Performance evaluation of HBsAg by Lumipulse HBsAg-HQ: The agreement with HBsAg by Architect HBsAg-QT and the effectiveness in predicting liver tissue pathological states of chronic hepatitis B patients

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Conflict of interest

None declared

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Abstract

Background. A novel high-sensitivity HBsAg quantification assay, Lumipulse HBsAg-HQ, was developed. However, its performance in practical application has not yet been adequately investigated.

Objectives. The aim of the study was to evaluate the agreement of serum HBsAg by Lumipulse HBsAg-HQ (HBsAg-HQ) with HBsAg by Architect HBsAg-QT (HBsAg-QT) and comparatively investigate the efficacy of serum HBsAg-HQ and HBsAg-QT in predicting the liver tissue pathological states of chronic hepatitis B (CHB) patients.

Material and methods. A total of 147 HBeAg-positive and 128 HBeAg-negative patients were enrolled. HBsAg-HQ and HBsAg-QT were measured using CLEIA Lumipulse G1200 and CMIA Abbott Architect I2000 automatic analyzer, respectively. The Scheuer standard was used for the pathological diagnosis of liver tissue samples.

Results. In both HBeAg-positive and HBeAg-negative patients, HBsAg-HQ was significantly positively correlated with HBsAg-QT ($r = 0.913$ and $r = 0.959$, respectively), the overall disagreement rates between HBsAg-HQ and HBsAg-QT were 2.72% (4/147) and 4.69% (6/128), respectively. In HBeAg-positive patients, the area under the ROC curve (AUC) of HBsAg-HQ and HBsAg-QT for predicting the grade $\geq G3$ (0.686 and 0.684, respectively) and stage $\geq S4$ (0.739 and 0.745, respectively) were the greatest compared with other pathological states; the optimal cutoffs of HBsAg-HQ and HBsAg-QT for predicting the grade $\geq G3$ were $<2.244 \times 10^7$ mIU/mL and $<3.589 \times 10^7$ mIU/mL, and those for predicting the stage $\geq S4$ were 7.328×10^6 mIU/mL and $<6.194 \times 10^6$ mIU/mL, respectively.

Conclusions. HBsAg-HQ is highly correlated and in agreement with HBsAg-QT in both HBeAg-positive and HBeAg-negative patients; HBsAg-HQ and HBsAg-QT are very valuable in predicting the grade $\geq G3$ and stage $\geq S4$ in HBeAg-positive patients.

Key words: hepatitis B surface antigen, performance evaluation, Lumipulse HBsAg-HQ, pathology, non-invasive diagnosis

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Introduction

The detection of serum hepatitis B surface antigen (HBsAg) is the leading hallmark for screening and diagnosing hepatitis B virus (HBV) infection. In the past 10 years, serum HBsAg quantification assays have been developing rapidly. Among them, Architect HBsAg-QT (Abbott Laboratories, Chicago, USA) and Elecsys HBsAg II (Roche Diagnostics GmbH, Mannheim, Germany) have been widely studied and preliminary applied.^{1–4} Several studies have demonstrated that the levels of serum HBsAg are different during the various phases of natural history and are associated with the liver tissue pathological states of chronic HBV infection.^{5–8} Furthermore, some evidence has reinforced its value in predicting drug efficacy and evaluating the prognosis in patients with chronic hepatitis B (CHB).^{9,10}

Recently, a novel high-sensitivity linearized HBsAg quantification assay, Lumipulse HBsAg-HQ (Fujirebio Inc., Tokyo, Japan), was developed.¹¹ The original studies on this assay indicated that the detection of serum HBsAg by Lumipulse HBsAg-HQ (HBsAg-HQ) could potentially assist in diagnosing occult hepatitis B infection^{12–15} and showed consistent results with the qualitative and quantitative detection of serum HBsAg by Architect HBsAg-QT (HBsAg-QT) and HBsAg by Elecsys HBsAg II (HBsAg-E II).^{16,17} However, the correlation between the serum HBsAg-HQ levels and the natural history and the liver tissue pathological states of chronic HBV infection have not yet been adequately investigated.¹⁸

Objectives

The objective of this study is to further evaluate the agreement of serum HBsAg-HQ with HBsAg-QT and comparatively investigate the effectiveness of serum HBsAg-HQ and HBsAg-QT in predicting the liver tissue pathological states of CHB patients.

