A Study of Liver Biopsies and Liver Disease Among Hemophiliacs

By Louis M. Aledort, Peter H. Levine, Margaret Hilgartner, Philip Blatt, Joel A. Spero, Judith D. Goldberg, L. Bianchi, Valeur Desmet, Peter Scheuer, Hans Popper, and Paul D. Berk

Hepatic histologic materials (biopsy or autopsy) and associated clinical data from 155 hemophiliacs were collected by an ad hoc hemophilia study group and analyzed retrospectively in an effort to determine the spectrum of liver disease in this population and to examine the relationship between the severity of liver disease and treatment history. Clinical information on the frequency of complications from 126 biopsies in 115 hemophilic patients provided a unique opportunity to assess the safety of liver biopsy in such patients. The incidence of cirrhosis (15%) and chronic active hepatitis (7%) was lower than previously reported. The frequency of severe liver disease (chronic active hepatitis or cirrhosis) in patients receiving large pooled concentrates was no greater than in patients treated principally with cryoprecipitate or plasma. The risks of liver biopsy in this setting are relatively high: clinically significant hemorrhage followed 12.5% of the procedures.

In the past ten years there has been increasing recognition of, and concern over, the high incidence of abnormal liver function test results, as well as markers for hepatitis B, in hemophiliacs. Several early studies, reviewing deaths and in some instances autopsy material from hemophiliacs, reported essentially no deaths from liver disease. However, recent studies demonstrating that a large majority of hemophilic patients have biochemical evidence of hepatic dysfunction correspond in time with the introduction of concentrated antihemophilic factor replacement materials for these patients and have led to concern about the use of products prepared from pooled plasma.

Increasing numbers of liver biopsies are being performed on hemophiliacs throughout the world. The published results, based principally on a small series, emphasize the severity of the pathologic lesions observed as well as the safety of the procedure. However, we were aware of many biopsy specimens with minimal findings and of at least two unreported deaths following the procedure. Accordingly, an ad hoc group sought to collect and review all available liver biopsy samples on hemophiliacs in an attempt to determine (1) the spectrum of liver disease in hemophiliacs, (2) whether the nature and severity of the liver disease depended on the type and magnitude of prior transfusion therapy, and (3) whether safety warranted the continued performance of liver biopsies on hemophiliacs.

Materials and Methods

Patient population. A small group, designated the Ad Hoc Hemophilia Study Group (AHHSG) initiated this study by contacting all major hemophilia treatment centers listed by the World Federation of Hemophilia in the United States and Western Europe. Additional centers were identified and contacted if they had published data on liver biopsies in hemophilia or were personally known to the investigators. Institutions were asked to submit for review all liver biopsy samples on hemophiliacs in an attempt to determine (1) the spectrum of liver disease in hemophiliacs, (2) whether the nature and severity of the liver disease depended on the type and magnitude of prior transfusion therapy, and (3) whether safety warranted the continued performance of liver biopsies on hemophiliacs.

From the Departments of Medicine (Polly Annenberg Levee Hematology Center) and Biostatistics, and the Stratton Laboratory for the Study of Liver Disease, Mt Sinai School of Medicine, New York; the Department of Medicine, University of Massachusetts Medical School, Worcester, the Department of Pediatrics, Cornell University Medical Center, New York; the Department of Medicine, University of North Carolina, Chapel Hill; the Department of Medicine (Division of Coagulation), University of Pittsburgh School of Medicine and Central Blood Bank of Pittsburgh; the Departments of Pathology of the Universities of Basel, Switzerland, and Louvain, Belgium; and the Department of Histopathology at the Royal Free Hospital and School of Medicine, London.

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Address reprint requests to Dr Louis M. Aledort, Mt Sinai School of Medicine, Fifth Ave and 100th St, New York, NY 10029.

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RESULTS

Demography. Of the patients entered into the study, 80% were factor VIII deficient and 17% factor IX deficient. Three percent of all autopsy cases were unclassified. This older autopsy material, derived from well-known clinicians with a particular interest in hemophilia, antedated the time when specific factor VIII and IX assays were available. Using each institution's local classification based on factor level and clinical symptoms, 79% were classified as severe, 15% mild, and 2% moderate. Collectively, the 115 patients who underwent biopsies and the 40 patients autopsied were comparable with respect to type and severity of hemophilia. Liver disease as a clinical complication of hemophilic care was unrecognized during the period when most of the autopsy material was collected. In addition, availability of tests for hepatitis B markers followed that era. Hence, no analysis was attempted of the scanty laboratory data available on the autopsied patients.

