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Original Article

Total serum bile acids predict therapy for HBeAg-negative chronic hepatitis B patients with borderline ALT and high HBV DNA

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Abstract

Introduction: The introduction of antiviral therapy in chronic hepatitis B (CHB) infection depends on precise evaluation of hepatic lesions. Total serum bile acids (TSBAs) are highly sensitive in monitoring liver dysfunction. We evaluated the predictive role of TSBAs for hepatic lesions in CHB patients with borderline alanine aminotransferase (ALT) and high level of hepatitis B virus (HBV) DNA copies.

Methodology: 328 CHB patients were enrolled, 241 were hepatitis B e antigen (HBeAg)-positive and 87 were HBeAg-negative. Patients were further divided into two entities according to inflammation/fibrosis evaluated by liver biopsy, low-grade (inflammation grade < 2 and fibrosis stage < 2) and high-grade (inflammation grade ≥ 2 or/and fibrosis stage ≥ 2) cohorts. TSBAs were compared with noninvasive tools including aspartate aminnotransferase (AST)-to-platelet ratio index (APRI), fibrosis-4 (FIB-4) and red cell distribution width (RDW)-to-platelet ratio (RPR) to predict high-grade hepatic lesions in CHB subgroups.

Results: TSBAs, APRI, FIB-4 and RPR were statistically different between low- and high-grade patients in HBeAg-positive cohort. Only TSBAs showed significant difference between low and high grade in HBeAg-negative patients. Similarly, APRI, FIB-4 and RPR were correlated with different division of inflammation/fibrosis only in HBeAg-positive while TSBAs were correlated with inflammation/fibrosis levels in both HBeAg-positive and HBeAg-negative groups. Of the four indicators, the receiver operating characteristic (ROC) curve analysis showed that TSBAs have the maximum AUC (area under the curve) in HBeAg-negative group but the minimum in HBeAg-positive cohort.

Conclusions: TSBAs can be used for predicting antiviral therapy in CHB patients with HBeAg-negative, borderline ALT and high HBV DNA.

Key words: total serum bile acids; chronic hepatitis B; antiviral therapy; hepatic lesions; noninvasive model.

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Introduction

Hepatitis B virus (HBV) infection is the leading cause of liver cirrhosis and hepatocellular carcinoma (HCC). The estimated data from 120 countries showed that the global prevalence of hepatitis B surface antigen (HBsAg) in 2016 was 3.9% (95% uncertainty interval [UI] 3.4-4.6), corresponding to 291,992,000 (251,513,000-341,114,000) infections [1]. In China, the mortality rate of HBV-related HCC increased from 12.88 per 100,000 people in 1990 to 16.42 per 100,000 people in 2016 [2]. The benefit of timely and effective antiviral therapy has been demonstrated in reducing endpoints of cirrhosis and HCC. Thus, it is important to evaluate the indicators for HBV treatment since not all patients with chronic hepatitis B (CHB) progress to life-threatening complications [3].

Currently, a panel of non-invasive indices and tools are used to evaluate hepatic lesions and categorize patients to determine who are eligible for treatment [4]. The American Association for the Study of Liver Diseases (AASLD) and Asian-Pacific Association for the Study of the Liver (APASL) guidelines provide good evidence that patients with borderline alanine aminotransferase (ALT) > 2 ULN should have therapy after 3-6 months of observation [5,6]. However, the indication is less clear for those with borderline ALT levels (≤ 2 ULN), even for those who are considered at high risk of fibrosis, i.e., hepatitis B e antigen (HBeAg)-positive, high HBV DNA and normal ALT but > 40 years of age. Liver biopsy is recommended for evaluation of inflammation and fibrosis via histological grading, although it is invasive and has up to 30% sampling error [7]. On the other hand, biochemical and radiological methods such as aspartate aminotransferase (AST)-to-platelet ratio index (APRI), fibrosis-4 (FIB-4), red blood cell distribution width-to-platelet ratio (RPR) and FibroScan have considerably reduced the need for liver biopsy [4,8]. However, these non-invasive tools still remain to be validated, especially in those with HBeAg-negative CHB patients

where they may not provide an accurate correlation with hepatic lesions.