Material and methods

Patients

A total of 275 Chinese patients with chronic HBV infection who were hospitalized in the Shanghai Public Health Clinical Center of Fudan University (Shanghai, China) between August 2012 and July 2015 were prospectively enrolled, among whom 147 and 128 patients were hepatitis B e antigen (HBeAg)-positive and HBeAg-negative, respectively. The diagnoses of all the patients were in accordance with the standards set forth in the Asian-Pacific clinical practice guidelines for the management of hepatitis B (2015 update).¹⁹ Patients with other forms of viral hepatitis,

drug-induced liver injuries, hereditary liver diseases, schistosomiasis japonica infection, autoimmune diseases, endocrine and metabolic diseases, and blood system diseases were excluded. Patients who had been treated with interferon alpha, nucleoside(s), steroids, or licorices were also excluded.

All patients provided written consent prior to a liver biopsy, and all clinical investigations were conducted according to the principles expressed in the 1995 Declaration of Helsinki.

Histological assessment

Ultrasound-assisted liver biopsies were performed using a 1-second liver biopsy needle (16G). The biopsies were collected immediately after the procedure and transferred into plastic tubes for freezing. One pathologist, who was blinded to all biochemical, serologic and virological parameters, was assigned to review all biopsy specimens. A biopsy length of at least 10 mm was required for inclusion in this study. The pathological diagnosis of liver tissues was performed independently by 1 experienced pathologist. The pathological diagnosis referred to the Scheuer standard,²⁰ in which a grade is used to describe the intensity of the necro-inflammatory activity, and a stage is used as a measure of fibrosis and architectural alteration. The grades include 5 levels, G0–G4, and the stages include 5 levels, S0–S4.

Laboratory assays

Serum samples used for measurements were taken within 1 week before and 1 week after liver biopsy and stored at -40°C . Serum HBsAg-HQ and HBsAg-QT were quantified using chemiluminescence enzyme immunoassay (CLEIA) in a Lumipulse G1200 automated analyzer (Fujirebio Inc., Tokyo, Japan) and chemiluminescence microparticle immunoassay (CMIA) in an Abbott Architect I2000 automated analyzer (Abbott Laboratories, Chicago, USA), respectively. The HBsAg-HQ reagents were provided by Fujirebio Inc., and the HBsAg-QT reagents were purchased from Abbott Laboratories. The linear detection range of the HBsAg-HQ was from 5 to 150 000 mIU/mL. If the serum exceeded the upper detection limit, it was diluted 1,000 times and remeasured. Similarly, the linear detection range of the HBsAg-QT was 50 to 250 000 mIU/mL, and if the serum exceeded the upper detection limit, it was diluted 500 times and remeasured.

The serum HBeAg was measured using CMIA Abbott Architect I2000 automated analyzer, with a lower detection limit of 1 S/CO. The serum HBV DNA was measured using Bio-Rad Icyler PCR System (Bio-Rad Laboratories, Inc., USA), and the polymerase chain reaction (PCR) kits were obtained from Qiagen Shenzhen Co. Ltd. (Shenzhen, China). The linear detection range of HBV DNA was 5×10^2 IU/mL to 5×10^7 IU/mL.

Statistical analysis

Statistical analyses were performed using MedCalc v. 15.1 (MedCalc Software, Broekstraat, Mariakerke, Belgium). A paired-samples t-test was used to compare the differences between the serum HBsAg-HQ and HBsAg-QT. Pearson correlation, linear regression analysis and Bland-Altman plots were used to evaluate the agreement between the HBsAg-HQ and HBsAg-QT quantitation. Spearman's correlation analysis was used to analyze the correlation of the serum HBsAg-HQ and HBsAg-QT levels with the liver tissue pathological grade and stage. The receiver operating characteristic (ROC) curve was used to assess the effectiveness of serum HBsAg-HQ and HBsAg-QT for predicting the different liver tissue pathological states. The paired-samples DeLong non-parametric test was used to compare the differences in the area under the ROC curve (AUC) between the serum HBsAg-HQ and HBsAg-QT for predicting the same liver tissue pathological states. A 2-sided p-value of less than 0.05 was considered to be significant.

