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Prospective Comparison of Fibroscan, King's Score and Liver Biopsy for the Assessment of Cirrhosis in Chronic Hepatitis C Infection

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Abstract and Introduction

Abstract

Historically, liver biopsy (LB) was the sole method to evaluate the severity of hepatic fibrosis in patients with chronic hepatitis C infection. However, LB is expensive and associated with a risk of severe complications. Therefore, noninvasive tests have been developed to assess the severity of liver fibrosis. The accuracy of Fibroscan (FS) and King's score (KS) was evaluated individually and in combination using liver histology as the reference standard. One hundred and eighty-seven patients were identified who had undergone a biopsy with a diagnosis of chronic hepatitis C virus (HCV) mono-infection (HCV RNA-positive by RT-PCR), attending King's College Hospital (*n* = 88) or the Royal Free Hospital (*n* = 99) (London) between May 2006 and December 2007. Liver fibrosis was scored using the Ishak method; significant fibrosis was defined as Ishak fibrosis stage F3–F6, and cirrhosis defined as Ishak fibrosis F5–F6. The diagnostic accuracy of each test was assessed by area under receiver operator characteristic curves (AUROC). Median age was 49 years (43–54) and 115 (61%) were male. The AUROC for FS, KS and FS + KS for the diagnosis of Ishak F3–F6 were 0.83, 0.82 and 0.85, respectively and for the diagnosis of cirrhosis (≥F5) were 0.96, 0.89 and 0.93, respectively. The negative predictive values for the diagnosis of cirrhosis using the optimal cut-off results for fibrsocan (10.05 kPa), KS (24.3) and the two combined (26.1) were 98%, 91% and 94%, respectively. The noninvasive markers and, particularly, FS were effective tests for the prediction of cirrhosis in chronic hepatitis C. Both KS and FS also had clinical utility for the prediction of Ishak fibrosis stages F3–F6.

Introduction

Hepatitis C virus (HCV) infection is associated with a wide spectrum of liver disease. Progressive fibrosis is the hallmark of worsening chronic hepatitis C infection (CHC). Cirrhosis leads to the complications of end-stage liver disease including liver failure, ascites, variceal bleeding, porto-systemic hepatic encephalopathy and hepatocellular carcinoma. At presentation, 10–20% of chronic hepatitis C patients are cirrhotic and between 20% and 30% of noncirrhotic patients will develop it within one or more decades.[1–3] Patients with more severe hepatic fibrosis at baseline have a lower rate of sustained virological response in comparison with patients with a lower stage of fibrosis.[4]

Historically, liver biopsy (LB) has been the only accepted method to evaluate the severity of hepatic fibrosis in CHC-infected patients. However, it can be associated with life-threatening complications and pain.[5,6] Furthermore, it is subject to inter-observer variability and sampling error and this can lead to either under- or over-staging of disease.[7–9] Therefore, there is a need for noninvasive tests that accurately and safely measures severity of liver fibrosis. There has been a proliferation in the number of noninvasive tests of liver fibrosis over the last decade some of which have been shown to have clinical utility[10–13]

Fibroscan (FS) or transient elastography [FibroScan (FS); Echosens, Paris, France] is a noninvasive method for measuring liver stiffness. The stiffer the tissue, the faster the shear wave propagates. Studies have confirmed that FS reflects hepatic fibrosis in CHC and has a good correlation with histological staging systems.[13–15] The King's score (KS) is a simple noninvasive test that was developed to predict the likelihood of significant fibrosis or cirrhosis at the bedside or in the clinic. It was derived from a training set of 602 consecutive CHC-infected patients seen at King's College Hospital (KCH) and was validated in a further 102 patients.[16]

A previous study has suggested that a combination of noninvasive tests may be the best strategy to improve the diagnostic accuracy of noninvasive tests.[15] The aim of this study was to determine the diagnostic accuracy for predicting stage of fibrosis of FS and KS individually and in combination in consecutive patients with CHC against LB.

Patients and Methods

One hundred and ninety-nine (199) patients with a diagnosis of CHC infection attending KCH and the Royal Free Hospital (RFH) (London, UK) between May 2006 and December 2007 were identified. Inclusion criteria were as follows: all patients positive for anti-HCV antibodies using a third-generation enzyme immunoassay, positive HCV RNA by reverse-transcription polymerase chain reaction and CHC mono-infection with an available LB that had been performed no more than 6 months before the FS. Exclusion criteria were body mass index (BMI) >30 kg/m^2 and patients who were currently on anti-viral therapy or who had completed anti-viral therapy, Patients with co-existent liver disease, e.g. chronic hepatitis B infection, autoimmune liver disease and human immunodeficiency virus (HIV), patients with a history of high alcohol consumption, in men this was defined as patients consuming >80 g alcohol per day, and in women was defined as patients consuming >60 g of alcohol per day. HCV genotypes were measured prior to commencing treatment if patients agreed to have it. The study protocol conformed to the ethical guidelines of the 1975 Helsinki declaration.

Clinical Parameters

The following data were recorded from all patients at the time of FS: age, gender, HCV genotype, alcohol consumption, time since LB, current or previous use of anti-viral therapy, diabetes mellitus and BMI. BMI was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2).

Biochemical Parameters

The following data were recorded from patients at the time of LB: full blood count, international normalized ratio (INR) and routine liver function tests including bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase and gamma-glutamyl transferase. Routine biochemical assessment of renal function was performed. Only AST, INR and platelets were documented for the study. At KCH, platelets were measured using Advia (Siemens, Deerfield, IL, USA, reference range 150–450 × 10^9/L), and AST was measured using Advia 2400 (Siemens, reference range 10–50 IU/L). INR was measured using Stago Star Evolution (Diagnositica Stago, Asniéres sur seine, France, reference range 0.9–1.2). At RFH, platelets were measured with Advia 2120 (Siemens, reference range 150–450 × 10^9/L), AST was measured with Roche MODULAR analyzer (Roche diagnostics, Indianapolis, IN, USA, reference range <37) and INR was measured using Stago Star Evolution (Diagnositica Stago, reference range 0.9–1.2). The assays were run in separate laboratories. Percutaneous LBs were performed provided patients had platelets >80 × 10^9/L, INR <1.3 and haemoglobin >10 g/dL. Blood products were given to achieve these targets where necessary. Where coagulation or platelets remained low, transjugular LB was performed: three cores of tissue were taken to provide adequate tissue for histological analysis as previously described.[17]

