Correlation Between Liver Biopsy and Fibrotest in The Evaluation Of Hepatic Fibrosis in Patients with Chronic Hepatitis C

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LA TUNISIE MEDICALE - 2010 ; Vol 88 (n°08) : 573 - 578

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RÉSUMÉ

Buts: Déterminer la valeur diagnostique du Fibrotest en comparaison avec la biopsie hépatique dans l'évaluation de la fibrose hépatique chez les patients atteints d'hépatite chronique C.

Méthodes: Cette étude prospective menée sur 2 ans (2006-2007) a inclu des patients consécutifs atteints d'hépatite chronique C et naifs au traitement. Le Fibrotest et la biopsie hépatique ont été réalisés. les courbes ROC (Receiver operating characteristics), la sensibilité, la specificité, les valeurs prédictives positive et negative ont été utilisées pour évaluer la valeur diagnostique du Fibrotest par rapport à la classification de METAVIR.

Résultats: Nous avons inclu 65 patients: 28 hommes et 37 femmes (âge moyen : 50 years); 92% de nos patients avaient un génotype 1. A l'histologie, les résultats de la fibrose étaient : 3. 1% F0; 24. 6% F1; 32. 3%F2; 29. 2%F3 et 10. 8 %F4. La valeur diagnostique du Fibrotest dans la détection de la fibrose significative (F2-F4) était de 0. 87. Un score >0. 5 avait une sensibilté de 85. 1%, une specificité de 72. 2%, une valeur prédictive positive de 88. 9%, et une valeur prédictive négative de 65 %. La valeur diagnostique du Fibrotest dans la detection de la cirrhosis (F4) était de 0. 85. Il a été noté 13/65 cas de discordance (20%) pour la fibrose, 4 cas ont été attribués à la biopsie et 6 cas au Fibrotest. La discordance était inexpliquée dans 3 cas. La taille de la biopsie <15mm [OR=2. 82, 95% CI, 1. 3–6. 07; p =0. 008] et le stade de fibrose F0, F1, F2 [OR =3. 35 , 95% CI, 1. 1–10. 2; p =0. 03] étaient considérés comme facteurs de risque de discordance en analyse multivariée.

Conclusion: cette étude prospective confirme la bonne valeur diagnostique du Fibrotest par rapport à l'examen histologique du foie.

Mots-clés

hepatite C-fibrose-foie -biopsie-marqueurs biologiques

SUMMARY

Aim: To assess the diagnostic value of Fibrotest in comparison with liver biopsy, for the evaluation of hepatic fibrosis in patients with chronic hepatitis C.

Methods: This prospective study included in 2 years (2006-2007), consecutive patients with chronic hepatitis C naive to treatment. Fibrotest and liver biopsy were performed. Receiver operating characteristics (ROC) curves , the sensitivity, specificity, positive and negative predictive values were used to assess the diagnostic value of Fibrotest in comparison with the METAVIR classification.

Results: We recruited a total of 65 patients: 28 males and 37 females (mean age: 50 years); 92% of the patients had genotype 1. The histological fibrosis results were: 3. 1% F0; 24. 6% F1; 32. 3%F2; 29. 2%F3 and 10. 8 %F4. The diagnostic value of Fibrotest in the detection of significant fibrosis (F2-F4) was 0. 87. A score >0. 5 has a sensitivity of 85. 1%, a specificity of 72. 2%, a positive predictive value of 88. 9%,and a negative predictive value of 65%. The diagnostic value of Fibrotest in the detection of cirrhosis (F4) was0. 85. There were 13/65 cases of discordance (20%) for fibrosis, 4 cases were attributable to biopsy and 6 cases to Fibrotest. The discordance was unexplained in 3 cases. the size of biopsy<15mm [OR=2. 82, 95% CI, 1. 3–6. 07; p =0. 008] and the stage of fibrosis F0,F1,F2 [OR =3. 35 , 95% CI, 1. 1–10. 2; p=0. 03] were considered as risk factors of discordance in multivariate analysis.

Conclusion: This prospective study confirmed the good diagnostic value of Fibrotest as compared with the histological analysis of liver biopsy.