ALT is an enzyme released from damaged liver cells and therefore is an indicator of hepatocellular integrity. However, CHB patients with normal ALT may have debates and clinical dilemmas in monitoring hepatic injury especially in the HBeAg-negative cohort [9]. Bile acids are synthesized in the liver, excreted into the bile, and passed into the intestine where they are reabsorbed and transported back to the liver by the portal system. The enterohepatic circulation of bile acids is a highly efficient process based on efficient reabsorption by the liver. The total bile acids leaked into the peripheral circulation constitute total serum bile acids (TSBAs), which therefore reflects the synthetic, excretory and re-absorptive function of the liver. Thus, TSBAs have early sensitivity in mirroring both the initial phase as well as the longitudinal development in liver diseases [10]. In this study, we aim to investigate the predictive value of TSBAs for hepatic injury in CHB patients with normal or slightly elevated ALT and high HBV DNA. We compared the predictive performance of TSBAs both in HBeAg-positive and HBeAg-negative CHB patients with traditional non-invasive methods including APRI, FIB-4 and RPR.

Methodology

Patients

This is a retrospective cross-sectional study of diagnostic test accuracy. We included 328 CHB patients in the Second Hospital of Anhui Medical University between 2012 and 2019. This hospital is a nonprofit academic medical center focused on integrated health care, education, and research. It was first accredited by the Joint Commission International (JCI) in 2017, with the catchment area of Anhui Province and served more than 2 million patients in 2021. CHB was defined as persistent presence of serum HBsAg for > 6 months. According to the AASLD and APASL recommendations, the inclusion criteria were as follows: (1) patients with age ≥ 18 years and who were treatment-naive; (2) ALT ≤ 2 ULN; (3) HBeAg-positive patients with HBV DNA ≥ 20000 IU/mL; (4) HBeAg-negative patients with HBV DNA ≥ 2000 IU/mL. The exclusion criteria were as follows: (1) coinfection with other hepatotropic viruses (hepatitis A/C/D/E virus) or human immunodeficiency virus (HIV); (2) coexistence of autoimmune hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, drug hepatitis, primary biliary cirrhosis, HCC, or any other type of cancer. (3) patients who were pregnant, and (4) who had hematological diseases or other diseases that could interfere with liver function tests. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee for the Second Hospital of Anhui Medical University (NO. 2019SHAMU0013), and all patients had signed informed consent.

Routine laboratory tests

Fasting serum samples were collected before liver biopsy and the initiation of antiviral therapy. Serum ALT (IU/L), AST, (IU/L), gamma-glutamyl transpeptidase (GGT, IU/L), alkaline phosphatase (ALP, IU/L), albumin (ALB, g/L), globulin (GLB, g/L), cholesterol (CHO, μmol/L) and TSBAs (μmol/L) were detected by AU5800 biochemistry analyzer (Beckman Coulter, California, USA). Blood routine test was performed by an automated hematology analyzer (XT-2000i, Sysmex, Kobe, Japan). HBV DNA was measured using the real-time polymerase chain reaction (Mx3000p, Agilent Technologies, California, USA) with the limit detection at 500 IU/mL. Serological markers of HBV (HBsAg, antibody against HBsAg (anti-HBs), HBeAg, antibody against HBeAg (anti-HBe), antibody against hepatitis B core antigen (anti-HBc)) were measured using commercially available kits with chemiluminescence apparatus (ARCHITECT i2000SR; Abbott diagnostics, Illinois, USA).

Liver biopsy

An ultrasonography-guided percutaneous liver biopsy was performed with a 16-gauge needle before antiviral therapy in all CHB patients. Biopsy specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin and reticular fiber staining or Masson's staining on each section. These specimens were evaluated by two independent and experienced pathologists who were unaware of the patients' identity and clinical information. Histologic characteristics of liver inflammation grade (G) and fibrosis stage (S) were determined using the Batts-Ludwig system [11,12]. This system is simple and easily reproducible via a clear graphic demonstration of what is meant by each grade and stage, and is most appropriate for management of individual patients included in this study. Inflammation was graded according to the amount of portal/periportal and lobular activity (G0-G4, count whichever is greater). Fibrosis was staged as follows: stage 0 (S0), the absence of fibrosis; stage 1 (S1), fibrous portal expansion; stage 2 (S2), periportal or rare portal-portal septa; stage 3 (S3), fibrous septa with architectural distortion; and stage 4 (S4), cirrhosis. G0-1 and S0-1 were considered to be

low-grade hepatic lesions (< G/S2), indicating no or mild inflammation and fibrosis, respectively; G2-4 and S2-4 were considered to be high-grade (≥ G/S2) which indicates moderate to severe inflammation and fibrosis, respectively.

Noninvasive prediction methods and calculation formulae

Fibrosis indices were calculated as below and were then compared to the biopsy results:
APRI = (AST (IU/L)/ULN of AST)/platelet count (10^9/L) × 100 [13];
FIB-4 = (age (years) × AST (IU/L))/(platelet count (10^9/L) × (ALT (IU/L))1/2) [14];
RPR = RDW (%)/platelet count (10^9/L) [15].
The ULN of ALT was 40 IU/L for females and 50 IU/L for males.

