

Serum Ferritin Level Predicts Advanced Hepatic Fibrosis among U.S. Patients with Phenotypic Hemochromatosis

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Background: DNA-based *HFE* gene testing can confirm hereditary hemochromatosis in most people of Northern European descent. However, liver biopsy is important to detect cirrhosis.

Objective: To develop noninvasive criteria to predict the presence or absence of advanced hepatic fibrosis or cirrhosis in Americans with hemochromatosis.

Design: Cross-sectional study.

Setting: Six tertiary care referral clinics.

Patients: 182 patients with phenotypically defined hemochromatosis.

Measurements: Liver histopathology and serum ferritin, aspartate aminotransferase, and alanine aminotransferase levels. Multivariate logistic regression analysis was used to examine factors associated with cirrhosis (defined as bridging fibrosis or unequivocal cirrhosis on biopsy).

Results: Cirrhosis was present in 40 of 182 (22%) patients in the overall group and in 35 of 147 (24%) of C282Y homozygotes.

Only 1 of 93 patients with a serum ferritin level less than 1000 $\mu\text{g/L}$ had cirrhosis compared with 39 of 89 patients with serum ferritin levels greater than 1000 $\mu\text{g/L}$ ($P < 0.001$). No C282Y homozygotes or C282Y/H63D compound heterozygotes with serum ferritin levels less than 1000 $\mu\text{g/L}$ had cirrhosis. Elevated serum aminotransferase levels ($P = 0.001$) and serum ferritin levels greater than 1000 $\mu\text{g/L}$ ($P = 0.001$), but not age older than 40 years ($P = 0.2$), were independently associated with cirrhosis. In a multivariate model, the probability of cirrhosis was 7.4% among patients with serum ferritin levels less than 1000 $\mu\text{g/L}$ compared with 72% among patients with serum ferritin levels greater than 1000 $\mu\text{g/L}$ after adjustment for age and elevated serum liver enzyme levels.

Conclusions: Patients with hemochromatosis and serum ferritin levels less than 1000 $\mu\text{g/L}$ are unlikely to have cirrhosis. Liver biopsy to screen for cirrhosis may be unnecessary in such patients, regardless of age or serum liver enzyme levels.

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Hereditary hemochromatosis is a common inherited disorder among people of Northern European descent. Screening studies using phenotypic criteria have found a prevalence of 1:200 to 500 and a carrier frequency of nearly 10% (1). Patients with phenotypic expression of this disorder may have evidence of iron deposits in many organs, including the liver, heart, skin, joints, anterior pituitary, and pancreas. Involvement of these organs can lead to cirrhosis and liver failure, cardiomyopathy, skin pigment changes, arthropathy, impotence, and diabetes, respectively. Long-term survival among patients with hemochromatosis who have cirrhosis at the time of diagnosis is statistically significantly lower than that in an age- and sex-matched control group primarily because of a statistically significantly increased risk for hepatocellular carcinoma and liver failure (2, 3). Therefore, it is important to ascertain the presence or absence of advanced hepatic fibrosis or cirrhosis in patients with hemochromatosis, because this information has important prognostic value and will probably change management. In the absence of obvious decompensated cirrhosis, liver biopsy is the only reliable means to identify cirrhosis among patients with hemochromatosis.

The discovery of the hemochromatosis gene, *HFE*, in 1996 has allowed identification of a genotype (C282Y/C282Y) that is most likely to lead to clinically significant iron overload (4). Two mutations were initially described among patients with hemochromatosis: a cysteine to ty-

rosine substitution at amino acid 282 (C282Y) and a histidine to aspartate substitution at amino acid 63 (H63D). The homozygous C282Y mutation was subsequently found in 80% to 90% of persons of Northern European descent with a typical hemochromatosis phenotype. Given the high prevalence of C282Y homozygosity in patients with the hereditary hemochromatosis phenotype, testing for the presence of this mutation has largely replaced liver biopsy as the confirmatory test in patients suspected of having hemochromatosis. Liver biopsy is used primarily to detect significant fibrosis for prognosis and to ascertain the need for hepatocellular carcinoma screening. This has led to inquiry into ways to predict the presence or absence of advanced fibrosis (bridging fibrosis or cirrhosis) by using clinical or laboratory tests in patients in whom the diagnosis of hemochromatosis has been confirmed by *HFE* gene testing.

