
Title Page

Title: Application strategies of serum HBV DNA detection in HBV infection patients: a retrospective study of 5611 specimens

Shortened title: Clinical detection pathway of HBV DNA

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Abstract

The detection of HBV DNA plays a critical role in determining the level of viral replication in HBV infected patients. However, how to select appropriate HBV DNA detection method, low-sensitivity (ls) and hypersensitivity (hs), remains

unclear. In this study, HBsAg, HBeAg, ALT, AST and hs HBV DNA titers in serum of 5611 cases with suspected HBV infection were reviewed. Besides, the dynamic changes of HBV DNA and HBsAg in 85 chronic hepatitis B (CHB) patients receiving 3 H J ,) dr entecavir (ETV) were observed. The results showed the positive rate of HBV DNA was 32.8%, of which low viral load (20~500 IU/ml) accounted for 51.8%. In the 5611 cases, when the HBsAg<1000 IU/ml, the proportion of low viral load was 76.3%. Moreover, in patients receiving antiviral treatment, when HBsAg<2000 IU/ml 3 H J ,) dr. HBsAg<3500 IU/ml (ETV), the proportion of patients with low viral load was 79.5% or 78.0%, respectively. We developed a strategy of serum HBV DNA detection in HBV infection patients. When

Hepatitis B virus (HBV) infection remains a major public health concern worldwide. Globally, approximately 2 billion people have evidence of HBV infection, with nearly 240 million chronic carriers of HBsAg [1]. In China, hepatitis B is still a widely prevalent infectious disease [2,3] and the average annual cost of HBV-related diseases among hospitalized patients was about \$11,989 [4]. Therefore, to select the appropriate indicators which followed the principles of health economics is crucial in diagnosis, treatment and monitoring of HBV infection.

Several clinical indications such as HBV DNA, HBsAg, HBeAg, ALT and AST have been applied for the detection and assessment of HBV infection. Among them, the serum HBV DNA quantified by real-time fluorescent quantitative PCR is a key factor to determine the level of viral replication in different phases of HBV infection, the selection of patients for treatment and the efficacy of antiviral therapy. [5]. Moreover, patients with chronic HBV infection should detect HBV DNA every 6-12 months and the low limit of detection of HBV DNA assays was better less than 20 IU/ml [6]. Therefore, a simple and convenient method for HBV DNA detection with low-sensitivity (ls) based on nucleic acid lysis principle has been developed to detect relatively high concentrations of HBV DNA.

Meanwhile, to detect relatively low concentrations of HBV DNA, a method with hypersensitivity (hs) based on magnetic-bead technology has also been established. Although the low limit of detection of latter method was 20 IU/ml, the detection time and cost were 4 to 5 times higher than the former. Meanwhile, repeated detection of HBV DNA, especially hs HBV DNA, occupies most of the detection costs of patients. Moreover, the nucleic acid amplification test requires

skilled operators and high quality management, which restricts its widespread application in China, especially in some primary hospitals.

In the previous studies, we reviewed the correlation between serum markers and genotype of HBV in 49,164 patients [7] and initially revealed the clinical significance of HBsAg in 116,455 patients [8]. Recently, HBV serum markers and ALT/AST detections have been widely used in clinical practice owing to their advantages, such as high automation level, low cost and rapid detection. However, whether the combination of HBV serum markers and ALT/AST levels could be made to predict preliminary the concentration of HBV DNA in patients with HBV infection and develop an appropriate clinical detection guide pathway for ls HBV DNA and hs HBV DNA, still remains to be figured out.

In this study, we reviewed the levels of serum HBsAg, HBeAg, HBV DNA, ALT and AST in 5611 inpatients and outpatients with HBV infection, and followed up the dynamic changes of HBsAg and HBV DNA in 85 chronic hepatitis B (CHB) patients receiving antiviral therapy. Besides, the consistency between ls and hs HBV DNA detection methods was also analyzed in detail. Our results would provide a laboratory basis for HBV DNA clinical detection pathways.

Materials and methods

Patients

A total of 5611 specimens (including outpatients and inpatient suspected of HBV infection) were collected between January 1, 2016 and August 1, 2017 from

different departments at the First Affiliated Hospital of Fujian Medical University including 952 HBsAg negative and 4659 HBsAg positive patients. According to

155 females with a median age of 39 years (range: 12-77 years). All the specimens were without hemolysis or lipoidemia and stored at -20 °C. According to CLSI document EP09-A3 (method comparison and bias estimation using patient samples) [9], the certainty of statistical analysis could be improved with increased number of samples although the minimum number needed for comparative studies is only 40. Further, all study protocols were approved by the Institutional Medical Ethics Review Board of the First Affiliated Hospital of Fujian Medical University (IEC-FOM-013-1.0). Patients provided written or verbal consent prior to inclusion in the study.

