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Transient Elastography with Serum Hepatitis B Surface Antigen Enhances Liver Fibrosis Detection

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Background: The aim of this study was to explore transient elastography (TE) with quantitative hepatitis B surface antigen (qHBsAg) for detecting advanced hepatic fibrosis.





Material/Methods: This was a single-center prospective real-life analysis of 111 treatment-naïve chronic hepatitis B (CHB) patients enrolled into the Establishment of Non-invasive Diagnosis Criteria and Model of Hepatitis B Virus-related Cirrhosis Study.

Results: There were significant correlations between TE, qHBsAg, and fibrosis. Both qHBsAg and TE were identified as independent predictors for advanced fibrosis. In receiver operating characteristic curve (ROC) analysis, TEqHBsAg (combination of TE and qHBsAg) resulted in the highest area under the receiver-operator curve (AUC) (0.912), mainly due to increased specificity. Using the optimal cut-off, TEqHBsAg provided a sensitivity of 86.7%, and increased specificity from 78.7% to 85.1%.

Conclusions: Combining TE with qHBsAg enhances specificity in identifying advanced fibrosis in treatment-naïve CHB patients.

MeSH Keywords: **Diagnosis • Hepatitis B Surface Antigens • Hepatitis, Chronic • Liver • Transients and Migrants**

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Background

It is estimated that approximately 400 million individuals are living with chronic hepatitis B (CHB) worldwide, with 75% of cases in the Asia-Pacific region [1,2]. CHB leads to severe sequelae in about 5% of infected adults and in a larger proportion of children, and is responsible for 0.5–1.2 million deaths per year. These statistics show the need for additional measures, since intensified treatment and follow-up may reduce the burden of this disease.

Liver biopsy (LB) is normally recommended for staging of fibrosis [3]. However, it is an invasive procedure hampered by patient distress, serious complications, sampling bias, variability in interpretation, and difficulty of repetition for fibrosis monitoring [4,5]. As a novel non-invasive assessment, transient elastography (TE) has been validated as a surrogate marker in many studies [6–8], and has gained widespread use in many regions due to its advantages of accuracy, simplicity, and rapid results [9–11]. However, TE has a suboptimal specificity due to overestimation caused by non-fibrotic histological features. At least 1 study has examined combining TE with readily available non-invasive markers for predicting fibrosis [12], but the effect was marginal.

Hepatitis B surface antigen (HBsAg) has been the criterion standard in detecting active HBV infection since its discovery, and is traditionally measured qualitatively. The recent availability of quantitative assays has reinforced its value in monitoring prognosis and treatment response in patients with CHB [13–15]. A few studies have investigated the correlation between quantitative HBsAg (qHBsAg) levels and the stage of fibrosis [16–18]. The limited available data show a potential value of qHBsAg as a disease-specific marker in fibrosis staging. However, the value of combining TE with qHBsAg in fibrosis detection remains unclear.

The aim of this study was to evaluate the predictive value of combining TE with qHBsAg in identifying advanced fibrosis in CHB patients.

Material and Methods

Patients

Consecutive treatment-naïve CHB patients who were enrolled into the Establishment of Non-invasive Diagnosis Criteria and Model of Hepatitis B Virus-related Cirrhosis Study were assessed at the Japan-China Friendship Hospital in Beijing, China, between March 2012 and December 2013. The diagnosis of CHB was in accordance with the 2010 guidelines for the prevention and treatment of chronic hepatitis B of the Chinese Medical

Association. Inclusion criteria for patients were: 1) age 18–65 years, 2) serum HBsAg-positive for at least 6 months, and 3) persistently or intermittently abnormal aminotransferase (aspartate aminotransferase or alanine aminotransferases) values. Patients with the following conditions were excluded from the study: 1) current or previous antiviral therapy, 2) current or previous liver function decompensation, 3) aminotransferase normalization therapy within the 2 preceding weeks, 4) absolute contraindications to LB [platelets (PLT) $<60 \times 10^9/L$, international normalized ratio >1.35], 5) body mass index ≥ 30 , and 6) presence of other liver diseases (hepatitis C virus, hepatitis D virus, or human immunodeficiency virus co-infection; autoimmune hepatitis; suspected or proven hepatocellular carcinoma; or average alcohol consumption >20 g daily for men, or >10 g average for women). All patients gave written informed consent. The study protocol conformed to the ethics guidelines of the Declaration of Helsinki and was approved by our Institutional Ethics Committee.