Key-words

hepatitis C-fibrosis-liver-biopsy-Biological Markers

Assessment of the presence and severity of liver fibrosis is critical to determine therapeutic strategies, prognosis and the potential risks for complications in patientswith chronic hepatitis C virus (HCV). The consensus in many conference statements recommends liver biopsy in the management of almost all patients with chronic hepatitis C. The statements have also underlined the necessity for developing reliable noninvasive tests [1], since liver biopsy is an invasive procedure and has limitations [2-8]. Alternative noninvasive methods have thus been developed, particularly biochemical markers of fibrosis which can be measured on a blood sample and correlated with pathology scores [9-16]. A proprietary fibrosis score Fibrotest®, based on biochemical serum markers has been described that could substantially reduce the number of biopsies performed for the management of HCV infection [12]. The score is computed by accessing a proprietary website and entering the patient's age, sex, and results for serum α2macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin and gammaglutamyl-transpeptidase. Investigators from France reported that with appropriate cutoff values, the Fibrotest®score in HCV patients gave either a 100%negative predictive value for the absence of clinically significant fibrosis or a 91% positive predictive value for its presence [12]. Fibrotest® has been validated by several studies in patients with chronic HCV infection [17- 23] but very few independent studies have been reported [10, 11, 22] and several interrogations persist concerning discrepancies between hepatic fibrosis estimates by Fibrotest® and pathology findings [21, 23]. The purpose of this study was to compare Fibrotest® versus liver biopsy histology in a Tunisian population of patients with chronic HCV infection.

MATERIALS AND METHODS

The study was approved by a local ethics committee and all patients gave prior informed consent for being included.

Patients

This prospective study included in 2 years (2006-2007), consecutive patients with chronic HCV infection (patients with a positive serological test by at least a second-generation ELISA and HCV RNA detectable by PCR) and who had an indication for liver biopsy. The recruited patients had a blood sample for biochemistry (Fibrotest®). Exclusion criteria were co infection with HIV, hepatitis B virus, other liver disease, non-interpretable liver biopsy, renal failure, renal transplantation and serum taken after any antiviral therapy.

Liver biopsy

Transparietal liver biopsy was performed under local anesthesia. Liver biopsy specimens were fixed in formalin, paraffin-embedded, and stained with haematoxylin eosin, Masson's trichrome and picrosirius red forcollagen. All samples were analyzed with the METAVIR group scoring system, by one pathologist who was unaware of patient characteristics. Every biopsy specimen was staged on a scale of F0 to F4: F0=no fibrosis, F1=portal fibrosis without septa, F2=few septa, F3=numerous septa without cirrhosis, andF4=cirrhosis.

Fibrotest®

Fasting blood samples were obtained and the biochemical tests (α2-macroglobulin, haptoglobin, gamma-glutamyl-transpeptidase (GGT), total bilirubin, apolipoprotein A1) were performed by a laboratory regularly using the tests and meeting quality control standards. Fibrotest® scores were determined using the algorithm proposed by BioPredictive® by an investigator who was unaware of the results of the liver biopsy. The results were interpreted after transforming the biochemistry score into an equivalent histological fibrosis stage, as shown in Table 1.

Table 1 : Fibrotest® interpretation by BioPredictive®.

Fibrotest® score	Estimate of fibrosis stage
0,00-0,21	F0
0,22-0,27	F0-F1
0,28-0,31	F1
0,32-0,48	F1-F2
0,49-0,58	F2
0,59-0,72	F3
0,73-0,74	F3-F4
0,75-1,00	F4

Discordance

The biochemistry and histology stages were compared patient by patient. A significant discordance between Fibrotest® and biopsy stages was defined as a difference of two points or more between the two methods. If the difference (biochemistry stage-biopsy stage) was positive, the Fibrotest® was considered to overestimate the fibrosis in comparison with the histological results. If the difference was negative, the Fibrotest® was considered to underestimate the fibrosis.