Measurement of serum HBsAg and HBeAg

The serum level of HBsAg and HBeAg were detected by Architect I2000 analyzer (Abbott Laboratories, Chicago), based on a chemiluminescent microparticle immunoassay, according to the P D Q X I D 5 P W X C L H T 1 protocol. The detection values of HBsAg ranges from 0.05 to 250 IU/ml. A 1:500 dilution was performed in samples with HBsAg>250 IU/ml. Although this commercial HBeAg kit is not marketed as a quantitative assay, it produces a signal-to-cutoff (S/CO) ratio that is linear within a restricted range [10]. The internal quality control included the negative (0.5 S/CO) and positive (2.0 S/CO) HBeAg serum was provided by Controls & Standards Biotechnology (Beijing, China). The samples of HBeAg levels in the range 0.8-1.2 S/CO were retested in duplicates to ensure the accuracy of the HBeAg results. Specimens with HBsAg • , 8 P O R U • + % 6 1 \$ J 2 are considered reactive.

Measurement of ALT and AST

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The serum levels of ALT and AST were detected by an automatic biochemistry analyzer cobas8000 (Roche Diagnostics, Switzerland) according to manufacturer's instructions on the principle of Enzyme Kinetics with standard substance and quality control substance from American Bio-Rad Laboratories (Hercules). The upper limit of normal (ULN) for ALT and AST was 40 U/L and 35 U/L respectively.

Quantitation of low-sensitivity and hypersensitivity HBV DNA

For low-sensitivity HBV DNA kit, a nucleic acid lysis buffer was used to allow rapid lysis and release of HBV DNA from 5 $\times$ 10³ IU/ml. For hypersensitivity HBV DNA kit, the magnetic-bead technology was used to extract HBV DNA from 200 $\times$ 10³ IU/ml. Kits were provided by Sansure Biotech Inc. (Hunan, China) as per W K H P D Q X I D F W X U H H F V H B V Q D N W W X F W W P Q V. Extracted HBV DNA was subsequently amplified by real-time PCR using Genome Diagnostic HBV quantification kit (Sansure Biotech Inc., China) which was performed on Roche Lightcycler 480 analyzer (Roche Corporation, Basel, Switzerland). The quality control substance was bought from Controls & Standards Biotechnology (Beijing, China). The detection limit for ls HBV DNA was 500 IU/ml and the linear range was 500 IU/ml~1.0 E+09 IU/ml. The detection limit for hs HBV DNA was 20 IU/ml and the linear range was 20 IU/ml~1.0 E+09 IU/ml.

To further distinguish two methods, HBV DNA from 20 IU/ml to 500 IU/ml (1.3 log₁₀ IU/ml~2.7 log₁₀ IU/ml) was defined as low viral load. According to CLSI document EP09-A3, 500 specimens were collected and measured by two methods as described above to assess their relevance and bias.

(61/250) was between 20 IU/ml and 500 IU/ml, indicating that the coincidence rate of negative results of the two methods is 100% when the limit of detection was 500 IU/ml. Moreover, linear regression and deviation analysis were performed on 250 positive samples (HBV DNA >500 IU/ml) with gradient concentrations. Our results showed that the correlation coefficient was 0.955 (Fig.1A) and the average deviation was -0.22% (Fig.1B).

Distribution of HBV DNA in 5,611 specimens

The distribution of the HBV DNA titers in 5,611 specimens was showed in Fig.2. The percentage of HBV DNA positive specimens was 32.8% (1840/5611) and the HBV DNA positive level mostly ranged from 20 IU/ml to 500 IU/ml (51.7% of the positive specimens and 17.0% of total specimens) (Fig.2A). During 20 IU/ml~500 IU/ml HBV DNA, 20 IU/ml~150 IU/ml accounted for 65.9% (628/953), 150 IU/ml~300 IU/ml accounted for 21.5% (205/953), 300 IU/ml~500 IU/ml accounted for 12.6% (120/953). There was no significant difference in HBeAg positive ratio (46.0%~51.9%) among different gradient intervals of low viral load (Fig.2B).

Serological characteristics of different HBsAg or HBeAg

To further explore the distributed characteristics of serum HBV DNA under different HBsAg or HBeAg levels, the relationship among them was analyzed. A poor correlation between HBsAg and HBV DNA titers ($R^2=0.159$, $P<0.001$) was observed (Fig.3A). Further, patients were divided into 10 groups according to HBsAg titer. The levels of HBV DNA, HBeAg and ALT/AST increased as HBsAg level elevated, while the proportion of low viral load decreased especially

when HBsAg concentration was lower than 1000 IU/ml (83.86% : 48.80%) (Table 2).

As shown in Fig.3B, the correlation between HBeAg and HBV DNA was stronger than that between HBsAg and HBV DNA ($R^2=0.276$, $P<0.001$). The patients were divided into 6 groups according to the level of HBeAg (0~0.5, 1~10, 10~100, 100~500, 500~1000 and >1000 S/CO). It can be inferred that, as HBeAg titer gradual increased, HBV DNA and ALT/AST levels also increased gradually while the proportion of low viral load decreased. In addition, when HBeAg>100 S/CO, the proportion of low viral load decreased significantly (65.19% : 28.28%) and the abnormal rate of ALT/AST increased significantly (22.68% : 44.59%) (Table 3). All these results suggested that the relationship between HBV DNA and the interval of HBsAg or HBeAg could be applied to make a preliminary prediction of HBV DNA.

