NON-INVASIVE SERUM FIBROSIS MARKERS FOR SCREENING AND STAGING CHRONIC HEPATITIS C VIRUS (HCV) PATIENTS IN A LARGE U.S. COHORT

Scott D. Holmberg¹, Mei Lu², Loralee B. Rupp³, Lois E. Lamerato³, Anne C. Moorman¹, Vinutha X. Vijayadeva⁴, Joseph A. Boscarino⁵, Emily W. Henkle⁶, Stuart C. Gordon², for the Chronic Hepatitis Cohort Study (CHeCS) Investigators*

¹Division of Viral Hepatitis, Centers for Disease Control and Prevention Atlanta, GA
²Henry Ford Health System (HFHS) Data Coordinating Center, Detroit, MI
³Center for Health Services Research, HFHS, Detroit, MI
⁴Kaiser Permanente-Center for Health Research, Honolulu, HI
⁵Geisinger Health System, Danville, PA
⁶Kaiser Permanente- Center for Health Research Northwest, Portland, OR

Correspondence: Dr. Scott Holmberg; CDC Mailstop G-37; 1600 Clifton Rd; Atlanta, GA 30333 USA.  em: sdh1@cdc.gov

*See listing of CHeCS Investigators at end of article
ABSTRACT

Background: Liver biopsy remains critical for staging liver disease in hepatitis C virus (HCV)-infected persons, but is a bottleneck to evaluation, follow-up and treatment of HCV. Our analysis sought to validate ‘APRI’ (aspartate aminotransferase [AST]-to-platelet ratio index) and ‘FIB-4,’ an index from serum fibrosis markers (alanine aminotransferase [ALT], AST, and platelets plus patient age) to stage liver disease.

Methods: Biopsy results from HCV patients in the Chronic Hepatitis Cohort Study (‘CHeCS’) were mapped to a F0-F4 equivalent scale; APRI and FIB-4 scores at the time of biopsy were then mapped to the same scale.

Results: We identified 2,372 liver biopsies from HCV-infected patients with contemporaneous laboratory values for imputing APRI and FIB-4. Fibrosis stage distributions by the equivalent biopsy scale were: 267 (11%) F0; 555 (23%) F1; 648 (27%) F2; 394 (17%) F3; and 508 (21%) F4. Mean APRI and FIB-4 values significantly increased with successive fibrosis levels (p<0.05). The areas under the curves using receiver operating characteristic curves (AUROC) analysis distinguishing severe (F3-F4) from mild-to-moderate fibrosis (F0-F2) were: 0.80 (0.78, 0.82) for APRI; and 0.83 (0.81, 0.85) for FIB-4. There was a significant difference between the AUROCs of FIB-4 and APRI (p < 0.001); 88% of persons who had a FIB-4 score > 2.0 were at stage F2 or higher.

Conclusion: In a large observational cohort, FIB-4 was good at differentiating five stages of chronic HCV infection. It can be useful in screening patients who need biopsy and therapy; for monitoring less advanced disease patients; and for longitudinal studies.

Summary: Authors review two (FIB-4 and APRI) systems relying on more easily obtainable serum and cell (platelet) biomarkers for liver biopsies to stage liver disease. In turn, this would be an important step in opening treatment and care of uncomplicated HCV-infected patients by infectious disease specialists.
INTRODUCTION

Staging HCV infection is still mainly based on degree of histologic fibrosis in a liver biopsy sample, but there are many problems in relying on biopsy. Although percutaneous liver biopsy is usually a safe procedure, it is costly and does carry a small risk for complication (1). There can easily be sampling errors, because only approximately .002% of the organ is biopsied, and inter- and intra-observer discrepancies of 10% to 20% in assessing hepatic fibrosis have been reported (2,3). In addition, liver biopsy is performed or arranged for by a small number of specialists, creating a “bottleneck” in staging and treating HCV-infected patients. The procedure is uncomfortable if not painful, and some patients demur from getting the procedure and, thus, evaluation for therapy. Further, as biopsy is usually performed once on a patient, the ability to monitor a patient’s liver fibrosis would benefit from an index based on serum fibrosis markers comparable to determining CD4+ cell counts as used for evaluating and monitoring HIV patients.

