Journal of Viral Hepatitis

Comparison of liver biopsy and non-invasive techniques for liver fibrosis assessment in patients infected with HCV-genotype 4 in Egypt.

Journal:	*Journal of Viral Hepatitis*
Manuscript ID:	JVH-00005-2014.R1
Manuscript Type:	Original Paper
Date Submitted by the Author:	12-Apr-2014
Complete List of Authors:	Bonnard, Philippe; AP-HP, Service de Maladies Infectieuses; INSERM, U707 Elsharkawy, Aisha; Faculty of medicine, Cairo University, Endemic medicine and hepatology department Zalata, Khaled.; mansoura, pathology Delarocque-Astagneau, Elisabeth; Pasteur Institute, Emerging diseases epidemiology Biard, Lucie; Pasteur Institute, Emerging diseases epidemiology Le Fouler, Lenaig; Institut Pasteur, Emerging Diseases Epidemiology Unit Hassan, Abobakr; Department of Tropical Diseases, National Hepatology and Tropical Medicine Research Institute Abdelhamid, Mohamed; El-Menia University, Microbiology Eldaly, Mai; Viral Hepatitis Research Laboratory, National Hepatology and Tropical Medicine Research Institute, Esmat, Mohammed Gamal; Department of Tropical Diseases, National Hepatology and Tropical Medicine Research Institute El-Kassas, Mohammed; Department of Tropical Diseases, National Hepatology and Tropical Medicine Research Institute Bedossa, Pierre; AP-HP, Pathology Carrat, Fabrice; INSERM, U707 Fontanet, Arnaud; Institut Pasteur, Epidemiology; Esmat, Gamal; Faculty of Medicine, Cairo University, Tropical Medicine Department
Keywords:	liver fibrosis, liver biopsy, hepatitis C virus, Egypt, serum markers

Comparison of liver biopsy and non-invasive techniques for liver fibrosis assessment in patients infected with HCV-genotype 4 in Egypt.

Bonnard P[1,2], Elsharkawy A[3], Zalata K[4], Delarocque-Astagneau E[5], Biard L[5], Le Fouler L[5], Hassan AB[3], Abdel-Hamid M[6.7], El-Daly M[7], Gamal ME[8], El Kassas M[8], Bedossa P[9], Carrat F[2], Fontanet A[5,10], Esmat G[8].

1: Infectious Diseases, Hôpital Tenon (AP-HP), Paris, France.

2: Unité INSERM U707, UPMC, Paris, France.

3: Department of Tropical Medicine and Hepatology, Faculty of Medicine, Cairo University, Egypt.

4: Department of Pathology, Mansoura Faculty of Medicine, Mansoura, Egypt.

5: Emerging Diseases Epidemiology Unit, Institut Pasteur, Paris, France.

6: Department of Microbiology and Immunology, Faculty of Medicine, Minia University, Minia, Egypt.

7: Viral Hepatitis Research Laboratory, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.

8: Tropical Medicine Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt.

9: Anatomopathology, Hôpital Beaujon (AP-HP), Clichy, France.

10: Conservatoire National des Arts et métiers, Paris, France

Corresponding author :

Dr Ph. Bonnard, Service de Maladies Infectieuses.

Hôpital Tenon (AP-HP), 4 rue de la Chine, 75020, Paris, France

Tel : +33 1 56 01 74 21 (+33 6 79 61 50 43),

Fax : +33 1 56 01 74 23 (+33 1 56 01 74 12)

e-mail : philippe.bonnard@tnn.aphp.fr

word count : abstract : 242

main text : 2914

running head : Liver fibrosis assessment in Egypt.

Abstract :

Background: In Egypt, as elsewhere, liver biopsy (LB) remains the gold standard to assess liver fibrosis in chronic hepatitis C (CHC), and is required to decide if a treatment should be proposed. Many of its disadvantages have led to develop non invasive methods to replace LB. These new methods should be evaluated in Egypt, where circulating virus genotype 4 (G4), elevated body mass index, and co-infection with schistosomiasis may interfere with liver fibrosis assessment.

Patients and methods: Egyptian CHC infected patients with G4 underwent a LB, an elastometry measurement (Fibroscan©), and serum markers (APRI, Fib4 and Fibrotest©). Patients had to have a LB ≥15 mm length or ≥ 10 portal tracts with two pathologists blinded readings to be included in the analysis. Patients with hepatitis B virus co-infection were excluded.

Results: 312 patients are reported. The performance of each technique for distinguishing F0F1 vs F2F3F4 was compared. The area under receiver operating characteristic curves was 0.70, 0.76, 0.71 and 0.75 for APRI, Fib-4, Fibrotest© and Fibroscan© respectively (no influence of schistosomiasis was noticed). An algorithm using the Fib4 for identifying patients with F2 stage or more reduced by nearly 90% the number of liver biopsies.

Conclusion: Our results demonstrated that non invasive techniques were feasible in Egypt, for CHC G4 infected patients. Because of its validity and its easiness to perform, we believe that Fib4 may be used to assess the F2 threshold, which decides if treatment should be proposed or delayed.

Key words: liver fibrosis,

liver biopsy,

serum markers,

hepatic elastography,

hepatitis C virus,

Egypt.