Guyader and colleagues (5) examined several clinical and laboratory variables associated with fibrosis in a cohort of patients with hemochromatosis in France ($n = 197$) and validated their findings from a group of patients with hemochromatosis in Canada ($n = 113$); all patients were C282Y homozygotes. No patients had severe fibrosis in the absence of the following: hepatomegaly, elevated serum aspartate aminotransferase (AST) levels, or serum ferritin levels greater than 1000 $\mu\text{g/L}$ (5). A study of 66 U.S. patients with variable phenotypic expression found that no patients younger than 40 years of age had advanced fibrosis (6). On

Context

Gene testing can confirm hereditary hemochromatosis in people of Northern European descent. Whether liver biopsy is needed to detect the adverse prognostic finding of cirrhosis is controversial.

Contribution

This multicenter cross-sectional study of 182 patients with phenotypically defined hemochromatosis found that the probability of cirrhosis among patients older than 40 years of age who had abnormal liver enzyme levels and serum ferritin levels less than 1000 $\mu\text{g/L}$ was only 7%.

Implications

Screening for cirrhosis with liver biopsy is probably not necessary in patients with hemochromatosis and serum ferritin levels less than 1000 $\mu\text{g/L}$.

—The Editors

the basis of these studies, several authors and practice guidelines have suggested that liver biopsy is unnecessary in C282Y homozygotes younger than 40 years of age with serum ferritin levels less than 1000 $\mu\text{g/L}$ (7, 8). Liver biopsy has been recommended for patients with other *HFE* genotypes and for patients older than 40 years of age.

Previous reports, however, suggested that U.S. patients with hemochromatosis have greater heterogeneity and less severe phenotypic expression than Europeans (9). Many possible reasons explain these differences in phenotypic expression, including differences in diet and alcohol consumption, as well as allelic differences in modifier genes, between American and European patients. Furthermore, some variables used to predict fibrosis in previous studies, such as hepatomegaly, are imprecise and difficult to reproduce. Thus, the criteria proposed by Guyader and colleagues may not apply to a large, heterogeneous population of U.S. patients with the hemochromatosis phenotype.

The previous U.S. study included a small sample size, did not include patients with a uniform phenotype, and did not use a multivariate regression analysis to determine whether serum ferritin level was an independent predictor of advanced hepatic fibrosis (6). Therefore, it is unclear whether the study's findings may be generalizable.

We sought to identify simple clinical and laboratory variables that predict advanced fibrosis in a geographically varied U.S. sample with phenotypically defined hemochromatosis after exclusion of patients with other risk factors for fibrosis, including alcohol intake, hepatitis C, or steatohepatitis.

METHODS**Patients**

We reviewed the records of patients evaluated for hereditary hemochromatosis at six tertiary referral clinics to identify potential study participants. The six centers (Uni-

versity of Washington Medical Center, Seattle, Washington; Mayo Clinic, Rochester, Minnesota; William Beaumont Hospital, Royal Oak, Michigan; Southern Iron Disorders Center, Birmingham, Alabama; Rochester General Hospital, Rochester, New York; and University of Vermont Medical Center, Burlington, Vermont) represented different geographical regions in the United States: the Northeast, Midwest, Southeast, and Northwest.

The following clinical and laboratory data were available at the time of diagnosis on all patients who were considered for the study: age, sex, pretreatment serum ferritin levels, and *HFE* mutation status. In addition, all patients had undergone liver biopsy and the pathology reports were available for review. All patients had increased stainable iron ($\geq 3+$) by using Perls Prussian blue stain with a periportal to pericentral gradient (10).

