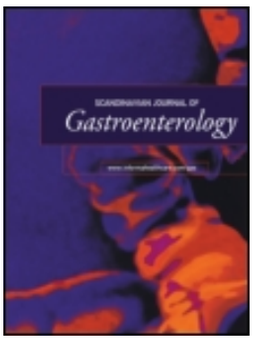

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ORIGINAL ARTICLE

Comparison of non-invasive assessment to diagnose liver fibrosis in chronic hepatitis B and C patients

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Abstract

Objective. Chronic viral hepatitis B and C cause liver fibrosis, leading to cirrhosis. Fibrosis assessment is essential to establish prognosis and treatment indication. We compared seven non-invasive tests, separately and in combination, in chronic hepatitis patients to detect early stages of fibrosis according to the Metavir score in liver biopsy. ***Material and methods.*** Galactose and methacetin breath tests (GBT and MBT), biomarkers (hyaluronic acid (HA), aspartate aminotransferase platelet ratio index (APRI), FibroTest, and Fib-4) and transient elastography (TE) were evaluated in 89 patients. Additionally, 31 healthy controls were included for evaluation of breath tests and biomarkers. ***Results.*** Serum markers (HA, APRI, FibroTest, and Fib-4) and elastography significantly distinguished non-cirrhotic (F0123) from cirrhotic (F4) patients ($p < 0.001$, $p = 0.015$, $p < 0.001$, $p = 0.005$, $p = 0.006$, respectively). GBT, HA, APRI, FibroTest, Fib-4, and TE detected F01 from F234 ($p = 0.04$, $p = 0.011$, $p = 0.009$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively). A combination of different tests (TE, HA, and FibroTest) improved the performance statistically, area under the curve (AUC) = 0.87 for F234, 0.92 for F34, and 0.90 for F4. ***Conclusion.*** HA, APRI, FibroTest, Fib-4, and TE reliably distinguish non-cirrhotic and cirrhotic patients. Except for MBT, all tests discriminate between mild and moderate fibrosis. As single tests: FibroTest, Fib-4, and TE were the most accurate for detecting early fibrosis; combining different non-invasive tests increased the accuracy for detection of liver fibrosis to such an extent and thus might be acceptable to replace liver biopsy.

Key Words: *Breath tests, fibrosis, non-invasive diagnostics, serum markers, transient elastography*

Introduction

Chronic viral hepatitis B (CHB) and C (CHC) are global public health problems leading to liver fibrosis and ultimately to cirrhosis, decompensated liver disease, and hepatocellular carcinoma. At present, the golden standard to assess amount of liver fibrosis, caused by any form of liver disease, is a liver biopsy using the Ishak [1] or Metavir [2,3] fibrosis scoring systems. However, biopsy is prone to sampling error and substantial intra- and inter-observer variability leading to over or under staging of fibrosis [4] and this procedure also has significant morbidity, including infections, major bleeding, ascites leakage and post-procedure pain, and can lead to mortality [5]. This hampers the primary determination of fibrosis and optimal management of therapy. Consequently, there is a need for non-invasive methods to accurately diagnose the presence of liver fibrosis and cirrhosis (Metavir stage ≥F2), especially as decision to start therapy.

Most previous studies on non-invasive diagnostics, including breath tests, serum biomarkers, and transient elastography (TE), reliably determined the

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presence or absence of cirrhosis, but did not discriminate between the earlier stages of fibrosis very well [6–8].

Breath tests measure various metabolic functions and rely on processing of an administered ^{13}C-labeled substrate (stable isotope), which can be detected in expired air. Expired $^{13}CO_2$ reflects the residual functional liver mass [9].

^{13}C-galactose breath test (GBT) measures galactose oxidation capacity of the liver in a cytosolic pathway in which galactose kinase is the rate limiting enzyme in the metabolism leading to $^{13}CO_2$ [10].

Methacetin enables quantitative evaluation of the cytochrome P450 IA2 dependent liver function in polymorphic mitochondria. After ingestion of ^{13}C-methacetin, the liver metabolises ^{13}C-methacetin into acetaminophen and $^{13}CO_2$ [11].

With respect to the serological tests, HA is an unbranched high-molecular-weight polysaccharide and a component of the extracellular matrix. The HA levels in serum are increased in advanced liver diseases, which is caused by increasing amounts of fibroblasts and hepatic stellate cells [12,13].

The aspartate aminotransferase (AST) platelet ratio index (APRI) score consists of the AST-to-platelet ratio, two inexpensive laboratory tests which are routinely performed in all patients [8,14–16].

The FibroTest is an algorithm consisting of age, sex, and five serum markers: alpha-2 macroglobulin (α2M), haptoglobin, total bilirubin, gamma-glutamyltransferase (γGT), and apolipoprotein A1 (ApoA1) [17].

The Fib-4 index is a simple and inexpensive algorithm consisting of age and the routine laboratory tests AST, alanine aminotransferase (ALT), and platelets [18,19]. In addition, the correlation of histology with TE has been evaluated [20,21].

TE measures liver stiffness in kilopascals [22]. With TE, a larger sample size of the liver is examined than in biopsy, which ensures a reduction in sampling error [6,23]. In previous studies, TE has shown good discriminative value for the presence or absence of cirrhosis (F4). Although there is significant overlap among F1, F2, and F3, the accuracy of discrimination is reasonable [24].