Histological Evaluation

Paraffin-embedded LB specimens of more than 10 mm or >10 portal tracts were analysed by an experienced histopathologist (AQ, FG, APD). The number of LB fragments was not routinely recorded for the purpose of the study. The histopathologists were unaware of patient clinical data, other than the knowledge of chronic HCV infection. LB specimens were stained with hematoxylin and eosin, and fibrosis stage was analysed examining LB specimens stained with reticulin. Histological fibrosis stage was scored using the method described by Ishak.[18] Significant fibrosis was defined as Ishak fibrosis stage F3–F6, and cirrhosis was defined as Ishak fibrosis stage F5–F6. The METAVIR (M) classification has been used in a number of studies, a conversion factor was introduced to standardize results taking into account the relative frequencies of each stage of fibrosis using the method described by Poynard and colleagues:[19] Ishak F0 = METAVIR F0, Ishak F1–2 = METAVIR F1, Ishak F3 = METAVIR F2, Ishak F4–5 = METAVIR F3 and Ishak F6 = METAVIR 4.[20] The length of biopsy was

recorded and a sensitivity analysis was performed to determine the effect of biopsy length on the accuracy of the noninvasive tests performed using a cut-off of 10 mm. LB examination was performed by senior operators using the Menghini technique[21] using the following LB needles (e.g. Hepafix; Braun, Melsungen, Germany) or a transjugular approach as previously described.[17]

Fibroscan (FS) Measurement

This was performed on the right lobe of the liver in the intercostals spaces with the patient in the supine position with the right arm in maximum abduction. The transducer tip was smeared with coupling gel and placed on the skin at the level of the right lobe of the liver. On locating the target area, i.e. an area of liver 6 cm depth with no major vascular structures measurements were started after pressing the probe button. The measurement depth was between 25 and 65 mm. Ten valid measurements were required for a successful acquisition with a greater than 60% success rate being needed to allow inclusion in the final analysis. The results were expressed in kilopascals (kPa). The value recorded was the median measurement of the readings. Measurements were only considered reliable if 10 validated measurements were recorded. Patients, in who FS measurement was not reliable, were not included in the analysis. Patients with BMI>30 kg/m^2 were excluded because of the failure of FS to achieve accurate acquisitions in these patients as previously described.[13,22]

King's Score

The results of FS were compared to KS and in combination. The KS is a simple noninvasive test utilizing the contracting elements of age, AST and INR all of which are multiplied together and divided by the platelet count.

$$\mathrm{KS} = \mathrm{Age\,(years)} \times \mathrm{AST\,(IU/L)} \times \mathrm{INR/Platelets}\,(\times 10^9/\mathrm{L})$$

The accuracy of each test alone and in combination was compared for different stages of liver fibrosis by calculating area under receiver operator characteristics curves (AUROC).

Statistical Methods

Data are expressed as median with interquartile range unless stated otherwise. Statistical analysis was performed using the Statistical Program for Social Sciences (SPSS 13.0 for Windows; SPSS Inc., Chicago, IL, USA). The endpoints in this study were the accuracy of FS, KS and combined FS + KS in determining the presence of Ishak F0–1 *vs* F2–6, Ishak F3–6 and cirrhosis Ishak F5–6. The chi-squared test or Fischer's exact test was used to compare categorical variables. Continuous variables were compared using the Mann–Whitney *U* test. A two-sided *P*-value <0.05 was considered to be statistically significant. Correlations between noninvasive tests and Ishak fibrosis stage were calculated using Pearson's correlation and box-plots were calculated. The top and bottom of the boxes are the first and third quartiles, and the length of the box corresponds to the interquartile range, and the line through the box represents the median.

The diagnostic accuracy of each test to determine stage of liver fibrosis was performed calculating area under receiver operating characteristics (AUROC) curves. AUROC curves measure the sensitivity and specificity over the range of the variable. An ideal test produces an AUROC of 1.00, whereas prediction no better than chance is associated with an AUROC of 0.5, statistical significance from an AUROC of 0.5 was reported. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each test alone and in combination were then performed. To eliminate any spectrum bias that may occur when there is a higher prevalence of any particular stage of fibrosis, AUROC curve analysis was also performed comparing individual stages of Ishak fibrosis, i.e. F0 *vs* F1, F1 *vs* F2, F2 *vs* F3, F3 *vs* F4, F4 *vs* F5 and finally F5 *vs* F6. Standardization of AUROC was performed based on prevalence of fibrosis stages and by calculating the difference between mean fibrosis stage of advanced fibrosis minus the mean fibrosis stage of nonadvanced fibrosis in converted METAVIR units (DANA).[19] Because the METAVIR classification has been used in a number of studies, a conversion factor was introduced to determine the performance of the fibrosis test and to standardize the fibrosis stage for the cohort: Ishak F0 = METAVIR F0, Ishak F1–2 = METAVIR F1, Ishak F3 = METAVIR F2, Ishak F4–5 = METAVIR F3 and Ishak F6 = METAVIR 4.[16] The adjusted AUROC (Ad AUROC) was calculated using the formula: Ad AUROC = Ob AUROC + (0.1056) × (2.16 – DANA), as described by

Poynard and colleagunes.[19]

Results

Between May 2006 and December 2007, 199 consecutive patients were identified who fulfilled inclusion criteria. Twelve patients (6%) were excluded for the following reasons; patient declining LB (*n* = 2), unable to find LB result (*n* = 1), unable to acquire transient elastography reading (*n* = 1), unreliable liver stiffness reading (*n* = 8). All patients in whom FS measurement was unsuccessful were overweight (BMI > 25 kg/m^2). The median age was 49 years (43–54), and there were 111 men (59%). The median length of LB samples was 15 mm (13–17 mm). The distribution of Ishak fibrosis stage was as follows: F0, *n* = 6; F1, *n* = 51; F2, *n* = 41; F3, *n* = 28; F4, *n* = 11; F5, *n* = 11, F6, *n* = 39. Fifty patients (27%) had cirrhosis based on Ishak fibrosis stage. Adjusting for METAVIR units, the frequencies were F0 = 6 (3%), F1 = 92 (49%), F2 = 28 (15%), F3 = 22 (12%) and F4 = 39 (21%). The mean fibrosis stage for advanced fibrosis using the METAVIR score was (0.15 × 2) + (0.12 × 3) + (0.21 × 4)/0.48 = 3.13. The mean fibrosis stage for nonadvanced fibrosis was 0.49/0.52 = 0.94. Thus, for this cohort the DANA = 3.13 – 0.94 = 2.19). The genotype was available for 148 patients: genotype 1 (*n* = 81, 55%), genotype 2 (*n* = 10, 7%), genotype 3 (*n* = 43, 29%), genotype 4 (*n* = 12, 8%) and genotype 5 (*n* = 2, 1%). Twenty-one per cent of patients were cirrhotic. The baseline characteristics for the study population are summarized in Table 1, and the baseline characteristics according to liver centre are shown in Table 2. The correlations between Ishak fibrosis stages and noninvasive tests, alone and in combination were FS, (*r* = 0.72, *P* < 0.0001), KS (*r* = 0.66, *P* ≤ 0.0001) and FS + KS (*r* = 0.71, *P* < 0.0001). The box-plots are shown in Figs 1–3.