BACKGROUND:

The histological assessment of liver fibrosis is a crucial part of the evaluation of patients infected with hepatitis C virus (HCV). Liver fibrosis scoring system uses the METAVIR score which ranges from F0 (no fibrosis) to F4 (cirrhosis) [1]. This evaluation is important because liver fibrosis determines whether to introduce or to delay antiviral treatment (treatment is usually proposed to patients who are ≥ F2 [2]). The gold standard for liver fibrosis evaluation is the liver biopsy (LB) [3, 4]. However, it has numerous side effects [4, 5], and suffers from inter-observer discrepancies [6, 7] For that reason, non invasive techniques, more acceptable to the patient, have been developed [8, 9].

The ideal non invasive technique should be valid, painless, reproducible, easy-to-learn, easy-to-perform, and cheap. Among these new techniques, serum markers and liver elastometric measurement are the most commonly used. However, the diffusion of these non invasive methods is reduced in resources-limited countries where they were not locally evaluated. In addition, in these countries, alternatives cheaper than LB would are needed.

In Egypt, where the HCV prevalence is the highest in the world [10], the National recommendations require that HCV-infected patients undergo a LB at the initial evaluation, and that a treatment is proposed to patients with a fibrosis rate ≥F2. Having a cheaper and more acceptable alternative to LB is critical in a country where six million individuals are chronically infected with HCV [11]. However, these new non invasive methods should be locally evaluated, since circulating virus genotype (genotype 4), elevated body mass index highly prevalent in Egypt and co-infections with schistosomiasis may interfere with liver fibrosis assessment.

PATIENTS AND METHODS:

Patients: The study was conducted at the National Hepatology and Tropical Medicine Research Institute (Cairo, Egypt). To be included, patients had to be aged 18 or more and to be infected by HCV (presence of serum HCV RNA using polymerase chain reaction assay). Pregnant women, patients infected with a non-4 genotype, or with a positive HBs antigen, or with a contra-indication to LB, or with a Child-Pugh score B or C, or patients refusing a possible treatment were not included in the study.

Ethical statement: All the patients first received information on the study from their referring physician and were asked to sign an informed consent form. Standard management of HCV patients in Egypt includes LB to determine if a treatment is indicated. Needle biopsy of the liver was performed in the standard manner in our study. The patients also underwent non-invasive (elastography) and semi-invasive investigations (serum markers of fibrosis). When found eligible for treatment, all patients enrolled in this study initiated a 48-week regimen of pegylated-interferon and ribavirin under the national treatment program (Egyptian National Control Strategy for Viral Hepatitis (2008-2012)).

Data collection: The following information was collected: Age, gender, height, weight, alcohol and chicha consumption, presence of ascites or hepatic encephalopathy. Patients first underwent a blood intake to rule out a contra-indication to the LB. Then, they had the liver stiffness measurement, the biological samples collection and the LB within 15 days.

Liver histology : Patients underwent LB by trained hepatologists with a 16-G true cut biopsy needle (HS, Italy). The samples were fixed in formalin, paraffin embedded. Slides were stained with hemaotxylin-eosin ans Sirius red. The slides were read by two pathologists, the second reader being unaware of the first reader's findings. Both readers were blinded to the results of the alternative methods of fibrosis assessment. The size of the biopsy and the number of portal tracts were noted. To be included in the analysis, the LB must be over 15mm or include at least10 portal tracts (except for the "F4" stage for which LB could be fragmented). Liver fibrosis and necrotico-inflammatory activity were assessed with the METAVIR scoring system [1]. Fibrosis was therefore scored on a scale from 0 to 4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis), and activity on a scale from 0 to 3 (A0 = none, A1 = mild, A2 = moderate, A3 = severe). In case of discrepancy of 1 fibrosis point between the two readings, the highest value was chosen. In case of discrepancy of 2 fibrosis points between the two readings,,a third one was requested by an independent pathologist. If the third reading confirmed one of the previous ones, that result was kept in the analysis; if the third reading did not confirm any of the previous ones, the patient was not included in the analysis. Hepatic steatosis was classified as none

(<1%), mild (1-32%), moderate (33-66%) or severe (>66%) as already reported [12]. Finally, the presence of schistosomiasis (granuloma) was noted.

Virologic and biochemical analysis : The HCV serology was assayed using the Axsym HCV EIA (v3.0) test (Abbott Laboratories, Diagnostic Division, USA). HCV genotype was performed with Restriction Fragment Length Polymorphism technique at the 5'-untranslated region, using two combinations of restriction endonucleases (MvaI/HinfI and RsaI/HaeIII)(Boehringer-Manheim, Germany) [13, 14]. The patients were screened for HBsAg by using the Axsym HbsAg immunoassay test (Abbott Laboratories, Diagnostics Division, USA). All biochemical analysis were performed in Cairo at the Viral Hepatitis Research Laboratory (VHRL), NHTMRI. ALT (IU/l), AST (IU/l), bilirubin (µmol/l), alkaline phosphatase (IU/l), white blood cell count (cells/mm3), haemoglobin (g/l), platelets count (/l), prothrombin time (PT), creatinin (µmol/l), β-HCG (IU/l, for women of age to procreate), schistosomiasis serology, α2-macroglobulin (g/l, (α2-MG)), haptoglobin (g/l), apolipoprotein-A1 (g/l, (apo-A1)).