Clinical and laboratory assessment

Serum samples, TE, and LB were obtained in sequence on the same day in all cases. Relevant clinical variables included age, sex, PLT, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), total bilirubin (TBIL), albumin (ALB), FIB4 [(AST \times age)/(PLT \times ALT^{1/2})], APRI (AST/PLT), hepatitis B e antigen (HBeAg), HBV DNA, and HBsAg.

Serum HBsAg levels were quantified using the Architect HBsAg I200 assay (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's protocol. The detection value ranges from 0.05 to 250 IU/ml. Samples with an HBsAg level higher than 250 IU/ml require a 1:500 dilution. Levels of qHBsAg are expressed as \log_{10} IU/ml. A central clinical laboratory was used to analyze serum samples. All of the blood samples were collected, centrifuged, and immediately stored in a temperature-monitored storage device at -80°C .

Transient elastography

Fibroscan (EchoSens, Paris, France) examination was performed before LB by an experienced observer who was blinded to patient data. Liver stiffness was determined on the right lobe of the liver as previously described [19]. The results are expressed in kilopascals (kPa) and the median value of 10 acquisitions was considered for analysis, including only those cases with a success rate higher than 60% and an interquartile range (IQR)/result ratio <0.3 .

Histological assessment

LB was performed using 16G MAXCO needles (Bard Co., Murray Hill, NJ, USA) under ultrasound guidance. The specimens were

Table 1. Baseline characteristics of patients infected with HBV.

Index	Whole group	Stages of fibrosis		P value
		F0–F2 (n=75)	F3–F4 (n=27)	
Sex (male/female)	89/13	66/9	23/4	0.741
Age (year)*	39.4±11.3	37.0±10.4	45.8±11.6	0.000
ALT (IU/L)**	51.5 (35.0, 96.0)	49.0 (31.5, 93.5)	66.0 (43.0, 111.0)	0.052
GGT (IU/L)**	32.0 (21.0, 61.0)	27.0 (17.0, 37.5)	64.0 (33.0, 97.8)	0.000
TBIL (μmol/L)*	15.53±6.49	14.44±5.68	18.42±7.65	0.006
ALB (g/L)**	47.0 (45.0, 49.3)	47.0 (45.0, 50.0)	46.0 (44.3, 47.8)	0.724
PLT (10 ⁹ /L)*	179.93±64.08	198.92±58.66	134.22±53.21	0.000
HBeAg (negative/positive)	36/66	24/49	12/17	0.639
qHBsAg (log ₁₀ IU/ml)*	3.34±0.95	3.48±1.00	2.97±0.69	0.016
HBV DNA (log ₁₀ IU/ml)**	6.0 (3.0, 7.0)	6.0 (3.0, 8.0)	5.0 (3.0, 7.0)	0.118
TE (kPa)**	7.0 (5.0, 12.0)	6.1 (4.7, 8.0)	17.1 (11.0, 21.7)	0.000
FIB4**	1.02 (0.65, 2.12)	0.95 (0.58, 1.62)	1.56 (0.84, 3.21)	0.020
APRI**	0.20 (0.11, 0.43)	0.19 (0.11, 0.35)	0.31 (0.11, 0.68)	0.145

* Data are mean ±SD; ** Data are median (25%, 75%). ALT – alanine aminotransferase; GGT – γ-glutamyl transferase; TBIL – total bilirubin; ALB – albumin; PLT – platelets; HBeAg – hepatitis B e antigen; qHBsAg – quantitative hepatitis B surface antigen; HBV – hepatitis B virus; TE – transient elastography.

fixed, paraffin-embedded, and stained with hematoxylin and eosin and Masson's trichrome. Liver fibrosis was evaluated according to Metavir staging (F0–F4). F3 and F4 were defined as advanced fibrosis. Fibrosis staging was considered as reliable when liver specimen length was ≥10 mm and the portal tract number was ≥5. All liver biopsies were evaluated separately by 2 experienced pathologists, followed by a consensus reading in cases of discordance. The pathologists were blinded to clinical information.