In the biopsy material, biochemical abnormalities equal to or greater than twice the upper limit of normal were present in 47% of cases for SGOT, 57% for SGPT, and 9% for bilirubin. The percentage of cases with abnormal values for each test was substantially higher than previously reported. Twenty-four percent of the patients undergoing biopsies showed positive results for serum HBsAg, in contrast to the usually reported low incidence of 5% to 7% for all hemophiliacs. The higher incidence in our study may represent selection of patients for liver biopsy because of persistent antigenemia as part of the clinical picture. Splenomegaly, hepatomegaly, or both were found in 49% of the patients receiving biopsies. The median age was 28 years at biopsy and 26 years at autopsy.

Treatment history. Seventeen percent of all patients in the study were never exposed to concentrate, 20% had a cumulative lifetime total of <100,000 units of concentrate, and 53% had been exposed cumulatively to >100,000 units of concentrate prior to biopsy or autopsy. Exposure was unknown for 10% of the cases. In contrast to the total study population, only 5% of those who had liver biopsies had never received any concentrate, whereas 53% of the patients whose autopsy material was supplied had never received concentrate. This difference in the pattern of the replacement therapy between these two groups was statistically significant ($\chi^2 = 47.5, P = .0001$) and reflects the fact that the patients who underwent biopsies were current cases, whereas autopsy materials often reflected cases retrieved from older files. Available data were inadequate to examine differences in liver function or hepatitis B markers between biopsy and autopsy cases, again reflecting the paucity of modern test data submitted on the autopsied patients.

Biopsy findings. Although there was good agreement among the four pathologists with respect to the histologic features they observed in each case, there was a surprising amount of disagreement on the final diagnosis. Thus, all four pathologists agreed on a specific pathologic entity in only 55% of the cases, and at least three of four agreed in only 76% of the cases. There was considerable disagreement in the classification of cases representing CPH and CAH. Accordingly, at the consensus meeting, patients were further classified into those whose hepatic lesions were trivial, mild to moderate, or severe; those with cirrhosis (which superseded any coexistent diagnosis); and those with other pathologic features. The spectrum of histopathologic diagnoses that fell into each of these consensus categories is indicated in Table 1.

As shown in Table 2, 64% of all cases had trivial, mild, or moderate hepatic lesions, and only 7% had severe lesions. Fifteen percent had cirrhosis and 14% had other lesions.

**Table 1. Hepatic Disease in Hemophiliacs**

<table>
<thead>
<tr>
<th>Consensus Classification</th>
<th>Histologic Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic hepatitis</td>
<td></td>
</tr>
<tr>
<td>Trivial</td>
<td>Spotty liver cell injury</td>
</tr>
<tr>
<td>Mild/moderate</td>
<td>Chronic inflammatory infiltrate</td>
</tr>
<tr>
<td>Severe</td>
<td>Chronic lobular hepatitis (CLH)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Chronic persistent hepatitis (CPH)</td>
</tr>
<tr>
<td>Other conditions</td>
<td>Chronic active hepatitis (CAH)</td>
</tr>
<tr>
<td></td>
<td>Chronic active hepatitis</td>
</tr>
<tr>
<td></td>
<td>Acute viral hepatitis, drug-associated lesions, fatty liver, alcoholic liver disease, cancer, terminal ischemia (autopsy cases)</td>
</tr>
</tbody>
</table>
Within this classification the distribution of lesion types and severity was comparable for the autopsy and biopsy cases ($\chi^2 = 7.26, P = .06$). Among patients ultimately classified as severe, there was virtual unanimity among the four pathologists in classifying these cases as CAH in their initial review. The overall lower level of agreement in the initial review was seen to reflect principally subtle differences in the weight given by each pathologist to various histologic features in arriving at a specific diagnosis in the majority of cases with borderline disease. Such borderline cases are now known to be common in non-A, non-B hepatitis, a condition in which the value of the conventional classification into CPH and CAH based on the presence or absence of piecemeal necrosis has recently been questioned.15 Because of the lack of agreement on the diagnosis of CAH in patients with mild to moderate hepatic lesions, it is not possible to be more specific than the consensus diagnosis with respect to histologic severity. A comprehensive review of the histologic features in this patient population will be reported elsewhere.

It was of interest that no etiologic speculation was possible in many cases. However, features considered suggestive of non-A, non-B hepatitis16 (Table 3) were frequently noted to predominate in some HBsAg-positive patients in whom histologic features of hepatitis B virus (HBV) infection (eg, ground glass hepatocytes) were lacking. This may suggest that the non-A, non-B agent(s), rather than HBV, caused the ongoing liver disease in such cases, despite the serologic evidence of HBV infection.

For the total population, there was no association between the history of therapeutic products received and the histologic severity of the liver disease ($\chi^2 = .6$). Although no significant association was observed either among the 40 autopsied cases ($P = .8$). 21 of these had received no concentrate. Among the 15 autopsied patients known to have received concentrate, the incidence of severe chronic hepatitis or cirrhosis was 20%. Thus, the prevalence of severe liver disease is comparable for reported levels of any exposure to concentrate in both the biopsy and autopsy groups.