Thus, several indices constructed from non-invasive serum-based biomarkers of fibrosis—here called “serum fibrosis markers”—have been proposed and validated, usually within relatively small sets of treatment-naïve chronic hepatitis C patients (4). Most attention has centered on the aspartate aminotransferase (AST)-to-platelet ratio index (APRI)(5-8), and the FIB-4 index (9-11), that is calculated from AST, alanine aminotransferase (ALT), platelet count and patient age. More complicated indices using harder-to-obtain laboratory values (12-14) with or without transient elastography (15,16) have also been proposed. However, APRI and FIB-4 have been of more interest to clinicians because they are simple to calculate and readily available from hospital or clinic laboratories during usual patient care. That is, these simple calculations based on serum result would be useful to screen patients with high values needing biopsy and clinical follow-up and to provide a system for categorizing stage of illness. It is critical to determine which HCV patients have advanced fibrosis to gauge the urgency of treatment as well as the need for upper endoscopy for varices, every six-month ultrasounds for hepatocellular cancer screening, and closer clinical monitoring of cirrhotic patients.

The Chronic Hepatitis Cohort Study (CHeCS), a prospective, longitudinal, observational cohort study, was established to assess the clinical impact of chronic viral hepatitis B and C in the United States (17,18). CHeCS is a ‘dynamic’ multi-center cohort study conducted at four large, integrated health care systems located in Detroit, Michigan, Danville, Pennsylvania, Portland, Oregon, and Honolulu, Hawaii and represents a geographically, ethnically and clinically diverse US-based cohort of, currently, about 3,000 HBV-infected and 12,000 HCV-infected patients. Since CheCS is an observational study, the data collected from the electronic
The laboratory tests necessary for imputation of the serum fibrosis markers were not necessarily collected on the same date as the liver biopsy (but close in time). The goal of this analysis was to evaluate the capability of serum fibrosis markers, imputed from labs collected during the course of routine care and within 6 months of a biopsy, to accurately predict fibrosis level as interpreted by pathologists reading biopsies in an uncontrolled, real world setting.

METHODS

The Chronic Hepatitis Cohort Study (CHeCS)

The patients included in this study are the chronic hepatitis C subpopulation of the CHeCS cohort, the recruitment and baseline characteristics of which have been described (18). Briefly, the analysis included adults aged > 18 years from the four participating health care organizations (Geisinger Health System, Danville, PA; Henry Ford Health System, Detroit MI; Kaiser Permanente - Northwest, Portland, OR; Kaiser Permanente - Honolulu, HI) with at least one admission or outpatient provider, laboratory, or emergency department encounter from January 1, 2006 through December 31, 2010.

The study underwent ethical review and was approved by the institutional review boards at each study site and the Centers for Disease Control and Prevention (Atlanta, GA). Trained medical abstractors conducted the chart reviews to confirm chronic infection status, as well as to collected biopsy results. The study was restricted to confirmed chronic hepatitis C patients who had the requisite serum fibrosis markers and biopsy fibrosis readings within 6 months of each other.

Data collection and classification

Patient data were collected and analyzed from electronic medical records including: age (at time of liver biopsy); gender; race/ethnicity; annual income (derived from census tract data based on zip code or patient residence); serum alanine aminotransferase (ALT) level and aspartate aminotransferase (AST) levels (elevated values were relative to the upper limit of normal value specific to each laboratory that performed the test); and platelet counts. The laboratory data were largely collected via EMRs; in addition, lab values from external laboratories were captured through the chart abstraction. It was not a requirement that the all of the component lab values necessary for imputing the serum fibrosis markers were collected on the same day as each other; the serum fibrosis markers were imputed based on labs collected
up to within 7 days of each other. Lab tests after liver transplantation were excluded from this analysis.

**Histological liver assessment**

Liver biopsies were fixed in formalin and embedded in paraffin and were evaluated by pathologists for determination of fibrosis status. Fibrosis scores from different scoring systems (IASL, Batts Ludwig, Metavir, Ishak, Knodell, Scheuer) were mapped to a F0-4 equivalency scale. That is, fibrosis was ranked as: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. If patient had a liver transplant, the laboratory results and biopsies after the transplant were excluded from this analysis. If the patient had more than one biopsy, the most severe biopsy at the earliest date with available lab results was used for this analysis.

