BRIEF ARTICLE

Staging of liver fibrosis in chronic hepatitis B patients with a composite predictive model: A comparative study

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Abstract

AIM: To evaluate the efficacy of 6 noninvasive liver fibrosis models and to identify the most valuable model for the prediction of liver fibrosis stage in chronic hepatitis B (CHB) patients.

METHODS: Seventy-eight CHB patients were consecutively enrolled in this study. Liver biopsy was performed and blood serum was obtained at admission. Histological diagnosis was made according to the METAVIR system. Significant fibrosis was defined as stage score $\geqslant$ 2, severe fibrosis as stage score $\geqslant$ 3. The diagnostic accuracy of 6 noninvasive liver fibrosis models, including serum aspartate aminotransferase (AST) to platelet ratio index (APRI), FIB-4, Forn's index, Fibrometer, Hepascore, and Shanghai Liver Fibrosis Group's index (SLFG), was investigated.

RESULTS: The APRI, FIB-4 and Forn's index under receiver operating characteristic curve (AUROC) for significant fibrosis were 0.71, 0.75 and 0.79, respectively, with a diagnosis accuracy of 67%, 77% and 80%, respectively, and 0.80, 0.87 and 0.86, respectively, under the AUROC for severe fibrosis. The Hepascore, SLFG, and Fibrometer were 0.80, 0.83 and 0.85, respectively under the AUROC for significant fibrosis ($P < 0.01$). The diagnosis accuracy of Hepascore and SLFG was 86% and 88%, respectively. The Hepascore, SLFG, and Fibrometer were 0.95, 0.93, and 0.94, respectively, under the AUROC for severe fibrosis ($P < 0.01$).

CONCLUSION: The models containing direct serum markers have a better diagnostic value than those not containing direct serum markers.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects 350 million individuals worldwide. At least one million people chronically infected with HBV would die of chronic liver diseases each year[1]. Thus, it is important to prevent the progression of early liver fibrosis to

cirrhosis[2]. Although liver biopsy is the gold standard for the assessment of fibrosis, it has several disadvantages, such as poor patient compliance, sampling error, limited usefulness for dynamic surveillance, and poor intra- and inter-observation concordance[3-5]. Considering these limitations, noninvasive histology predictors are urgently needed[6].

Since single fibrosis surrogate cannot measure fibrosis, an alternative approach combined with a number of parameters can generate algorithms capable of evaluating fibrosis. A number of noninvasive models containing serum markers, such as serum aspartate aminotransferase (AST) to platelet ratio index (APRI), FIB-4, Forn's index, Fibrometer, Hepascore, Shanghai Liver Fibrosis Group's index (SLFG) have been studied worldwide[7-12]. Additionally, except for SLFG, little has been known about the role of these models in predicting fibrosis stage of chronic hepatitis B (CHB) because most studies were performed in chronic hepatitis C (CHC). China has a high prevalence of CHB, and most hepatocellular carcinomas result from chronic HBV infection. Therefore, we carried out this study to identify the best practical noninvasive model of liver fibrosis in CHB.

MATERIALS AND METHODS

Patients
Seventy-eight consecutive eligible CHB patients who underwent a liver biopsy in March 2006-August 2008 at Zhongshan Hospital, Fudan University, Shanghai, China, were included in this study. Blood serum was collected and stored at -80℃ for further test. Chronic HBV infection was diagnosed based on positive surface antigen of HBV (HBsAg) and fluctuated alanine aminotransferase. Exclusion criteria included chronic liver disease due to other causes or co-infection with hepatitis D, clinically overt cirrhosis, previous or concomitant anti-HBV therapy, alcohol consumption exceeding 20 g/d in men and exceeding 10 g/d in women. Data were retrospectively analyzed. The study protocol was approved by the Institutional Review Board in our hospital. Written informed consent was obtained from each patient.

