Original Article
Noninvasive predictive models of liver fibrosis in patients with chronic hepatitis B

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Abstract: Objective: The aim of the present study was to establish noninvasive diagnostic models for liver fibrosis and assess their predictive accuracy (AC). Methods: A total of 349 patients with chronic hepatitis B virus infection were evaluated, who underwent liver biopsy and pathologic examination at Beijing Ditan Hospital affiliated to Capital Medical University. Patients were subdivided in disease-immune tolerant (n = 125) and immune reactive HBeAg positive (n = 224) groups. Diagnostic models were based on independent markers of liver fibrosis. Receiver operating characteristic (ROC) curves were used to set cutoff values and determine the diagnostic value of the models. Results: Wang I and Wang II models were constructed using independent disease markers. Wang I model cutoff values ≤ 1.75 and > 5.84 were used to identify patients in the immune tolerant phase with or without significant fibrosis. The area under the ROC curve (AUC) for this model was 0.866 (95% CI, 0.790, 0.942) and an AC of 92.0% was obtained. Wang II model cutoff values ≤ 3.79 and > 7.06 were used to identify immune reactive HBeAg-positive patients with or without significant fibrosis. AUC was 0.872 (95% CI, 0.824, 0.920), with an AC of 88.0%. Conclusions: Both Wang models enabled noninvasive liver fibrosis assessment with reliable predictive power and reproducibility for diagnosis of fibrosis in immune tolerant and immune reactive HBeAg-positive patients. With further development, these models may provide a clinical alternative to liver biopsy.

Keywords: Hepatitis B virus, liver fibrosis, noninvasive diagnosis, model, receiver operating characteristic (ROC) curve

Introduction

More than 400 million people worldwide are estimated to have contracted hepatitis B virus (HBV). Liver fibrosis is a serious consequence of chronic liver disease [1], and about 100 million people die because of chronic liver disease secondary to HBV infection and its complications each year [2, 3].

Persistent HBV infection leads to inflammatory infiltration and necrosis of liver parenchyma, resulting in fibrous hyperplasia. The severity of liver fibrosis correlates with patient prognosis and determines the treatment strategy required. Therefore, accurate assessment of liver inflammation and fibrosis is an important step toward reducing or reversing liver fibrosis, and improving the prognosis of patients with chronic hepatitis B (CHB) infection.

Pathologic examination of a liver biopsy is currently the gold standard diagnostic technique for the assessment of liver fibrosis [4]. However, the procedure is invasive and not readily repeatable. It also carries the risk of clinical complications, sampling errors, interpretation errors, and poor patient compliance [5-8]. For these reasons, blood biochemical markers and serum fibrosis markers are commonly used as surrogates to detect liver fibrosis. However, these diagnostic techniques are limited in sensitivity (SN) and specificity (SP) [9]. In recent years, a number of clinical serologic markers, imaging, and noninvasive diagnostic models of liver fibrosis have emerged. These include the Forns' score [10], APRI (aspartate aminotransferase-platelet count ratio index) [11-13], FibroTest [14], Fibrometer [15], and Hepascore [16]. However, most of these models have focused on chronic hepatitis C (CHC) and alcoholic liver

Table 1. Univariate analysis of markers associated with significant fibrosis in the immune tolerant phase

