*Am. J. Trop. Med. Hyg.*, 82(3), 2010, pp. 454–458
doi:10.4269/ajtmh.2010.09-0088


# Comparison of Elastography, Serum Marker Scores, and Histology for the Assessment of Liver Fibrosis in Hepatitis B Virus (HBV)-Infected Patients in Burkina Faso

Philippe Bonnard,* Roger Sombié, Francois-Xavier Lescure, Alain Bougouma, Jean Baptiste Guiard-Schmid, Thierry Poynard, Paul Calès, Chantal Housset, Patrice Callard, Catherine Le Pendeven, Joseph Drabo, Fabrice Carrat, and Gilles Pialoux

*Maladies Infectieuses, Hôpital Tenon (AP-HP), UPMC Univ Paris 06, UPMC Paris Liver Center, Paris, France; Hépatogastroentérologie, CHU Yalgado Ouedraogo, Ouagadougou, Burkina Faso; Hépatogastroentérologie, Hôpital Pitié Salpêtrière (AP-HP), UPMC Univ Paris 06, UPMC Paris Liver Center, Paris, France; Hépatogastroentérologie, CHU Angers, Angers, France; Biochimie, Hôpital Tenon (AP-HP), UPMC Univ Paris 06, UPMC Paris Liver Center, Paris, France; Anatopathologie, Hôpital Tenon (AP-HP), Paris, France; Virologie, Hôpital Tenon (AP-HP), Paris, France; Medecine Interne, CHU Yalgado Ouedraogo, Ouagadougou, Burkina Faso; Unité INSERM U707, UPMC Univ Paris 06, UPMC Paris Liver Center, Paris, France*

*Abstract.* Liver fibrosis (LF) must be assessed before talking treatment decisions in hepatitis B. In Burkina Faso, liver biopsy (LB) remains the "gold standard" method for this purpose. Access to treatment might be simpler if reliable alternative techniques for LF evaluation were available. The hepatitis B virus (HBV)-infected patients who underwent LB was invited to have liver stiffness measurement (Fibroscan) and serum marker assays. Fifty-nine patients were enrolled. The performance of each technique for distinguishing F0F1 from F2F3F4 was compared. The area under receiver operating characteristic (AUROC) curves was 0.61, 0.71, 0.79, 0.82, and 0.87 for the aspartate transaminase to platelet ratio index (APRI), Fib-4, Fibrotest, Fibrometre, and Fibroscan. Elastometric thresholds were identified for significant fibrosis and cirrhosis. Combined use of Fibroscan and a serum marker could avoid 80% of biopsies. This study shows that the results of alternative methods concord with those of histology in HBV-infected patients in Burkina Faso. These alternative techniques could help physicians to identify patients requiring treatment.

## INTRODUCTION

The prevalence of hepatitis B virus (HBV) infection in Burkina Faso is about 15%.[1] The primary infection is frequently asymptomatic, sometimes occurring by mother–child transmission, and hepatitis B is often diagnosed when already at an advanced stage. Access to treatment is limited both by drug costs and by the need for liver fibrosis assessment before starting treatment. Needle biopsy of the liver (NBL) is the standard technique, and is the only method available in Burkina Faso. In principle, it is offered systematically to patients with active HBV replication, as shown by HBV polymerase chain reaction (PCR) positivity. However, NBL is invasive, potentially painful, and carries a risk of serious complications.[2] Furthermore, fibrosis can be either under- or overestimated by NBL.[3] Needle biopsy of the liver is also clearly unsuitable for monitoring disease progression, especially in untreated patients. Patients with active HBV replication do not always have NBL, and many therefore remain untreated. Likewise, as it is the patient who must pay for what can be a very lengthy treatment, NBL showing that treatment is warranted is no guarantee that treatment will effectively start. An alternative method for evaluating liver fibrosis is therefore needed to optimize patient management in resource-limited countries. This would also simplify access to treatment.