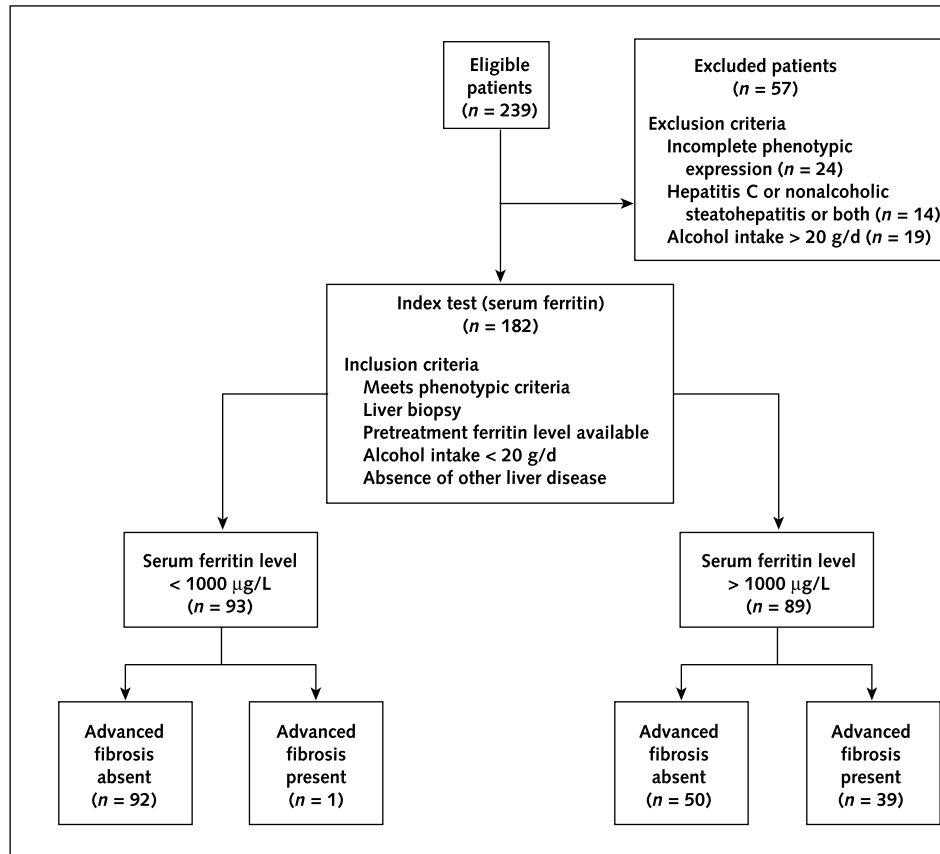
Inclusion criteria were 1) phenotypic hemochromatosis (on the basis of hepatic iron concentration of ≥ 4000 $\mu\text{g/g}$ dry weight [9] or hepatic iron index of ≥ 1.9 , calculated as hepatic iron concentration [$\mu\text{mol/g}$] divided by age [years] [11], or >4 g of iron removed by quantitative phlebotomy [7]); 2) no other known causes of chronic liver disease; 3) absence of secondary cause for iron overload; 4) available pretreatment serum ferritin value; 5) alcohol intake less than 20 g/d; and 6) no risk factors for or histologic evidence of nonalcoholic steatohepatitis (body mass index < 33 kg/m^2 , serum triglyceride level < 5.65 mmol/L [< 500 mg/dL], and no type 2 diabetes mellitus).

The institutional review board at each participating institution approved the study. Two investigators who were blinded to the laboratory and clinical data reviewed the reports of pathologists' interpretations of liver biopsy specimens. The following information was recorded: degree of stainable iron (0 to 4+); presence or absence of cirrhosis (bridging fibrosis was classified as cirrhosis) on the basis of the description by the attending pathologist; and evidence of other liver diseases, such as fatty change, steatohepatitis, or chronic hepatitis. Serum iron, transferrin-iron saturation, ferritin, and aminotransferase activity (AST and alanine aminotransferase [ALT]) were also recorded if available. Because reference ranges for serum AST and ALT varied among the different centers and normal ranges varied within sites depending on reagent kits used, serum ALT and AST values greater than 75 U/L were defined as elevated since this value represented the high end of the reported normal ranges. In most cases, the time frame between serum ferritin measurement and liver biopsy was less than 2 months and only baseline (pretreatment) ferritin levels were used. Hepatic iron concentration was measured by atomic absorption spectrophotometry or colorimetry as previously described (10).

***HFE* Mutation Analysis**

Analysis of the C282Y and H63D mutation was performed according to the method described by Feder and colleagues (4). Patients were classified as C282Y homozy-

Figure 1. The use of serum ferritin in predicting presence or absence of advanced hepatic fibrosis among 182 patients with the hemochromatosis phenotype.



Overall numbers of patients evaluated for participation, patients excluded from participation (and reasons for exclusion), as well as patients with elevated serum ferritin levels and patients with and without advanced fibrosis are shown.

gotes, C282Y/H63D compound heterozygotes, or as having other *HFE* mutations (C282Y/WT, H63D/H63D, or H63D/WT).

