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Prospective evaluation of transient elastography for the diagnosis of hepatic fibrosis in Asians: comparison with liver biopsy and aspartate transaminase platelet ratio index

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SUMMARY

Background
Transient elastography (TE) is a reliable non-invasive predictor of hepatic fibrosis, but data on TE in Asians are limited.

Aim
To evaluate prospectively the accuracy of TE for diagnosis of hepatic fibrosis in Asians compared with APRI (aspartate transaminase to platelet ratio index).

Methods
One hundred and twenty consecutive patients who underwent liver biopsy were enrolled. TE (Fibroscan) was performed by two independent operators. Fibrosis was graded by two independent pathologists using the METAVIR classification. Area under receiver operating curves (AUROC) were used to evaluate the accuracy of TE and APRI in diagnosing significant fibrosis (F ≥ 2) and cirrhosis (F4).

Results
Predominant aetiologies were hepatitis B (48%), non-alcoholic steatohepatitis (14%) and hepatitis C (8%). TE was unsuccessful in five patients (4.2%) because of small inter-costal space (three patients), obesity and ascites. There was good correlation between TE and fibrosis (r = 0.606). AUROC for diagnosis of significant fibrosis was 0.856 (95% CI 0.779–0.932) for TE and 0.673 (95% CI 0.568–0.777) for APRI. AUROC for diagnosis of cirrhosis was 0.924 (95% CI 0.857–0.990) for TE and 0.626 (95% CI 0.437–0.815) for APRI. Optimal TE value was 9.0 kPa for diagnosis of significant fibrosis and 16.0 kPa for cirrhosis with specificity/sensitivity/PPV/NPV/accuracy of 82.6%/85.2%/80.9%/86.7%/84.1% and 88.9%/82.7%/32.0%/98.8%/83.2%, respectively.

Conclusions
Transient elastography is a reliable predictor of hepatic fibrosis in Asians. Failure of TE in Asians is commonly because of small inter-costal space. TE is superior to APRI for non-invasive diagnosis of hepatic fibrosis and cirrhosis.

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BACKGROUND

Early identification of hepatic fibrosis is important to guide treatment decisions in the management of patients with chronic liver disease. In patients with chronic viral hepatitis, histological staging of fibrosis is recommended by the American and European Associations for the Study of Liver Diseases to identify patients at risk of progressive liver disease and those eligible for anti-viral therapy.[1–3] In patients with non-alcoholic fatty liver disease (NAFLD), the presence of hepatic fibrosis provides important prognostic information for the management of the patient.[4] The accepted gold standard for diagnosis of hepatic fibrosis is with a liver biopsy.[5] However, the limitations of liver biopsy are well documented – it is an invasive procedure with a small risk of morbidity and mortality (up to 3% and 0.03% respectively); it is prone to sampling error as well as inter- and intra-observer variations in histological interpretation and it is poorly accepted by patients, especially for repeat assessments.[6–11]

These limitations fuel the need to find alternative, non-invasive methods of assessing hepatic fibrosis that are reliable, accurate and importantly, acceptable to the patient. This need is more pressing presently in view of promising developments in anti-fibrotic therapies, which will increase the emphasis on non-invasive serial assessments of hepatic fibrosis to assess regression of fibrosis. Several simple serum markers have been reported to be useful surrogate markers of hepatic fibrosis. These include the aspartate transaminase to alanine transaminase (AST/ALT) ratio (AAR),[12] AST to platelet ratio index (APRI),[13] age-platelet count index (API)[14] and many others. Among these, APRI has been widely studied because of its simplicity and universal applicability. APRI has been reported to be capable of predicting significant fibrosis and cirrhosis in 51% and 81% of patients with chronic hepatitis C (CHC), respectively.[13] The predictive ability of non-invasive surrogate markers was improved using algorithms involving a combination of simple and specialized serum markers (e.g. Forns Index,[15] FibroTest,[16, 17] FibroIndex,[18] Fibrosure,[19] etc.). However, the widespread applicability of these tests is limited, as they are proprietary tests involving complex mathematical calculations. Furthermore, their accuracy is affected by concomitant medical conditions including haemolysis, cholestasis and transaminitis.

Recently, the measurement of liver stiffness by transient elastography (TE) has been shown to be an accurate predictor of histological fibrosis in patients with CHC.[20–22] TE has also been validated as a reliable predictor of fibrosis in patients with primary biliary cirrhosis, primary sclerosing cholangitis,[23] HIV/HCV co-infection[24] and hepatitis C recurrence after liver transplantation.[25] The experience with TE is less extensive in chronic hepatitis B (CHB), as most studies on TE were performed in Western populations where CHC is predominant. Some of these studies included subpopulations of patients with CHB infection, but the number of these patients was small.[26–28] These studies suggest that TE performs equally well in the prediction of hepatic fibrosis in patients with various aetiologies of liver disease. However, validation studies in these homogenous patient groups have resulted in a varied spectrum of different diagnostic cut-off values. As such, it is difficult to implement a standardized cut-off value for diagnosis of significant fibrosis by TE in a heterogenous clinical setting. More information is needed in this field to assess the diagnostic accuracy of TE, especially in different patient populations and different aetiologies of liver disease, before valid recommendations can be made towards the adoption of TE in routine clinical practice.

