Early hepatitis B viral DNA clearance predicts treatment response at week 96

Xiao-Yu Fu, De-Ming Tan, Cui-Mei Liu, Bin Gu, Li-Hua Hu, Zhong-Tian Peng, Bin Chen, Yuan-Lin Xie, Huan-Yu Gong, Xiao-Xuan Hu, Lian-Hui Yao, Xiao-Ping Xu, Zheng-Yuan Fu, Lang-Qiu He, Si-Hai Li, Yun-Zhu Long, De-Hui Li, Ji-Long Gu, Shi-Fang Peng

Yun-Zhu Long, The Central Hospital of Zhuzhou, Zhuzhou 412007, Hunan Province, China
De-Hui Li, The First People’s Hospital of Changde, Changde 415003, Hunan Province, China
Ji-Long Gu, The Central Hospital of Shaoyang, Shaoyang 422000, Hunan Province, China

Author contributions: All authors contributed to the study.

Supported by the National High Technology Research and Development Program (863 Program), No. 2012AA022605.

Institutional review board statement: This study was reviewed and approved by the Medical Ethics Committee of Xiangya Hospital, Central South University, Hunan, China.

Clinical trial registration statement: This is an observational study rather than a clinical trial. All patients received nucleoside (acid) analogues according to the guidelines.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors declare no conflict of interest.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Unsolicited manuscript
Abstract

AIM
To investigate whether hepatitis viral DNA load at 24 wk of treatment predicts response at 96 wk in patients with chronic hepatitis B.

METHODS
A total of 172 hepatitis B envelope antigen (HBeAg)-positive chronic hepatitis B patients who received initial treatment at 16 tertiary hospitals in Hunan Province, China were enrolled in this study. All patients received conventional doses of lamivudine and adefovir dipivoxil, telbivudine, entecavir dispersible tablets, or entecavir conventional tablets for 96 wk. Patients who used other antiviral drugs or antitumor and immune regulation therapy were excluded. Patients were stratified into three groups according to their viral DNA load at 24 wk: < 10^4 IU/mL (group 1), 10^4-10^6 IU/mL (group 2), and > 10^6 IU/mL (group 3). Correlations of 24-wk DNA load with HBeAg negative status and HBeAg seroconversion at 96 wk were analyzed. Receiver operating characteristic curve analysis was used to test the predictive value of the HBV DNA load at 24 wk for long-term response.

RESULTS
The rates of conversion to HBeAg negative status and HBeAg seroconversion rates were 53.7% and 51.9%, respectively, in group 1; 35.21% and 32.39% in group 2; and 6.38% and 6.38% in group 3. The receiver operating characteristic curves for the three subgroups revealed that the lowest DNA load (< 10 IU/mL) was better correlated with response at 96 wk than a higher DNA load (10-10^3 IU/mL). Nested PCR was used for amplifying and sequencing viral DNA in patients with a viral DNA load > 200 IU/mL at 96 wk; resistance mutations involving different loci were present in 26 patients, and three of these patients had a viral DNA load 10-10^3 IU/mL at 96 wk.

CONCLUSION
Hepatitis B viral DNA load at 24 wk of antiviral treatment in patients with chronic hepatitis B is a predictor of the viral load and response rate at 96 wk.
The ultimate goal of treatment of HBV infection would be functional cure, meaning a similar life expectancy of chronic HBV patients to that of patients who have self-resolution of their infection. As this clinical outcome cannot be measured in the short term, Liang et al[10] proposed apparent virological cure, which is based on the stable off-drug suppression of HBV viremia and antigenemia, and normalization of alanine aminotransaminase (ALT) and other laboratory tests. It was suggested that virological cure should be the goal of future therapies in all patients with chronic HBV infection. Hepatitis B envelope antigen (HBeAg) is an independent indicator of active viral DNA replication. It is directly correlated with disease progression to advanced stages such as cirrhosis and HCC. Therefore, sustained HBeAg seroconversion is a satisfactory result after treatment of HBeAg-positive chronic hepatitis B, and it is associated with improved long-term prognosis[5,6]. A rapid decline in hepatitis B surface antigen (HBsAg) and HBeAg titers during treatment implies a high rate of HBeAg seroconversion, as has been documented with interferon treatment[12,13]. These features are valuable in predicting the therapeutic effects in chronic HBV infection. However, very few studies using nucleoside (acid) analogue therapy have reported correlations between the changes in HBsAg and HBeAg titers and seroconversion of HBeAg.[8,14]