Alteration of HBV DNA and HBsAg in CHB patients during antiviral therapy

To evaluate the relationship between HBV DNA and HBsAg in the process of antiviral therapy, a total of 44 CHB patients receiving 3 H J ,) and 41 CHB patients receiving ETV therapy were enrolled. As shown in Fig.4, the level of HBV DNA and HBsAg gradually declined with antiviral therapy. For CHB patients with VR or PVR received 3 H J ,) therapy, the mean level of HBV DNA was 2.7 log₁₀ IU/ml when HBsAg decreased to 2000 IU/ml (3.3 log₁₀ IU/ml) (Fig.4A, 4B). During 3 H J ,) therapy, the HBV DNA titer in 79.5% (35 of 44) patients fluctuated from 1.3 to 2.7 log₁₀ IU/ml when HBsAg<2000IU/ml,

especially in PVR groups. However, for CHB patients received ETV therapy, HBsAg declined more slowly compared to PegIFN treatment. The mean level of HBV DNA was $2.7 \log_{10}$ IU/ml when HBsAg decreased to 3000 IU/ml ($3.5 \log_{10}$ IU/ml) or 3500 IU/ml ($3.55 \log_{10}$ IU/ml) in VR or PVR groups (Fig.4C, 4D), respectively. During ETV therapy, the HBV DNA titer in 78.0% (32 of 41) patients ranged from 1.3 to $2.7 \log_{10}$ IU/ml when HBsAg<3500 IU/ml, especially in PVR groups.

Furthermore, to evaluate the values for HBsAg to predict low viral load, ROC curves were conducted. The AUC was 0.918 for HBsAg ($P<0.001$) in CHB patients with low viral load (Fig. 5A). Meanwhile, the AUC was 0.873 for HBsAg ($P<0.001$) in CHB patients receiving ETV therapy (Fig. 5B). The optimal cut-off values to predict low viral load by HBsAg were 3056 and 3513 IU/ml in CHB patients with low viral load and ETV therapy, with high sensitivity and specificity at 75.0%/92.3% and 77.5%/85.9%, respectively.

Suggestion for ls/hs HBV DNA detection pathways

Given the correlations among HBsAg, HBeAg, ALT, AST and HBV DNA, a clinical detection pathway for ls HBV DNA/hs HBV DNA detection was proposed in Fig.6. Based on the levels of HBsAg, 952 of 5611 serum samples may not be considered to detect HBV DNA. When the level of HBsAg was 0.05-1000 IU/ml, HBV DNAs were mostly <20 IU/ml as the levels of ALT, AST and HBeAg were normal and hs HBV DNA should be detected directly if one of the levels of ALT, AST and HBeAg was abnormal. While the levels of HBsAg

were at a higher degree, Is HBV DNA can be detected directly since HBV DNA was usually ≥ 500 IU/ml or < 20 IU/ml and hs HBV DNA was suggested only when HBeAg was weakly positive (1 to 100 S/CO). Moreover, hs HBV DNA should be selected when HBsAg < 3500 IU/ml during ETV therapy or HBsAg < 2000 IU/ml during 3 H J ,) therapy. According to the detection pathway mentioned above, only about 35% of the 5611 specimens need to detect hs HBV DNA, 34% need to directly detect Is HBV DNA and 31% may not consider HBV DNA detection.

Discussion

The serological, virological and biochemical markers of HBV infection play important roles in the assessment of infection status, disease progression prediction and efficacy judgment. Moreover, appropriate longitudinal long-term follow-up for these markers is also necessary [5,6]. Serological and biochemical indicators have been widely used in clinical due to their advantages, such as short detection time, easy operation, highly automation and low technical requirements of the laboratory [11,12]. Quantification of serum HBV DNA is a crucial factor in the evaluation of patients with HBV infection and in the assessment of the efficacy of antiviral therapy [13]. Moreover, the detection limit of HBV DNA was better less than 20 IU/ml according to new guidelines [6]. In recent years, researchers have pay much more attention to the detection of low viral load in CHB patients, such as patients with liver cirrhosis and/or hepatocellular carcinoma with any detectable HBV DNA level regardless of ALT levels who

should start antiviral therapy. Taken the off-treatment of nucleoside (acid) drugs in CHB patients into consideration, a highly sensitive method should be developed to detect the low viral load. Therefore, a hypersensitive method for HBV DNA detection based on magnetic-bead technology in addition to the routine low-sensitive HBV DNA detection have been carried out [14]. However, in the absence of a good clinical detection pathway, clinicians cannot select personalized indicators, such as HBV serological parameters, liver function, HBV DNA and especially hs HBV DNA which greatly increased the cost of medical care. In addition, hs HBV DNA cannot be carried out routinely in the majority of grass-roots hospital laboratory. Therefore, how to develop a suitable clinical detection pathway is a problem needed to be solved.

In this study, HBV DNA levels in 500 patients with HBV infection were measured by rapid lysis method and magnetic-bead technology respectively. It is found that there was a good consistency and the correlation coefficient was 0.955 and the average deviation was -0.22% when the HBV DNA concentration ≥ 500 IU/ml. Consistent with the literature, the rapid lysis method cannot detect while the magnetic-bead technology partially detected (24.4%) as the DNA concentration < 500 IU/ml [15]. Hence, the result of the rapid lysis method was not necessarily verified by the magnetic-bead technology with high costs when the HBV < 500 IU/ml. Therefore, a hypersensitive magnetic-bead technology is recommended only when the concentration of HBV DNA is below 500 IU/ml.