Data analysis

Qualitative data among groups were compared using the chi-squared test. Student's t test, ANOVA, or the Kruskal-Wallis test were used for quantitative data. Spearman's correlation coefficient (r) was used for correlation analysis. Logistic regression analyses were performed to investigate the significant predictors of advanced fibrosis. The accuracy of the parameters was assessed by calculating the area under the receiver-operator curve (AUC) and the magnitude of the effects was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Specificities were compared using the chi-squared test. Statistical significance was taken as 2-sided $P < 0.05$. All statistical analyses were performed using SPSS software version 17.0.

Results

Baseline characters

Of the 111 patients included in this study, LB failed in 5 patients because the liver specimen length was <10 mm, while TE failed in 4 patients with narrow intercostal spaces or high body mass index. LB and TE were successfully performed in 102 patients (mean age 39.4±11.3 years, 89 males). Advanced fibrosis (F3 and F4) was confirmed in 27 (26.5%) patients. The mean qHBsAg was 3.34±0.95 (log₁₀IU/ml). The median TE was 6.95kPa. Age, GGT, TBIL, TE, and FIB4 were higher in patients with advanced fibrosis than in patients without advanced fibrosis, while qHBsAg and PLT were lower in patients with advanced fibrosis. There was no significant difference in male sex, ALT, ALB, HBeAg, HBV DNA, or APRI between the 2 groups (Table 1).

Correlation between qHBsAg, transient elastography, and stage of fibrosis

There was a significant association between TE and fibrosis severity ($r=0.642$, $P < 0.001$) in the whole group. HBeAg (+) patients had a stronger correlation between TE and fibrosis severity ($r=0.686$, $P < 0.001$) than HBeAg (–) patients ($r=0.587$, $P=0.002$; Figure 1).

