



## CLINICAL RESEARCH STUDY

AJM Theme Issue: Gastroenterology

# Assessment of FIBROspect II to Detect Hepatic Fibrosis in Chronic Hepatitis C Patients

 Atif Zaman, MD, MPH,<sup>a</sup> Hugo R. Rosen, MD,<sup>a</sup> Ken Ingram, PA-C,<sup>a</sup> Christopher L. Corless, MD, PhD,<sup>a</sup> Esther Oh,<sup>b</sup> Katie Smith<sup>b</sup>
<sup>a</sup>Oregon Health & Science University, Portland; and <sup>b</sup>Prometheus Laboratories, San Diego, Calif.

**ABSTRACT**

**BACKGROUND:** The degree of liver fibrosis in patients with Hepatitis C (HCV) provides important prognostic information; however, the only current method available to obtain this information is by performing a liver biopsy. Liver biopsies are invasive, associated with complications, and costly. There has been recent interest in developing a panel of serum markers that can reliably predict the presence of fibrosis and, thus, obviate the need for a liver biopsy. Our objective was to prospectively validate a panel of serum fibrosis markers (FIBROspect<sup>SM</sup> II) that has been recently developed.

**METHODS:** Serum was obtained from 108 consecutive HCV (15% with HCV/ETOH) patients seen in a hepatology clinic at a single tertiary care center at the time of liver biopsy. The performance of FIBROspect II (consisting of 3 fibrosis markers: hyaluronic acid, tissue inhibitor of metalloproteinases 1, and alpha-2-macroglobulin) in differentiating mild (F0-F1) from significant (F2-F4) fibrosis was assessed by comparing the panel results with performed liver biopsy.

**RESULTS:** The prevalence of significant fibrosis in the study group was 36.1%. The diagnostic value of the serum marker panel to detect significant fibrosis as assessed by area under the receiver operating characteristic (ROC) curve was 0.826. Performance characteristics are as follows: sensitivity 71.8%, specificity 73.9%, positive predictive value 60.9%, negative predictive value 82.3%, and overall accuracy of 73.1%.

**CONCLUSION:** This prospective study supports the clinical utility of serum markers in detecting fibrosis and validates the performance of FIBROspect II in a prospective cohort of patients. The high negative predictive value of the test provides a reliable alternative to rule out severe fibrosis. © 2007 Elsevier Inc. All rights reserved.

**KEYWORDS:** Hepatitis C; Hepatic fibrosis

The majority of chronic liver diseases are typified by hepatic injury and inflammation, which then leads to fibrogenesis.<sup>1</sup> Fibrogenesis is considered a dynamic process characterized by the formation of the constituents of the extracellular matrix, which is a mixture of glycoproteins and proteoglycans in a complex network. Fibrosis is felt to be a

physiologic mechanism, initially beneficial in helping to limit the extension of the inflammatory reaction, but as injury persists it is detrimental to the liver. Collagen and matrix proteins that constitute fibrosis are largely produced by activated stellate cells. Over time, the inflammation seen in the liver due to chronic injury can fluctuate, worsening or improving over time. However, fibrosis, in a setting of chronic persistent injury, is believed to be progressive and largely irreversible.<sup>2,3</sup> Ultimately, progressive fibrosis leads to architectural distortion of the liver, and cirrhosis. Therefore, the progression of fibrosis determines prognosis in patients with chronic liver disease.

Currently, the gold standard for determining the degree of hepatic fibrosis is a liver biopsy. Several scoring systems

Funding for this study was given by Prometheus Laboratories, which included only the cost of serum fibrosis testing, shipping and handling costs, and statistical support. No other financial support was given.

Requests for reprints should be addressed to Atif Zaman, MD, MPH, Division of Gastroenterology and Hepatology, Oregon Health & Science University, Mailcode: PV310, 3181 SW Sam Jackson Park Road, Portland, Oregon 97201.