The aim of this study was to compare seven non-invasive diagnostics, the GBT and ^{13}C-methacetin breath test (MBT), four biochemical serum markers (hyaluronic acid (HA), APRI, FibroTest, and Fib-4) and TE, separately and in combination, to access liver fibrosis in a single study population. The reference for these non-invasive tests was Metavir staging in the liver biopsy with an adequate length of ≥20 mm.

Methods

Study population

Mono-infected CHB or CHC patients referred for liver biopsy to our out-patient clinic between February 2007 and November 2007 were invited to participate in this study. Exclusion criteria were alcohol intake >20 g/day, co-infection with HIV or hepatitis D, or the presence of hepatocellular carcinoma. Additionally, healthy controls were included for the breath tests and serological tests. The study protocol was approved by the institutional review board of Erasmus MC. All subjects provided written informed consent prior to enrollment.

Liver biopsy

Two well-experienced hepatologists performed all biopsies. To reduce complications, during this procedure abdominal ultrasound was used to identify liver parenchymal and vascular structures. Biopsies were taken with a 14G true-cut needle and required a routinely length ≥20 mm. After embedding biopsies in paraffin, lengthwise sections were cut and stained with picrosirius red. Two expert hepatopathologists scored all specimens (double read) for different fibrosis categories using Metavir scoring: F0: no fibrosis, F1: portal fibrosis without septa, F2: few septa, F3: numerous septa without cirrhosis, and F4: cirrhosis [2]. No biopsies were obtained from controls.

Breath tests

Both breath tests required overnight fasting before the test. For GBT, the participant received a dose containing 495 mg unlabeled (^{12}C-) galactose/kg bodyweight (VWR, Prolabo, Amsterdam, The Netherlands) and 5 mg/kg ^{13}C-labeled galactose (99% APE, Sigma–Aldrich, Zwijndrecht, The Netherlands) in 200 ml water. For MBT, the dose was 2 mg/kg ^{13}C-methacetin (99% APE, Campro, Berlin; *N*-(4-methoxy-^{13}C-phenyl) acetamide) in 200 mL water. At baseline (T = 0 min) four breath samples were collected, followed by duplicate breath samples at 10, 20, 30, 40, 60, 90, 120, 150, and 180 min after drinking the substrate.

To exclude any influence of variations in CO_2 production during breath tests, participants were at rest, fasting and not allowed to smoke.

The $^{13}CO_2/^{12}CO_2$ isotope ratio in the breath samples was analyzed by isotope ratio mass spectrometry (ABCA Sercon, UK). The percentage of ^{13}C exhaled was calculated assuming a CO_2 production rate of 9 mmol/h/kg. Results were expressed as cumulative

proportion of ^{13}C administered dose recovered over time using area under the curve (AUC) calculations and were categorized by Metavir classification. Elapsed time between GBT and MBT was at least 1 week to exclude any influence of previous ^{13}C-substrates. In all patients, the breath tests were performed within 6 months after liver biopsy irrespective of treatment.

Serum markers

Blood samples were obtained from all patients on the day of biopsy. Blood was taken from healthy controls preceding the breath test. Serum ALT, AST, albumin, γGT, and total bilirubin were determined in all participants. Alkaline phosphatase, trombocytes, and prothrombin time (PTT) were additionally determined in patients, but not in controls. Serum HA was measured (Corgenix, Broomfield, CO, USA) in all participants (normal range: 0–75 ng/ml).

APRI was calculated in serum of all patients as follows: AST/(ULN) × 100/platelets(10^9/L). FibroTests were performed on all serum samples from patients and controls. This FibroTest was based on sex, age, and five serum markers: α2M (Dako Diagnostics, Enschede, The Netherlands), haptoglobin, total bilirubin, γGT (Roche Diagnostics, Maizy, France) and ApoA1 (Beckman Coulter, Wiener Neudorf, Austria). α2M, haptoglobin, total bilirubin, and γGT were determined on a Modular P800 system (Roche) and ApoA1 on an Immage 800 system (Beckman). FibroTest results ranged between 0 and 1 [25].

Fib-4 was measured in the serum of all patients as follows: age(years) × [AST(U/L)/platelets (10^9/L) × $\sqrt{}$ALT(U/L)] [19]. For both FibroTest and Fib-4, increasing outcomes corresponded to more severe fibrosis stages.

Transient elastography

TE (FibroScan®, EchoSens, Paris, France) preceded the biopsy in the same session [22,26]. TE measured low-frequency elastic waves (50 Hz) through a medium and the speed of these waves was positively correlated with stiffness of the liver. A success rate of >60% was considered reliable in 10 validated measurements with interquartile range (IQR) <30% of the median [27].

Statistics

The data were analyzed using SPSS version 16.0 for Windows. Clinical and laboratory data were shown as mean with 95% confidence interval. Diagnostic results between patients were compared using the non-parametric Wilcoxon–Mann–Whitney *U*-test or Student's *T*-test with two-sided p values ≤0.05 were regarded as significant. Analysis of variance (ANOVA) was used to compare continuous variables. Diagnostic performances of non-invasive tests were expressed by sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and the area under the receiver operating characteristic (ROC) curve. To assess the association between non-invasive diagnostics and histology, linear and binary logistic regression analyses were performed. SAS version 9.2 was used to perform statistical comparison of ROC with Proc Logistic and ROC statement test.

