

# Noninvasive Prediction of Cirrhosis in C282Y-Linked Hemochromatosis

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**The aim of the present study was to examine the predictive accuracy of noninvasive clinical and biochemical variables associated with cirrhosis among patients with C282Y homozygous hemochromatosis. Sixteen clinical and laboratory variables were recorded at the time of diagnosis in 193 Canadian C282Y homozygous patients. All patients underwent percutaneous liver biopsy and 27 (14%) had biopsy specimen–proven cirrhosis. Prediction of cirrhosis was assessed first by univariate regression analysis. Variables significantly related to cirrhosis were then evaluated by stepwise linear multivariate regression. Receiver operating characteristic curve analysis of the most informative variables from multivariate analysis was then used to devise a clinically applicable index for the noninvasive prediction of cirrhosis. This index was then validated in 162 C282Y homozygous patients in France. Ferritin, blood platelets, and aspartate transaminase (AST) level were selected for the clinical index. The combination of ferritin levels of 1,000  $\mu\text{g/L}$  or greater, platelet levels of  $200 \times 10^9/\text{L}$  or less, and AST levels above the upper limit of normal led to a correct diagnosis of cirrhosis in 77% of Canadian patients. In the French patients, this led to a correct diagnosis of cirrhosis in 90%. In conclusion, in C282Y homozygous patients, a combination of easily measured laboratory variables (ferritin, platelets, AST) can be used to make the diagnosis of cirrhosis in approximately 81% of cases, reducing the need for liver biopsy. (HEPATOLOGY 2002;36: 673-678.)**

Previously, the diagnosis of hemochromatosis was dependent on the phenotypic expression of iron overload. Liver biopsy was an important diagnostic tool because it allowed measurement of hepatic iron concentration. The discovery in 1996 of the C282Y mutation of the HFE protein (a substitution of tyrosine for cysteine at the 282 amino acid position) on the short arm of chromosome 6 has led to a noninvasive method of diagnosing hemochromatosis.<sup>1</sup> Worldwide, 69% to 100% of patients with typical phenotypic hemochromatosis are C282Y homozygotes.<sup>1-6</sup> In referral centers, within pedigrees with iron overload, close to 100% of typical patients are C282Y homozygotes.<sup>1,4</sup>

Genetic testing has thus replaced liver biopsy in the diagnosis of hemochromatosis in many C282Y homozy-

gotes. The role of liver biopsy in these patients remains in the assessment of possible cirrhosis. The advantages of diagnosing cirrhosis in all liver disease patients includes the implementation of screening for portal hypertension therapy and hepatocellular carcinoma, and planning for liver transplantation. A previously published study by Guyader et al.<sup>7</sup> showed that the absence of severe fibrosis could be accurately predicted based on a combination of clinical and biochemical variables. However, this model included a subjective measure of fibrosis, hepatomegaly, and was not able to accurately predict the presence of cirrhosis. The aim of the present study was to improve the positive prediction of cirrhosis in C282Y homozygotes by using objective, noninvasive, laboratory variables.

## Patients and Methods

**Patients.** Patients were selected from a database of HFE genotyped patients based on the following criteria: (1) homozygosity for the C282Y mutation; (2) availability of clinical and laboratory data, and liver biopsy performed allowing for accurate assessment of cirrhosis; and (3) absence of hepatitis C virus antibodies, hepatitis B surface antigen, or a history of significant alcohol ingestion ( $>60$  g of ethanol/d). This study was performed on

Abbreviation: AST, aspartate transaminase.

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Received March 20, 2002; accepted June 11, 2002.

Supported by the Canadian Liver Foundation, the Canadian Institute for Health Research, and the Richard and Penny Wilson Foundation.

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0270-9139/02/3603-0019\$35.00/0

doi:10.1053/jhep.2002.35343

ambulatory patients, which excluded decompensated cirrhosis.