The APRI and Fib-4 scores were calculated as described in the princeps publication, as APRI = AST [-fold upper limit of normal] x 100/PLT [10^9/l], and Fib-4 = age (years) x AST[IU/l] / (PLT[10^9/l] x (ALT[IU]$^{1/2}$) [15, 16]. The scores were calculated by Biopredictive for Fibrotest© (FT), blindly to the results from histology and elastography [17].

Hepatic elastography: A Fibroscan© FS 512 probe was used (Echosens, Paris, France), as described elsewhere [18]. The device was implanted in NHTMRI thanks to a grant from the Agence Nationale de Recherche sur le Sida (ANRS). Physicians received a one-week FibroScan© (FS) training followed by a week's training six months later. All the patients underwent the examination with a normal probe (no XL probe was used). The success rate [19] of the examination was calculated as the ratio between the number of measurements validated by the machine and the total number of attempted measurements. The liver stiffness corresponds to the median value of the validated measurements. The interquartile range (IQR) stands for the interval around the median that contains 50% of the valid measurements. To be considered interpretable and valid, the examination must include at least 10 measurements, with a SR of at least 66%, and the IQR must not exceed 33% of the results of the examination.

Statistical analysis: Continuous variables were described using a descriptive analysis (means, medians, SDs, ranges, and quartiles). The performance of FS, FT, APRI, and Fib4 was assessed with receiver operating characteristic (ROC) curves. A patient was considered positive or negative according to whether the non-invasive technique value was greater than, lesser than, or equal to a given cut-off value. The ROC curve is a plot of sensibility (Se) versus (1-Specificity (Sp)) for all possible cut-off values. The most commonly used accuracy index is the area under the ROC curve (AUROC), values close to 1.0 indicating high diagnostic accuracy. For each technique (FS, FT, APRI, and Fib4), sensibility and specificity were calculated for

each threshold. The optimal cut-off values used for each test were determined by maximizing the Youden index (Se+Sp-1). Confidence interval (95%) for sensitivity and specificity were computed for these cut-off values [20], as well as positive and negative predictive values were computed for these cut-off values. By using these cut-off values, the agreement between APRI, Fib-4, FT, FS and liver fibrosis for the diagnosis of two stages is reported. The diagnosis of significant fibrosis (F≥2) was assessed by comparing pooled F2F3F4 patients with F0F1patients. Agreement of cirrhosis (F4) was assessed by comparing F0F1F2F3 patients with F4 patients.

Applicability of Fibroscan was estimated as the proportion of patients without missing assessment of elastometry and with reliable FS results. The AUROC was corrected for applicability as proposed by Poynard et al., considering that unreliable elastometry assessment was equivalent to classifying patients as random (AUROC=0.5) [21]. Additionally, Obuchowski's measure was also computed in order to provide a complete assessment of the non-invasive markers in the diagnosis of fibrosis stage, with METAVIR staging as categorical five-point scale gold standard [22-24]. Obuchowski's measures were weighted on the relative proportion of the 5 fibrosis stages in the sample. They were also computed with a penalty function to take into account the differences between stages.

Statistical analysis was implemented with STATA software (IC 10.0, College Station, TX, USA) and R software (version 2.15.2).

RESULTS :

Patients : From February 2009 to November 2010, 500 patients were considered eligible for the study (figure 1). Among them, 26 (5.2%) had some post hoc non inclusion criteria (missing age, HCV RNA negative, non-4 genotype, Child Pugh B, or HBV infection). Of the 474 patients included in the study. 316 (66%) had a LB with at least 10 portal tracts or a length≥15mm, or a cirrhosis and were included in the analysis (66%). Twenty patients had a LB with a discrepancy of 2 fibrosis stages and required a third blinded reading. Despite this third reading, 4 LB were excluded from the final analysis (see Patients and Methods). Eventually, the analysis included these 312 patients (flow chart is shown in figure 1).

The search for factors associated with LB exclusion (n = 157), failed to find explanatory epidemiological factors (age, gender, BMI, presence of steatosis). The table 1 reports the clinical and biological characteristics of the patients included in the analysis (n=312) and the patients not included because the LB did not meet inclusion criteria (n=157). These characteristics and epidemiological factors are reported in table 1.

No side effects requiring hospitalization were reported.The distribution pattern of fibrosis, activity and steatosis are shown in table 2. Granulomatous lesions consistent with hepatic schistosomiasis were detected on 4 (1.5%) LB.

The box plots of values of different techniques against each stage of fibrosis (METAVIR) (A=Fib4, B=APRI, C=Fibrotest, D=Fibroscan) are reported in figure 2. Area under receiver operating characteristics (AUROC) curves of the alternative techniques versus the gold standard for the threshold F0F1 vs F2F3F4 and F0F1F2F3 vs F4 are shown on figure 3. AUROC (and 95%>CI), sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of each cutoff are reported in table 3.