**Indices bases on serum fibrosis markers**

All patients’ laboratory data (alanine aminotransferase [ALT], aspartate aminotransferase [AST], platelet count) were collected through electronic medical records. If multiple laboratory values were available, the results closest to the time of biopsy were used. APRI, FIB-4, and, for purposes of comparison, AST/ALT ratio were calculated when the laboratory assessments were within 7 days of each other and within 6 months of the biopsy.

- **AST/ALT**  
  Aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio

- **APRI**  
  \[ \frac{\text{AST level} \, (/\text{ULN}^*)}{\text{Platelet count}} \times 100 \]  
  (*where ULN= upper limit of normal for that laboratory)

- **FIB-4**  
  \[ \text{Age (years)} \times \text{AST (U/L)} \times \text{Platelet count (10}^9/L) \times [\text{ALT (U/L)}]^{1/2} \]

**Statistical Methods:**

Each serum fibrosis marker was evaluated for normality, but as they were not normally distributed, log transformation was used for the analysis. To determine the association of each index based on serum fibrosis markers with biopsy fibrosis staging, Generalized Estimating Equation (GEE) was used-- instead of the analysis of variance (ANOVA)—as it provides a robust estimation with less restriction on the underlying distribution of data. The analysis tested for the mean differences among five biopsy fibrosis stages (the overall group effect), followed by
pair-wise comparisons between fibrosis levels if an overall group effect was detected at p<0.05. The mean and its 95% CI were calculated for each biopsy group.

In addition, the predictive ability of each index from serum fibrosis markers to differentiate advanced fibrosis (F3 and F4) from mild-to-moderate fibrosis (F0 through F2) was measured by the areas under the curves using receiver operating characteristic curves (AUROC) analysis. Nonparametric Mann-Whitney U test was used to assess statistically significant differences of AUROC between the three indices based on serum fibrosis markers. For each marker, the optimal cutoff point was identified to minimize misclassification with calculation of sensitivity and specificity. The same analysis was repeated using the indices when the laboratory assessments were within 7 days of each other and within 3 months of the biopsy.

RESULTS

A total of 10,473 patients had confirmed chronic HCV and, after excluding 41 patients with only biopsy after liver transplant, 4,313 (41%) had unique fibrosis staging by liver biopsy. Of them, 2,372 (55%) had calculable APRI, FIB-4, and AST/ALT scores within 6 months of the biopsy date. These patients were a mean age of 50 years at the time of biopsy and, like all hepatitis C patients in the CHeCS (18), were more likely male (61%), white (65%) and, for those in health plans, enrolled a mean of 7.4 years (88.7 months) (Table 1)

Overall correlation of APRI and FIB-4 with successive stages of liver fibrosis

The fibrosis stage distributions by the equivalent scale were: 267 (11%) F0; 555 (23%) F1; 648 (27%) F2; 394 (17%) F3; and 508 (21%) F4 (Table 2). Biomarker values were significantly associated overall fibrosis stage levels (p<0.01). The mean and its 95% CIs were mutually exclusive of each other indicating significant mean differences among biopsy fibrosis levels (p<0.05) (Table 2).

The AST/ALT ratios, used by some clinicians and in other analyses (19), were calculated for purposes of comparison (Figure), but clearly performed less well than either APRI or FIB-4 (Figure).

The ability of indices from serum fibrosis markers for predicting severe fibrosis in liver histology (F3-F4): The AUROCs and their 95% CIs in distinguishing severe fibrosis (F3-F4) from mild-to-moderate fibrosis (F0-F2) were 0.80 (0.78, 0.82) for APRI, 0.83 (0.81, 0.85) for FIB-4, and 0.64 (0.61, 0.66) for AST/ALT ratio (p < 0.001; Figure). There was a significant difference between the AUROCs of FIB4 and APRI, and between APRI and AST/ALT ratio (Figure). The optimal cutoff point for APRI was 0.81 (sensitivity 75%, specificity 74%), 1.81 for FIB4
(sensitivity 74%, specificity 77%), and 0.82 for ALT/AST ratio (sensitivity 62%, specificity 60%). Of 981 patients with FIB-4 > 2.0, 862 (87.9%) had a biopsy reading F2 or higher. Restricting to laboratory values obtained within 3 months of the biopsy, AUROCIs and their 95% CIs in distinguishing severe fibrosis (F3-F4) from mild-to-moderate fibrosis (F0-F2) were 0.81 (0.79, 0.83) for APRI, 0.84 (0.82, 0.86) for FIB4, and 0.66 (0.63, 0.68) for AST/ALT ratio.