Of the 288 C282Y homozygous patients in the Canadian database, 193 were eligible for analysis by using these criteria. The primary reason for exclusion was the absence of a liver biopsy specimen in 91 patients. An additional 4 patients lacked sufficient clinical or laboratory data. Patients diagnosed without genetic testing, or participants in an ongoing population screening project, were not included. A total of 113 of the patients in the present study were previously included in the validation cohort of the study by Guyader et al.<sup>7</sup>

**Methods.** The following data were recorded at the time of diagnosis: age, sex, serum ferritin, transferrin saturation, serum aspartate transaminase (AST), alanine transaminase, mean corpuscular volume, blood platelets, international normalized ratio, serum albumin, and bilirubin. The upper limit of normal for AST in Canadian patients and French women was 40 U/L, and 50 U/L in French men. The proband case was the first diagnosed case in the family, and discovered cases were other family members subsequently found to have hemochromatosis. Patients were considered diabetic if they required long-term treatment with insulin or oral hypoglycemic agents to control hyperglycemia. The presence of arthritis was determined by history and physical examination. Cardiac disease was based on history and/or clinical signs of congestive heart failure or arrhythmias requiring medical therapy. Data from liver biopsy specimen included hepatic iron concentration (normal, <36  $\mu\text{mol/g}$  of dry liver weight) and hepatic iron index (hepatic iron concentration/age in years). The presence of cirrhosis was established by an experienced pathologist familiar with chronic liver disease and iron overload. Cirrhosis was defined as widespread destruction of normal liver structure by fibrosis and the formation of regenerative nodules.<sup>8</sup>

**Genetic Studies.** Analysis of the C282Y mutation was performed by polymerase chain reaction amplification before restriction fragment length polymorphism analysis as previously described.<sup>9</sup>

**Statistical Analysis.** Statistical analysis was performed by logistic regression and receiver operating characteristic curves by using the MedCalc software package (version 6.0, Mariakerke, Belgium). Values were considered significant with *P* less than .05. The independent effect of significant variables from univariate analysis (*P* < .05) were assessed by using multivariate regression. A second, simplified model for clinical use was then constructed based on a decision tree combining variables selected in multivariate analysis. Optimization of thresholds for this model was based on sensitivity, specificity,

and the area under receiver operating characteristic curves.

**Validation of Predictive Models.** These results were validated in a second population of 162 homozygous C282Y French patients. The selection criteria used was the same as that applied to the Canadian patients. From a database of 246 French C282Y homozygotes, 84 were excluded because of the absence of a liver biopsy specimen (*n* = 84), significant alcohol consumption (*n* = 55), or viral hepatitis (*n* = 7).

## Results

**Patients.** The main clinical and laboratory data of the 193 Canadian patients are given in Table 1. Of these, 166 patients (86%) had no cirrhosis and 27 (14%) had biopsy specimen-proven cirrhosis. The median age was 49 years (25th-75th percentile, 39-60 years), 68 (35.2%) patients were women, and 125 (64.8%) were men. Patients were identified by family screening in 81 cases (42%). The remaining 112 (58%) presented with clinical symptoms suggestive of hepatic disease and/or iron overload or an asymptomatic increase in serum iron values (transferrin saturation, serum ferritin).

**Univariate Analysis.** Comparison of clinical and laboratory features were performed between those with and without cirrhosis (Table 1). Hepatic iron concentration