Results

Clinical characteristics of the patients

There was no significant difference in the male-to-female ratio ($p = 0.490$) between the HBeAg-positive and HBeAg-negative patients; however, there was a significant difference in the average age ($p = 0.000$) between the HBeAg-positive and HBeAg-negative patients. The difference in alanine transaminase (ALT) ($p = 0.947$) between the HBeAg-positive and HBeAg-negative patients was not statistically significant. The differences in the serum HBsAg-HQ, HBsAg-QT and HBV DNA (all $p = 0.000$) between the HBeAg-positive and HBeAg-negative patients were all statistically significant. There was a significant difference ($p = 0.046$) in the frequency of the different pathological grades, but there was no significant difference ($p = 0.469$) in the frequency of the different pathological stages between the HBeAg-positive and HBeAg-negative patients (Table 1).

In the HBeAg-positive patients, the serum HBsAg-HQ and HBsAg-QT had a significant negative correlation with the serum ALT ($r = -0.258$; $p = 0.002$ and $r = -0.254$; $p = 0.002$). In the HBeAg-negative patients, the serum HBsAg-HQ and HBsAg-QT did not have a significant correlation with the serum ALT ($r = -0.094$; $p = 0.291$ and $r = -0.067$; $p = 0.454$) (Fig. 1A–D). In both the HBeAg-positive and HBeAg-negative patients, the serum HBsAg-HQ and HBsAg-QT levels were significantly positively correlated with the serum HBV DNA ($r = 0.524$; $p = 0.000$ and $r = 0.501$; $p = 0.000$ in the HBeAg-positive patients, and $r = 0.350$; $p = 0.000$ and $r = 0.390$; $p = 0.000$ in the HBeAg-negative patients) (Fig. 2A–D).

Comparison between HBsAg-HQ and HBsAg-QT levels

Regardless of serum HBeAg state and HBsAg levels, there was no significant difference between the serum HBsAg-HQ and HBsAg-QT ($p = 0.691$). Grouping the patients according to serum HBeAg state and HBsAg levels showed that there were no significant differences between serum HBsAg-HQ and HBsAg-QT in patients with both HBeAg-positive ($p = 0.853$) and HBeAg-negative ($p = 0.647$) and in patients with both higher HBsAg levels (HBsAg-QT $\geq 100\,000$ mIU/mL) ($p = 0.942$) and lower HBsAg levels (HBsAg-QT $< 100\,000$ mIU/mL) ($p = 0.089$) (Table 2).

Correlation and agreement between HBsAg-HQ and HBsAg-QT

Independent of the serum HBeAg state and the HBsAg levels, the serum HBsAg-HQ had a significantly positive correlation with HBsAg-QT ($r = 0.955$; $p = 0.000$) (Fig. 3A, Table 3). The linear regression analysis showed that $\text{HBsAg-HQ} = 0.892 + 0.866 \times \text{HBsAg-QT}$ ($t = 53.087$; $p = 0.000$). The Bland-Altman analysis showed that, compared to the serum HBsAg-QT levels, the serum HBsAg-HQ levels had an upward bias of $0.04 \log_{10}\text{mIU/mL}$ with a 95% limit of agreement (LOA) of -0.61 to 0.69 mIU/mL . The disagreement rates of $\leq 95\%$ LOA and $\geq 95\%$ LOA were 0.73% (2/275) and 2.91% (8/275), respectively, and that the

Table 1. Clinical characteristics of the patients according to their serum HBeAg state

Characteristics	HBeAg-positive (n = 147)	HBeAg-negative (n = 128)	χ^2/t^b	p-value*
Gender (male:female)	93:54	87:41	0.478	0.490 ^a
Age [mean $\pm$ SD (range)], [years]	35.48 $\pm$ 11.29 (15–72)	43.88 $\pm$ 12.51 (19–78)	5.851	0.000 ^b
Serum ALT [mean $\pm$ SD (range)], $\times$ ULN [#]	3.22 $\pm$ 6.34 (0.25–37.85)	3.18 $\pm$ 5.31 (0.18–30.45)	0.067	0.947 ^b
Serum HBsAg-HQ [mean $\pm$ SD (range)], [$\log_{10}$ mIU/mL]	6.932 $\pm$ 0.764 (4.724–8.176)	5.830 $\pm$ 0.930 (1.906–7.101)	10.647	0.000 ^b
Serum HBsAg-QT [mean $\pm$ SD (range)], [$\log_{10}$ mIU/mL]	6.915 $\pm$ 0.856 (4.375–8.097)	5.773 $\pm$ 1.053 (1.778–7.226)	9.777	0.000 ^b
Serum HBV DNA [median (range)], [$\log_{10}$ IU/mL]	6.498 $\pm$ 1.554 (UD $\rightarrow$ 7.699)	3.901 $\pm$ 1.380 (UD $\rightarrow$ 7.644)	14.557	0.000 ^b
Pathological grade (G1:G2:G3)	75:33:39	84:22:22	6.164	0.046 ^a
Pathological grade (S1:S2:S3:S4)	56:38:21:32	58:28:21:21	2.533	0.469 ^a

[#] ULN – upper limit of normal = 40 IU/L; * HBeAg-positive vs HBeAg-negative; ^a χ^2 test; ^b independent samples t-test.

