Modeling Hepatic Fibrosis in African American and Caucasian American Patients With Chronic Hepatitis C Virus Infection

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Assessment of histological stage is an integral part of disease management in patients infected with the hepatitis C virus (HCV). The aim of this study was to develop a model incorporating objective clinical and laboratory parameters to estimate the probability of severe fibrosis (*i.e.*, Ishak fibrosis ≥ 3) in previously untreated African American (AA) and Caucasian American (CA) patients with HCV genotype 1. The Ishak fibrosis scores of 205 CA and 194 AA patients enrolled in the Viral Resistance to Antiviral Therapy of Chronic Hepatitis C study (Virahep-C) were modeled using simple and multiple logistic regression. The model was then validated in an independent cohort of 461 previously untreated patients with HCV. The distribution of fibrosis scores was similar in the AA and CA patients as was the proportion of patients with severe fibrosis (35% vs. 39%, $P = .47$). After accounting for the number of portal areas in the biopsy, patient age, serum aspartate aminotransferase, alkaline phosphatase, and platelet count were independently associated with severe fibrosis in the overall cohort, and the relationship with fibrosis was similar in both the AA and CA subgroups. The area under the receiver operating characteristic curve (AUROC) of the Virahep-C model (0.837) was significantly better than in other published models ($P = .0003$). The AUROC of the Virahep-C model was 0.851 in the validation population. In conclusion, a model consisting of widely available clinical and laboratory features predicted severe hepatic fibrosis equally well in AA and CA patients with HCV genotype 1 and was superior to other published models. The excellent performance of the Virahep-C model in an external validation cohort suggests the findings are replicable and potentially generalizable. (HEPATOLOGY 2006; 44:925-935.)

Abbreviations: HCV, hepatitis C virus; Virahep-C, Viral Resistance to Antiviral Therapy of Chronic Hepatitis C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AUROC, area under the receiver operating characteristic curve.

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The prevalence of infection with hepatitis C virus (HCV) and the proportion with HCV genotype 1 are significantly higher among African Americans (AAs) than among Caucasian Americans (CAs).[1] In addition, there are differences in the proliferative response of peripheral lymphocytes exposed to HCV antigens *in vitro,* which may lead to less severe liver disease and a poorer response to interferon therapy in AAs with HCV than in CAs with HCV.[2,3] However, information on the severity and rates of hepatic fibrosis progression among AAs with HCV has been limited because of the small number of AA patients enrolled in previous studies.[4-8]

Assessment of the severity of hepatic fibrosis is an integral part of disease management in patients with chronic HCV infection. Patients with minimal or mild fibrosis appear to progress slowly, and antiviral treatment can be safely delayed or withheld, particularly among patients with HCV genotype 1.[9] On the other hand, patients with more severe fibrosis (e.g., bridging or septal fibrosis) may progress to cirrhosis and should be prioritized for antiviral treatment.[10] Liver biopsy is the conventional means of assessing hepatic fibrosis, but this invasive procedure is expensive, potentially dangerous, and prone to sampling error. Therefore, accurate, noninvasive means for estimating the severity of hepatic fibrosis are needed to help with the management of the large number of HCV patients worldwide.[10] Panels of serum markers that reflect hepatic fibrogenesis and fibrolysis have been proposed to estimate hepatic fibrosis severity.[11-13] However, simple indices that incorporate widely available biochemical parameters such as serum aspartate aminotransferase (AST) level and platelet count appear to have performance characteristics similar to the more complex serum marker panels.[14-16] The utility of indices incorporating routine laboratory tests to estimate hepatic fibrosis severity in a large AA group with HCV has not been reported.

The Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) study was a multicenter study aimed at identifying the mechanisms of nonresponse and antiviral resistance among AA and CA patients with previously untreated HCV genotype 1 infection.[17] The aim of the current analysis was to compare hepatic fibrosis severity between the 194 AA and 205 CA patients enrolled in Virahep-C in order to identify factors associated with severe fibrosis in the two racial groups and to devise a simple model incorporating readily available clinical and laboratory features to estimate the presence of severe hepatic fibrosis. This model was developed using the Virahep-C cohort and subsequently validated in an independent cohort of 461 well-characterized, previously untreated patients with chronic hepatitis C seen at the NIH clinical center.[18]

Patients and Methods

Patient Population. The Virahep-C study was conducted at 8 clinical centers in the United States with analyses performed by the Data Coordinating Center at the University of Pittsburgh (Pittsburgh, PA).[17] Inclusion criteria for the main trial included having detectable HCV RNA in serum, being HCV genotype 1, having had no previous interferon therapy, and being between 18 and 70 years of age. All subjects had to identify themselves by race as either Black/African American or White/Caucasian American, but not both, and as having been born in the United States. Another inclusion criterion was having had a pretreatment liver biopsy within 18 months of screening whose results were consistent with chronic hepatitis C. Patients with cirrhosis or transition to cirrhosis were required to have no evidence of decompensation or hepatocellular carcinoma on imaging and a serum alpha-fetoprotein level of less than 100 ng/mL within 6 months of entry. Exclusion criteria included pregnancy, detectable hepatitis B surface antigen or anti-HIV, serum creatinine greater than 1.5 $\times$ the upper limit of normal, anemia, and a platelet count of less than 75,000 cells/mL. Subjects with evidence of recent alcohol or drug abuse or severe psychiatric disorder were excluded, as were subjects who reported consuming more than 2 alcoholic beverages per day. All study participants signed a written informed consent approved by the local institutional review board. Targeted enrollment for this study was 200 AA patients and 200 CA patients with HCV genotype 1. The current analysis focused on the 194 AA and 205 CA Virahep-C participants who had an interpretable liver biopsy.