The aim of our study was to validate prospectively the accuracy of TE in the diagnosis of hepatic fibrosis in an Asian population with various aetiologies of liver disease, in comparison with APRI using liver biopsy as the gold standard.

PATIENTS AND METHODS

We prospectively studied 120 consecutive patients who were admitted for liver biopsy to the Department of Gastroenterology and Hepatology, Singapore General Hospital between January 2006 and July 2007. Indications for liver biopsy included histological assessment of fibrosis prior to initiation of anti-viral treatment for chronic viral hepatitis, staging of fibrosis in patients with suspected non-alcoholic steatophepatitis (NASH) and histological confirmation of patients with clinically suspected autoimmune hepatitis, primary biliary cirrhosis or unexplained transaminitis. We included a group of patients with no known underlying liver disease who underwent hepatic surgery for various indications. The purpose of recruiting this group was to include patients with no expected fibrosis, to evaluate the accuracy of TE across all

fibrosis grades, thus avoiding selection bias favouring patients with established fibrosis. Similarly, we included a group of patients with cirrhosis who were scheduled for resection of hepatocellular carcinoma (HCC). Patients with decompensated cirrhosis (significant ascites, encephalopathy or variceal bleeding) were excluded from the study. Patients with viral hepatitis (CHC or CHB) were all treatment-naïve. Patients with HCC were all in Child Pugh class A, with single tumours <3 cm in diameter. Informed consent was obtained from all patients prior to entry into the study. The study was approved by the Institutional Review Board of the Hospital.

All patients underwent TE measurement by FibroScan (Echosens, Paris) within 24 h of their liver biopsy or surgery. Details of the mechanics of TE have been previously described.[21] Briefly, an ultrasonic transducer probe mounted on the axis of a vibrator is placed in the inter-costal space over the right lobe of the liver. A vibration pulse of mild amplitude and low frequency is transmitted to the liver by the transducer, inducing an elastic shear wave through the liver. Pulse-echo ultrasonic acquisitions are simultaneously performed to follow the propagation of the shear wave and measure its velocity, which provides an indication of the stiffness of the liver. The faster the shear wave propagates, the stiffer the liver. Each vibration pulse provides a liver stiffness measurement (LSM) measured in kilopascals, which indicates the stiffness of the liver. The TE result is reported as the median value of the LSMs.

Each patient underwent two sets of TE measurements. These were performed by two independent, trained operators who were blinded to the result obtained by the other. The measurement was performed over the right lobe of the liver with the patient lying in the supine position, with the right arm fully abducted. At least 10 valid TE readings were measured in each patient, and the median LSM value was recorded. Measurements with a success rate of <60% were deemed as failures and excluded from analysis. We considered the median value of LSM to be representative only if the inter-quartile range (IQR) was <30% of the median value and those with IQR >30% median were excluded from analysis.

Biochemical and clinical parameters were obtained from the patients during the admission for the liver biopsy or surgery. Variables recorded included patient age, gender, aetiology of liver disease, indication for liver biopsy or surgery, body mass index (BMI), liver function test, serum creatinine and haematological indices including platelet count and prothrombin time. APRI was calculated according to the formula: $[(\text{AST}/\text{ULN})/(\text{Platelet count} \times 10^9/\text{L})] \times 100$.[13] APRI values ≤0.50 and >1.50 were evaluated for prediction of significant fibrosis and APRI values of ≤1.00 and >2.00 were evaluated for prediction of cirrhosis.[13]

Liver biopsy was performed via the percutaneous route in 95 patients (79%). This was performed using 16G disposable biopsy needles (Hepafix, Braun, Germany) via the standard Menghini technique. Radiologically guided biopsies were obtained in 12 patients (10%), via transjugular route in four patients (3%) and during surgery in nine patients (8%). The liver specimens (median length 15 mm, range 2–40 mm) were stained with H&E and Sirius Red for assessment of fibrosis. Only specimens with more than five portal tracts were accepted for analysis. The specimens were graded for fibrosis according to the METAVIR classification[6]: no fibrosis (F0), portal fibrosis without septa (F1), portal fibrosis with few septa (F2), numerous septa without cirrhosis (F3), cirrhosis (F4). The METAVIR classification was chosen, as it provides a reliable classification of fibrosis across aetiologies and also to maintain consistency with other studies. The specimens were read by two independent, experienced histopathologists who were blinded to the LSM result. Cases in which there was a disparity in fibrosis grade between the two pathologists were resolved by consensus agreement. Steatosis was graded by the histopathologist as none, mild (<30%) or severe (≥30%).

