Fibrotest-Actitest™: The Biochemical Marker of Liver Fibrosis – The Israeli Experience

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Abstract

Background: The Fibrotest-Actitest™ is a six-parameter scoring system that allows quantification of liver fibrosis and inflammation. This test has been validated by several studies in hepatitis B and C viruses and alcoholic liver disease, with a high correlation between the liver biopsy and the results of the FT-AT (AUROC between 0.78 and 0.95). The FT-AT was introduced in Israel (Rambam Laboratory) in March 2005.

Objectives: To assess the results of HCV patients who underwent the test during the period March 2005 to February 2006.

Methods: Serum was taken and brought to the central laboratory performing the tests within 4 hours. Six parameters were evaluated using commercial kits approved by the designer of the test (Biopredictive): total bilirubin, gamma-glutamyltransferase, alpha-2 macroglobulin, haptoglobin, alanine aminotransferase, and apolipoprotein-A1. The results were sent to the website of Biopredictive (France), which provided the FT-AT score online using a patented formula.

Results: Of the 325 patients tested, only 4 were not interpretable because of hemolysis. Patients' age ranged from 7 to 72 years (median 42); 54% were female. Liver biopsy was performed in 81 patients and was compared with the results of the Fibrotest. Findings were as follows: 27% of the patients were F0, 19% F1, 20% F2, 17% F3 and 17% F4; 18% were A0, 32% A1, 28% A2 and 22% A3. The AUROC curve comparing the Fibrotest with liver biopsy with a cutoff point at F2 and A2 for significant fibrosis and inflammation was 0.85 and 0.79 respectively.

Conclusion: Fibrotest is a simple and effective method to assess liver fibrosis and inflammation and can be considered an alternative to liver biopsy in most patients with HCV.

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Assessment of the presence and severity of liver fibrosis and inflammation is critical to determine therapeutic strategies, prognosis and the potential risks for complications in patients with chronic liver disease. Liver biopsy, the gold standard for the assessment of necro-inflammatory activity and fibrosis, is invasive and is associated, even in expert hands, with rare but severe side effects such as bleeding or pneumothorax [1]. Moreover, it carries a sampling error of one or two stages in a third of the cases, as demonstrated by Regev et al. [2]. In the past decade, many investigators have proposed non-invasive tests to replace the liver biopsy using either a single biochemical marker or a combination of tests. An ideal non-invasive marker for the evaluation of liver fibrosis should accurately predict the presence or absence of significant fibrosis (high sensitivity, specificity, positive and negative predictive values). In addition, it should be readily available and reproducible with low inter-laboratory variability and with applicability in liver disease of various etiologies. Although liver biopsy does not fulfill many of these criteria, it has remained the gold standard mainly because of the absence of better alternatives.

Recently, there has been a renewed interest in non-invasive markers of fibrosis because of evolving novel therapies for hepatitis C and B. The most extensively studied test is the Fibrotest-Actitest™ [see 3 for review]. It is a score composed of six blood parameters – including alpha-2 macroglobulin, haptoglobin, total bilirubin, gamma-glutamyltransferase, apolipoprotein-A1, and alanine aminotransferase. These tests were chosen because of their importance in liver fibrogenesis and fibrolysis. As the fibrotic process begins, there is a down-regulation of haptoglobin and apolipoprotein-A1, together with an up-regulation of the α2-macroglobulin molecule [4-6].

The Fibrotest has been validated in more than 1500 patients with hepatitis C [3], hepatitis B [7,8], non-alcoholic steato-hepatitis and alcoholic liver disease [9]. One criticism was that most of these studies came from the group that invented the method. However, three independent studies have also confirmed the accuracy of the Fibrotest [10-12]. We present the results of one year experience with this test in Israel, adding to the pool of independent studies on Fibrotest.