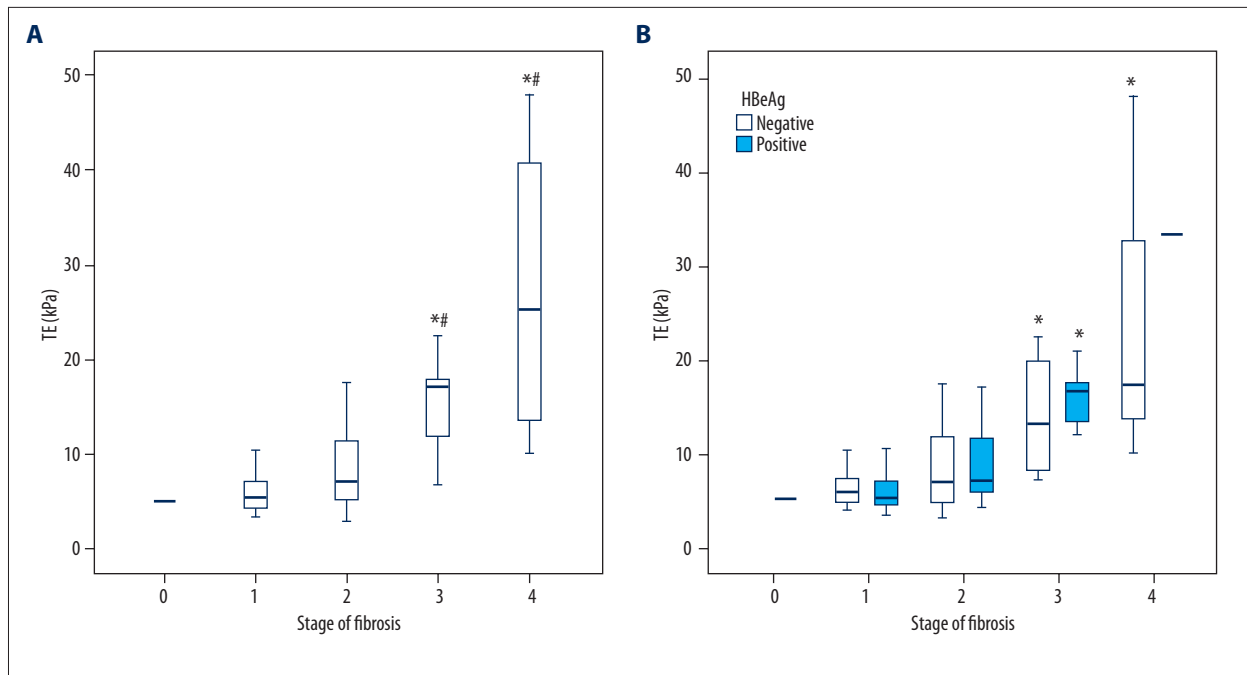


Figure 1. (A, B) Distribution according to fibrosis state of liver stiffness in CHB patients. There was a strong correlation between TE ($r=0.642$, $P=0.000$) and fibrosis severity. The correlation remained significant in both HBeAg(+) and HBeAg(-) patients, but was stronger in HBeAg(+) patients ($r=-0.686$, $P=0.000$). * $P<0.05$, vs. F1; # $P<0.05$, vs. F2.

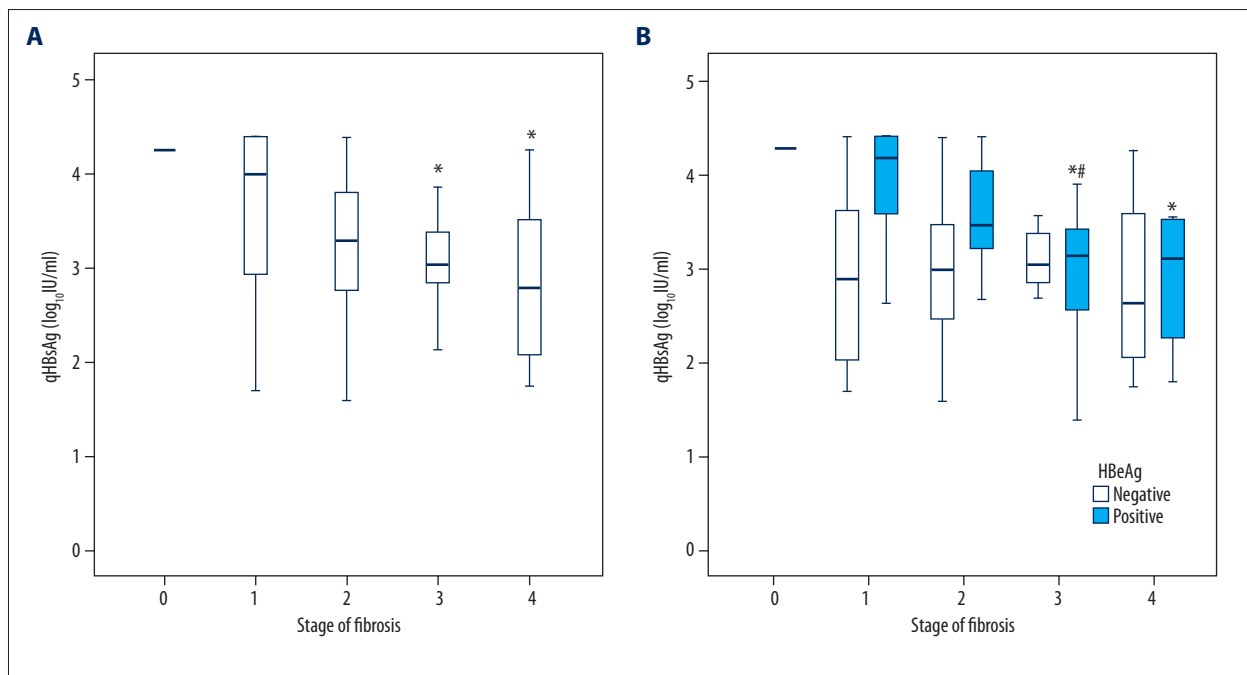


Figure 2. (A, B) Distribution according to fibrosis state of qHBsAg values in CHB patients. qHBsAg was correlated with stage of fibrosis in the entire group of CHB patients ($r=-0.395$, $P=0.000$). There were stronger correlations in HBeAg(+) patients ($r=-0.564$, $P=0.000$), but no significant correlation in HBeAg(-) patients. * $P<0.05$, vs. F1; # $P<0.05$, vs. F2.