Statistical analysis

All statistical analyses were performed using SPSS software (version 14; SPSS Inc., Chicago, IL, USA). Bivariate Spearman rank correlation coefficient was used to analyse the correlation between LSM and histological fibrosis grade. Qualitative and quantitative differences between subgroups were compared using Mann–Whitney *U*- and Student's *t*-test (including one-way ANOVA where appropriate) respectively. The degree of inter- and intra-observer variability in TE measurement and pathological grading was assessed using the kappa statistic. The diagnostic performance of TE and APRI were assessed by receiving operator characteristic (ROC) curves. The area under the ROC curves (AUROC) was used to assess the accuracy of the diagnostic test and to identify optimal cut-off points for the diagnosis of significant fibrosis (defined as METAVIR score

F ≥ 2) and cirrhosis (METAVIR score F4). Optimal cut-off values were chosen based on a maximum sum of sensitivity and specificity, and positive and negative predictive values (PPV and NPV) and accuracy of the chosen cut-off value for each group was calculated. A two-sided *P*-value of <0.05 was deemed significant.

RESULTS

Patient characteristics

A total of 120 patients were enrolled in the study. Demographic characteristics, biochemical parameters and disease aetiologies are summarized in Table 1. There were 69 males (57.5%) and 51 females, with a median age of 49.5 years. Mean BMI was 24.0 kg/m^2. The aetiology of liver disease was CHB in 58 patients (48.3%), NAFLD in 17 (14.2%) and CHC in nine (7.5%). The aetiology of liver disease in the remaining patients included autoimmune hepatitis, primary biliary cirrhosis, drug-induced hepatitis, alcoholic hepatitis and HCC (Table 1). Four of the five HCC patients had underlying CHB infection, the aetiology of liver disease in the remaining patient was not known. Eighteen patients (15%) were classified under miscellaneous aetiology – these included haemangioma, benign liver cysts, liver metastases, graft-versus-host disease, Wilson disease, CMV hepatitis, nonspecific hepatitis and nodular regenerative hyperplasia.

Liver stiffness and relationship with histological fibrosis grade

A total of 240 TE measurements were performed for the 120 patients enrolled. Five patients (4.2%) were excluded from the analysis because of failure to obtain a satisfactory TE result (success rate <60%). Three of the failures were because of narrow inter-costal spaces in small-sized females (mean BMI 20.8), one because of obesity (BMI 34.4) and one for reasons of ascites. Median duration of TE examination was 4.3 min (range 1.6–17.5 min). LSM readings ranged from 3.8 to 58.1 kPa, with a mean success rate (valid/total LSM readings) of 84.6% and a median IQR of 16% (range 5.1–56.3%). Eight patients had IQR >30% and were excluded from the analysis. The distribution of METAVIR scores are shown in Table 1. In all, 55.8% of patients had no or mild fibrosis (F0 or F1 respectively) and 44.2% had significant fibrosis and cirrhosis (F2–F4).

Table 1. Baseline patient characteristics (n = 120)

Demographics	
Male gender	69 (57.5%)
Age (years)	49.5 (20–79)
Race (% Chinese/Malay/Indian/Others)	86.7/8.3/4.2/0.8
Body mass index (kg/m^2)	24.0 (13.8–36.3)
Aetiology of liver disease (%)	
Chronic hepatitis B	58* (48.3)
Non-alcoholic fatty liver disease	17 (14.2)
Autoimmune hepatitis/primary biliary cirrhosis	10 (8.3)
Chronic hepatitis C	9 (7.5)
Drug-induced liver injury	5 (4.2)
Hepatocellular carcinoma (HCC)	5* (4.2)
Alcoholic hepatitis	2 (1.7)
Miscellaneous	18 (15.0)
Biochemical parameters	
Albumin (g/L)	38 (18–47)
Bilirubin (μmol/L)	15 (4–501)
Alkaline phosphatase (U/L)	85 (8–1083)
Alanine transaminase (U/L)	79.5 (12–1336)
Aspartate transaminase (U/L)	57 (17–1249)
Gamma glutamyl transferase (U/L)	66 (10–1359)
Alfafetoprotein (U/L)	4.4 (1–518)
Creatinine (mmol/L)	80 (41–1103)
Haemoglobin (g/dL)	13.7 (8.3–16.8)
Platelets (10^3/mm^3)	217 (19–638)
Prothrombin time (s)	10.0 (8.8–18.5)
METAVIR stage (%)	
F0	28 (23.3)
F1	39 (32.5)
F2	21 (17.5)
F3	20 (16.7)
F4	12 (10)

Values presented as n (%) or median (range), unless otherwise indicated.
* Four patients with HCC had underlying chronic HBV, the aetiology was not known in one patient.