Multivariate analysis suggested a statistical link between high BMI (>30) and high elastometry (OR=2.9, p=0.05), and a trend between having a high elastometry and steatosis > 33% (OR=2.94, p=0.068).

Among the 312 elastometry assessments, 39 (12.5%) did not meet the usual reliability criteria for the FS (SR>66% and IQR<1/3 the median stiffness). A new analysis was performed, restricted to the 273 patients whose assessments were reliable, showing similar results compared to the analysis with 312 patients (data not shown). When a random assignment was given to FS with non interpretable values (correction for applicability: see Patients and Methods), the AUROC value for FS was reduced to 0.69 for F0F1 vs F2F3F4 and 0.83 for F0F1F2F3 vs F4.

Obuchowski's measure for the diagnosis of fibrosis stages ranged from 0.74 (APRI) to 0.80 (FS) (table 4). They were not different across the 4 tests except for APRI

compared to Fib4 (0.74 vs 0.78, p=.0009) and APRI compared to FS (0.74 vs 0.0, p=0.004) (table 5).

Fib4 came across as valid and very simple to determine (based only on age, liver enzymes, and platelets). Using the thresholds displayed in Table 3 for differentiating patients with low fibrosis (F0F1), and severe fibrosis (F4), we categorized the patients as not needing treatment, needing a liver biopsy to decide on treatment indication, or requiring treatment (Figure 4).

With this algorithm, patients are dispatched into three categories: ❶ Patients with a Fib4 $\leq$1: no LB is proposed to confirm this result, no treatment is required, and patients are invited for follow-up with Fib4 one year later. Only a few patients of this class have a major indication to treatment, as 9.4% of them are F4. ❸ Patients with a Fib4 $\geq$ 1.27: no LB is proposed to confirm this result and a treatment is proposed. A few patients among them (17.6%) have no or mild liver fibrosis and will undergo a treatment without an urgent need. ❷ Patients with 1 < Fib4 < 1.27: a LB is proposed to assess liver fibrosis. With this algorithm, only 11% of the patients undergo a LB.

DISCUSSION :

Recent guidelines from an international panel suggested that alternative techniques for liver fibrosis assessment in HCV-G4 patients should be studied [25]. This ANRS-funded study showed that it was possible to use such techniques in the Egyptian context.

The main result of this study shows that in this context of HCV-genotype 4 infected population, non invasive techniques can be used for liver fibrosis assessment. Because they are easy-to-calculate, unlicensed, and they don't need further costs for accreditation or maintenance, we believe that APRI and Fib4 could be introduced in Egypt. Of the two tests, Fib4 performed well on our study, while APRI did not have so good performances as already reported [15]. Furthermore, we think that in this epidemic context, the ability of the tests to stage F2 vs F3 or F0 vs F1 is not a crucial point; the priority is to know whether a patient has severe fibrosis and should undergo immediate treatment or to know if he doesn't have any liver fibrosis (F0F1) and have no indication to treatment. These serum markers have already been used in this purpose [26], and we believe that this is probably the most appropriate way to use them in this heavy epidemic context.

Fibroscan did well, although not as efficient at grade F2 and F4 stages than already published studies [27]. This lower statistical correlation between the FS with grades of fibrosis could be increased by using an XL probe in daily practice in Egypt. This should be a particular subject to pay attention for, as 97/312 (31%) of the patients included in this study have a BMI>30. No influence of positive schistosomiasis serology was noticed, and a very small number of patients with schistosomiasis histological lesions were included. Noteworthy, we found that high BMI (>30) was linked to high elastometry (OR=2.9, p=0.05). This statistical linkage may be explained by a relation between BMI and fibrosis and steatosis in Egyptian population as already demonstrated by others [28]. Wong et al. [29] already suggested that this finding could be linked with non alcoholic fatty liver diseases. In our study, we only found a trend between steatosis and high measurements of elastography (OR=2.94, p=0.065); in contrary to what was already reported, we did not find a particular high proportion of patients with steatosis [30]. However, this trend suggests that in a population wherein a high proportion of patients have a BMI>30 (31% in our study), elastometry should be difficult to use because it could overestimate liver fibrosis in a high proportion of patients. Despite the high BMI of included patients, no influence of BMI was reported for serum markers.

FT was not as good as reported in previous publications at the F2 stage [27]. We believe that this may be linked to the fact that the use of tests that need some measurements which are not routinely performed in Egypt or other resources-limited countries (apolipoprotein-A1, α2-macroglobulin) is hazardous.