Transient elastography (Fibroscan [FS], Echosens, France) is a reproducible, non-invasive and rapid method for measuring liver stiffness. It is based on sonographic measurement of the propagation velocity of an elastic wave induced by the device itself: the faster the wave, the stiffer the medium. Studies of patients with hepatitis C virus (HCV) infection have shown that wave velocity correlates with the degree of liver fibrosis, and that elastography can accurately detect both early fibrosis[4] and advanced fibrosis.[5] However, few data are available on the use of this method in patients with hepatitis B, especially in sub-Saharan Africa.

Scores based on serum markers have been developed to estimate liver fibrosis without the need for NBL. Here, we compared the performance of Fibrotest (Biopredictive, Paris, France), Fibrometre (Biolivescale, Angers, France), APRI (the aspartate transaminase to platelet ratio index), and FIB-4, by comparison with histology, in HBV-infected patients in Burkina Faso.[6–9]

## PATIENTS AND METHODS

**Patients.** All the patients were over 18 years of age and were infected by HBV, as shown either by HBV PCR positivity or by HBsAg positivity (in patients on treatment). The patients were managed at Yalgado Ouedraogo university hospital (Ouagadougou, Burkina-Faso). Patients were not eligible for the study if they had a clinical diagnosis of liver cancer or if they were pregnant. Despite a low prevalence of gastrointestinal schistosomiasis in this urban area and in Ouagadougou, patients who had a past history of schistosomiasis were excluded from the study and patients who had visible granuloma on liver biopsy were not included in the analysis.

**Ethics 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 HBV PCR-positive patients in Burkina Faso includes liver biopsy, to determine if treatment is indicated. Needle biopsy of the liver was performed in the standard manner in this study. The patients also underwent non-invasive (elastograpy) and semi-invasive investigations (serum markers of fibrosis). Patients who were serologically screened for HCV and HIV infection received relevant information before testing, and received the results from their referring physician. Patients with HIV co-infection could be referred to the relevant hospital department. The scientific committee prepared an ethics statement that was signed by all participants in this research.

**Data collection.** The following clinical information was collected: age, weight, size, alcohol consumption, ongoing treatment,

* Address correspondence to Philippe Bonnard, Maladies Infectieuses et Tropicales Hôpital Tenon (AP-HP), 4 rue de la Chine 75020 Paris cedex 20, France. E-mail: philippe.bonnard@tnn.aphp.fr

date of diagnosis of HBV infection, prior assessment of liver fibrosis, ongoing anti-HBV treatment, and HIV or HCV coinfection. Patients underwent liver stiffness measurements and blood tests on the same day.

**Liver histology.** Patients who had already had liver biopsy in the year before the study could be enrolled without the need for a second biopsy. The other patients underwent NBL prospectively. Needle biopsy of the liver was done by a trained hepatologist, with a 15-G Hepafix needle (Braun Medical, Melsungen, Germany). The samples were fixed in paraffin and stained with Sirius red and hematin-eosin. The slides were all read by two experienced 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, and a consensus interpretation was reached if there was a discrepancy. The size of the biopsy specimens was noted. Liver fibrosis and necroinflammatory activity were assessed with the METAVIR scoring system.[10] 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). All the patients had undergone NBL as part of their routine hepatitis B management less than 1 year before study entry, or underwent NBL less than 6 months after elastography and calculation of fibrosis scores based on serum markers.

**Virologic and biochemical analyses.** The HBV DNA was assayed in France using the Abbott PCR real-time assay (M2000sp and M2000rt (Applied 7500, Abbott, IL) with a detection limit of 10 IU/mL.

The patients were screened for HIV and HCV infection by using the Tecan Genesis RSP 150 platform (Zurich, Switzerland) and Biorad reagents (Hercules, CA). The HIV seropositivity was confirmed with the BioMérieux Vidas Duo Quick test (Marcy l'Etoile, France).