Statistical Analysis

We compared the demographic and clinical features and laboratory values of the patients with and without advanced hepatic fibrosis or cirrhosis. The presence or absence of advanced fibrosis or cirrhosis was examined as the outcome variable in a multivariate logistic regression model. We followed a commonly used heuristic in which 10 to 15 outcome events (bridging fibrosis or cirrhosis) are needed to examine each predictor variable properly. Therefore, we decided a priori to include three predictor variables at a time: age (<40 years and \geq 40 years), abnormal AST or ALT level, and serum ferritin level (<1000 $\mu\text{g/L}$ and \geq 1000 $\mu\text{g/L}$). The model was examined for interactions among the potential risk factors. Adjusted probabilities of absence of bridging fibrosis or cirrhosis were calculated from the logistic regression model. A receiver-operating characteristic (ROC) curve was constructed by using different cutoff values for serum ferritin levels to calculate a range of sensitivity and specificity. In addition, both positive and negative likelihood ratios were calculated for sev-

eral predetermined thresholds, as well as strata of serum ferritin levels. Statistical analyses were conducted by using the Stata software program (Stata Corp., College Station, Texas).

Role of the Funding Source

The funding source had no role in the design, conduct, or reporting of the study or in the decision to publish the manuscript.

RESULTS

Of 284 patients referred to the study, 45 patients were excluded because of lack of liver biopsy; an additional 57 (20%) patients were excluded because of the following reasons: incomplete phenotypic expression (that is, patients did not meet any of three phenotypic criteria [hepatic iron concentration < 4000 $\mu\text{g/g}$ dry weight, hepatic iron index < 1.9, or < 4 g of iron removed by quantitative phlebotomy]) ($n = 24$), another obvious cause of liver disease (nonalcoholic steatohepatitis, hepatitis C, or other) ($n = 14$), or average alcohol intake greater than 20 g/d ($n = 19$). Of 239 eligible patients, 182 (65%) met all inclusion and exclusion criteria (Figure 1).

Table 1. Demographic Characteristics, Serum Liver Enzyme Levels, and Liver Biopsy Findings of the Study Sample (n = 182)*

Characteristic	Value
Mean age \pm SD, y	51 \pm 12.2
Men, n (%)	135 (74)
C282Y/C282Y homozygotes, n (%)	147 (81)
C282Y/H63D compound heterozygotes, n (%)	15 (8)
C282Y/wild type, H63D/wild type, or H63D/H63D, n (%)	7 (4)
Wild type for both C282Y or H63D mutations, n (%)	13 (7)
Bridging fibrosis or cirrhosis, n (%)	40 (22)
Elevated ALT level, n (%)	31 (23)
Elevated AST level, n (%)	21 (14)

* All patients met phenotypic criteria for hemochromatosis. ALT = alanine aminotransferase; AST = aspartate aminotransferase.

Demographic characteristics of the study sample are shown in Table 1. The mean age of the patients was 51 years, and all patients were self-identified as white. A total of 171 of 182 (94%) patients were defined as having the hemochromatosis phenotype on the basis of hepatic iron concentration of 4000 $\mu\text{g/g}$ dry weight or greater or hepatic iron index greater than 1.9. Hepatic iron concentration was unavailable in the other 11 patients. Each of these patients had a hepatic iron stain compatible with hereditary hemochromatosis, and all patients had greater than 4 g of mobilizable iron stores. A total of 147 of 182 (81%) patients were C282Y homozygotes; 15 of 182 (8%) were C282Y/H63D compound heterozygotes; and the remaining 20 (11%) were heterozygous for C282Y or H63D, homozygous for H63D, or had neither mutation. Bridging fibrosis or cirrhosis was seen in 40 of 182 (22%) patients. Serum ALT levels were available in 135 patients and elevated in 31 (23%) patients. Serum AST levels were available in 153 patients and elevated in 21 (14%) patients.

The clinical and laboratory data for patients with bridging fibrosis or cirrhosis are shown in Table 2. Serum ferritin levels less than 1000 $\mu\text{g/L}$ were strongly associated with the absence of bridging fibrosis or cirrhosis. Only 1 of 40 (2%) patients with bridging fibrosis had a serum ferritin level less than 1000 $\mu\text{g/L}$, compared with 65% of patients without bridging fibrosis or cirrhosis. Age at diagnosis and the proportion of patients older than 40 years of age did not differ between patients with and those without bridging fibrosis or cirrhosis. However, the only patient with a serum ferritin level less than 1000 $\mu\text{g/L}$ and bridging fibrosis or cirrhosis was older than 40 years of age.

