



CORRELATIONS BETWEEN NON-INVASIVE EXPLORATIONS AND LIVER BIOPSY IN THE DETERMINATION OF LIVER FIBROSIS IN CHRONIC VIRAL HEPATITIS C

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Abstract. Introduction. An adequate diagnosis of fibrosis in viral hepatitis C is essential for treatment decisions and disease prognostic. Because of the limitations of liver biopsy, noninvasive explorations of fibrosis are frequently employed nowadays. This study's aim was that of evaluating the correlation between the currently available noninvasive standardized tests (FibroTest and FibroScan) and liver biopsy. **Material and methods** The study cluster consisted of n=26 naïve patients with chronic viral hepatitis C, which underwent antiviral treatment in the National Institute for Infectious Diseases "Prof. Dr. Matei Balș", Bucharest, Romania, during December 2007 – December 2008. Fibrosis was assessed before commencement of the combined treatment (Pegylated Interferon and Ribavirin) by means of liver biopsy, FibroScan and FibroTest and at 48 weeks through FibroScan and FibroTest, verifying the statistic correlation of the results acquired through the three methods. **Results.** The values obtained through FibroScan ranged between min 4.9 kPa and max 49.1kPa, respecting the validation criteria IQR<30%, SR>60%. The cut-off values were: 7.1kPa for F2, 9.5 kPa for F3 and 12.5 kPa for F4. FibroScan and FibroTest results have correlated in a 92% percentage. When the results of FibroScan and FibroTest coincided, biopsies were statistically correlated at AUROC 0.79 for F>= 2, 0.91 for F>=3 and 0.97 for F>=4. The frequency of grounds for discrepancy of the three evaluation methods was: LB 5%, Fibroscan 2%, Fibrotest 1%. **Conclusions.** FibroScan is a new, simple, noninvasive method which is available at the patient's bedside for quick evaluation of liver fibrosis degree with accuracy similar to that of the FibroTest, especially for the assessment of significant hepatic fibrosis (F2-F4). The combined use of FibroScan and FibroTest results for the evaluation of hepatic fibrosis degree can permit avoiding liver biopsy in a considerable number of patients with chronic viral hepatitis C.

Keywords: hepatic fibrosis, noninvasive tests, FibroScan, FibroTest, chronic viral hepatitis C.

Abbreviations: AUROC (AUC) - area under the receiver operating characteristic curve, IQR – interquartile range, Fs – FibroScan, FT-AT – FibroTest ActiTest, LSM - liver stiffness measurement, Se – sensibility, Sp – specificity, SR – success rate, SVR – sustained viral response

Background.

Assessing liver fibrosis in patients with liver disease is traditionally performed by liver biopsy, an imperfect gold standard. In 2003, in "Hepatology", Bedossa et al. published a study correlating Fibrotest with liver biopsy with an important discordance factor for biopsy: three fragments of biopsy taken from 3 different places in the liver of the same patient in the same day revealed 3 different stages in Metavir scale: one fragment indicated F2, one F3 and one F4, while Fibrotest indicated all three segments as F4 stage fibrosis. Correlating the patients' analyses, the most probable fibrosis score was F4, as statistic assays showed.

Starting from this data, our study used all available options for the assessment of liver fibrosis status: Liver biopsy, Fibrotest and Fibroscan for the same patient in the same day. Our assessment was done at baseline using all three tests, at week 48 of combined PegIFN and Ribavirin therapy, and at 6 months after the end of therapy (SRV), only through the two non-invasive tests.

In this article we analyze the preliminary baseline results, assessing all three tests: liver biopsy, Fibrotest and Fibroscan.

Non-invasive techniques, liver stiffness measurements (LSM) by Fibroscan® and biomarkers

[FibroTest® (FT)], are widely used in countries where they are available, and as such, in Romania, since October 2008. The non-invasive techniques are authorized to be employed for receiving therapy from CAS (the national health insurance company), provided the 2 analyses concord, therefore liver biopsy is not mandatory for receiving treatment. As such, it is essential to identify factors associated with the accuracy of these techniques.

As for liver biopsy, the "gold standard" in evaluating the hepatic stage of fibrosis for chronic hepatitis, as it has been considered, recent studies have shown that its AUROC accuracy is only 80% for hepatic fibrosis. [Poynard 2007, EASLD].

Liver biopsy, due to its risks and limitations, is no longer considered mandatory as the first-line indicator of liver injury, and several markers have been developed as non-invasive alternatives. [2, 3, 4]

Moreover, liver biopsy is an invasive procedure, unsuitable for tight monitoring [4–6]. In addition, fragmented or small-sized specimens may cause the underestimation of fibrosis.

Methods.