All biochemical analyses were done at Tenon hospital in Paris, with the exception of the platelet count (PLT) and the prothrombin time (PT), that were done on the day of sampling at Ouagadougou university hospital. The following analytes were measured with a ci8000 automat (Abbott, IL): urea, aspartate transaminase (AST), alanine transaminase (ALT), bilirubin, g-glutamyl transpeptidase (γ-GT) activity, apolipoprotein-A1 (apo-A1), α2 macroglobulin (α2-MG), and haptoglobin. Platelets were measured with an ABX Pentra 60 automat (Montpellier, France), and the PT with a Diagnostica Stago Start 4 (Parsippany, NJ). Hyaluronic acid (HA) was measured with an enzyme-linked immunosorbent assay (ELISA) method (Corgenix, CO). The APRI and FIB-4 scores were calculated as described in the princeps publication, as APRI = AST [-fold upper limit of normal] × 100/PLT [109/l], and FIB-4 = age (years) × AST [IU/l]/(PLT [$10^9$/l] × (ALT [IU/l])½).

The scores were calculated by Biopredictive for Fibrotest (FT), and by Biolivescale for Fibrometer (FM), blindly to the results of histology and elastography.

**Hepatic elastography.** We used a Fibroscan FS 512 (Echosens, Paris, France), as described elsewhere.[11] The device was made available for a 1-week period in the Liver Unit of Ouagadougou university hospital. The success rate of the examination is calculated as the ratio between the number of measurements validated by the machine and the total number of attempted measurements during the same examination. The median value of the validated measurements is taken to represent liver stiffness. The interquartile range (IQR) represents the interval around the median that contains 50% of valid measurements. To be considered interpretable and valid, the examination must include at least 10 measurements with a success rate of at least 66%, and the IQR must not exceed 33% of the result of the examination. Fibroscan (FS) was done on the same day as serum marker assays.

**Statistical analysis.** A descriptive analysis was used to study the distribution of continuous variables (means, medians, SDs, ranges, and quartiles) and the frequencies of categorical variables. The diagnostic performance of FS, FT, FM, APRI, and FIB-4 was assessed with receiver operating characteristic (ROC) curves. A patient was considered positive or negative according to whether the noninvasive marker value was greater than, less than, or equal to a given cut-off value. Connected with any cut-off value is the probability of a true positive (sensitivity) and a true negative (specificity). The ROC curve is a plot of sensitivity versus 1 minus specificity 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. Optimal cut-off values for liver stiffness were chosen to maximize the sum of sensitivity and specificity, often optimizing sensitivity. Positive and negative predictive values were computed for these cut-off values. The fibrosis stage given by Biopredictive was used for FT. By using these cut-off values, the agreement between FS, FT, and liver biopsy (LB) for the diagnosis of significant fibrosis ($F \geq 2$) was assessed by comparing pooled F2–F4 patients with F0/F1 patients; agreement on the diagnosis of severe fibrosis ($F \geq 3$) was assessed by comparing F3–F4 patients with F0–F2 patients; agreement on cirrhosis ($F \geq 4$) was assessed by comparing F0–F3 patients with F4 patients. Statistical analyses were implemented with SPSS 14.0 software (SPSS, Inc., Chicago, IL).

## RESULTS

Fifty-nine patients were enrolled during the week in which the Fibroscan device was available. Only two patients were excluded, because they had undetectable HBV DNA without treatment, and biopsy was not therefore considered to be warranted. The patients' clinical and biological characteristics are shown in Table 1. Alcohol consumption was assessed by interview. Twenty percent of patients ($N = 12$) declared occasional alcohol consumption (beer), and none more than 10 g/day. None of the patients was infected by HIV and three patients (5%) carried anti-HCV antibodies, but all of them had negative HCV PCR. No patients were excluded because of signs of schistosomiasis on liver biopsy. The median interval between

TABLE 1
Clinical and biological characteristics of the patients*

| | |
|---|---|
| Sex ratio (M/F) | 2.2/1 |
| Age (y) | 35 ± 9 |
| BMI (kg/m$^2$) | 24 ± 5 |
| AST (IU/L) | 53 ± 88 |
| ALT (IU/L) | 64 ± 120 |
| Total bilirubin (μmol/L) | 15 ± 12 |
| γ-GT (IU/L) | 50 ± 95 |
| α2-MG (g/L) | 2.8 ± 0.8 |
| Apo-A1 (g/L) | 1.5 ± 0.2 |
| Haptoglobin (g/L) | 0.83 ± 0.4 |
| Hyaluronic acid (μg/L) | 27 ± 31 |
| Urea (mmol/L) | 3 ± 1 |
| PT (%) | 96 ± 6 |
| Platelets ($10^9$/L) | 209 ± 80 |

*Results are expressed as means ± SD; BMI = body mass index.