Results

This study included 89 patients and 31 healthy controls; 48 CHB patients (35 men, mean age 37 years, mean body mass index (BMI) 25.4), 41 CHC patients (27 men, mean age 47 years, mean BMI 25), and 31 controls (19 men, mean age 35 years, mean BMI 25). No patients were excluded because of short biopsy length. All liver biopsies of the patients were scored using the Metavir system by two expert pathologists, nine patients were scored as F0 (no fibrosis), 36 as F1 (mild fibrosis), 14 as F2 (moderate fibrosis), 14 as F3 (moderate to severe fibrosis), and 16 as F4 (cirrhosis). All cirrhotic patients were classified as Child Pugh A. Additional demographic, viral, and biochemical characteristics of the study population were shown in Table I. Because of logistic or medical reasons, not all patients underwent all non-invasive tests.

Breath tests

None of the participants sustained any adverse reaction, such as diarrhea or abdominal pain, as a result of the galactose or methacetin intake. The numbers of participants per test are shown in Table II. For GBT, the mean of cumulative recoveries in controls was higher than in patients ($p < 0.001$). GBT distinguished F01 from F234 ($p = 0.04$), but did not reliably distinguish F0123 from F4 ($p = 0.12$). Using linear regression, cumulative recovery (AUC) of GBT was on average 0.29 lower in men than in women ($p = 0.002$) and was inversely related with age (0.27; $p < 0.001$). MBT did not significantly distinguish F01 from F234 or F0123 from F4 (Table II). Using linear regression, MBT was positively correlated with BMI (0.24; $p = 0.011$), data not shown.

Serum markers

Because no biopsies were taken from the controls, AST, ALT, albumin, and total bilirubin were

Table I. Demographic, viral, and biochemical characteristics of CHB and CHC patients and controls.

		Metavir (n = 89)				
	Controls (n = 31)	F0 (n = 9)	F1 (n = 36)	F2 (n = 14)	F3 (n = 14)	F4 (n = 16)
Demography**						
Male	19 (61%)	5 (56%)	23 (64%)	12 (86%)	10 (71%)	12 (75%)
Age (years)*	35 ± 11	36 ± 11	36 ± 11	44 ± 14	48 ± 11	48 ± 9
Height (cm)*	175 ± 10	171 ± 9	172 ± 10	174 ± 7	170 ± 12	176 ± 13
Weight (kg)*	77 ± 14	70 ± 12	74 ± 15	73 ± 12	78 ± 10	80 ± 13
BMI*	25 ± 4	24 ± 3	25 ± 4	24 ± 4	27 ± 4	26 ± 4
Etiology**						
CHB	–	6 (67%)	20 (56%)	10 (71%)	7 (50%)	5 (31%)
CHC	–	3 (33%)	16 (44%)	4 (29%)	7 (50%)	11 (69%)
Biochemistry*,†						
AST (U/L)	30 ± 10	35 ± 10	50 ± 42	66 ± 45	52 ± 22	68 ± 46
ALT (U/L)	29 ± 22	53 ± 25	84 ± 99	111 ± 111	78 ± 48	55 ± 40
Albumin (g/L)	46 ± 2	46 ± 2	46 ± 3	45 ± 3	44 ± 2	42 ± 5
Total bilirubin (μmol/L)	12 ± 6	14 ± 9	12 ± 9	13 ± 6	13 ± 7	16 ± 10
γGT (U/L)	31 ± 32	31 ± 11	54 ± 53	48 ± 38	82 ± 91	125 ± 153
Alkaline phosphatase (U/L)	–	69 ± 16	69 ± 16	74 ± 18	69 ± 17	87 ± 40
Trombocytes (*10E9/L)	–	228 ± 57	216 ± 59	230 ± 69	188 ± 44	140 ± 85
PTT (s)	–	12 ± 1	12 ± 1	12 ± 1	12 ± 1	14 ± 2

Abbreviations: BMI = body mass index; CHB = chronic viral hepatitis B; CHC = chronic viral hepatitis C; AST = aspartate aminotransferase; ALT = alanine aminotransferase; γGT = gamma-glutamyltransferase; PTT = prothrombin time.
*Mean ± standard deviation.
**Numbers (percentage of subgroup).
†Normal reference ranges: AST: <37 U/L for men, <31 U/L for women; ALT: <41 U/L for men, <31 U/L for women; albumin: 35–50 g/L; total bilirubin: <17 μmol/L; γ-GT: <50 U/L; alkaline phosphatase: <120 U/L; trombocytes: 150–400 × 10^9/L; PTT: 10.9–13.3 s.

measured to confirm them as healthy. HA was performed on all participants (Table II and Figure 1). HA distinguished F0123 from F4 ($p < 0.001$) with a corresponding AUC mean (95% CI) of 0.86 (0.75–0.96) (Figure 2C) and F01 from F234 ($p = 0.011$) with AUC of 0.72 (0.61–0.82) (Figure 2A and Table III). Despite the significance, almost all measurements in the range F0–F3 were within normal values (≤75 ng/mL). APRI scores were calculated only for patients (Table II). APRI significantly distinguished F0123 from F4 ($p = 0.015$), F012 from F34 ($p = 0.013$), and F01 from F234 ($p = 0.009$), despite some overlap between the various stages of fibrosis.

