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Assessment of asymptomatic liver fibrosis in alcoholic patients using fibroscan: prospective comparison with seven non-invasive laboratory tests

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SUMMARY

Background
Systematic screening for liver fibrosis in heavy-drinking patients is a challenge.

Aims
To assess Fibroscan for non-invasive diagnosis of asymptomatic liver fibrosis in alcohol abuse patients, to determine diagnostic liver stiffness cut-off values and to compare performance of Fibroscan with seven non-invasive laboratory tests.

Methods
One hundred and three alcoholic patients were studied. Liver fibrosis was staged by METAVIR system. Fibroscan, Fibrotest, Fibrometer, Hepascore, APRI, PGA, PGAA and hyaluronic acid tests were performed. Liver stiffness cut-offs were determined using receiver-operating characteristic (ROC) curves.

Results
Liver stiffness was correlated with fibrosis ($r = 0.72$, $P < 0.014$), with median at 5.7, 6.3, 8.4, 15 and 47.3 kPa for F0 ($n = 8$), F1 ($n = 18$), F2 ($n = 24$), F3 ($n = 20$) and F4 ($n = 33$) stage fibrosis respectively. For Fibroscan, areas under ROC curves (AUROCs) were 0.84 (95% CI: 0.73–0.95) ($F \geq 1$), 0.91 (0.85–0.98) ($F \geq 2$), 0.90 (0.82–0.97) ($F \geq 3$) and 0.92 (0.87–0.98) ($F = 4$), yielding diagnostic stiffness cut-offs of 5.9 ($F \geq 1$), 7.8 ($F \geq 2$), 11 ($F \geq 3$) and 19.5 (F4) kPa. Sensitivity, specificity, PPV and NPV were 80%, 90.5%, 93% and 70% for $F \geq 2$, and 85.7%, 84.2%, 68.6% and 87.9% for $F = 4$. Performance of Fibroscan was higher than seven laboratory tests, for which AUROCs ranged from 0.66 to 0.77 ($F \geq 1$), from 0.54 to 0.82 ($F \geq 2$), from 0.43 to 0.88 ($F \geq 3$) and from 0.56 to 0.89 ($F = 4$), with significant difference only vs. APRI ($P < 0.001$) and Hepascore ($P = 0.04$). Combining Fibroscan with each tests did not improve performance.

Conclusions
Fibroscan is effective to assess liver fibrosis in alcoholic patients. Instant screening of liver fibrosis in heavy drinkers is feasible without liver biopsy.

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INTRODUCTION

Chronic, excessive alcohol consumption is a major public health problem. Alcoholic liver disease (ALD) is characterized by a spectrum of damage, ranging from hepatic steatosis to cirrhosis.[1] In people consuming more than 50 g of alcohol per day for more than 5 years, hospital-based studies using systematic liver needle biopsy (LNB) have estimated the prevalence of severe fibrosis and cirrhosis at between 15% and 40%.[2–6] However, it is probable that severe fibrosis and (above all asymptomatic) alcoholic cirrhosis are under-diagnosed in routine clinical practice,[7] notably because the invasiveness[8–10] and potential morbidity and mortality of LNB make it poorly accepted by patients.[11, 12] Equally, early diagnosis would be essential to motivate patients undergoing alcohol withdrawal treatment and to implement preventive measures for cirrhosis complications.[2]

Several non-invasive laboratory tests have been developed to assess liver fibrosis including the PGA and PGAA index [a combination of prothrombin time (PT), gammaglutamyl transferase (GGT), apolipoprotein A1 (Apo-A1) and alpha-2 macroglobulin (A2M) levels],[3, 13] serum hyaluronic acid (HA) levels,[12, 13] PT alone[14, 15] and the aspartate aminotransferase (ASAT) to platelet ratio index (APRI).[16] More complex assays [such as Fibrotest (FT)] are based on a statistical approach. The FT has been validated in chronic viral hepatitis C (HCV)[17] and was recently assessed in alcoholic fibrosis.[5] The Fibrometer[6] (FM) and Hepascore[18] (HS) are based on a similar methodology and the FM has been specifically used in a study on alcoholic fibrosis study. A recently developed, non-invasive technique based on ultrasound-based transient elastography [Fibroscan (FS)] allows evaluation of liver fibrosis by measuring liver stiffness.[19] This technique has been widely validated in HCV patients,[19–22] but has not yet been extensively studied in ALD.[23]

The primary objective of this study was to evaluate the performance of FS for the non-invasive diagnosis of asymptomatic liver fibrosis in chronic alcohol abusers. The secondary objectives were to determine stiffness cut-off values for the non-invasive diagnosis of each stage of fibrosis in ALD and to assess the value of FS relative to seven non-invasive laboratory assays.