Baseline Assessment. All eligible subjects underwent a complete history and physical examination. The presumed duration of HCV infection was estimated from information provided in patient interview about the earliest parenteral exposure to HCV such as injection drug use or blood transfusion. Subjects were asked how much alcohol and cigarettes they consumed both currently and in the past.[19] Pretreatment complete blood count with platelets and serum AST, alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, and creatinine levels as well as alpha-fetoprotein, international normalized ratio (INR), fasting serum iron studies and cholesterol and triglyceride levels were determined at local clinical laboratories. Serum HCV RNA levels were determined at a central laboratory (SeraCare BioServices, Gaithersburg, MD) using the COBAS Amplicor Hepatitis C Virus Monitor Test, version 2.0 assay (sensitivity 600 IU/mL; Roche Molecular Diagnostics, Alameda, CA). Samples testing negative using this quantitative assay were tested in duplicate with the Amplicor assay (sen-

sitivity 50 IU/mL; Roche). HCV genotyping was performed using the VERSANT HCV Genotype Assay (Bayer, Tarrytown, NY), a line probe hybridization assay.

Liver Histopathology. All available liver biopsy specimens were reviewed by a hepatopathologist (D.K.) who was unaware of any clinical and demographic information. Masson trichrome and hematoxylin and eosin stains were reviewed for hepatitis stage and grade according to the criteria of Ishak et al.[20] and the METAVIR scoring system.[21] To control for biopsy size, the length of the biopsy was measured with a hand ruler, and all portal areas on one cross section were counted. Width was categorized on the basis of the apparent average width of the biopsy core as less than 0.55 mm, 0.55-1.1 mm, or more than 1.1 mm. Severe fibrosis was defined as an Ishak fibrosis score of 3 or more, which corresponds to a METAVIR fibrosis stage of F2 or higher.

National Institutes of Health Validation Cohort. The laboratory and clinical parameters of 494 previously untreated patients with chronic hepatitis C seen at the NIH Clinical Center between 1980 and 2003 were recently described.[18] A subgroup of 461 patients who had laboratory parameters obtained within 6 months of their liver biopsy were utilized for validation of the Virahep-C fibrosis model. Patient ethnicity was self-reported by the patients when seen at the NIH. The same hepatopathologist (D.K.) scored all the Virahep-C and NIH liver biopsy samples. HCV genotyping was done in 331 subjects, including 272 patients with genotype 1.

Data Analyses. Racial differences in the distributions of baseline laboratory results and demographic factors were tested using Student *t* test or the Wilcoxon rank sum test for continuous variables and either Pearson's chi-square test for nominal variables or the Jonckheere-Terpstra test for ordinal categorical variables. Multiple logistic regression was employed to model Ishak fibrosis ≥ 3 as a function of pretreatment laboratory results and demographic factors. To assess and correct for potential biopsy inadequacy in estimating fibrosis severity (e.g., understaging), the number of portal areas (10 or fewer vs. at least 11) was included in the multiple logistic regression model. To predict severe fibrosis in the model, the number of portal areas was included in the constant term to adjust for adequacy of biopsy size.

The area under the receiver operating characteristic (AUROC) curve was computed for the Virahep-C fibrosis model and compared to other published models including the AST to platelet ratio index (APRI), the AST/ALT ratio, and the cirrhosis discriminant score (CDS).[15,22-27] The AUROCs were compared using the method of DeLong et al.[28,29] The APRI (AST level/[ULN]/platelet count [10^9/L] $\times$ 100) optimally predicts Ishak fibrosis of at least 3 with a cutoff of 0.80.[15] An AST/ALT ratio greater than 1.0 has been proposed as a useful marker of cirrhosis (Ishak fibrosis ≥ 5) but is a poor predictor of severe fibrosis.[22,23] The CDS is a combination of the platelet count, ALT/AST ratio, and INR with a cutoff of at least 8 that has a high specificity for severe fibrosis.[24] Finally, the age-platelet index (AP index) combines patient age and platelet count into a score of 1-10.[25] To compare the AUROCs of independent samples (*i.e.*, AA vs. non-AA, CA vs. AA), a Wilcoxon rank sum test was used. For all analyses, *P* values less than .05 were interpreted as statistically significant. Both SAS and the R statistical programming language were used.

Results

Virahep-C Patient Population. HCV RNA levels were similar in the 194 AA and 205 CA patients (Table 1). The distribution of parenteral risk factors was similar between AAs and CAs (injection drug use 48% vs. 52%, blood products 22% vs. 24%, unknown 13% vs. 14%, intranasal cocaine 8% vs. 4%, and other 8% vs. 7%) as was the estimated duration of infection. Pretreatment serum AST, ALT, albumin, and bilirubin levels were significantly lower in AA patients than in CA patients. In contrast, serum alkaline phosphatase and ferritin levels were significantly higher. Female AA patients had a higher body weight and BMI than female CA patients, but these parameters were not statistically significantly different between AA and CA men. Diabetes mellitus and hypertension were significantly more common among AA patients than among CA patients.

A broad distribution of hepatic fibrosis scores was observed in the Virahep-C population (Fig. 1). The Ishak fibrosis scores were not significantly different between the AA and CA patients ($P = .45$). In addition, the proportion of patients with Ishak fibrosis ≥ 3 (39% CA vs. 35% AA, $P = .47$) was not significantly different between the two racial groups.