Statistical analysis

SPSS version 11. 5 was used for the statistical analysis. The performance of Fibrotest® was determined using the receiving operating characteristic (ROC) curve method. The area under the receiver operating curve (AUROC) was used to establish the cut-off levels discriminating between fibrosis stages. These cut-off levels were defined as the biochemical test value giving the best sensitivity and specificity. The sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively) were established for each cut-off. Univariate analysis and multivariate analysis by multiple logistic regression were carried out to determine the factors associated with significant discordance (P values <0. 05 were considered significant).

RESULTS

The Characteristics of included patients are given in Table 2.

We recruited a total of 65 patients: 28 males and 37 females (57%), with a mean age of 50±9. 2 years (29-65ans). Most of the patients were infected with genotype 1 (92 %) and had a high viral load (>500 000 UI/ml) (78%).

Table 2 : Characteristics of included patients

Number of patients	65
Age years	50 ± 9,2
Male n (%)	28 (43%)
Female n (%)	37 (57%)
Genotype n (%)	
1	60 (92 %)
2	4(6%)
3	1(2%)
Baseline viral load >500000UI/ml,n (%)	51 (78 %)
Quality of liver biopsy	
Biopsy size mm mean (SD)	17,67 (6,07)
≥15mm and ≥5 Portal tracts n (%)	48 (73,8%)
≥25mm et ≥5 Portal tracts n (%)	9 (13,8%)
Histological fibrosis results n (%)	
No fibrosis F0	2 (3.1%)
Portal fibrosis F1	16(24%)
Few septa F2	21(32%)
Many septa F3	19(29%)
Cirrhosis F4	7(10%)
Fibrotest® Markers (normal range)	
Gamma glutamyl t ranspeptidase IU/l(<45)	72,12 (8-512)
Total bilirubin Ìmol/l(<17)	12,11 (4,7-57,8)
α2 macroglobulin g/l(1,34-2,69)	3,2 (1,08-5,19)
Haptoglobin g/l (0,34-2,13)	0.8 (0,05-1,9)
Apolipoprotein A1 g/l(1,04-2,02)	1,18 (0,61-1,89)
Fibrotest® results n (%)	
F0	7(10,7%)
F0-F1	3 (4,6%)
F1	23(3%)
F1-F2	7(10,7%)
F2	11(17%)
F3	9(13.8%)
F3-F4	3(4.6%)
F4	23(35,4%)

Liver biopsy

The liver biopsy sample measured 17. 67±6. 07 mm on average (range 10-35 mm) and contained, on average 10. 47±5. 29portal spaces (range 2-25). 87. 6% of the biopsy samples (57/65) measured more than 15mm. The histological fibrosis results were: 2/65 (3. 1%) F0; 16/65(24. 6%) F1; 21/65 (32. 3%) F2; 19/65 (29. 2%) F3; 7/65 (10. 8 %) F4. Thus, 27. 6% were considered to have insignificant fibrosis (F0-F1), 72% significant fibrosis (F2-F3- F4) and 40 % severe fibrosis (F3-F4).

Fibrotest®

The mean fibrosis score was 0. 58 ± 0. 24 (range 0. 05 – 0. 96). The Fibrotest® results were: 7/65 (10. 7%) F0; 3/65(4. 6%) F0-F1; 2/65(3%) F1; 7/65 (10. 7%) F1-F2; 11/65 (17%) F2; 9/65 (13. 8%) F3; 3/65 (4. 6%) F3-F4and 23/65 (35. 4%) F4. The diagnostic values of Fibrotest® for the estimation of fibrosis staging using the METAVIR score as gold standard are given in Table 3

Table 3 : Diagnostic values of biochemical markers of fibrosis for the estimation of fibrosis staging.