**Table 1. Clinical and Laboratory Data of Canadian Patients**

	No Cirrhosis	Cirrhosis	<i>P</i> Value
No. of patients	166	27	
Age (yr)	49 (38-60)	49 (43-61)	.32
Sex (M/F)	100/66	25/2	.001
Proband/discovered	87/79	25/2	<.0001
Ferritin ( $\mu\text{g/L}$ )	626.5 (271-1,141)	3,096 (1,845-4,000)	<.0001
Transferrin saturation (%)	72.8 (54-85)	91.4 (83-97)	<.0001
AST (ULN)	0.65 (0.52-0.90)	1.5 (1.16-1.94)	<.0001
ALT (ULN)	0.69 (0.45-1.1)	1.44 (0.97-1.77)	<.0001
Bilirubin ( $\mu\text{mol/L}$ )	12.6 (10-16)	18.5 (13-27)	<.0001
Albumin (g/L)	41.7 (40-43)	42.6 (38.7-44)	.27
INR	1.0 (0.98-1.0)	1.0 (1.0-1.3)	.63
Platelets ( $\times 10^9/\text{L}$ )	238 (202-279)	149 (106-184)	<.0001
MCV (fL)	95 (92-99)	98 (94-101)	.01
Diabetes (yes/no)	19/139	10/15	.001
Arrhythmia (yes/no)	3/142	0/17	.63
Arthritis (yes/no)	61/100	13/12	.19
HIC ( $\mu\text{mol/L}$ )	179 (120-240)	465 (333-562)	<.0001
HII (HIC/age ratio)	4 (2.2-5.7)	10 (5.6-12)	<.0001

NOTE. Values are given as median (25th-75th percentile). Ranges are expressed in parentheses. Normal hepatic iron concentration <36  $\mu\text{mol/g}$  dry liver weight. *P* values are those for the slope of the univariate regression equation for each variable.

Abbreviations: M, male; F, female; ULN, upper limit of normal; INR, international normalized ratio; MCV, mean corpuscular volume; HIC, hepatic iron concentration; HII, hepatic iron index.

**Table 2. Multiple Linear Regression Analysis to Predict Cirrhosis in Hemochromatosis Patients**

I	$X_i$	$\beta$	95% Confidence Interval	SE	T	P
1	Ferritin	0.00009	0.00004	0.00002	4.8	.000003
2	Platelets	-0.001	0.0005	0.0003	-3.7	.001
3	AST	0.004	0.002	0.001	3.3	.001
4	Diabetes*	0.14	0.11	0.05	2.5	.01
5	Constant	0.1				

NOTE. Equation of prediction was:  $Y$  predicted (probability of having cirrhosis) =  $P[\text{cirrhosis}(X_1, X_2, X_3, X_4)] = (\beta_0 + \sum \beta_i X_i) = (0.1 + 0.00009 \text{ ferritin} - 0.001 \text{ platelets} + 0.004 \text{ AST} + 0.14 \text{ diabetes}_{(0,1)})$ .

\*Absence of diabetes, 0; presence of diabetes, 1.

and hepatic iron index were not included in the analysis because these variables were dependent on liver biopsy specimens. Univariate analysis was performed for each variable (using logistic regression for continuous variables and linear regression for frequency data). Alanine transaminase, AST, bilirubin, ferritin, diabetes mellitus, blood platelets, mean corpuscular volume, transferrin saturation, proband, and male gender were significantly associated with the diagnosis of cirrhosis ( $P < .05$ ).

**Multivariate Analysis.** The independent effect of factors found to be significant in univariate analysis were assessed by multivariate analysis. By using the multiple regression model ferritin ( $P < .0001$ ), AST ( $P = .0007$ ), blood platelets ( $P = .0013$ ), and diabetes ( $P = .0098$ ) were selected as predictors of cirrhosis. The parameter estimation from this analysis is given in Table 2.