Univariate Models to Predict Ishak Fibrosis ≥ 3. Simple logistic regression models were used to assess the relationship of demographic, pretreatment laboratory, and clinical factors with severe hepatic fibrosis. Both hypertension and diabetes mellitus were positively associated with severe fibrosis, whereas smoking and alcohol consumption were not (Table 2). Age was positively associated with severe hepatic fibrosis, but race and gender were not. The probability of severe fibrosis increased with increasing values of serum AST, ALT, alkaline phosphatase, total bilirubin, ferritin, and INR. In contrast, the probability of severe fibrosis decreased with increasing platelet count and cholesterol and albumin levels.

Table 1. Baseline Features of the Virahep-C Study Population

	Caucasian Americans		African Americans		
		n		n	*P* *
Demographics					
Age (yrs)	48 (43, 52)	205	49 (45, 52)	194	.0810”
% Male	65.4	134	64.4	125	.9281
Years of infection **	27 (19, 33)	158	25 (17, 32)	143	.1396”
Laboratory parameters & histology					
Log HCV RNA	6.3±0.8	205	6.2±0.7	192	.3754^
% Genotype					
1, NOS	7.8	16	5.7	11	.3778
1a	57.8	118	46.4	90	.0288
1b	28.4	58	46.4	90	.0003
1a/1b	5.9	12	1.5	3	.0447
Serum ALT (IU/L)[1]	74 (51, 138)	205	59.5 (40, 90)	194	<.0001‘
Serum AST (IU/L)[1]	52 (37, 87)	205	51 (34, 69)	194	.0164‘
Alk phosphatase (IU/L)[1]	77 (61, 95)	205	81 (63, 107)	194	.0364‘
Albumin (g/dl)	4.2±0.3	205	4.0±0.3	194	<.0001^
Total bilirubin (mg/dL)[1]	0.7 (0.5, 0.9)	205	0.6 (0.4, 0.8)	194	.0005^
INR	1.0 (0.9, 1.1)	204	1.0 (0.9, 1.1)	193	.8569”
White blood cell count (10^3/mL)[1]	6.2 (4.9, 7.4)	204	5.8 (4.7, 7.5)	194	.0710^
Platelet count (10^3 / mL)[1]	206.5 (161.5, 244)	204	214.5 (168, 268)	194	.1044^
Ferritin (mg/dl)[1]	149 (75, 292)	203	244 (109, 413)	193	.0001^
% Iron/ TIBC × 100	34.1 (26.7, 46.1)	202	33.9 (25.7, 41.3)	189	.1163”
Total cholesterol (mg/dL)[1]	174 (153, 197)	202	175 (152, 196)	191	.7661^
Triglycerides (mg/dL)[1]	97.5 (75, 137)	184	110 (76, 165)	182	.0420^
Ishak fibrosis score					.4473~
0	10.7	22	10.3	20	
1	23.4	48	26.8	52	
2	27.3	56	28.4	55	
3	20.0	41	17.0	33	
4	9.3	19	12.4	24	
5	3.9	8	2.1	4	
6	5.4	11	3.1	6	
% Ishak score≥ 3	38.5	79	34.5	67	.4683
Total HAI Inflammation	9 (7, 11)	205	8 (7, 10)	194	.2919”
Days between biopsy and baseline	103 (25, 241)	205	66 (19, 200)	194	.2325”
Clinical features					
Male weight (kg)	87.3 (78.0, 99.8)	134	90.3 (79.8, 103.9)	123	.2467”
Female weight (kg)	68.5 (60.3, 82.1)	71	82.1 (73.0, 96.6)	69	<.0001”
BMI (kg/m^2)					
Males[2]	28.4 (25.8, 31.4)	134	28.5 (26.2, 32.4)	123	.1759^
Females[2]	25.0 (22.3, 31.7)	69	31.6 (26.9, 35.3)	68	<.0001‘
% Diabetes	4.4	9	15.5	30	.0004
% Hypertension	21.0	43	43.3	84	<.0001
% Current smoker	34.5	70	41.5	78	.1859
Alcohol Use (drinks/day)					
<1	88.6	178	87.7	164	.9174
1 to 2	7.0	14	5.4	10	.6527
≥ 2	4.5	9	59.1	13	.4047

NOTE. All statistics reported as “mean ± SD”, “median (interquartile range), or “%”.

Abbreviations: NOS, Not otherwise specified

*For continuous or ordinal variables, P refers to comparisons of African American vs Caucasian American using a pooled variance estimator t-test (^), Satterthwaite method t-test (‘), a Wilcoxon rank sum test (“), or an Exact Jonckheere-Terpstra test (~). For categorical variables, P refers to a continuity adjusted chi-square test. T-tests conducted on transformed scales are noted:

[1]Transformation: natural logarithm;

[2] Transformation: 1 – reciprocal

**Estimated from patient interview of earliest parenteral risk factor

Liver Biopsy Size and Probability of Severe Fibrosis. Because previous reports have demonstrated that small liver biopsy samples may underestimate the severity of hepatic fibrosis, severe fibrosis was analyzed as a function of biopsy size.[30,31] The median length, width, and number of portal areas in the biopsies were similar in AA and CA patients (Table 3). However, the number of portal areas was significantly associated with severe fibrosis,