Variable	Modeling (n = 125)	No significant fibrosis (n = 87)	Significant fibrosis (n = 38)	*P*-value
Patients (n, %)	125 (100.0%)	87 (69.6%)	38 (30.4%)	
Male (n, %)	82 (65.6%)	57 (65.5%)	25 (65.8%)	0.976
Age (y)	33.79 ± 8.86	32.17 ± 8.94	37.50 ± 7.54	0.002
BMI (kg/m^2)	23.03 ± 3.59	22.51 ± 3.24	24.23 ± 4.08	0.013
WBC (10^9/L)	5.57 ± 1.33	5.56 ± 1.40	5.59 ± 1.18	0.894
RBC (10^{12}/L)	4.84 ± 0.59	4.90 ± 0.59	4.70 ± 0.57	0.070
Hb (g/L)	145.63 ± 18.13	145.75 ± 18.16	145.34 ± 18.32	0.907
Platelets (10^9/L)	188.77 ± 49.00	199.87 ± 44.78	163.37 ± 49.33	< 0.001
ALT (IU/L)	26.94 ± 9.94	26.05 ± 8.09	28.99 ± 7.27	0.057
AST (IU/L)	23.30 ± 5.11	22.18 ± 4.34	25.85 ± 5.83	< 0.001
AST/ALT	0.93 ± 0.31	0. 93 ± 0.33	0.93 ± 0.24	0.953
Total bilirubin (μmol/L)	15.62 ± 10.61	15.33 ± 8.39	16.28 ± 14.59	0.645
Direct bilirubin (μmol/L)	4.09 ± 2.38	4.15 ± 2.54	3.96 ± 2.00	0.683
Albumin (g/L)	45.18 ± 3.86	45.75 ± 3.97	43.90 ± 3.32	0.013
Globulin (g/L)	30.25 ± 4.14	29.79 ± 4.29	31.28 ± 3.64	0.065
Albumin/globulin	1.52 ± 0.25	1.56 ± 0.26	1.42 ± 0.21	0.004
GGT (IU/L)	18.92 ± 13.73	15.38 ± 6.36	27.03 ± 21.01	0.002
PTA (%)	84.41 ± 11.27	86.07 ± 11.00	80.59 ± 11.09	0.012
Ln (HBVDNA)	15.16 ± 3.72	16.23 ± 3.26	12.69 ± 3.58	< 0.001

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; GGT = gamma-glutamyltransferase; Hb = hemoglobin; PTA = prothrombin activity; RBC = red blood cell; WBC = white blood cell.

Table 2. Univariate analysis of markers associated with significant fibrosis in the immune reactive HBeAg-positive phase

Variable	Modeling (n = 224)	No significant fibrosis (n = 95)	Significant fibrosis (n = 129)	*P*-value
Patients (n, %)	224 (100.0%)	95 (42.4 %)	129 (57.6%)	
Male (n, %)	163 (72.8%)	70 (73.3%)	93 (72.1%)	0.791
Age (y)	34.26 ± 9.04	31.63 ± 7.66	36.20 ± 9.51	< 0.001
BMI	24.41 ± 3.95	23.65 ± 3.59	24.96 ± 4.13	0.013
WBC (10^9/L)	5.57 ± 1.59	5.81 ± 1.59	5.39 ± 1.57	0.050
RBC (10^{12}/L)	4.87 ± 0.54	5.01 ± 0.56	4.76 ± 0.50	0.001
Hb (g/L)	148.23 ± 17.66	149.90 ± 17.99	147.01 ± 17.38	0.226
Platelets (10^9/L)	184.99 ± 54.40	208.13 ± 52.26	167.95 ± 49.60	< 0.001
ALT (IU/L)	173.03 ± 269.34	105.89 ± 175.16	222.48 ± 313.11	< 0.001
1N < ALT ≤ 2N	56.01 ± 11.68	56.07 ± 11.77	55.93 ± 11,67	0.947
ALT > 2N	340.07 ± 356.88	249.96 ± 300.5	373.20 ± 372.00	0.141
AST (IU/L)	105.57 ± 182.86	59.13 ± 93.31	139.77 ± 221.54	< 0.001
AST/ALT	0.68 ± 0.42	0. 65 ± 0.50	0.70 ± 0.36	0.396
Total bilirubin (μmol/L)	20.93 ± 25.15	16.82 ± 16.93	23.96 ± 29.50	0.023
Direct bilirubin (μmol/L)	8.47 ± 19.51	5.95 ± 13.52	10.32 ± 22.82	0.075
Albumin (g/L)	44.30 ± 4.63	45.17 ± 4.03	43.65 ± 4.95	0.012
Globulin (g/L)	31.71 ± 4.30	31.34 ± 3.66	31.98 ± 4.72	0.256
Albumin/globulin	1.42 ± 0.24	1.46 ± 0.21	1.39 ± 0.25	0.033
GGT (IU/L)	58.87 ± 71.28	32.08 ± 34.83	78.61 ± 83.88	< 0.001
PTA (%)	82.87 ± 12.89	87.45 ± 13.82	79.49 ± 11.06	< 0.001
Ln (HBVDNA)	15.98 ± 2.81	17.02 ± 1.71	15.22 ± 3.20	< 0.001

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; GGT = gamma-glutamyltransferase; Hb = hemoglobin; PTA = prothrombin activity; RBC = red blood cell; WBC = white blood cell.