PATIENTS AND METHODS

Patients

Patients with alcohol abuse consulting the Departments of Hepato-Gastroenterology, Alcoholism and Internal Medicine at the Amiens University Hospital, France, for detoxification and/or in-patient rehabilitation were prospectively included from April 2005 to January 2007. The inclusion criteria targeted patients aged 18 or over who admitted to chronic consumption of more than 50 g/day of alcohol for more than 5 years[3–6] and who agreed to undergo LNB to detect ALD. The mean alcohol intake and duration of alcohol abuse were estimated according to the patient's declarations and those of his/her close relatives, as recorded by a senior physician. The exclusion criteria were as follows: existence of non-ALD (chronic viral hepatitis C and B), refusal to undergo LNB, absence of consent, ongoing pregnancy, failure to obtain valid transient elastography measurements, severe acute alcoholic hepatitis (AH; Maddrey score ≥32) and the presence of known or decompensated alcoholic cirrhosis (ascites, oesophageal varices, PT <70% or imaging evidence of cirrhosis) as the clinical diagnosis does not require further investigations.

Study methodology

The following data were recorded: age, gender, weight, height, body mass index (BMI), alcohol intake (g/day), the duration of alcohol abuse (years) and the presence or absence of clinical signs of chronic liver disease. A blood sample was taken to measure the following laboratory parameters: PT, blood cell and platelet counts, ASAT/ALAT, alkaline phosphatase, GGT, total and free bilirubin, cholesterol, triglycerides, albumin, gammaglobulins, serum iron, transferrin saturation, ferritin, viral hepatitis B and C, HIV, anti-nuclear, anti-smooth muscle, anti-mitochondrial and anti-LKM1 antibodies, HA, A2M, Apo-A1 and haptoglobin. Ultrasonically guided LNB was performed according to good clinical practice.[8–10, 24]

The FS, LNB, blood biochemistry profile and non-invasive, diagnostic laboratory tests for liver fibrosis (FT, FM, HS, PGA and PGAA index, APRI and HA) were all performed on the same day. The FS was carried out by an experienced clinician.[25] Histological examination was performed by an independent pathologist blinded to the other results.

Liver stiffness measurements

The Fibroscan (Echosens, Paris, France) is a recently developed technique for non-invasive liver stiffness measurement.[19] A painless mechanical impulse (described as a 'flick') is delivered to an intercostal space over the liver, producing a wave of mechanical deformation. A low-frequency ultrasound transducer monitors the wave's progression through the organ and yields the propagation speed, which is related to the milieu's mechanical properties – the harder the tissue, the higher the speed. The technique allows estimation of liver stiffness in kilopascals (kPa), which in turn is correlated with liver fibrosis.[19] The FS probe is applied perpendicularly to the skin. The pressure exerted by the operator is standardized by a visual indicator. Ten repeated measurements are performed. The final result is the median of all valid measurements performed. The test results were only considered in the final analysis when the elastographic measurement success rate was <60%, with at least 10 valid measurements.

Non-invasive laboratory tests for liver fibrosis

The Fibrotest (Biopredictive, Paris, France) score was determined using the following equation:[22] 4.467 log[A2M (g/L)] − 1.357 log[haptoglobin (g/L)] + 1.017 log[GGT (IU/L)] + 0.0281 × [age (years)] + 1.737 × log[bilirubin (μmol/L)] − 1.184 × [Apo-A1 (g/L)] + 0.301 × gender (female = 0, male = 1) − 5.540. The Fibrometer (BioLiveScale, Angers, France) score was calculated as follows:[6] −0.169 PT (%) + 0.015 A2M (mg/dL) + 0.032 HA (μg/L) − 0.140 age (years) + 16.541. The PGA and PGAA indices were calculated by adding the four laboratory parameters (PT, GGT, Apo-A1 and A2M) scored on a 0–4 scale according to published methods.[3, 13] The APRI was calculated as described elsewhere.[16] Blood hyualuronic acid levels were measured with an immunoenzymatic assay. Lastly, the HS logistic regression model was calculated using the following equation:[18] $y = \exp[-4.185818 - (0.0249 \times \text{age}) + (0.7464 \times \text{gender}) + (1.0039 \times \text{A2M}) + (0.0302 \times \text{HA}) + (0.0691 \times \text{bilirubin}) - (0.012 \times \text{GGT})]$. The HS is defined as $y/1 + y$.

Histological analysis

Liver needle biopsies were performed percutaneously with an 18-gauge needle (Bard Monopty, Covington, GA, USA). Samples were fixed in 10% formalin for 24 h, embedded in paraffin, sliced and stained with haematoxylin–phloxin–saffron, Masson's trichromic and Perls reagent.