The median LSM value correlated well with histological fibrosis grade (Spearman correlation coefficient, r = 0.606, P < 0.001). Inter-observer agreement for TE was good (r = 0.939, P < 0.001), with a kappa co-efficient of 0.71 between the two operators. There was 94% agreement between the two pathologists in the METAVIR grading (kappa co-efficient 0.84). In the seven cases where they disagreed, the discrepancy involved only one METAVIR grade (five were between F0 and F1, and two between F2 and F3). For those cases, they subsequently reviewed the specimens using Orcein and Victoria blue stains to reach a consensus.

Table 2. LSM according to liver fibrosis groups (n = 120)

	Group 1	Group 2	Group 3
Fibrosis groups	No significant fibrosis	Significant fibrosis	Cirrhosis
METAVIR	F0–F1	F2–F3	F4
No. patients (%)	67 (55.8%)	41 (34.2%)	12 (10%)
TE failures	2	1	2
IQR > 30% median	4	3	1
Median LSM score	6.9	12.2	24.8
Minimum LSM	3.8	4.7	11.9
25th percentile LSM	5.6	9.6	19.5
75th percentile LSM	8.1	19.0	39.4
Maximum LSM	29.5	42.1	58.1

LSM, liver stiffness measurements; TE, transient elastography; IQR, inter-quartile range.

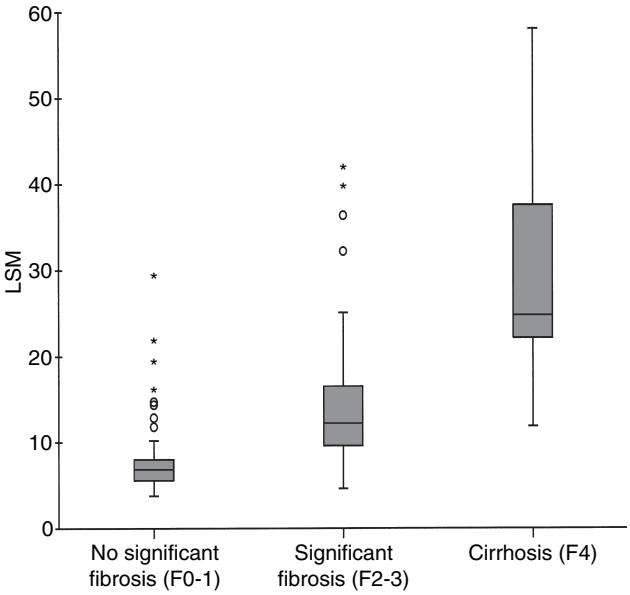

Figure 1. Boxplot of liver stiffness measurement (LSM) according to fibrosis groups.

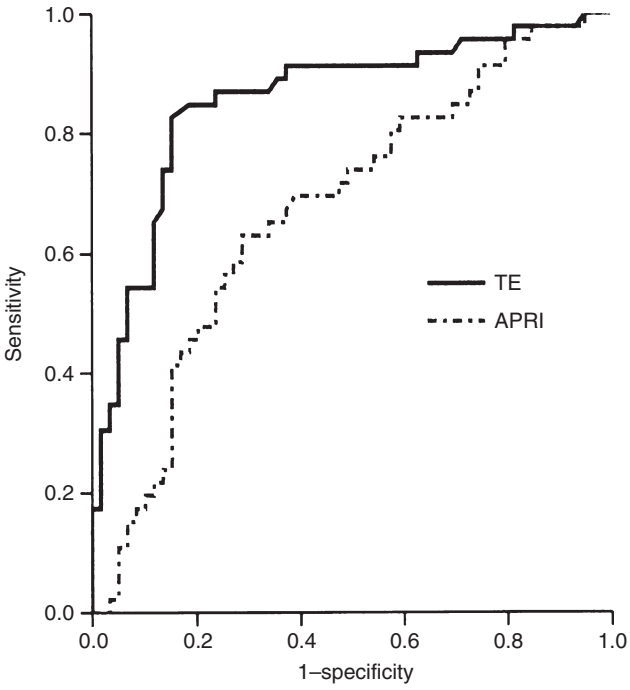

Figure 2. Receiving operator characteristic curves of transient elastography and aspartate transaminase to platelet ratio index for predicting significant fibrosis.

From a clinical perspective, the primary diagnostic utility of TE would be its ability to identify patients with significant fibrosis (defined as METAVIR F ≥ 2) and those with cirrhosis (METAVIR F4). Hence, the diagnostic accuracy of TE was assessed in three groups of patients – those with no clinical fibrosis (METAVIR F0–F1), those with significant fibrosis (METAVIR F2–F3) and a third group with cirrhosis (METAVIR F4). The corresponding LSM values and ranges for these three groups are shown in Table 2, and are graphically represented in Figure 1.