Serum qHBsAg showed an inverse correlation with fibrosis severity ($r=-0.395$, $P<0.001$) and TE ($r=-0.383$, $P=0.001$) in all patients infected with HBV. The inverse correlations

between qHBsAg and fibrosis severity ($r=-0.564$, $P<0.001$), or TE ($r=-0.628$, $P<0.001$) were stronger in HBeAg (+) patients, but not statistically significant in HBeAg (-) patients (Figures 2, 3).

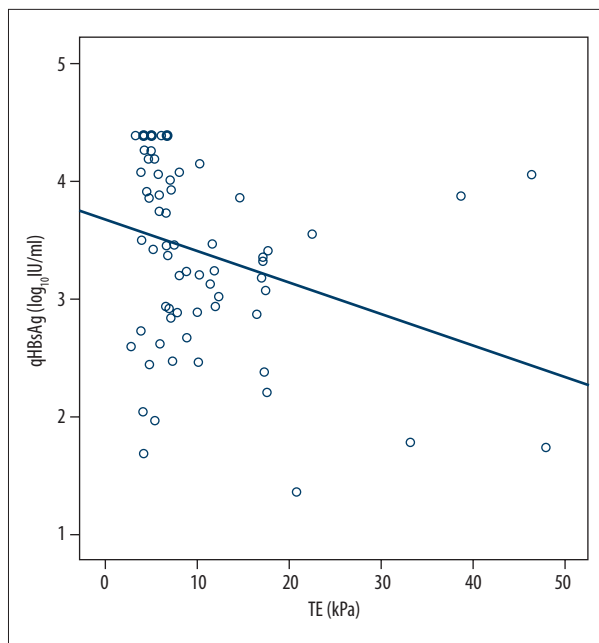


Figure 3. Correlation between serum qHBsAg and TE in CHB patients. There was a correlation between qHBsAg and TE in the entire group of HBV-infected patients ($r=-0.383$, $P=0.001$).

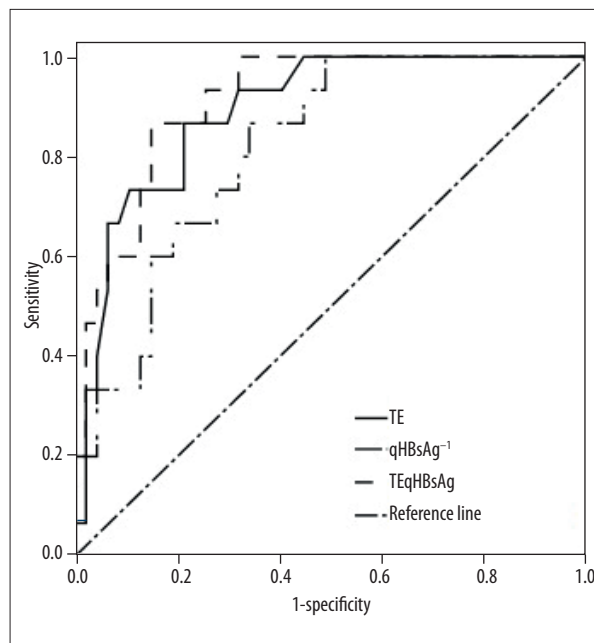


Figure 4. ROC curves for the performance of qHBsAg⁻¹, TE, and TEqHBsAg for identifying advanced fibrosis in CHB patients. TEqHBsAg resulted in the highest AUC (0.912), followed by TE (0.892) and qHBsAg⁻¹ (0.752). ROC analysis of TEqHBsAg suggested that a cut-off of 3.385 exhibited a sensitivity of 86.7% and a specificity of 85.1%, with a PPV of 85.3% and an NPV of 86.5%.

Table 2. Logistic regression analyses exploring qHBsAg and TE as predictors of advanced fibrosis.

	β	OR (95% CI)	P value
qHBsAg	-1.199	0.301 (0.097, 0.932)	0.037
TE	1.169	3.219 (1.387, 7.472)	0.006

qHBsAg – quantitative hepatitis B surface antigen; TE – transient elastography.