Liver histology and quantification of fibrosis
Liver tissue was obtained by sono-guided percutaneous biopsy (Bard®, Magnum®, 18G, USA) and stained with hematoxylin-eosin-safran and Masson's trichrome. Fibrosis staging (F) and inflammatory activity (A) were decided according to the METAVIR system. Fibrosis staging was divided into F0-F4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = periportal fibrosis with few septa, F3 = septal fibrosis with many septa, and F4 = cirrhosis). Inflammatory activity was divided into A0-A3 (A0 = no histologic necroinflammatory activity, A1 = minimal activity, A2 = moderate activity, A3 = severe activity). The activity was assessed by integrating the severity and intensity of piecemeal (periportal) and lobular necrosis[13]. According to the American Association for the Study of Liver Disease Practice

Guidelines, we defined significant fibrosis as METAVIR fibrosis with a score $\geq$ 2 (F2, 3, 4) and severe liver fibrosis as METAVIR fibrosis with a score $\geq$ 3 (F3, 4)[14].

Serum parameters
Following parameters, including AST, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), bilirubin, total cholesterol, urea, prothrombin time (PT), prothrombin index (PI), hemoglobin and platelet count (PLT) were assayed. AST, ALT, GGT, bilirubin, total cholesterol, urea were tested with Hitachi 7600, Japan. PT and PI were tested with Sysmex CA7000, Japan. Hemoglobin and PLT was tested with Sysmex routine blood test pipeline, Japan. The reference value was 0-75 IU/L for ALT and AST. Serum α_2-macroglobulin (A2M) (GenWay Biotech, San Diego, USA) and hyaluronic acid (HA) (Shanghai High Medical Biotech, Shanghai, China) concentrations were measured by enzyme linked immunosorbent assay.

APRI, FIB-4, Forn's index, SLFG, Hepascore, and Fibrometer were detected according to the following formulas: APRI = [AST (/ULN)/PLT (10^9/L)] × 100, FIB-4 = [age (yr) × AST (U/L)]/{[PLT (10^9/L)] × (ALT (U/L)]$^{1/2}$}, Forn's index = 7.811 - 3.131 × ln [PLT (10^9/L)] + 0.781 × ln [GGT (U/L)] + 3.467 × ln [age (yr)] - 0.014 × [cholesterol (g/L)], SLFG = 10 × e^Y/(1+e^Y), Y = -13.995 + 3.220 × lg [A2M (g/L)]+ 3.096 × lg [age (yr)] + 2.254 × lg [GGT (U/L)] + 2.437 × lg [HA (ng/mL)], Hepascore = e^Y/(1+e^Y), Y = - 4.185818 - [0.0249 × age (yr)] + [0.7464 × sex (male = 1, female = 0)] + [1.0039 × A2M (g/L)] + [0.0302 × HA (ng/mL)] + [0.0691 × TB (μmol/L)] - [0.0012 × GGT (U/L)]; Fibrometer = - 0.007 × PLT (10^9/L) - 0.049 × PI (%) + 0.012 × AST (U/L) + 0.005 × A2M (g/L) + 0.021 × HA (ng/mL) - 0.270 × urea (mmol/L) + 0.027 × Age (yr) + 3.718.

Statistical analysis
Statistical analysis was performed using Spearman's two-tail test and univariate analysis. $P < 0.05$ was considered statistically significant. Sensitivity, specificity, positive and negative predictive values (NPV and PPV) were calculated by using cutoffs according to the original studies. The overall diagnostic performance of scores was evaluated by area under ROC curves (AUROCs). We used DANA method, which was developed by Poynard *et al*[15,16], to adjust the observed AUROCs in our study. All the AUROCs were adjusted to a standard DANA of 2.5 using the formula: Adjusted AUROC (AdAUC) = Observed AUROC + 0.1056 × (2.5 - Observed DANA). The AUROCs were compared with the method of Hanley-McNeil[17].