AUROC analysis for TE

The AUROC for significant fibrosis (METAVIR F ≥ 2) was 0.856 (95% CI 0.779–0.932) and the optimal cut-off value for the identification of significant fibrosis was 9.0 kPa (Figure 2). The corresponding AUROC for cirrhosis (METAVIR F4) was 0.924 (95% CI 0.857–0.990) with an optimal cut-off value identified as 16 kPa (Figure 3). The sensitivity, specificity, PPV, NPV, positive likelihood ratio, negative likelihood ratio, and accuracy for the chosen cut-off limits are presented in Table 3.

The LSM value of >9.0 kPa correctly identified 38 of the 46 patients with METAVIR F ≥ 2 (82.6% sensitivity). Of the eight patients who were incorrectly classified, five had F2 fibrosis and three had F3 fibrosis, with LSM values ranging from 4.7 to 9.0 kPa. Of the 47 patients who had a LSM >9 kPa, 38 were correctly identified to have significant fibrosis (80.9% PPV). In total, 67 patients did not have significant fibrosis on histology (F0 or F1). Nine of these patients had inaccurately elevated LSM

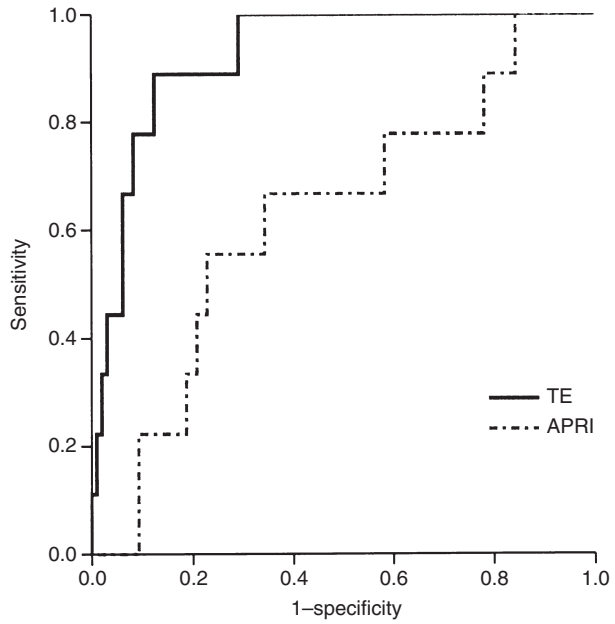

Figure 3. Receiving operator characteristic curves of transient elastography and aspartate transaminase to platelet ratio index for predicting cirrhosis.

readings ranging from 10.2 to 29.5 kPa (three with METAVIR F0 and six with METAVIR F1). Univariate analysis was performed to attempt to identify factors related to the false elevation of LSM values in this subset of patients with no significant fibrosis. This was felt to be of clinical importance, as this would be the subgroup of patients who may have been unnecessarily commenced on anti-viral treatment for CHB or CHC based on the TE result. Mean bilirubin (22.3 ± 32.7 vs. 71.6 ± 145.8 U/L, $P = 0.026$), ALT (89.2 ± 49.8 vs. 223.6 ± 362.2 U/L, $P = 0.01$), AST (61.0 ± 40.1 vs. 193.5 ± 341.9 U/L, $P = 0.008$) and PT (9.9 ± 0.5 vs. 10.6 ± 1.5 s, $P = 0.026$) were significantly different between those correctly diagnosed and those inaccurately diagnosed by TE. BMI and steatosis were not found to be significantly associated with TE inaccuracy. However, when subjected to multivariate analysis, none of the above factors was identified as an independent predictor of TE inaccuracy.

The diagnostic performance of 16 kPa as the selected cut-off for the diagnosis of cirrhosis is shown in Table 3. This cut-off value maintained adequate sensitivity (88.9%) and specificity (82.7%), correctly identifying eight of nine patients with F4 METAVIR grade on histological analysis and excluding 81 of 98 patients with METAVIR <F4. An LSM of <16 kPa was 98.8% accurate in predicting the absence of cirrhosis in 81 of 82 patients. However, it fared poorly in correctly identifying patients with cirrhosis (32.0% PPV). This was because of an elevated LSM value beyond

Table 3. Diagnostic performance of TE and APRI to detect significant fibrosis (F ≥ 2) and cirrhosis (F4)

	Significant fibrosis (F ≥ 2)		Cirrhosis (F4)	
	TE	APRI	TE	APRI
Optimal cut-off value	9.0 kPa	>1.50	16.0 kPa	>2.00
AUROC (95% CI)	0.856 (0.779–0.932)	0.673 (0.568–0.777)	0.924 (0.857–0.990)	0.626 (0.437–0.815)
Sensitivity (%)	82.6	28.6	88.9	18.2
Specificity (%)	85.2	83.6	82.7	87.9
Positive predictive value (%)	80.9	58.3	32.0	14.3
Negative predictive value (%)	86.7	59.3	98.8	90.6
Positive likelihood ratio	5.581	1.744	5.139	1.504
Negative likelihood ratio	0.204	0.854	0.134	0.931
Accuracy (%)	84.1	59.1	83.2	80.9

TE, transient elastography; APRI, aspartate transaminase to platelet ratio index; AUROC, area under receiver operating curves.