DISCUSSION

In a large observational ‘real world’ cohort of chronic hepatitis C patients, FIB-4 was superior to APRI and much superior to a simple AST/ALT ratio at distinguishing severe fibrosis from mild-to-moderate fibrosis. Both FIB-4 and APRI had excellent predictive ability when the serum fibrosis marker(s) could be collected up to within six months of the biopsy. FIB-4 scores were strongly associated with patient status within five stages of HCV infection determined by biopsy. To our knowledge, this is the largest such analysis of these serum fibrosis marker scores as derived from a US population of chronic hepatitis C patients.

There are several reasons why using FIB-4 would be helpful in guiding patient monitoring and care. Current guidelines for antiviral treatment for HCV recommend, among other things, liver biopsy confirmation of substantial fibrosis or cirrhosis (20, 21). In limited studies to date, high FIB-4 scores (e.g. > 2.25) appear to discriminate between these severe stages (F3, F4) and low or moderate stages (F0-F2) of fibrosis (13,22). Use of FIB-4 may obviate the need for liver biopsy for uncomplicated earlier stage HCV patients. Further, determining who of the 30-40% of hepatitis C patients will progress to cirrhosis end stage liver disease, hepatocellular carcinoma and death has been problematic (23): a noninvasive serum fibrosis marker score would avert this difficulty in monitoring patients’ disease progression.

Therapeutic decisions about when to start antiviral therapy or not are not the only reason that clinicians may want a non-invasive way to monitor and assess liver disease. It is critical to determine which HCV patients have advanced fibrosis to gauge the need for upper endoscopy for varices, every six month ultrasounds for hepatocellular carcinoma, and close clinical monitoring of cirrhotic patients.

There are other advantages to using FIB-4 or other serum fibrosis marker indices to initially stage and follow HCV patients. First, liver biopsy is usually performed or arranged for (to be done by radiologist) by a liver specialist, requiring the patient to seek care from such a specialist. As there are over 3 million HCV-infected patients in the United States, but fewer than 2,000 board-certified hepatologists, there is a scarcity of clinicians qualified to diagnose, follow and treat HCV patients. Although liver biopsy is not required for treatment, in the CHeCS HCV-
infected population, 38.4% had had a biopsy between 2001 and 2010 (18). Requiring biopsy to justify antiviral therapy creates a bottleneck that may lead to many HCV-infected patients not seeking or receiving care, as in this population (17,18). There is growing interest and attention from the perspective of health care advocates and hepatologists that hepatitis C care can and should be provided by internists, infectious disease specialists, family practitioners and other clinicians (24). Ease of monitoring would be especially helpful in systems such as Project ECHO in New Mexico, that has demonstrated the utility and effectiveness of guiding non-specialist clinicians by teleconference and other telecommunication in caring for HCV patients in remote, rural or hard-to-access areas (25). Still, even if non-specialists can manage uncomplicated HCV infection, it is important to note that management of late-stage, cirrhotic patients, especially those who may decompensate with antiviral therapy, should continue to be managed by hepatologists and others with experience in treating such patients.

Studies of the natural history, timing and success of treatment of chronic HCV have been hampered by a lack of a relatively easy non-invasive staging system, such as CD4-cell count and viral load as used for HIV. Clinically, it is hard to monitor the progress of an individual patient without performing multiple biopsies. Thus, another advantage of using FIB-4 will be to allow longitudinal studies of the natural history of HCV, risk of and preventive factors for liver disease progression. As liver biopsies are usually performed only once on a patient, understanding the progression of HCV infection have been limited to studies of the few patients who have multiple biopsies (26) or by meta-analysis of several small studies (22,27). Longitudinal analysis of the effects of antiviral drug therapy, alcohol use (or cessation) and other factors that may impact HCV disease progression is important, but requires a way of monitoring progression similar to that seen with HIV (CD4+ cell levels).

