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Non-invasive tests for fibrosis and liver stiffness predict 5-year survival of patients chronically infected with hepatitis B virus

V. de Lédinghen*,†, J. Vergniol*, C. Barthe*, J. Foucher*,‡, F. Chermak*, B. Le Bail†,§, W. Merrouche* & P.-H. Bernard‡

*Centre d'Investigation de la Fibrose hépatique, Hôpital Haut-Lévêque, CHU Bordeaux, Pessac, France.
†INSERM U1053, Université Bordeaux Segalen, Bordeaux, France.
‡Service d'Hépato-Gastroentérologie, Hôpital Saint-André, CHU Bordeaux, Bordeaux, France.
§Service d'Anatomie-Pathologique, Hôpital Pellegrin, CHU Bordeaux, Bordeaux, France.

Correspondance to:
Dr V. de Lédinghen, Service d'Hépato-Gastroentérologie, Centre d'Investigation de la Fibrose hépatique, Hôpital Haut-Lévêque, 33604 Pessac, France.
E-mail: victor.deledinghen@chu-bordeaux.fr

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SUMMARY

Background
Liver stiffness and non-invasive tests predict overall survival in chronic hepatitis C. However, in patients chronically infected with hepatitis B virus (HBV), only the association between liver stiffness and the risk of hepatocellular carcinoma has been published.

Aim
To evaluate the 5-year prognostic value of liver stiffness, non-invasive tests of liver fibrosis, and liver biopsy, to predict overall survival in chronic hepatitis B.

Methods
In a consecutive cohort, we prospectively assessed fibrosis, with liver stiffness, FibroTest, APRI, FIB-4 and liver biopsy (if indicated). We examined death and liver transplantation during a 5-year follow-up, and factors associated with overall survival.

Results
A total of 600 patients (men 64%, mean age 42 years, inactive carriers 36%) with chronic hepatitis B were included. At 5 years, 25 patients were dead (13 liver-related deaths) and four patients had liver transplantation. Overall survival was 94.1% and survival without liver-related death 96.3%. No liver-related death was observed in inactive carriers. Survival was significantly decreased in patients diagnosed with severe fibrosis, whatever the non-invasive method used ($P < 0.0001$), or liver biopsy ($P = 0.02$). Patients' prognosis decreased as liver stiffness and FibroTest increased. In multivariate analysis, FibroTest and liver stiffness had the highest hazard ratio with survival. The association persisted after adjustment on age, necro-inflammatory histological activity presumed by ActiTest and treatment.

Conclusions
Liver stiffness measurement or FibroTest can predict survival in chronic HBV infection. Thus, these tools may help physicians to early assess prognosis and discuss specific treatments, such as liver transplantation.

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INTRODUCTION

The evaluation of liver fibrosis is a key step to manage a chronic liver disease and to assess its prognosis, as complications mainly occur in patients with advanced stages. Portal hypertension, ascites or hepatocellular carcinoma are associated with a shorter survival. Early assessment of the risk of bad prognosis helps the physician to manage patients with cirrhosis and to decide for a liver transplantation. Known risk factors for disease progression in chronic hepatitis B can be broadly divided into host, and viral factors. Host factors include age, gender, genetic susceptibility, family history, obesity and immune status. Viral factors can include HBeAg status, hepatitis B virus (HBV) DNA level, genotype, mutants and co-infection.

Liver biopsy does not satisfy quality criteria as a surrogate endpoint marker because of its complication rate, sampling error, intra- and interobserver variability, expense and patient reluctance to undergo serial monitoring.[1, 2]

Different scores such as the Child-Pugh score or the MELD score (model of end stage liver disease) are correlated with the prognosis of advanced cirrhosis and help the physicians to take care of cirrhotic patients.[3, 4] In the past decade, non-invasive markers have been developed as an alternative for liver biopsy, to evaluate the severity of chronic liver diseases, such as FibroTest (Biopredictive, Paris, France; FibroSure-Labcorp, Burlington, NC, USA). It has a high degree of accuracy and reproducibility in predicting bridging fibrosis and cirrhosis in patients with chronic liver diseases.[5] In chronic hepatitis B, its performance is highly significant, with area under the ROCs (AUROCs) of 0.84–0.90 and 0.85–0.87 for the diagnosis of significant fibrosis and cirrhosis respectively.[6, 7] FibroTest has a 5-year prognostic value similar to that of liver biopsy for the prediction of cirrhosis decompensation and survival in patients with chronic hepatitis C virus (HCV),[8–10] chronic hepatitis B[10] and alcoholic liver disease.[11] The aspartate aminotransferase (AST) to platelet ratio index (APRI),[12] and the FIB-4 score,[13] have significant diagnostic performance for fibrosis and cirrhosis, and are significantly associated with mortality in patients with chronic HCV infection.[14] To our knowledge, no study has evaluated the association of APRI or FIB-4 score with the long-term survival in HBV.

Liver stiffness measurement (LSM) using M probe of FibroScan (Echosens, Paris, France) is a non-invasive method for the diagnosis of liver fibrosis. It has a high degree of accuracy and reproducibility in predicting bridging fibrosis and cirrhosis in patients with chronic liver diseases.[15–20] In chronic hepatitis B, its performance is highly significant, with AUROCs of 0.81–0.87 and 0.85–0.93 for the diagnosis of significant fibrosis and cirrhosis respectively.[7, 21–23] There is a relationship between the value of liver stiffness and various complications of cirrhosis, such as oesophageal varices, variceal bleeding, portal hypertension, ascites and hepatocellular carcinoma.[24] Moreover, LSM can predict survival in chronic hepatitis C.[9] In HBV patients, the risks of hepatocellular carcinoma development, liver-related events and liver-related mortality increase with LSM.[25–27] To our knowledge, there is no study evaluating a relationship between 5-year overall survival and liver stiffness in patients with chronic hepatitis B.