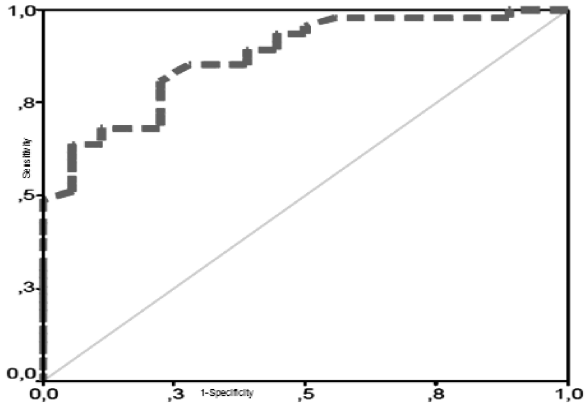
Stage	**AUROC [CI 95%]**	**p**	**Cut-off**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
F0-F1 versus F2-F4	0.87 [0.78-0.96]	0.0001	0.5	85.1	72.2	88.9	65
F0-F2 versus F3-F4	0.76 [0.64-0.88]	0.0001	0.52	92.3	53.8	57.1	91.3
F0-F3 versus F4	0.85 [0.72-0.97]	0.002	0.75	85.7	70.7	26.1	97.6

AUROC: area under the curve.
PPV: positive predictive value.
NPP: negative predictive value.

Several hypotheses were tested:

1-Significant fibrosis: F0-F1 versus F2-F4 (Figure 1)
AUROC = 0. 87[0. 78-0. 96] (p = 0. 0001)
The best cut-off was 0. 5: sensitivity 85. 1%, specificity 72. 2%, PPV 88. 9%, NPV 65%.
2-Severe fibrosis F0-F2 versus F3-F4
with AUROC = 0. 76 [0. 64-0. 88] (p =0. 0001).
The best cut-off was 0. 52: sensitivity 92. 3%, specificity 53. 8%, PPV 57. 1%, NPV 91. 3%.
3-Cirrhosis F0-F3 versus F4
with AUROC = 0. 85[0. 72-0. 97] (p =0. 002).
The best cut-off was 0. 75: sensitivity 85. 7%, specificity 70. 7%, PPV 26. 1%, NPV 97. 6%.
4- Adjacent stages F1 versus F2 with AUROC = 0. 83 [0. 7-0. 96] (p =0. 001) and F3 versus F4with AUROC = 0. 76 [0. 55-0. 97] (p =0. 04).

Figure 1 : Area under the curve (AUROC) for the Fibrotest® versus liver biopsy to discriminate for significant fibrosis (F0-F1 vs F2-F4).AUROC = 0.87[0.78-0.96]
(p = 0.0001)



The discordance

Considering greater than 2 points difference, there were 13/65 cases of discordance (20%) for fibrosis (12 overestimations and 1 underestimations by Fibrotest® compared with the biopsy).

Imputation of discordances

The causes of failure among the 13 discordant cases were considered attributable to biopsy in 4cases and to Fibrotest in 6 cases. The discordance was unexplained in 3 cases.

Among the 4 errors attributable to biopsy, 3 were false negatives and 1 was false positive. Among the false negative, the remarkable elements was a biopsy of poor quality in 1 patient (biopsy size: 10mm and 2 portal tracts), a cirrhosis in Fibrotest® suggested by clinical, biochemical, radiological, and endoscopic features in 2 patients. The false positive was due to a fragmented biopsy in 1 patient.

All the causes of Fibrotest® failure was false positives.

The remarkable elements were a low haptoglobin<0, 2g/due to acute hemolysis (n=2), a high α2-macroglobulin>4g /l without a concomitant increase in haptoglobin due to inflammation (n=2), a high GGT due to extrahepatic cholestasis (n=1) and to obesity (n=1).

Risk factors of discordance

Univariate and multivariate analyses were carried out to determine the factors associated with discordances.

In univariate analysis (table4), the male gender [OR=2. 97, 95% CI, 1. 01–8. 67; P =0. 03], the size of biopsy<15mm [OR=4. 51, 95% CI, 1. 71–11. 92; P =0. 003], and the stage of fibrosis F0, F1, F2 [OR=8, 95% CI, 1. 1–57. 86; P =0. 008], were considered as risk factors of discordance.

In multivariate analysis, two risk factors remained significant as shown in table5, the size of biopsy<15mm [OR=2. 82, 95% CI, 1. 3–6. 07; P =0. 008] and the stage of fibrosis F0, F1, F2 [OR =3. 35, 95% CI, 1. 1–10. 2; P =0. 03].