In the controls, the FibroTest results did not differ from F0 ($p = 0.127$), as expected (Table II and Figure 1B). FibroTest discriminated very well between F0123 and F4 ($p < 0.001$). Also, F01 was significantly different from F234 ($p < 0.001$) with an AUC of 0.80 (0.71–0.89) (Table III). After exclusion of severe fibrosis and cirrhosis, patients with F01 differed from F2 ($p = 0.024$). Even F1 by itself was different from F2 ($p = 0.032$). The optimal cut-off for several fibrosis stages with corresponding sensitivity, specificity, PPV, and NPV were listed in Table IV. Using 0.75 as cut-off value in detecting cirrhosis, sensitivity, and NPV were 1 for both CHB and CHC. F34 was detected with a high sensitivity of 97% and 91% in CHB and CHC, respectively, and F234 was detected with a sensitivity of 85% and 74%. Using linear regression, the FibroTest correlated positively with histology ($p < 0.001$).

Fib-4 scores were calculated in all patients (Table II and Figure 1C). The values ranged from 0.28 to 15.25. Fib-4 differentiated among several fibrosis stages: F0123 from F4 ($p = 0.005$), F012 from F34 ($p = 0.013$), and F01 from F234 ($p < 0.001$). Also, no and mild fibrosis (F01) was statistically significant from moderate fibrosis (F2) ($p = 0.03$). Fib-4 correlated positively with FibroTest (63.7%), TE (58%), and histology (60%). No patients with F34 had Fib-4 value <0.8; therefore the NPV was 100%. Cut-off values of 1.45 and 3.25 for predicting severe fibrosis had been determined by Sterling et al. [19]. Using 3.25 as cut-off in our data set detecting severe fibrosis showed a sensitivity of 37.7% and PPV 100% (Table IV). Using 1.45 for excluding fibrosis, the specificity was 69.9% and NPV was 76.2%. 63.4% of all Fib-4 results in CHC were ranged above cut-off value 3.25 or under 1.45, so patients could thus be prevented from undergoing biopsy with 19% misclassification rate. In CHB, cut-off at 3.25 showed specificity of 97.2% and NPV of 79.5%, whereas cut-off at 1.45 showed specificity of 80.6% and NPV of 87.9%. Using these cut-off values, only 14% of the samples were misclassified compared with liver biopsy, and 77% of the non-invasive tests correspond to biopsies.

Table II. Outcome of the non-invasive diagnostics in controls and patients, according to Metavir stages.

		Metavir (n = 89)				*p*-Value**	*p*-Value**
Total	Controls (n = 31)	F0 (n = 9)	F1 (n = 36)	F2 (n = 14)	F3 (n = 14)	F4 (n = 16)	≥F2	≥F4
GBT*	**20.4** ± 3.4 (n = 31)	**17.8** ± 3.9 (n = 8)	**16.5** ± 4.2 (n = 34)	**15.3** ± 3.1 (n = 14)	**15.2** ± 3.7 (n = 11)	**14.3** ± 5.3 (n = 15)	0.04	0.118
MBT*	**68.2** ± 15.5 (n = 30)	**74.3** ± 17.3 (n = 9)	**70.8** ± 17.0 (n = 31)	**68.1** ± 15.3 (n = 14)	**63.6** ± 12.1 (n = 11)	**63.0** ± 21.7 (n = 15)	0.083	0.189
HA*	**23.8** ± 27.7	**27.4** ± 18.5	**41.1** ± 61.7	**31.8** ± 30.2	**65.8** ± 75.7	**182.4** ± 190	0.011	<0.001
APRI*	–	**0.4** ± 0.2	**0.7** ± 0.6	**0.8** ± 0.5	**0.8** ± 0.3	**2.0** ± 1.8	0.009	0.015
FibroTest*	**0.11** ± 0.09	**0.21** ± 0.17	**0.21** ± 0.17	**0.33** ± 0.21	**0.45** ± 0.20	**0.55** ± 0.26	<0.001	<0.001
Fib-4		**0.85** ± 0.41	**1.03** ± 0.53	**1.4** ± 0.77	**1.60** ± 0.46	**5.01** ± 4.70	<0.001	0.005
TE*	–	**6.4** ± 1.0 (n = 8)	**7.6** ± 4.6 (n = 34)	**10.2** ± 5.6 (n = 11)	**14.1** ± 7.0 (n = 13)	**22.9** ± 16.2 (n = 15)	<0.001	0.006

*Mean ± standard deviation.
**Control group excluded from statistical analysis.
P ≤ 0.05 is significant.

Transient elastography

Elastography was performed in 81 patients. Eight patients were excluded from TE due to failure of adequate measurements. TE distinguished between F0123 and F4 (p = 0.006), between F012 and F34 (p < 0.001), as well as between F01 and F234 (p < 0.001) (Table II and Figure 1D). Even after exclusion of F4, patients with F23 showed increased elasticity compared with F01 (p = 0.001). The corresponding AUC mean with threshold F34 was 0.87 (0.79–0.96) and with threshold F4 was 0.89 (0.82–0.96) (Figure 2 and Table III). TE was confounded by age (p = 0.010). Cut-off values for several fibrosis stages, subdivided by etiology, were defined by Verveer (unpublished data) and validated in our patient population (Table IV).