RESULTS

Patient characteristics
The mean age of the 78 patients (66 males, 12 females) was 32.6 ± 12.3 years. The mean length of liver biopsies was 18.2 ± 3.4 mm, and the liver specimen length was longer than 15 mm. Significant fibrosis was found in 32

Table 1 Main characteristics of patients studied

	Patients (*n* = 78)	FOF1 (*n* = 46)	F2F3F4 (*n* = 32)	*P* value (FOF1 *vs* F2F3F4)
Age (mean ± SD, yr)	32.6 ± 12.3	29.6 ± 12.0	36.9 ± 11.4	0.009
Men (*n*, %)	66 (84.6)	38 (82.6)	28 (87.5)	0.113
CHB family history (*n*, %)	29 (37.2)	18 (39.1)	11 (34.4)	0.104
WBC (mean ± SD, 10^9/L)	5.3 ± 1.4	5.5 ± 1.2	4.9 ± 1.6	0.060
Hb (mean ± SD, g/L)	142.6 ± 15.7	145.6 ± 13.4	138.3 ± 17.8	0.044
PLT (mean ± SD, 10^9/L)	170.2 ± 51.5	185.9 ± 40.7	147.6 ± 57.3	0.002
TB [median (interquartile range), μmol/L]	15.4 (11.5-20.6)	14.7 (10.6-19.1)	16.7 (12.1-24.1)	0.087
CB [median (interquartile range), μmol/L]	5.7 (4.0-7.8)	5.5 (3.9-7.1)	6.4 (4.5-10)	0.057
ALT [interquartile median (range), U/L]	115 (55-241)	93.5 (32-240)	132 (76-263)	0.165
AST [interquartile median (range), U/L]	67.5 (38-121)	56 (30-95)	86.5 (41-152)	0.042
GGT [interquartile median (range), U/L]	52.5 (27-76)	36.5 (21-59)	66.5 (46-94)	0.006
Alb (mean ± SD, g/L)	42.4 ± 5.1	44.0 ± 4.8	40.2 ± 4.6	0.001
PT (mean ± SD, s)	12.0 ± 1.1	11.5 ± 0.9	12.6 ± 0.9	< 0.001
PI (mean ± SD, s)	1.00 ± 0.08	1.03 ± 0.07	0.95 ± 0.06	< 0.001
TC (mean ± SD, mmol/L)	3.8 ± 0.8	3.8 ± 0.8	3.7 ± 0.9	0.135
HA [interquartile median (range), ng/mL]	125 (75-224)	88 (49-129)	167 (116-382)	< 0.001
A2M (mean ± SD, g/L)	2.96 ± 0.58	2.73 ± 0.48	3.28 ± 0.55	< 0.001
Lg HBV-DNA (mean ± SD)	6.0 ± 1.9	5.9 ± 2.0	6.1 ± 2.1	0.314
HBeAg positive (*n*, %)	55 (70.5)	32 (69.6)	23 (71.9)	0.223
Liver specimen length (mean ± SD, mm)	18.2 ± 3.4	18.4 ± 3.3	17.9 ± 3.6	0.254
METAVIR A stage (*n*, %)				
A0	4 (5.1)			
A1	41 (52.5)			
A2	32 (41.1)			
A3	1 (1.3)			
METAVIR F stage (*n*, %)				
F0	13 (16.7)			
F1	33 (42.3)			
F2	13 (16.7)			
F3	10 (12.8)			
F4	9 (11.5)			

SD: Standard deviation; WBC: Leucocyte; Hb: Hemoglobin; TB: Total bilirubin; CB: Conjugated bilirubin; Alb: Albumin; TC: Total cholesterol.

patients (41.0%), severe fibrosis in 19 patients (24.4%), and early cirrhosis in 9 patients (11.5%), respectively. Main features of the patients are summarized in Table 1.

Correlation between non-invasive model and fibrosis stage

METAVIR fibrosis stages were significantly correlated with APRI, FIB-4, Forn's index, Fibrometer, Hepascore and SLFG. A better correlation was observed between Fibrometer (*r* = 0.69), SLFG (*r* = 0.68) and Hepascore (*r* = 0.62) (*P* < 0.001). The box-plots of fibrosis scores are shown in Figure 1. A correlation was also found between scores and histological activity, especially between SLFG (*r* = 0.55), Fibrometer (*r* = 0.54) and APRI (*r* = 0.51) (*P* < 0.001).