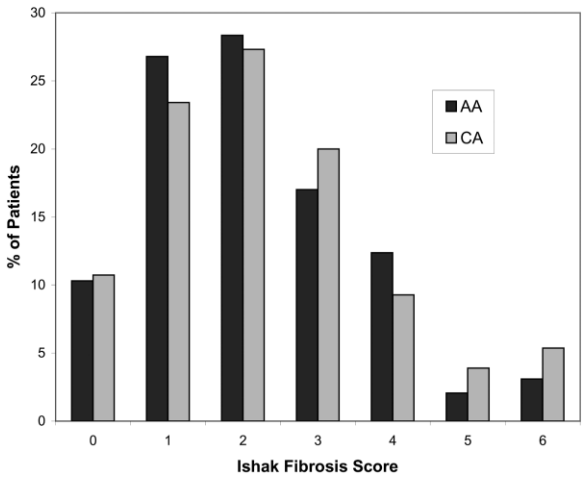

Fig. 1. Distribution of Ishak fibrosis scores in AA and CA patients with HCV genotype 1 enrolled in the Virahep-C study.

with biopsies having fewer than 11 portal areas less likely to demonstrate severe fibrosis (Fig. 2). Although liver biopsy length was strongly associated with the number of portal triads ($r = 0.853$, $P < .0001$), univariate and multivariate analyses did not show a significant relationship between biopsy length and the probability of severe fibrosis on (data not shown).

Multivariable Models to Predict Ishak Fibrosis ≥ 3. The results of the simple logistic regression analyses were used to guide the construction of the multiple logistic regression model. To make use of all the biopsies available and to simultaneously adjust for the relationship between the number of portal areas and fibrosis score, the number of portal areas was included as a dichotomous variable (< 11 vs. ≥ 11). Because the number of portal areas was related to sampling artifact (*i.e.*, smaller biopsies may understage fibrosis), the coefficient for the number of

Table 2. Single Explanatory Factor Models for Predicting Severe Hepatic Fibrosis in the Virahep-C Cohort

Single Explanatory Factor*	Ishak Fibrosis 0 - 2 (n = 253)	Ishak Fibrosis 3-6 (n = 146)	Odds Ratio (95 % CI)	P **
Age (years)	47 (42, 51)	50 (46, 53)	1.72 (1.36-2.17)	<.0001
% Male	62.5	69.2	1.35 (0.87-2.08)	.1756
% Caucasian American	49.8	54.1	1.19 (0.79-1.79)	.4072
Years of infection	24 (16, 32) n = 193	27.5 (22.5, 33) n = 108	1.53 (1.18-1.97)	.0011
Log HCV RNA	6.2 ± 0.7 n = 253	6.3 ± 0.7 n = 144	1.10 (0.90-1.36)	.3545
ALT (IU/mL) [1]	55 (41, 84)	82 (59, 138)	1.90 (1.52-2.39)	<.0001
AST (IU/mL) [1]	45 (31, 59)	74 (50, 106)	2.67 (2.06-3.47)	<.0001
AST/ALT ratio [1]	0.7 (0.6, 0.9)	0.8 (0.7, 1.0)	1.36 (1.10-1.67)	.0040
Alk phosphatase (IU/mL) [1]	75 (59, 90)	92 (73, 118)	1.97 (1.56-2.49)	<.0001
Albumin (g/dl)	4.2 ± 0.3	4.0 ± 0.4	0.69 (0.56-0.86)	.0007
Total bilirubin (mg/dL) [1]	0.6 (0.4, 0.8)	0.7 (0.5, 0.9)	1.75 (1.39-2.21)	.0001
INR	1.0 (0.9, 1.0) n = 252	1.0 (1.0, 1.1) n = 145	1.74 (1.39-2.19)	<.0001
WBC count (10^3/mL)	6.1 (4.8, 7.5) n = 252	5.7 (4.7, 7.3) n = 146	0.93 (0.76-1.15)	.5031
Platelet count (10^3/mL)	228.5 (189, 278) n = 252	176 (142, 214) n = 146	0.39 (0.30-0.51)	<.0001
Ferritin (mg/dL) [1]	174 (77, 303) n = 251	263 (136, 413) n = 145	1.63 (1.30-2.05)	.0001
% Iron/TIBC x 100	32.2 (25.4, 41.8) n = 248	36.6 (27.8, 46.6) n = 143	1.11 (0.90-1.37)	.3113
Cholesterol (mg/dL) [1]	179 (159, 200)	164 (146, 193)	0.72 (0.58-0.89)	.0025
Triglycerides (mg/dL) [1]	100 (74, 146.5) n = 236	109 (79, 149) n = 130	1.09 (0.88-1.34)	.4532
Biopsy to baseline (days)	70 (21, 227)	90 (21, 217)	1.06 (0.86-1.29)	.5874
No. of portal tracts	14 (8, 21)	18.5 (13, 26)	1.37 (1.12-1.68)	.0023
% Portal tracts 10	63.6	84.2	3.06 (1.83-5.11)	<.0001
Biopsy length (mm)	13 (9, 22) n = 251	13 (10, 20) n = 146	0.93 (0.76-1.15)	.5111
% Biopsy width ≥ 0.55 mm	83.7 n = 251	88.4 n = 146	1.48 (0.81-2.72)	.2040
Weight (kg)	84.4 (73.5, 95.9) n = 252	85.3 (77.1, 99.3) n = 145	1.26 (1.02-1.54)	0.0288
Male	87.1 (78.5, 99.3)	90.5 (79.8, 104.6)	1.24 (0.96-1.59)	0.0944
Female	78.9 (64.1, 88.5)	77.6 (68.5, 92.1)	1.17 (0.82-1.66)	0.3877
BMI (kg/m^2) [2]	28.3 (24.8, 32.6) n = 250	28.9 (26.0, 32.3) n = 144	1.13 (0.92-1.39)	0.2418
Male[2]	28.5 (25.9, 31.7)	28.7 (26.2, 32.0)	1.10 (0.86-1.42)	0.4500
Female[2]	27.2 (23.6, 34.2)	29.8 (25.6, 33.3)	1.18 (0.82-1.70)	0.3713
% Diabetes	6.7	15.1	2.46 (1.26-4.81)	0.0083
% Hypertension	24.9	43.8	2.35 (1.53-3.63)	0.0001
% Current smoker	37.7 n = 247	38.2 n = 144	1.02 (0.67-1.56)	0.9150
% Alcohol use	24.4 n = 246	19.0 n = 142	0.73 (0.44, 1.21)	0.2224