Combination of non-invasive methods

Using ordinal logistic regression, the FibroTest and Fib-4 by themselves both showed an AUC of 0.80 for F01 versus F234. TE showed an AUC of 0.83, which was not significantly different from AUC of FibroTest or Fib-4 (p = 0.20, p = 0.34, respectively). Several combinations of non-invasive tests had been tested (Table III). Combinations with breath tests or APRI did not improve AUC (data not shown). Fib-4 and TE combination increased the AUC to 0.88 (0.80–0.96) for F01 versus F234. By contrast, addition of GBT, MBT, HA, APRI or FibroTest to Fib-4 and TE did not or slightly improve the AUC for ≥F2, or improved it only slightly.

HA, FibroTest, Fib-4, and TE were good markers for detecting severe fibrosis ≥F3. These tests, expressed as AUC, were significantly higher than both GBT and MBT (HA: p = 0.009 and p = 0.0003; FibroTest: p = 0.039 and p = 0.011; Fib-4: p = 0.018, and p = 0.0008; TE: p = 0.0005 and p = <0.0001, respectively). The best combination, consisting of HA, FibroTest, and TE, increased AUC to 0.92 for ≥F3. The percentage of patients classified correctly with concordant test results was 58%. The number of misclassification was only 4%. If only two out of three tests had to be conclusive, the number of correctly classified patients increased to 80% with a sensitivity of 52%, specificity of 96%, PPV of 88%, and NPV of 80%.

Cirrhosis detection ≥F4 was very accurate using TE (AUC of 0.89). TE combined with HA increased AUC to 0.91, although not significant. Using this combination, 73% of all patients were classified correctly with a specificity of 75% and a sensitivity of 60%. Misclassification with concordant test results was only 5%.