The aim of this prospective study was to evaluate the 5-year prognostic value of liver stiffness, FibroTest, APRI, FIB-4 and liver biopsy for predicting survival in patients with chronic hepatitis B.

PATIENTS AND METHODS

Patients

From April 2003 to December 2010, we prospectively collected data on a large cohort of consecutive patients addressed to our centre for chronic hepatitis B. At baseline, we noted the clinical, biological, endoscopic and radiological parameters, and any liver decompensation in the past. During the follow-up, we collected the natural history events, such as liver complications, liver transplantation or death. Study patients belonged to a prospective hospital-based cohort of the Hepatology Unit of Haut-Lévêque Hospital (University Hospital of Bordeaux, Pessac, France). In this cohort study, we targeted follow-up consultations and exams every 6 or 12 months or closer. The end of the follow-up was 31 March 2011. Final analysis of the data was done in February 2012.

We included all consecutive patients with an age over 18 and chronic hepatitis B of any severity. The determination of chronic hepatitis B was made using standard diagnostic criteria: positive HBs antigen for more than 6 months. Inactive carriers were defined as patients with negative HBe antigen, normal alanine aminotransferase (ALT) level (at least four times a year), and HBV-DNA <20 000 IU/mL (at least twice in the past 5 years). Exclusion criteria were all other causes of chronic liver disease. Patients with HIV infection or ascites were excluded. HBV treatment was given according to EASL guidelines.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Patients were enrolled after written informed consent was obtained.

Clinical and biological parameters

For all patients, the following parameters were determined at the time of liver stiffness. Clinical parameters

included weight, height, past history of ascites or bleeding varices and hepatocellular carcinoma. Biological parameters included AST, ALT, γ-glutamyl-transpeptidase (GGT), total bilirubin, platelet count, prothrombin time and different scores: FibroTest, APRI and FIB-4. In patients with cirrhosis, oesophageal varices were examined by upper gastrointestinal endoscopy. Since ascites is a physical limitation to the technique because elastic waves do not propagate through liquids, patients with ascites were excluded.

FibroTest and ActiTest scores were computed on the Biopredictive website (www.biopredictive.com).[28] APRI index was calculated as follows: aspartate transaminase (× upper limit of normal) × 100/platelet count (giga/L).[29] FIB-4 score was calculated as follows: [age (in years) × AST (U/L)]/[platelet count (giga/L) × ALT (U/L)$^{1/2}$].[13] For each non-invasive test, we used the most usual published cut-off for severe fibrosis or cirrhosis (F3F4 or F4 according to Metavir score).

Liver stiffness measurement

Liver stiffness measurement was performed with the patient lying in dorsal decubitus with the right arm in maximal abduction, on the right lobe of the liver, through intercostal spaces. The operator, assisted by a time-motion ultrasound image, located a liver portion at least 6 cm thick and free of large vascular structures. When the target area had been located, he pressed the probe button to commence the measurements. The measurement depth was between 25 and 65 mm using the M probe of FibroScan. Ten validated measurements were performed on each patient. The results were expressed in kilopascals (kPa). Only procedures with at least 10 validated measurements and an interquartile range (IQR) inferior to 30% of the median value were considered reliable.[17, 30] Measurement of liver stiffness was performed in our unit by specialised nurses.

Liver biopsies

Patients with possible indication of treatment or with discordance between FibroTest and LSM (less than 10% of cases) had a liver biopsy,[19] which was processed with standard techniques. One experienced pathologist (Brigitte Le Bail), unaware of the biochemical markers, evaluated the stage of fibrosis and grade of activity according to the METAVIR scoring system.[31]

Follow-up

If needed, patients had a conventional treatment of their diseases during the follow-up (oral nucleoside/nucleotide therapy). The overall 5-year survival (whatever the cause of death, and including liver transplantation) was the 'a priori' main end-point used to compare the prognostic value of the different methods. Survival time was calculated from the date of liver stiffness to the endpoint date (March 31, 2011). This interval was censored at the time of last follow-up. Each year, for patients who had not been seen at our hospital in the previous 12 months, we found out whether they were living and, if not, the date and the cause of death. For patients who were still alive, we either interviewed the patients or obtained information through their physicians. For patients who died outside our hospital, we obtained information about the date and cause of death from their physicians or family. The survival without liver-related death (including death related to liver disease and liver transplantation) was also assessed.

Statistical analysis

Tests were used as follows: chi-squared test for qualitative comparisons, Mann–Whitney test for quantitative comparisons, AUROC curves for the diagnostic and univariate prognostic analyses.[32] The prognostic AUROCs were estimated by the empirical (nonparametric) method of Delong *et al.*,[33] or by binomial approach when events were less than 29 cases, and compared using the paired method. Comparisons between diagnostic AUROCs were performed using the Obuchowski method to take into account the risk of multiple testing and the spectrum effect.[34–36]

The Obuchowski measure allows to compare two biomarkers with a single test, avoiding appropriate correction for the type I error when comparing two biomarkers for different stages or grades.[36] This measure is a multinomial version of the AUROC. With N (=5) categories of the gold standard outcome (histological fibrosis stage) and AUROCst, the estimate of the AUROC of diagnostic tests for differentiating between categories s and t, the Obuchowski measure, is a weighted average of the $N(N–1)/2$ (=10) different AUROCst corresponding to all the pairwise comparisons between two of the N categories. Each pairwise comparison has been weighted to take into account the distance between activity grades (i.e. the number of units on the ordinal scale). A penalty function proportional to the difference in METAVIR units between grades was defined: the penalty function was 0.25 when the difference between stages was 1, 0.50 when the difference was 2, 0.75 when the difference was 3 and 1 when the difference was 4. The Obuchowski measure can be interpreted as the probability that the non-invasive index will correctly rank two randomly chosen patient samples from different activity grades according to the weighting scheme, with a penalty for misclassifying patients. The

overall Obuchowski measure is not equivalent to a usual AUROC curve as the measurements are weighted according to the distance between stages.