Overall diagnostic performance of serum markers

The mean levels of AST, GGT, PT, PI, A2M and HA were higher in patients with F2-F4 fibrosis than in those with F0-F1 fibrosis (*P* < 0.01). The mean levels of hemoglobin, PLT and albumin were lower in patients with F2-F4 fibrosis than in those with F0-F1 fibrosis (*P* < 0.01). Multiple regression analysis showed that A2M and HA were the independent factors for significant fibrosis (A2M, OR = 5.36, 95% CI: 1.58-18.13, *P* = 0.007; HA, OR = 1.01, 95% CI: 1.00-1.02, *P* = 0.007). AUROC

was used to evaluate the overall diagnostic performance of scores (Table 2).

The APRI, FIB-4, Forn's index, Hepascore, SLFG and Fibrometer were 0.71, 0.75, 0.79, 0.80, 0.83, and 0.85 respectively under the AUROC for F0-F4 (Figure 2A), and 0.75, 0.79, 0.83, 0.84, 0.86, and 0.88 respectively under the adjusted AUROC for F0-F4 with DANA method. The AUROC for Fibrometer, SLFG and Hepascore was better than that for APRI and FIB-4 (*P* < 0.01).

The APRI, FIB-4, Forn's index, Hepascore, SLFG and Fibrometer were 0.80, 0.87, 0.86, 0.95, 0.93, and 0.94 under the AUROC for F0-F4 (Figure 2B). The Hepascore, Fibrometer and SLFG levels were significantly higher than the APRI level under the AUROC for F0-F4 (*P* < 0.01).