Table 3. Inflammation grade and fibrosis stage in the immune tolerant and immune reactive HBeAg-positive phases

Grade	Immune tolerant phase (n, %)	Immune reactive HBeAg-positive phase (n, %)
Inflammation grade		
G0	0 (0.0%)	0 (0.0%)
G1	79 (63.2%)	75 (33.5%)
G2	36 (28.8%)	79 (35.3%)
G3	10 (8.0%)	64 (28.6%)
G4	0 (0.0%)	6 (2.7%)
Fibrosis stage		
S0	4 (3.2%)	2 (0.9%)
S1	83 (66.4%)	93 (41.5%)
S2	20 (16.0%)	82 (36.6%)
S3	15 (12.0%)	35 (15.6%)
S4	3 (2.4%)	12 (5.4%)

Table 4. Multiple regression of markers associated with significant fibrosis in the immune tolerant phase

Variable	Immune tolerant Phase (n = 125)		
	β	OR§ (95% CI)	P
PLT	-0.015	0.985 (0.974-0.996)	0.010
GGT	0.071	1.073 (1.007-1.143)	0.029
Ln [HBV DNA]	-0.226	0.798 (0.701-0.909)	0.001
AST	0.154	1.166 (1.052-1.293)	0.003

§OR is the risk of significant fibrosis in immune tolerant phase patients. 95% CI: 95%, Confidence Interval of the difference.

disease, and are less accurate when used in patients with CHB [11, 17].

Further studies have established more specific diagnostic models for the prediction of fibrosis in patients with CHB, including the Shanghai Liver Fibrosis Group model [18], Hui et al. model [19], S index [20], and FibroIndex [21]. These models were developed primarily for patients with CHB associated with elevated aminotransferases, but these techniques have not yet been sufficiently validated, and thus are not widely used.

Patients in the immune tolerant phase usually display normal liver function, and as a result, many remain inadequately treated, despite previous studies demonstrating that fibrosis occurs in 30-40% of patients during this phase [22]. Timely and accurate recognition of fibrosis

is essential to improve the prognosis of liver disease. Antiviral and antifibrosis treatments can delay and reduce the incidence of liver cirrhosis and cancer [23]. However, models of the immune tolerant- and immune reactive HBeAg-positive phases have not yet been developed. We, therefore, aimed to establish two individual models to aid the early detection of liver fibrosis in the immune tolerant and immune reactive HBeAg-positive phases of HBV. These models were based on routine laboratory tests and liver biopsy results. The objective was to improve predictive accuracy, define a noninvasive method for screening therapeutic targets, and establish a basis for appropriate antiviral treatment and efficacy evaluation.

Materials and methods

Patient selection

A total of 349 patients with CHB treated at the Capital Medical University, Beijing Ditan Hospital from January 2009 to December 2011 were included in the study; they were diagnosed according to the American Association for the Study of Liver Diseases CHB prevention and treatment guidelines [24]. The included patients had a history of hepatitis B or had been hepatitis B surface antigen (HBsAg) positive for more than 6 months, and were HBeAg seropositive and/or had high HBV DNA levels. Exclusion criteria included coexisting viral infections and other forms of liver diseases or hepatic fibrosis-related diseases such as rheumatism, systemic lupus erythematosus, chronic obstructive pulmonary disease, renal failure, hematological diseases, or tumors. Patients receiving antiviral therapy in the year before hepatic biopsy, those receiving medical treatment within 3 months, or dialysis, blood transfusion or the administration of blood products within 1 week of hepatic biopsy were also excluded. The study protocol was approved by the ethics committee of the Capital Medical University, Beijing Ditan Hospital and written informed consent was obtained from each patient.

Patients were grouped according to disease phase: immune tolerant or immune reactive HBeAg positive. The immune tolerant phase of HBV infection is characterized by hepatitis B e antigen (HBeAg) positivity, high levels of HBV replication (reflected by high serum HBV DNA

Table 5. Multiple regression of markers associated with significant fibrosis in the immune reactive HBeAg-positive phase.