Fibrosis and inflammatory activity were evaluated according to the METAVIR system,[26] as validated in ALD:[27] for fibrosis, F0 = absence of fibrosis, F1 = minimal portal fibrosis without septa, F2 = portal fibrosis with a few septa, F3 = septal fibrosis, F4 = cirrhosis; for inflammatory activity, A0 = absence of activity, A1 = slight activity, A2 = moderate activity, A3 = severe activity. Perisinusoidal fibrosis (PSF) was scored from 0 to 3 according to the Brunt classification:[28] 0 = absence of PSF; 1 = minimal PSF; 2 = moderate PSF; 3 = severe PSF. Hepatic steatosis was evaluated on a 0–3 scale according to the percentage of hepatocytes containing fatty vacuoles: 0 = absence of steatosis, 1 = steatosis ≤30%, 2 = 31–59%, 3 = ≥60%.[29] AH damage was also noted (liver cell necrosis, polymorphonuclear leukocyte infiltration, Mallory bodies), together with the presence of haemosiderin deposits revealed by the Perls stain.[30]

Statistical analysis

Quantitative demographic data were expressed as means ± standard deviation and range and the extent of histological damage was expressed as a percentage. Median stiffness was calculated for each stage of histological damage and box plots were used to show the distribution of stiffness values (in log units) according to METAVIR score, PSF and steatosis. Spearman's correlation coefficient was used. Univariate analysis and multiple regression analysis were used to study the relationship between liver stiffness and fibrosis, inflammatory activity, steatosis, PSF and laboratory parameters (PT, blood and platelet counts, ASAT /ALAT, alkaline phosphatase, GGT, total and free bilirubin, cholesterol, triglycerides, albumin, gammaglobulins, serum iron, transferrin saturation, ferritin, HA, A2M, Apo-A1 and haptoglobin). Only parameters significantly associated with liver stiffness in univariate analysis were included in a multiple regression analysis model.

The diagnostic performance of FS was evaluated by using receiver-operating characteristic (ROC) curves and by calculating the area under the ROC curve (AUROC). ROC curves were drawn for the detection of individuals with a METAVIR fibrosis score of 1 or more ($F \geq 1$), 2 or more ($F \geq 2$), 3 or more ($F \geq 3$) or cirrhosis ($F = 4$). The optimal liver stiffness cut-off

values for detection of the various fibrosis stages were determined from ROC curves according to the best compromise between sensitivity and specificity, thus enabling calculation of the corresponding positive predictive values (PPVs) and negative predictive values (NPVs). The diagnostic performance of the various non-invasive laboratory tests was studied by determining the AUROCs for each METAVIR fibrosis stage. Lastly, the association between FS and each non-invasive laboratory test for the diagnosis of fibrosis was analysed by ordinal regression. ROC curves were then plotted and AUROC were calculated for each stage of liver fibrosis and for each association. AUROCs for each non-invasive technique and for FS were compared.[31]

The *a priori* limit of significance for the alpha risk was set at $P < 0.05$. All statistical analyses were performed using SPSS software, version 11 (SPSS Inc., Chicago, IL, USA). The study was approved by the Regional Investigational Review Board and was performed in accordance with the Declaration of Helsinki (as revised in 2000). All patients gave their written, informed consent.

RESULTS

Description of the study population

One hundred and sixty patients met the inclusion criteria, but 57 were not included, mostly because of refusal to participate, and two failures to record valid elastography measurements (1.9%). The final analysis was therefore performed on a total of 103 patients. The demographic, laboratory and morphological characteristics of the patients included are summarized in Table 1. The mean BMI was 27.7 ± 5.9 kg/m^2 (range: 16–38 kg/m^2). The mean daily alcohol intake was 128.4 ± 78.3 g/day (50–400), with a mean duration of

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

Characteristics	Results (mean ± s.d.) Included patients (n = 103)	Non-included patients (n = 57)	P
Males n (%)	76 (74%)	43 (75.4)	0.85
Age (years)	52.6 ± 9.6	50.5 ± 9.9	0.196
BMI	27.7 ± 5.9	31	0.58
Alcohol intake (g/day)	128.4 ± 78.3	137.5 ± 79.1	0.47
Duration of alcohol abuse (years)	18.7 ± 8.8	14.9 ± 6.8	0.07
White blood cells (4–10.10^3/mm^3)	7092 ± 2482	6952.8 ± 2394	0.74
Mean corpuscular volume (μ^3)	97.9 ± 7.7	95.4 ± 13.6	0.15
Platelets (150–400.10^3/mm^3)	250.5 ± 126	230 ± 123	0.35
PT (70–100%)	87 ± 11.8	87.8 ± 10.2	0.65
ASAT (<35 IU/L)	79.8 ± 78.7	72.6 ± 67.6	0.57
ALAT (<50 IU/L)	61.7 ± 59.3	55.6 ± 48.8	0.52
Gamma-GT (<51 IU/L)	484.9 ± 811.9	374.3 ± 545	0.38
Alkaline phosphatase (<117 IU/L)	114 ± 122.6	104.5 ± 104.5	0.64
Total bilirubin (<21 μmol/L)	17.6 ± 42	13.3 ± 7.6	0.65
Gamma globulins (10–20 g/L)	11.3 ± 3.5	9.9 ± 3.7	0.052
Serum albumin (37–53 g/L)	38.5 ± 7.2	40.4 ± 7.3	0.18
Total cholesterol (<5.2 mmol/L)	5.1 ± 2.4	5.15 ± 1.8	0.99
Triglycerides (<1.8 mmol/L)	1.7 ± 1.3	1.4 ± 0.9	0.23
Hyaluronic acid (<75 μg/L)	80.4 ± 106	66.4 ± 111	0.66
Apolipoprotein A1 (1.1–2.0 g/L)	1.56 ± 0.45	1.6 ± 0.48	0.82
Haptoglobin (0.34–2.0 g/L)	1.53 ± 0.81	2.18 ± 1.21	0.051
α2 Macroglobulin (1.1–2.5 g/L)	2 ± 0.86	1.8 ± 0.66	0.32
Serum iron (9–30.4 μmol/L)	19.7 ± 11.1	19.5 ± 9.8	0.91
Transferrin saturation (20–40%)	37.8 ± 21.75	40.8 ± 18	0.46
Serum ferritin (20–300 μg/L)	641.9 ± 580.5	523.4 ± 338.1	0.22