The prevalence of bridging fibrosis or cirrhosis did not significantly differ between patients who were C282Y homozygous and those in other *HFE* genotype groups. No patients in the large subgroup of C282Y homozygotes or C282Y/H63D compound heterozygotes with serum ferritin levels less than 1000 $\mu\text{g/L}$ had bridging fibrosis or cirrhosis. Conversely, 38 of 82 (46%) patients in this group with serum ferritin levels greater than 1000 $\mu\text{g/L}$ had bridging fibrosis or cirrhosis. Multivariate logistic re-

gression analysis demonstrated that normal serum aminotransferase levels and serum ferritin levels less than 1000 $\mu\text{g/L}$ were independently associated with absence of bridging fibrosis or cirrhosis in the overall study sample ($P = 0.001$), but age older than 40 years was not ($P = 0.2$). Among a reference group with patients older than 40 years of age and abnormal serum liver enzyme levels, patients with serum ferritin levels less than 1000 $\mu\text{g/L}$ had a 92.6% (95% CI, 59.7% to 99.1%) probability of absence of bridging fibrosis or cirrhosis, compared with a probability of only 28.0% (CI, 14.3% to 47.3%) among patients with serum ferritin levels of 1000 $\mu\text{g/L}$ or greater (Table 3).

The ROC curve for serum ferritin is shown in Figure 2. The area under the curve (C-statistic), which indicates the overall accuracy of the test, was 0.91 (CI, 0.89 to 0.93). When different serum levels of ferritin were used, the greatest accuracy in predicting bridging fibrosis or cirrhosis was obtained with a serum level of 1000 $\mu\text{g/L}$. For serum ferritin level of 1000 $\mu\text{g/L}$ or greater as a diagnostic test for bridging fibrosis or cirrhosis, we calculated 97.5% sensitivity (CI, 95.3% to 99.7%) and 65.0% specificity (CI, 35.1% to 41.9%). Positive and negative likelihood ratios were calculated for several threshold values of serum ferritin (Table 4). For example, for a serum ferritin level of 1000 $\mu\text{g/L}$ or greater as a diagnostic test for bridging fibrosis or cirrhosis, we calculated a positive likelihood ratio of 1.5 and a negative likelihood ratio of 0.04. In addition, several stratum-specific likelihood ratios were calculated (Table 4).

We also examined for the effect of partial verification bias that may have occurred if patients with serum ferritin levels greater than 1000 $\mu\text{g/L}$ were more likely to have undergone liver biopsy. A sensitivity analysis was conducted by using data from the 45 patients meeting eligi-

Table 2. Comparison between Patients with and without Bridging Fibrosis or Cirrhosis*

Characteristic	Patients with Bridging Fibrosis or Cirrhosis (n = 40)	Patients without Bridging Fibrosis or Cirrhosis (n = 142)
Age at diagnosis, y	52 \pm 12	50 \pm 12
Age < 40 years, n (%)	5 (13)	31 (22)
Men, n (%)	35 (88)	100 (74)
Serum transferrin-iron saturation, n (%)	37 (91.21 \pm 1.7)	134 (80.6 \pm 17.9)
Serum ferritin level, $\mu\text{g/L}$	4411 \pm 1158	957 \pm 646
Hepatic iron concentration, $\mu\text{g/g}$ (dry weight) [†]	22 903 \pm 14 366	9990 \pm 6514
Hepatic iron index [‡]	8.2 \pm 4.6	3.7 \pm 2.6
Serum ferritin level \leq 1000 $\mu\text{g/L}$, n (%)	1 (2.5)	92 (65)
Elevated ALT level, n/n (%)	16/29 (55)	15/106 (14)
Elevated AST level, n/n (%)	16/36 (44)	5/117 (4)
C282Y/C282Y homozygotes, n (%)	35 (88)	112 (79)

* All patients met phenotypic criteria for hemochromatosis. Values with a plus/minus sign are expressed as mean \pm SD. ALT = alanine aminotransferase; AST = aspartate aminotransferase.

[†] Data available for 171 patients.

[‡] Data available for 172 patients.

Table 3. Adjusted Probability of the Absence of Bridging Fibrosis or Cirrhosis in a Reference Group with the Hemochromatosis Phenotype

Characteristic	Adjusted Probability (95% CI), %*	P Value
Age		
>40 y†	28.0 (14.3–47.3)	0.2
≤40 y	51.4 (24.2–77.6)	
Serum liver enzyme level‡		
Abnormal††	28.0 (14.3–47.3)	0.001
Normal	70.7 (53.1–83.7)	
Serum ferritin level		
≥1000 μg/L†	28.0 (14.3–47.3)	0.001
<1000 μg/L	92.6 (59.7–99.1)	

* Adjusted probabilities are derived from a logistic regression model with age, serum liver enzyme levels, and serum ferritin level as predictors of the absence of bridging fibrosis or cirrhosis.