Sensitivity, specificity, positive and negative predictive values

NPV and PPV for the diagnosis of significant fibrosis are presented in Table 3. Cutoffs were chosen for each model as previously described. This analysis could not be performed for Fibrometer since no cutoff was provided in the study by Calès *et al*[10]. When different cutoffs were used for each model, the percentage of classifiable subjects was 45%-78%, with a diagnostic accuracy of
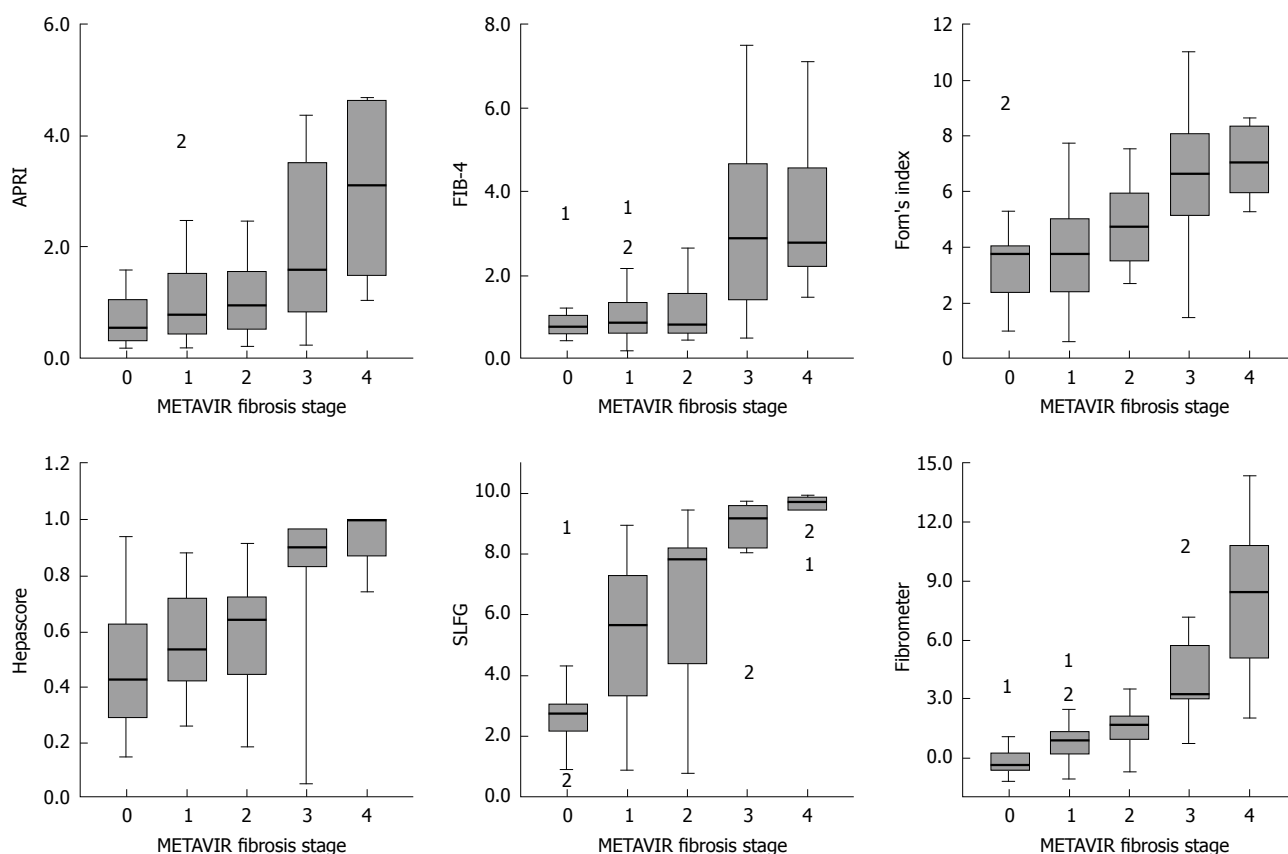
Figure 1 Model values according to METAVIR fibrosis stages. The top and bottom of each box are the 25th and 75th centile interval, the line through the box is the median and the error bars are the 5th and 95th centile interval. 1 and 2 indicate the extreme values.
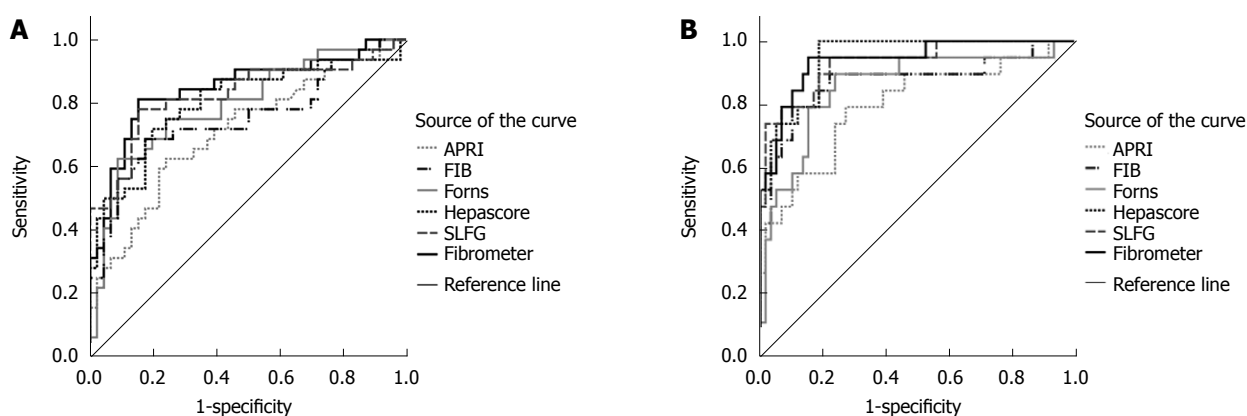

Figure 2 ROC curves for the 6 fibrosis models to discriminate METAVIR fibrosis stages F0-1 from F2-4 (A) and F0-2 from F3-4 (B).

67%-86% (Table 4). Significant fibrosis (F2-4) was predicted in 13%-32% of patients with their PPV ranged 62%-88%. Since lower cutoffs were originally described to rule out significant fibrosis, attention must be paid to NPV ranging 76%-85%. The best positive predictive value (PPV = 0.88) for significant fibrosis was observed when SLFG > 8.7.