PT, prothrombin time; ASAT, aspartate aminotransferase.

alcohol abuse of 18.7 ± 8.8 years (6–46). The mean PT, platelet count, albumin and total bilirubin values were within the respective normal reference ranges. Lastly, no significant differences in terms of clinical or laboratory parameters were observed between the populations of included and non-included patients.

Liver histology results

Liver biopsies measured 12.2 ± 3 mm, with an average of 7.8 ± 2.7 portal tracts. According to the METAVIR fibrosis classification, 33 (32%) patients had cirrhosis and 77 (74.7%) had fibrosis stage ≥2. PSF was severe for 32 (31.1%) patients (Table 2). The necrotic/inflammatory activity was generally low and was scored as A1 for 66 (64.1%) patients. Histological foci of asymptomatic AH were seen in 21 patients (20.4%). ASAT and bilirubin were higher for AH patients with 134.2 ± 113.2 vs. 22.3 ± 19.2 IU/L ($P = 0.0002$), and 14 ± 90 vs. 11 ± 5.9 μmol/L respectively. PT did not differ according to the presence or absence of AH, with 86 ± 13.5 vs. 89 ± 1.4 ($P = 0.42$). Lastly, considering results which were histologically close to the norm, eight (7.8%) and 24 (23.3%) patients were free of portal fibrosis and PSF respectively.

Table 2. Histological results and corresponding liver stiffness values

Histological lesions	Liver stiffness (kPa)			
	n (%)	Median	Mean ± s.d.	Range
Fibrosis (METAVIR)				
F0	8 (7.8)	4.9	5.7 ± 1.6	4.6–9.4
F1	18 (17.5)	5.9	5.7 ± 1.2	3.9–7.9
F2	24 (23.3)	8.9	12.6 ± 10.3	2.8–46.4
F3	20 (19.4)	14.7	19.2 ± 12.4	6.1–49.6
F4	33 (32)	45	40.8 ± 20.3	13.7–72
Activity (METAVIR)				
A0	24 (23.3)	5.9	8.6 ± 5.9	2.8–24.5
A1	66 (64.1)	11.8	18.8 ± 16.9	31–72
A2	11 (10.7)	35.8	39.7 ± 24.9	17.1–66.4
A3	2 (1.9)	58	58 ± 11.9	49.6–66.4
Perisinusoidal fibrosis				
0	24 (23.3)	5.3	7.7 ± 8.1	4.6–35.8
1	37 (35.9)	7.8	12.6 ± 11.5	2.8–46.4
2	10 (9.7)	12.6	18.6 ± 17.9	6.9–62.1
3	32 (31.1)	22.3	30.2 ± 20.7	3.1–72
Steatosis				
0	18 (17.5)	10.6	15.4 ± 12.4	4.4–46.4
1	61 (59.3)	8.1	16.7 ± 17.7	2.8–66.4
2	23 (22.3)	18.5	22.5 ± 19.7	3.9–72
3	1 (0.9)	45	–	–

Results of FS liver stiffness measurements and comparison with liver histology

The recorded liver stiffness values were between 2.8 and 75 kPa, with a mean of 18.6 ± 18.9 kPa. The mean measurement success rate was 81.9 ± 23.8%, with an average of 11.8 ± 2 valid measurements. The median stiffness values were 4.9, 5.9, 8.9, 14.7 and 45 kPa for F0, F1, F2, F3 and F4 METAVIR fibrosis scores respectively (Table 2). In univariate analysis, 12 parameters were significantly correlated with liver stiffness (Table 3). No correlation was observed with hepatic steatosis ($r = 0.064$, $P = 0.52$) or the following laboratory parameters: ASAT, ALAT, white blood cell and platelet counts, mean corpuscular volume, cholesterol, TG, serum iron and transferrin saturation and serum ferritin. In a multiple regression analysis (liver stiffness vs. METAVIR fibrosis and activity, sinusoidal fibrosis, GGT, bilirubin, Apo-A1, haptoglobin, 2 macroglobulin, PT, alkaline phosphatase, albumin and gamma globulin), the METAVIR fibrosis score was the only histological parameter significantly associated with liver stiffness ($P = 0.014$). PT ($P < 0.001$) and alkaline phosphatase ($P = 0.006$) were also associated with liver stiffness. Box plots were drawn to show the

Table 3. Correlations with liver stiffness in univariate analysis

Parameters	*r*	*P*
Fibrosis (METAVIR)	0.79	<0.001
Activity (METAVIR)	0.48	<0.001
Sinusoidal fibrosis (BRUNT)	0.52	<0.001
GGT (IU/L)	0.33	0.003
Bilirubin (μmol/L)	0.36	0.001
Apolipoprotein A1 (g/L)	−0.38	<0.001
Haptoglobin (g/L)	−0.26	0.015
Alpha-2 macroglobulin (g/L)	0.42	<0.001
PT (%)	−0.58	<0.001
Alkaline phosphatase (IU/L)	0.39	<0.001
Albumin (g/L)	−0.24	0.012
Gamma globulins (g/L)	0.32	0.02

GGT, gammaglutamyl transferase; PT, prothrombin time.