Comparison of diagnostic performance of qHBsAg⁻¹, TE and TEqHBsAg for advanced fibrosis

In a logistical regression analysis that included qHBsAg, TE, FIB4, and APRI, only qHBsAg and TE remained as independent predictors for advanced fibrosis (Table 2). Therefore, qHBsAg, and TE, and the combination of TE and qHBsAg (TEqHBsAg, TE divided by qHBsAg), were used for the following diagnostic analysis.

TEqHBsAg resulted in the highest AUC (0.912), followed by TE (0.892) and qHBsAg⁻¹ (0.818) in the entire cohort (all $P<0.001$), mainly due to increased specificity (Table 3, Figure 4). Using the optimal cutoff of 8.950, TE provided a sensitivity of 86.7% and specificity of 78.7%. The TEqHBsAg cutoff of 3.385 led to the same sensitivity, but a higher specificity (85.1% vs. 78.7%, $P=0.202$). Likewise, the PPV and NPV were improved, at 85.3%

and 86.5% for TEqHBsAg, as opposed to 80.3% and 85.5% for TE, respectively. When taking the cutoff values that provided a sensitivity of 100.0%, the specificity was increased from 55.3% to 68.1% ($P=0.062$).

In a sub-analysis, we stratified the patients according to HBeAg status. TEqHBsAg still had the highest AUC compared to qHBsAg⁻¹ and TE in both groups. In HBeAg (-) patients, the combination of qHBsAg⁻¹ and TE improved the specificity of the diagnosis. When cutoff values corresponding to a sensitivity of 85.7% were chosen, the specificity was increased from 70.6% to 82.4% ($P=0.165$). In HBeAg (+) patients, TEqHBsAg had the same specificity as TE at the optimal cutoff, but a higher specificity (76.7% vs. 60.0%, $P=0.039$) when taking the cutoff values provided a sensitivity of 100.0% (Table 4).

Table 3. Comparison of the performance of qHBsAg⁻¹, TE, and TEqHBsAg for the diagnosis of advanced fibrosis in CHB patients.

	AUC (95% CI)	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P value
qHBsAg ⁻¹	0.818 (0.711–0.926)	0.2969	86.7	66.0	71.8	83.2	0.000
		0.2584	100.0	51.1	67.1	100.0	
TE	0.892 (0.809–0.976)	8.950	86.7	78.7	80.3	85.5	0.000
		6.65	100.0	55.3	69.1	100.0	
TEqHBsAg	0.912 (0.841–0.983)	3.385	86.7	85.1	85.3	86.5	0.000
		2.221	100.0	68.1	75.8	100.0	

AUC – area under the receiver-operator curve; PPV – positive predictive value; NPV – negative predictive value; qHBsAg – quantitative hepatitis B surface antigen; TE – transient elastography; TEqHBsAg – TE divided by qHBsAg.

Table 4. Comparison of the performance of qHBsAg⁻¹, TE, and TEqHBsAg for the diagnosis of advanced fibrosis in patients with different HBeAg status.

	AUC (95% CI)	Cut-off value	Sensitivity (%)	Specificity (%)	P value
HBeAg (-)					
qHBsAg-1	0.630 (0.384–0.876)	0.3464	71.4	64.7	0.325
		7.100	100.0	64.7	
TE	0.849 (0.684–1.000)	8.450	85.7	70.6	0.008
		3.385	85.7	82.4	
TEqHBsAg	0.882 (0.712–1.000)	3.385	85.7	82.4	0.004
HBeAg (+)					
qHBsAg-1	0.929 (0.802–1.000)	0.3039	87.5	86.7	0.000
		0.2584	100.0	73.3	
TE	0.906 (0.767–1.000)	11.700	87.5	90.0	0.000
		6.65	100.0	60.0	
TEqHBsAg	0.933 (0.834–1.000)	3.663	87.5	90.0	0.000
		2.221	100.0	76.7	

AUC – area under the receiver-operator curve; HBeAg – hepatitis B e antigen; qHBsAg – quantitative hepatitis B surface antigen; TE – transient elastography; TEqHBsAg – TE divided by qHBsAg; ALT – alanine aminotransferase.