One other limitation of this study is the proportion of LB performed which did not fulfill the inclusion criteria chosen for the study. By requiring strict criteria for the LB (size> 10mm, at least 15 portal tracts, two blinded readers, with a third reader in case of discrepancy of more than two fibrosis stages) we required ideal conditions for this gold standard. This was necessary to compare the efficiency of alternative techniques to the reference technique. However, in the context of routine clinical practice, this study shows that 33% (157/469) of the biopsies do not meet such criterias. These conditions are probably not the ones which the clinicians are used to work with. In most of the centers, only one reading is provided and the physician decides to treat or not according to this single reading. We found a higher proportion of patients with none or minimal fibrosis (F0-F1) among patients with an improper LB than among patients with LB meeting the inclusion criteria of the protocol (69% vs. 56%, p = 0.005). This may explain the lower platelet count and higher ALT values among the enrolled patients

Finally, we believe that Fib4 could be an interesting alternative to know if the patient should be treated or not. We presented an algorithm including only Fib4 to assess liver fibrosis in Egypt because it is the most usable technique in such a particular situation. Furthermore, Fib4 is effective to detect a patient with cirrhosis who will need a priority treatment, but who will also need a special monitoring for the detection of hepatocarcinoma and oesophageal varices. By using this algorithm, less than 10% of the decisions (7.6%) resulting from it are not adequate with the real liver fibrosis stage of the patient. If the aim of non invasive fibrosis markers is to reduce but not substitute the need for liver biopsy [31], our Fib4 algorithm saves nearly 90% of the liver biopsies. The results we propose concerning Fib4 seem to be validated in a larger egyptian prospective set of patients who underwent LB with a unique pathologist reading in « real life »[28], suggesting that this algorithm could be used in clinical practice. The use of Fib4 could be extended in a future study, whether to consider if this test can be a predictor of the evolution of chronic hepatitis C, in patients who don't undergo an anti HCV treatment.

We propose that patients who did not undergo anti HCV treatment after a single Fib4<1 should be yearly assessed with Fib4. This will help to detect patients with rapid liver fibrosis and patients with a liver fibrosis underevaluated by the former test and who actually need treatment.

ACKNOWLEDGEMENTS:

The authors want to acknowledge all the patients included in the study.

We would like to adress our special ackowledgements to Enas Mohamed Abd Elwahed, Amany Mohamed Sayed, Rita Rafaat Gad, and Nehal Alsaqqa for the help they provide during the data collection.

The study was funded by a grant from the French Agence Nationale de Recherche sur le Sida et les hépatites virales (ANRS 12184).

CONFLICT OF INTERESTS:

None.

DECLARATION OF FUNDING INTERESTS:

None.

REFERENCES :

1. Bedossa, P., Poynard, T., *An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group.* Hepatology, 1996. **24**(2): p. 289-93.
2. Ghany, M.G., et al., *Diagnosis, management, and treatment of hepatitis C: an update.* Hepatology, 2009. **49**(4): p. 1335-74.
3. Strader, D.B., et al., *Diagnosis, management, and treatment of hepatitis C.* Hepatology, 2004. **39**(4): p. 1147-71.
4. Bravo, A.A., S.G. Sheth, and S. Chopra, *Liver biopsy.* N Engl J Med, 2001. **344**(7): p. 495-500.
5. West, J. and T.R. Card, *Reduced mortality rates following elective percutaneous liver biopsies.* Gastroenterology. **139**(4): p. 1230-7.
6. Rousselet, M.C., et al., *Sources of variability in histological scoring of chronic viral hepatitis.* Hepatology, 2005. **41**(2): p. 257-64.
7. Regev, A., et al., *Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection.* Am J Gastroenterol, 2002. **97**(10): p. 2614-8.
8. Mehta, S.H., et al., *Exceeding the limits of liver histology markers.* J Hepatol, 2009. **50**(1): p. 36-41.
9. Castera, L., *Transient elastography and other noninvasive tests to assess hepatic fibrosis in patients with viral hepatitis.* J Viral Hepat, 2009. **16**(5): p. 300-14.
10. Deuffic-Burban, S., et al., *Expected increase in hepatitis C-related mortality in Egypt due to pre-2000 infections.* J Hepatol, 2006. **44**(3): p. 455-61.
11. Breban, R., et al., *Towards realistic estimates of HCV incidence in Egypt.* J Viral Hepat, 2013. **20**(4): p. 294-6.
12. Brunt, E.M., et al., *Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions.* Am J Gastroenterol, 1999. **94**(9): p. 2467-74.
13. Constantine, N.T., M. Abdel-Hamid, and D. Oldach, *Rapid genotyping of hepatitis C virus.* N Engl J Med, 1995. **333**(12): p. 800.
14. McOmish, F., et al., *Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey.* J Clin Microbiol, 1994. **32**(4): p. 884-92.
15. Wai, C.T., et al., *A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C.* Hepatology, 2003. **38**(2): p. 518-26.
16. Vallet-Pichard, A., V. Mallet, and S. Pol, *FIB-4: a simple, inexpensive and accurate marker of fibrosis in HCV-infected patients.* Hepatology, 2006. **44**(3): p. 769; author reply 769-70.
17. Imbert-Bismut, F., et al., *Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study.* Lancet, 2001. **357**(9262): p. 1069-75.
18. Ziol, M., et al., *Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C.* Hepatology, 2005. **41**(1): p. 48-54.
19. Al-Hamoudi, W., et al., *Treatment of genotype 4 hepatitis C recurring after liver transplantation using a combination of pegylated interferon alfa-2a and ribavirin.* Dig Dis Sci, 2011. **56**(6): p. 1848-52.
20. Robin, X., et al., *pROC: an open-source package for R and S+ to analyze and compare ROC curves.* BMC Bioinformatics, 2011. **12**: p. 77.
21. Poynard, T., et al., *FibroTest and Fibroscan performances revisited in patients with chronic hepatitis C. Impact of the spectrum effect and the applicability rate.* Clin Res Hepatol Gastroenterol, 2011. **35**(11): p. 720-30.
22. Lambert, J., et al., *How to measure the diagnostic accuracy of noninvasive liver fibrosis indices: the area under the ROC curve revisited.* Clin Chem, 2008. **54**(8): p. 1372-8.
23. Carrat, F., *Integrating the applicability rate into the performance evaluation of noninvasive staging of liver fibrosis: a step towards a change from the "accuracy" to the "clinical" paradigm.* Clin Res Hepatol Gastroenterol, 2011. **35**(11): p. 691-2.