NBL and elastometry/blood tests (performed the same day) was 4 months [0.5–10]. Eleven patients (19%) had already been tested for liver fibrosis (all by NBL) and 55% of these biopsies ($N = 6$) had shown fibrosis ≥ F3.

Seventeen patients (29% of the study population) were already on treatment (median duration 10 months, range 1–24 months); it consisted of lamivudine alone in 15 cases and lamivudine plus tenofovir in two cases. Thirteen of the treated patients had HBV viral load below 10 IU/mL; one patient (treated for 1 month) had HBV viral load > $28.10^6$ IU/mL, and another patient (treated for 4 months) had HBV viral load 1500 IU/mL.

Forty-eight patients (81% of the study population) underwent NBL prospectively. None developed complications necessitating hospitalization. All the biopsies were considered to be interpretable, and the mean length of the tissue cores was 21 ± 6 mm. The distribution of liver fibrosis stages was as follows: 5 patients were F0 (8%), 13 (22%) F1, 19 (32%) F2, 8 (14%) F3, and 14 (24%) F4. No signs of iron overload, granuloma, or alcohol-related disease were seen. However, four patients had moderate steatosis (median 22.5%, range 10–30).

Box-plots of serum marker values at each Metavir stage of fibrosis are shown in Figure 1, whereas Figure 2 shows the ROC curves for the different methods and the different Metavir stages of fibrosis (F0F1 versus F2F3F4 and F0F1F2F3 versus F4).

Table 2 shows the AUROC curves for each serum fibrosis marker score and for each considered cutoff. There was no difference between FT, FM, and FS for any stage of fibrosis. In contrast, APRI and FIB-4 performed less well than the other methods for identifying Metavir ≥ F2 fibrosis.

We determined the best performance of each test by searching the highest value of the "sensibility" + "specificity" sum. The performance of the different tests for detecting different fibrosis thresholds (≥ F2 and ≥ F4) is shown in Table 3, with the corresponding sensitivities, specificities, and positive and negative predictive values. The best techniques for identifying patients who might qualify for treatment (fibrosis ≥ F2) were Fibroscan, FT, and FM. The techniques with the best performance for advanced fibrosis (≥ F4) were Fibroscan, FT, FM, and FIB-4.

## DISCUSSION

Alternatives to biopsy for assessing liver fibrosis are especially crucial in sub-Saharan Africa, as access to therapy for