E-mail address: zamana@ohsu.edu

to assess fibrosis have been proposed, including the Knodell score,<sup>4</sup> the Ishak score,<sup>5</sup> and the Metavir score.<sup>6</sup> The Metavir system has been carefully validated in chronic hepatitis C patients and is used increasingly in practice. None to mild fibrosis is generally considered F0-F1, and F2-F4 is considered significant fibrosis—where F4 is cirrhosis. Liver biopsies, however, are invasive and have associated morbidity including pain, intraperitoneal hemorrhage, intrahepatic or subcapsular hematomas, hemobilia, bile peritonitis, and pneumothorax. Complications requiring hospitalization occur in 1%-3% of patients, and mortality rates are between 1 in 10,000 and 1 in 12,000.<sup>7</sup> The diagnostic limitations of a percutaneous liver biopsy include inter- and intra-observer variability of the fibrosis staging and sampling error. Although factors that improve the diagnostic accuracy of a liver biopsy include use of a Trucut 15-gauge needle rather than Menghini-type needles, multiple passes, biopsy core sizes  $\geq 2$  cm, and use of pathologists familiar with liver biopsy readings,<sup>7,8</sup> even in the ideal situation a single pass percutaneous liver biopsy incorrectly stages fibrosis in 20% of patients.<sup>9</sup>

Because of the invasiveness of a liver biopsy and the issues outlined above, there has been great interest in developing noninvasive markers of fibrosis. Recently, a panel of fibrosis markers has been developed and consists of hyaluronic acid (HA), tissue inhibitor of metalloproteinases 1 (TIMP-1), and alpha-2-macroglobulin with very good performance characteristics in predicting the absence/presence of significant hepatic fibrosis.<sup>10</sup> However, this serum panel was developed using banked serum samples. Furthermore, this serum panel has not been validated using samples from an independent cohort. Therefore, the aim of this study is to prospectively validate this panel of serum markers (FIBRO*Spect*II<sup>®</sup>) using a cohort of consecutive patients seen in a hepatology clinic at a university hospital.

## METHODS

### Study Subjects

Consecutive patients with hepatitis C seen in the hepatology clinic at Oregon Health and Science University were asked to participate in this study. All patients were anti-HCV (hepatitis C) positive and had detectable plasma HCV-RNA by polymerase chain reaction. The study was approved by the Institutional Review Board at Oregon Health and Science University. The period of enrollment was between October 2001 and June 2003. All patients at the time of liver biopsy underwent a blood draw for a serum sample. All serum samples were shipped frozen to Prometheus Laboratories for analysis.

## Serum Fibrosis Marker Assays

Serum levels of 3 fibrosis markers (FIBRO*Spect* II<sup>®</sup>, Prometheus Laboratories, San Diego, Calif) were determined by technologists blinded to clinical, laboratory and histological findings. Serum hyaluronic acid was measured in an enzyme-linked sandwich assay using HA-binding protein (Corgenix, Westminster, Conn). Tissue inhibitor of metalloproteinase-1 (TIMP-1) was measured by a sandwich ELISA (Amersham Pharmacia Biotech, Piscataway, NJ). Alpha2-macroglobulin was measured by nephelometry (Beckman Coulter, Brea, Calif). The analytical performance of these 3 assays has been validated in a clinical laboratory, with intra- and inter-assay variability of 2%-11% and 1%-11%, respectively.

## CLINICAL SIGNIFICANCE

- Liver biopsy is an expensive and an invasive procedure, but it is the current gold standard for determining hepatic fibrosis in hepatitis C patients.
- The serum fibrosis panel FIBRO*Spect* II can effectively identify people who do not have fibrosis, but it may not be useful in differentiating between intermediate stages of fibrosis.

## Histologic Analysis

All liver biopsies were read by a single pathologist (C.C.) using the Metavir scoring classification where the stage of fibrosis was assessed on a 5-point scale (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = numerous septa without cirrhosis, 4 = cirrhosis). Significant fibrosis was considered to be stage  $\geq 2$ . A random sample of 1/3 of the liver biopsies was re-read by the pathologist to verify the reproducibility of the histologic findings. Excellent reproducibility of the histologic findings was observed with a  $\kappa = 0.8$ . Therefore, the original Metavir scores were used in the final analysis. A liver biopsy was considered to be adequate if the core sample was  $> 15$  mm and had more than 5 portal tracts in the specimen; all study samples were considered adequate by these criteria.

## Statistical Analysis

Patient baseline characteristics are summarized using descriptive statistics and reported as mean  $\pm$  standard deviation or proportions. The FIBRO*Spect* II index (0-100) was generated for each sample from a logistic regression model of the 3 markers that was previously established to discriminate F0-F1 from F2-F4 (Metavir) fibrosis.<sup>10</sup> The diagnostic value of the algorithm in the study cohort was assessed by area under the ROC curve. Samples with an index  $\geq 42$  were classified as consistent with significant fibrosis, and those with an index from 0 to 41 were considered to be consistent with no/mild fibrosis to determine clinical performance of the algorithm. Statistical analyses were performed with SAS software version 8.0 (SAS Institute Inc., Cary, NC) and SPSS software version 11.5 (SPSS Inc., Chicago, Ill).