Table 1. Baseline characteristics of 187 study patients

Characteristics	***n* = 187**
Age	49 (43–55)
Sex (men),%	111 (59)
BMI (kg/m^2)	26 (22–30)
AST (IU/L)	49 (36–82)
INR	1.0 (0.99–1.1)
Platelets (×10^9/L)	212 (165–259)
Liver biopsy (mm)	15 (13–17)
Ishak fibrosis score (%)	
F0	6 (3.2)
F1	51 (27.3)
F2	41 (21.9)
F3	28 (15)
F4	11 (5.9)
F5	11 (5.9)
F6	39 (20.9)
METAVIR	
F0	6
F1	92

F2	28
F3	22
F4	39
Genotype (%)	
1	81 (42.4)
2	10 (5.2)
3	43 (22.5)
4	12 (6.3)
5	2 (1)

BMI, body mass index; AST, aspartate aminotransferase; INR, international normalized ratio.

Table 2. Baseline characteristics with chronic hepatitis C infection from Royal Free Hospital (RFH) (*n* = 99) and King's College Hospital (KCH) (*n* = 88)

Variable	RFH	KCH	*P*
Age (years)	51 (44–57)	47 (42–53)	0.02
Sex (men),%	65 (65%)	50 (55%)	0.16
BMI (kg/m^2)	23.2 (21.1–26.3)	26.8 (22.5–29.7)	0.11
AST (IU/L)	47 (34–67)	53 (37–96)	0.11
INR	1 (1–1.1)	0.99 (0.94–1.1)	0.006
Platelets (×10^9/L)	214 (176–264)	204 (159–244)	0.13
Liver biopsy (mm)		15 (13–17)	
Ishak fibrosis score (%)			
F0	3 (3)	3 (3.4)	0.04
F1	36 (36)	15 (17)	
F2	23 (23)	18 (21)	
F3	11 (11)	17 (19)	
F4	2 (2)	9 (10)	
F5	5 (5)	6 (7)	
F6	19 (19)	20 (23)	
Genotype (%)			
1	41 (55)	40 (52)	0.06
2	2 (3)	8 (10)	
3	19 (25)	24 (31)	
4	7 (9)	5 (6.5)	

5	2 (2)	0

BMI, body mass index; AST, aspartate aminotransferase; INR, international normalized ratio.

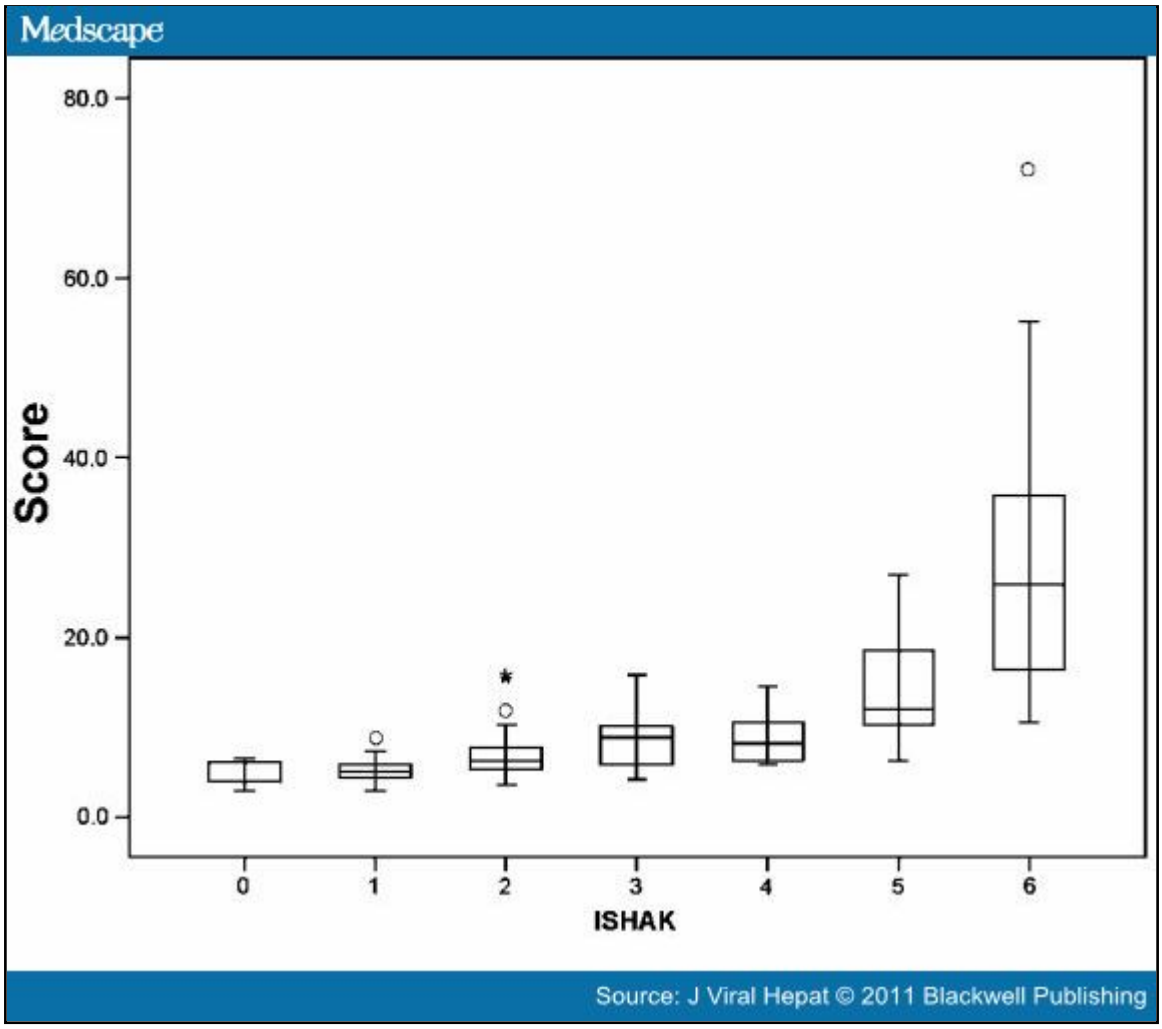

Figure 1. Box-plot for Fibroscan for each Ishak fibrosis stage.