Transient elastography (FibroScan) may soon be approved for use in the United States, and this technology appears to be superior to FIB4 or other serum fibrosis marker calculations for later stage (F3, F4) hepatic fibrosis and cirrhosis, but also equally or less useful in the diagnosis of moderate or less liver fibrosis (4, 28). Besides its expense, the applicability (80%) of elastography is not as good as that for serum fibrosis markers, and unreliable results—i.e., not meeting manufacturer’s recommendations—have been reported for 16% (29). Problems are caused by patient obesity, limited operator experience, or if a patient has eaten a meal within the previous 3 hours (4,29,30). In any case, serum fibrosis markers will for the near future remain more readily available, reliable, and less expensive to the widening group of physicians who are treating chronic hepatitis C.

Limitations to this analysis include variability in these serum fibrosis markers at various
stages of liver disease (fibrosis). In terms of assessing liver disease severity, it has not been demonstrated that assessment of structure (biopsy) is more reliable than indices derived from liver injury (ALT, AST) and hematologic (platelet) tests. However, even assuming that liver histology is the “gold standard,” it is subject to inter- and intra-observer discrepancies of 10% to 20% in those reading biopsy specimens (2,3). Thus, we did not—and could not—rely on central reading of over 2,000 biopsies at the four sites; we wanted to investigate performance of non-invasive serum fibrosis markers and biopsy as performed in a wide range of real-world settings and situations. Nonetheless, biopsies were somewhat over represented in men and white persons compared to HCV prevalence in these groups in the general population (31), and so this factor must be considered when generalizing from these data.

While assigning multiple fibrosis staging systems to a single category (F0 through F4) may result in misclassification, presumably roughly equal numbers of specimens were incorrectly categorized to a higher or lower stage. However, such variability may have limited clinical applicability. Based on our analysis, a FIB-4 score of 1.81 provides the best sensitivity and specificity for distinguishing stages F3 and F4 from lesser stages of liver fibrosis. As a simpler guide, a threshold FIB-4 score 2.0 or greater would identify 88% of those at F2 or higher stage of liver fibrosis, who are appropriate for further evaluation, including biopsy, and treatment.

In summary, this analysis suggests that use of FIB-4 will facilitate screening, identification and treatment of HCV patients needing liver biopsy and antiviral therapy, be accessible to non-hepatologist clinicians who do or wish to care for patients with chronic HCV infection, and provide a reasonable staging system for the analysis of HCV infection and the factors that accelerate (e.g., alcohol use) and stop or retard (e.g., antiviral therapy) disease progression. Accordingly, the CHeCS Investigators are currently analyzing several outcomes—such as mortality, hospitalization, and efficacy of antiviral drug therapy—stratified by patients’ FIB-4 levels.
APPENDIX

The CHeCS Investigators include the following investigators and sites: Scott D. Holmberg, Eyasu H. Teshale, Philip R. Spradling, and Anne C. Moorman, Division of Viral Hepatitis, National Centers for HIV, Viral Hepatitis, STD, and TB Prevention (NCHHSTP), Centers for Disease Control and Prevention (CDC), Atlanta, Georgia; Stuart C. Gordon, David R. Nerenz, Mei Lu, Lois Lamerato, Loralee B. Rupp, Nonna Akkerman, Nancy J. Oja-Tebbe, Chad M. Cogan, and Dana Larkin, Henry Ford Health System, Detroit, Michigan; Joseph A. Boscarino, Zahra S. Daar, Robert E. Smith, Patrick J. Curry, Brandon D. Geise, and Joe B. Leader; Geisinger Health System, Danville, Pennsylvania; Cynthia C. Nakasato, Vinutha Vijayadeva, Kelly E. Sylva, John V. Parker, and Mark M. Schmidt, Kaiser Permanente- Hawaii, Honolulu, Hawaii; Emily M. Henkle, Tracy L. Dodge, Erin M. Keast, and Lois Drew, Kaiser Permanente- Northwest, Portland, OR.

ACKNOWLEDGMENTS

CHeCS is funded by the CDC Foundation that received unrestricted grants from: Abbott Laboratories; Genentech, a member of the Roche Group; Janssen Pharmaceutical Companies of Johnson & Johnson; and Vertex Pharmaceuticals.