Statistical analysis

The data were expressed as a median along with the range of lower-upper quartiles (between 25th and 75th percentiles). Mann-Whitney U test was used to compare age, ALT, AST, GGT, ALP, ALB, GLB, TSBAs, CHO, HBV DNA, platelet/neutrophil/lymphocyte counts, RDW and APRI/FIB-4/RPR indices between HBeAg-positive and HBeAg-negative groups, and between low-grade (< G/S2) and high-grade (≥ G/S2) cohorts. The sample rates of hierarchical G and S panels between HBeAg-positive and HBeAg-negative groups were analyzed by Pearson's $\chi 2$ test or Fisher's exact test. Spearman rank-order correlation was employed to test the association between TSBAs, APRI, FIB-4, RPR and inflammation grades or fibrosis stages. Receiver operating characteristic (ROC) analysis was performed for TSBAs, APRI, FIB-4 and RPR. AUC (area under the curve), sensitivity and specificity at defined points of the curve were determined. Statistical analyses were performed using the SPSS version 22.0 (Chicago, IL) and MedCalc version 19.4 (Ostend, Belgium). A two-tailed $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of CHB patients

The demographic and clinical characteristics of the 328 CHB patients who were enrolled in the study are shown in Table 1. Patients with HBeAg-positive had significantly higher HBV DNA [7.44 (6.66-7.89) vs. 5.13 (4.66-5.67) log10 IU/mL, $p < 0.001$], TSBAs [7.90 (4.45-12.35) vs. 3.96 (3.44-4.45) μmol/L, $p = 0.001$], platelet [182 (147-214) vs. 148 (112-184) × 10^9/L, $p <$

Table 1. Baseline characteristics of the CHB patients.

	HBeAg-positive (n = 241)	HBeAg-negative (n = 87)	*p*
Age (Y)	32.00 (26.00, 38.00)	41.00 (36.50, 47.00)	*< 0.001*
Male (%)	163 (67.63%)	56 (64.37%)	*0.579*
HBV DNA (log10 IU/mL)	7.44 (6.66, 7.89)	5.13 (4.66, 5.67)	*< 0.001*
ALT (IU/L)	44.00 (27.00, 61.50)	41.00 (28.00, 62.00)	*0.681*
AST (IU/L)	30.00 (23.00, 40.00)	33.00 (26.00, 43.00)	*0.099*
GGT (IU/L)	20.00 (14.00, 30.50)	20.00 (14.00, 28.00)	*0.932*
ALB (g/L)	40.30 (37.80, 43.00)	41.70 (37.90, 44.80)	*0.105*
GLB (g/L)	25.90 (23.70, 28.90)	26.40 (23.40, 29.40)	*0.513*
ALP (IU/L)	70.00 (59.00, 91.50)	73.00 (62.00, 96.00)	*0.385*
TSBAs (μmol/L)	7.90 (4.45, 12.35)	3.96 (3.44, 4.45)	*0.001*
CHO (μmol/L)	4.12 (3.61, 4.67)	1.37 (0.75, 2.54)	*0.065*
PLT (10^9/L)	182.00 (147.00, 214.0)	148.00 (112.00, 184.00)	*< 0.001*
N (10^9/L)	2.91 (2.34, 3.50)	2.66 (2.28, 3.39)	*0.198*
L (10^9/L)	2.02 (1.64, 2.44)	1.71 (1.37, 2.15)	*< 0.001*
RDW (%)	12.90 (12.50, 13.45)	12.90 (12.60, 13.30)	*0.879*
Model			
APRI	0.40 (0.29, 0.65)	0.54 (0.39, 0.85)	*< 0.001*
FIB-4	0.83 (0.60, 1.27)	1.43 (1.07, 2.15)	*< 0.001*
RPR	0.07 (0.06, 0.09)	0.09 (0.07, 0.11)	*< 0.001*
Inflammation grade			
G0-1	182 (75.52%)	61 (70.11%)	*0.324*
G2	49 (20.33%)	21 (24.14%)	*0.458*
G3	9 (3.73%)	5 (5.75%)	*0.535**
G4	1 (0.42%)	0 (0%)	*1.00**
Fibrosis stage			
S0-1	177 (73.44%）	45 (51.72%）	*< 0.001*
S2	41 (17.01%)	30 (34.48%)	*0.001*
S3	12 (4.98%)	9 (10.35%)	*0.08*
S4	11 (4.56%)	3 (3.45%)	*1.00**

* Fisher's exact test; Underlined *p* values are statistically significant. CHB: chronic hepatitis B; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALB: albumin; GLB: globulin; ALP: alkaline phosphatase; TSBAs: total serum bile acids; CHO: cholesterol; PLT: platelets; N: neutrophils; L: lymphocytes; RDW: red cell distribution width; APRI: ALT-to-platelet ratio; FIB-4: fibrosis index based on the four factors; RPR: RDW-to-platelet ratio.