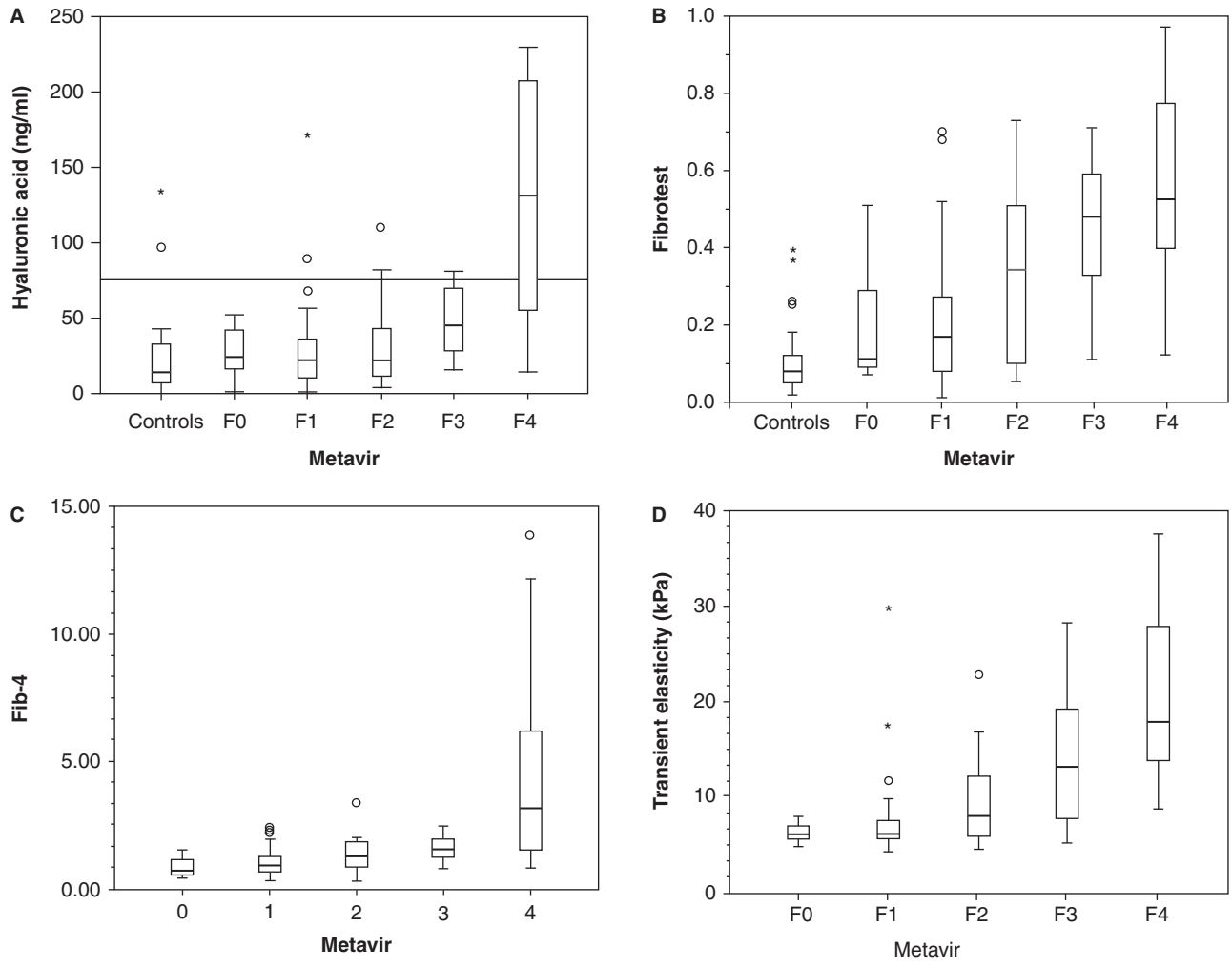

Figure 1. Boxplots of (A) hyaluronic acid, (B) FibroTest, (C) Fib-4, and (D) transient elastography scores performed in controls and patients by Metavir stage. Horizontal line in (A) shows upper limit of normal range (75 ng/mL).

Discussion

The assessment of liver fibrosis is important for evaluation of the disease progression and is relevant for therapeutic management. Liver biopsy is used to estimate fibrosis stage, but is prone to sample- and observer variability due to the distribution of fibrosis within the liver, and complicated by morbidity and mortality. Therefore, liver biopsy is not a perfect golden standard, resulting in false positive and false negative diagnosis [28,29]. There is thus a need for non-invasive methods to diagnose accurately the presence of liver fibrosis and cirrhosis.

In this study, we have shown that all serum markers and TE determine significant fibrosis and cirrhosis with good discriminative value.

Most participants attended both GBT and MBT. In contrast to previous studies on breath tests [7,9–11], our results were disappointing. Limitation of GBT, previously described in literature, was the influence of portal blood flow, which was resolved by saturating the liver with high doses of unlabeled galactose. GBT distinguished reliably between F01 and F234, but not between other fibrosis stages, probably due to metabolic function overcapacity of the liver [9]. In our study, MBT did not reliably distinguish between any fibrosis stages. MBT was positively correlated with BMI although other studies did not support this [30,31]. Significant differences in GBT measurements after 60 min and in MBT after 30 min have been described [10,32], but analyzing these earlier time points in our data did not result in significance.

HA is a very sensitive marker to exclude cirrhosis, if 75 ng/mL is measured as upper limit of normal (ULN). Several studies have been performed with HA, using other cut-off values to exclude cirrhosis. However, in our data set these previously described

cut-offs were less sensitive in excluding cirrhosis [33,34]. Serum marker Fib-4 also takes ALT and age into account, beside AST and platelet count in the APRI score, and is therefore much more accurate in diagnosing fibrosis. Fib-4 has already been tested in CHB and CHC co-infected and mono-infected patients separately [35]. In our study, we validated Fib-4 in an external data set for both CHB and CHC. Despite small sample sizes, we were also able to distinguish between CHB and CHC results for other serum markers and TE. Differences between CHB and CHC can be explained by the presence of

