis and milder forms of liver disease and support the continued use of concentrates in transfusion-requiring hemophiliacs. At the same time, although the results of this study indicate a predominantly benign hepatic histology in patients with intermittent enzyme abnormalities, any form of liver disease is obviously undesirable and continued efforts to prepare hepatitis-free products should remain an issue of high priority.

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#### REFERENCES

1. Mannucci PM, Capitanio A, del Ninno E, et al: Asymptomatic liver disease in haemophiliacs. *J Clin Pathol* 28:620, 1974
2. Webster WP, Blatt PM, Lesesne HR, Roberts HR: Liver function tests in hemophiliacs, in Fratantoni JC, Aronson DL (eds): *Unsolved Therapeutic Problems in Hemophilia. Proceedings of a Conference sponsored by Bureau of Biologics, FDA, Division of Blood Diseases and Resources, NHLBI, National Hemophilia Foundation, Bethesda, Md, March 1-2, 1976. DHEW Publication no. (NIH) 77-1089, 1977, p 35*
3. Hasiba UW, Spero JA, Lewis JH: Chronic liver dysfunction in multi-transfused hemophiliacs. *Transfusion* 17:490, 1977
4. Hilgartner MW, Giardina P: Liver dysfunction in patients with hemophilia. *Scand J Hematol (Suppl)* 30:6, 1977
5. Levine PH, McVerry BA, Attock B, Dormandy KM: Health of the intensively treated hemophiliac, with special reference of abnormal liver chemistries and splenomegaly. *Blood* 50:1, 1977
6. Cederbaum AI, Blatt PM, Levine PH: Abnormal serum transaminase levels in patients with hemophilia A. *Arch Intern Med* 142:481, 1982
7. Hasiba U, Eyster ME, Gill FM, Kajani M, Lewis JH, Lusch CJ, Prager D, Rice SA, Shapiro SS: Liver dysfunction in Pennsylvania's multitransfused hemophiliacs. *Dig Dis Sci* 25:776, 1980
8. Lesesne HR, Morgan JE, Blatt PM, Webster WP, Roberts HR: Liver biopsy in hemophilia A. *Ann Intern Med* 86:703, 1977
9. Spero JA, Lewis JH, Van Thiel DH, Hasiba U, Rabin BS: Asymptomatic structural liver disease in hemophilia. *N Engl J Med* 298:1373, 1978
10. Preston FE, Triger DR, Underwood JCE, Bardhan G, Mitchell VE, Stewart RM, Blackburn EK: Percutaneous liver biopsy and chronic liver disease in Haemophiliacs. *Lancet* 2:592, 1978
11. Mannucci PM, Ronchi G, Rota L, Colombo M: A clinicopathologic study of liver disease in hemophilia. *J Clin Pathol* 31:779, 1978
12. Leevy CM, Popper H, Sherlock S (eds): *Diseases of the Liver and Biliary Tract*. Washington, DC, Government Printing Office, (DHEW Publication no (NIH) 77-725), 1977
13. Lesesne HR, Blatt PM, Taylor RE, White GC, Johnson G: Cirrhosis, variceal bleeding and distal splenorenal shunt in hemophilia A. *South Med J* (in press)
14. Spero JA, Lewis JH, Fisher SE, Hasiba U, Van Thiel DH: The high risk of chronic liver disease in multitransfused juvenile hemophiliac patients. *J Pediatr* 94:875, 1979