S. C.G. receives grant/research support from Abbott Pharmaceuticals, Bristol-Myers Squibb, Exalenz BioScience, Gilead Pharmaceuticals, GlaxoSmithKline, GlobelImmune, Intercept Pharmaceuticals, Merck, Roche Pharmaceuticals, Tibotec, Vertex Pharmaceuticals, and Zymogenetics; serves as a consultant for Achillion, Bristol-Myers Squibb, CVS Caremark, Gilead Pharmaceuticals, Merck, Salix Pharmaceuticals, Johnson and Johnson, and Vertex; and serves on the Data Monitoring Board for Tibotec.
References


Figure (legend): The predictive ability of three non-invasive methods for severe fibrosis. The areas under the curves using receiver operating characteristic (AUROC) analysis in distinguishing severe fibrosis (Stages F3 and F4) from mild-to-moderate fibrosis (Stages F0 through F2) were: 0.80 (0.78, 0.82) for APRI, 0.83 (0.81, 0.85) for FIB-4, and 0.64 (0.61, 0.66) for AST/ALT ratio.
Table 1: Characteristics of CHeCS* hepatitis C-infected participants who had available biopsy, laboratory, and demographic information

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>Persons studied (N=2,372)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
</tr>
<tr>
<td>Portland, OR</td>
<td>785 (33%)</td>
</tr>
<tr>
<td>Honolulu, HI</td>
<td>358 (15%)</td>
</tr>
<tr>
<td>Detroit, MI</td>
<td>704 (30%)</td>
</tr>
<tr>
<td>Danville, PA</td>
<td>525 (22%)</td>
</tr>
<tr>
<td>Age at Biopsy (yrs)</td>
<td></td>
</tr>
<tr>
<td>Mean (S.D.)</td>
<td>50.1 (8.96)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>51.0 (16, 78)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>931 (39%)</td>
</tr>
<tr>
<td>Male</td>
<td>1441 (61%)</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
</tr>
<tr>
<td>American Indian</td>
<td>42 (2%)</td>
</tr>
<tr>
<td>Asian</td>
<td>98 (4%)</td>
</tr>
<tr>
<td>Black</td>
<td>419 (18%)</td>
</tr>
<tr>
<td>Hawaiian/PI</td>
<td>74 (3%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>209 (9%)</td>
</tr>
<tr>
<td>White</td>
<td>1530 (65%)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>98 (4%)</td>
</tr>
<tr>
<td>Median Household Income</td>
<td></td>
</tr>
<tr>
<td>&lt;$15,000</td>
<td>50 (2%)</td>
</tr>
<tr>
<td>$15-30,000</td>
<td>380 (16%)</td>
</tr>
<tr>
<td>$31-50,000</td>
<td>1161 (50%)</td>
</tr>
<tr>
<td>$51-75,000</td>
<td>579 (25%)</td>
</tr>
<tr>
<td>≥ $75,000</td>
<td>161 (7%)</td>
</tr>
</tbody>
</table>

*CHeCS,’ Chronic Hepatitis Cohort Study
Table 2: Correlation of HCV disease stage by invasive (liver biopsy staging) and non-invasive (APRI and FIB-4 scores), Chronic Hepatitis Cohort Study (CHeCS), 2,372 biopsies, 2008-2011*

<table>
<thead>
<tr>
<th>DEGREE OF FIBROSIS (STAGE)</th>
<th>Liver Biopsy Scoring Systems</th>
<th>Non-invasive Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IASL</td>
<td>Metavir</td>
</tr>
<tr>
<td>No fibrosis (F0) (N= 267)</td>
<td>No fibrosis (0)</td>
<td>F0</td>
</tr>
<tr>
<td>Fibrous portal expansion (F1) (N= 555)</td>
<td>Mild-portal Fibrosis (1)</td>
<td>F1</td>
</tr>
<tr>
<td>Few bridges or septa (F2) (N=648)</td>
<td>Moderate fibrosis (2)</td>
<td>F2</td>
</tr>
<tr>
<td>Numerous bridges or septa (F3) (N=394)</td>
<td>Severe fibrosis (3)</td>
<td>F3</td>
</tr>
<tr>
<td>Cirrhosis (F4) (N= 508)</td>
<td>Cirrhosis (4)</td>
<td>F4</td>
</tr>
</tbody>
</table>

* Abbreviations used: APRI, aspartate aminotransferase (AST)-to-platelet ratio index; FIB-4, a fibrosis index that combines three standard biochemical values (platelets, alanine aminotransferase [ALT], AST) plus patient age; IASL, International Association for the Study of the Liver; 95% CIs, 95 percent confidence intervals.  † Score differences between stages, p < 0.05