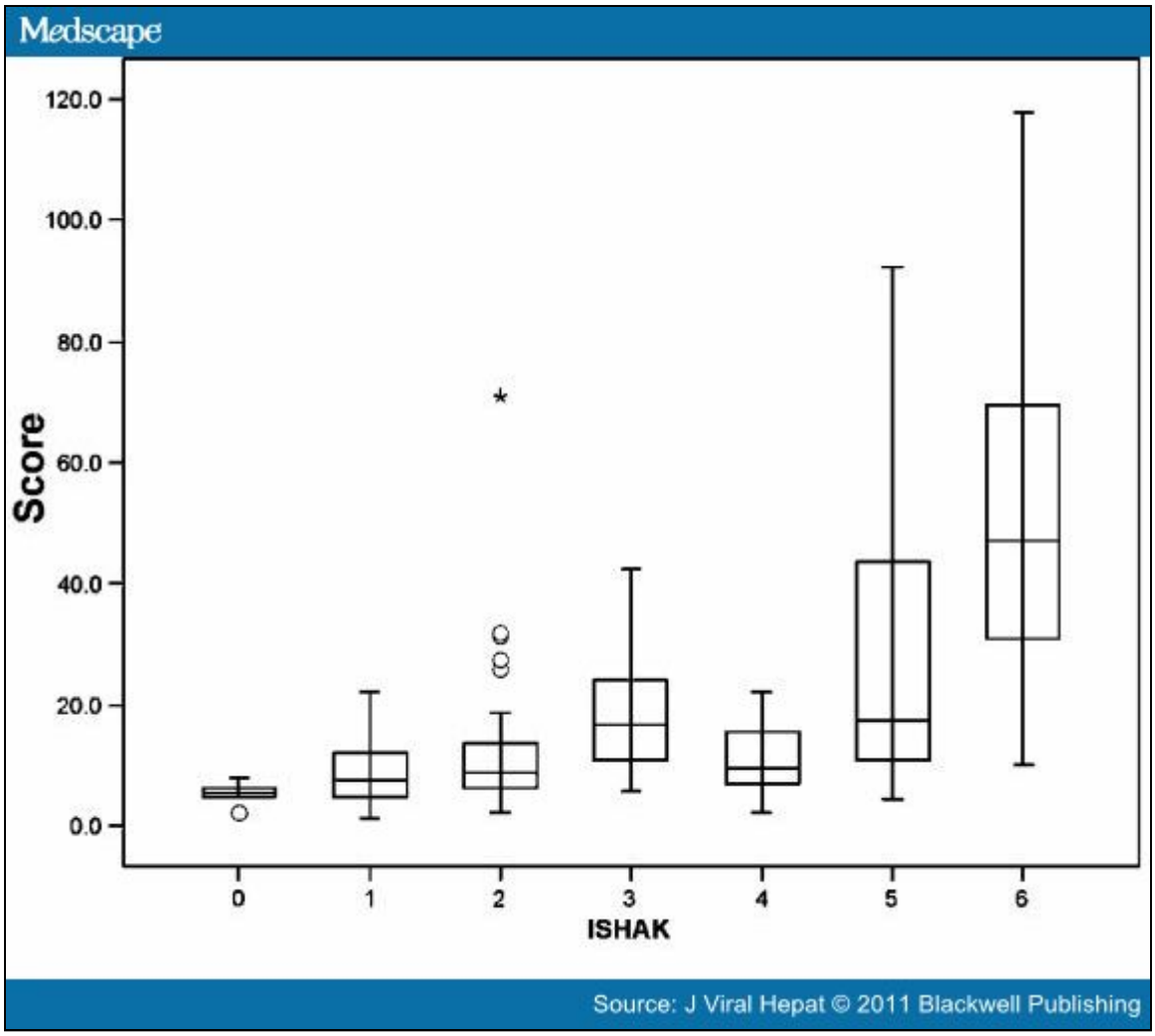

Figure 2. Box-plot for King's score for each Ishak fibrosis stage.

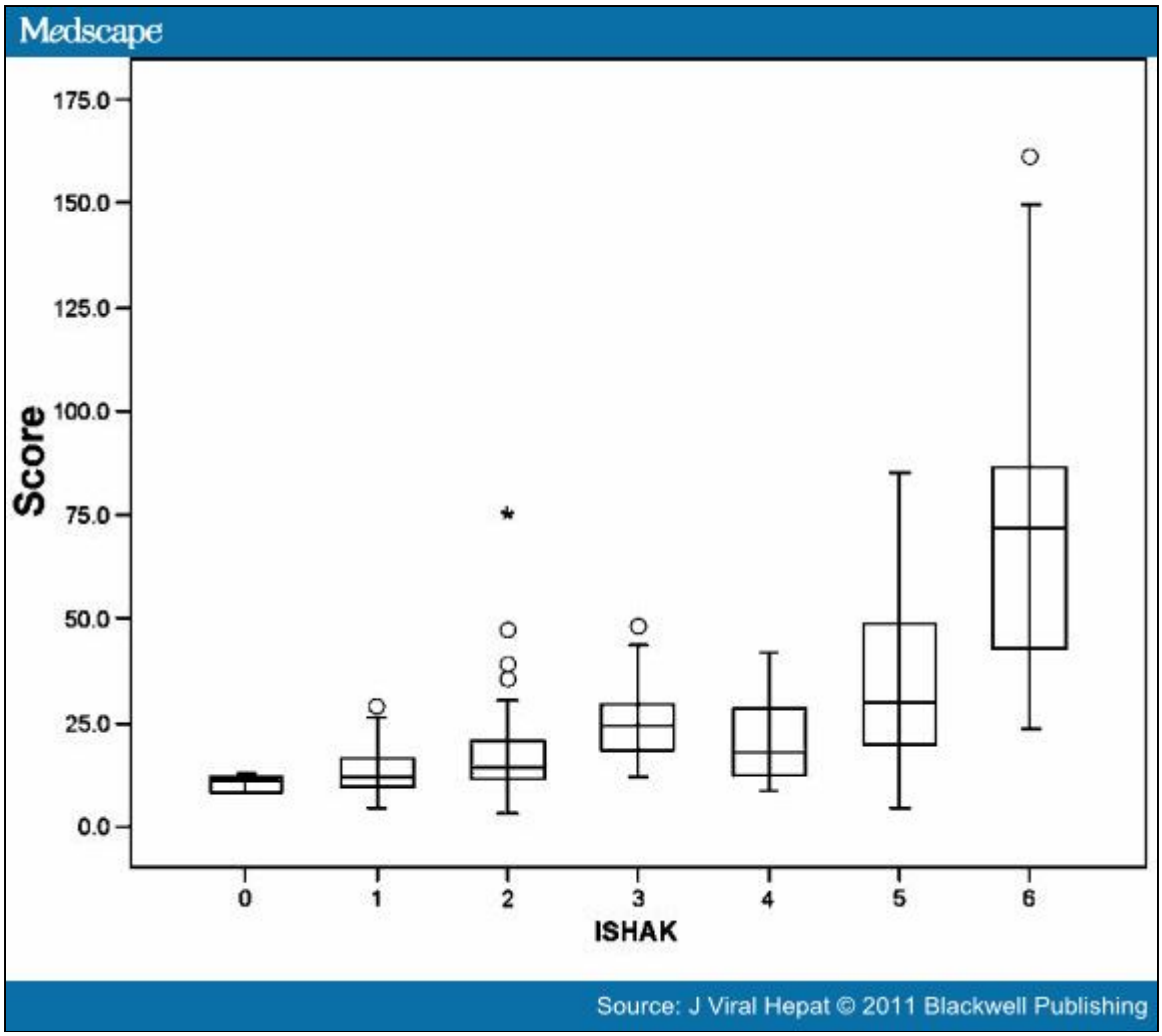

Figure 3. Box-plot for combined Fibroscan + King's score.