16 kPa in 19 patients who did not have cirrhosis on histology and two cirrhotic patients who had LSM <16 kPa (11.7 and 11.9 kPa).

AUROC analysis for APRI

The correlation between APRI and METAVIR fibrosis was weak, but significant (correlation coefficient 0.286, $P = 0.002$). APRI >1.50 accurately identified the presence or absence of significant fibrosis in 59.1% of patients, and was chosen as the optimal cut-off for prediction of the presence of significant fibrosis. Similarly, APRI >2.00 was accurate for prediction of presence of cirrhosis in 80.9%. The AUROC for detection of significant fibrosis by APRI was 0.673 (95% CI 0.568–0.777) compared to 0.856 (0.779–0.932) for TE. The corresponding AUROC for cirrhosis was 0.626 (95% CI 0.437–0.815) for APRI compared to 0.924 (0.857–0.990) for TE (Table 3).

Agreement between TE and APRI

Based on the chosen cut-off values for diagnosis of significant fibrosis, TE agreed with APRI in 65 of 105 patients (61.9%) (Table 4a). Of the 32 patients diagnosed with significant fibrosis by TE but not by APRI, 25 had METAVIR F ≥ 2 on liver biopsy. Eight patients were classified as having significant fibrosis by APRI but not by TE, but only one had METAVIR F ≥ 2 on liver biopsy. Hence, in the group of 40 patients where there was discordance between APRI and TE in the diagnosis of significant fibrosis, TE correctly classified 32 patients (80%) when compared against the gold standard (liver biopsy) in contrast to APRI, which was correct in only 20%.

The agreement between TE and APRI was better for the diagnosis of cirrhosis, agreeing in 83 of 105 cases (79%) (Table 4b). TE and APRI failed to agree in the diagnosis of cirrhosis for 22 patients. Seventeen patients were classified as cirrhotic by TE, but not by APRI. Of these, 11 patients were correctly diagnosed by APRI (no cirrhosis on histology), but not by TE, and six cirrhotics were correctly identified by TE, but not by APRI. Five patients were classified as cirrhotic by APRI although they had LSM <16 kPa. Histological review revealed the absence of cirrhosis in all five patients. Hence, APRI was more accurate than TE in identifying patients with cirrhosis, but TE was more accurate in excluding cirrhosis.

Table 4. Comparison between TE and APRI in the diagnosis of significant fibrosis and cirrhosis

	Diagnosis of significant fibrosis by APRI		
	Fibrosis absent (APRI ≤ 1.5)	Fibrosis present (APRI > 1.5)	Total
(a) Significant fibrosis			
Diagnosis of significant fibrosis by TE			
Fibrosis absent (LSM ≤ 9)	50	8	58
Fibrosis present (LSM > 9)	32	15	47
Total	82	23	105
	Diagnosis of cirrhosis by APRI		
	Cirrhosis absent (APRI ≤ 2.0)	Cirrhosis present (APRI > 2.0)	
(b) Cirrhosis			
Diagnosis of cirrhosis by TE			
Cirrhosis absent (LSM ≤ 16)	75	5	80
Cirrhosis present (LSM > 16)	17	8	25
Total	92	13	105

TE, transient elastography; APRI, aspartate transaminase to platelet ratio index.

DISCUSSION

On the basis of this prospective analysis of 120 Asian patients, we have found that TE using Fibroscan is a reliable, non-invasive method for identification of patients with significant hepatic fibrosis. TE is readily reproducible and its measurement is operator-independent, with low inter- and intra-observer variability. We have identified the value of 9.0 kPa as the optimal cut-off for the non-invasive diagnosis of clinically significant fibrosis (METAVIR ≥ F2) in Asian patients, with an AUROC of 0.856 (95% CI 0.779–0.932), sensitivity of 82.6%, specificity of 85.2%, PPV and NPV of 80.9% and 86.7% respectively.