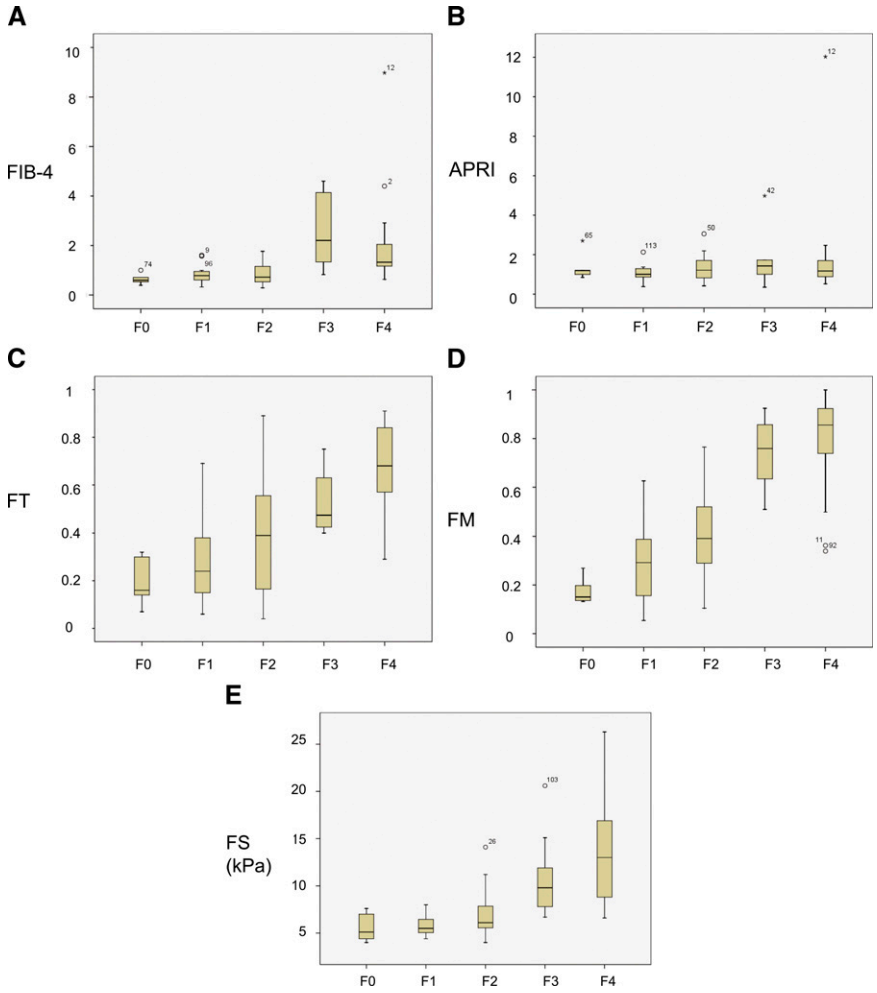


FIGURE 1. Box plots of **A**, (Fib4); **B**, aspartate transaminase to platelet ratio index (APRI); **C**, Fibrotest (FT); **D**, Fibrometer (FM); and **E**, Fibroscan (FS). The upper and lower limits of the boxes are the first and third quartiles. The size of the box represents the interquartile range containing 50% of the values (the median is represented by the horizontal line). The upper and lower limits represent the maximal and minimal values. This figure appears in color at www.ajtmh.org.

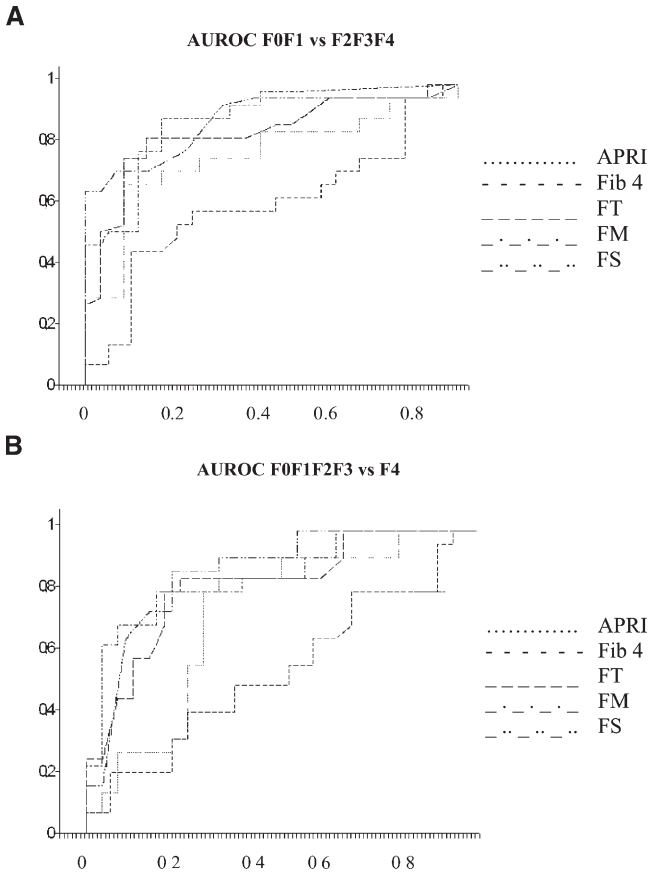


FIGURE 2. Receiver operating characteristic (ROC) curves for fibrosis scores (API, FIB-4, Fibrotest [FT], Fibrometer [FM], and Fibroscan [FS]) at different stages of fibrosis: **A**, F0F1 v/s F2F3F4 and **B**, F0F1F2F3 v/s F4.