Variable	Immune reactive HBeAg-positive phase (n = 224)		
	β	OR§ (95% CI)	P
PLT	-0.011	0.989 (0.982-0.997)	0.004
GGT	0.021	1.021 (1.010-1.033)	0.000
Ln [HBVDNA]	-0.258	0.773 (0.660-0.904)	0.001
TBIL	-0.019	0.981 (0.965-0.997)	0.021
RBC	-1.475	0.229 (0.106-0.496)	0.000
PTA	-0.052	0.950 (0.921-0.979)	0.001
BMI	0.160	1.173 (1.054-1.305)	0.003

§OR is the risk of significant fibrosis of immune reactive HBeAg-positive phase patients. 95% CI: 95% Confidence Interval of the difference.
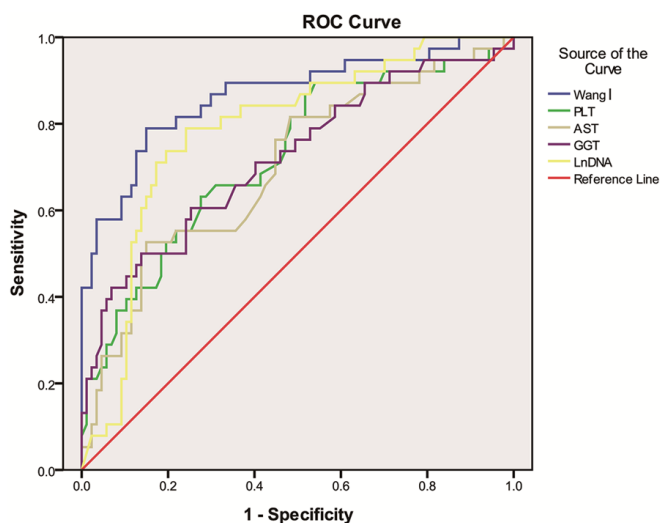

Figure 1. ROC curve generated from the diagnosis of significant liver fibrosis by modeling patients in the immune tolerant phase using the Wang I index.

blood cells, red blood cells (RBC), platelets (PLT), hemoglobin, alanine aminotransferase (ALT), AST, total bilirubin (TBIL), direct bilirubin, albumin (ALB), globulin, A/G, gamma-glutamyltransferase (GGT), prothrombin activity (PTA) levels, and HBV marker levels were measured using a Sysmex automated blood cell analyzer (XE 5000; SYS-MEX, Kobe City, Japan) and a 7180 automatic biochemical analyzer (7180; Hitachi, Tokyo, Japan). Coagulation tests were performed using an automatic coagulation analyzer. The body mass index (BMI) and AST/ALT were calculated.

Histopathologic examination of liver biopsy

Biopsies were performed by Doppler ultrasound guided positioning of a 16G biopsy needle (16G-9-20T; Cook, USA). Each biopsy was completed within 1 s, measured at least 1.0 cm in length, and contained more than four portal tracts. The specimens were fixed in 10% formaldehyde solution, embedded in paraffin, and serially sectioned for hematoxylin and eosin, Masson trichrome, and reticular fiber staining. The stage of liver fibrosis was assessed according to the Scheuer (S) classification scale, where S0-S1 indicated no significant liver fibrosis, and S2-S4 indicated significant liver fibrosis [30]. The sections were independently examined by a pathologist.

Statistical analysis and Wang models establishment

Statistical analysis was carried out using SPSS version 18.0 software (SPSS Inc., Chicago, IL). In all tests, $P < 0.05$ was considered statistically significant. HBV DNA measurements were converted to natural logarithms to fit a normal distribution. Univariate analysis of continuous variables was carried out to determine significant differences between groups using t-tests and analysis of variance. The X^2-test was used to compare categorical variables between groups. Multivariate analysis was carried out to select predictors of CHB using stepwise logistic regression.

The regression equations were used to define Wang models for the prediction of fibrosis. The

levels), normal-to-low levels of aminotransferases, mild or no liver necroinflammation, and no or slow fibrosis [25-28]. The immune reactive HBeAg-positive phase is characterized by HBeAg positivity, relatively low levels of viral replication (reflected by lower serum HBV DNA levels), increased or fluctuating levels of aminotransferases, moderate or severe liver necroinflammation, and more rapid fibrosis [25-29].