Patients and Methods

A total of 325 patients with chronic hepatitis C were consecutively enrolled at 10 liver centers throughout the country – Shaare Zedek (Jerusalem), Rabin/Beilinson (Petah Tikva), Sheba (Tel Hashomer), Bnei Zion (Haifa), Hadassah (Jerusalem),

FT-AT = Fibrotest-Actitest
HCV = hepatitis C virus

Western Galilee (Nahariya), Tel Aviv Sourasky (Tel Aviv), Kaplan (Rehovot), Soroka (Beer Sheva), and HeEmek (Afula). All patients had chronic HCV infection without liver complications such as ascites or upper gastrointestinal bleeding, documented by positivity of HCV antibodies and HCV-RNA in the serum. Signed informed consent was obtained from each patient. Demographic, clinical and biochemical data were recorded anonymously in the database.

Liver biopsy

Liver biopsies were performed in 81 patients within a month from the FT-AT. The biopsies were examined at each center and analyzed by the local pathologist for fibrosis stage and activity grade according to the Metavir scoring system [13]. Fibrosis was staged on a scale of 0 to 4: with F0 indicating no fibrosis, F1 portal fibrosis without septa, F2 few septa, F3 numerous septa without cirrhosis, and F4 cirrhosis. Activity grading by the Metavir system (based on the intensity of necro-inflammatory activity, mainly on necrosis) was scored as follows: with A0 indicating no activity, A1 mild activity, A2 moderate activity, and A3 severe activity.

Fibrotest-Actitest

In March 2005, the Fibrotest-Actitest was introduced in Israel at the Rambam Laboratory after validation of the methods used for the test by its designer (Biopredictive, France). The different kits used for the Fibrotest were those recommended by Biopredictive, namely turbidimetry from Dako using polyclonal anti-human α2-macroglobulin, immunoturbidimetry from Roche for apolipoprotein-A1 and haptoglobin, enzymatic colorimetric assays for GGT, the Diazo method for bilirubin, and the method according to the International Federation of Clinical Chemistry for ALT. The sera were separated upon arrival to the laboratory, stored at -18˚C up to 5 days. The ALT, GGT, and bilirubin were performed on the first day. All the tests were done by the automated Integra 400 (Roche Diagnostics, USA).

The serum samples were collected in 5 ml tubes without anticoagulant. There was no need for fasting. The sera were sent from several centers and reached the laboratory within 4 hours.

The results were sent to the Biopredictive website (biopredictive.com) together with the age and gender of the patient. The score of the Fibrotest and Actitest was calculated according to a patented formula and given online. Fibrosis using FT was staged on a scale of 0 to 4 with respect to Metavir fibrosis staging. Fibrosis was staged by FT scoring as follows: F0 for a score of 0–0.21, F0-F1 for 0.22–0.27, F1 for 0.28–0.31, F1-F2 for 0.32–0.48, F2 for 0.49–0.58, F3 for 0.59–0.72, F3-F4 for 0.73–0.74, and F4 0.75–1. Necro-inflammatory activity using AT was graded on a scale of 0 to 3 with respect to Metavir activity grading. Activity by AT scoring was graded as follows: A0 for 0–0.17, A0-A1 for 0.18–0.29, A1 for 0.30–0.36, A1-A2 for 0.37–0.52, A2 for 0.53–0.60, A2-A3 for 0.61–0.62, and A3 for 0.63–1 [14].

GGT = gamma-glutamyltransferase
ALT = alanine aminotransferase

Table 1. Characteristics of study patients

	All patients (n=325)	Patients with liver biopsy (n=81)
Age (yrs)	42 ± 6	40 ± 9
Gender	53% female	51% female
ALT (IU/L)	49 ± 60	45 ± 42
Bilirubin (mg/dl)	0.7 ± 0.4	0.8 ± 0.3
Haptoglobin (g/L)	0.9 ± 0.5	0.8 ± 0.4
α2-macroglobulin (g/L)	2.5 ± 0.7	2.7 ± 0.6
Apolipoprotein A1 (g/L)	1.33 ± 0.2	1.2 ± 0.2
GGT (IU/L)	43 ± 40	46 ± 39

Table 2. Results of the Fibrotest and the Actitest

Fibrotest		Actitest	
F0	27%	A0	18%
F1	19%	A1	32%
F2	20%	A2	28%
F3	17%		
F4	17%	A3	22%

Statistical analysis

The diagnostic values of FT and AT compared to the Metavir fibrosis and activity scores were assessed by logistic regression, and area under the receiver operating characteristic curves (AUROC) was obtained. The main endpoints were the AUROC for the diagnosis of significant fibrosis (F2-F4 vs. F0-F1) and moderate to severe activity (A2-A3 vs. A0-A1).