HBV infection depends on prior diagnosis of liver fibrosis. Thus, accurate assessment of fibrosis is a prerequisite for improving the management of HBV-infected patients in resource limited countries. Until recently, the reference method was needle biopsy, even in Europe. However, for reasons stated in the introduction, not all patients with positive HBV PCR are biopsied, despite this being recommended in clinical practice guidelines. Liver fibrosis is under assessed mainly for reasons of poor access to the necessary technical resources, and also poor access to screening. Physicians are faced with a dilemma when, regardless of the results of liver fibrosis assessment, their patients will not have access to modern drugs because of their cost or because they are not locally available. Access to a reliable method of liver fibrosis assessment is therefore a precondition for large clinical trials of anti-HBV therapy. Local guidelines in Burkina Faso recommend

TABLE 2

AUROC curves for APRI, FIB-4, FT, FM, and FS according to the Metavir fibrosis stage*

| | F0F1 vs. F2F3F4 | F0F1F2F3 vs. F4 |
|---|---|---|
| APRI | 0.61 (0.46–0.76) | 0.5 (0.32–0.68) |
| FIB-4 | 0.71 (0.57–0.84) | 0.74 (0.60–0.87) |
| FT | 0.79 (0.66–0.91) | 0.85 (0.74–0.96) |
| FM | 0.82 (0.71–0.94) | 0.86 (0.74–0.97) |
| FS | 0.87 (0.79–0.96) | 0.88 (0.80–0.97) |

*AUROC = area under the receiver operating characteristic curves; APRI = aspartate transaminase to platelet ratio index; FT = Fibrotest; FM = Fibrometer; FS = Fibroscan.

TABLE 3

Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) of the different methods of fibrosis assessment, with optimal cutoffs based on histological findings*

| | Cutoff | Se (%) Sp (%) | PPV (%) NPV (%) |
|---|---|---|---|
| Metavir fibrosis stage ≥ F2 | | | |
| APRI | 1.00 | 55 50 | 69 29 |
| FIB-4 | 0.8 | 74 60 | 83 52 |
| FT | 0.37 | 77 80 | 88 56 |
| FM | 0.35 | 80 70 | 87 59 |
| FS | 7.3 | 75 85 | 90 55 |
| Metavir fibrosis stage ≥ F4 | | | |
| APRI | 1.2 | 50 51 | 23 75 |
| FIB-4 | 1.05 | 92 70 | 46 94 |
| FT | 0.5 | 83 77 | 50 94 |
| FM | 0.68 | 83 84 | 58 94 |
| FS | 11 | 71 88 | 71 88 |

*APRI = aspartate transaminase to platelet ratio index; FT = Fibrotest; FM = Fibrometer; FS = Fibroscan.

treatment of stage ≥ F2 disease. In this specialized center, 455 patients were managed for chronic hepatitis B at the time of the study, of whom only 63 (14%) were on treatment. The need for LB is an obstacle to treatment, as it is difficult to perform; in addition, treatment, if indicated, may not be possible, for reasons of cost. Thus, many patients are not assessed for fibrosis and are therefore deprived of the chance of treatment. If the distribution of fibrosis stages observed in the study population (70% of patients ≥ F2) also applied to all the patients managed in the study center, then 320 patients would qualify for treatment. Although this is a very approximate figure, it does provide an idea of the potential benefit of introducing a non-invasive method of fibrosis evaluation.

We chose not to exclude patients already on treatment, as the mean interval between the outset of treatment (immediately after LB) and elastography/serum marker assays was only 8 months. The few published data on changes in HBV-related fibrosis on treatment[12] show that such changes can occur, when measured after 49 months on average. In addition, although fibrosis can improve on treatment, it can also deteriorate. We therefore considered that the inclusion of some treated patients could not, after such a short treatment period, have a significant influence on the comparison of LB and the alternative methods.