Some authors[8,10] have proposed that patients with an undetectable HBV DNA level after the first 24 wk of antiviral therapy could have high HBeAg seroconversion rates and low drug resistance. Due to the lack of adequate data regarding these markers, no cutoff standards have been set. The sensitivity of the HBV test in patients with chronic hepatitis B has also been called into question. According to the clinical management guidelines of the European Association for the Study of the Liver Diseases[5] for chronic hepatitis B, a highly sensitive HBV DNA test should be lower than the lowest detection limit of real-time quantitative PCR (10-15 IU/mL).[9]

In this study, we aimed to further determine the response of chronic hepatitis B to antiviral treatment by using a highly sensitive HBV DNA detection kit (lower limit of detection < 10 IU/mL). Specifically, we wished to determine whether the hepatitis B viral DNA load at 24 wk of antiviral treatment is an accurate predictor of the viral load and HBeAg seroconversion rate at 96 wk, and whether our results with a more sensitive assay would differ from previously reported results.

MATERIALS AND METHODS

Study design and population

Between December 2013 and March 2014, 172 consecutive HBeAg-positive patients who were newly diagnosed with chronic hepatitis B and received initial treatment at 16 tertiary hospitals in Hunan Province, China were enrolled in this prospective observational study. All patients were advised that they would have long-term and regular medication use and regular follow-up, and that there was the possibility of developing viral resistance and having adverse reactions to the drugs. All patients signed written informed consent forms before the start of treatment. This study was performed in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Xiangya Hospital, Central South University, Hunan, China. Baseline HBV DNA of all patients was ≥ 10^6 IU/mL, and ALT values were ≥ 2 upper limit of normal (ULN).

The following inclusion criteria were applied: patients met the diagnostic criteria for chronic hepatitis B, had no previous use of any anti-HBV drugs or other antiviral agents, had ALT values > 2 ULN, had HBV DNA > 10^6 IU/mL, and did not have clinically decompensated liver cirrhosis. The following patients were excluded: patients with other hepatotropic viral infections such as hepatitis C and hepatitis D, those with HIV infection, and those who used other antiviral drugs or antitumor and immune regulation therapy.

Treatment

Patients were given individualized antiretroviral regimens based on the recommendations in the guideline[5] and their disease conditions and financial situations, including conventional doses of one of the following treatments for 96 wk: 100 mg/d lamivudine (LAM; GlaxoSmithKline, United Kingdom); 10 mg/d adefovir dipivoxil (ADV; Chia Tai Tianqing Pharmaceutical Group Co., Ltd., Jiangsu Province, China); 600 mg/d tenofovir (Td, Novartis, Basel,
Switzerland) once daily; 0.5 mg/d entecavir dispersible tablets (ETV; Chia Tai Tianqing Pharmaceutical Group Co., Ltd.); or 0.5 mg/d entecavir tablets (ETV; Bistrol-Myers Squibb, New York, NY). In patients who received telbivudine (33 patients), the regimen was adjusted according to the response-guided therapy to optimize the treatment as follows: if HBV DNA was greater than 300 IU/mL at 24 wk, adefovir dipivoxil was added to the regimen; this adjustment was made in 11 patients.

**Clinical and laboratory data collection**

ALT, HBV DNA, HBsAg, HBeAg, and anti-HBe were measured in each patient before treatment and at 24, 48, 72, and 96 wk after treatment. An automated biochemical analyzer (Olympus AU640, Olympus, Japan) was used for the measurement of ALT. A chemiluminescent microparticle immunoassay (Abbott i2000, AltaVista, VA, United States) was used to detect HBsAg, HBeAg, and anti-HBe. Real-time fluorescence-based quantitative PCR was used to detect HBV DNA in a gene amplification laboratory authenticated by the Ministry of Health, China. Highly sensitive magnetic bead-based detection reagent was purchased from Hunan Shengxiang Biotechnology Co., Ltd (Northeast Gate, Hunan Province, China). The lower limit of detection with this reagent kit is 10 IU/mL, with comparable sensitivity and specificity to those with the COBAS TaqMan HBV assay for HBV DNA detection (Roche)[15,16]. A real-time PCR 7500 system was purchased from Applied Biosystems Inc. (Carlsbad, CA). The reference range of ALT was 0–40 U/L. HBsAg > 0.05 IU/mL, HBeAg > 1.0 s/co, and HBeAb < 1.0 s/co were considered positive results. Normalization of ALT was considered a biochemical response. The lowest detection limit of HBV DNA was < 10 IU/mL, and HBV DNA < 10^3 IU/mL was considered a complete virological response.