DISCUSSION

Noninvasive models have been proposed for the assessment of liver fibrosis. The diagnostic performance of

APRI, FIB-4, Forn's index, Fibrometer, Hepascore and SLFG was evaluated for the assessment of liver fibrosis in CHB patients. These models are mainly based on two kinds of serum markers, direct and indirect. Direct serum markers are directly linked to the modifications in extracellular matrix (ECM) metabolism. Indirect serum markers have no direct link with liver fibrosis but reflect liver dysfunction or other phenomena caused by fibrosis. We focused on the serum markers of fibrosis. The main end-point of our study was to evaluate the global diagnostic performance of models by comparing their AUROCs. Our study indicated that models containing

Table 2 AUROC for F0F1 *vs* F2-4 and F0-2 *vs* F3-4

	F0-1 *vs* F2-4				F0-2 *vs* F3-4		
	AUROC	SD	95% CI	AdAUC	AUROC	SD	95% CI
APRI	0.71	0.06	0.59-0.83	0.75	0.80	0.06	0.67-0.92
FIB-4	0.75	0.06	0.63-0.87	0.79	0.87	0.06	0.76-0.99
Forn's index	0.79	0.05	0.69-0.90	0.83	0.86	0.06	0.75-0.96
Hepascore	0.80	0.05	0.70-0.91	0.84	0.95	0.02	0.90-0.99
SLFG	0.83	0.05	0.73-0.93	0.86	0.93	0.03	0.87-0.99
Fibrometer	0.85	0.05	0.75-0.94	0.88	0.94	0.03	0.88-0.99

Table 3 Sensitivity, specificity, predictive values and likelihood ratios of scores according to different cutoffs for the diagnosis of significant fibrosis

Score	Cutoff	%	Significant fibrosis (F2-4)					
			Sen	Spe	PPV	NPV	+LR	-LR
APRI	< 0.50	27	0.84	0.35	0.47	0.76	1.29	0.46
	> 1.50	32	0.47	0.80	0.62	0.69	2.35	0.66
FIB-4	< 1.45	65	0.63	0.85	0.74	0.76	4.20	0.44
	> 3.25	13	0.25	0.96	0.80	0.65	6.25	0.78
Forn's index	< 4.20	46	0.75	0.63	0.59	0.78	2.03	0.40
	> 6.90	18	0.34	0.96	0.85	0.68	8.50	0.69
Hepascore	< 0.50	23	0.88	0.50	0.55	0.85	1.74	0.26
	> 0.84	27	0.50	0.91	0.80	0.72	5.56	0.55
SLFG	< 3.00	23	0.91	0.33	0.48	0.83	1.36	0.27
	> 8.70	22	0.47	0.96	0.88	0.72	11.75	0.55

Sen: Sensitivity; Spe: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; +LR: Positive likelihood ratio; -LR: Negative likelihood ratio.

Table 4 Percentage of classifiable subjects, correct prediction, diagnostic accuracy and biopsies that could be avoided *n* (%)

Models	Cut-offs	Classifiable subjects	Correct prediction	Diagnostic accuracy	Biopsy avoided
APRI	< 0.50	21 (27)	16 (76)	67%	31 (40)
	> 1.50	25 (32)	15 (62)		
FIB-4	< 1.45	51 (65)	39 (76)	77%	47 (60)
	> 3.25	10 (13)	8 (80)		
Forn's index	< 4.20	36 (46)	28 (78)	80%	40 (51)
	> 6.90	14 (18)	12 (85)		
Hepascore	< 0.50	18 (23)	15 (85)	82%	32 (41)
	> 0.84	21 (27)	17 (80)		
SLFG	< 3.00	18 (23)	15 (83)	86%	30 (38)
	> 8.70	17 (22)	15 (88)		

direct serum markers (Fibrometer, SLFG, Hepascore) performed much more accurately than models containing only indirect serum markers (APRI, FIB-4, Forn's index).