24. Obuchowski, N.A., M.J. Goske, and K.E. Applegate, *Assessing physicians' accuracy in diagnosing paediatric patients with acute abdominal pain: measuring accuracy for multiple diseases.* Stat Med, 2001. **20**(21): p. 3261-78.
25. Khattab, M.A., et al., *Management of hepatitis C virus genotype 4: recommendations of an international expert panel.* J Hepatol, 2011. **54**(6): p. 1250-62.
26. Usluer, G., et al., *Comparison of non-invasive fibrosis markers and classical liver biopsy in chronic hepatitis C.* Eur J Clin Microbiol Infect Dis, 2012. **31**(8): p. 1873-8.
27. Castera, L., et al., *Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C.* Gastroenterology, 2005. **128**(2): p. 343-50.
28. Khairy, M., et al., *Non-invasive prediction of hepatic fibrosis in patients with chronic HCV based on the routine pre-treatment workup.* Hepat Mon, 2012. **12**(11): p. e6718.
29. Wong, G.L., et al., *Association between anthropometric parameters and measurements of liver stiffness by transient elastography.* Clin Gastroenterol Hepatol, 2013. **11**(3): p. 295-302 e1-3.
30. Kamal, S.M. and I.A. Nasser, *Hepatitis C genotype 4: What we know and what we don't yet know.* Hepatology, 2008. **47**(4): p. 1371-83.
31. Sebastiani, G. and A. Alberti, *Non invasive fibrosis biomarkers reduce but not substitute the need for liver biopsy.* World J Gastroenterol, 2006. **12**(23): p. 3682-94.

Table 1: Baseline characteristics of included and non included patients.

	157 patients with LB which did not conform to criteria	312 patients with LB which did conform to criteria	p
Age (years)	38.3 +/- 11	39.4 +/- 10.7	0.3
Sex (male)	108 (69%)	202 (65%)	0.38
Body Mass Index (kg/m^2)	27.3 +/- 4	27.9 +/- 4	0.13
Positive schistosomiasis serology (%)	29	36	0.19
Albumin (g/l)	43 +/- 5	42.7 +/- 5	0.72
Prothrombin time (%)	90 +/- 10	89 +/- 11	0.37
Total bilirubin (µmol/l)	12.2 +/- 4.5	12.7 +/- 4.8	0.29
γ-Glutamyl-transpeptidase (IU/l)	51 +/- 61	53 +/- 47	0.66
Proportion of F0F1 (%)	69	56	0.005
Platelet count (10^3/mm^3)	236 +/- 64	218 +/- 62	0.035
ALT (x ULN)	1.38 +/- 1	1.61 +/- 1.3	0.04

Quantitative variables are expresses as mean+ SD

Table 2: Liver fibrosis, activity and steatosis in the included patients.

Fibrosis (n=312)		Activity (n=266)		Steatosis (n=312)	
stage	n (%)	stage	n (%)	stage	n (%)
0	2 (0.6)	0	3 (1)	none	208 (66.7)
1	106 (34)	1	103 (39)	mild	85 (27.2)
2	102 (32.7)	2	120 (45)	moderate	18 (5.8)
3	49 (15.7)	3	40 (15)	severe	1 (0.3)
4	53 (17)				

According to Metavir

Table 3: AUROC curves, Se, Sp, PPV and NPV of the different methods of fibrosis assessment with optimal cutoffs.

	stage	cut-off	AUROC	Se (%)	Sp (%)	PPV (%)	NPV (%)
APRI	≥ F2	0.5	0.70 (0.64-0.76)	63 (49-73)	69 (56-78)	80	50
	F4	0.76	0.76 (0.69-0.83)	66 (44-77)	75 (58-83)	35	92
Fib4	≥ F2	1	0.76 (0.70-0.81)	67 (56-75)	74 (58-83)	83	54
	F4	1.27	0.81 (0.75-0.87)	85 (68-92)	66 (49-74)	34	96
FT	≥ F2	0.37	0.74 (0.69-0.80)	78 (65-85)	64 (44-74)	80	61
	F4	0.81	0.77 (0.69-0.84)	49 (27-61)	93 (74-97)	60	90
FS	≥ F2	7.8	0.71 (0.64-0.76)	53 (39-61)	83 (67-90)	85	48
	F4	10.4	0.88 (0.82-0.93)	86 (70-94)	81 (47-88)	47	97

Table 4: Obuchovski's measure with 95% confidence interval (CI95%).

tests	Obuchovski's measure	CI 95%
APRI	0.74	0.69-0.78
Fib4	0.78	0.74-0.82
FT	0.76	0.69-0.82
FS	0.8	0.78-0.86

Table 5: p values comparing Obuchowski's measures among non invasive methods.

tests	APRI	Fib4	FT
APRI	-	-	-
Fib4	0.0009	-	-
FT	0.64	0.25	-
FS	0.004	0.51	0.12

Figure 1: flow chart of the patients included in the study.