For survival analyses, time-dependent Kaplan–Meier analysis for survival curves, the log-rank test for univariate comparisons and the Cox proportional hazard model for multivariate analysis were performed. We also used the Cox model including the two best prognostic factors (FibroTest and LSM) to assess their performance by AUROCs. Because of the limited number of events, only overall 5-year survival was assessed. Because of possible colinearity effect between FibroTest and age, and known association between stiffness age and necro-inflammatory activity, a multivariate analysis was performed adjusted on age and ActiTest. We used liver stiffness values expressed in log values to perform statistical analysis and comparisons.

We used two-sided statistical tests for all analyses. Due to the number of biomarkers comparisons ($n = 4$) a P value <0.01 was considered as significant, between 0.01 and 0.05 as borderline, greater than 0.05 as not significant. Statistical analyses were performed using Number Cruncher Statistical Systems software[37] and R software [library(nonbinROC)and library(ROCR)].[38]

The predetermined factors associated with prognosis were fibrosis as the main factor, and three covariates: HBV treatment, age and necro-inflammatory grade at baseline presumed by ActiTest. Treatment was defined as at least one treatment given during the follow-up.

As biopsy was performed in 214 subjects only, a sensitivity analysis of survival was performed including biopsy stage, FibroTest and LSM.

All the variables have been introduced initially in the multivariate analysis as continuous variables for continuous data: age and biomarkers. If using the continuous quantitative value in the Cox regression model, the maximum likelihood procedure cannot reach convergence (no completion), a semi-quantitative value of the continuous data was used in the multivariate model.

RESULTS

Characteristics of patients

A total of 650 patients were recruited (Figure 1). Among these patients, 23 (3.5%) were excluded for not interpretable LSM: liver stiffness failure (no valid shot) in eight patients (1.2%), less than 10 valid measurements in seven patients (1.1%), IQR/liver stiffness >0.30 in eight patients (1.2%). Moreover, eight patients (1.3%) had no FibroTest (two missing and six not applicable due to high ALT level). At least, 19 patients had no follow-up (3.1%). Therefore, 600 patients were evaluated. APRI and FIB4 were missing for 12 patients out of these 600 patients (platelet count or AST or ALT level missing).

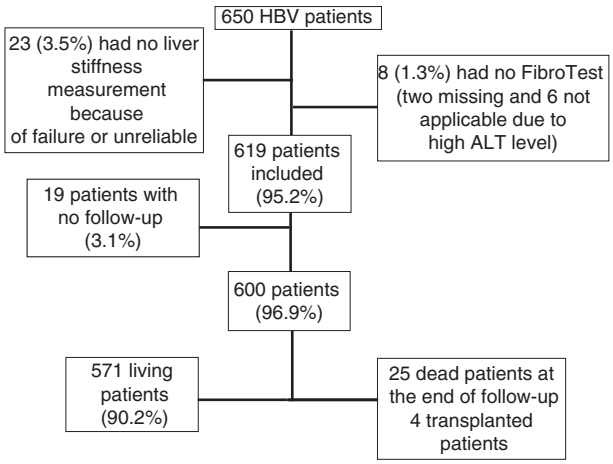


Figure 1 | Chart-flow of the study population of patients chronically infected by HBV.

Median follow-up was 49.7 months (IQR 32.2–63.2). Clinical and biological characteristics of the patients are indicated in Table 1. A majority of them were men (64%), and mean age was 42.5 ± 15.2 years. Most of patients (56.5%) had normal ALT level (<40 IU/L in women, <50 IU/L in men). Only 13% of patients had ALT level >2 × ULN. Ninety-four patients (15.7%) had liver stiffness >9 kPa (cut-off value for severe fibrosis or cirrhosis in HBV patients with normal ALT level), 46 patients (7.7%) had FibroTest >0.74, 10 patients (1.7%) had APRI >2, and 27 patients (4.6%) had FIB-4 score >3.25. Liver biopsy was available in 214 patients (35.7%) and showed severe fibrosis or cirrhosis in 86 cases (40.2%). Median liver biopsy samples length was 22 mm.[16–30]

Accuracy of biomarkers for the diagnosis of fibrosis stage

As indicated in Table 2, liver stiffness and FibroTest had comparable diagnostic accuracy for the diagnosis of fibrosis stage, but higher accuracy in comparison with other biomarkers, when biopsy was taken as the reference.