Assessments

Baseline patient characteristics including gender, age, height, and weight were recorded. Within 1 week of liver biopsy, blood samples were collected from all subjects, and white

Table 6. Diagnostic measures of patients in the immune tolerant phase

Wang I index	Total (n = 125)	S0-S1 (n = 87)	S2-S4 (n = 38)	SN (%)	SP (%)	PPV (%)	NPV (%)	AC (%)	AUROC (95% CI)
1.75				89.5	66.7	54.0	93.5	73.6	0.866 (0.790, 0.942)
> 1.75	63	29	34						
≤ 1.75	62	58	4						
5.84				57.9	96.6	88.0	84.0	84.8	
> 5.84	25	3	22						
≤ 5.84	100	84	16						

AC = accuracy; AUROC = area under the receiver operating characteristic curve; NPV = negative predictive value; PPV = positive predictive value; SN = sensitivity; SP = specificity.

markers identified by univariate analysis were further analyzed using forward stepwise multivariate logistic regression, and were sequentially entered into the model to identify significant variables. Markers were considered predictive when P < 0.05, and insignificant when P > 0.1.

Receiver operating characteristic (ROC) curves were plotted and area under the curve (AUC) values calculated. Sensitivity and specificity were determined, and the maximum Youden index (SN + SP—1) corresponding to the best upper and lower limit cutoff values was combined with the positive predictive value (PPV), negative predictive value (NPV). Accuracy (AC) was defined as the degree to which the diagnostic results conformed to the actual results, and was applied to evaluate the model's diagnostic value.

Results

Diagnostic model for the immune tolerant phase

The characteristics of patients with immune tolerant and immune reactive disease are shown in **Tables 1** and **2**, respectively [31]. Patients were further stratified according to significant fibrosis presence or absence (**Table 3**).

The 125 patients in the immune tolerant phase were diagnosed as having either no significant fibrosis (87 patients, 69.6%) or significant liver fibrosis (38 patients, 30.4%). Among the 224 patients in the immune reactive HBeAg-positive phase, 95 patients (42.4%) had no significant liver fibrosis, and significant liver fibrosis was present in 129 patients (57.6%).

Univariate analysis identified 9 markers, including age, BMI, PLT, AST, ALB, A/G, GGT, PTA, ln [HBVDNA] that differed significantly between

these two groups (**Table 1**). Multiple analysis identified 4 independent predictors of fibrosis: PLT, AST, GGT, and ln [HBVDNA] (**Table 4**). The following multiple regression equation was constructed based on their relative regression coefficients:

A = 0.153—0.015 × PLT + 0.154 × AST + 0.071 × GGT-0.226 × ln (HBVDNA).

'A' was converted to a score from 0-10 using the following formula:

$$\text{Wang I} = 10 \times \frac{e^{A}}{1 + e^{A}}.$$

Diagnostic model for the immune reactive HBeAg-positive phase

Univariate analysis identified 12 variables that differed significantly between patients with fibrosis from those without similarly. These included age, BMI, RBC, PLT, ALT, AST, TBIL, ALB, ALB/globulin, GGT, PTA, and ln [HBVDNA]. Multivariate forward stepwise logistic regression identified 7 independent predictors of fibrosis: RBC, PLT, TBIL, GGT, PTA, ln [HBVDNA], and BMI (**Table 5**). The following multiple regression equation was constructed to predict significant fibrosis:

C = 13.657—1.475 × RBC—0.011 × PLT—0.019 × TBIL + 0.021 × GGT—0.052 × PTA—0.258 × ln (HBVDNA) + 0.160 × BMI.

'C' was converted to a score from 0-10 using the following formula:

$$\text{Wang II} = 10 \times \frac{e^{C}}{1 + e^{C}}.$$

Diagnostic value of the Wang I index in immune tolerant patients

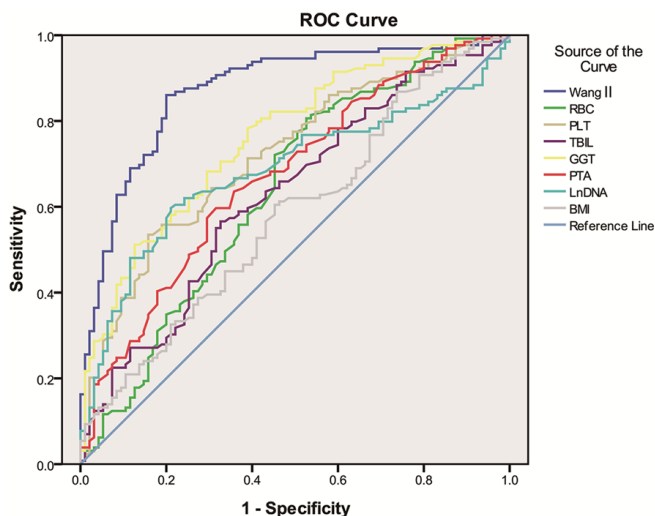The ROC curve demonstrated that the Wang I index predicted significant fibrosis among the

Figure 2. ROC curve generated from the diagnosis of significant liver fibrosis by modeling patients in the immune reactive HBeAg-positive phase using the Wang II index.