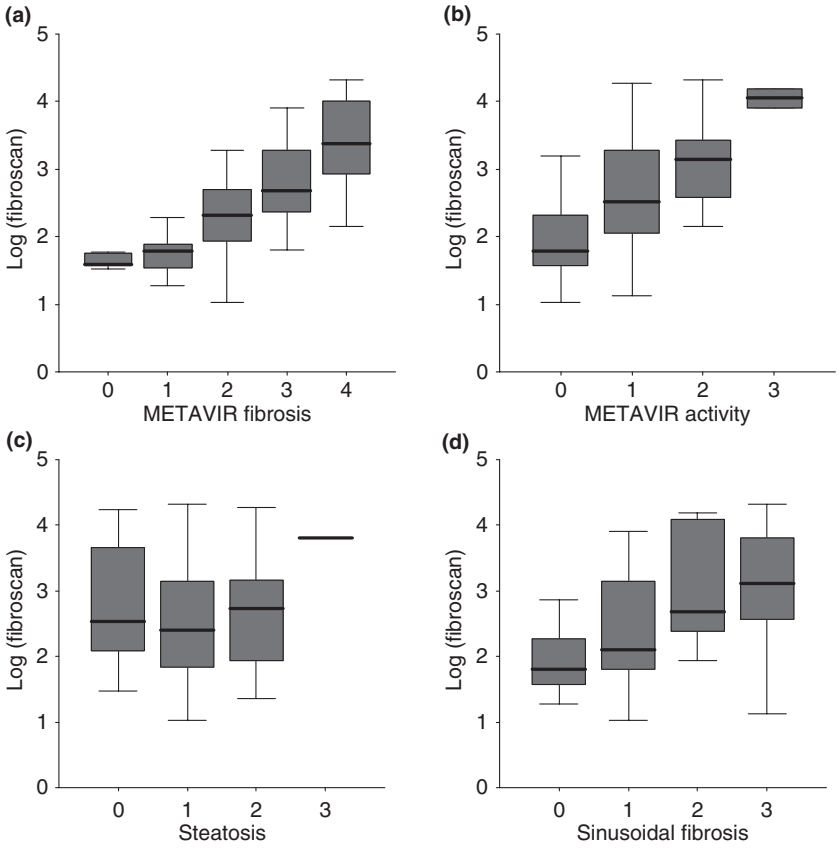

Figure 1. Box plots of liver stiffness (log units) as a function of METAVIR fibrosis, inflammatory activity, steatosis and perisinusoidal fibrosis. The median is represented as a horizontal line within the box and its lower and upper edges represent the first and third quartiles respectively. The brackets below and above the box enclose 95% of the entire set of stiffness values. (a) Liver stiffness and METAVIR fibrosis. (b) Liver stiffness and METAVIR activity score. (c) Liver stiffness and steatosis. (d) Liver stiffness and perisinusoidal fibrosis.

distribution of the observed liver stiffness values as a function of the METAVIR fibrosis stage (Figure 1a), inflammatory activity (Figure 1b), steatosis (Figure 1c) and PSF (Figure 1d).

In 21 patients with histological signs of asymptomatic AH, the mean liver stiffness score was 12.5 ± 19.2 vs. 11.35 ± 18.7 kPa for patients without AH (n = 82), P = 0.52. Frequencies of liver fibrosis (F0–F4), and liver stiffness for each stage of fibrosis were not different between AH and non-AH groups (data not shown).

Determination of liver stiffness cut-off values for the diagnosis of liver fibrosis according to the METAVIR classification

Receiver-operating characteristic curves for stiffness values were plotted to compare F0 vs. F1234 ($F \geq 1$, Figure S1, journal website), F01 vs. F234 ($F \geq 2$, Figure S1, journal website), F012 vs. F34 ($F \geq 3$, Figure S1, journal website) and F0123 vs. F4 ($F = 4$, Figure S1, journal website). The AUROCs were 0.84 (95% CI: 0.73–0.95) for $F \geq 1$, 0.91 (0.85–0.98) for $F \geq 2$, 0.90 (0.82–0.97) for $F \geq 3$ and 0.92 (0.87–0.98) for $F = 4$. According to the ROC curves, the liver stiffness cut-offs selected for $F \geq 1$, $F \geq 2$, $F \geq 3$ and $F = 4$ were 5.9, 7.8, 11 and 19.5 kPa respectively; the corresponding sensitivities, specificities, PPVs and NPVs are summarized in Table 4.