Fig. 1. Correlations of the serum HBsAg-HQ and HBsAg-QT with serum ALT in HBeAg-positive (A, B) and HBeAg-negative (C, D) patients

Fig. 2. Correlations of the serum HBsAg-HQ and HBsAg-QT with serum HBV DNA in HBeAg-positive (A, B) and HBeAg-negative (C, D) patients

overall disagreement rate was 3.64% (10/275) between the serum HBsAg-HQ and HBsAg-QT (Fig. 3B, Table 3).

In the HBeAg-positive patients, the serum HBsAg-HQ had a significantly positive correlation with HBsAg-QT

($r = 0.913$; $p = 0.000$) (Fig. 1C, Table 3), and HBsAg-HQ = $1.296 + 0.815 \times \text{HBsAg-QT}$ ($t = 26.929$; $p = 0.000$). The overall disagreement rate was 2.72% (4/147) between the serum HBsAg-HQ and HBsAg-QT (Fig. 1D, Table 3).

Table 2. Comparison between the serum HBsAg-HQ and HBsAg-QT levels

Study population	N	HBsAg-HQ ($\pm$ SD)	HBsAg-QT ($\pm$ SD)	t-test	p-value
Overall	275	6.419 $\pm$ 1.007	6.383 $\pm$ 1.109	0.397	0.691
HBeAg-positive	147	6.932 $\pm$ 0.764	6.915 $\pm$ 0.856	0.185	0.853
HBeAg-negative	128	5.830 $\pm$ 0.930	5.773 $\pm$ 1.053	0.459	0.647
HBsAg-QT $\geq$ 5.000 log ₁₀ mIU/mL	244	6.655 $\pm$ 0.752	6.660 $\pm$ 0.790	0.072	0.942
HBsAg-QT <5.000 log ₁₀ mIU/mL	31	4.564 $\pm$ 0.823	4.206 $\pm$ 0.806	1.732	0.089

The measurement units for HBsAg-HQ and HBsAg-QT were both log₁₀ mIU/mL.

Fig. 3. Correlation and agreement between the serum HBsAg-HQ and HBsAg-QT

The measurement units of HBsAg-HQ and HBsAg-QT were both log₁₀ mIU/mL. A, C and E show the scatter diagrams of the correlation between serum HBsAg-HQ and HBsAg-QT in the overall, HBeAg-positive and HBeAg-negative patients; B, D and F show the Bland–Altman plots of the agreement between the serum HBsAg-HQ and HBsAg-QT in the overall, HBeAg-positive and HBeAg-negative patients, in which the upper and lower horizontal solid lines represent the upper and lower limits of the 95% LOA, respectively, and the middle horizontal solid lines represent the average of the serum HBsAg-HQ and HBsAg-QT difference. The horizontal dotted lines represent an average value of 0 for the serum HBsAg-HQ and HBsAg-QT differences.

In HBeAg-negative patients, the serum HBsAg-HQ had a significantly positive correlation with HBsAg-QT ($r = 0.959$; $p = 0.000$) (Fig. 1E, Table 3); HBsAg-HQ = 0.963

+ $0.843 \times$ HBsAg-QT ($t = 37.740$; $p = 0.000$). The overall disagreement rate was 4.69% (6/128) between serum HBsAg-HQ and HBsAg-QT (Fig. 1F, Table 3).