0.001] and lymphocyte [2.02 (1.64-2.44) vs. 1.71 (1.37-2.15) × 10^9/L, $p < 0.001$) counts than that of HBeAg-negative cohort, whereas the indices of APRI [0.40 (0.29-0.65) vs. 0.54 (0.39-0.85), $p < 0.001$], FIB-4 [0.83 (0.60-1.27) vs. 1.43 (1.07-2.15), $p < 0.001$] and RPR [0.07 (0.06-0.09) vs. 0.09 (0.07-0.11), $p < 0.001$] were significantly lower in HBeAg-positive patients.

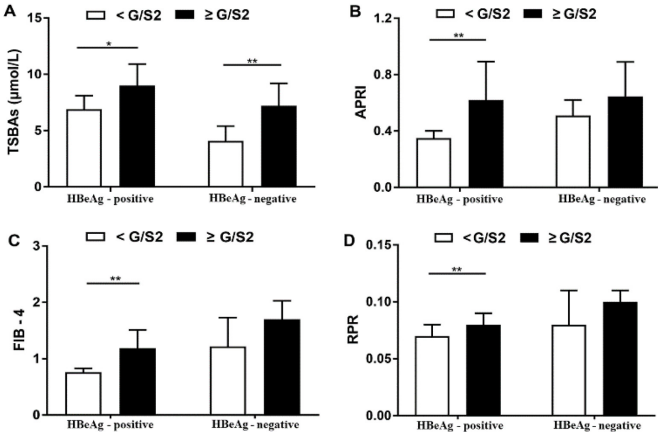
The distribution of G0-4 and S0-4 subgroups were as follows: HBeAg-positive CHB patients, G0-1, 182 (75.52%); G2, 49 (20.33%); G3, 9 (3.73%); G4, 1 (0.42%); S0-1, 177 (73.44%); S2, 41 (17.01%); S3, 12 (4.98%); S4, 11 (4.56%). HBeAg-negative, G0-1, 61 (70.11%); G2, 21 (24.14%); G3, 5 (5.75%); G4, 0 (0%); S0-1, 45 (51.72%); S2, 30 (34.48%); S3, 9 (10.35%); S4, 3 (3.45%). There were significant differences in S0-1 ($p < 0.0001$) and S2 ($p = 0.0001$) between the two groups, whereas no significant difference was observed in the distribution of S3, S4 and any G levels (Table 1).

TSBAs have dominant relevance to inflammation/fibrosis levels in HBeAg-negative patients

The 328 CHB patients, including 241 with HBeAg-positive and 87 with HBeAg-negative, were further divided into two entities low-grade (< G/S2) and high-grade (≥ G/S2) according to the liver biopsy. TSBAs levels in < G/S2 (n = 159) and ≥ G/S2 (n = 82) HBeAg-positive patients were 6.90 (3.90-11.20) and 9.00 (5.05-16.90) μmol/L ($p = 0.005$), and in < G/S2 (n = 38) and ≥ G/S2 (n = 49) HBeAg-negative were 4.10 (2.68-6.23) and 7.20 (4.05-11.50) μmol/L ($p < 0.001$), respectively. On the other hand, APRI, FIB-4 and RPR indices of ≥ G/S2 subgroups were significantly higher than that of < G/S2 in HBeAg-positive cohort ($p < 0.001$). However, the three non-invasive predictive tools had no significant difference between < G/S2 and ≥ G/S2 in HBeAg-negative patients (Figure 1).