We included 26 naïve patients with chronic hepatitis C, who fulfilled all the criteria for receiv-

Patient ID	LB		FibroScan values				Fibrotest	
	Baseline		Baseline				Baseline	
	Fibrosis stage	BMI (kg/m ²)	Stiff. (kPa)	Stiff.	IQR (kPa)	SR (%)	F	A
1	F1	20.4	7.0	F1	1.2	100	F1	A2
2	F2	23.1	7,9	F2	1.3	96	F2	A1
3	F3	22.4	10,1	F3	2.0	100	F3	A3
4	F4	21.0	11,4	F3	1.9	100	F2-F3	A1-A2
5	F0		7,9	F2	1.6	96	F2	A1
6	F3	20.1	10,1	F3	1.8	100	F2-F3	A3
7	F4	23.7	11.4	F3	1.9	100	F2-F3	A1-A2
8	F2	25.7	7,9	F2	2.1	96	F2	A1
9	F0	25.4	8,8	F2	2.1	100	F3	A1-A2
10	F4	24.7	16,6	F4	1.7	100	F3	A1
11	F3	25	3,6	F0	1.6	96	F3-F4	A2-A3
12	F3	22.1	9,5	F3	1.5	100	F3-F4	A1-A2
13	F4	23	21,2	F4	1.6	96	F4	A2-A3
14	F4	22.9	31,2	F4	1.9	100	F4	A3
15	F4	20.1	21,2	F4	1.9	100	F4	A2-A3
16	F4	21.1	31,2	F4	2.0	100	F4	A3
17	F3	23.2	10,3	F3	2.1	100	F4	A1-A2
18	F4	24.2	12,6	F3-F4	2.1	100	F4	A2
19	F4	23.2	14,3	F4	1.9	96	F4	A2
20	F3	24	27,7	F4	1.8	100	F4	A3

Table I. Patients included in the study – baseline assessment

ing the antiviral therapy: Pegylated Interferon plus Ribavirin from the national program of therapy:

- High viral load
- Raised ALT level, but not higher than 5 times normal
- Neutrophil count not lower than 1500,
- Thrombocyte number not lower than 90000,
- Fibrosis higher than F2 on liver biopsy, or on the two non invasive tests.

At screening, biopsy was performed and in the same day the patients underwent Fibrotest analyses and Fibroscan, with a delay of maximum 48 hours. (see table I.)

Liver stiffness measurements

The patients were studied using the non-invasive method of transient elastography (Fibroscan, Echosens, Paris, France). The stiffness results are expressed in kiloPascals (kPa). The maneuver was performed by a trained physician (more than 100 measurements), blinded to all other characteristics, and according to the manufacturers' recommendations.

The recommended criteria were: success rate greater than 60% (SR60), at least 10 valid liver stiffness measurements (V10) and interquartile range/median LSM <30% (IQR30);[7-8,15]

Biochemical markers

FibroTest, ActiTest and SteatoTest (Biopredictive, Paris, France) were performed according to the published recommendations. [25,27]

FT-AT is a non-invasive blood test that links the quantitative results of six serum biochemical markers – alpha2-macroglobulin, haptoglobin, GGT, total bilirubin, apolipoprotein A1 and alanine amino transferase (ALT) – to the patient's age and gender in a patented artificial intelligence algorithm (USPTO 6,631,330) in order to generate an assessment of the fibrosis stage and necroinflammatory degree of the liver.[14] It is this continuous linear biochemical assessment of fibrosis stage and necroinflammatory activity degree that provides a numerical quantitative estimation of liver fibrosis ranging from 0 to one, corresponding to the well-established METAVIR scoring system of stages F0 to F4 and of degrees A0 to A3 (see Figure 1).[10]

Furthermore, the service provided by Biopredictive includes several security algorithms which permit the identification of patients at high risk of false positive and false negative results. Patients with a high-risk profile include statuses such as: hemo-

lysis, acute inflammation and Gilbert syndrome, as well as errors in units.

In our study of FT, we used a security algorithm profile excluding Gilbert's disease, hemolysis, acute inflammation profiles and extremes values (one percentile) of FT components [7-8,15].

Liver histology

Liver biopsies were obtained using 16 G disposable needles (Hepafix; B. Braun, Melsungen, Germany). The liver specimens (median 17 mm, range 12–54 mm) were stained with H&E. Necroinflammatory activity and liver fibrosis were scored according to Ishak [33] and METAVIR [34] scales.

We excluded from analysis all specimens shorter than 1.5 cm and/or with less than 11 portal tracts.

The liver biopsies were analyzed double blind by two anatomopathologist specialists and the results were concordant.

Definition of the strength of concordance and statistical analysis

The strength of concordance between LSM and FT was assessed using the area under the receiver operating characteristic curve (AUC). [15-17] The AUC combines the sensitivity (Se) and specificity (Sp) of a given quantitative marker for the diagnosis of a specific definition of fibrosis. The disease was defined as advanced (or bridging) fibrosis.

Se is usually assessed in patients with advanced fibrosis (i.e. stages F2, F3, F4 in the METAVIR scoring system) [18] while **Sp** is assessed particularly for non-advanced fibrosis (i.e. stages F0, F1).