125 patients of the immune tolerant group. An area under the ROC curve (AUC) of 0.866 was obtained for Wang I index, which was significantly higher than the AUC values for individual markers ($P < 0.05$; **Figure 1**). At 89.5% SN and 96.6% SP, the Wang I index negative cutoff value was 1.75 and the positive cutoff value 5.84. With Wang I index ≤ 1.75, the NPV was 93.5%, indicating that 58 of 62 patients with a Wang I index ≤ 1.75 had no significant liver fibrosis (**Table 6**). A SN of 89.5% was obtained, indicating that 34 of 38 patients with significant fibrosis, confirmed by liver biopsy, had a Wang I index > 1.75. The diagnostic AC was 73.6%.

A patient with a Wang I index > 5.84 was suspected to have significant fibrosis. As shown in **Table 6**, the PPV using this cutoff value was 88.0% i.e. 22 of 25 patients with a Wang I index > 5.84 had significant fibrosis. The corresponding SP was 96.6%. Among the 87 patients in whom liver fibrosis absence was confirmed by liver biopsy, 84 individuals had a Wang I index ≤ 5.84, indicating a diagnostic AC of 84.8%.

Overall, 80 of 125 patients (64.0%) were diagnosed correctly, and 7 patients (5.6%) were diagnosed incorrectly. The diagnostic AC was 92.0%. The application of this model would have enabled 69.6% of patients (87 patients) to avoid liver biopsy. Among the remaining 38 (30.4%) patients with a Wang I index between 1.75 and 5.84, the presence of significant fibro-

sis ought to be determined by liver biopsy or other detection methods.

Diagnostic value of the Wang II index in immune reactive patients

The Wang II index predicted liver fibrosis in 224 patients in the immune reactive HBeAg-positive group. The AUC for Wang II (0.872) was significantly higher than that obtained for any of the individual markers ($P < 0.05$; **Figure 2**). With 90.7% SN and 91.6% SP, the negative and positive cutoff values for significant fibrosis were 3.79 and 7.06, respectively.

With Wang II index ≤ 3.79, the SN and NPV were 90.7% and 84.4%, respectively (**Table 7**), and the absence of fibrosis was predicted correctly in 65 of the 77 patients (84.4%). Among the 129 patients with fibrosis confirmed by biopsy, 117 (90.7%) individuals had a Wang II index > 3.79; an AC of 81.2% was obtained.

When the Wang II index cutoff value was > 7.06, the SP and PPV were 91.6% and 91.0%, respectively (**Table 7**). Fibrosis was predicted correctly in 91.0% (81/89) of patients. Among the 95 patients in which no fibrosis was detected by biopsy, 87 patients (91.6%) had a Wang II index ≤ 7.06. The AC was 75.0%.

Overall, 146 of 224 patients (65.2%) were diagnosed correctly. The diagnostic AC of the Wang II model was 88.0%. The application of this model would enable 166 (74.1%) patients to avoid liver biopsy. There were 58 patients (25.9%) with a Wang II index between 3.79 and 7.06 who would still require liver biopsy.

Diagnostic ACs of noninvasive markers for predicting liver fibrosis

A comparison of different models, including the Wang I and Wang II indexes, S index [17], Zeng model [18], model proposed by Hui *et al* [19], APRI [12] and APAG is summarized in **Table 8**. Although the SN and SP of APAG were slightly higher than the values obtained for the Wang I and Wang II indexes, its PPV, NPV and AUC were significantly lower than in Wang models. The Wang I index had the best NPV (93.5%) in diagnosing no significant liver fibrosis, while the Wang II index had best PPV (91%) in diagnosing

Table 7. Diagnostic measures of patients in the immune reactive HBeAg-positive phase

Wang II index	Total (N = 224)	S0-S1 (N = 95)	S2-S4 (N = 129)	SN (%)	SP (%)	PPV (%)	NPV (%)	AC (%)	AUROC (95% CI)
3.79				90.7	68.4	79.6	84.4	81.2	0.872 (0.824, 0.920)
> 3.79	147	30	117						
≤ 3.79	77	65	12						
7.06				62.8	91.6	91.0	64.4	75.0	
> 7.06	89	8	81						
≤ 7.06	135	87	48						

AC = accuracy; AUROC = area under the receiver operating characteristic curve; NPV = negative predictive value; PPV = positive predictive value; SN = sensitivity; SP = specificity.