Direct serum markers are useful for assessing the speed of liver fibrogenesis. HA, a component of ECM, is a glycosaminoglycan synthesized by hepatic stellate cells and degraded by liver sinusoidal cells[18]. A2M is a protease inhibitor with its concentration increased due to stellate cell activation and liver fibrosis[19]. Studies have demonstrated that HA and A2M levels are correlated with hepatic fibrosis in patients with CHB or CHC[11,20-22], which is consistent with the findings in our study. Multiple

regression analysis in our study showed that HA and A2M were the independent factor for significant fibrosis and had a better diagnostic accuracy.

It has been reported that APRI under the AUROC for significant fibrosis is 0.76 (95% CI: 0.74-0.79)[23]. It has been shown that the accuracy of APRI under the AUROC for significant fibrosis is 0.63 and 0.72 in CHB patients[24,25]. Zhang *et al*[26] showed that APRI has low diagnostic accuracy of liver fibrosis and APRI combined HA can achieve a better diagnostic accuracy of liver fibrosis. FIB-4 has a high diagnostic accuracy of severe fibrosis[27]. Mallet *et al*[28] reported that FIB-4 is 0.81 under the AUROC for severe fibrosis. In our study, the FIB-4 was 0.87 under the AUROC for severe fibrosis. The reported Forn's index is 0.76 under the AUROC for significant fibrosis[29]. In our study, the Forn's index was 0.79 under the AUROC for significant fibrosis. The diagnostic value of APRI, FIB-4 and Forn's index was much lower in CHB patients than in CHC patients.

Calès *et al*[10] have developed Fibrometer for the diagnosis of significant fibrosis and found that its diagnostic performance is stable in patients with different chronic liver diseases[30,31]. Hepascore has been used in diagnosis of significant and severe fibrosis[12]. In our study, the Hepascore was 0.80 and 0.95 under the AUROC for significant and severe fibrosis. SLFG is the first developed model in CHB patients. In our study, the NPV and PPV of SLFG under AUROC are similar to the reported

data[11]. These results indicat that Fibrometer, SLFG and Hepascore can be used in diagnosis of liver fibrosis. However, these noninvasive models should be validated in a larger number of CHB patients.

Broadly speaking, no true noninvasive model could exactly reflect liver fibrosis. Transient elastography (fibroScan) is another noninvasive method to detect the mean liver stiffness for diagnosing fibrosis. However, it is expensive and may be limited in those with narrow intercostal spaces, morbid obesity or significant ascites[32,33]. These noninvasive models can be used in clinical management of CHB by offering an attractive alternative to liver biopsy.

In our study, since the sample size was small, further study is needed before these models are used in clinical practice. Validating against not only histological stage scores but also digital image analysis and clinical outcomes may also be a better choice.

In conclusion, serologic models containing direct serum markers of Hepascore, SLFG, and Fibrometer have better diagnostic values in CHB patients than those containing only indirect serum markers of APRI, FIB-4, Forn's index.

COMMENTS

Background
Clinically, developing noninvasive methods for diagnosing liver fibrosis is important. Noninvasive models have been established most in hepatitis C patients.

Research frontiers
Noninvasive models have been proposed for the assessment of liver fibrosis. These models have been evaluated at many medical centers in chronic hepatitis C (CHC), but few in chronic hepatitis B (CHB). In this study, the efficacy of 6 noninvasive models was evaluated and the more valuable models were identified for predicting liver fibrosis in CHB.

Innovations and breakthroughs
The efficacy of 6 noninvasive models was evaluated in a cohort of Chinese patients with CHB. The results indicate that their efficacy is not influenced by ethnic and virus factors. This study also showed that the serologic models containing direct serum markers, such as hyaluronic acid (HA) and α_2-macroglobulin (A2M) have better diagnostic values in CHB patients than in those not containing direct serum markers.

Applications
Noninvasive models can be used in diagnosis of liver fibrosis in patients with CHB in China or in Asia.

Terminology
DANA method: A method used to adjust the differences caused by the prevalence of fibrosis stages. Standard prevalence is defined as a prevalence of 0.20 for each of the five stages.

Peer review
The study adds information that helps establishment of strategies against liver fibrosis diagnosis using noninvasive methods. The study was scientifically designed. The manuscript is logical and readable.

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