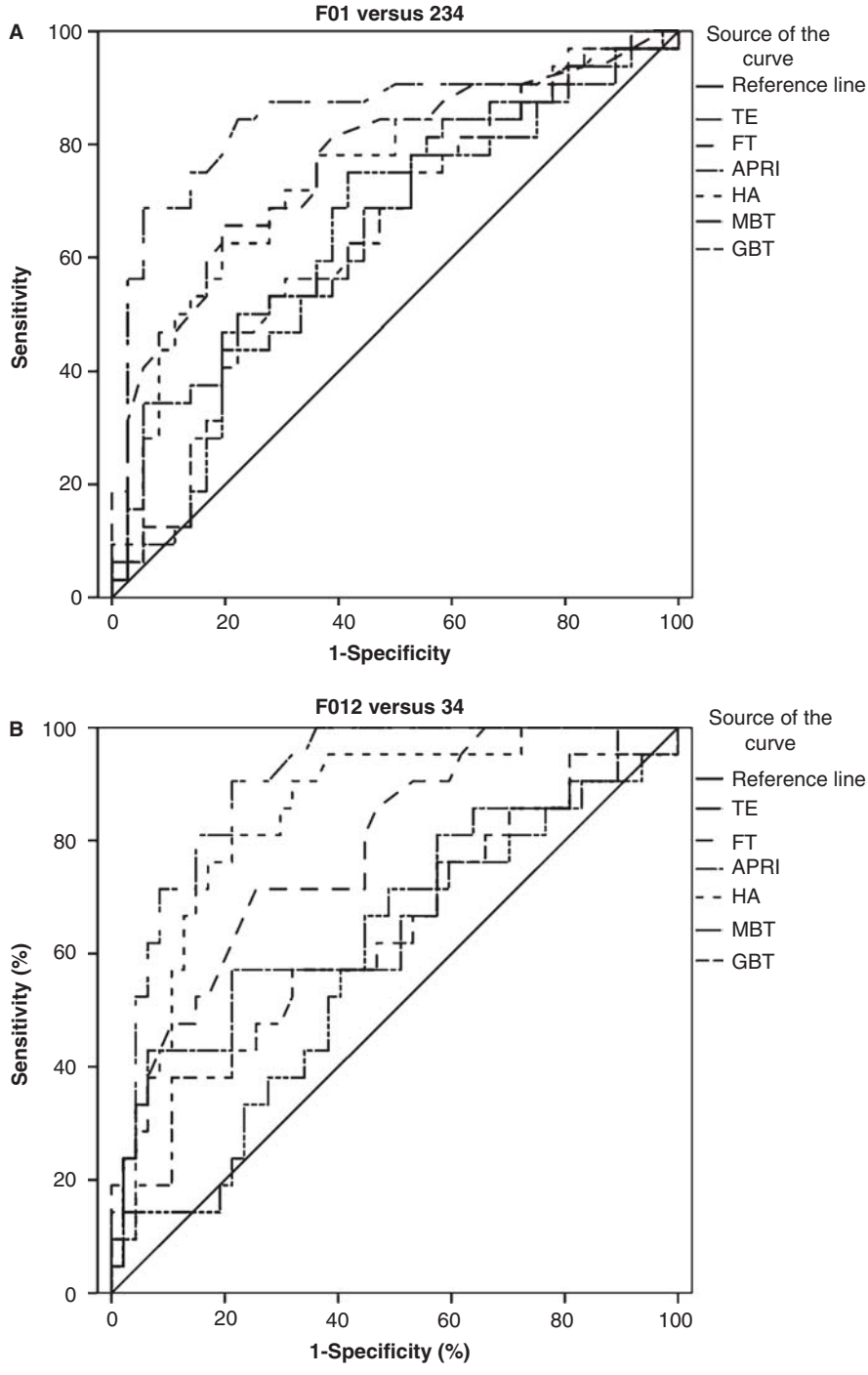

Figure 2. Receiver operator characteristic (ROC) curves from all non-invasive tests with (A) threshold F01 vs. F234, (B) F012 vs. F34, and (C) F0123 vs. F4. Patients with missing data were not included. Diagonal segments are produced by ties.

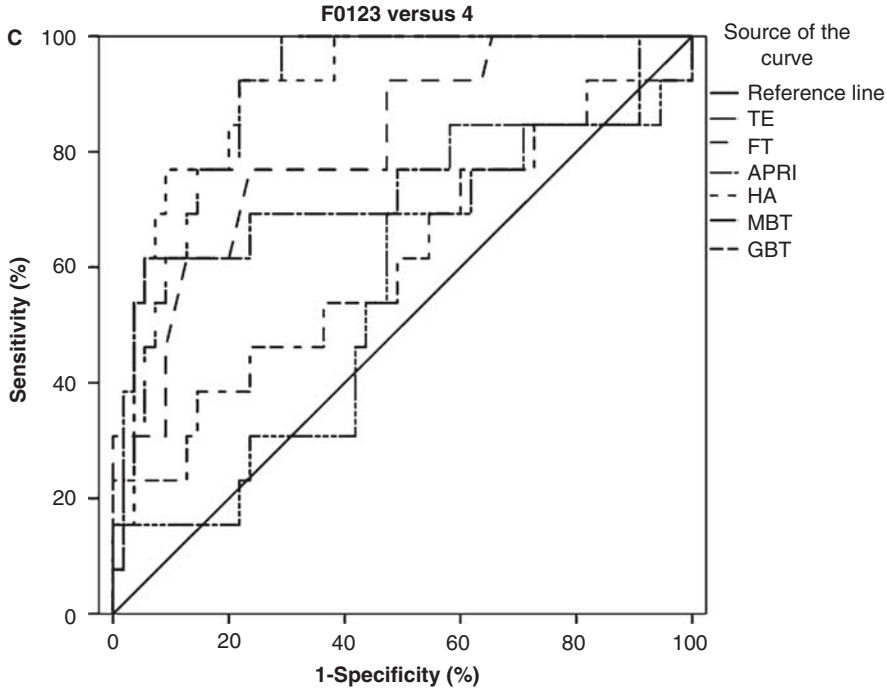

Figure 2. (*Continued*).

inflammation due to additional diseases like rheumatoid arthritis. These influences are observed more frequently in CHC patients, due to the more frequent extra-hepatic inflammatory implications in CHC patients compared with CHB patients.

Besides the non-invasive tests, we have taken into account some other tests have been described recently. Enhanced liver fibrosis (ELF), an algorithm consisting of tissue-inhibitor of matrix-metalloproteinase-1 (TIMP-1), HA, and aminoterminal propeptide of type III collagen [36] or YKL-4 [37] are also being studied in comparison with liver biopsy. In addition, acoustic radiation force impulse (ARFI) [38] is another promising technique with elastography.

For both FibroTest and TE, it is important to choose a cut-off value with a high NPV, which results in high specificity for measuring early fibrosis stages. However, in detecting cirrhosis, a high PPV without false negative results is preferable, due to the risk of hepatocellular carcinoma. Of all tested non-invasive diagnostics, TE performed the best as single test in our study. Because of non invasiveness of the test and good correlation with biopsy, this may a very useful test for follow-up [39].

Table III. AUC and 95% CI for GBT, MBT, HA, APRI, FibroTest, Fib-4, and TE, according to Metavir stages.