Table 3. Pearson correlation and agreement between the serum HBsAg-HQ and HBsAg-QT

Study population	N	r	p-value	Bias	95% LOA	Inconsistency rate (%)		
						≤95% LOA	≥95% LOA	Sum
Overall	275	0.955	0.000	0.04	−0.61–0.69	2/275(0.73)	8/275(2.91)	10/275(3.64)
HBeAg-positive	147	0.913	0.000	0.02	−0.67–0.70	2/147(0.68)	3/147(2.04)	4/147(2.72)
HBeAg-negative	128	0.959	0.000	0.06	−0.55–0.66	1/128(0.78)	5/128(3.91)	6/128(4.69)
HBsAg-QT ≥5.000	244	0.929	0.000	−0.01	−0.58–0.57	2/244(0.82)	4/244(0.16)	6/244(0.25)
HBsAg-QT <5.000	31	0.861	0.000	0.36	−0.49–1.20	0/31(0.00)	2/31(6.45)	2/31(6.45)

The measurement units for HBsAg-HQ and HBsAg-QT were both $\log_{10}$ mIU/mL; bias – the average of serum HBsAg-HQ and HBsAg-QT differences; LOA – limit of agreement.

Fig. 4. Correlation of the serum HBsAg-HQ and HBsAg-QT levels with the liver tissue pathological grade and stage

The measurement units of HBsAg-HQ and HBsAg-QT were both $\log_{10}$ mIU/mL. The box-plots represent the serum distributions of HBsAg-HQ and HBsAg-QT in different pathological grades and stages in the HBeAg-positive (A, B) and HBeAg-negative patients (C, D), in which the top and bottom of the box indicates the upper and lower quartile, and the horizontal line in the box indicates the median. The upper and lower horizontal lines outside the box indicate the 95% quintile, and the circle indicates the extreme value.

In patients with higher HBsAg levels (HBsAg-QT $\geq 100\,000$ mIU/mL), the serum HBsAg-HQ had a significantly positive correlation with HBsAg-QT ($r = 0.929$; $p = 0.000$) (Table 3), such that $\text{HBsAg-HQ} = 0.762 + 0.885 \times \text{HBsAg-QT}$ ($t = 39.150$; $p = 0.000$). The overall disagreement rate was 0.25% (6/244) between the serum HBsAg-HQ and HBsAg-QT levels (Table 3). In patients with lower HBsAg levels (HBsAg-QT $< 100\,000$ mIU/mL), the serum HBsAg-HQ had a significantly positive correlation with HBsAg-QT ($r = 0.861$; $p = 0.000$) (Table 3), with $\text{HBsAg-HQ} = 0.869 + 0.879 \times \text{HBsAg-QT}$ ($t = 9.098$; $p = 0.000$). The overall disagreement rate was 6.45% (2/31) between the serum HBsAg-HQ and HBsAg-QT (Table 3).

Correlation of HBsAg-HQ and HBsAg-QT with pathological grade and stage

In HBeAg-positive patients, the serum HBsAg-HQ and HBsAg-QT were significantly negatively correlated with the pathological grade ($r_s = -0.298$; $p = 0.000$ and $r_s = -0.320$; $p = 0.000$, respectively) and stage ($r_s = -0.366$; $p = 0.000$ and $r_s = -0.373$; $p = 0.000$, respectively) (Fig. 4A,B). In HBeAg-negative patients, the serum HBsAg-HQ and HBsAg-QT levels were not significantly correlated with the pathological grade ($r_s = -0.127$; $p = 0.155$ and $r_s = -0.073$; $p = 0.411$, respectively) and stage ($r_s = -0.045$; $p = 0.615$ and $r_s = -0.011$; $p = 0.903$, respectively) (Fig. 4 C,D).

Performance of HBsAg-HQ and HBsAg-QT in predicting pathological states

In HBeAg-positive patients, all AUCs of the serum HBsAg-HQ and HBsAg-QT for predicting the pathological grades $\geq G2$ and $\geq G3$, and stages $\geq S2$, $\geq S3$ and $\geq S4$ were significantly greater than the area under the diagonal reference line (all $p < 0.01$). Of these, only the AUCs of serum HBsAg-HQ and HBsAg-QT for predicting pathological stage $\geq S3$ and $\geq S4$ were >0.70 (Table 4, Fig. 5). In the HBeAg-negative patients, all the AUCs of serum HBsAg-HQ and HBsAg-QT for predicting pathological grades $\geq G2$ and $\geq G3$, and stages $\geq S2$, $\geq S3$ and $\geq S4$ were not significantly greater than the area under diagonal reference line (all $p > 0.05$) (Table 4).