Survival

At the beginning of the study, 11 patients (1.8%) had a hepatocellular carcinoma. At the end of follow-up, six of them were still alive. The overall number of death/transplantation was 29 (4.8%) of 600, 17 liver-related and 12

Table 1 | Characteristics of the 600 patients with chronic hepatitis B infection

Characteristics (n = 600)	Results
(A) Clinical characteristics	
Males – n (%)	385 (64.2)
Age – years ± S.E.	42.5 ± 15.2
Body mass index – kg/m² ± S.E.	24.3 ± 3.9
Median tobacco use – pack-year (range)	0 (0–5)
Median alcohol use – drink/week (range)	0 (0–4)
HBV characteristics – n (%)	575 (95.8)
Positive HBe antigen – n (%)	94 (16.3)
Negative HBe antigen (pre-C mutant) – n (%)	272 (47.4)
Inactive carriers – n (%)	209 (36.3)
Fibrosis according to liver biopsy – n (%)	214 (35.7)
F0	13 (6.1)
F1	61 (28.5)
F2	54 (25.2)
F3	36 (16.8)
F4	50 (23.4)
Oesophageal varices – n (%)	
No endoscopy	489 (81.5)
Varices stage 0 or 1	103 (17.2)
Varices stage 2 or 3	8 (1.4)
Past history of ascites – n (%)	4 (0.7)
Hepatocellular carcinoma at inclusion – n (%)	11 (1.8)
Liver stiffness measurement	
Median – kPa (IQR)	5.4 (4.4–7.6)
>9 – n (%)	94 (15.7)
>20 – n (%)	25 (4.2)
>30 – n (%)	13 (2.2)
>40 – n (%)	6 (1)
>50 – n (%)	5 (0.8)
(B) Biological characteristics [median (IQR)] (missing specified)	
Platelet count (giga/L)	218 (181–257)
Prothrombin time (%)	92 (85–99)
Total bilirubin (μmol/L)	10 (8–14)
GGT (IU/L)	25 (17–42)
AST (IU/L)	29 (24–39)
ALT (IU/L)	32 (22–51)
FIB-4 (missing n = 7)	0.93 (0.62–1.52)
APRI (missing n = 8)	0.33 (0.25–0.48)
ActiTest	0.17 (0.09–0.33)
FibroTest	0.26 (0.14–0.47)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl-transpeptidase.

Table 2 | Overall accuracy (weighted AUROCs) of biomarkers for the diagnosis of fibrosis stages in patients with chronic hepatitis B infection

	wAUROC	Comparison: Z-test (P significance)		
	m (S.E.)	FibroTest	LSM	FIB-4
FibroTest	0.832 (0.014)			
LSM	0.825 (0.014)	0.68		
FIB-4	0.796 (0.012)	0.03	0.06	
APRI	0.769 (0.010)	0.0002	0.03	0.06

LSM, liver stiffness measurement.

A total of 209 of 600 patients had biopsy and all the four biomarkers performed.

The Obuchowski measure estimates the overall accuracy (weighted AUROC) that is the weighted mean of the 10 pairwise comparisons stage by stage (F0 vs. F1, F0 vs. F2, F0 vs. F3, F0 vs. F4, F1 vs. F2, F1 vs. F3, F1 vs. F4, F2 vs. F3, F2 vs. F4, and F3 vs. F4).

Table 3 | Causes of the 29 deaths or transplantations during the follow-up of 600 patients with chronic hepatitis B infection

Liver-related deaths (n = 17)	Deaths unrelated to liver disease (n = 12)
Hepatocellular carcinoma (n = 7)	Non liver cancer (n = 6)
Hepatic failure (n = 4)	Cardiovascular disease (n = 2)
Variceal bleeding (n = 1)	Infection (n = 1)
Hepatorenal syndrome (n = 1)	Unknown (n = 3)
Liver transplantation (n = 4)	

not liver-related deaths. Among these 29 patients, 25 patients were men, and 13 patients (44.8%) had LSM ≤9 kPa at inclusion. All transplanted patients received liver transplantation for HCC.

At inclusion, 209 patients (36.3%) were inactive carriers, 272 patients (47.4%) had chronic hepatitis with negative HBe antigen, and 94 patients (16.3%) had chronic hepatitis with positive HBe antigen. During follow-up, 452 patients (75.3%) did not receive any treatment for HBV infection.

Among the 94 patients with liver stiffness >9 kPa, 53 patients (56.4%) did not receive any treatment (six deaths in this group), and 41 patients received treatment (10 deaths in this group). Among the 506 patients with liver stiffness ≤9 kPa, 399 patients (78.8%) did not receive any treatment (eight deaths in this group), and 107 patients received treatment (five deaths in this group).

Details of the causes of deaths are given in Table 3. In the overall population (600 patients), the 5-year overall survival was 0.94 (95% CI 0.91–0.96), and the 5-year

survival without liver-related death or transplantation was 0.96 (95% CI 0.94–0.98).

Only three deaths were observed in the 209 inactive carriers. All these deaths were unrelated to the liver (lung and pancreas cancers).

Prediction of survival

The survival probability of patients classified according to liver stiffness, FibroTest, APRI, FIB-4 and liver biopsy is detailed in Figure 2 and their specific association with survival in Table 4.