DISCUSSION

Between 1991 and 2008, a total of 14 validated biomarkers have been identified for assessment of Liver Fibrosis [28-32], five of them were patented: Fibrotest®, FibroSpect II, Enhanced Liver Fibrosis, FibroMeter, and HepaScore.

Fibrotest® invented by Thierry Poynard and team in 2001[12], was the most used patented panel and the most evaluated.

Recently, Poynard et al [33] analyzed in their overview of evidence-based data, a total of 38 different populations published in numerous articles between 2001 and 2008 [28-32] including 7985 subjects with both Fibrotest® and biopsy (4600 hepatitis C, 1580 hepatitis B, 267 non alcoholic fatty liver disease, 524 alcoholic liver disease, and 1014 mixed) and find that for Fibrotest®, the mean diagnostic value for the diagnosis of advanced fibrosis assessed using standardized area under the ROC curves was 0. 84 (95%confidence interval 0. 83-0. 86), without a significant difference between the causes of liver disease.

One criticism of this test was that most of the studies that validated it were performed by the inventor of the Fibrotest® but three independent studies have confirmed results obtained by Poynard's group [10, 11, 22]. In the study by Castera et al [10], the Fibrotest® was compared to transient elastography (the Fibroscan), APRI and liver biopsy in patients with chronic hepatitis C. The AUROC for significant fibrosis (F>2) were 0. 83, 0. 85 and 0. 78 for the Fibroscan, the Fibrotest®, and the APRI, respectively. The study by Cales et al [11], compared the Fibrotest®, with 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 Fibrotest® (the difference was not statistically significant).

In the study by Halfon and collaborators [22] on504 patients with chronic hepatitis C, the AUROC for significant fibrosis was 0. 79. The present study confirms the results of these independent studies. We showed that the AUROC for significant fibrosis (more than F2) was 0. 87 in agreement with the aforementioned studies.

The different meta-analyses also demonstrated that Fibrotest® like biopsy has lower diagnostic value to discriminate between two adjacent stages than between two extreme stages [25-32].

In our study, the diagnostic value of Fibrotest® in the adjacent stages F1 versus F2 and F3 versus F4 was respectively 0. 83 and 0. 76. Fibrotest® 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.

Discordance analysis between Fibrotest® and liver biopsy was assessed by several studies and among the discordances observed, the cause of failure is frequently due to biopsy failure [22, 23, 34-37]. In the study conducted by Poynard et al [23], discordance was observed in 154 of 537 patients (29%), including 12% for fibrosis staging only; 12% for activity grading only and 24 patients (4. 5%) for both and was attributable to biopsy failure in 97 (18%), to Fibrotest® failure in 13 patients (2. 4% P<0. 001 versus biopsy) and non attributable in 44 patients (8. 2%). For fibrosis staging, the most frequent biopsy errors were false negatives (4. 5%) associated with smaller biopsy size and steatosis. False positives of fibrosis staging (3. 5%) were associated with fragmented biopsies. The most frequently identified cause of Fibrotest® failure was false negatives attributable to inflammation, with an isolated increase in haptoglobin (4 cases), one false-positive Fibrotest® result was attributable to hemolysis, and one to post transplantation fibrosing cholestasis.

In the Halfon et al. study [22], discordance between Fibrotest® and biopsy results for fibrosis staging was observed in 92 of the 504 patients (18%). Discordances were attributable to biopsy in 19 cases (4%), to Fibrotest® in 27cases (5%) and undetermined in 46 cases (9%).

In our study, discordance between Fibrotest® and biopsy results for fibrosis staging was observed in 13 of the 65 patients (20%). Discordances were attributable to biopsy in 4cases and to

Fibrotest® in6 cases, 3 cases had unexplained discrepancy. The male gender, the size of biopsy<15mm and the stage of fibrosis F0, F1, F2 were considered as risk factors of discordance.

In conclusion, this study suggests that Fibrotest® could be used as an alternative to liver biopsy for the assessment of fibrosis stage in patients with chronic hepatitis C. Biopsy could be useful when Fibrotest® is not applicable or discordant.

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