Discussion

Fibrosis staging has long been suggested to be crucial in the management of CHB, and the recent availability of non-invasive assessment of liver fibrosis reinforced its value in monitoring prognosis and treatment response in patients with CHB. As a non-invasive approach for evaluating liver fibrosis, TE has gained widespread use due to its advantages, which include accuracy, simplicity and rapid results; however, it is hampered by the overestimation caused by non-fibrotic histological features. Recently, as advances have been made in the development of qHBsAg assays, qHBsAg was recognized as an important marker in monitoring the natural history of CHB [20]. Seto et al. [16] suggested that high serum HBsAg levels ($\geq 25,000$ IU/ml) can

accurately predict mild fibrosis (Ishak fibrosis score ≤ 1) in HBeAg (+) CHB patients with ALT $\leq 2 \times$ ULN. The current study investigated the predictive value of the combination of TE and qHBsAg in identifying advanced fibrosis. Our study is a single-center prospective real-life analysis of treatment-naïve CHB patients.

As expected, there was a significant association between TE and fibrosis severity in our research. The AUC, sensitivity, and specificity provided by TE in our study were comparable with those found in other studies [12]. We confirmed the inverse correlation between qHBsAg and severity of fibrosis in treatment-naïve CHB patients. The exact mechanism for the inverse relationship between the HBsAg levels and the stages of fibrosis is unclear. It was hypothesized that enhanced immune

clearance of HBsAg, intracellular block of HBsAg secretion, or diminishing ability of the host to support viral replication may be involved. Consistent with publications by Seto et al. [16] and Martinot-Peignoux et al. [18], the relationship was only statistically significant in HBeAg (+) patients. It could be speculated that HBeAg (+) status reflects a lack of immune control, whereas HBeAg (–) status means the host exerts some level of immune control over the infection. In the immune-tolerance phase, HBsAg remains high and fibrosis is minimal. When entering the immune clearance phase, with repeated immune-mediated damage, more fibrosis develops and HBsAg decreases. However, Tseng et al. [21] suggested that high HBsAg levels can predict disease progression in HBeAg (–) patients. The role of qHBsAg in patients with different HBeAg statuses awaits further clarification.

Previous papers on the subject of combining TE with easily available serum markers have shown that the diagnostic accuracy increased, but that the specificity was not significantly enhanced. Because one of these publications suggested that FIB4 and APRI were independent predictors of advanced liver fibrosis, we included qHBsAg, TE, FIB4, and APRI in the logistical regression analysis. Only qHBsAg and TE remained as independent predictors, so we did not perform further analysis of FIB4 and APRI. In our study, the combination with qHBsAg enhanced the specificity of TE in identifying advanced fibrosis in all groups. TEqHBsAg resulted in the highest AUC (0.912) compared to TE and qHBsAg, in which a cutoff of 3.385 led to a sensitivity of 86.7% and a specificity of 85.1% in the whole group. Specificities were increased by combining TE with qHBsAg⁻¹, when taking both optimal cutoff and cutoff values providing a sensitivity of 100.0%. Although the improvement in specificity was marginal, it may be of clinical importance if this effect reaches statistical significance in a larger sample. In both HBeAg (–) and HBeAg (+) patients, the accuracy of TE in predicting advanced fibrosis was improved by the combination.

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The accuracy of TEqHBsAg was higher in HBeAg (+) patients. Using the lower cutoff of TEqHBsAg (2.221) to reach 100% sensitivity, 81.1% of patients classified in the advanced fibrosis group were diagnosed correctly, and the specificity was significantly increased, from 60.0% to 76.7%. The improvement in specificity seen with TE reinforces its value in treatment decision-making, prognosis, and treatment response monitoring. The advantages of non-invasive nature and accuracy of TEqHBsAg shown in this study may make it a promising procedure for use in the surveillance of CHB patients.

The small number of patients in this study requires validation of the results with a larger cohort, and prevented further analysis by subgroups, such as different clinical stages of patients. Genotype information, which may affect qHBsAg and TEqHBsAg, was not available. The possible value of combining other parameters was not fully investigated. The value of TEqHBsAg in detecting advanced fibrosis should be confirmed in a larger cohort of patients with adequate representation of individual genotypes and ethnic groups.

Conclusions

The combination of qHBsAg with TE enhanced the specificity for identifying advanced fibrosis, and may be a promising procedure for CHB surveillance.

Acknowledgments

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Conflicts of interest

All of the authors declare that they have no conflicts of interest regarding this paper.

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