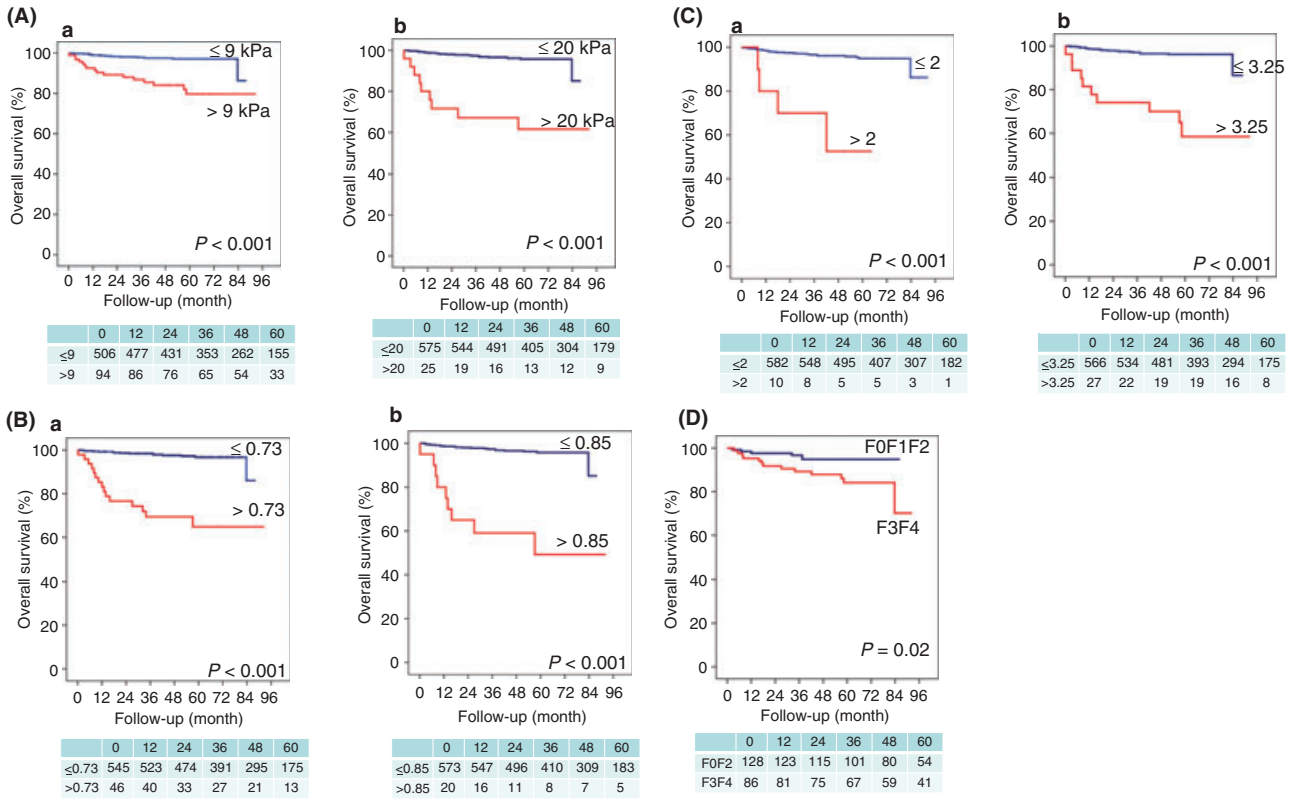
For FibroTest and liver stiffness, two cut-offs were evaluated for predicting survival: 0.73 and 0.85 for Fibrotest, 9 and 20 kPa for liver stiffness, according to previous published results.[8] As indicated in Figure 2A, B, overall survival was decreased according to liver stiffness and FibroTest values. The 5-year overall survival was 97.1% in patients with liver stiffness <9 kPa, and 61.5% in patients with liver stiffness >20 kPa, and 96.8% for FibroTest $\leq$0.73, and 49.2% in patients with FibroTest >0.85.

In univariate and multivariate analysis, FibroTest and liver stiffness had the higher prognostic values. The association persisted after adjustment on age, necro-inflammatory histological activity presumed by ActiTest, HBV-DNA and treatment.

When the prognostic performances were expressed using AUROC (Figure 3), there was high significance for Fibrotest 0.82 (95% CI 0.71–0.89), and liver stiffness 0.80 (0.70–0.87) without significant difference between these biomarkers.

DISCUSSION

To our knowledge, no studies reported on the association between liver histology, APRI, FIB-4 scores, liver stiffness and survival in HBV patients.[39] Only FibroTest has been shown to be associated with survival in chronic hepatitis B.[40, 41] Our study is the first showing that liver stiffness has a prognostic value at 5 years for overall survival in patients with chronic HBV infection. Moreover, to our knowledge, this is the first study evaluating the prognosis value of different non-invasive tests for overall survival in patients with chronic HBV infection. The present study shows that liver stiffness and FibroTest can predict similar survival in HBV patients.



	0	12	24	36	48	60
≤9	506	477	431	353	262	155
>9	94	86	76	65	54	33

	0	12	24	36	48	60
≤20	575	544	491	405	304	179
>20	25	19	16	13	12	9

	0	12	24	36	48	60
≤2	582	548	495	407	307	182
>2	10	8	5	5	3	1

	0	12	24	36	48	60
≤3.25	566	534	481	393	294	175
>3.25	27	22	19	19	16	8

	0	12	24	36	48	60
≤0.73	545	523	474	391	295	175
>0.73	46	40	33	27	21	13

	0	12	24	36	48	60
≤0.85	573	547	496	410	309	183
>0.85	20	16	11	8	7	5

	0	12	24	36	48	60
F0F2	128	123	115	101	80	54
F3F4	86	81	75	67	59	41

Figure 2 | Overall survival probability (Kaplan–Meier analysis) of 600 patients chronically infected by HBV according to: (A) liver stiffness (a: cut-off of 9 kPa; b: cut-off of 20 kPa), (B) Fibrotest (a: cut-off of 0.73; b: cut-off of 0.85), (C) blood tests (a: APRI; b: FIB-4) and (D) Liver biopsy.