The results of our study are comparable with those of the previous studies of TE.[21–28] In a recent systematic review assessing TE for prediction of F ≥ 2 fibrosis, a combined AUROC of 0.83 (95% CI 0.02–

1.00) with summary sensitivity of 63.8% (95% CI 49.6–75.9%) and specificity of 86.5% (95% CI 79.8–91.2) at a cut-off threshold of 7.1–8.8 kPa was reported.[29] Hence, our results demonstrate that TE maintains its accuracy in Asian patients with hepatic fibrosis from different aetiologies.

In comparison with previous studies on TE, our study provides several unique findings. First, we provide information on the diagnostic accuracy of Fibroscan in an Asian population with variable aetiologies of liver disease. TE has been well studied as a predictor of hepatic fibrosis and cirrhosis predominantly in Western populations with CHC. In a systematic review of Fibroscan for prediction of CHC-related fibrosis, only two of the 11 studies identified were performed in predominantly Asian populations.[29] While the process of CHC-related fibrosis is not expected to be different between Western and Asian populations, several inherent differences may affect the accuracy and clinical performance of TE. As the average BMI in the Asian population is lower than that of the Western population, the problems of obesity limiting the success of TE can be expected to be lower. Obesity (BMI > 28) has been consistently reported as a factor limiting the success of TE measurements in western studies.[22, 30, 31] In our study, despite obtaining a failure rate similar to Western studies,[31] we encountered only one TE failure because of obesity (BMI > 34). Conversely, we found that in Asians, narrow inter-costal space is an important factor contributing to failure to obtain a TE reading. This is especially true in small-sized Asian females. This may represent a potential limitation when using the standard Fibroscan probe (which was designed for the Western population) in small-sized Asian patients.

Second, a majority of our study population had underlying CHB infection. There are limited data on TE for the assessment of hepatic fibrosis in patients with CHB. Coco *et al.*[28] reported their experience with TE in an Italian cohort, which included 79 patients with CHB. Fraquelli *et al.*[30] included 16 patients with CHB in a study performed in a similar population. Some information about TE in Asian patients with CHB was provided by Ogawa *et al.*,[27] who studied the diagnostic performance of TE in 68 Asian patients with CHB and Kawamoto *et al.*,[32] who included 10 patients with CHB in their study. While the underlying mechanism of progressive fibrosis in chronic viral hepatitis is expected to be similar, several differences between patients with CHB and patients with CHC may affect the diagnostic accuracy of TE. First, patients with CHB more commonly experience wide fluctuations in transaminase levels because of disease activity, whereas the levels are relatively more stable in CHC. At least two studies have proposed that significant hepatic necroinflammatory activity affects the accuracy of TE.[28, 30] Second, regenerative nodules in CHB are generally larger with more widespread piecemeal necrosis, which may affect the elastic property of the liver.[33] Third, patients with CHC (but not CHB) often have a component of hepatic steatosis, which may affect the LSM reading.[30] Hence, the direct application of validated TE results from a CHC population to a CHB population may result in inaccuracy. Thus, more data are required before TE can be applied in the routine management of CHB patients. In our study, 58 patients (48.3%) had CHB, thus providing an important contribution to the growing information in this specific patient population. In a separate analysis of the CHB cohort (n = 58), the AUROC for F ≥ 2 fibrosis was 0.870 (95% CI 0.774–0.967) with sensitivity 90.9%, specificity 73.3%, PPV 71.4%, NPV 91.7% at a cut-off threshold of 8.3 kPa.

Our study was designed to include sufficient numbers of patients without significant hepatic fibrosis (F0 and F1). This was to assess the accuracy of TE in patients without fibrosis and to investigate the reasons for inaccurate elevations of TE in these patients. We found that even amongst patients without fibrosis (i.e. METAVIR F0), TE readings may be elevated in up to 10% (three of 28), possibly because of various factors such as marked transaminitis (ALT and AST > 1000 U/L) in a patient with LSM of 11.1 kPa, diffuse macrovesicular steatosis in another patient with LSM of 10.2 kPa and hypoalbuminemia (albumin 25 U/L) in the third patient with a LSM of 19.4 kPa.

Several factors were found to affect the accuracy of TE for diagnosis of significant fibrosis. These included hyperbilirubinaemia, transaminitis, and prolonged prothrombin time. These factors were found to cause a significant overestimation of TE values, leading to false positive identification of fibrosis. However, on multivariate analysis, no single predictive factor was identified. The effect of transaminitis on accuracy of TE has been previously reported.[28] In this study by Coco *et al.*, TE values were elevated during flares of transaminitis in patients without fibrosis, and subsequently returned to normal after resolution of the hepatitic event. The authors postulated that the inflammatory infiltrate and oedema in the liver

during episodes of hepatitis exacerbations may have an impact on the TE value. In our study, ALT levels beyond five times the upper limit of normal were found to be significantly correlated to the inaccuracy of TE ($P = 0.001$), whereas the relationship was not significant at lower levels of ALT elevation. Similarly a bilirubin level >100 μmol/L correlated significantly with inaccurate diagnosis of significant fibrosis by TE ($P = 0.01$). This may be because of transient increased stiffness of the liver caused by parenchymal collapse during massive necrosis and cholestasis. Hence, we advise caution in interpreting the validity of TE measurements when performed in patients with significant transaminitis (ALT > 5 × ULN) or hyperbilirubinaemia (bilirubin > 100 μmol/L), as this may lead to overestimation of fibrosis grade in 15–20% of cases. In such patients, serial measurements are recommended to reassess the TE value after resolution of the transaminitis or hyperbilirubinaemia.