† Patients in the reference group were older than age 40 years and had abnormal serum liver enzyme levels and abnormal serum ferritin levels.

‡ Serum liver enzyme levels were considered normal if both aspartate and alanine aminotransferase levels were less than 75 U/L.

bility criteria who had not undergone liver biopsy. The 45 patients were added to the 182 patients in the original study sample, and the sensitivity, specificity, and likelihood ratios were recalculated in an expanded study group ($n = 227$). The proportion of patients with serum ferritin levels less than 1000 μg/L (29 of 45, 64%) and greater than 1000 μg/L (16 of 45, 36%), and the probability of bridging fibrosis or cirrhosis based on the original data were used to reconstruct our 2×2 table. By using this method, we derived a sensitivity of 97.8%, specificity of 67%, and negative likelihood ratio of 0.03; these estimates are very close to those obtained in the original sample (97.5%, 65%, and 0.04, respectively), indicating limited verification bias.

DISCUSSION

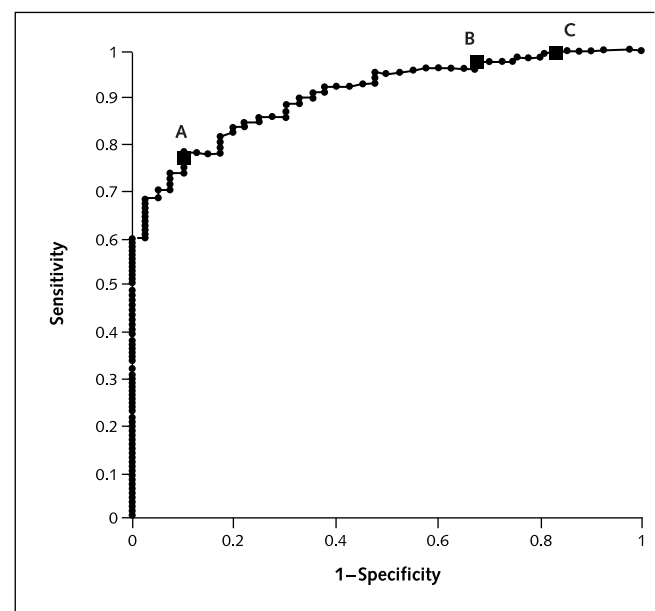
The current study examined the relationship between predictors of advanced fibrosis among patients expressing the phenotype of hemochromatosis but uncomplicated by substantial concomitant alcohol intake or risk factors for other common chronic liver diseases. We found that bridging fibrosis or cirrhosis is rare in this sample with serum ferritin levels less than 1000 μg/L. These findings applied not only to the C282Y homozygotes but also to C282Y/H63D compound heterozygotes. Since only 11% of the patients had other *HFE* genotypes, the study was not sufficiently powered to examine the use of serum ferritin in predicting the presence or absence of bridging fibrosis or cirrhosis in patients with other *HFE* genotypes. Among C282Y homozygotes or C282Y/H63D compound heterozygotes, no patient with a serum ferritin less than 1000 μg/L had bridging fibrosis or cirrhosis, regardless of age. Elevated serum ALT or AST levels were also independently associated with advanced hepatic fibrosis. However, serum AST and ALT levels lacked negative predictive value and specificity for excluding hepatic fibrosis because almost half

of the study sample with advanced hepatic fibrosis had AST or ALT levels below the threshold level of 75 U/L. Similarly, age older than 40 years was not independently associated with bridging fibrosis or cirrhosis independent of serum ferritin level less than 1000 μg/L.

These findings are likely to affect the management of patients with hereditary hemochromatosis. Current recommendations suggest that liver biopsy be considered among C282Y homozygotes older than 40 years of age and in all patients with other genotypes (7, 8). Most experts agree that the presence of the C282Y homozygous genotype is adequate for confirming the diagnosis of hemochromatosis in individuals with elevated serum iron markers without conditions associated with secondary iron overload (8). Therefore, liver biopsy would be considered only in C282Y homozygotes to determine cirrhosis or advanced hepatic fibrosis, whereas biopsy would be useful both to confirm the diagnosis of hemochromatosis and to identify cirrhosis among patients with other *HFE* genotypes. Our data suggest that performing a liver biopsy is unnecessary for prognostic reasons in C282Y homozygotes and C282Y/H63D compound heterozygotes with serum ferritin levels less than 1000 μg/L and that age is not an important variable in deciding to perform liver biopsy for prognostic reasons. Our study also demonstrates that normal serum liver enzyme levels lack negative predictive value and specificity compared with serum ferritin level in making decisions about which patients with hemochromatosis on the basis of gene testing should undergo liver biopsy for prognosis.