**Clinical Predictive Model of Cirrhosis.** Variables selected by multivariate analysis (ferritin, AST, blood platelets, and diabetes) were combined to produce a clinically applicable model. Receiver operating characteristic curve analysis was used to select cut-off thresholds with the greatest discriminate power to determine patients with cirrhosis. In the case of ferritin, values between 1,000 and 1,767  $\mu\text{g/L}$  gave the best compromise in terms of sensitivity and specificity (ferritin = 1,000  $\mu\text{g/L}$ : sensitivity 100%, specificity 72%; ferritin 1,767  $\mu\text{g/L}$ : sensitivity 82%, specificity 84%) for the prediction of cirrhosis (Fig. 1). A threshold of 1,000  $\mu\text{g/L}$  was chosen to maximize the positive prediction of cirrhosis and for ease of use in clinical practice. Serum ferritin was the most powerful variable for the prediction of cirrhosis. Twenty-seven of 75 patients (36%) with ferritin levels of 1,000  $\mu\text{g/L}$  or greater had a diagnosis of cirrhosis. Similar analyses were performed for platelets, diabetes, and AST values to determine the combination of variables providing the greatest predictive accuracy for the diagnosis of cirrhosis (Table 3). Among the group with ferritin levels of 1,000  $\mu\text{g/L}$  or greater, excluding patients with platelets greater than

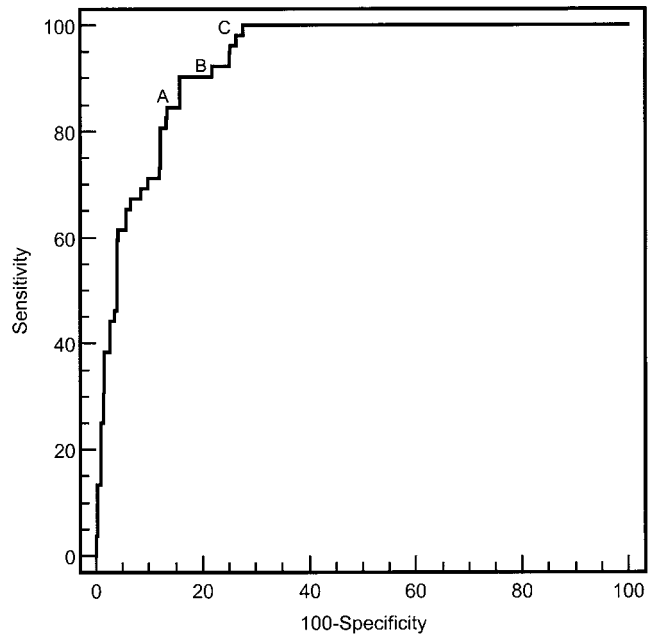


Fig. 1. Receiver operating characteristic curve for serum ferritin in the prediction of cirrhosis ( $n = 355$ ). The area under the curve was 0.93 (95% confidence interval, 0.90-0.96). Serum ferritin levels of (A) 1,767, (B) 1,572, and (C) 1,000  $\mu\text{g/L}$  are shown.

$200 \times 10^9/\text{L}$  and a normal AST level led to the accurate prediction of cirrhosis in 17 of 22 patients (77%; Fig. 2).

Diabetes has been shown to be associated with the development of liver fibrosis in patients with viral hepatitis and alcohol-induced and non-alcohol-induced steatohepatitis.<sup>10,11</sup> In the present study, the presence of diabetes correlated with cirrhosis. However, this variable did not appear in the final clinical model because its inclusion did not improve diagnostic accuracy above that of the other 3 easily measured laboratory values.

**Table 3. Receiver Operator Characteristic Curve Results for the Prediction of Cirrhosis in Hemochromatosis Patients**

Thresholds	Area Under Curve (95% CI)	Sensitivity	Specificity	+LR
Ferritin $\geq 1,000 \mu\text{g/L}$				
Canadian	0.91 (0.86-0.95)	100	71.7	3.53
French	0.95 (0.91-0.98)	100	73.0	3.70
Combined	0.93 (0.90-0.96)	100	71.9	3.56
Platelet $\leq 200 \times 10^9/\text{L}$				
Canadian	0.83 (0.77-0.88)	81.5	77.1	3.56
French	0.89 (0.84-0.94)	76.0	87.6	6.12
Combined	0.85 (0.81-0.89)	78.8	81.8	4.34
AST $\geq \text{ULN}$				
Canadian	0.89 (0.84-0.93)	81.5	81.9	4.51
French	0.85 (0.79-0.90)	48.0	94.9	9.33
Combined	0.86 (0.82-0.90)	65.4	87.7	5.34

Abbreviations: CI, confidence interval; LR, positive likelihood ratio; ULN, upper limit of normal.