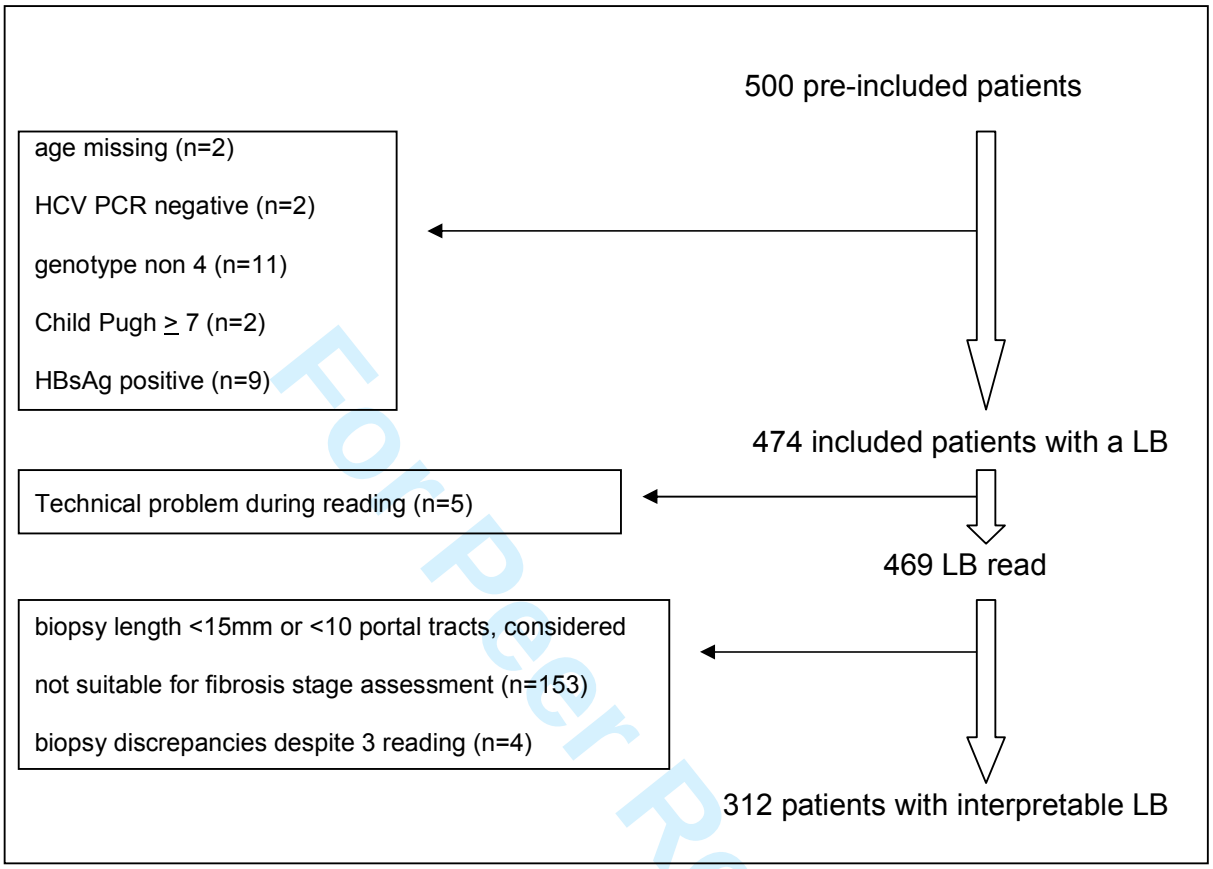

Figure 2: Box plots of values of different tests for each stage of METAVIR fibrosis (A=Fib4, B=APRI, C=FT, D=FS).