	≥F2 (95% CI)	≥F3 (95% CI)	≥F4 (95% CI)
GBT	0.62 *(0.50–0.75)*	0.60 *(0.47–0.74)*	0.61 *(0.44–0.79)*
MBT	0.62 *(0.49–0.74)*	0.62 *(0.49–0.75)*	0.58 *(0.42–0.74)*
HA	0.72 *(0.61–0.82)*	0.81 *(0.72–0.91)*	0.86 *(0.75–0.96)*
APRI	0.69 *(0.58–0.80)*	0.69 *(0.57–0.81)*	0.72 *(0.55–0.89)*
FibroTest	0.80 *(0.71–0.89)*	0.83 *(0.74–0.92)*	0.81 *(0.70–0.92)*
Fib-4	0.80 *(0.71–0.89)*	0.82 *(0.74–0.91)*	0.84 *(0.72–0.97)*
TE	0.83 *(0.74–0.93)*	0.87 *(0.79–0.96)*	0.89 *(0.82–0.96)*
HA + FibroTest	0.81 *(0.71–0.90)*	0.85 *(0.76–0.93)*	0.86 *(0.76–0.96)*
HA + Fib-4	0.80 *(0.71–0.90)*	0.83 *(0.75–0.92)*	0.86 *(0.74–0.98)*
HA + TE	0.85 *(0.76–0.94)*	0.91 *(0.85–0.98)*	0.91 *(0.84–0.97)*
FibroTest + Fib-4	0.83 *(0.74–0.91)*	0.86 *(0.78–0.94)*	0.87 *(0.76–0.97)*
FibroTest + TE	0.86 *(0.78–0.95)*	0.90 *(0.84–0.97)*	0.87 *(0.78–0.95)*
Fib-4 + TE	0.88 *(0.80–0.96)*	0.90 *(0.83–0.96)*	0.90 *(0.81–0.99)*
HA + FibroTest + TE	0.87 *(0.78–0.95)*	0.92 *(0.86–0.98)*	0.90 *(0.83–0.98)*
HA + FibroTest + Fib-4	0.83 *(0.75–0.92)*	0.86 *(0.78–0.94)*	0.87 *(0.77–0.98)*

Abbreviations: AUC = area under the curve; GBT = galactose breath test; MBT = methacetin breath test; HA = hyaluronic acid; APRI = aspartate aminotransferase platelet ratio index; TE = transient elastography.

Table IV. Diagnostic values of non-invasive tests with defined optimal cut-off.

	FibroTest (CHB, $n = 48$; CHC, $n = 40$)						Fib-4 (CHB, $n = 48$; CHC, $n = 41$)			Transient elastography (CHB, $n = 45$; CHC, $n = 36$)					
	≥F2		≥F3		≥F4		≥F3			≥F2		≥F3		≥F4	
	HBV	HCV	HBV	HCV	HBV	HCV	HBV	HCV		HBV	HCV	HBV	HCV	HBV	HCV
Cut-off*	0.31	0.31	0.58	0.58	0.75	0.75	<1.45	>3.25	<1.45	>3.25	7	5	10	10	14	14
Sens.	85	74	97	91	100	100	67	25	72	28	73	6	85	84	80	88
Spec.	68	76	25	41	8	24	81	97	70	100	84	100	73	76	75	73
PPV	76	74	80	68	77	64	53	75	65	100	86	100	91	80	97	88
NPV	79	76	75	78	100	100	88	80	76	64	70	57	62	81	27	73
LR+	2.66	3.09	1.30	1.55	1.09	1.31	3.44	8.93	2.38	∞	4.63	6	3.13	3.58	3.22	3.23
LR−	0.23	0.35	0.11	0.21	0	0	0.41	0.77	0.40	0.722	0.32	0.94	0.20	0.21	0.26	0.16

Abbreviations: Sens. = sensitivity; Spec. = specificity; PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR− = negative likelihood ratio.
Values defined by Poynard et al. for FibroTest, Sterling et al. for Fib-4, and Verveer (personal communication) for TE.

TE combined with FibroTest and HA increased the accuracy (AUC of 0.92) of predicting severe fibrosis and narrowed the confidence intervals.

In predicting F01 versus F234, Fib-4 combined with TE performed well, with AUC of 0.88. However, the combination of TE, FibroTest, and HA can also be used to distinguish F01 from F234 (AUC of 0.87) without significant difference. Unfortunately, elastography is not available in all hospitals. Therefore, the combination of FibroTest and Fib-4 is a good alternative, with AUC of 0.83. To distinguish between F012 and F34, the best combination was HA, FibroTest, and TE (AUC of 0.92). The combination of HA and TE (AUC of 0.91) was the best option to determine cirrhosis. Although AUC increases, differences are not significantly different and thereby would not be clinically very relevant.

Recent studies have reported using APRI and FibroTest [40,41] as non-invasive diagnostics. These studies support our data concerning the percentage of replacing biopsies in a larger patient cohort.

The AUCs of the non-invasive tests are good, but are not as effective for measuring the early stages of fibrosis (F0, F1, and F2). However, our study has generated promising hypotheses for further research in this field.

Although liver biopsy remains necessary to assess fibrosis in new patients, we recommend combining the first liver biopsy with non-invasive tests. Combinations of TE, HA, and FibroTest are accurate enough by themselves to detect moderate and severe fibrosis. Especially when these non-invasive tests correspond with initial histology, non-invasive diagnostic tests can help in future follow-up of disease management, especially when performing liver biopsy is not possible due to medical reasons, and thereby reducing the number of biopsies.

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