**DNA sequencing**

Detection of drug-resistance loci was carried out in each patient before the administration of antiviral therapy. At 96 wk of treatment, patients with HBV DNA > 200 IU/mL were selected for nested PCR, using their DNA as templates for detecting the presence of anti-drug mutations via PCR product sequencing. Primer sequences for amplification were A1: 5’-GCGGGGT TTTTCTTTAGA-3’ (203-221), A2: 5’-CGGGCAACGGGGTGAAAGCTT-3’ (1158-1138), B1: 5’-CTTGCTCTCAATTGTCCT-3’ (345-364), and B2: 5’-ACATACTTTCCCAATCAGTA-3’ (990-971). Primers A1 and A2 were used in the first round of PCR, and primers B1 and B2 were used in the second round. Reaction conditions of PCR were denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s, and subsequently, a final extension at 72 °C for 5 min. After PCR, 5 μL PCR product from each sample was separated by 2% agarose gel electrophoresis. Amplified DNA fragment was approximately 650 bp. Positive PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) using an ABI 3730xl DNA Analyzer.

**Statistical analysis**

SPSS 18.0 (SPSS, Chicago, IL) software was used for statistical analyses. Continuous quantitative data are presented as the means ± SD. Student’s t test was used for comparisons between groups. Chi-square (x^2) test was used for comparison of categorical data. Multivariate logistic regression was used to analyze correlations between clinical characteristics and the occurrence of conversion to HBeAg negative status/ HBeAg seroconversion at 96 wk. Receiver operating characteristic (ROC) curve analysis was used to test the prediction value of viral DNA load at 24 wk for long-term response. P < 0.05 was considered statistically significant. Statistical review of the study was performed by a biomedical statistician from Public Health of Xiangya Medicine College.

**RESULTS**

**Baseline characteristics in patients before antiviral therapies**

A total of 243 patients were enrolled in this study, of whom 172 were followed for 96 wk, and 71 were lost to follow-up. Thus, a total of 172 patients were included in the statistical analyses. Baseline clinical data of the 172 patients are given in Table 1. Patients were divided into three groups on the basis of their HBV DNA values at 24 wk: <10 IU/mL (group 1), 10–10^3 IU/mL (group 2), and >10^3 IU/mL (group 3). No significant differences in age, ALT values, HBV, HBsAg, or HBeAg were found. The ratio of male to female patients appeared higher in groups 1 and 2 than in group 3, but the difference was not statistically significant. Moreover, we performed a correlation analysis of gender and low viral DNA load at 24 wk and found no correlation (P = 0.833).

**Correlation between long-term treatment response with HBV DNA levels at 24 wk**

Treatment response-related variables were compared among patients grouped according to the 24-wk DNA load. As shown in Table 2, the rates of ALT normalization at 24 wk were as follows: group 1, 94.4%; group 2, 85.9%; and group 3, 40.4%. At 96 wk, the ALT normalization rates were: group 1, 100%; group 2, 93.0%; and group 3, 51.1%. Patients with HBV DNA < 10^3 IU/mL at 24 wk had significantly higher ALT return-to-normal rate at 96 wk than other patients (P < 0.01). Table 3 illustrates the correlation between the HBV DNA levels at 24 wk and the DNA response at 96 wk. Thus, 50 of the 54 (90.7%) group 1 patients had < 10 IU/mL DNA at 96 wk, and 100% were considered to have a complete response (< 10^3 IU/mL). In
Table 1  Baseline clinical data of patients before treatment