Diagnostic Accuracy of FS, KS and FS + KS

Liver stiffness measurements ranged from 3.0 to 73.5 kPa (median 6.8). The readings for KS ranged from 1.1 to 441.7 (median 12.5), and for the combined score FS + KS, the range of scores was 3.2–476.5 (median 19.8). The diagnostic accuracy of the tests, alone and in combination to predict liver fibrosis is shown in Table 3. The AUROC for ≥F2 was for FS, KS and FS + KS: 0.86, 0.79 and 0.82, respectively. The AUROC for ≥F3 was for FS, KS and FS + KS: 0.89, 0.83 and 0.86, respectively. The AUROC for cirrhosis (≥F5) for FS, KS and FS + KS was 0.97, 0.88 and 0.92, respectively. The AUROC curves of this analysis are shown in Figs 4–6. The best discriminator cut-offs to predict the worsening stages of liver fibrosis are shown in Table 4 together with sensitivity, specificity, PPV and NPV. The tests were also assessed to determine whether gender influenced their ability to predict significant fibrosis and cirrhosis. No statistical difference was demonstrated (data not shown). The tests were not good at the lower stages of fibrosis but were of increasingly accurate ≥Ishak F4.

Table 3. AUROC and 95% confidence intervals for FS, KS and FS + KS, according to Ishak fibrosis stage and AUROC comparison between tests

Ishak Stage	AUROC	95% CI	SE	*P*
F1				
FS	0.72	0.56–0.89	0.08	FS *vs* KS = 0.27
KS	0.84	0.75–0.93	0.05	FS *vs* FS + KS=0.16

FS + KS	0.84	0.75–0.92	0.04	KS *vs* FS + KS=0.85
F2				
FS	0.86	0.80–0.91	0.03	FS *vs* KS = 0.07
KS	0.79	0.72–0.86	0.04	FS *vs* FS + KS = 0.28
FS + KS	0.82	0.76–0.88	0.03	KS *vs* FS + KS = 0.003
F3				
FS	0.89	0.83–0.94	0.03	FS *vs* KS = 0.08
KS	0.83	0.77–0.89	0.03	FS *vs* FS + KS = 0.33
FS + KS	0.86	0.80–0.91	0.03	KS *vs* FS + KS= 0.006
F4				
FS	0.92	0.88–0.96	0.02	FS *vs* KS = <0.0001
KS	0.82	0.75–0.89	0.03	FS *vs* FS + KS = 0.002
FS + KS	0.85	0.79–0.92	0.03	KS *vs* FS + KS = 0.01
F5–6				
FS	0.97	0.94–0.99	0.01	FS *vs* FS + KS = 0.004
KS	0.88	0.82–0.94	0.03	FS *vs* FS + KS = 0.04
FS + KS	0.92	0.87–0.97	0.03	KS *vs* FS + KS = 0.004

FS, Fibroscan; KS, King's score, SE, standard error. **P*-value < 0.0001 for all tests for all stages of liver fibrosis.

Table 4. FS, KS and FS + KS cut-off values for the diagnosis of minimal fibrosis (F2–F6), significant fibrosis (F3–6) and cirrhosis (F5–6)

Method	Cut-off	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Prevalence	+LR	−LR
≥F2								
FS	6.75	68	91	96	49	74	7.6	0.6
KS	7.6	80	69	89	53	75	2.6	0.3
FS + KS	16.2	76	77	91	51	75	3.3	0.3
≥F3								
FS	8.85	74	88	85	78	49	6.3	0.3
KS	9.87	84	70	74	80	51	2.7	0.2
FS + KS	22.8	71	87	85	74	51	5.5	0.3
≥F5								

FS	10.05	93	88	68	98	22	7.4	0.07
KS	24.3	74	90	70	91	25	7.2	0.3
LSM + KS	26.1	86	79	58	94	25	4.2	0.2

FS, Fibroscan; KS, King's score.