For factors related to elastography, the end point was advanced fibrosis as defined using FT (above 0.48). [15]

For factors related to FT the end point was advanced fibrosis as defined as LSM above 7.1 kPa. [13,14]

The AUC was used as a measurement of discrimination and estimated using the empirical (non-parametric) method of DeLong et al. [19]. Statistical analysis was performed by SPSS (version 10.0, SPSS Inc., Chicago, IL, USA).

Results.

Among the 35 screened patients, 30% did not fulfill the recommended criteria for LSM interpretation, 4% did not fulfill the criteria for FT interpretation, 7% did not fulfill the criteria for liver biopsy, (these 7% do not include the patients

which refused biopsy) while 67% fulfilled all criteria. Patients with non-interpretable LSM were older, more often female, and had higher weight, BMI, abdominal and thoracic folds in comparison with patients with interpretable LSM.

The following factors were associated with lower strength of concordance only for one or two of the methods: male gender, BMI greater than 30kg/m², higher weight, abdominal fold over 30mm, thoracic fold over 15mm.

Concordance analysis among patients with recommended criteria

Concordances between LSM, FT and biopsy are detailed in figure 1.

LSM and FT, using biopsy as reference, had similar accuracy with a trend in favor of FT: AU-

recommendations were used. The methodology implied independent recommendations for LSM and FT. Indeed there was no relationship between the applicability of FT (mostly related to Gilbert's syndrome, hemolysis and acute sepsis) and the applicability of LSM (number of valid LSM, success rate and LSM/IQR).

The present study confirmed that an overweight status and BMI were associated with less applicability of LSM [26,35]. In a large population, Foucher et al. identified BMI as the only factor associated with non-applicability of FibroScan®. In overweight or obese patients, the fatty thoracic belt attenuates both elastic waves and ultrasound rendering liver stiffness measurement impossible. In these cases, no results were obtained with FibroScan® preventing the risk of false measurement. [35]

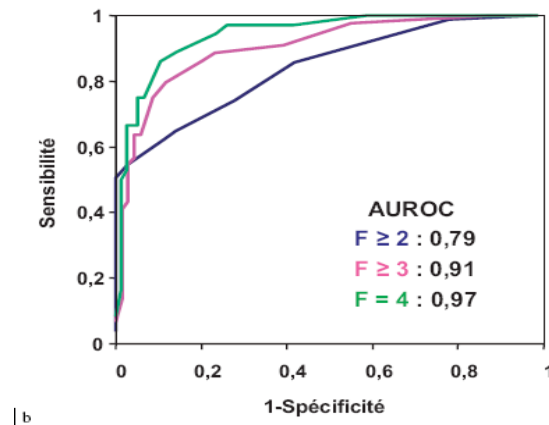


Figure 1. Correlation between the 2 non invasive tests and liver biopsy

ROC (0.72 vs 0.79;P=0.12)

Older age was significantly associated with lower concordance and this could be related to the following more rational factors that are also significantly associated with age: NAFLD, thoracic fold, waist circumference, BMI, serum glucose, ST, and AT.

Limitations of the study

A major limitation of the present study is the number of patients included – only 26.

The sample size of liver biopsies had the median value of 17.

Advantages of the study

A major advantage of the present study was the analysis of two tests (LSM and FT) simultaneously performed with liver biopsy.

Proof of concept

For the proof of concept, manufacturers'

Results on all three tests correlated for all F stages in 89% of cases. For F2 AUROC concordance is 0.79, for F3 – 0.91, for F4 – 0.97.

The causes for discordance among the three evaluation methods were: liver biopsy (in 5% of cases), Fibroscan (2%), Fibrotest (1%).

Conclusions.

FibroScan is a new, simple, non-invasive technique, available at the patient' bedside for rapid, prompt evaluation of the degree of liver fibrosis, presenting accuracy similar to that of FibroTest, especially for the evaluation of significant liver fibrosis (F2-F4). The combined use of FibroScan and FibroTest results for the assessment of hepatic fibrosis degree may permit the avoidance of hepatic biopsy in a large number of patients which chronic viral hepatitis C.

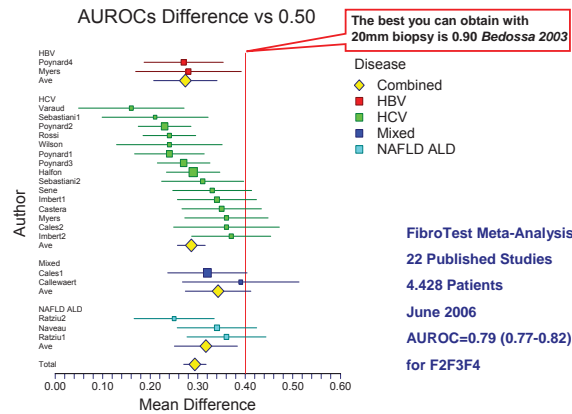


Figure 2. FibroTest Meta-Analysis

In conclusion, this study has validated the concept of using the strength of concordance between two non-invasive estimates of liver fibrosis for the identification of factors associated with variability and precautions of use (see figure. 2). Manufacturers' recommendations must be strictly followed.

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