*Measures displayed as "mean ± SD", "median (interquartile range)" or "%".

**Comparison of subjects with Ishak fibrosis 0-2 versus Ishak fibrosis 3-6. The odds ratios with 95% confidence intervals show the effect of a one standard deviation increase in the explanatory factor on the transformed scale, if a transformation was used.

[1] Natural log transform.

[2] 1- reciprocal transform.

Table 3. Comparison of Liver Biopsy Characteristics by Subject Race in the Virahep-C Cohort

Biopsy Characteristic	Caucasian American	African American	P ^
Biopsy length	(n = 204)	(n = 193)	
Median (IQR) (mm)	13 (9, 20)	14 (9, 20)	.4496[1]
% ≤ 15 mm	60.8	54.9	.2798
% > 15 to ≤ 25 mm	27.9	30.6	.6418
% > 25 mm	11.3	14.5	.4167
Biopsy width	(n = 204)	(n = 193)	
% < 0.55 mm	15.7	13.5	.6296
% 0.55-1.1 mm	81.4	84.5	.4954
% > 1.1 mm	2.9	2.1	.7517*
No. of portal tracts	(n = 205)	(n = 194)	
Median (IQR)	15 (10, 24)	15 (10, 23)	.8764[1]
% ≤ 10	29.8	27.8	.7544
% 11 to ≤ 20	39.0	38.1	.9381
% > 20	31.2	34.0	.6243

[1]Wilcoxon rank sum test Chi-square test for percentages, except for Fisher's exact test (denoted by *)

^Comparison of African American and Caucasian American subjects

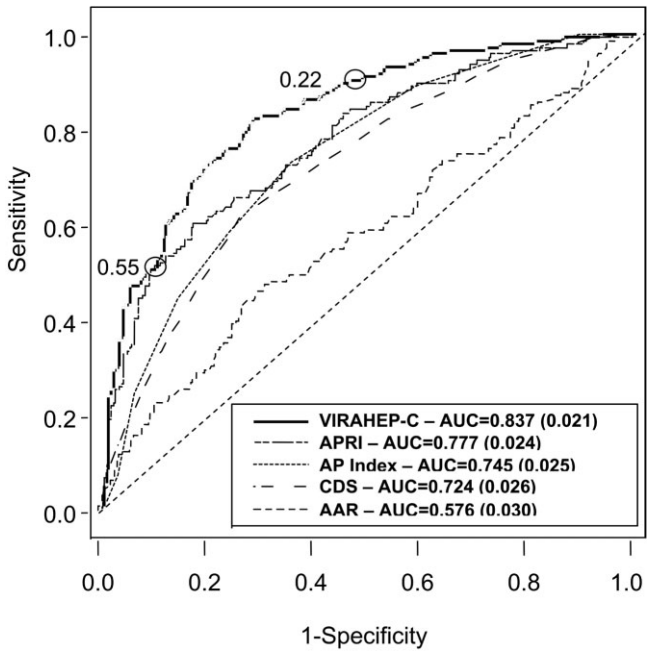

Fig. 3. Comparison of the Virahep-C model to other published models in estimating the presence of severe hepatic fibrosis. The AUROC of the Virahep-C model was significantly better at estimating the probability of severe fibrosis than the APRI (0.837 vs. 0.777, $P = .0003$) as well as the AST/ALT ratio (AAR), clinical discriminant score (CDS), and age-platelet index (AP).

portal areas was included in the intercept term of the final model, which assumed a biopsy of adequate size (*i.e.*, 11 or more portal areas). This analysis revealed that serum AST, platelet count, alkaline phosphatase, and age were independently associated with severe fibrosis in the overall study group. The final logistic regression model was:

Probability of observing severe fibrosis on an adequate biopsy = $1/(\exp[-y) + 1)$ with $y = -5.17 + 0.20 \times$ race $+ 0.07 \times$ age (years) $+ 1.19 \times \ln$(AST [IU/L]) $- 1.76 \times \ln$(platelet count [103/mL]) $+ 1.38 \times \ln$(alkaline phosphatase [IU/L]).

For the category of race in the model, CA was designated as 1 and AA as 0. Age was expressed in years, AST in IU/L, platelet count in 10^3/mL (*i.e.*, 210,000 = 210), and alkaline phosphatase in IU/L. In multivariable analysis, AA race was not significantly associated with severe hepatic fibrosis ($P = .45$), but race was left in the model because the main study was designed to investigate racial differences in patients with chronic hepatitis C, and CA patients exhibited a trend toward more severe fibrosis. Interactions of race with patient'sage, AST, platelet count, and alkaline phosphatase were not statistically significant.