Despite the many potential confounding factors, certain serum markers could be used in routine practice. It is important to note that the distribution of fibrosis stages was homogeneous in our study population, ruling out a selection bias. It is unlikely that these data reflect the distribution of fibrosis stages in the general HBV-infected population in Burkina Faso: in particular, stages F0/F1 are likely to be more frequent than in our study population. Fibrotest and FM accurately detected significant fibrosis (≥ F2), which often indicates treatment. Furthermore, FT, FM, and FIB-4 were sensitive methods for extensive fibrosis (≥ F4), which warrants screening for liver cancer, portal hypertension, and esophageal varices, and possibly indicates bleeding prophylaxis with beta-blockers. The APRI score did not correlate very well with Metavir fibrosis stages.

Hepatic elastography was simple: among the patients who underwent NBL, only one patient (1.7%) was not included in the analysis because of difficulties in obtaining valid FS values. The FS accurately detected both significant and extensive fibrosis. The two thresholds identified in our study (7.3 kPa for ≥ F2 and 11 kPa for ≥ F4) correlate with those identified in

France for HBV-infected patients,[13] suggesting that this device could be used in Africa with the same thresholds.

Thus, although simple serum markers (FIB-4 and APRI) performed less well than more sophisticated markers for identifying fibrosis stage F2, this study suggests that FT and FM could nonetheless be used in routine practice in sub-Saharan Africa (the different components of the serum markers are not routinely measured, and the score is expensive to calculate in the Burkina Faso context), and liver stiffness measurement by FS could be the best means of identifying the different stages of fibrosis.

More than half the patients who had already been biopsied at study entry had advanced-stage fibrosis (≥ F3), suggesting that biopsy is only offered in the later stages of hepatitis B. Possible reasons include poor access to screening or to PCR, and the cost of screening, biopsy, and treatment.

Some biopsies could be spared if these markers were used in daily practice. Indeed, four (36%) of the 11 patients already biopsied before study entry had fibrosis stage F1. These "negative" biopsies could be avoided by the use of FS, as all four of these patients had FS < 7.3 kPa. In contrast, FT missed one of these four cases, FM and FIB-4 two, and APRI three.

In practice, an HBV-infected patient with a liver stiffness value below 7.3 kPa does not require further investigations (serum markers or biopsy), or treatment, because this cutoff has a negative predictive value of 90% for hepatic fibrosis ≥ F2. We found that the most accurate serum markers (Fibrotest and Fibrometre) and elastography agreed on the presence of fibrosis stage ≥ F2 in 48/59 patients (81%). If LB were to be practiced only if the two alternative approaches disagreed on stage ≥ F2 fibrosis, 81% of biopsies among patients qualifying for treatment could be avoided.

This study shows that for the first time alternative methods for assessing liver fibrosis are feasible in patients with isolated HBV infection in Burkina Faso, and probably elsewhere in sub-Saharan Africa. Introduction of these techniques in Burkina Faso could simplify and improve access to treatment.

Received February 15, 2009. Accepted for publication November 16, 2009.

Acknowledgments: We thank Olga Lompo (Pathology Department, Ouagadougou university hospital), Lassana Sangaré (Virology Department, Ouagadougou university hospital), and Soraya Fellahi (Biochemistry Department, Tenon Hospital [AP-HP], Paris) for their involvement in this study. We also thank David Young for translating the French manuscript.

Financial disclosure: The authors (BP, SR, LFX, BA, GSJB, HC, CP, LC, DJ, CF, and GP) who took part in this research declare that they have no financial relationship with the manufacturers of the devices involved in the study and that they received no funding from the manufacturers to carry out this research. Thierry Poynard is the inventor of the FibroTest and has a capital interest in Biopredictive, the company marketing FibroTest; the royalties belong to the French public health organization Assistance Publique Hôpitaux de Paris. Paul Calès has a potential conflict of interest because of stock ownership in a company (BioLiveScale) marketing Fibrometre, created under the auspices of Angers University, France.