Results

Sera from 325 consecutive patients were tested. Only 6 (1.8%) were not interpretable because of hemolysis (very low level of haptoglobin with high level of bilirubin). There were more females (54%) than males. The median age was 42 years, with a range of 4 to 77. Table 1 gives the patients' demographic and biochemical characteristics.

Table 2 presents the distribution of the Fibrotest-Actitest among the patients: 27% were F0, 19% F1, 20% F2, 17% F3, and 17% F4; 18% were A0, 32% A1, 28% A2, and 22% A3.

The comparison between FT-AT and liver biopsy was done in 81 patients. The results are shown in Figure 1 for FT and Figure 2 for AT. The AUROC for significant fibrosis with a cutoff at F2 was 0.85. The AUROC for significant inflammation with a cutoff at A2 was 0.79.

There were 10 discordant cases (12.3%), defined by a difference of at least two fibrosis stages between the liver biopsy and the FT. Fibrosis staging by FT was higher than fibrosis staging by histology in six cases. In five of these cases, the length of the biopsy was small (less than 15 mm) and it was fragmented in four. In the four other cases, the fibrosis staging by FT was lower than on histology.

Discussion

Non-invasive assessment of liver fibrosis has gained much attention in the liver community in recent years. Since the

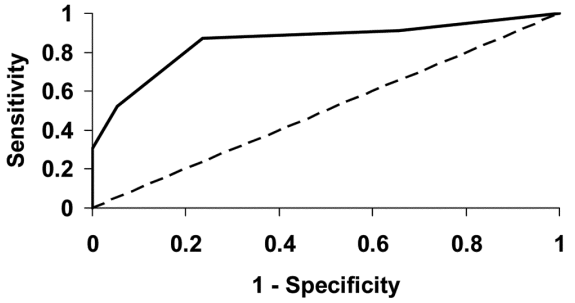

Figure 1. Area under the curve (AUROC) for the Fibrotest vs. liver biopsy to discriminate for fibrosis more than F2. AUROC = 0.85

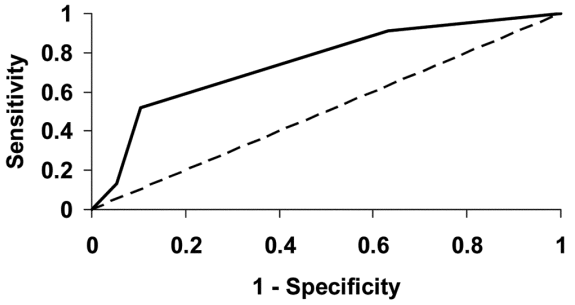

Figure 2. Area under the curve (AUROC) for the Actitest vs. liver biopsy to discriminate for inflammation more than A2. AUROC = 0.79

publication of the first article by Imbert-Bismuth and team in 2001 [15], several groups have tried to evaluate liver fibrosis by non-invasive methods. Single markers, including thrombocyte count [16], prothrombin time [17], hyaluronic acid [18], type IV collagen, N-terminal propeptide of type III procollagen (PIII-P) [19], have been evaluated and found not sufficiently accurate to predict the amount of liver fibrosis. Therefore, several markers combining multiple factors have been tried. The list includes the Fibrotest-Actitest [3], the APRI (**A**spartate aminotransferase/**P**latelets **R**atio **I**ndex) [20], the Forns score [21], the ELF (**E**uropean **L**iver **F**ibrosis) score [22], and more recently the Hepascore [23] and the Fibrometer score [11].