Analysis of liver stiffness cut-off values for the diagnosis of liver fibrosis in patients with biopsy specimens longer than 15 mm

Twenty one patients had a biopsy specimen longer than 15 mm (Table 5). The AUROCs were 0.80 (95% CI: 0.62–0.97) for $F \geq 1$, 0.90 (0.76–1.04) for $F \geq 2$, 0.97 (0.90–1.04) for $F \geq 3$ and 0.95 (0.87–1.03) for $F = 4$. According to the ROC curves, the liver stiffness cut-offs selected for $F \geq 1$, $F \geq 2$, $F \geq 3$ and $F = 4$ were 3.7, 8.2, 12.7 and 18.7 kPa respectively. The corresponding sensitivities, specificities, PPVs and NPVs were 90%, 100%, 100% and 35% for $F \geq 1$; 87.5%, 99.9%, 100% and 69% for $F \geq 2$; 92.3%, 87.5%, 92% and 66% for $F \geq 3$; and 82%, 90%, 90% and 69% for $F = 4$.

Table 4. Determination of liver stiffness cut-offs for METAVIR fibrosis diagnosis

METAVIR fibrosis	Stiffness cut-off (kPa)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUROC	95% CI
$F \geq 1$	5.9	83	86	97.6	35.3	0.84	0.73–0.95
$F \geq 2$	7.8	80	90.5	93	70	0.91	0.85–0.98
$F \geq 3$	11	86.7	80.5	81.8	84.3	0.90	0.82–0.97
$F = 4$	19.5	85.7	84.2	68.6	87.9	0.92	0.87–0.98

PPV, positive predictive values; NPV, negative predictive values; AUROC, area under ROC curve.

Table 5. Liver stiffness cut-offs for METAVIR fibrosis diagnosis in patients with biopsy specimens ≥15 mm ($n = 21$)

METAVIR fibrosis	Stiffness cut-off (kPa)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUROC	95% CI
$F \geq 1$	3.7	90	100	100	35	0.80	0.62–0.97
$F \geq 2$	8.2	87.5	99.9	100	69	0.90	0.76–1.04
$F \geq 3$	12.7	92.3	87.5	92	66	0.97	0.90–1.04
$F = 4$	18.7	82	90	90	69	0.95	0.87–1.03

PPV, positive predictive values; NPV, negative predictive values; AUROC, area under ROC curve.

Comparison between FS and seven non-invasive laboratory tests

For each of the seven non-invasive laboratory tests, ROC curves were calculated for METAVIR fibrosis stages $F \geq 1$, $F \geq 2$, $F \geq 3$ and $F = 4$ respectively (Figure S1, journal website). Ultrasound-based transient elastography yielded higher AUROC values than any of the non-invasive laboratory tests, regardless of the fibrosis stage considered. The APRI score yielded the lowest AUROC. According to the Hanley and McNeil method, the differences were significant only for FS vs. APRI score (for $F \geq 2$, $F \geq 3$, $F = 4$), FS vs. HS (for $F \geq 2$ and $F = 4$), FS vs. PGA score (for $F \geq 1$ and $F \geq 2$) and FS vs. FT (for $F \geq 2$) (Table 6).

Table 6. Areas under ROC curves and 95% CIs for Fibroscan and comparison with seven non-invasive laboratory tests

	Liver fibrosis (METAVIR)			
	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F = 4$
Fibroscan	0.84 (0.73–0.95)	0.91 (0.85–0.98)	0.90 (0.82–0.97)	0.94 (0.87–0.98)
Fibrometer	0.72 (0.57–0.87)	0.82 (0.72–0.93)	0.88 (0.80–0.95)	0.85 (0.74–0.96)
Fibrotest	0.77 (0.63–0.90)	0.79 (0.69–0.90)*	0.80 (0.70–0.91)	0.84 (0.72–0.97)
Hepascore	0.70 (0.51–0.89)	0.76 (0.64–0.88)†	0.83 (0.74–0.93)	0.76 (0.63–0.90)†
Hyaluronic acid	0.76 (0.58–0.94)	0.80 (0.70–0.92)	0.83 (0.74–0.92)	0.80 (0.68–0.92)
PGA	0.66 (0.50–0.82)*	0.78 (0.68–0.89)‡	0.84 (0.74–0.94)	0.89 (0.82–0.97)
PGAA	0.74 (0.60–0.88)	0.81 (0.71–0.91)	0.86 (0.76–0.96)	0.83 (0.73–0.93)
APRI	0.76 (0.58–0.95)	0.54 (0.4–0.68)§	0.43 (0.30–0.56)§	0.56 (0.38–0.73)§

Comparison vs. Fibroscan: * $P = 0.04$; † $P = 0.02$; ‡ $P = 0.03$; § $P < 0.001$.