The frequency of exposure to various levels of concentrate, consensus histologic diagnosis, and severity of hemophilia were examined within all age groups, and possible associations among these variables were explored (Table 5). Other than an increased proportion of mild hemophiliacs among patients beyond the age of 40, presumably reflecting reduced survival of severe cases, no association between age, severity of hemophilia, product exposure, and histologic severity was discernible, although the number of childhood cases <10 years old was small.

Sequential histologic examinations. The single patient on whom three biopsies were performed showed a progression from acute hepatitis, with evidence of transition to chronicity to mild chronic hepatitis (CPH) to severe chronic hepatitis (CAH) over a three-year period. Of the 11 patients examined twice, five were unchanged—three with mild no exposure to concentrate. Of these, four (67%) had severe hepatitis or cirrhosis. By contrast, only five of 26 patients (19%) with exposure to <100,000 units of concentrate, and 18 of 72 patients (25%) with exposure to >100,000 units of concentrate had severe chronic hepatitis or cirrhosis. Thus, from this selected group of patients on whom biopsies were performed for some indication of suspected liver disease, no apparent increase in severity of disease is seen with increased exposure ($P = .6$). Although no significant association was observed among the 40 autopsied cases ($P = .8$). 21 of these had received no concentrate. Among the 21 autopsied patients known to have received concentrate, the incidence of severe chronic hepatitis or cirrhosis was 20%. Thus, the prevalence of severe liver disease is comparable for reported levels of any exposure to concentrate in both the biopsy and autopsy groups.

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**Table 4. Consensus Diagnosis for Patients Who Underwent Biopsies, by Exposure to Concentrate**

<table>
<thead>
<tr>
<th>Exposure to Concentrate</th>
<th>No Exposure</th>
<th>&lt; 100,000 U</th>
<th>≥ 100,000 U</th>
<th>Not Enough Data</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Trivial, mild to moderate</td>
<td>2</td>
<td>33.3</td>
<td>18</td>
<td>69.2</td>
<td>47</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td>16.7</td>
<td>2</td>
<td>7.7</td>
<td>6</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>3</td>
<td>50.0</td>
<td>3</td>
<td>11.5</td>
<td>12</td>
</tr>
<tr>
<td>Other lesions</td>
<td>0</td>
<td>—</td>
<td>3</td>
<td>11.5</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>100.0</td>
<td>26</td>
<td>100.0</td>
<td>72</td>
</tr>
</tbody>
</table>
Table 5. Frequency of Exposure to Concentrate, Consensus Histologic Diagnosis, and Level (Severity) of Hemophilia Within Age Groups

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Exposure to concentrate</th>
<th>Consensus diagnosis</th>
<th>Level (severity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>&lt;10 yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>10.0</td>
<td>0</td>
</tr>
<tr>
<td>&lt;100,000</td>
<td>5</td>
<td>50.0</td>
<td>5</td>
</tr>
<tr>
<td>≥100,000</td>
<td>4</td>
<td>40.0</td>
<td>15</td>
</tr>
<tr>
<td>Not enough data</td>
<td>0</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100.0</td>
<td>22</td>
</tr>
</tbody>
</table>

CPI-1, one with severe CAH, and one with cirrhosis—and one each showed evolution from acute hepatitis to chronic hepatitis with evidence of chronicity, from the latter diagnosis to mild CAH, and from mild chronic hepatitis (borderline CPH/CAH) to cirrhosis. By contrast, in two patients, initial biopsies showing mild to moderate CAH improved to milder hepatic lesions (CPH). In the final patient, mild CPH improved to a trivial lesion showing only minimal spotty necrosis. As in the total population (see below), changes in the results of hepatic biochemical tests did not predict alterations in hepatic history.

**Biopsy Sequelae.** Study participants reported that 12.5% of the biopsy procedures led to a prolongation of the planned hospitalization or to an appreciable increase in coagulation factor replacement beyond what had been planned for the biopsy in order to control hemorrhage. No deaths occurred as a result of the 126 biopsy procedures on 115 patients reported to the AHHSG.

**Biochemical and serologic studies.** Seventy-eight percent of the biopsy cases considered to have cirrhosis and 70% of the cases with severe hepatic lesions had SGOT activities at least two times normal compared with 37% of those with trivial and mild lesions and 45% of those with other lesions ($\chi^2 = 11.8, P = .008$). Similarly, the proportion of cases with abnormal SGPT activities (at least two times normal) appears higher in those cases with severe lesions (89%) and cirrhosis (68%) compared to those with trivial and other lesions (51% to 60%; $\chi^2 = 6.0, P = .11$). Only 13% of trivial cases were HBsAg positive, compared with 27% of other lesions, 40% of severe lesions, and 61% of cirrhosis ($\chi^2 = 19.7, P = .0002$). Although there are statistically significant differences in hepatic test abnormalities or the presence of HBsAg between the different histologic groups, blood testing in the individual patient did not, however, reliably predict either the type or severity of the hepatic histologic lesions.