Table 8. Comparisons of noninvasive models for predicting liver fibrosis

Noninvasive models	Cutoff value	Patients classified n (%)	Ishak Fibrosis Stage 0-1 (n)	Stage 2-4 (n)	SN (%)	SP (%)	PPV (%)	NPV (%)	AUROC
Wang I index (N = 125)	≤ 1.75	62 (46.0%)	58	4	89.5	66.7	54.0	93.5	0.866
	> 5.84	25 (20.0%)	3	22	57.9	96.6	88.0	84.0	
Wang II index (N = 224)	≤ 3.79	77 (34.4%)	65	12	90.7	68.4	79.6	84.4	0.872
	> 7.06	89 (39.7%)	8	81	62.8	91.6	91.0	64.4	
S index (N = 386) [17]	< 0.1	61 (15.8%)	40	21	90.4	24.0	60.9	65.6	0.686
	≥ 0.5	103 (26.7%)	23	80	36.5	86.2	77.7	50.9	
Zeng model (N = 200) [18]	< 3.0	43 (21.5%)	37	6	94.8	44.1	70.1	86.1	0.840
	> 8.7	45 (22.5%)	4	41	35.3	95.2	91.1	51.6	
APAG (N = 250)	< 0.27	39 (16%)	ND	ND	95	29	62	82	0.792
	> 0.80	43 (17%)	ND	ND	28	95	87	52	
			Stage 0-2 (n)	Stage 3-6 (n)					
Model of Hui et al (N = 147) [19]	≤ 0.15	55 (37.4%)	52	3	93	49	41	95	0.803
	> 0.5	27 (18.4%)	10	17	41	90	63	79	
APRI (N = 192) [12]	≤ 0.5	55 (28.6%)	47	8	91.0	47.0	61.0	86.0	0.80
	> 1.50	42 (21.9%)	5	37	41.0	95.0	88.0	64.0	

S index = 1000 × GGT/(PLT × ALB^2); APRI = [AST level (ULN)/Platelet counts (10^9/L)] × 100. ND = not described.

significant liver fibrosis. Interestingly, the AUCs of Wang I and Wang II indexes were the largest compared with all other models. These results indicated that the Wang I and Wang II indexes more accurately predicted liver fibrosis than other models.

Discussion

Serum HBV DNA levels, aminotransferase levels, and histologic findings have all been used as indicators of HBV presence and antiviral treatment efficacy. However, the extent of liver fibrosis is considered the key factor in determining the effectiveness of antiviral treatment in patients during the immune tolerant phase of HBV infection with normal serum ALT levels [24].

Liver biopsy is widely regarded as the gold standard diagnostic technique for assessing liver fibrosis, but the procedure is not conducive for monitoring histologic changes because of its invasiveness. In this study, the Scheuer S2-S4 histological classification was used to assess the severity of fibrosis in biopsies, and routine laboratory tests were used as screening markers. Based on their ALT levels, patients with CHB were divided into immune tolerant- and immune reactive HBeAg-positive groups. Two non-invasive diagnostic models, namely Wang I and Wang II, were developed to assess liver fibrosis in these two experimental groups.

We found that the application of different diagnostic models to both the immune tolerant and immune reactive HBeAg-positive phases of CHB improved predictive AC. For patients in the immune tolerant phase, the Wang I model would have enabled 69.6% of patients to avoid liver biopsy. In the immune reactive HBeAg-positive phase of CHB, using the Wang II model,

74.1% of patients were assessed as having liver fibrosis at an AC of 88.0%.

The previously reported noninvasive diagnostic model for liver fibrosis in HBV infection did not distinguish between the immune tolerant and immune reactive HBeAg-positive phases [18-21]. We found that independent predictors of liver fibrosis in the immune tolerant phase differed from those in the immune reactive HBeAg-positive phase. The Wang models improved diagnostic efficiency, SN, and SP, especially in the immune tolerant phase of CHB, suggesting that they may be suitable for assessing long-term disease progression and guiding antiviral and anti-fibrosis treatment decisions.