To elucidate the clinical value of hs HBV DNA, we reviewed the titers of HBV DNA in 5,611 patients and found that the positive rate of HBV DNA detection

was 32.8%, and the HBV DNA of low viral load in the positive specimens accounted for 51.7%. Therefore, in order to minimize the occurrence of false negative, hypersensitive technology is necessary to be applied to detect HBV DNA for specific patients with low viral load in the natural history of HBV infection or in the process of antiviral therapy. Our study showed that HBV DNA titer of 20 IU/ml~150 IU/ml accounted for 65.9%, 150 IU/ml~300 IU/ml accounted for 21.5%, 300 IU/ml~500 IU/ml accounted for 12.6% in low viral load. Therefore, it can be inferred that a large number of lower concentrations (1~20 IU/ml) may exist in 67.2% of HBV DNA negative samples which determined by the magnetic-bead technology owing to its sensitivity limitation. We believed that with the development of higher sensitive technology, such as digital PCR [16,17], will promote the clinical application value of HBV DNA. Although there was no significant difference in HBeAg positive ratio (46.1%~51.9%) among different gradient intervals of low viral load, the positive rate of HBeAg increased slightly with the increase of viral load.

To determine whether HBV DNA was linearly related to HBsAg and HBeAg, a regression analysis between HBsAg, HBeAg and HBV DNA had been performed, respectively. The correlation coefficients between HBsAg and HBV DNA, HBeAg and HBV DNA were 0.16 and 0.28 respectively, which was lower than the reported 0.69 and 0.74 [21,22]. The difference of the population and the number of samples was also attributed to the inconsistency. Comparatively, the results in our study were more representative since a large scale of samples in different phases of HBV infection was collected. However, with the increasing of HBsAg or HBeAg levels, the detectable rate and concentration of HBV DNA also increased while low viral load decreased. Meanwhile, when HBsAg>1000 IU/ml or HBeAg>100 S/CO, the proportion of low viral load decreased significantly. Therefore, the relationship between HBV DNA and the interval of HBsAg or HBeAg can be applied to make a preliminary prediction of HBV DNA.

To further investigate the effect of antiviral therapy on HBsAg or HBV DNA titers, 85 CHB patients in different treatment time were followed up. The results showed that the levels of HBV DNA and HBsAg gradually declined during antiviral therapy. Unlike the consequences in the 5611 population, low viral load began to appear when HBsAg decreased to 2000~3500 IU/ml which suggested that antiviral therapy would strongly inhibit HBV DNA replication and the changes of HBsAg might have a certain degree of hysteresis. Especially, ETV only inhibits the reverse transcription of the pgRNA but do not target the cccDNA directly. Thus, changes at transcriptional levels, particularly in the HBsAg secretory pathway, are not expected [23]. Therefore, the HBsAg cut-off value during antiviral therapy should be increased accordingly.

Based on the data and experimental results mentioned above, we first proposed ls/hs HBV DNA clinical detection pathway. According to the detection results of HBsAg, HBV DNA titer was stratified. Further, according to the levels of ALT/AST/HBeAg and the methods of antiviral therapy, we can determine whether to choose ls HBV DNA method, hs HBV DNA method or no detection. Therefore, on the basis of our detection pathway, only 1964 of 5611 serum samples needed to detect hs HBV DNA directly (953 cases were low viral load actually), 1907 patients could detect ls HBV DNA directly and 1739 patients may not need to be detected. Consequently, the development of appropriate detection pathway can greatly improve the efficiency of testing and reduce the unnecessary waste of medical resources, which is suitable for laboratories that cannot perform ls HBV DNA and/or hs HBV DNA. According to 2017 clinical practice guidelines on the management of hepatitis B virus infection and 2015 guideline of prevention and treatment for chronic hepatitis B (3,6), the presence of HBeAg, HBV DNA levels, ALT and AST values have been applied for assessment of HBV infection and selecting patients with antiviral therapy, the anti-HBe was not included in these guidelines. So, anti-HBe was not considered in our study. We will consider anti-HBe as a new marker for clinical detection pathway of CHB patients in future. It is worthy to mention that due to the differences in method selection and the definition of the diagnostic cut-off point, each laboratory should tailor their HBV DNA serological tests according to respective conditions and demands.

In summary, except for the limit of detection, there was a good comparability for hs HBV DNA and ls HBV DNA. Samples with low viral load in the clinical

occupied a large proportion. While in different levels of HBsAg or HBeAg, the distribution of HBV DNA was significantly uneven. Based on HBV serological indicators and ALT/AST levels, the methods for HBV DNA should be selected appropriately depending on the different population.

Abbreviations

HBV, hepatitis B virus; ls HBV DNA, low-sensitivity HBV DNA; hs HBV DNA, hypersensitivity HBV DNA; ALT, alanine transaminase; AST, aspartate transaminase; CHB, chronic hepatitis B; ETV, entecavir; VR, virological response; PVR, partial virological response; ROC, receiver operating characteristic curve; AUC, area under the curve.

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Competing interests

The authors declare that they have no competing interests.

Ethical approval

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The study was approved by the Institutional Medical Ethics Review Board of the First Affiliated Hospital of Fujian Medical University (IEC-FOM-013-1.0).

Patients provided written or verbal consent prior to inclusion in the study.