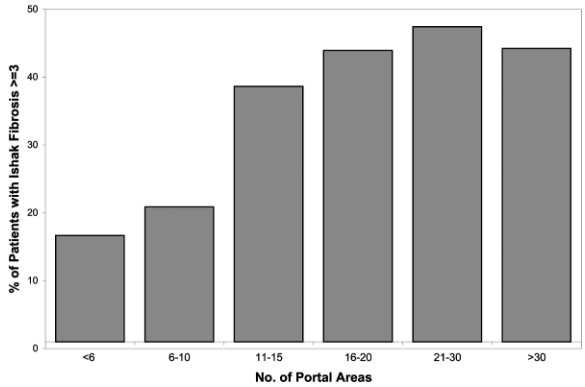

Fig. 2. Percentage of patients with severe fibrosis in relation to the number of liver biopsy portal areas. Patients with a liver biopsy that contained at least 11 portal areas had a significantly greater likelihood of having severe fibrosis than those with a biopsy containing fewer than 11 portal areas (43% vs. 20%, $P = .0009$). However, there was no significant increase in the likelihood of severe fibrosis among patients who had a biopsy that contained 11-15 portal triads compared to those whose biopsies had 16-20, 21-30, or >30 portal triads ($P = .717$).

The overall AUROC for the model was 0.837 (95% CI: 0.796-0.878; Fig. 3). To use the model, various cutoff values were selected to predict whether severe fibrosis was present. A model score (predicted probability) of at least 0.22 yielded 90% sensitivity for the presence of significant fibrosis with a positive predictive value (PPV) of 53% (Table 4). Similarly, a model score of ≤ 0.55 yielded 90% specificity for the absence of significant fibrosis, with a negative predictive value of 76%. If a model score of ≤ 0.22 was used to predict the absence of severe fibrosis, 150 (38%) of the Virahep-C subjects would be so classified with 90% accuracy. Similarly, using a model score greater than 0.55 for predicting severe fibrosis would accurately classify 99 (25%) of the Virahep-C patients with 75% accuracy. The model would not be used to predict fibrosis in the remaining 149 (37%) subjects, whose model scores

Table 4. Use of the Virahep-C Fibrosis Model to Predict Ishak Fibrosis ≥ 3 in the Overall Group, African Americans, and Caucasian Americans

Cutoff	n (%)	Ishak 0-2 n (%)	Ishak 3-6 n (%)	Sensitivity %	Specificity %	PPV %	NPV %
AA & CA	398	252	146				
≤ 0.22	150(38)	135 (54)	15 (10)		54		90
> 0.22	248 (62)	117 (46)	131 (90)	90		53	
≤ 0.55	299 (75)	227 (90)	72 (49)		90		76
> 0.55	99 (25)	25 (10)	74 (51)	51		75	
AA	194	127	67				
≤ 0.22	78 (40)	72 (57)	6 (9)		57		92
> 0.22	116 (60)	55 (43)	61 (91)	91		53	
≤ 0.55	152 (78)	117 (92)	35 (52)		92		77
> 0.55	42 (22)	10 (8)	32 (48)	48		76	
CA	204	125	79				
≤ 0.22	76 (37)	69 (55)	7 (9)		55		91
> 0.22	128 (63)	56 (45)	72 (91)	91		56	
≤ 0.55	145 (71)	110 (88)	35 (44)		88		76
> 0.55	59 (29)	15 (12)	44 (56)	56		75	

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; AA, African American; CA, Caucasian American.

were between 0.22 and 0.55, because of the large probability of misclassification.

The utility of the Virahep-C model for both racial groups was confirmed by fitting it separately for the AA and CA subgroups. The AUROC was 0.845 for AA and 0.847 for CA ($P = .90$), indicating the Virahep-C model is useful in estimating severe fibrosis in both racial groups. In addition, the cutoff values for 90% sensitivity and 90% specificity for severe fibrosis were similar for each race (data not shown).

Other Published Models of Ishak Fibrosis ≥ 3. The AUROC for the 398 Virahep-C patients in the Virahep-C fibrosis model was 0.837 (95% CI: 0.796-0.878) (Fig. 3). In comparison, the APRI model gave an AUROC of 0.777 (95% CI: 0.729-0.825), so that the new Virahep-C model predicted severe hepatic fibrosis significantly better than the APRI ($P = .0003$).[28] The AUROCs for the age-platelet index, clinical discriminant score, and AST/ALT ratio were 0.745 (95% CI: 0.697-0.793), 0.724 (95% CI: 0.674-0.774), and 0.576 (95% CI: 0.516-0.635), respectively, indicating the Virahep-C model was significantly better in this patient sample.

Use of Virahep-C Fibrosis Model in NIH Cohort. The predictive accuracy of the Virahep-C fibrosis model was assessed in an independent cohort of 461 patients with chronic hepatitis C who were evaluated at the NIH Clinical Center.[18] The results of the Virahep-C model were evaluated separately in the AA (16%) and non-AA (84%) groups, the latter of which included CA (81%) and Asian American (3%) patients. In the NIH cohort, the AA patients were significantly older than the non-AA patients, and as expected, the HCV genotype 1 was more common in the AA group (Table 5). The overall distribution of Ishak fibrosis scores and the proportion with an Ishak fibrosis score of at least 3 were not significantly different between AA and non-AA. However, as in the Virahep-C population, the serum ALT and albumin levels were significantly lower and the serum alkaline phosphatase levels were significantly higher in the AA group than in the non-AAgroup.