Table 4 | Prognostic value of biopsy and biomarkers for predicting mortality or liver transplantation in patients with chronic hepatitis B

Estimate	Univariate analysis		Multivariate analysis			
Prognostic aim			Independent prognostic value of each estimate adjusted for age, ActiTest and treatment. Only one estimate per model		Independent prognostic value of the two most predictive estimates, Stiffness and FibroTest adjusted for treatment and baseline viral load	
	HR (95% CI)	*P*	HR (95%CI)	*P*	HR (95%CI)	*P*
Stage METAVIR biopsy F0F2 vs. F3F4 (*n* = 214)	1.6 (1.1–2.3)	0.02	1.4 (0.96–2.1)	0.07	–	–
Liver stiffness (log) *n* = 600	35 (14–87)	<0.0001	19 (7–56)	<0.0001	6.8 (1.6–28.7)	0.009†
FibroTest *n* = 600	185 (39–869)	<0.0001	29 (4–220)	<0.0001	17.5 (2–152)	0.009‡
APRI (missing = 8)	16 (5–52)	<0.0001	NA*	NA*	–	–
FIB-4 (missing = 7)	23 (9–63)	<0.0001	14 (4–47)	<0.0001	–	–
ActiTest *n* = 600	14 (4–51)	<0.0001	–	–	–	–
Age (per year) *n* = 600	1.07 (1.04–1.10)	<0.0001	–	–	–	–
Treatment *n* = 600	3.3 (1.5–7.1)	0.002	–	–	2.36 (0.85–6.56)	0.10
Baseline viral load (log) (missing = 60)	0.90 (1.0–1.0)	0.31	0.97 (0.81–1.17)	0.79	1.03 (0.97–1.23)	0.71

HR, hazard ratio; 95% CI, 95% confidence interval; NA, not applicable; *P*, significance.

* Two few patients dead with high APRI for a multivariate analysis.

† When modelling was performed in 600 patients (without entering baseline viral load) HR = 5.5 (1.5–20.2); *P* = 0.009.

‡ When modelling was performed in 600 patients (without entering baseline viral load) HR = 41 (6–270); *P* = 0.001.

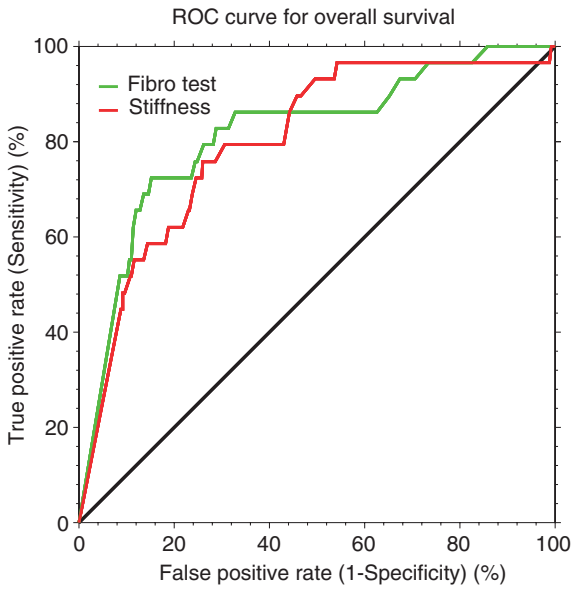


Figure 3 | Prognostic values for overall survival of FibroTest and liver stiffness in 600 patients chronically infected by HBV. There was no significant difference between the two AUROCs: Fibrotest 0.82 (95%CI 0.71–0.89), and liver stiffness 0.80 (0.70–0.87) (*P* = 0.73).

The second main finding that liver stiffness and FibroTest had better prognosis values than liver biopsy, the classical gold standard for staging, was expected as the prognosis gets worse as these quantitative fibrosis estimates increased. This cannot be the case for the METAVIR cirrhosis as there is a unique stage 'F4' for cirrhosis. A better prognostic value of FibroTest vs. biopsy had been already observed by Ngo *et al.*[8] and Vergniol *et al.*[9] for HCV patients.

The third main finding is the ranking of non-invasive methods for prognosis purpose. From the results, liver stiffness and FibroTest had better prognostic values than FIB4 and APRI score. The result confirms previous results published in HCV patients.[9] We have not evaluated other biomarkers such as Fibrometer, ELF and Hepascore. However, we cannot compare liver stiffness and FibroTest since liver biopsy was missing in a large number of patients.

Since ascites is a physical limitation to the technique because elastic waves do not propagate through liquids, patients with ascites at baseline were excluded. The prognosis of those patients is known to be bad, and our results may have been underestimated comparing to the reality. Moreover, we used only M probe of FibroScan for the assessment of liver stiffness. New methods as

ARFI or Aixplorer need to be evaluated as prognosis factors. Another limitation of this study is that our centre is a tertiary centre. Therefore, patients may not be representative of a larger HBV patient population. However, in this study, 23% of biopsied patients had cirrhosis and a majority of patients had F0F1F2 fibrosis.

HBe Ag status was available in more than 95% of subjects, but we did not collect HBV-DNA in all cases at inclusion (60 missing data). The association of non-invasive markers and HBV-DNA as prognosis model should be of interest. Moreover, the relationship between Fibrotest or liver stiffness and clinical events related to cirrhosis (hepatocellular carcinoma, ascites, etc.) needs to be further evaluated.

The ALT and AST are surrogates of hepatic necroinflammation, which tend to fluctuate significantly in chronic hepatitis B and might not reflect the fibrosis staging as accurately as in other liver diseases.[15] Therefore, in patients with ALT level >2 or 3 × ULN, our results need to be further evaluated.