Although some may be promising, the most extensively used test is the FT-AT, which has been validated in more than 1500 patients in clinical trials [3] and is used in 13 countries with more than 100,000 tests performed until now (T. Poynard, personal communication, 2006). The correlation found between histology and the FT, according to the AUROC analysis, varies between 0.79 and 0.90 [3,10-12]. It has been validated in hepatitis C, hepatitis B, human immunodeficiency virus-HCV co-infected patients [24], and most recently in non-alcoholic steato-hepatitis and alcoholic liver disease [9]. It has been shown to be superior to the APRI [25]. It does, however, have some drawbacks: it should be used cautiously in patients with hemolysis and Gilbert syndrome because of their effect on haptoglobin and bilirubin, respectively; it should not be used in patients with an acute inflammatory syndrome because of the effect on α2-macroglobulin and haptoglobin. Nevertheless, it can be interpreted in more than 95% of patients with chronic liver disease, as in our study where the test could be interpreted in 98% of the patients.

One criticism of this test was that most of the studies that validated it were performed by the inventor of the Fibrotest, Thierry Poynard. Recently, three independent studies have confirmed previous results obtained by Poynard's group, with similar results [10-12]. In the study by Halfon and collaborators [12] on 504 patients with hepatitis C, the AUROC for significant fibrosis was 0.79. Discordance between liver biopsies and FT results was noted in 18% of the patients, which was attributed to the biopsy in 4% of the cases, to the FT in 5%, and undetermined in 9%. The biopsies were smaller than 15 mm and/or poly-fragmented, suggesting cirrhosis, which was underestimated by the biopsy and truly evaluated by the FT.

The FT failures were related to hemolysis, Gilbert syndrome, or inflammatory syndromes. In the study by Castera et al. [10], the FT was compared to transient elastography (the Fibroscan), APRI and liver biopsy. The Fibroscan is a new technique that allows measurement of liver stiffness. It uses an ultrasound transducer probe mounted on the axis of a vibrator; vibrations are transmitted by the transducer, inducing an elastic shear wave that propagates through the underlying tissue. The velocity of the shear wave, which is related directly to tissue stiffness, is measured: the stiffer the issue, the faster the shear wave propagates. The results are expressed in kilopascals. The AUROC for significant fibrosis (F>2) were 0.83, 0.85 and 0.78 for the Fibroscan, the FT, and the APRI, respectively. However, there are some limitations to the Fibroscan. It cannot be used in obese patients or in patients with ascites, since the probe cannot be applied accurately on the patient. Moreover, its sensitivity for low stages of fibrosis (F0, F1 and F2) seems inadequate, since it cannot differentiate between these three stages [see Figure 1A in ref 10]. The third paper, by Cales et al. [11], compared the FT with a new score called the Fibrometer, which includes platelets, prothrombin index, AST, α2-macroglobulin, hyaluronic acid, urea and age. The AUROC for significant fibrosis in 383 patients with viral hepatitis was 0.88 for the Fibrometer and 0.81 for the FT (the difference was not statistically significant).

The present study confirms the results of these independent studies. We showed that the AUROC for significant fibrosis (more than F2) was 0.85, in agreement with the aforementioned studies. The number of discordant patients was relatively small (10 patients, i.e., 12.3%). The discordance could be attributed to small liver biopsies in 5 of the 10 cases. Thus, FT seems accurate in more than 90% of cases.

A major problem with investigating these new non-invasive biomarkers is that there is no true gold standard. Liver biopsy cannot overcome the heterogeneity of the fibrotic process and therefore can be misleading in true estimation of total liver fibrosis [2]. It seems that a liver biopsy should have a length of at least 25 mm to be representative of the whole process of the liver. An ideal, but impossible study would be to perform

AST = aspartate aminotransferase

laparoscopy with two biopsies of 20 mm to reach a total length of 40 mm.

In conclusion, we have shown that the Fibrotest-Actitest is an easy test to perform and enables an accurate evaluation of the amount of fibrosis and inflammation in HCV patients in 90% of cases. It should be discussed with the patients and the medical team caring for them as an alternative to liver biopsy.

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