In HBeAg-positive patients there were no significant differences for predicting all the same pathological states

(all $p > 0.05$) between the AUCs of the serum HBsAg-HQ and HBsAg-QT (Table 4). In the HBeAg-negative patients, there were no significant differences for predicting the same pathological states except for predicting the pathological stage $\geq S4$ between the AUCs of serum HBsAg-HQ and HBsAg-QT ($p = 0.046$ for predicting the pathological stage $\geq S4$, $p > 0.05$ for predicting the other pathological states) (Table 4).

The optimal cut-offs of serum HBsAg-HQ and HBsAg-QT for predicting a pathological grade $\geq G3$ were $< 2.244 \times 10^7$ mIU/mL and $< 3.589 \times 10^7$ mIU/mL, respectively, and the corresponding sensitivity, specificity were 87.18% and 97.44%, and 43.52% and 38.89%, respectively. The optimal cut-offs of serum HBsAg-HQ and HBsAg-QT for predicting the pathological stage $\geq S4$ were $< 7.328 \times 10^6$ mIU/mL and $< 6.194 \times 10^6$ mIU/mL, respectively, and the corresponding sensitivity, specificity were 81.25% and 75%, and 64.35% and 67.83%, respectively (Table 5).

Table 4. AUCs of serum HBsAg-HQ and HBsAg-QT for predicting the different liver tissue pathological states

Pathological states	HBsAg	HBeAg-positive					HBeAg-negative				
		AUC*	SE [#]	Z [^]	p-value	95% CI	AUC*	SE [#]	Z [^]	p-value	95% CI
$\geq G2$	HBsAg-HQ	0.651 ^a	0.046	3.290	0.001	0.568~0.727	0.573 ^f	0.051	1.425	0.154	0.482~0.660
	HBsAg-QT	0.670 ^a	0.045	3.755	0.000	0.588~0.745	0.547 ^f	0.052	0.781	0.435	0.451~0.629
$\geq G3$	HBsAg-HQ	0.686 ^b	0.047	3.957	0.000	0.604~0.760	0.584 ^g	0.064	1.317	0.188	0.493~0.671
	HBsAg-QT	0.684 ^b	0.046	4.038	0.000	0.602~0.758	0.558 ^g	0.068	0.778	0.437	0.463~0.641
$\geq S2$	HBsAg-HQ	0.659 ^c	0.049	3.266	0.001	0.576~0.735	0.492 ^h	0.054	0.145	0.885	0.402~0.582
	HBsAg-QT	0.660 ^c	0.050	3.213	0.001	0.577~0.736	0.514 ^h	0.053	0.156	0.876	0.418~0.598
$\geq S3$	HBsAg-HQ	0.712 ^d	0.042	5.045	0.000	0.632~0.784	0.572 ⁱ	0.052	1.391	0.164	0.481~0.659
	HBsAg-QT	0.720 ^d	0.041	5.327	0.000	0.640~0.791	0.540 ⁱ	0.053	0.645	0.519	0.444~0.623
$\geq S4$	HBsAg-HQ	0.739 ^e	0.046	5.159	0.000	0.660~0.808	0.552 ^j	0.064	0.810	0.418	0.461~0.640
	HBsAg-QT	0.745 ^e	0.045	5.476	0.000	0.666~0.813	0.508 ^j	0.065	0.058	0.954	0.414~0.593

95% CI – 95% confidence interval; * AUC – area under the ROC curve; [#] SE – standard error; Z[^] – independent samples DeLong non-parametric test;

^{a-j} paired-samples DeLong non-parametric test; ^a Z = 1.005, p = 0.315; ^b Z = 0.071, p = 0.943; ^c Z = 0.034, p = 0.973; ^d Z = 0.391, p = 0.696; ^e Z = 0.238, p = 0.812;

^f Z = 1.511, p = 0.131; ^g Z = 1.131, p = 0.258; ^h Z = 0.211, p = 0.833; ⁱ Z = 1.826, p = 0.068; ^j Z = 1.992, p = 0.046.