Authors' addresses: Philippe Bonnard, Francois-Xavier Lescure, Jean Baptiste Guiard-Schmid, and Gilles Pialoux, Maladies Infectieuses et Tropicales, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France, E-mails: philippe.bonnard@tnn.aphp.fr, xavier.lescure@tnn.aphp.fr, guiardschmidjb@bf.afro.who.int, and gilles.pialoux@tnn.aphp.fr. Roger Sombié and Alain Bougouma, Hépatogastroentérologie, CHU Yalgado Ouedraogo, Burkina Faso, E-mails: docsombf@yahoo.fr and alain .bougouma@sante.gov.bf. Thierry Poynard, Hépatogastroentérologie, Hôpital Pitié Salpêtrière, 47 Bd de l'Hôpital, 75013 Paris, France, E-mail: tpoynard@teaser.fr. Paul Calès, Hépatogastroentérologie, CHU Angers, 4 rue Larrey, 49933 Angers, France, E-mail: paul.cales@univ-angers.fr. Chantal Housset, Biochimie, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France, E-mail: chantal.housset@tnn.aphp.fr. Patrice Callard, Anatomopathologie, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France, E-mail: patrice.callard@tnn.aphp.fr. Catherine Le Pendeven, Virologie, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France, E-mail: catherine.le-pendeven@tnn.aphp.fr. Joseph Drabo, Médecine Interne, CHU Yalgado Ouedraogo, Ouagadougou, Burkina Faso, E-mail: j_drabo@hotmail.com. Fabrice Carrat, Unité INSERM U707, Faculté de Médecine Saint Antoine, 27 rue de Chaligny, Paris, France, E-mail: fcarrat@u707.jussieu.fr.

## REFERENCES

1. Collenberg E, Ouedraogo T, Ganamé J, Fickenscher H, Kynast-Wolf G, Becher H, Kouyaté B, Kräusslich HG, Sangaré L, Tebit DM, 2006. Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban Burkina Faso: a comparative analysis. *J Med Virol 78:* 683–692.
2. Cadranel JF, Rufat P, Degos F, 2000. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology 32:* 477–481.
3. ter Borg F, ten Kate JJ, Cuypers HT, Leentvaar-Kuijpers A, Oosting J Wertheim-van Dillen PM, Honkoop P, Rasch MC, de Man RA, van Hattum J, Chamuleau RA, Tytgat GN, Jones EA, 2000. A survey of liver pathology in needle biopsies from HbsAg and anti-Hbe positive individuals. *J Clin Pathol 53:* 541–548.
4. Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, de Lédinghen V, Marcellin P, Dhumeux D, Trinchet JC, Beaugrand M, 2005. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology 41:* 48–54.
5. Ganne-Carrié N, Ziol M, deLedinghen V, Douvin C, Marcellin P, Castera L, Dhumeux D, Trinchet JC, Beaugrand M, 2006. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology 44:* 1511–1517.
6. Imbert-Bismuth F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T, 2001. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection. *Lancet 357:* 1069–1075.
7. Calès P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konaté A, Gallois Y, Ternisien C, Chevailler A, Lunel F, 2005. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology 42:* 1373–1381.
8. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS, 2003. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology 38:* 518–526.
9. Vallet-Pichard A, Mallet V, Pol S, 2006. FIB-4: a simple, inexpensive and accurate marker of fibrosis in HCV-infected patients. *Hepatology 44:* 769.
10. 1994. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology 20:* 15–20.
11. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V, 2005. Prospective comparison of transient elastography, fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology 128:* 343–350.
12. Hui CK, Leung N, Shek TWH, Yao H, Lee WK, Lai JY, Lai ST, Wong WM, Lai LS, Poon RT, Lo CM, Fan ST, Lau GK, Hong Kong Liver Fibrosis Study Group, 2007. Sustained disease remission after spontaneous HbeAg seroconversion is associtaed with reduction in fibrosis progression in chronic hepatits B Chinese patients. *Hepatology 46:* 690–698.
13. Marcellin P, Ziol M, Bedossa P, Douvin C, Poupon R, de Ledinghen V, Beaugrand M, 2008. Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. *Liver Int 9:* 242–247.