Limitations of our proposed criteria for liver biopsy in

Figure 2. The receiver-operating characteristic curve of serum ferritin levels as a diagnostic test for advanced hepatic fibrosis among patients with hemochromatosis.



Point A indicates the calculated values for a serum ferritin level cutoff value of 1500 μg/L, point B for 1000 μg/L, and point C for 500 μg/L.

Table 4. Likelihood Ratios of Test Results: Serum Ferritin Levels and Advanced Hepatic Fibrosis

Serum Ferritin	Fibrosis Present*	Fibrosis Absent	Likelihood Ratio (95% CI)
$\mu\text{g/mL}$	<i>n</i>		
Strata			Stratum-Specific
>1500	31	23	4.8 (2.5–9.1)
1000–1499	8	27	1.1 (0.4–2.5)
500–999	1	61	0.06 (0.00–0.4)
<500	0	31	0
Total	40	142	
Levels			Threshold-Specific
>1500	31	23	4.8 (2.5–9.1)
<1500	9	119	0.27 (0.1–0.6)
>1000	39	50	2.8 (0.4–20.8)
<1000	1	92	0.04 (0.005–0.3)
>500	40	111	1.28
<500	0	31	0

*Fibrosis defined as bridging fibrosis or cirrhosis.

patients with hemochromatosis are that they were derived from retrospective data and that some patients were excluded from the study on the basis of biopsy findings, a type of selection bias. However, only five patients were excluded solely because of histologic evidence of another complicating liver disease. All five had nonalcoholic steatohepatitis. Upon further review, four of five were noted to be obese (body mass index $> 34 \text{ kg/m}^2$), a major risk factor for this disorder that would have been clinically apparent. Nonalcoholic steatohepatitis was strongly suspected in the sixth patient before biopsy because of severe hyperlipidemia, moderate obesity, diabetes mellitus, and abnormal liver enzymes levels. The remaining patients were excluded from the study group on the basis of a history of excess alcohol ingestion or serologic evidence of chronic hepatitis C. Thus, all but one patient would have been identified as having a risk factor for concomitant other liver disease before liver biopsy. Recent studies suggest that only a minority of patients with the homozygous C282Y mutation have substantial hepatic iron overload (12). Although our data are from several referral centers and ascertainment bias must be considered, the large number of cases with hemochromatosis associated with the homozygous C282Y mutation suggests that a higher proportion of the individuals with this mutation may develop iron overload than suggested in the study by Beutler and colleagues. (12).

Another potential limitation of our study is the possibility of partial verification bias. Partial verification bias occurs when only a selected sample of patients (patients with serum ferritin levels $> 1000 \mu\text{g/L}$) undergoes the reference test (liver biopsy). Partial verification bias may inflate sensitivity estimates and reduce specificity estimates. We were somewhat limited in our ability to test for possible verification bias since our inclusion criteria required a liver biopsy and since no independent clinical or laboratory

methods other than liver biopsy are available to confirm advanced hepatic fibrosis. We tested for partial verification bias by adding the 45 patients who did not have liver biopsy in a sensitivity analysis. The recalculated sensitivity, specificity, and likelihood ratio were very close to those derived from the original sample, providing evidence for the absence of significant verification bias.

Furthermore, evidence suggested that the potential bias would have to be great to significantly alter the observed negative likelihood ratio of 0.04 closer to 0.5 or 1.0 (values that alter disease probability very little). Even in the highly unlikely situation that none of the patients in the nonbiopsied group who had serum ferritin levels greater than $1000 \mu\text{g/L}$ ($n = 29$) had cirrhosis and that all the patients with serum ferritin levels less than $1000 \mu\text{g/L}$ ($n = 16$) had cirrhosis, the negative likelihood ratio would increase to 0.74. This finding suggests that a threshold serum ferritin value of $1000 \mu\text{g/L}$ has a robust predictive value even after adjustment for possible verification bias.