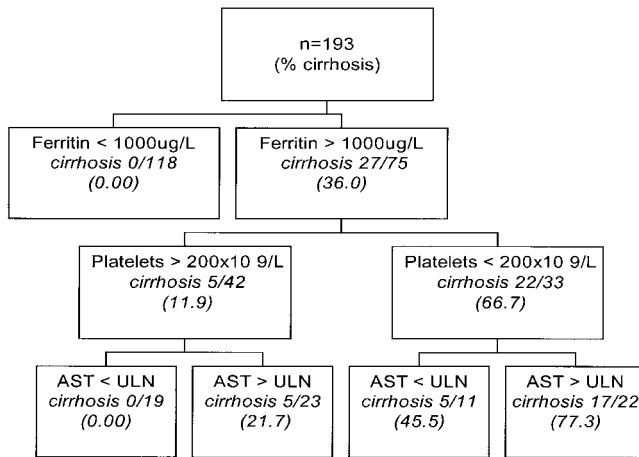


Fig. 2. Prediction of cirrhosis in 193 Canadian C282Y homozygotes by using ferritin, platelet, and AST values. ULN, upper limit of normal.

**Validation of the Clinical Model.** This model was applied to the 162 French C282Y homozygotes (Fig. 3). A comparison of Canadian and French patients is shown in Table 4. Within the subgroup with ferritin levels of 1,000  $\mu\text{g/L}$  or greater, platelets of  $200 \times 10^9/\text{L}$  or less, and AST values above the upper limit of normal, 9 of 10 (90%) were correctly identified as having cirrhosis.

Finally, when serum ferritin values, serum AST values, platelets, and diabetes mellitus were entered in stepwise multivariable analysis by using the same methods as that in the Canadian population, serum ferritin, serum AST, and platelets, but not diabetes mellitus, were selected in the model. The prediction model using both the Canadian and French patients is shown in Fig. 4.

**Discussion**

The present study tested the diagnostic accuracy of clinical and laboratory variables for the prediction of cirrhosis among patients with C282Y homozygous hemochromatosis. It has been previously established that such noninvasive markers can accurately predict the absence of cirrhosis among these patients.<sup>7</sup> The results of the present study show that an accurate diagnosis of cirrhosis can also be made in a significant proportion of C282Y homozygous patients.

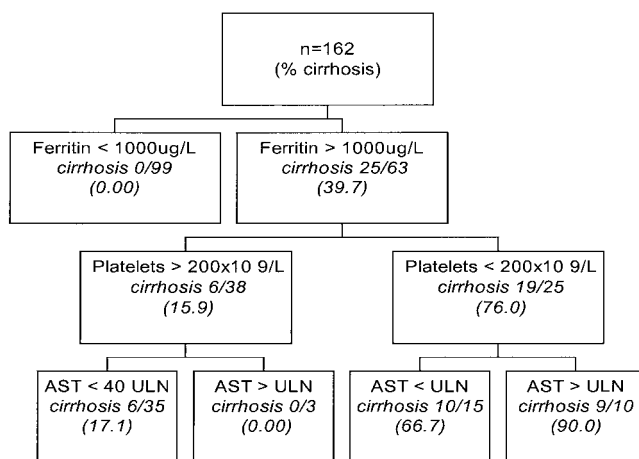


Fig. 3. Validation of the model in 162 French C282Y homozygotes. ULN, upper limit of normal.