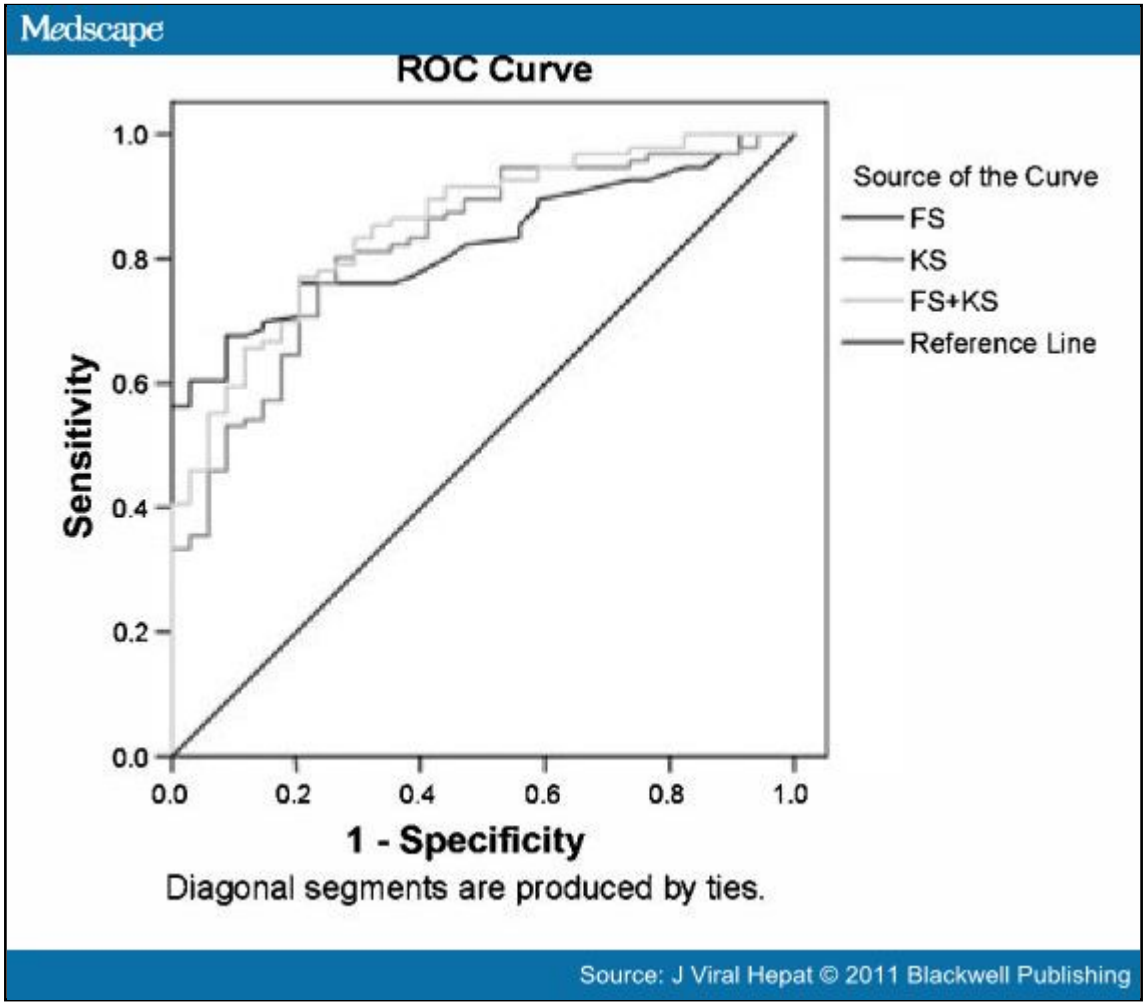

Figure 4. AUROC curve of Fibroscan (FS), King's score (KS) and combined FS + KS for the prediction of hepatic fibrosis stage ≥F2.

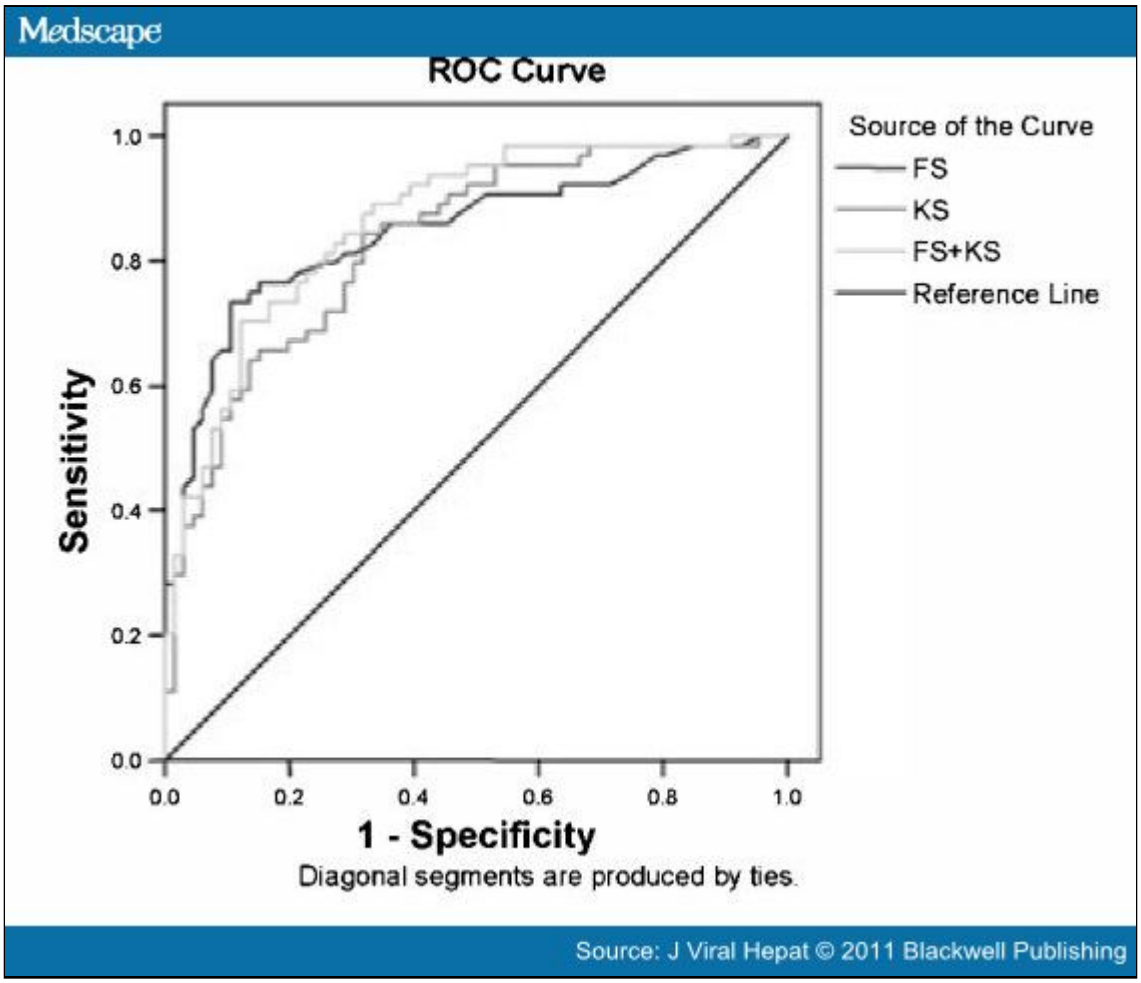

Figure 5. AUROC curve of Fibroscan (FS), King's score (KS) and combined FS + KS for the prediction of hepatic fibrosis stage ≥F3.