## RESULTS

One hundred eight consecutive hepatitis C patients seen at the Oregon Health and Science University Hepatology

**Table 1** Demographic, Laboratory and Histologic Characteristics of Hepatitis C Patients Undergoing a Liver Biopsy

	Study Cohort (n = 108)
Age (years)	44 (24-60)
Male (%)	70 (65%)
Female	38 (35%)
ALT in IU/L (SD)	81 (76)
AST in IU/L (SD)	59 (46)
Total bilirubin in mg/dL (SD)	1.1 (3.6)
Albumin in mg/dL (SD)	3.9 (0.3)
Metavir fibrosis stage	
0	14 (13%)
1	55 (51%)
2	25 (23%)
3	12 (11%)
4	2 (2%)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; SD = standard deviation.

Clinic were enrolled in this prospective validation study. Demographic and baseline characteristics are summarized in **Table 1**. The mean age of the cohort was  $44 \pm 7.9$  years with a male predominance (65%). Fifteen percent (15%) of the cohort also had a physician-reported diagnosis of alcohol-related liver disease. The mean ALT was 81 IU/L (range 16-519) and mean AST IU/L was 59 (range 17-341). There was no correlation between ALT and AST levels, and FIBROSpect II results. Overall, nearly 2/3 of subjects had no/mild fibrosis, while only 13% of subjects had advanced fibrosis (stage 3 or 4) on liver biopsy.

### Performance Characteristics of FIBROSpect II Index

**Table 2** describes the clinical performance characteristics of FIBROSpect II. The sensitivity and specificity were 71.8% and 73.9%, respectively, in a study population with an overall prevalence of significant fibrosis of 36.1%. For this prevalence, the positive and negative predictive values (PPV and NPV) were 60.9% and 82.3%, respectively. **Figure 1** describes how the performance characteristics of the assay vary based on the prevalence of significant fibrosis (F2-F4). As the baseline prevalence decreases, the NPV increases. Thus, at a baseline prevalence of 20% significant fibrosis, the NPV is over 90%. The overall accuracy of the assay does not change over a wide prevalence range, nor does the sensitivity or specificity. The likelihood for a positive result is 2.75 (95% CI, 1.79-4.33), and 0.38 (95% CI, 0.22-0.61) for a negative result. When the cutoff of significant fibrosis was changed to F3-F4, the sensitivity, specificity, positive predictive value, and negative predictive value changed to 81.8%, 62.9%, 20%, and 96.8%, respectively.

The ROC curve of the test cohort is described in **Figure 2**. The area under the curve (AUC) was 0.826, and as a pre-

dictive tool it was significantly higher than chance alone (ie, AUC = 0.5). FIBROSpect II test is based on a logistic regression index (range 0-100) determined by the predictive model. **Figure 3** describes the frequency of Metavir fibrosis stages over the range of FIBROSpect II index scores in the study population. Low index values (0-19) correctly identified no/mild fibrosis (F0-F1) in 90% of cases. On the other hand, a very high index score (80-100) correctly identified significant fibrosis (F2-F4) in nearly 80% of cases.