The correlation between TSBAs, APRI, FIB-4, RPR and pathological inflammation/fibrosis levels was also analyzed in HBeAg-positive and HBeAg-negative groups respectively. Of the four indicators, TSBAs had the maximum Spearman's correlation coefficient (rs = 0.38, p < 0.001) in HBeAg-negative patients but the minimum (rs = 0.18, p = 0.005) in HBeAg-positive

Figure 1. Comparisons of TSBAs, APRI, FIB-4 and RPR levels between < G/S2 and ≥ G/S2 subgroups in both HBeAg-positive and HBeAg-negative CHB patients. *p < 0.01; **p < 0.001.



cohort. The non-invasive tools APRI, FIB-4 and RPR were positively correlated with inflammation/fibrosis levels (APRI, $r_s = 0.45$, $p < 0.001$; FIB-4, $r_s = 0.35$, $p < 0.001$; RPR, $r_s = 0.31$, $p < 0.001$) in CHB patients with HBeAg-positive. In contrast, no significant correlation was observed between APRI ($r_s = 0.17$, $p = 0.110$), FIB-4 ($r_s = 0.12$, $p = 0.276$), RPR ($r_s = 0.08$, $p = 0.475$) and inflammation/fibrosis levels in HBeAg-negative group (Table 2). Thus, these results suggested that TSBAs have dominant relevance to inflammation/fibrosis levels in HBeAg-negative subjects as compared with traditional non-invasive tools APRI, FIB-4, RPR.

TSBAs have good performance in predicting ≥ G/S2 lesions in HBeAg-negative CHB.

The ROC curves of TSBAs and the other three non-invasive indices for predicting ≥ G/S2 hepatic lesions in HBeAg-positive and HBeAg-negative CHB were shown in Figure 2, and the diagnostic accuracy are compared in Table 3. In the HBeAg-positive group, the calculated AUC (95% CI) of TSBAs [0.61 (0.55-0.67)] was lower than that of APRI [0.77 (0.71-0.82)], FIB-4 [0.71 (0.65-0.77)] and RPR [0.69 (0.63-0.75)]. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TSBAs were 60.98%, 57.86%, 42.74% and 74.19% respectively, with an optimal cut-off value of 8.00 μmol/L. In

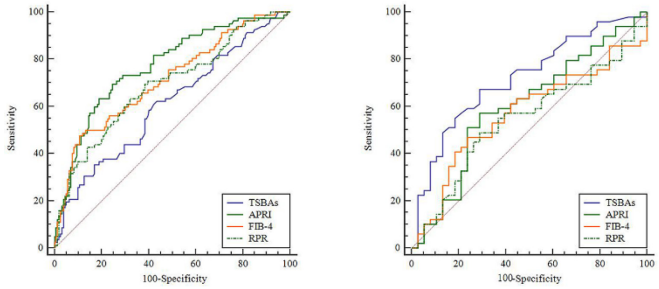
Table 2. Correlation of TSBAs, APRI, FIB-4 and RPR with hepatic lesions.

Non-invasive tools	HBeAg-positive		HBeAg-negative	
	Spearman's *r*	*p*	Spearman's *r*	*p*
TSBAs (μmol/L)	0.18	*0.005*	0.38	*< 0.001*
APRI	0.45	*< 0.001*	0.17	*0.110*
FIB-4	0.35	*< 0.001*	0.12	*0.276*
RPR	0.31	*< 0.001*	0.08	*0.475*

TSBAs: total serum bile acids; APRI: AST-to-platelet ratio; FIB-4: fibrosis index based on the four factors; RPR: RDW-to-platelet ratio. Underlined *p* values are statistically significant.

contrast, TSBAs had the maximum AUC [0.72 (0.61-0.81)] in the HBeAg-negative group, surpassing APRI [0.60 (0.50-0.70)], FIB-4 [0.57 (0.46-0.68)] and RPR [0.54 (0.43-0.64)]. Accordingly, the sensitivity, specificity, PPV and NPV of TSBAs were 67.35%, 71.05%, 75.00% and 72.79% respectively, with an optimal cut-off value of 5.40 μmol/L. Thus, TSBAs have good performance in predicting high-grade (≥ G/S2) lesions in CHB patients with HBeAg-negative.

Figure 2. Receiver operating characteristic (ROC) curves of TSBAs, APRI, FIB-4 and RPR in the prediction of ≥ G/S2 hepatic lesions in HBeAg-positive and HBeAg-negative CHB patients.



Discussion

Accurate assessment of hepatic lesions is pivotal for the introduction of antiviral therapy in CHB patients. Liver biopsy is undoubtedly the gold standard for determining liver injury based on inflammation and fibrosis grading, especially in CHB patients with borderline ALT levels. However, sampling limitations and complications of invasive procedure need to be balanced against the benefits of liver puncture [7]. Thus, the non-invasive methods that allow for a more rational, evidence-based approach to aid clinical decisions are preferred and are more acceptable. The classical algorithmic models, APRI, FIB-4 and RPR, have been widely used for hepatic diseases to estimate liver fibrosis, predict antiviral therapy and reflect adverse prognosis [16].