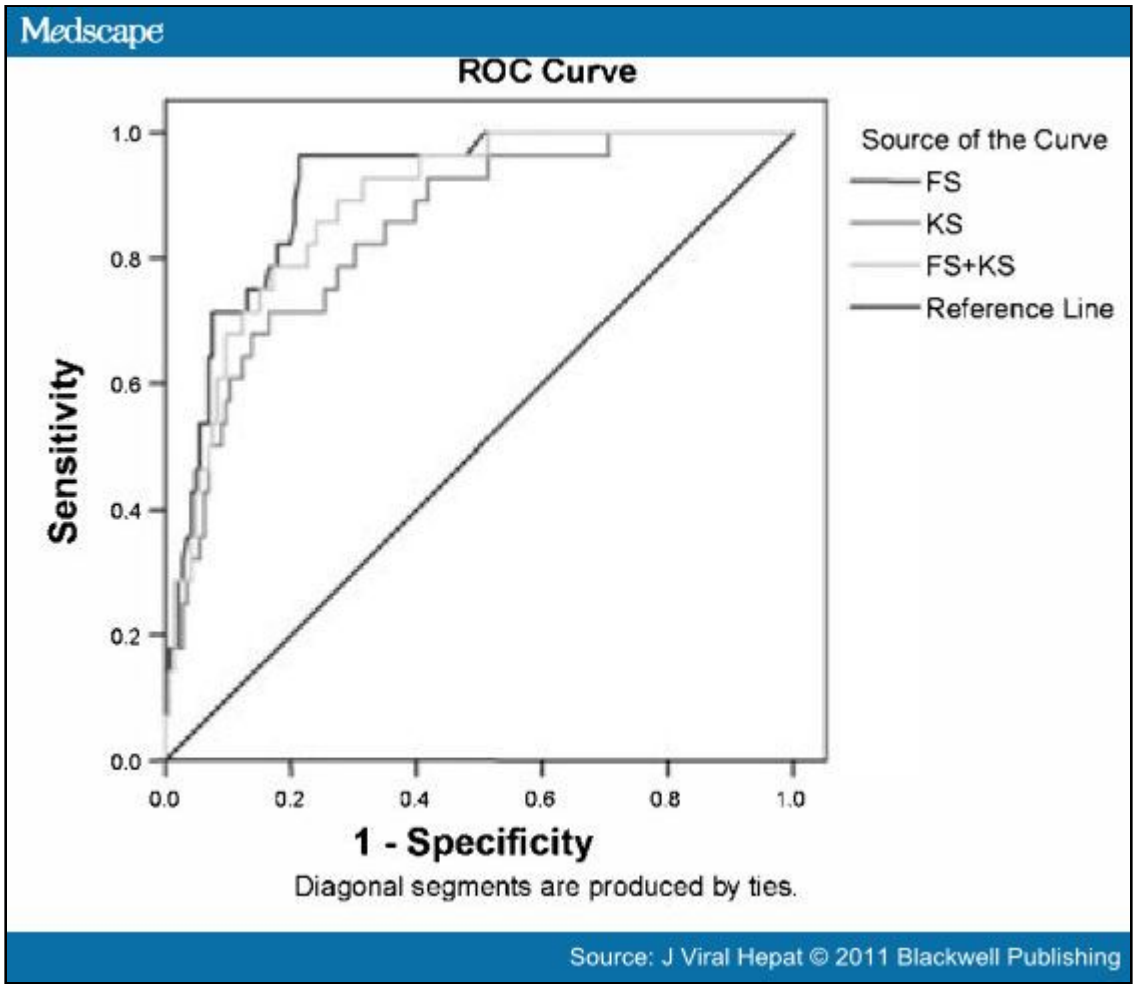

Figure 6. AUROC curve of Fibroscan (FS), King's score (KS) and combined FS + KS for the prediction of hepatic fibrosis stage F5–6 (cirrhosis).

Using METAVIR fibrosis (MF) stage significant fibrosis (≥MF2), the AUROC for FS, KS, and FS + KS were 0.89 (0.83–0.94, SE 0.03, *P* < 0.0001, standardized ROC = 0.89), 0.83 (0.77–0.89, SE = 0.03, *P* < 0.0001, Standardized ROC = 0.89) and 0.86 (0.80–0.91, SE 0.03, *P* < 0.0001, standardized ROC = 0.84), respectively. There was no statistical difference between the accuracy of FS *vs* KS (*P* = 0.08) alone. Using MF stage cirrhosis (MF4), the AUROC for FS, KS and FS + KS were 0.98 (0.96–0.99, SE 0.02, *P* < 0.0001, Ad AUROC = 0.98), 0.92 (0.88–0.97, SE = 0.01, *P* < 0.0001, Ad AUROC = 0.92) and 0.95 (0.92–0.98, SE 0.02, *P* < 0.0001, Ad AUROC = 0.92), respectively. There was a statistically significant difference between FS *vs* KS (*P* = 0.005).

Effect of Aspartate Aminotransferase on Accuracy of KS and Combined FS + KS

An analysis was performed to assess the impact of normal and elevated AST on the diagnostic accuracy of each noninvasive modality. Sixty-seven patients had normal AST level. The AUROC for ≥F3 fibrosis for FS, KS and FS + KS was 0.80 (0.65–0.95, *P* = 0.002), 0.83 (0.68–0.99, *P* = 0.001) and 0.88, respectively (0.75–1.0, *P* ≤ 0.0001). The AUROC for patients with elevated AST (*n* = 120) for ≥F3 for FS, KS and FS + KS was 0.84 (0.76–0.93, *P* < 0.0001), 0.79 (0.69–0.89, *P* < 0.0001) and 0.84 (0.75–0.92, *P* < 0.0001), respectively.

Sixty-seven patients had normal AST level with cirrhosis. The AUROC for ≥F5 fibrosis for FS, KS and FS + KS was 0.68 (0.54–0.79, *P* = 0.57), 0.96 (0.91–1.0, *P* = 0.12) and 0.94, respectively (0.88–1.0, *P* = 0.13). The AUROC for patients with elevated AST (*n* = 120) for ≥F5 for FS, KS and FS + KS was 0.89 (0.83–0.95, *P* < 0.0001), 0.78 (0.67–0.88, *P* < 0.0001) and 0.84 (0.76–0.92, *P* < 0.0001), respectively. The results are shown in Table 5.

Table 5. Performance of FS, KS and combined FS + KS in discriminating between significant fibrosis (F3–6) and cirrhosis (F5–6) according to normal or raised aspartate aminotransferase (AST)

Ishak Stage	AUROC	95% CI	SE	P
F3–6				
Normal AST				
FS	0.80	0.65–0.95	0.08	0.002
KS	0.83	0.68–0.99	0.08	0.001
FS + KS	0.88	0.75–1.0	0.07	<0.0001
Raised AST				
FS	0.84	0.76–0.93	0.04	<0.0001
KS	0.79	0.69–0.89	0.05	<0.0001
FS + KS	0.84	0.75–0.92	0.04	<0.0001
F5–6				
Normal AST				
FS	0.68	0.54–0.82	0.07	0.54
KS	0.98	0.93–1.0	0.02	0.11
FS + KS	0.96	0.89–1.0	0.03	0.12
Raised AST				
FS	0.96	0.92–1.0	0.18	<0.0001
KS	0.83	0.73–0.94	0.05	<0.0001
FS + KS	0.90	0.83–0.98	0.04	<0.0001

FS, Fibroscan; *N*, number; AUROC, area under the receiver operator characteristic curve; SE, standard error; F, fibrosis; KS, King's score.