Table 7. Areas under ROC curves and 95% CIs for Fibroscan alone and its combinations with non-invasive laboratory tests

	Liver fibrosis (METAVIR)			
	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F = 4$
Fibroscan (FS)	0.84 (0.73–0.95)	0.91 (0.85–0.98)	0.90 (0.82–0.97)	0.94 (0.87–0.98)
FS and Fibrometer	0.82 (0.72–0.93)	0.91 (0.85–0.98)	0.92 (0.86–0.98)	0.92 (0.86–0.98)
FS and Fibrotest	0.86 (0.77–0.95)	0.91 (0.84–0.97)	0.91 (0.85–0.97)	0.94 (0.87–0.98)
FS and Hepascore	0.84 (0.73–0.95)	0.91 (0.84–0.98)	0.92 (0.85–0.98)	0.92 (0.85–0.98)
FS and hyaluronic acid	0.85 (0.75–0.95)	0.92 (0.86–0.98)	0.91 (0.85–0.97)	0.90 (0.83–0.97)
FS and PGA	0.84 (0.74–0.94)	0.91 (0.84–0.97)	0.90 (0.84–0.97)	0.93 (0.88–0.98)
FS and PGAA	0.83 (0.74–0.93)	0.90 (0.84–0.97)	0.91 (0.85–0.98)	0.93 (0.87–0.98)

Comparison of FS with each non-invasive laboratory test

The correlations between FS and each non-invasive laboratory test are summarized in Table 6. Each pair of techniques was evaluated according to its AUROCs for the $F \geq 1$, $F \geq 2$, $F \geq 3$ and $F = 4$ fibrosis, but none yielded better diagnostic performance than FS alone (Table 7).

DISCUSSION

Chronic, excessive alcohol consumption is a public health problem in France. In this study, the alcohol-related inclusion criterion was an alcohol intake exceeding 50 g/day for more than 5 years – a cut-off frequently used in other studies.[3–5] In contrast, patients with obvious cirrhosis were excluded, as the aim of this study was to validate transient elastography for the diagnosis of liver fibrosis in asymptomatic, heavy-drinking patients. Overall, the mean age, gender ratio, alcohol intake and duration of alcohol abuse of the study population were comparable to the values reported in previous series[3–5] and therefore confirmed the reported prevalence of alcohol-related liver damage −19.4% of our patients had grade F3 fibrosis and 32% had cirrhosis.

The use of the FS for the non-invasive diagnosis of liver fibrosis has been widely validated in patients with chronic viral hepatitis C,[19–22] with a significant correlation between liver stiffness and grades of portal and periportal fibrosis[19–22] and fibrosis area.[32, 33] The FS is currently being validated for chronic hepatitis B,[34] HIV–HCV co-infection,[35] cholestatic intrahepatic diseases,[36, 37] non-alcoholic steatohepatitis[38] (NASH) and the diagnosis of cirrhosis complications.[39–41] This study is one of the first to evaluate the diagnostic performance of FS in a population of heavy drinkers at risk of ALD. A significant, multivariate correlation was demonstrated between fibrosis and liver stiffness, while alcoholic steatosis had no influence on elastography measurements, as also demonstrated in viral hepatitis C.[20, 22]

Stiffness cut-off values of 7.8 and 11 kPa were determined for the diagnosis of fibrosis $F \geq 2$ and $F \geq 3$ respectively, with corresponding NPVs of 70% and 84.3%. The NPV in the present series was better than those observed for HCV (ranging from 48% to 56%).[20, 22] The cut-off of 19.5 kPa for the alcoholic cirrhosis stage was close to the value of 17.6 kPa reported by Foucher *et al.*[39] in a study comprising 12.5% of patients with ALD. Moreover, Ganne-Carrie *et al.*[40] reported a cut-off of 21.5 kPa for the diagnosis of cirrhosis in 122 patients with ALD or NASH. The cut-offs reported in series exclusively comprising HCV patients are lower, between 12.5 and 14.6 kPa.[20, 22] Applying a cut-off of 12.8 kPa to our population of alcoholic patients yielded a sensitivity of 100%, but a lower specificity (75.4%) with a PPV and an NPV of 63.8% and 96.3% respectively. The median stiffness in our cirrhotic patients was high, with a value of 47.3 vs. 20–30 kPa in viral hepatitis C liver diseases.[20, 22] This confirms the findings of a study in which alcoholic cirrhotic patients had a median value of 52.4 vs. 23 kPa for patients with viral cirrhosis.[39] This dissimilarity may be explained by the different spatial distribution of alcoholic fibrosis, which develops in centrolobular and perisinusoidal regions as well as in periportal zones.[27] In studies on other liver diseases, the cut-offs for the diagnosis of cirrhosis were also higher than for viral cirrhosis, with 17.3 kPa for biliary tract diseases[36] and 17.5 kPa for NASH.[42] The

cause of cirrhosis is therefore primordial in the choice of a stiffness cut-off for the diagnosis of this condition using the FS, as the distribution of hepatic fibrosis differs for viral liver disease, ALD, NASH and biliary tract diseases.[43]