The Virahep-C model had an AUROC of 0.851 in the NIH cohort. Using a cutoff of less than 0.22 to exclude severe fibrosis in the NIH cohort gave a specificity of 65% and a negative predictive value of 85%. Using a cutoff greater than 0.55 to identify patients with severe hepatic fibrosis resulted in a sensitivity of 51% with a PPV of 81%. The Virahep-C model predicted severe hepatic fibrosis in the NIH cohort better than the AST/ALT ratio (AUROC = 0.556, $P < .0001$), AP index (AUROC = 0.722, $P < .0001$), and the APRI (AUROC = 0.83, $P = .057$). The Virahep-C model also performed significantly better in the AA subgroup than in the non-AA subgroup (AUROC 0.952 vs. 0.832, $P < .0001$). In addition, the Virahep-C model predicted severe hepatic fibrosis significantly better in the AA subgroup ($P < .001$) than did the AST/ALT ratio (0.563), the AP index (0.754), and the APRI (0.881). In the non-AA subgroup, the Virahep-C model was significantly better than the AST/ALT ratio (0.544) and the AP index (0.714) and predicted fibrosis as well as the APRI model (AUROC 0.832 vs. 0.830, $P = .85$).

Discussion

The primary aim of this analysis was to compare the severity of hepatic fibrosis prior to treatment in large

Table 5. Description of the National Institutes of Health External Validation Cohort of Previously Untreated Patients With HCV

Feature	Total NIH Cohort n = 461	Non-AA n = 388	AA n = 73	p**
Age (years)	43.6 (37.9, 50.9)	42.7 (37.4, 50.2)	45.9 (41.1, 53.1)	.0050"
% Male	59.4	58.0	67.1	.1841
% Genotype 1	82.2	79.9	93.1	.0274
BMI (kg/m^2)[2]	26.3 (23.6, 29.6)	26.0 (23.4, 29.3)	28.4 (25.6, 32.6)	.001^
Serum AST (IU/L)[1]	57 (37, 89)	57.0 (37, 89.5)	56 (36, 83)	.4181^
Serum ALT (IU/L)[1]	84 (51, 145)	88.0 (52.5, 154)	63 (47, 117)	.0018^
Alkaline phosphatase (IU/L)[1]	77 (60, 94)	76 (59, 91)	89 (72, 131)	.0001‘
Serum albumin (g/dL)	4.2 ± 0.4 n = 453	4.2 ± 0.4 n = 380	4.0 ± 0.5 n = 73	.0076‘
Total bilirubin (mg/dL)[1]	0.7 (0.5, 0.9) n = 459	0.7 (0.5, 0.9) n = 386	0.7 (0.6, 1.0) n = 73	.0708^
Platelet count (10^3/mL)[1]	213 (175, 255)	212.5 (176, 256)	213 (173, 252)	.6947^
Ishak fibrosis score				.0379~
0	18.9	11.0	20.4	
1	23.0	21.9	23.2	
2	19.3	17.8	19.6	
3	16.5	21.9	15.5	
4	10.0	13.7	9.3	
5	7.4	6.9	7.5	
6	5.0	6.9	4.6	
% Ishak score 3	38.8	36.9	49.3	.0611
# portal triads	18 (12, 25)	18 (12, 26)	16 (12, 22)	.0645"
% portal triads > 11	82.0	82.5	79.5	.6523

NOTE. All statistics reported as "mean ± SD", "median (interquartile range)" or "%".

Non-AA category includes Caucasian Americans and Asians.

*HCV genotype was available for 331 of the 461 patients (genotype not specified, n = 130).

**Non-AA versus AA comparisons. For continuous variables, P refers to comparisons of African American vs Non-African American groups using a pooled variance estimator *t* test (^), Satterthwaite method *t* test (‘), a Wilcoxon rank sum test (“), or an Exact Jonckheere-Terpstra test (~). For categorical variables, P refers to a continuity adjusted chi-square test. *t* tests conducted on transformed scales are noted:

[1]Transformation: natural logarithm;

[2]Transformation: 1 – reciprocal.

groups of AA and CA patients with chronic hepatitis C, HCV genotype 1. The distribution of Ishak fibrosis scores was similar in the two racial groups, as was the proportion of patients with severe fibrosis and cirrhosis (Fig. 1). However, AA patients had significantly lower serum AST, ALT, and bilirubin levels and higher serum alkaline phosphatase levels than did CA patients (Table 1). Of interest, similar racial differences in laboratory parameters were found in an independent patient population (Table 5), and similar findings have also been reported in previous retrospective analyses of AA cohorts with chronic hepatitis C.[4,5] Despite the AA patients consistently having serum ALT and AST levels that were lower than those of CA patients, this and other prospective multicenter trials have found similar levels of hepatic inflammation and fibrosis in AA and CA treatment candidates with HCV genotype 1.[7,8] Taken together, these studies suggest that the severity of underlying liver disease may be underestimated by the ALT and AST levels in AA patients.[4-6]

Why the AA patients in the Virahep-C and NIH cohorts had higher serum alkaline phosphatase levels than the CA patients is not clear. Alkaline phosphatase levels are usually normal in patients with chronic hepatitis C, unless there is advanced fibrosis or cirrhosis. In the Virahep-C patients, serum alkaline phosphatase level was positively associated with subject age and INR ($P = .006$) and inversely associated with platelet count and albumin level ($P < .0001$), suggesting that alkaline phosphatase level correlates to some extent with disease severity. Other studies have demonstrated that serum gamma glutamyltranspeptidase (GGTP), another marker of cholestasis, is also positively associated with severe fibrosis in chronic hepatitis C patients.[14] In fact, serum GGTP levels have been incorporated into several noninvasive algorithms of fibrosis.[14,32] Alkaline phosphatase elevation may also reflect a higher prevalence of insulin resistance or the effects of diabetes, hypertension, or other comorbid conditions among AA than among CA with chronic hepatitis C.[33-38]