References

Table 1. Population characteristics in different groups

	HBeAg positive		HBeAg negative		HBsAg negative
	Chronic infection	Chronic hepatitis	Chronic infection	Chronic hepatitis	
No.	1146	585	2333	595	952

Male/female

Table 2. The characteristics of serological indicators at different HBsAg titers

HBsAg (IU/ml)		No.	Detectable HBV DNA		Low viral load rate ^b	HBeAg positive rate	ALT/AST abnormal rate
			Rate	Value ^a			
0.00	0.04	952	0.42%	1.75±0.37	100.00%	0.00%	13.34%
0.05	99	618	30.10%	2.44±0.95	70.43%	12.62%	20.06%
100	499	546	32.05%	2.43±0.88	72.00%	18.13%	18.86%
500~1000		679	33.87%	2.22±0.70	83.91%	24.89%	17.23%
1000	1499	506	33.20%	2.95±1.15	46.43%	39.33%	21.94%
1500	1999	428	34.81%	2.80±1.50	45.03%	39.72%	19.86%
2000~2999		615	37.56%	2.91±1.43	44.98%	40.65%	23.58%
3000~4999		514	38.91%	3.26±1.61	35.50%	50.39%	23.54%
5000~10000		433	57.04%	3.68±1.83	35.22%	57.27%	34.87%
>10000		320	78.44%	5.05±2.24	13.55%	80.63%	48.44%

^aData are reported as the mean±SD (log₁₀ IU/ml). ^bThe ratio of low viral load (20~500IU/ml) populations/detectable HBV DNA populations. ALT, alanine transaminase; AST, aspartate transaminase.

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Table 3. The characteristics of serological indicators at different HBeAg levels

HBeAg S/CO	No.	Detectable HBV DNA		Low viral load rate ^b	ALT/AST abnormal rate
		Rate	Value ^a		
0-0.5	3528	20.58%	2.84±1.32	57.71%	17.35%
0.5-1.0	352	21.59%	2.61±1.06	67.11%	17.90%
1~10	623	44.78%	2.49±1.17	70.25%	23.76%
10-100	582	58.25%	2.67±1.32	65.19%	22.68%
100-500	222	65.32%	3.72±1.72	28.28%	44.59%
500-1000	132	87.88%	4.20±1.89	20.69%	57.58%
>1000	172	93.02%	5.78±1.94	0.63%	63.37%

^aData are reported as the mean±SD (log₁₀ IU/ml). ^bThe ratio of low viral load (20~500IU/ml) populations/detectable HBV DNA populations. ALT, alanine transaminase; AST, aspartate transaminase.

FIGURE LEGENDS

Fig.1. The consistency comparison of ls and hs HBV DNA testing methods. A. The correlation between ls and hs HBV DNA. B. Bland-Altman plot examination for

calculating absolute difference between methods. Is HBV DNA, low-sensitivity HBV DNA; hs HBV DNA, hypersensitivity HBV DNA.

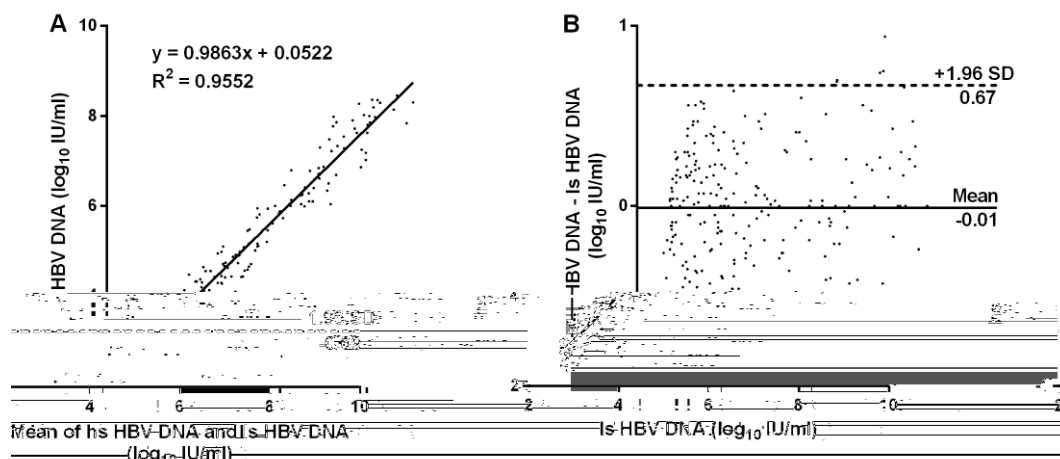


Fig.2. The distribution of HBV DNA titers (IU/ml). A, In 5611 patients, samples with HBV DNA <20 IU/ml occupied 67.2%, 20 IU/ml~500 IU/ml (low viral load) accounted for 17.0%, other levels shared the rest portion. B, HBeAg positive rate in different gradients of low viral load patients was shown. There was no significant difference in the positive rate of HBeAg among different gradient intervals. The percent of HBeAg positive patients was shown in the histogram.