**Table 4. Main Clinical and Laboratory Data of Canadian and French Patients**

	Canadian	French	P Value
No. of patients	193	162	
Age (yr)	49 (39-60)	46 (36-56)	.25
Sex (M/F)	125/68	100/62	.40
Cirrhosis (yes/no)	27/166	25/137	.29
Proband/screening	112/81	119/43	.09
Ferritin ( $\mu\text{g/L}$ )	774 (289-1794)	780 (499-1691)	.09
Transferrin saturation (%)	76.9 (59-89)	80 (72-85)	<.001
AST (ULN)	0.7 (0.55-1.05)	0.6 (0.39-0.88)	.08
ALT (ULN)	0.79 (0.5-1.25)	0.6 (0.4-0.8)	.02
Albumin (g/L)	42 (39-43)	0.5 (0.4-0.7)	.49
Platelets ( $\times 10^9/\text{L}$ )	230 (185-274)	235 (203-279)	.24
INR	1.0 (1.0-1.1)	1.1 (1.0-1.1)	.04
Diabetes (yes/no)	29/154	39/95	.16
HIC ( $\mu\text{mol/g dry wt}$ )*	221 (136-357)	277 (190-389)	.44
HII (HIC/age)	4.5 (2.6-6.9)	6.2 (4.0-9.1)	.39

NOTE. Values are given as median (25th-75th percentile). Ranges are expressed in parentheses.

Abbreviations: M, male; F, female; ULN, upper limit of normal; INR, international normalized ratio; HIC, hepatic iron concentration; HII, hepatic iron index.

\*Normal hepatic iron concentration  $<36 \mu\text{mol/g dry liver weight}$ .

Finally, when serum ferritin values, serum AST values, platelets, and diabetes mellitus were entered in stepwise multivariable analysis by using the same methods as that in the Canadian population, serum ferritin, serum AST, and platelets, but not diabetes mellitus, were selected in the model. The prediction model using both the Canadian and French patients is shown in Fig. 4.

Ferritin was the most significant variable for the positive and negative prediction of cirrhosis. In regard to the negative prediction of cirrhosis, our results are consistent with our previous study, with no patient with a ferritin level less than 1,000  $\mu\text{g/L}$  having a biopsy specimen diagnosis of cirrhosis. This represented 61% of the total

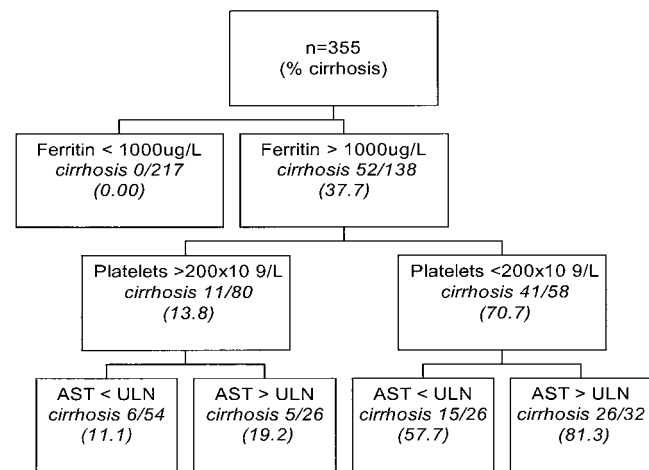


Fig. 4. Prediction of cirrhosis in 355 C282Y homozygotes by using combined Canadian and French patients. ULN, upper limit of normal.

number of patients. These findings provide additional evidence in support of the current AASLD guidelines that recommend that such patients not undergo liver biopsy procedure (provided they are <40 years of age with normal transaminase levels).<sup>12</sup> It should be noted that the median age of our patients was 49, and there were no cirrhotic patients older than 40 years, with a ferritin level of less than 1,000  $\mu\text{g/L}$ .