Table 5. Optimal cut-offs of serum HBsAg-HQ and HBsAg-QT for predicting different pathological states and the corresponding diagnostic parameters in HBeAg-positive patients

Pathological states	HBsAg	Cut-off	SEN (%)	SPE (%)	YI	PPV (%)	NPV (%)	ACC
$\geq G2$	HBsAg-HQ	<7.216	73.61	58.67	0.323	63.1	69.8	0.329
	HBsAg-QT	<7.555	88.89	46.67	0.356	61.5	81.4	0.429
$\geq G3$	HBsAg-HQ	<7.351	87.18	43.52	0.307	35.8	90.4	0.262
	HBsAg-QT	<7.555	97.44	38.89	0.363	36.5	97.7	0.342
$\geq S2$	HBsAg-HQ	<7.622	89.01	42.86	0.319	71.7	70.6	0.423
	HBsAg-QT	<7.555	84.62	51.79	0.364	74.0	67.4	0.414
$\geq S3$	HBsAg-HQ	<7.315	86.79	53.19	0.400	51.1	87.7	0.388
	HBsAg-QT	<7.555	98.11	44.68	0.428	50.0	97.7	0.477
$\geq S4$	HBsAg-HQ	<6.865	81.25	64.35	0.456	38.8	92.5	0.313
	HBsAg-QT	<6.792	75.00	67.83	0.428	39.3	90.7	0.300

The measurement units of HBsAg-HQ and HBsAg-QT were both log₁₀ mIU/mL; SEN – sensitivity; SPE – specificity; YI – Youden index; PPV – positive predictive value; NPV – negative predictive value; ACC – accuracy.

Fig. 5. ROC curves of serum HBsAg-HQ and HBsAg-QT for predicting a pathological grade $\geq$ G3 and stage $\geq$ S4 in HBeAg-positive patients

Discussion

Choi et al. confirmed that the qualitative results of serum HBsAg-HQ highly agreed with those of the serum HBsAg-QT, with a concordance rate of 99.8% ($k = 1.00$; 95% CI, 0.99–1.00). Of the 315 HBsAg-QT-positive samples, 314 were HBsAg-HQ-positive. Of the 685 HBsAg-QT-negative samples, 684 were HBsAg-HQ-negative.¹⁶ Recently, Yang et al. evaluated and compared the agreement of the qualitative and quantitative results of serum HBsAg-HQ with HBsAg-QT and HBsAg-EII.¹⁷ The results showed that of the 2,043 samples tested, 1,844 samples gave negative results for both HBsAg-HQ and HBsAg-QT, and 172 samples yielded positive results for both HBsAg-HQ and HBsAg-QT. Of the remaining 27 samples that had inconsistent HBsAg results, 3 were HBsAg-QT-positive and 24 were HBsAg-HQ-positive. Among these, none of the 3 HBsAg-QT-positive samples were subsequently confirmed to be positive. Twenty of the 24 HBsAg-HQ-positive samples were confirmed to be positive, 3 were confirmed to be negative and 1 gave an indeterminate result and therefore was excluded from the specificity calculation for HBsAg-HQ. The specificity was 99.84% for HBsAg-HQ and 99.84% for HBsAg-QT. Yang et al. also found that, of the 112 tested samples, HBsAg-HQ displayed an excellent correlation with both HBsAg-QT and HBsAg-EII ($r = 0.985$ and $r = 0.990$); the Bland-Altman analyses demonstrated that, compared to HBsAg-QT and HBsAg-EII, HBsAg-HQ had an upward bias of $0.19 \log_{10}$ IU/mL with a 95% LOA of -0.01 to $0.39 \log_{10}$ mIU/mL and $0.07 \log_{10}$ IU/mL with a 95% LOA of -0.12 to $0.25 \log_{10}$ mIU/mL, respectively.¹⁷

In this study, the serum HBsAg-HQ was significantly correlated with HBsAg-QT. The Bland-Altman analyses showed that, compared to the serum HBsAg-QT, the HBsAg-HQ had a slight bias of $0.04 \log_{10}$ mIU/mL with a 95% LOA of -0.61 to $0.69 \log_{10}$ mIU/mL; the overall disagreement rate was 3.64%. Further analyses of the grouping according to the HBeAg state (HBeAg-positive and HBeAg-negative) and the HBsAg levels (higher HBsAg levels (HBsAg-QT ≥ 100 000 mIU/mL) and lower HBsAg levels (HBsAg-QT < 100 000 mIU/mL)) also showed similar results. This further demonstrated that the serum HBsAg-HQ levels were highly correlated and highly agreed with HBsAg-QT.