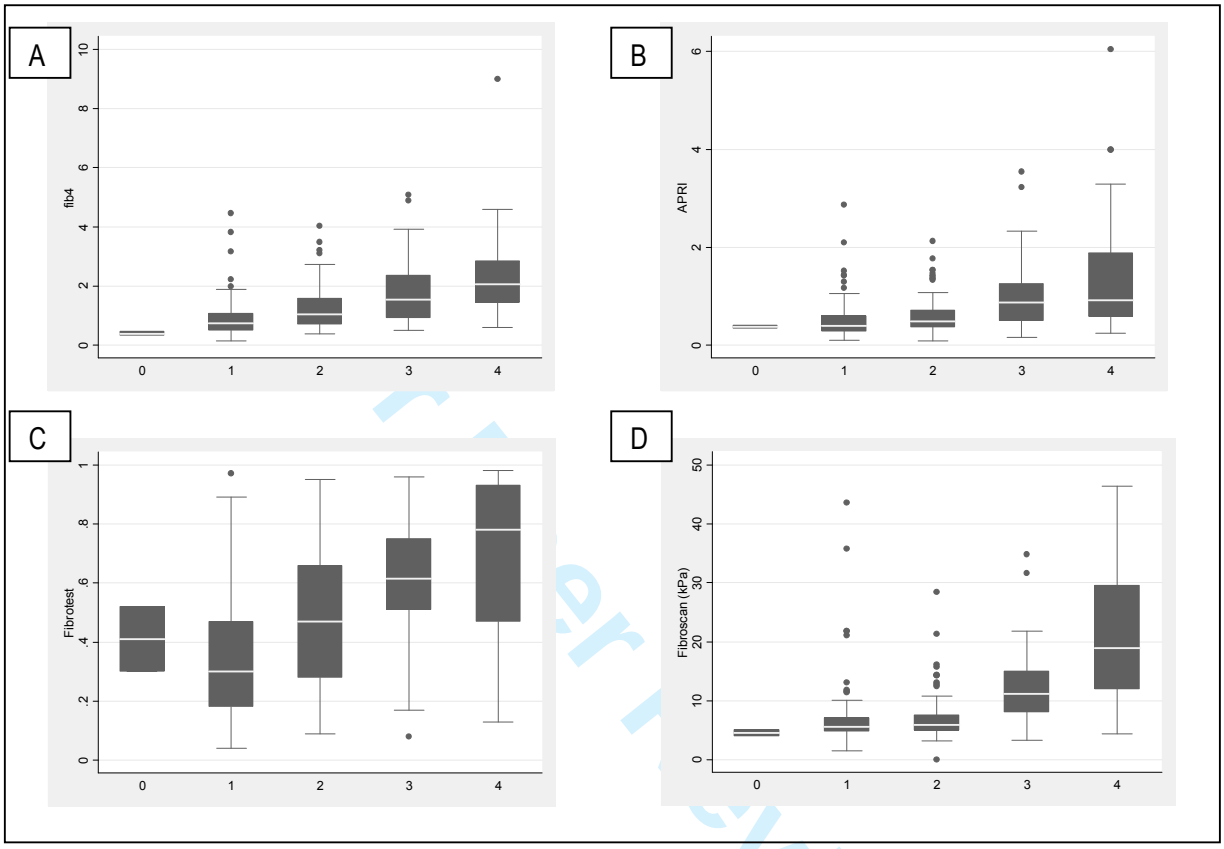

Figure 3: AUROC curves of each tests vs LB for the two thresholds (F0F1 vs F2F3F4 and F0F1F2F3 vs F4)

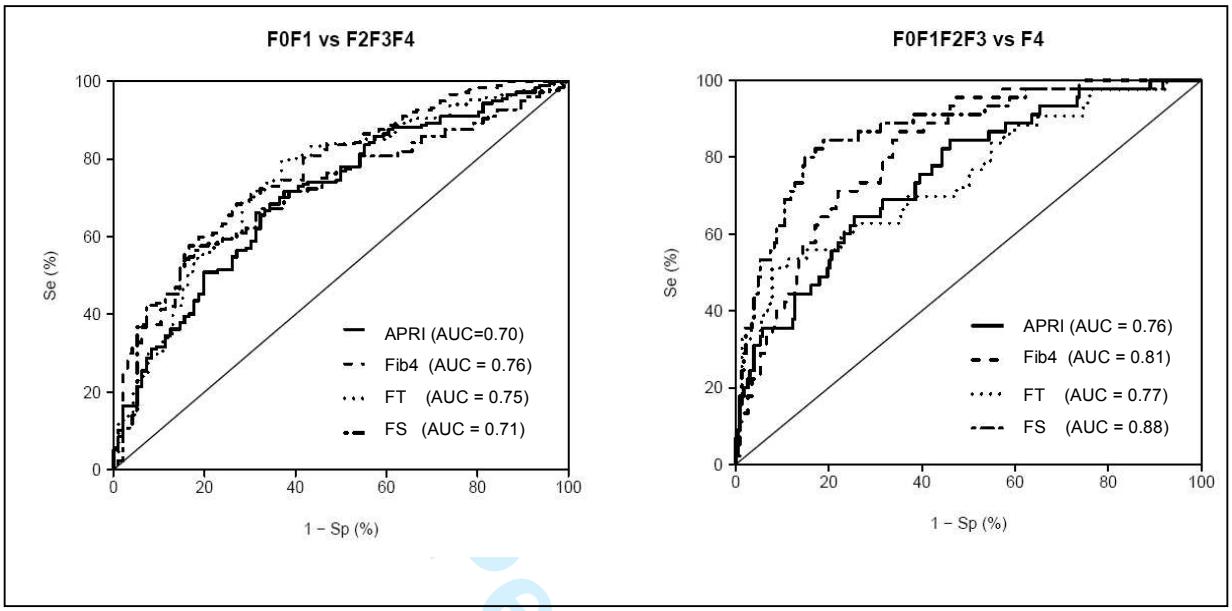

Figure 4 : proposition of an algorithm using Fib4 to classify liver fibrosis of HCV-infected patients.