Regarding the positive prediction of cirrhosis, the diagnostic accuracy of an elevated ferritin level was significantly improved with the addition of a platelet count of  $200 \times 10^9/\text{L}$  or less and AST level above the upper limit of normal. Among the Canadian patients, using a ferritin level of 1,000  $\mu\text{g/L}$  or greater as the sole criterion, only 36% of patients identified (27 of 75) had diagnosed cirrhosis. The predictive accuracy improved to 66.7% (22 of 33) with the addition of platelet counts, and to 77.3% (17 of 22) when all 3 variables were included. Similar results were noted when the predictive model was externally validated by using an independent population. The model was found to have good positive predictive accuracy. Among patients with ferritin levels of 1,000  $\mu\text{g/L}$  or greater, 40% (25 of 63) had cirrhosis. This diagnostic accuracy improved to 90% (9 of 10) with the inclusion of all 3 variables.

In both populations there were a subset of patients with an elevated ferritin level and reduced platelet count but a normal AST level. Eleven Canadian patients (5 with cirrhosis) and 8 French patients (5 with cirrhosis) were among this group. As well, among patients with an elevated ferritin level but platelet count greater than  $200 \times 10^9/\text{L}$  and a normal AST level, no Canadian patients had cirrhosis. However, 6 of 35 French patients in this subset had cirrhosis. All scoring systems arise as a compromise between simplicity of use and discriminant ability. Adjusting these thresholds to classify the cirrhotic patients in these subgroups would have resulted in a loss of predictive accuracy of the model as well as clinical applicability.

Our current model using 3 laboratory variables—ferritin, platelets, and AST—shows an improvement in the positive prediction of cirrhosis. The previous model included only 2 variables—ferritin and AST levels—in addition to the presence of hepatomegaly. Hepatomegaly was excluded from the current model. This is a subjective measure of hepatic fibrosis, vulnerable to interobserver variability.<sup>13</sup> Also, it is well documented that many patients with cirrhosis have small livers. The addition of platelet count is likely a significant factor in the improved accuracy of this model. Low platelet count may be a more reliable marker of portal hypertension than liver size. Thrombocytopenia in patients with cirrhosis has histori-

cally been attributed to hypersplenism.<sup>14</sup> The liver is also the primary site of production of thrombopoietin, a potent stimulator of megakaryocyte growth and platelet production.<sup>15</sup> It has been shown that among patients with cirrhosis, thrombopoietin levels are significantly decreased compared with controls.<sup>16</sup> The significance of thrombocytopenia in the prediction of cirrhosis in patients with hepatitis C has been recognized recently as an independent marker of cirrhosis among patients with hepatitis C.<sup>17</sup> Ferritin level was the most significant variable in the prediction of cirrhosis and the addition of platelet count and AST values improved the prediction. However, we would not recommend the prediction of cirrhosis by using only platelets and alanine transaminase in hemochromatosis.

In the current study, all patients with a history of significant alcohol intake were excluded. Before the exclusion of these patients, the 2 groups differed significantly in the prevalence of excessive drinkers (22% in the French population vs. 4% in the Canadian). Alcohol use is a significant, independent cofactor in hepatic fibrosis. Therefore, the inclusion of these patients in the previous study may have confounded the results. Furthermore, alcoholic siderosis has been misdiagnosed as hemochromatosis before genetic testing.

As is the nature of all predictive indices, our model uses data obtained at one point in time to predict the presence or absence of disease at that time. Given the chronic nature of C282Y homozygous hemochromatosis and changing clinical circumstances, ongoing re-evaluation with the use of follow-up data is important in the proper application of the model. This concept is particularly relevant in the case of patients who meet some but not all of the laboratory criteria.

This model does not apply to compound heterozygotes, H63D homozygotes, or patients with non-HFE-related iron overload.

The prognostic role of liver biopsy in a variety of liver diseases has been widely advocated by hepatologists. This is based on the premise that screening for hepatocellular carcinoma, portal hypertension, and planning for transplantation may be implemented in cirrhotic patients. However, this practice is not universally accepted and hemochromatosis patients may be cared for by hematologists, general internists, geneticists, and public health physicians. The decision regarding the appropriateness of liver biopsy in an individual patient is dependent on the practitioner. This laboratory index is not intended to replace clinical judgment, but should be considered a guideline to the clinical decision making of an individual physician.

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