### DISCUSSION

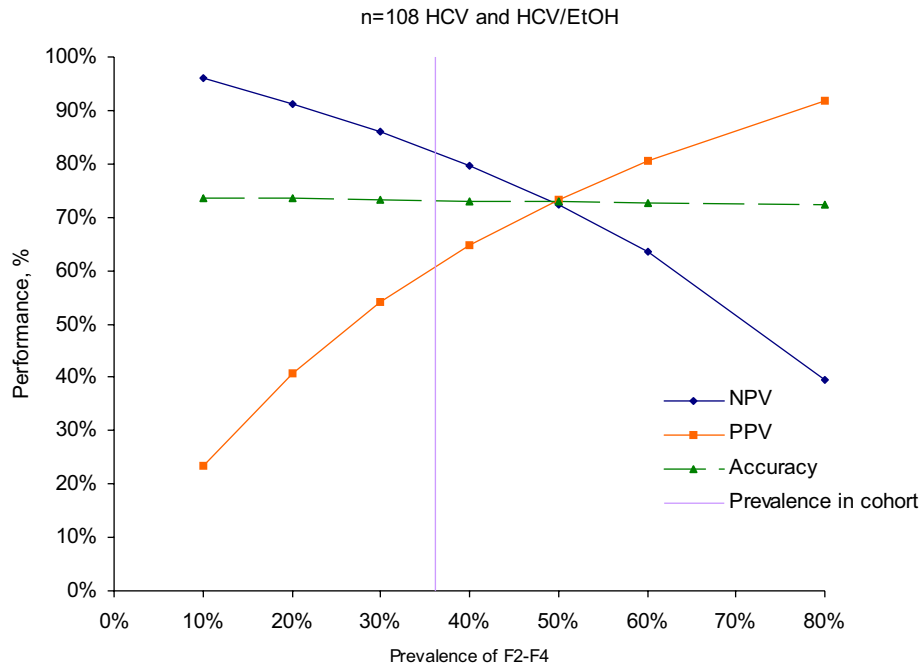
The current study validates the performance characteristics of the FIBROSpect II assay using a US cohort of HCV patients. Serum was collected prospectively at the time of liver biopsy. The overall performance of the diagnostic test was very good based upon the area under the ROC curve (a value of 0.826). Negative and positive predictive values were good to very good, depending upon the baseline prevalence of significant fibrosis (F2-F4) of the population. In general, the lower the baseline prevalence of significant fibrosis is, the better the negative predictive value of the test. Furthermore, extremely low and high index values of the test were excellent at correctly predicting the presence or absence of significant fibrosis. In our cohort, 19 patients had very low index value (<20) and in these patients a liver biopsy could be avoided. These findings are similar to a study by Patel et al<sup>10</sup> where the FIBROSpect II assay had similar performance characteristics—AUC of 0.831 and positive and negative predictive values of 74.3% and 75.8%, respectively. Thus, our study further validates this serum fibrosis panel, but using prospectively collected samples.

Numerous surrogate markers for hepatic fibrosis have been studied. There are direct biochemical markers of fibrosis, such as procollagen type III N-terminal peptide (PIIINP) and hyaluronic acid (HA). Because pathological accumulation of extracellular matrix is a result of alterations

**Table 2** Clinical Performance of the FIBROSpect II Algorithm Using an Index Threshold of 42 to Classify Each Subject as Consistent with Mild (F0-F1) or Significant (F2-F4) Fibrosis

	Biopsy		
	F2-F4	F0-F1	
FIBROSpect II positive*	28	18	46
FIBROSpect II negative*	11	51	62
Total	39	69	108
Prevalence of F2-F4	36.1%	95% CI	
Sensitivity	71.8%	55.1%	85.0%
Specificity	73.9%	61.9%	83.8%
PPV	60.9%	45.3%	74.9%
NPV	82.3%	70.5%	90.8%
Accuracy	73.1%	63.8%	81.2%

PPV = positive predictive value; NPV = negative predictive value.  
\*At an index threshold of 42.

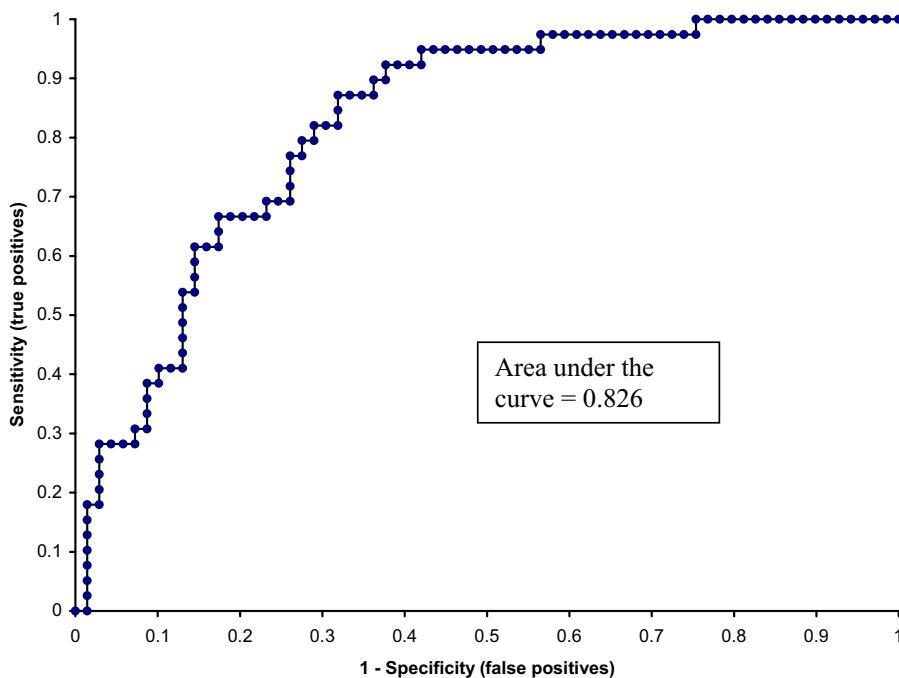


**Figure 1** Performance characteristics of the cohort in terms of negative predictive value (NPV), positive predictive value (PPV) based on the prevalence of significant fibrosis (F2-F4).