Effect of Liver Biopsy on Accuracy of KS and Combined FS + KS

Finally, the authors assessed the effect of LB length on diagnostic accuracy. For a LB length <15 mm, the accuracy in detecting significant fibrosis (F3–F6) for FS, KS and FS + KS was 0.84 (0.70–0.98, SE 0.07, P = 0.001), 0.84 (0.70–0.98, SE 0.07, P = 0.001) and 0.87 (0.74–0.99, SE 0.07, P < 0.001), respectively *vs* FS, 0.81 (0.70–0.91, SE 0.05, P < 0.0001), KS, 0.83 (0.72–0.93, SE 0.05, P < 0.0001) and FS + KS0.86 (0.76–0.95, SE 0.05, P < 0.0001) for LBs >15 mm. For a LB length <15 mm, the accuracy in detecting cirrhosis (F3–F6) for FS, KS and FS + KS was 0.98 (0.93–1.0, SE 0.02, P = 0.001), 0.94 (0.87–1.0, SE 0.04, $P \leq 0.0001$) and 0.97 (0.92–1.0, SE = 0.03, P < 0.0001), respectively *vs* FS, 0.94 (0.88–1.0, SE = 0.03, P < 0.0001), KS, 0.82 (0.71–0.9, SE 0.06, P < 0.0001) and FS + KS 0.88 (0.79–0.97, SE 0.05, P < 0.0001) for LBs >15 mm.

Discussion

The United Kingdom is moving away from the decision to treat chronic hepatitis C based on the presence of moderate or severe fibrosis. In the United Kingdom, in August 2006, the National Institute of Clinical Excellence (NICE) issued updated guidance on treatment for patients with chronic hepatitis C infection permitting treatment

of all chronic HCV-infected patients to be offered anti-viral therapy irrespective of fibrosis stage dramatically reducing the need for LB.[23]

This study evaluated the performance of FS, KS and FS + KS in 187 consecutive patients with CHC. The diagnostic performance of each test was good with clinical utility (AUROC > 0.8) for all three tests alone and in combination across the range of fibrosis studied. The combined use of FS and KS offered the most accurate test for ≤F2, but this failed to reach statistical significance in comparison with FS or KS alone. For all stages of fibrosis ≥F3 fibrosis, FS alone was the best test. Twenty-seven per cent of patients had cirrhosis based on Ishak fibrosis, in comparison with 27%,[14] 25.1%[15] and 19.5%[13] reported in previous FS studies.

The AUROC curves and cut-offs from the present study compare favourably to those from previous studies. The AUROC for significant fibrosis and cirrhosis defined in the studies by Ziol, Castera and Foucher were 0.79 and 0.97, 0.83 and 0.95, and 0.80 and 0.96, respectively. The optimal cut-off value for ≥F3 (8.9 kPa) was similar to those described (Foucher, 8.1 kPa, Ziol, 8.7 kPa; and Castera, 7.1 kPa), but the cut-off for cirrhosis (10.1 kPa) was lower than that described in similar studies (Foucher, 17.6 kPa; Ziol, 14.5 kPa; and Castera, 12.5 kPa). At the less severe grades of fibrosis, the PPV was high, whereas for cirrhosis the noninvasive tests were stronger with a high NPV. The main strength of FS is its high PPV and specificity at lower stages of fibrosis and its high NPV together with excellent sensitivity and specificity in detecting cirrhosis. The KS has the advantage that no additional equipment is required, and it is quick and easy to calculate. This may be particularly advantageous in parts of the world where FS is not routinely available. But, the test is not without its problems. There may not be standardization between different assays for AST, INR and platelets used between hospitals. Thus, the cut-off derived from one institution may differ from that of a neighbouring institution. In addition, age is taken as a surrogate marker of disease duration, and this assumption may be prone to error. Furthermore, medication or conditions that lead to prolongation of the INR, e.g. warfarin treatment, or low platelets or other factors that may lead to a rise in AST (e.g. steatohepatitis) may lead to an inaccurate assessment of disease severity. The problems with standardization of routine biochemical, haematological and coagulation tests have been described previously.[19]

The use of LB in chronic HCV is declining. Not only is LB associated with complications including bleeding, pain, pneumothorax, haemothorax, bile peritonitis, haemobilia, kidney and intestinal puncture and death,[5,24] but also is associated with problems of sampling error because a LB sample is only 1/50 000th of total liver volume. Regev *et al.* [7] showed a difference of 33% in fibrosis stage in left and right lobe LBs, while Siddique *et al.* [25] showed that even from biopsies taken from the same puncture site, 45% of patients could display at least one fibrosis stage of difference. Further problems occur because of intra-and inter-observer variability assessing fibrosis stage.[26] The length of LB can also be crucial in determining diagnostic accuracy. Regev showed only 65% of 15 -mm-staged liver fibrosis stage correctly, this increasing to 75% for 25mm sample. The length of LB may also be significant because fibrosis is likely to be under-staged as LB length diminishes.[9] More recently, work has been performed showing that length of LB and the number of fragments should be taken into account when evaluating the diagnostic accuracy of a test using AUROC curves.[19] This will obviously have an effect on the accuracy of LB as the gold standard and may explain in part why no test is likely to be perfect. For this reason, a sensitivity analysis on the effect on LB length above or below 15 mm was performed. This failed to demonstrate any statistical difference between the two groups in this study population. A potential advantage of FS is that it effectively samples an area of liver stiffness that is approximately of 1 cm diameter and 2 cm long, leading to a volume 100 times larger than a standard LB specimen.

As has been confirmed from previous studies, AST appears to have an impact on FS readings with elevation of transaminases being mirrored by increase in liver stiffness. It has been postulated that high transaminases are a reflection of hepatitis activity, and thus points to oedema and inflammatory infiltrate contributing to the transient elastographic measure.[27] For patients with normal AST, the KS had greater diagnostic accuracy than FS alone. However, the majority of patients had abnormal AST and thus, overall FS performed better, particularly for cirrhosis. Sebastiani *et al.* assessed the performance of AST-to-platelet ratio index (APRI), Forn's index, AST-to-ALT ratio (AAR), Fibrotest and Fibroindex in CHC patients with normal (n = 80) and elevated ALT (n = 164) in discriminating patients with significant fibrosis (METAVIR ≥ F2).[28] They found that performance across

all the tests was better in patients with elevated ALT. Interestingly, our study showed that KS was less accurate in patients with elevated AST. A possible explanation is that patient age and prolonged INR may counterbalance the effect of a lower AST, thereby preserving accuracy.

In summary, FS and KS have clinical utility in identifying patients with significant fibrosis and cirrhosis. For the lower stages of fibrosis (≤F2), the combination of FS + KS had the best AUROC curve, but it was not statistically different to FS or KS alone. FS alone was the best test for predicting ≥F3 fibrosis. FS measures liver stiffness and not fibrosis, and thus factors that cause inflammation or add to oedema may elevate FS readings. All tests perform better at the more severe scale of fibrosis, and overall FS is a superior test, but in circumstances where FS is not available, KS is a suitable alternative.

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Abbreviations

AAR, AST-to-ALT ratio; ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; AUROC, area under receiver operator characteristic curve; BMI, body mass index; CHC, chronic hepatitis C; FS, Fibroscan; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalized ratio; MF, METAVIR fibrosis; KS, King's score; LB, liver biopsy; PPV, positive predictive value; NPV, negative predictive value; KCH, King's College Hospital; RFH, Royal Free Hospital; ROC, receiver operator characteristic.

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