Seto et al. comparatively investigated the changes in the serum HBsAg-HQ and HBsAg-EII in different phases in the natural history of chronic HBV infection.¹⁸ The changes in the serum HBsAg-HQ in different phases were consistent with previous research based on the same criterion for the division of the natural history of chronic HBV infection.^{5,6,21–23} Unexpectedly, the serum HBsAg-HQ levels were significantly higher than the HBsAg-EII levels in either the immune tolerance or the activation phase of the HBeAg-positive patients. However, the serum HBsAg-HQ levels were similar to the HBsAg-EII levels in either the immune escape or control phase of the HBeAg-negative patients. However, the reason why the serum HBsAg-HQ levels in HBeAg-positive patients were significantly higher than HBsAg-E levels is not clear. The investigators speculated that this could possibly be due to the enhanced detection of minor viral populations with “a” determinant mutations in patients with higher viral loads. However, Yang et al. reported that the S gene mutations within the “a” determinant, such as T126A, T126S, Q129H, Q129R, T140S, and G145E, did not affect the correlation and agreement observed between the serum HBsAg-HQ and HBsAg-QT and HBsAg-EII.¹⁷

In this study, regardless of the serum HBeAg state and HBsAg levels, the difference between the serum HBsAg-HQ and HBsAg-QT levels was not significant. Further analyses of the grouping according to the HBeAg state and HBsAg levels also showed similar results. Our results agreed with the study by Yang et al. but failed to find a serum HBsAg-HQ level that was not significantly higher than HBsAg-QT.¹⁷ This result was not consistent with the findings of a previous study conducted by Seto et al.¹⁸

For the cause-specific indexes, several studies have shown that serum HBsAg and HBV DNA in HBeAg-positive patients and HBV DNA but HBsAg in HBeAg-negative patients are valuable in predicting liver tissue pathological states.^{7,24–29} However, the predictable optimal pathological state of serum HBsAg and HBV DNA in the HBeAg-positive patients was not consistent between the different studies,^{7,24–27} although for the HBV DNA in the HBeAg-negative patients, there was an agreement in the pathological grade $\geq$ G2 and stage $\geq$ S2.^{28,29} The results of this study were consistent with the findings by Cheng et al. and Jia

et al., but not those of Martinot-Peignoux et al., Xun et al. and Seto et al.^{7,24–27} Furthermore, we did not observe significant differences in the AUCs for predicting the same liver tissue pathological states between the serum HBsAg-HQ and HBsAg-QT in the HBeAg-positive patients. This study further demonstrated that, in the HBeAg-positive patients, serum HBsAs for predicting the liver tissue pathological states was valuable. Importantly, the efficacies of serum HBsAg-HQ for predicting the liver tissue pathological states were highly consistent with those of HBsAg-QT.

The results of this study showed that, in HBeAg-positive patients, the optimal cut-off of serum HBsAg-HQ for predicting a pathological grade $\geq G3$ was $< 7.351 \log_{10}$ mIU/mL (2.244×10^7 mIU/mL), with a difference of $-0.204 \log_{10}$ mIU/mL (-0.625 mIU/mL) from HBsAg-QT. The corresponding sensitivity, specificity, and positive and negative predictive values were 87.18%, 43.52%, 35.8%, and 90.4%, respectively. The optimal cut-off of serum HBsAg-HQ for predicting a pathological stage $\geq S4$ was $< 6.865 \log_{10}$ mIU/mL (7.328×10^6 mIU/mL), with a difference of $0.073 \log_{10}$ mIU/mL (1.183 mIU/mL) from HBsAg-QT. The corresponding sensitivity, specificity, and positive and negative predictive values were 81.25%, 64.35%, 38.8%, and 92.5%, respectively. This suggests that, in HBeAg-positive patients, the optimal cut-offs of serum HBsAg-HQ for predicting pathological grade $\geq G3$ and stage $\geq S4$ should be highly consistent with those of HBsAg-QT, and serum HBsAg-HQ and HBsAg-QT should be very valuable for predicting pathological grade $\geq G3$ and stage $\geq S4$.

In conclusion, this study further evaluated the agreement between the serum HBsAg-HQ and HBsAg-QT levels and comparatively investigated the effectiveness of serum HBsAg-HQ and HBsAg-QT in predicting the liver tissue pathological states of CHB. The results showed that the serum HBsAg-HQ was highly correlated and agreed with the HBsAg-QT in both the HBeAg-positive and HBeAg-negative patients, regardless of whether they presented with higher or lower HBsAg levels. Furthermore, serum HBsAg-HQ and HBsAg-QT had good predictive efficacy on the pathological grade $\geq G3$ and stage $\geq S4$ in HBeAg-positive patients, but did not have predictive efficacy on the pathological states in HBeAg-negative patients.

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