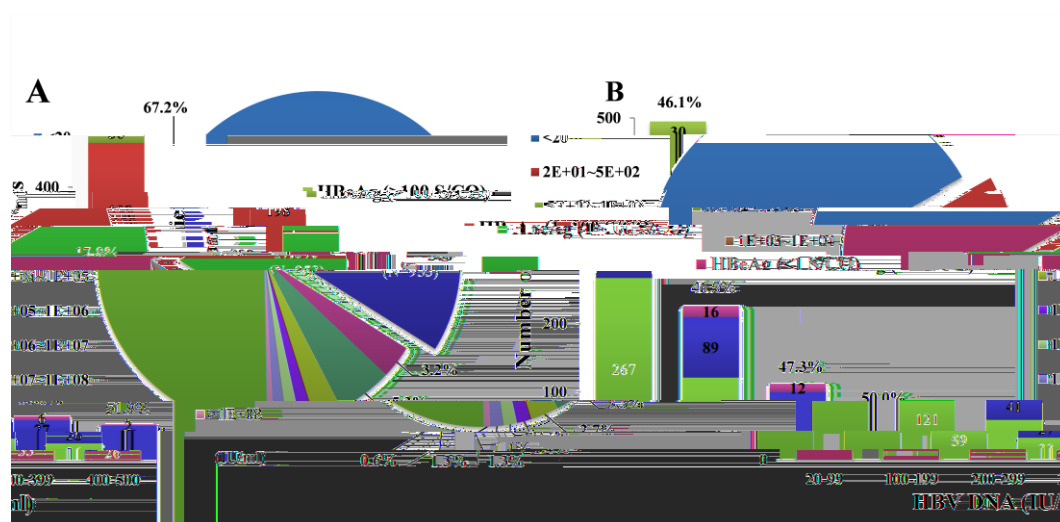


Fig.3. Correlation between serum HBsAg, HBeAg and HBV DNA. A, the relation between serum HBV DNA and HBsAg; B, the relation between serum HBV DNA and HBeAg.

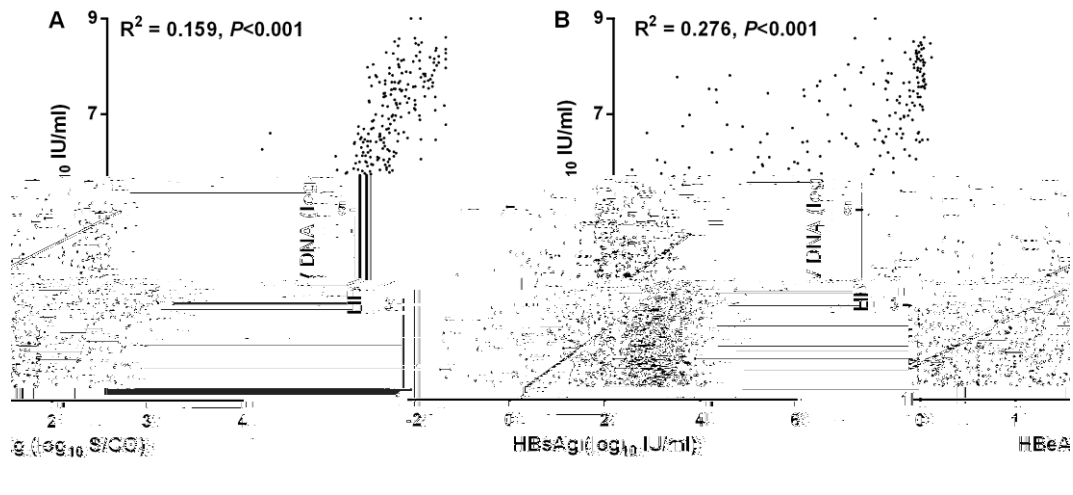


Fig.4. Dynamic changes of HBV DNA and HBsAg from baseline to 48 weeks in CHB patients with VR (A) or PVR (B) received 3 H J ,) therapy and with VR (C) or PVR (D) received ETV therapy. CHB, chronic hepatitis B; VR, virological response; PVR, partial virological response.

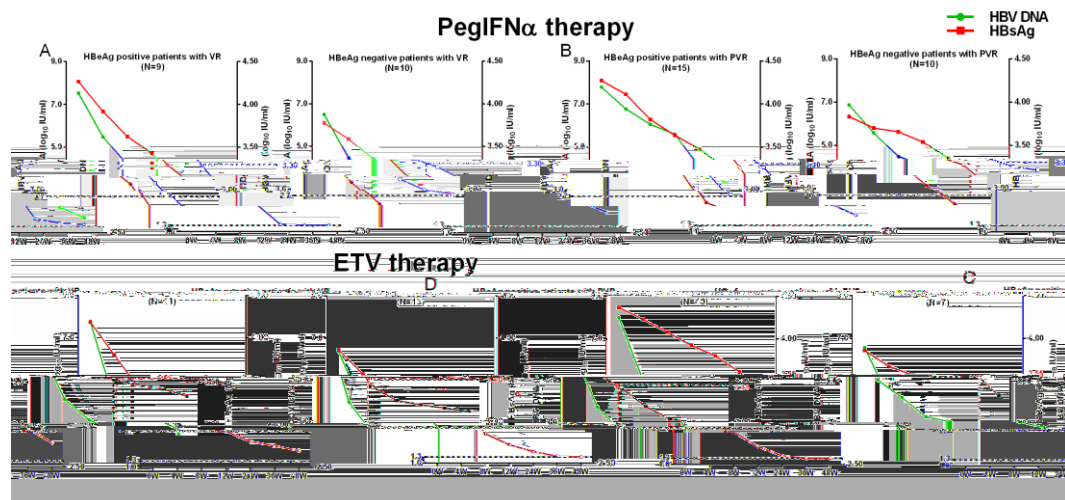


Fig.5. Receiver operating characteristic (ROC) curve analysis HBsAg between high HBV DNA (>500 IU/ml) and low HBV DNA (<500 IU/ml) groups. A, CHB