Previous studies have reported that the duration of HCV infection, alcohol consumption, and smoking are important cofactors for fibrosis progression in chronic hepatitis C. In this cohort, these three parameters were distributed similarly between the AA and CA patients and were not significantly associated with severe hepatic fibrosis. However, duration of infection is difficult to estimate, and errors in estimation may obscure its association with

fibrosis. In this cohort, patient age was significantly associated with the presence of fibrosis and may have served as a surrogate for disease duration. Smoking and alcohol were also not significantly associated with fibrosis in this cohort. Most enrolled patients drank little or no alcohol. Thus, the Virahep-C cohort cannot be used to assess the role of moderate to severe alcohol intake on hepatic fibrosis, and the absence of a significant association should not be interpreted as indicating alcohol intake has no effect on hepatic fibrosis progression in chronic hepatitis C.

An important aim of this analysis was to develop a model to estimate the presence of severe hepatic fibrosis in patients with chronic hepatitis C based on readily available and objective laboratory markers. A model incorporating age, serum AST, platelet count, and alkaline phosphatase yielded an AUROC of 0.837 in predicting severe fibrosis. These factors have been consistently found to be associated with advanced fibrosis.[23,26,39-45] The Virahep-C model was significantly more accurate in predicting severe hepatic fibrosis than were several other published models including the APRI (Fig. 3). The Virahep-C fibrosis model was also better at predicting no severe fibrosis.[46] Specifically, using a cutoff of 0.55 led to an estimation that 25% of Virahep-C patients had severe fibrosis, which had a positive predictive value of 75%, whereas with a cutoff of less than 0.22 an estimated 38% of Virahep-C patients did not have severe fibrosis, for which the negative predictive value was 90% (Table 4). Overall, using the Virahep-C model could have led to the avoidance of liver biopsy in up to 64% of patients so that only one-third would have required biopsy to accurately determine the presence of severe hepatic fibrosis. When the Virahep-C fibrosis model was tested in an independent cohort of untreated patients with chronic hepatitis C, a similar AUROC was obtained, though the model performed better in AA than in non-AA patients. In addition, the Virahep-C model was significantly better than the other published models in the NIH cohort, although the difference with APRI was of borderline significance ($P = .057$).

Previous studies have suggested that a biopsy that contains at least 11 portal areas and exceeds 25 mm in length and 1.4 mm in width is required to avoid understaging of hepatic fibrosis in hepatitis C.[30,31] In addition, recent studies have suggested that models of hepatic fibrosis perform better in patients whose liver biopsy sample was larger.[47,48] When an indicator for the number of portal tracts was incorporated into the Virahep-C model, this variable was not only a significant predictor of fibrosis in univariate analysis but also an independent predictor in multiple regression analysis. These data demonstrate that the odds of severe fibrosis is lower with a smaller liver biopsy (*i.e.*, one with fewer than 11 portal areas) than with a larger biopsy. The Virahep-C fibrosis model compensated for variability in biopsy sample size when predicting severe hepatic fibrosis by including in the constant the coefficient for biopsy size. This allowed use of all the Virahep-C biopsy data instead of having to discard the scores from smaller biopsies. Future studies should also assess the effect of biopsy size on model performance.

Among the strengths of the current analysis was that it included a large number of both AA and CA patients who were well characterized and previously untreated. The patient cohort included the full spectrum of disease severity of chronic hepatitis C, short of advanced cirrhosis. The study used biopsy readings from one expert hepatopathologist with careful determination of biopsy size and number of portal areas. Furthermore, the availability of an appropriate validation set provided assurance that the findings are replicable and potentially generalizable. Shortcomings of this study were that patients with moderate to severe alcohol use were excluded as were patients with HIV coinfection, factors that may significantly affect disease progression. In addition, pretreatment laboratory values were obtained locally at the 8 participating sites. Furthermore, liver biopsies were obtained up to 18 months before the laboratory determinations. However, routine liver biochemistries and complete blood counts are commonly analyzed using standardized instruments. In addition, the model performed well in the independent patient cohort, for whom routine laboratory studies were obtained at a single clinical laboratory and generally within a few days of liver biopsy.[18] Comparison of the Virahep-C fibrosis model to other serum fibrosis marker panels that presumably reflect hepatic fibrogenesis and fibrolysis more directly is of interest and planned for the future.[11-13,47] In addition, comparison of the Virahep-C fibrosis model to other noninvasive imaging modalities is of interest but was not feasible when the study was initiated in 2001.[49]

In summary, the distribution and severity of hepatic fibrosis was similar between AA and CA patients enrolled in the Virahep-C study despite significant differences in several baseline clinical and laboratory parameters. The Virahep-C cohort was used to develop a noninvasive model for predicting severe hepatic fibrosis. This model included age at the time of liver biopsy and laboratory tests (*i.e.*, serum AST, alkaline phosphatase, platelet count) routinely performed for patients with chronic hepatitis C. Overall, the model performed well in the Virahep-C population as well as in an independent cohort of previously untreated patients. In addition, the model was significantly better at predicting severe fibrosis than were other published models of routine laboratory parameters.

The Virahep-C fibrosis model may prove to be of value to practicing clinicians attempting to determine the severity of HCV. A web site for use of the model will be available at http://www.virahepc.org.

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