In summary, we conclude that liver biopsy is unnecessary to determine bridging fibrosis or cirrhosis in patients with a serum ferritin level less than $1000 \mu\text{g/L}$, normal serum AST and ALT levels, and the characteristic phenotype associated with hemochromatosis, particularly among C282Y homozygotes and C282Y/H63D compound heterozygotes, without excess alcohol intake or risk factors for other liver disease. Conversely, liver biopsy should be considered for patients with serum ferritin levels greater than $1000 \mu\text{g/L}$ because of significantly increased risk for advanced fibrosis.

From University of Washington, Seattle, Washington; Mayo Clinic, Rochester, Minnesota; Rochester General Hospital and University of Rochester, Rochester, New York; Southern Iron Disorders Center, Birmingham, Alabama; University of Vermont, Burlington, Vermont; Houston Veterans Administration Medical Center, Baylor College of Medicine, Houston, Texas; Health Services Research and Development Service, Veterans Affairs Puget Sound Health Care System, Seattle, Washington; and William Beaumont Hospital, Royal Oak, Michigan.

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COMMENTARY

This article presents information on the accuracy of a test whose results are expressed as a continuous variable (that is, the results can take on any value). Many studies provide the sensitivity, specificity, and likelihood ratios for a single value chosen to distinguish normal from abnormal (the cut-point). If authors choose only one value to describe the performance of a test, they miss an opportunity to help physicians maximize diagnostic information. In this article, the authors exemplify the value of providing likelihood ratios for different ranges of test results (Table 4). With this information, physicians can calculate the post-test probability of cirrhosis for a result that falls into any one of the ranges.

To appreciate the value of stratum-specific information on test performance, consider a patient who has a serum ferritin level of 1800 $\mu\text{g/mL}$. The likelihood ratio for a serum ferritin level greater than 1500 $\mu\text{g/mL}$ is 4.8. If the authors had only given the likelihood ratio for a single cut-point of greater than 1000 $\mu\text{g/mL}$, the physician would have to use 2.8 as the likelihood ratio. Suppose the patient's pretest odds of cirrhosis were 1:1 (corresponding to a 50% probability of cirrhosis). According to the odds ratio form of Bayes theorem*, the post-test odds would be 4.8:1 (an 83% probability of cirrhosis) if the physician used the stratum-specific likelihood ratio. If he or she used the likelihood ratio corresponding to a cut-point of greater than 1000 $\mu\text{g/mL}$, the post-test odds would be 2.8:1 (a 74% probability). These probabilities correspond to a 17% and a 26% probability, respectively, of being wrong in diagnosing cirrhosis on the basis of a serum ferritin level of 1800 $\mu\text{g/mL}$. In this case, using a stratum-specific likelihood ratio reduces the chance of diagnostic error.

Table 4 also has some interesting lessons to teach about interpreting a negative test result. For example, imagine a patient typical of those in this study. They would have a 20% pretest probability of having cirrhosis (1:4 odds). If such a patient had a serum ferritin level of 700 $\mu\text{g/mL}$, the likelihood ratio would be 0.06 (95% CI, 0 to 0.4), and the post-test odds of cirrhosis would be 1.5:100 (or a 1.5% probability). You would probably be satisfied that you had ruled out cirrhosis. Suppose your next patient with hemochromatosis has multiple findings of chronic liver disease. Your estimated pretest probability of cirrhosis would be much higher than for the average patient, say 75% (3:1 odds). Now, the post-test odds after a serum ferritin level of 700 $\mu\text{g/mL}$ are 18:100 (a 15% probability). Despite the low serum ferritin level, you might still suspect cirrhosis. This example shows that a negative test result often does not rule out disease if the pretest probability is high. In fact, because the upper limit of the confidence interval for a serum ferritin level of 700 $\mu\text{g/mL}$ is 0.4, a serum ferritin level of 700 $\mu\text{g/mL}$ is compatible with a post-test probability of cirrhosis as high as 55%.

This commentary has three lessons. First, simply characterizing a test result as falling above or below a single cut-point gives less information than specifying the actual value of the test result (assuming that the literature gives stratum-specific test performance data). Second, the post-test probability after a negative test result can be high if the pretest probability is high, even with a very accurate test. Third, it's important to know the 95% CI for a measure of test performance such as the likelihood ratio, so that one doesn't put too much confidence in a particular test result.

—The Editors

*The odds ratio form of Bayes' theorem states that the post-test odds = pretest odds \times likelihood ratio. The likelihood ratio is the frequency of a finding in patients with a disease divided by its frequency in patients who don't have the disease.

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