patients with 3 H J ,) 1 . W;B;C;D;E;F;G;H;I;J;K;L;M;N;O;P;Q;R;S;T;U;V;W;X;Y;Z;AA;AB;AC;AD;AE;AF;AG;AH;AI;AJ;AK;AL;AM;AN;AO;AP;AQ;AR;AS;AT;AU;AV;AW;AX;AY;AZ;BA;BB;BC;BD;BE;BF;BG;BH;BI;BJ;BK;BL;BM;BN;BO;BP;BQ;BR;BS;BT;BU;BV;BW;BX;BY;BZ;CA;CB;CC;CD;CE;CF;CG;CH;CI;CJ;CK;CL;CM;CN;CO;CP;CQ;CR;CS;CT;CU;CV;CW;CX;CY;CZ;DA;DB;DC;DD;DE;DF;DG;DH;DI;DJ;DK;DL;DM;DN;DO;DP;DQ;DR;DS;DT;DU;DV;DW;DX;DY;DZ;EA;EB;EC;ED;EE;EF;EG;EH;EI;EJ;EK;EL;EM;EN;EO;EP;EQ;ER;ES;ET;EU;EV;EW;EX;EY;EZ;FA;FB;FC;FD;FE;FF;FG;FH;FI;FJ;FK;FL;FM;FN;FO;FP;FQ;FR;FS;FT;FU;FV;FW;FX;FY;FZ;GA;GB;GC;GD;GE;GF;GG;GH;GI;GJ;GK;GL;GM;GN;GO;GP;GQ;GR;GS;GT;GU;GV;GW;GX;GY;GZ;HA;HB;HC;HD;HE;HF;HG;HH;HI;HJ;HK;HL;HM;HN;HO;HP;HQ;HR;HS;HT;HU;HV;HW;HX;HY;HZ;IA;IB;IC;ID;IE;IF;IG;IH;II;IJ;IK;IL;IM;IN;IO;IP;IQ;IR;IS;IT;IU;IV;IW;IX;IY;IZ;JA;JB;JC;JD;JE;JF;JG;JH;JI;JJ;JK;JL;JM;JN;JO;JP;JQ;JR;JS;JT;JU;JV;JW;JX;JY;JZ;KA;KB;KC;KD;KE;KF;KG;KH;KI;KJ;KK;KL;KM;KN;KO;KP;KQ;KR;KS;KT;KU;KV;KW;KX;KY;KZ;LA;LB;LC;LD;LE;LF;LG;LH;LI;LJ;LK;LL;LM;LN;LO;LP;LQ;LR;LS;LT;LU;LV;LW;LX;LY;LZ;MA;MB;MC;MD;ME;MF;MG;MH;MI;MJ;MK;ML;MM;MN;MO;MP;MQ;MR;MS;MT;MU;MV;MW;MX;MY;MZ;NA;NB;NC;ND;NE;NF;NG;NH;NI;NJ;NK;NL;NM;NN;NO;NP;NQ;NR;NS;NT;NU;NV;NW;NX;NY;NZ;OA;OB;OC;OD;OE;OF;OG;OH;OI;OJ;OK;OL;OM;ON;OO;OP;OQ;OR;OS;OT;OU;OV;OW;OX;OY;OZ;PA;PB;PC;PD;PE;PF;PG;PH;PI;PJ;PK;PL;PM;PN;PO;PP;PQ;PR;PS;PT;PU;PV;PW;PX;PY;PZ;QA;QB;QC;QD;QE;QF;QG;QH;QI;QJ;QK;QL;QM;QN;QO;QP;QQ;QR;QS;QT;QU;QV;QW;QX;QY;QZ;RA;RB;RC;RD;RE;RF;RG;RH;RI;RJ;RK;RL;RM;RN;RO;RP;RQ;RR;RS;RT;RU;RV;RW;RX;RY;RZ;SA;SB;SC;SD;SE;SF;SG;SH;SI;SJ;SK;SL;SM;SN;SO;SP;SQ;SR;SS;ST;SU;SV;SW;SX;SY;SZ;TA;TB;TC;TD;TE;TF;TG;TH;TI;TJ;TK;TL;TM;TN;TO;TP;TQ;TR;TS;TT;TU;TV;TW;TX;TY;TZ;UA;UB;UC;UD;UE;UF;UG;UH;UI;UJ;UK;UL;UM;UN;UO;UP;UQ;UR;US;UT;UU;UV;UW;UX;UY;UZ;VA;VB;VC;VD;VE;VF;VG;VH;VI;VJ;VK;VL;VM;VN;VO;VP;VQ;VR;VS;VT;VU;VV;VW;VX;VY;VZ;WA;WB;WC;WD;WE;WF;WG;WH;WI;WJ;WK;WL;WM;WN;WO;WP;WQ;WR;WS;WT;WU;WV;WW;WX;WY;WZ;XA;XB;XC;XD;XE;XF;XG;XH;XI;XJ;XK;XL;XM;XN;XO;XP;XQ;XR;XS;XT;XU;XV;XW;XX;XY;XZ;YA;YB;YC;YD;YE;YF;YG;YH;YI;YJ;YK;YL;YM;YN;YO;YP;YQ;YR;YS;YT;YU;YV;YW;YX;YY;YZ;ZA;ZB;ZC;ZD;ZE;ZF;ZG;ZH;ZI;ZJ;ZK;ZL;ZM;ZN;ZO;ZP;ZQ;ZR;ZS;ZT;ZU;ZV;ZW;ZX;ZY;ZZ