In our study, we decided to use published FibroTest and liver stiffness cut-offs for severe fibrosis and cirrhosis. To test clinically relevant cut-offs, we chose the previously published values for severe fibrosis and cirrhosis,[19, 22, 42] doing the hypothesis they may be associated with a worse prognosis. To have a sensitive test, we decided to evaluate a cut-off for severe fibrosis instead of a cut-off for cirrhosis. Therefore, we hypothesise that all patients with cirrhosis were included in the analysis. We also studied the prognosis value of a liver stiffness higher than 20 kPa and FibroTest >0.85, known to be associated with liver-related complications.[5, 8, 24] Our results show that, in the group of patients with cirrhosis, an increasing liver stiffness or FibroTest value is associated with a worse prognosis, emphasising the concept of non-invasive prediction of survival in cirrhotic patients with liver stiffness or FibroTest. The evolution of liver stiffness or FibroTest values over time is of major importance. However, this point was not evaluated in our study and needs further evaluation.

In our study, we decided not to test the Child-Pugh score as a prognosis factor. Indeed, the prognosis value of Child-Pugh score is well established in cirrhotic patients. Natural history of HBV patients is related to the amount of fibrosis, we therefore chose to test only non-invasive techniques for the diagnosis of fibrosis.

Few data exist showing that liver stiffness and FibroTest may decrease under treatment,[43, 44] suggesting it may be correlated with an improved survival. In our study, we found that liver stiffness and FibroTest were accurate prognostic factors independently of treatment. Only 25% of our patients received any treatment. Therefore, we could not evaluate the effect of treatment on survival. The interaction between treatment, liver stiffness and survival needs further evaluation.

Death and liver transplantation are sudden and objective criteria that can be precisely assessed. Since a large majority of the patients were treated in our centre, and almost all the events occurred in the southwest area of France, this allows us a sharp evaluation of survival using the Kaplan–Meier method. Our weakness of the present study was that the included population was not a random, community-based population. Another limitation is the absence of repeated measurements of standardised HBV-DNA, which has been associated with survival and occurrence of hepatocellular carcinoma. We have therefore not separated our population according to the standard classification using 'active' and 'inactive carriers'. We acknowledge that new studies should confirm the previously observed greater prognostic value of fibrosis biomarkers in comparison with viral load. In our population, HBV-DNA had no independent prognostic value (HBV-DNA was available in 540 cases). This could be due to a lack of power, but another rational explanation is that the REVEAL study had not assessed the baseline fibrosis stage using validated biomarker. At least, although non-invasive tests are important as prognostic indications of fibrosis, we need to keep in mind that hepatocellular carcinoma can develop in HBV independently of the presence of cirrhosis.

In this prospective study, we confirmed the prognostic value of liver stiffness and FibroTest on survival. This information is of a major importance, helping us to sharpen our various tools for the follow-up of our patients. Liver stiffness, as a good predictive factor of survival, may help the physician to evaluate earlier the severity of those diseases, to decide with stronger arguments of a liver transplantation or a portosystemic shunt, and to evaluate more precisely the surgical risk of our cirrhotic patients. It may also be used as a complement to the 'score foie' to decide of the emergency of a liver transplantation in a cirrhotic patient. LSM and/or FibroTest could replace liver biopsy for the evaluation of the disease, whatever the stage of the disease.

In our clinical practice, liver stiffness and FibroTest are already strong evidence to give information to patients and their family on the severity of the liver disease. Using these predictive data, it should help us in the future to caution our patients and their family of the importance of total alcohol abstinence or on the need

for serious compliance with anti-viral therapy, and to plan the timing of possible liver transplantation.

AUTHORSHIP

Guarantor of the article: None.

Author contributions: Julien Vergniol: study concept and design, analysis and interpretation of results. Juliette Foucher: acquisition of data, technical support. Faiza Chermak and Charline Barthe: acquisition of data. Pierre-Henri Bernard: acquisition of data. Brigitte Le Bail: acquisition of data, analysis and interpretation. Wassil Merrouche: acquisition of data, technical support. Victor de Lédinghen: study concept and design, drafting of the manuscript, statistical analysis, study supervision. All authors approved the final version of the manuscript.