A model termed PPT derived from three independent predictors of liver fibrosis, including platelet count (PLT), prothrombin time (PT) and total bile acid (TBA) was recently developed and showed an AUC of 0.83, indicating its superiority to other models such as APRI, FIB-4, age-AST model, AP index and APGA model [33]; however, the PPT model is at best comparable to the Wang methods described herein. In addition, the Wang models demonstrated a higher diagnostic AC than the S index [11, 17], Hui et al. prediction model [19], APAG index (including age, prothrombin time, albumin and gamma-glutamyltransferase), and Wong et al. model [32]. These models all have strengths and weaknesses. The S index model is relatively easy to use, but relies on a single parameter and is unable to distinguish between S1 and S2 fibrosis. Currently, this can only be achieved by pathologic examination. However, a combination of the S index with other markers may improve its predictive AC.

The Hui et al. model [19] applies strict requirements for > 10^5 copies/mL of HBV DNA to determine fibrosis. However, significant risk factors for cirrhosis and cancer can also occur at HBV DNA levels > 10^4 copies/mL [24]. The diagnostic model described by Wong et al. involves Fibroscan, a device that significantly improves the diagnostic AC with high SN and SP (> 90%) [32]. However, most hospitals do not have access to this equipment, limiting its widespread use in clinical practice. The APAG index model evaluates the influence of age, PLT, ALB and GGT on the development of fibrosis and has proved to be a convenient and accurate method. We attempted to improve the AC of this technique by adding several new parameters.

The Wang models in our study used conventional biochemical markers, such as PLTs, AST, GGT, PTA and BMI, all which have been previously validated as independent indicators of significant liver fibrosis [16, 24, 33]. PLTs are a useful measure of liver fibrosis and used in the FibroIndex, S index, APRI index, and the Fibrosis 4 model. HBV infection causes inflammation in the liver that continuously stimulates fibrosis and destroys the liver structure, resulting in reduced hepatic production of thrombopoietin I [34, 35]. The associated splenomegaly further reduces platelet production [34, 35].

We also observed that RBC counts were reduced in the immune reactive HBeAg-positive group. The hypersplenism secondary to liver fibrosis causes RBC damage via the nuclear-macrophage system [36]. Because RBCs supply oxygen to liver, their destruction causes liver tissue hypoxia, increasing the rate of liver cell injury, activating hepatic stellate cells, and promoting fibrosis. These findings suggest that RBCs constitute a reliable marker of liver fibrosis during the immune reactive HBeAg-positive phase of CHB.

We identified PTA and GGT as important parameters in the models, suggesting that these markers are closely related to the degree of liver fibrosis. The synthesis of clotting factors including prothrombin has been found to be significantly decreased in the presence of liver cell degeneration, necrosis and fibrosis [37]. Gamma-glutamyl transferase is a membrane-binding enzyme mainly distributed in the hepatocyte microsomal and canalicular bile. Inflammatory stimuli increase membrane permeability, resulting in increased GGT activity in the serum [38, 39]. Previous studies have found that GGT is a reliable predictor of CHC and CHB fibrosis [38, 40].

BMI is an important predictor of fibrosis in the Hui et al. model. In patients with CHC, high BMI, which is closely related to obesity and nonalcoholic fatty liver disease, reduces the antiviral response and increases the risk of fibrosis progression [41-43]. However, this marker has not been used in the CHC diagnostic models, and there are few reports of its diagnostic value in

patients with CHB [10, 11, 13-16, 44]. Our study suggests that obesity can contribute to liver fibrosis development. However, the relationship between BMI and fibrosis in CHB requires further investigation.

The main limitation of our models is that they were unable to define the precise stage of liver fibrosis. In addition, all new assessments of liver fibrosis should be combined with newly emerging noninvasive screening methods such as liver elasticity ultrasound (Fibroscan) [45] to further improve diagnostic AC. Further research, development, and validation of these models are required in a larger cohort of patients with CHB before the models can be introduced into clinical practice.

In conclusion, the Wang models provide reliable alternative to liver biopsy for assessing fibrosis. They may also help to evaluate the efficacy of antiviral and anti-liver fibrosis treatments. With further validation and development of these models in additional patient cohorts, their application may reduce the pain and risk associated with invasive liver examination, and conserve health care resources.

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Disclosure of conflict of interest

None.

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