: In this study, we showed that HBeAg-positive patients have significantly higher platelet counts than HBeAg-negative cohort, whereas ALT, AST and RDW (%) levels showed no significant difference between the two groups. This led to significantly lower APRI, FIB-4 and RPR values in the HBeAg-positive group, since all the three indices were calculated using platelet counts as denominators. These results are consistent with the findings of previous study [17] that compared PLT and FIB-4 between HBeAg-positive and HBeAg-negative groups. However, APRI was higher in HBeAg-negative cohort than that of HBeAg-negative patients in the same study [17]. The inconsistency may result from sampling differences as our study focused on CHB patients with ALT ≤ 2 ULN. It is very likely that the eligibility criteria also led to the lack of statistical difference in other liver function tests including GGT, ALB, GLB, ALP and CHO between HBeAg-positive and HBeAg-negative subjects (Table 1). Nevertheless, both TSBAs and HBV DNA were significantly higher in HBeAg-positive than that in HBeAg-negative cohort. It is also to be noted that HBeAg-positive patients were significantly younger than HBeAg-negative, just as it was found in the study by Goyal *et al.* [18]. One possible explanation is that HBV infection in older adults is usually mild and self-limiting, which is therefore prone to develop into HBeAg negative inactive carriers [19].

Mounting evidence has suggested that bile acid metabolism and HBV infection are interlinked by the sodium taurocholate co-transporting polypeptide (NTCP), which is responsible for sodium-dependent bile salts uptake by hepatocytes [20]. NTCP also functions as a cellular receptor for viral entry of HBV [20]. HBV infection may interfere with NTCP-mediated enterohepatic circulation of bile acids, since the molecular determinants critical for HBV entry overlap with that for bile acid transport [20, 21]. On the

Table 3. Diagnostic performance of TSBAs, APRI, FIB and RPR in hepatic lesions.

	AUC (95% CI)	Optimized Cutoff	Sen (%)	Spe (%)	PPV (%)	NPV (%)
HBeAg-positive						
TSBAs (μmol/L)	0.61 (0.55-0.67)	8.00	60.98	57.86	42.74	74.19
APRI	0.77 (0.71-0.82)	0.47	69.51	75.47	58.76	82.64
FIB-4	0.71 (0.65-0.77)	1.35	47.56	89.31	68.42	76.63
RPR	0.69 (0.63-0.75)	0.08	63.41	67.92	53.09	75.63
HBeAg-negative						
TSBAs (μmol/L)	0.72 (0.61-0.81)	5.40	67.35	71.05	75.00	72.79
APRI	0.60 (0.50-0.70)	0.62	57.14	71.05	71.05	55.10
FIB-4	0.57 (0.46-0.68)	1.79	46.94	76.32	69.70	51.85
RPR	0.54 (0.43-0.64)	0.10	48.98	71.05	66.67	50.00

TSBAs: total serum bile acids; APRI: AST-to-platelet ratio; FIB-4: fibrosis index based on the four factors; RPR: RDW-to-platelet ratio; AUC: aera under the curve; CI: confidence interval; Sen: Sensitivity; Spe: Specificity.

other hand, in the study by Kim *et al.* and Xun *et al*., bile acids were shown to increase HBV gene expression and inhibit interferon-alpha activity [22, 23]. This interaction may contribute to a better understanding of the increased TSBAs in CHB patients.

Our data demonstrated that the percentage of CHB patients with no or mild fibrosis (S0-1) is significantly higher in HBeAg-positive group than the negative control (73.44% *vs.* 51.72%, $p < 0.001$). In contrast, the percentage of moderate fibrosis (S2) was observed to be higher in HBeAg-negative *vs.* HBeAg-positive subjects (34.48% *vs.* 17.01%, $p = 0.001$). Besides, no significant difference was observed in the distribution of S3, S4, and all inflammation grades (G0 to 4) between the two groups (Table 1). These results are in agreement with our observation and that of other researchers that HBeAg seroconversion during the natural history of CHB is associated with a decline in serum HBV DNA levels and growth of liver fibrosis [24].