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REFERENCES

1. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449–57.
2. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. *Hepatology* 2009; **49**: 1017–44.
3. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646–9.
4. Kamath PS, Kim WR. The model for end-stage liver disease (MELD). *Hepatology* 2007; **45**: 797–805.
5. Poynard T. First-line assessment of patients with chronic liver disease with non-invasive techniques and without recourse to liver biopsy. *J Hepatol* 2011; **54**: 586–7.
6. Poynard T, Ngo Y, Munteanu M, Thabut D, Ratziu V. Noninvasive markers of hepatic fibrosis in chronic hepatitis B. *Curr Hepat Rep* 2011; **10**: 87–97.
7. Kim BK, Kim SU, Kim HS, *et al.* Prospective validation of FibroTest in comparison with liver stiffness for predicting liver fibrosis in Asian subjects with chronic hepatitis B. *PLoS ONE* 2012; **7**: e35825.
8. Ngo Y, Munteanu M, Messous D, *et al.* A prospective analysis of the prognostic value of biomarkers (FibroTest) in patients with chronic hepatitis C. *Clin Chem* 2006; **52**: 1887–96.
9. Vergniol J, Foucher J, Terrebonne E, *et al.* Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. *Gastroenterology* 2011; **140**: 1970–79, 1979. e1971–1973.
10. Morra R, Lebray P, Ingiliz P, *et al.* FibroTest has better diagnostic and prognostic values than the aspartate aminotransferase-to-platelet ratio index in patients with chronic hepatitis C. *Hepatology* 2008; **47**: 353–4; author reply 354–6.
11. Naveau S, Gaude G, Asnacios A, *et al.* Diagnostic and prognostic values of noninvasive biomarkers of fibrosis in patients with alcoholic liver disease. *Hepatology* 2009; **49**: 97–105.
12. Wai CT, Greenson JK, Fontana RJ, *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518–26.
13. Sterling RK, Lissen E, Clumeck N, *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317–25.
14. Nunes D, Fleming C, Offner G, *et al.* Noninvasive markers of liver fibrosis are highly predictive of liver-related death in a cohort of HCV-infected individuals with and without HIV infection. *Am J Gastroenterol* 2010; **105**: 1346–53.
15. de Ledinghen V, Vergniol J. Transient elastography for the diagnosis of liver fibrosis. *Expert Rev Med Devices* 2010; **7**: 811–23.
16. Wong VW, Vergniol J, Wong GL, *et al.* Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454–62.
17. Fraquelli M, Rigamonti C, Casazza G, *et al.* Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968–73.
18. de Ledinghen V, Douvin C, Kettaneh A, *et al.* Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**: 175–9.
19. Castera L, Vergniol J, Foucher J, *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343–50.
20. Friedrich-Rust M, Ong MF, Martens S, *et al.* Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960–74.
21. Marcellin P, Ziol M, Bedossa P, *et al.* Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. *Liver Int* 2009; **29**: 242–7.
22. Chan HL, Wong GL, Choi PC, *et al.* Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B. *J Viral Hepat* 2009; **16**: 36–44.
23. Vigano M, Paggi S, Lampertico P, *et al.* Dual cut-off transient elastography to assess liver fibrosis in chronic hepatitis B: a cohort study with internal validation. *Aliment Pharmacol Ther* 2011; **34**: 353–62.
24. Foucher J, Chanteloup E, Vergniol J, *et al.* Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403–8.
25. Fung J, Lai CL, Seto WK, Wong DK, Yuen MF. Prognostic significance of

liver stiffness for hepatocellular carcinoma and mortality in HBeAg-negative chronic hepatitis B. *J Viral Hepat* 2011; **18**: 738–44.
26. Jung KS, Kim SU, Ahn SH, *et al.* Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). *Hepatology* 2011; **53**: 885–94.
27. Kim SU, Lee JH, Kim do Y, *et al.* Prediction of liver-related events using fibroscan in chronic hepatitis B patients showing advanced liver fibrosis. *PLoS ONE* 2012; **7**: e36676.
28. Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069–75.
29. Forns X, Ampurdanes S, Llovet JM, *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986–92.
30. Castera L, Foucher J, Bernard PH, *et al.* Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010; **51**: 828–35.
31. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; **20**: 15–20.
32. Zhou X, Obuchowski N, McClish D. *J Statistical Methods in Diagnostic Medicine. 1st ed*. New York: John Wiley & Sons, 2002.
33. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; **44**: 837–45.
34. Lambert J, Halfon P, Penaranda G, Bedossa P, Cacoub P, Carrat F. How to measure the diagnostic accuracy of noninvasive liver fibrosis indices: the area under the ROC curve revisited. *Clin Chem* 2008; **54**: 1372–78.
35. Obuchowski NA. An ROC-type measure of diagnostic accuracy when the gold standard is continuous-scale. *Stat Med* 2006; **25**: 481–93.
36. Hillis SL, Obuchowski NA, Schartz KM, Berbaum KS. A comparison of the Dorfman-Berbaum-Metz and Obuchowski-Rockette methods for receiver operating characteristic (ROC) data. *Stat Med* 2005; **24**: 1579–1607.
37. Hintze J. *JNCSS 2003 User Guide*. Kaysville, UT: Number Cruncher Statistical Systems, 2003.
38. Kremer JM, Lee RG, Tolman KG. Liver histology in rheumatoid arthritis patients receiving long-term methotrexate therapy. A prospective study with baseline and sequential biopsy samples. *Arthritis Rheum* 1989; **32**: 121–7.
39. Taylor BC, Yuan JM, Shamliyan TA, Shaukat A, Kane RL, Wilt TJ. Clinical outcomes in adults with chronic hepatitis B in association with patient and viral characteristics: A systematic review of evidence. *Hepatology* 2009; **49**: S85–95.
40. Poynard T, Ngo Y, Perazzo H, *et al.* Prognostic value of liver fibrosis biomarkers: a meta-analysis. *Gastroenterol Hepatol (N Y)* 2011; **7**: 445–54.
41. Ngo Y, Benhamou Y, Thibault V, *et al.* An accurate definition of the status of inactive hepatitis B virus carrier by a combination of biomarkers (FibroTest-ActiTest) and viral load. *PLoS ONE* 2008; **3**: e2573.
42. Wong GL, Wong VW, Choi PC, Chan AW, Chan HL. Development of a non-invasive algorithm with transient elastography (Fibroscan) and serum test formula for advanced liver fibrosis in chronic hepatitis B. *Aliment Pharmacol Ther* 2010; **31**: 1095–103.
43. Vergniol J, Foucher J, Castera L, *et al.* Changes of non-invasive markers and FibroScan values during HCV treatment. *J Viral Hepat* 2009; **16**: 132–40.
44. Poynard T, Ngo Y, Marcellin P, Hadziyannis S, Ratziu V, Benhamou Y. Impact of adefovir dipivoxil on liver fibrosis and activity assessed with biochemical markers (FibroTest-ActiTest) in patients infected by hepatitis B virus. *J Viral Hepat* 2009; **16**: 203–13.