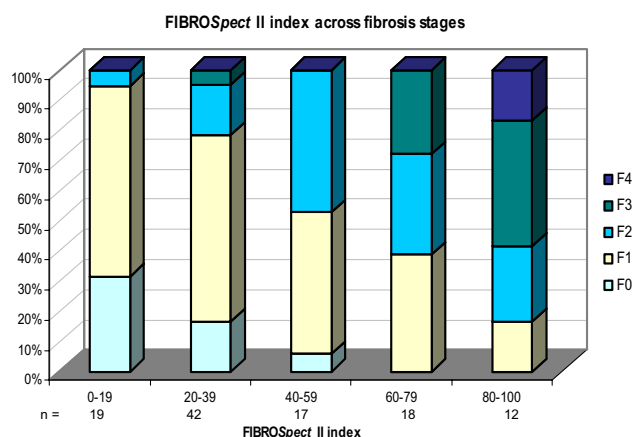
in the synthesis and degradation of matrix proteins, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) have also been studied as candidate markers. Matrix metalloproteinases constitute a family of zinc endopeptidases capable of degrading collagens, and TIMPs regulate its activity. Therefore, an imbalance in MMPs and TIMPs may be an important determinant of hepatic fibrosis. Proinflammatory markers such as LPS (li-

popolysaccharide), TNF-alpha (transforming growth factor), and HGF (human growth factor) have also been evaluated. Other markers that have been studied include gamma glutamyltranspeptidase (GTT), total bilirubin, aspartase aminotransferases (AST), platelet count, YKL-40, apolipoprotein A1, and alpha 2 macroglobulin.

Recently, well-described serum fibrosis marker panels in addition to FIBRO*Spect* II include Fibrotest and the AST-



**Figure 2** Receiver Operator Characteristic Curve of the FIBRO*Spect* II Index for differentiating Metavir Stage F0-F1 from F2-F4.



**Figure 3** The frequency of Metavir fibrosis stages over the range of FIBROSpect II index scores.

to-platelet ratio index (APRI). Forns et al used simple parameters including age, GTT, cholesterol level, and platelet count to predict hepatic fibrosis.<sup>11</sup> Two cutoff points were determined (a low point of 4.2 and a high point of 6.9). A score  $<4.2$  in 96% of patients accurately identified mild fibrosis. However, a score  $>6.9$  correctly diagnosed significant fibrosis (stage 2-4) only 30% of time. The APRI assay also uses more standard laboratory tests to assess hepatic fibrosis—the AST-to-platelet ratio index.<sup>12</sup> This index was able to predict significant fibrosis accurately in 51% and cirrhosis in 81% of patients with hepatitis C. Imbert-Bismut et al developed a panel of fibrosis markers<sup>13</sup> that consists of 5 biomarkers, including apolipoprotein A1, haptoglobin, alpha 2 macroglobulin, GGT, and total bilirubin. Their assay allowed the classification of 12% of patients as having no significant fibrosis (F0-F1 fibrosis) with a negative predictive value of 100%, and 34% of patients having significant fibrosis (F2-F4 fibrosis) with 90% positive predictive value.

At first glance, these studies suggest that the serum fibrosis panels are quite good for assessing the extent of liver fibrosis. However, there are some concerns regarding the currently available tests. Many of the assays have indeterminate values that limit their clinical utility. For example, the panel developed by Forns et al<sup>11</sup> has a lower and upper cutoff limit of 4.2 and 6.9, respectively. If the assay value falls between these 2 values, it is considered an indeterminate value. Ultimately, in their study, only 51% of patients could have their fibrosis classified. In addition, many of these tests were studied in patients referred to tertiary care centers or were a part of treatment trials for hepatitis. Therefore, the performance of these assays may be different in routine clinical practice.