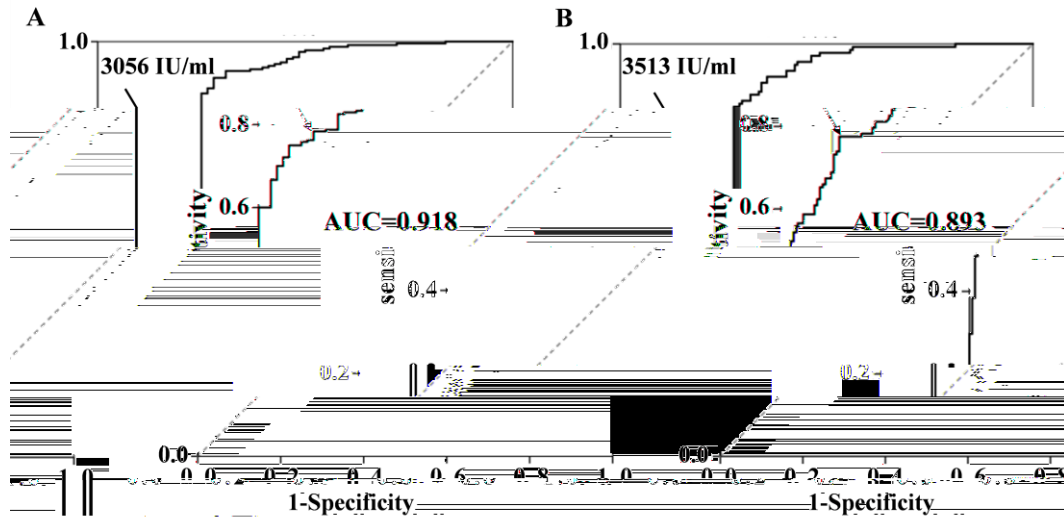
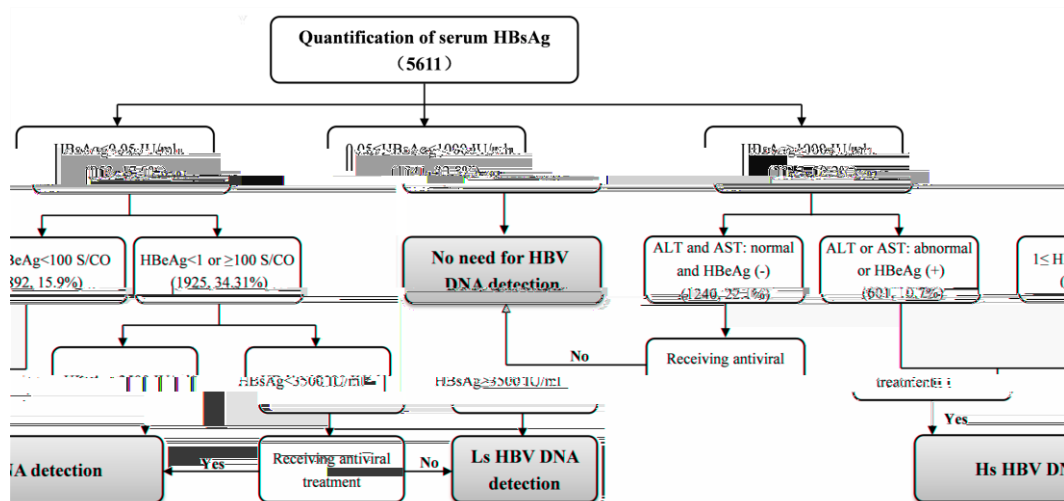


Fig.6 The clinical detection pathway for serum ls/hs HBV DNA (n, %). HBsAg quantification was recommended for initial clinical screening of HBV DNA. On one hand, if the initial screening result was negative (<0.05 IU/ml), HBV DNA detection should be unnecessary. If HBsAg level was 0.05 IU/ml $\sim$ 1000 IU/ml, whether detecting hs HBV DNA or not was decided by the levels of ALT, AST and HBeAg. On the other hand, if the HBsAg was much higher than 1000 IU/ml, ls HBV DNA was recommended. Only when HBeAg was 1 S/CO $\sim$ 100 S/CO, hs HBV DNA was recommended. Moreover, for more precise option, the method of antiviral therapy should be considered. Ls HBV DNA, low-sensitivity HBV DNA; Hs HBV DNA, hypersensitivity HBV DNA; ALT, alanine transaminase; AST, aspartate transaminase.



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*The HBsAg cut-off value should be decreased from 3500 to 2000 IU/ml during

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