**DISCUSSION**

This study reports the largest series of hepatic histologic materials in hemophiliacs assembled for review. It would have been preferable to examine a more homogeneous population, followed with current and standardized diagnostic modalities, treated prospectively according to well-defined protocols, and biopsied only under predetermined criteria. Nevertheless, the risks of performing additional biopsies prospectively appeared sufficiently formidable to encourage this retrospective analysis of available materials.

Accordingly, the goals of this review were (1) to define the spectrum of liver disease in hemophiliacs, (2) to examine the relationship between the severity of histologically documented disease and treatment history, and (3) to estimate the risk-benefit ratio for liver biopsy in this population.

Results of our histologic evaluation reveal that the incidence of cirrhosis in our large series is 15%, less than previously reported. In the noncirrhotic biopsy specimens, the incidence of severe necro-inflammatory disease, mainly CAH, was also lower than previously described. Most slides showed CPH, CLH, or mild borderline CAH. Many cases with serologic markers for hepatitis B lacked the histologic features of B hepatitis (eg, ground glass cells) and showed features of non-A, non-B hepatitis. The lack of agreement on diagnosis reflects a high proportion of cases with mild to moderate disease, with features suggestive of both CPH and mild CAH, as well as of a non-A, non-B etiology. These cases differ histologically from the more florid types of chronic hepatitis observed in autoimmune...
HBV-associated chronic liver disease, and it was from studies of the latter that the current diagnostic criteria for CPH and CAH largely evolved.\textsuperscript{12,13} Studies of histologic materials from patients with presumptive non-A, non-B disease, including the present biopsies and autopsies, have led hepatopathologists to recognize the need for new descriptive and diagnostic criteria,\textsuperscript{12,15} but no generally accepted schema is currently available. The consensus classification developed during this study is at best an interim measure with respect to precise histologic classification, but appears suitable for addressing the more clinically oriented goals of the present study.

The lack of severity of the histopathologic findings in the current materials may not be entirely reassuring. Some recent evidence suggests insidious progression of non-A, non-B hepatitis to cirrhosis,\textsuperscript{16} although other studies suggest the possibility of reversion toward normal hepatic architecture.\textsuperscript{20} Both progression and reversion, as well as a static picture, were observed in patients examined repeatedly in the present study. As in previous reports, hepatic biochemical and serologic tests did not predict the histologic lesions.

There was no association in this study between the treatment regimen and histologic severity. Specifically, there was no evidence of more severe liver disease in patients receiving concentrates prepared from large pools of donor plasma. Thus, at this time there appears to be no indication to alter current therapy patterns because of concern over plasma product-related liver disease. Whether pooled plasma-derived products may present a greater risk for the acquisition of acquired immune deficiency syndrome (AIDS) by hemophiliacs\textsuperscript{21} was not addressed in this study.

These data are of particular interest, as it is now clear that the use of large amounts of cryoprecipitate and concentrate leads to identical patterns of hepatitis B markers in recipients as well as produces similar patterns of biochemical abnormalities.\textsuperscript{2} This was not originally predicted since patients receiving cryoprecipitate or plasma are exposed to far fewer donors compared to those receiving concentrate. For example, the average factor VIII-deficient patient receives 40,000 units of factor per year.\textsuperscript{11} The maximum annual exposure to donors is 400 when plasma and/or cryoprecipitate are used, whereas a single vial of 1,000 units of factor VIII concentrate is derived from a pool of between 2,500 and 22,500 donors. These findings may be of particular importance when attempting to make rational treatment decisions for hemophiliacs at a time when serologic screening tests for type III human T cell leukemia virus—the presumptive cause of AIDS—are not widely available, nor their efficacy established.

The risks of liver biopsy in hemophiliacs are not insignificant. Despite the experience of the participating centers with both liver biopsy and hemophilia, one of every eight procedures (12.5\%) reported to the present study was complicated by prolonged hospitalization and/or requirements for appreciably increased factor use because of hemorrhage. Beyond this high morbidity rate, we are aware of two deaths from uncontrollable bleeding following liver biopsy at two centers, in New York and London, which did not contribute histologic materials to the study. Hence, based on our estimate of approximately 200 liver biopsies in hemophiliacs worldwide at the time of our review, the fatality rate from the procedure may approximate 1\%, compared to <0.01\% in nonhemophiliacs.\textsuperscript{22} This is of great concern. As the vast majority of biopsy specimens showed histologically unimpressive lesions, and as there is in any case no currently effective therapy for CAH,\textsuperscript{23} the information obtained by liver biopsy in this patient population only occasionally justifies the increased risk of this procedure.

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REFERENCES


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