There are several issues that need to be kept in mind regarding serum fibrosis markers. Performance characteristic parameters such as negative and positive predictive values are greatly affected by the baseline prevalence in the study population. In turn, the performance of these assays is dependent on the prevalence of hepatic fibrosis in the population being studied. In this study, the FIBROSpect II assay

was studied in patients referred to a tertiary care center. Therefore, the performance of this assay may be different in routine clinical practice. However, in clinical practice the prevalence of significant fibrosis is likely to be lower and thus, the assay will likely be more useful in excluding significant fibrosis. Also, these assays are generally good at differentiating between advanced fibrosis and minimal or no fibrosis but are poor at differentiating between the intermediate grades of fibrosis (F1-F3)—this is the case with the FIBROSpect II assay as well. In addition, these serum assays can only be as good as the gold standard for determining hepatic fibrosis, the liver biopsy. And, as mentioned above, the overall sensitivity of a single pass percutaneous liver biopsy is only 80%. In this study, all biopsy samples were considered adequate and, thus, sampling error was minimized. Another diagnostic limitation of a percutaneous liver biopsy includes inter- and intra-observer variability of the fibrosis staging. This was minimized in this study by using a single hepatopathologist. In addition, when a third of the biopsies were blindly read again, there was high agreement with the initial reading ( $\kappa = 0.8$ ). Finally, the FIBROSpect II assay was validated using a fibrosis cutoff where F2-F4 was considered significant fibrosis. This cutoff is useful when determining which hepatitis C patients to treat, where typically patients with F2 or higher fibrosis are felt to be appropriate candidates for interferon-based therapy. However, in practice F2 fibrosis or less is considered mild fibrosis. As shown in the results, when the significant fibrosis cutoff was changed to F3-F4, the sensitivity of the test increased but the specificity decreased.

In summary, this study validates the performance characteristics of a noninvasive serum fibrosis marker—FIBROSpect II using a prospective cohort of patients with chronic hepatitis C. Similar to other noninvasive fibrosis tests, FIBROSpect II may not be useful in differentiating between the intermediate stages of fibrosis. However, the high negative predictive value of this assay in low prevalence populations of F2-F4 fibrosis may allow clinicians to avoid a liver biopsy in certain situations, such as patients who refuse a biopsy, or have a contraindication (hemophiliacs). Simple laboratory tests such as prothrombin time, albumin, total bilirubin, and platelet counts in conjunction with an abdominal imaging study should continue to be utilized to identify overt cirrhosis. Furthermore, FIBROSpect II may be useful in assessing fibrosis progression in patients who have had a baseline liver biopsy in the past, including those patients with non-HCV-related liver diseases—although this must be studied prospectively before it can be recommended. It will also be important to prospectively validate these results using a community-based setting with different relative prevalence of different stages of HCV in order to further assess its clinical utility. Moreover, it will be of interest to determine how the FIBROSpect II might change in patients receiving antiviral therapy.

## References

1. Rockey DC. Antifibrotic therapy in chronic liver disease. *Clin Gastroenterol Hepatol*. 2005;3(2):95-107.
2. Schuppan D, Ruehl M, Somasundaram R, et al. Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis*. 2001;21:351-372.
3. Eng FJ, Friedman SL. Fibrogenesis I. New insights into hepatic stellate cell activation: the simple becomes complex. *Am J Physiol*. 2000;279:G7-G11.
4. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology*. 1981;1(5):431-435.
5. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. *J Hepatol*. 1995;22(6):696-699.
6. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology*. 1996;24(2):289-293.
7. Bravo A, Sheth S, Chopra S. Liver biopsy. *N Engl J Med*. 2001;344(7):495-500.
8. Colombo M, Del Ninno E, de Franchis R, et al. Ultrasound assisted percutaneous liver biopsy: superiority of the Trucut over the Menghini needle for the diagnosis of cirrhosis. *Gastroenterology*. 1988;95:487-489.
9. Poniachik J, Bernstein DE, Reddy KR, et al. The role of laparoscopy in the diagnosis of cirrhosis. *Gastrointest Endosc*. 1996;43:568-571.
10. Patel K, Gordon SC, Jacobson I, et al. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol*. 2004;41:935-942.
11. Forn X, Ampurdanes S, Llovet JM, et al. Identification of chronic hepatitis C without fibrosis by a simple predictive test. *Hepatology*. 2002;36:986-992.
12. Wai CT, Greenon JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003;38:518-526.
13. Imbert-Bismut F, Ratziu V, Pieroni L, et al. Biochemical markers of liver fibrosis: a prospective study. *Lancet*. 2001;357:1069-1075.