It is well documented that the non-invasive tools APRI, FIB-4 and RPR have the advantages of predicting liver fibrosis with high applicability (> 95%) and good interlaboratory reproducibility [25]. In this study, APRI, FIB-4 and RPR indices were statistically different between low-grade (< G/S2) and high-grade (≥ G/S2) subgroups, and were positively correlated with inflammation/fibrosis levels only in HBeAg-positive cohort. In contrast, TSBAs were observed to be significantly different between < G/S2 and ≥ G/S2, and correlated with inflammation/fibrosis levels both in HBeAg-positive and HBeAg-negative CHB patients. These results are consistent with metabonomic studies by Wang *et al.* [26] and Yin *et al.* [27] who showed that TSBAs were associated with pathological progression of CHB and therefore may be indicative of hepatitis B-induced cirrhosis. Interestingly, the Spearman's correlation coefficient of TSBAs was substantially higher in HBeAg-negative than that in HBeAg-positive. Furthermore, of the four indicators, TSBAs have the maximum AUC, PPV and NPV scores in the HBeAg-negative rather than HBeAg-positive subjects. It is to be noted that a higher NPV indicates that TSBAs can accurately rule out those who do not need treatment independent of liver biopsy. These data strongly suggest that TSBAs are superior to APRI, FIB-4 and RPR for predicting liver lesions in HBeAg-negative CHB patients.

This study has two limitations. Firstly, we studied a small number of CHB patients, especially the HBeAg-negative cohort. Secondly, this is a retrospective study which is limited to the data acquisition of TSBAs fluctuation after antiviral therapy. Thus, a prospective longitudinal study with sufficient sample size is needed to verify the indicative role of TSBAs in CHB-related hepatic lesions [28]. However, the prospect of this study is undoubtedly exciting in revealing that TSBAs are eligible for predicting ≥ G/S2 hepatic lesions in HBeAg-negative CHB patients with borderline ALT (≤ 2 ULN) and high HBV DNA. Early initiation of antiviral therapy is critical for CHB patients with these serological characteristics. Choi *et al*. have shown that untreated high viral load HBeAg-negative CHB patients without significant ALT elevation had higher risks of HCC and death/transplantation than treated active phase patients with elevated ALT [29].

Conclusions

Our results provide evidence that TSBAs have good performance in predicting high-grade (≥ G/S2) hepatic lesions in HBeAg-negative CHB patients with borderline ALT (≤ 2 ULN) and high HBV DNA. These findings can be used to optimize the antiviral therapy of CHB patients by concentrating on understanding TSBAs and traditional non-invasive methods such as APRI, FIB-4 and RPR.

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Authors' contributions

Li-wen Chen performed critical revision and final approval of the manuscript; Ran Xie collected the data, performed statistical analysis and wrote the manuscript; Jiao Li collected the data and performed statistical analysis; Hao Zhang conceived the study; Ling-mei Wang and Cheng-rong Huang coordinated the analysis of data.

References

1. Polaris Observatory Collaborators (2018) Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol 3: 383-403.
2. Liu J, Liang W, Jing W, Liu M (2019) Countdown to 2030: eliminating hepatitis B disease, China. Bull World Health Organ 97: 230-238.
3. European Association for the Study of the Liver (2017) EASL 2017 Clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 67: 370-398.
4. Li Y, Cai Q, Zhang Y, Xie Q, Xu N, Jiang X, Li J, Li X, Zhang Z (2016) Development of algorithms based on serum markers and transient elastography for detecting significant fibrosis and cirrhosis in chronic hepatitis B patients: significant reduction in liver biopsy. Hepatol Res 46: 1367-1379.

5. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH, American Association for the Study of Liver Diseases (2016) AASLD guidelines for treatment of chronic hepatitis B. Hepatology 63: 261-283.
6. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH (2016) Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 10: 1-98.
7. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD; American Association for the Study of Liver Diseases (2009) Liver biopsy. Hepatology 49: 1017-1044.
8. Wu X, Cai B, Su Z, Li Y, Xu J, Deng R, Wang L (2018) Aspartate transaminase to platelet ratio index and gamma-glutamyl transpeptidase-to-platelet ratio outweigh fibrosis index based on four factors and red cell distribution width-platelet ratio in diagnosing liver fibrosis and inflammation in chronic hepatitis B. J Clin Lab Anal 32: e22341.
9. Lin CL, Liao LY, Liu CJ, Yu MW, Chen PJ, Lai MY, Chen DS, Kao JH (2007) Hepatitis B viral factors in HBeAg-negative carriers with persistently normal serum alanine aminotransferase levels. Hepatology 45: 1193–1198.
10. Luo L, Aubrecht J, Li D, Warner RL, Johnson KJ, Kenny J, Colangelo JL (2018) Assessment of serum bile acid profiles as biomarkers of liver injury and liver disease in humans. PLoS One 3: e0193824.
11. Goodman ZD (2007) Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol 47: 598-607.
12. Batts KP, Ludwig J (1995) Chronic hepatitis: an update on terminology and reporting. Am J Surg Pathol 19: 1409–1417.
13. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS (2003) A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 38: 518–526.
14. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators (2006) Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 43: 1317–1325.
15. Chen B, Ye B, Zhang J, Ying L, Chen Y (2013) RDW to platelet ratio: a novel noninvasive index for predicting hepatic fibrosis and cirrhosis in chronic hepatitis B. PLoS One 8: e68780.
16. Huang R, Wang G, Tian C, Liu Y, Jia B, Wang J, Yang Y, Li Y, Sun Z, Yan X (2017) Gamma-glutamyl-transpeptidase to platelet ratio is not superior to APRI, FIB-4and RPR for diagnosing liver fibrosis in CHB patients in China. Sci Rep 7: 8543.
17. Liu DP, Lu W, Zhang ZQ, Wang YB, Ding RR, Zhou XL, Huang D, Li XF (2018) Comparative evaluation of GPR versus APRI and FIB-4 in predicting different levels of liver fibrosis of chronic hepatitis B. J Viral Hepat 25: 581-589.
18. Goyal SK, Jain AK, Dixit VK, Shukla SK, Kumar M, Ghosh J, Ranjan A, Gupta N, Tripathi M (2015) HBsAg level as predictor of liver fibrosis in HBeAg positive patients with chronic Hepatitis B virus infection. J Clin Exp Hepatol 5: 213-220.
19. Kemp L, Clare KE, Brennan PN, Dillon JF (2019) New horizons in hepatitis B and C in the older adult. Age Ageing 48: 32-37.
20. Yan H, Peng B, Liu Y, Xu G, He W, Ren B, Jing Z, Sui J, Li W (2014) Viral entry of hepatitis B and D viruses and bile salts transportation share common molecular determinants on sodium taurocholate cotransporting polypeptide. J Virol 88: 3273-3284.
21. König A, Döring B, Mohr C, Geipel A, Geyer J, Glebe D (2014) Kinetics of the bile acid transporter and hepatitis B virus receptor Na+/taurocholate cotransporting polypeptide (NTCP) in hepatocytes. J Hepatol 61: 867-875.
22. Kim HY, Cho HK, Choi YH, Lee KS, Cheong J (2010) Bile acids increase hepatitis B virus gene expression and inhibit interferon-alpha activity. FEBS J 277: 2791-2802.
23. Xun Z, Lin J, Yu Q, Liu C, Huang J, Shang H, Guo J, Ye Y, Wu W, Zeng Y, Wu S, Xu S, Chen T, Chen J, Ou Q (2021) Taurocholic acid inhibits the response to interferon-α therapy in patients with HBeAg-positive chronic hepatitis B by impairing CD8+ T and NK cell function. Cell Mol Immunol 18: 461-471.
24. Suslov A, Meier MA, Ketterer S, Wang X, Wieland S, Heim MH (2021) Transition to HBeAg-negative chronic hepatitis B virus infection is associated with reduced cDNA transcriptional activity. J Hepatol 74: 794-800.
25. Li Q, Li W, Huang Y, Chen L (2016) The gamma-glutamyl transpeptidase-to-platelet ratio predicts liver fibrosis and cirrhosis in HBeAg-positive chronic HBV infection patients with high HBV DNA and normal or mildly elevated alanine transaminase levels in China. J Viral Hepat 23: 912-919.
26. Wang X, Xie G, Zhao A, Zheng X, Huang F, Wang Y, Yao C, Jia W, Liu P (2016) Serum bile acids are associated with pathological progression of hepatitis B-induced cirrhosis. J Proteome Res 15: 1126-1134.
27. Yin P, Wan D, Zhao C, Chen J, Zhao X, Wang W, Lu X, Yang S, Gu J, Xu G (2009) A metabonomic study of hepatitis B-induced liver cirrhosis and hepatocellular carcinoma by using RP-LC and HILIC coupled with mass spectrometry. Mol Biosyst 5: 868-876.
28. Stauber RE, Fauler G, Rainer F, Leber B, Posch A, Streit A, Spindelboeck W, Stadlbauer V, Kessler HH, Mangge H (2017) Anti-HCV treatment with ombitasvir/paritaprevir/ritonavir ± dasabuvir is associated with increased bile acid levels and pruritus. Wien Klin Wochenschr 129: 848-851.
29. Choi GH, Kim GA, Choi J, Han S, Lim YS (2019) High risk of clinical events in untreated HBeAg-negative chronic hepatitis B patients with high viral load and no significant ALT elevation. Aliment Pharmacol Ther 50: 215-226.

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