We compared the diagnostic accuracy of TE against APRI for the non-invasive identification of significant hepatic fibrosis. APRI has previously been shown to be a reliable predictor of hepatic fibrosis in patients with CHC, with an AUROC of 0.80 (95% CI 0.74–0.87).[13] However, the poor performance of APRI in other study populations has cast doubts on its reliability.[34, 35] We chose to compare the accuracy of TE against APRI for two reasons. First, APRI can be easily calculated from simple blood tests that are routinely performed for patients during their admission for liver biopsy. Second, we were interested to study the performance of APRI in a population with predominant CHB infection to evaluate if it has any potential role in the non-invasive diagnosis of fibrosis and subsequent longitudinal follow-up of such patients. APRI is easily calculable for any patient, whereas TE may not be successful in all patients. In our study, TE was successful in over 95% of the study cohort. In these patients, TE is superior to APRI in the diagnosis of hepatic fibrosis, with a higher AUROC and superior sensitivity, specificity and accuracy. Hence, our results are in agreement with those of Wai *et al.*,[34] as we have found APRI to be of limited diagnostic utility in our predominantly HBV-positive population. In comparison, TE is a more accurate noninvasive predictor of hepatic fibrosis.

Our study primarily focused on the objective of assessing TE for identifying significant fibrosis, and was not sufficiently powered to evaluate its role for the diagnosis of cirrhosis as only 10% of the study population comprised cirrhotic patients ($n = 12$). Of these, LSM readings failed in two, one because of ascites and the other because of narrow inter-costal space. It has been shown that the diagnostic accuracy of TE is associated with the prevalence of fibrosis and cirrhosis in the population studied.[29, 30] As a result of the small numbers of cirrhotic patients in our study, the diagnostic accuracy of TE for cirrhosis suffered, with a PPV of only 32.0%. In addition, the optimal cut-off of 16 kPa identified in our study is higher than the values commonly reported.[29] As discussed earlier, marked transaminitis, hyperbilirubinaemia and prolonged prothrombin time are possible reasons for the high false positive rates for diagnosis of cirrhosis by TE. However, an important observation from our results is that a LSM of 16 kPa is very accurate in excluding the presence of cirrhosis, and can reliably do so in 99% of the patient cohort.

We acknowledge several limitations in our study. The use of liver biopsy as a reference standard is fraught with limitations of an 'imperfect gold standard bias'.[36] Even when liver biopsy is performed by an experienced physician and read by an expert pathologist, error in disease staging can occur in up to 20%.[37] The length of the biopsy specimen is another factor that has been shown to contribute to inaccuracy in interpretation of fibrosis.[10] The median length of the liver biopsy samples in our study was 15 mm. Although this is less than the optimal length of 25 mm suggested by Bedossa *et al.* for evaluation of fibrosis,[8] it is not markedly different from the median specimen length of 17 mm reported in a recent meta-analysis on TE.[38] Furthermore, when we performed a subgroup analysis, the specimen length was not found to have any significant effect on the accuracy of TE. This is consistent with the findings of the systematic review performed by Shaheen *et al.*,[29] in which the liver biopsy size did not affect the accuracy of TE result. The METAVIR grading system may not be the most appropriate scoring system to assess hepatic fibrosis in alcoholic hepatitis or NASH, where the distribution of fibrosis is predominantly pericellular rather than periportal. However, in a subgroup analysis, the results in patients with alcoholic hepatitis and NASH were not found to be significantly different from that of the overall cohort.

In conclusion, TE (Fibroscan) provides an accurate and reliable non-invasive diagnosis of significant hepatic fibrosis in Asian patients. Its performance in Asian patients with CHB is similar to that reported for Western patients with CHC. In comparison with the

Western population, TE failures because of obesity are not commonly encountered among Asian patients, but a potential concern is the problem of inadequate intercostal space to facilitate proper placement of the Fibroscan probe in small-sized Asian females. TE results should be interpreted with caution in patients with marked transaminitis and hyperbilirubinaemia, as these may be falsely elevated. TE has an important role in the clinical management of patients with chronic liver disease, given its ability to predict accurately the presence of hepatic fibrosis in a non-invasive manner.

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