Only two other studies have evaluated the performance of the FS exclusively in patients with ALD. Foucher *et al*. included 60 alcoholic patients investigated by LNB and transient elastography[44], but only the AUROC for the diagnosis of grade F4 fibrosis was reported (0.96). Another series comprising 105 heavy-drinking patients with LNB (fibrosis classification according to Brunt *et al*.) and transient elastography[45] reported a significant correlation between fibrosis and liver stiffness ($r = 0.77$, $P < 0.001$) and no correlation with steatosis, as in this study. The AUROCs for the diagnosis of grades $F \geq 2$ and $F = 4$ were 0.96 (95% CI: 0.90–0.99) and 0.90 (0.81–0.95) respectively. These two series did not exclude patients with known or obvious cirrhosis, thus explaining their high reported proportion of cirrhotic individuals (55.6% in one study).[44] Lastly, a third study examined 245 patients consulting alcohol abuse specialists, with measurement of liver stiffness. However, LNB was only performed for the 41 (18%) patients with a liver stiffness value greater than or equal to 13 kPa (the cut-off described for HCV); this led to the diagnosis of cirrhosis in 34 subjects, but no other conclusions could be drawn concerning diagnostic performance.[23] The AUROCs determined in this study for noncirrhotic fibrosis stages F1, F2 and F3 (0.84, 0.91 and 0.89 respectively) are close to those published for viral liver diseases[20, 22], but comparative data in the ALD field are lacking.

In this study, the diagnostic performance of FS was slightly better than the performance of the seven non-invasive laboratory tests and this difference was especially significant vs. APRI and HS. FS AUROCs were higher for all the METAVIR fibrosis stages studied, as found in another study in which the AUROC for F4 was 0.96 for FS, 0.84 for FT and 0.85 for HA.[44] The diagnostic performance of APRI was unsatisfactory in this study confirming previous reports[44, 46, 47] and this test should not be used for the diagnosis of alcoholic liver fibrosis. The various combinations of the FS with non-invasive laboratory tests did not improve diagnostic performance relative to FS alone, with no improvement in the AUROCs for fibrosis stages $F \geq 1$, $F \geq 2$ and $F = 4$. Overall, our results show that ultrasound-based transient elastography can be used on its own for the non-invasive diagnosis of alcoholic fibrosis, which has the advantage of not increasing costs.

A potential limitation of this study concerns the size of our liver biopsies, slightly smaller than the generally recommended optimal length of 15 mm,[48] which may have led to underestimation of fibrosis in the context of chronic viral hepatitis.[49, 50] In another study, only 16% of biopsies were greater than 20 mm in length.[12] In two FS studies, sensitivity analysis with longer biopsies always demonstrated better AUROCs.[21, 22] In this study, 20.4% of the samples were ≥15 mm and despite the size of the biopsies, high AUROC values were observed for FS. In our analysis of sensitivity in patients with biopsies ≥15 mm, AUROC was higher only for $F \geq 3$, and finally, the determination of stiffness cut-offs was similar to that demonstrated in the overall population. Another histological finding must be discussed: asymptomatic AH was detected in 21% of patients. Although AST levels were slightly higher than in patients without AH, no difference in liver stiffness was observed between the two groups or for each level of fibrosis. Asymptomatic AH may therefore have no impact on liver stiffness. Asymptomatic AH is common in heavy drinkers[51] and differs from severe acute AH with more a greater transaminase and bilirubin flare. These patients were excluded from the study. Lastly, the METAVIR system was used because it has been validated in large cohorts of patients with ALD[27, 51] and is much better known than other histological systems that are more complex to use in routine clinical practice.[52] In multiple regression analysis, the area of fibrosis was explained only by the METAVIR fibrosis score.[27]

The clinical management of alcoholic patients requires accurate assessment of liver damage including LNB.[48] In the field of viral hepatitis C, antiviral treatment is often initiated at $F \geq 2$.[53] In heavy drinkers, the presence of severe F3-stage fibrosis or cirrhosis prompts screening for portal hypertension and hepatocarcinoma.[8] The demonstration of less severe fibrosis could represent an additional motivational factor for patients undergoing alcohol withdrawal treatment. In this context, the availability of a non-invasive assay can improve care provision, relative to liver biopsy. The FS test has the advantage of being pain-free, easy-to-learn and fast[54] yielding an immediate result during the consultation. It can also be repeated to monitor changes in fibrosis during alcohol withdrawal treatment, but this aspect is yet to be studied. Furthermore, transient elastography examines 1/500 of the

liver volume vs. 1/50 000 for liver biopsy[19] reducing sampling errors.[55, 56] In contrast, obesity is a true limitation.[57] Non-invasive laboratory tests can also be repeated over time for monitoring purposes, but require the additional intervention of a nurse and a second medical visit to provide the results.

Overall, FS exhibited good levels of diagnostic performance for the screening of alcoholic liver fibrosis in heavy-drinking patients in our study. These results open up new perspectives in the field of ALD for systematic screening for liver fibrosis, longitudinal monitoring and, lastly, as a motivational aid in alcohol withdrawal treatment.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Figure S1. Receiver-operating characteristic curves for the Fibroscan and seven non-invasive biological assays.

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