| DNA expression level at 24-wk (IU/mL) | \( < 10 \) | \( 10^{-10} \) | \( > 10^3 \) | \( P \) value |
|--------------------------------------|----------|-----------|-----------|-----------|
| Number of cases                      | 54       | 71        | 47        |           |
| Gender (male/female)                 | 39/16    | 59/12     | 26/21     | 0.108     |
| Age (yr)                             | 36.79 ± 8.68 | 37.33 ± 6.92 | 35.82 ± 10.08 | 0.181     |
| ALT (U/L) median (range)             | 133 (80-897) | 367 (84-813) | 319 (82-965) | 0.212     |
| AST (U/L)                            | 206.75 ± 133.09 | 183 ± 147.19 | 177 ± 109.85 | 0.323     |
| PLT (10^3/L)                         | 203.02 ± 70.16 | 263.33 ± 96.15 | 176.86 ± 62.05 | 0.109     |
| Total bilirubin (\( \mu \)mol/L)    | 43.12 ± 8.24 | 45.39 ± 7.15 | 42.29 ± 7.29 | 0.838     |
| HBV DNA (log_{10} IU/mL) median (range) | 7.37 ± 0.49 | 7.59 ± 0.63 | 7.26 ± 0.37 | 0.785     |
| HBeAg (s/co) median (range)          | 3.78 (2.98-5.46) | 3.66 (3.03-5.71) | 3.49 (3.04-5.66) | 0.801     |

Table 2  Rates of alanine aminotransaminase normalization according to 24-wk DNA load

| DNA expression level at 24-wk (IU/mL) | \( < 10 \) | \( 10^{-10} \) | \( > 10^3 \) |
|--------------------------------------|----------|-----------|-----------|
| 24 wk                                | 94.40%   | 85.9%     | 40.4%     |
| 96 wk                                | 100%     | 93.0%     | 51.1%     |

Table 3  DNA expression at 24 wk and DNA response at 96 wk

| DNA expression level at 24 wk (IU/mL) | DNA response at 96 wk (IU/mL) | \( < 10 \) | \( 10^{-10} \) | \( > 10^3 \) |
|--------------------------------------|--------------------------------|----------|-----------|-----------|
| \( < 10 \)                           | No response                    | 100.00%  | 0         |
| \( 10^{-10} \)                       |                                | 85.92%   | 0         |
| \( > 10^3 \)                         |                                | 31.92%   | 39.68%    |

Table 4  DNA expression at 24 wk and HBeAg response at 96 wk

| HBeAg response at 96 wk | DNA expression level at 24 wk (IU/mL) | \( < 10 \) | \( 10^{-10} \) | \( > 10^3 \) | \( P \) value |
|-------------------------|--------------------------------------|----------|-----------|-----------|-----------|
| Rate of conversion to HBeAg negative status | 53.70% (50/94) | 35.21% (25/71) | 6.38% (3/47) | 0.012     |
| HBeAg conversion rate   | 51.85% (28/54) | 32.39% (23/71) | 6.38% (3/47) | 0.017     |

In order to determine whether the potency of nucleosides could have caused differences in viral suppression, we examined the anti-viral efficacies of different drugs. As illustrated in Table 5, of 54 patients with HBV DNA < 10 IU/mL at 24 wk, 5 (5/16 = 31.25%) received LAM + ADV, 9 (9/33 = 27.27%) received telbivudine, 21 (21/59 = 35.59%) received entecavir tablets, and 20 (20/64 = 31.25%) received entecavir dispersible tablets. No significant differences were found among different treatment groups with regard to HBV DNA below detection limits, ALT normalization rate, rate of conversion to HBeAg-negative status, and HBeAg conversion rate at 94 wk (\( P = 0.127 \)).

Table 5 Treatment efficacies of various antiviral therapies

| Viriological parameter at 96 wk | LAM + ADV | Telbivudine | Entecavir tablets | Entecavir dispersible tablets | \( P \) value |
|---------------------------------|-----------|-------------|------------------|-----------------------------|-----------|
| HBV DNA below detection (< 1000 IU/mL as a reference) | 68.75% | 66.67% | 79.66% | 78.13% | 0.089 |
| ALT normalization rate         | 81.25%  | 75.76%     | 86.44%           | 85.94%                      | 0.096     |
| Rate of conversion to HBeAg negative status | 31.25% | 36.36% | 35.59% | 34.38% | 0.615 |
| HBeAg seroconversion rate      | 25.00%  | 33.33%     | 32.20%           | 31.25%                      | 0.203     |

ROC curve analysis

ROC curve analysis was conducted for each group to determine the predictive values of viral DNA load at 24 wk for HBeAg negative conversion or seroconversion at 96 wk (Figure 1, Table 6). The area under the curve in patients with \( < 10 \) IU/mL HBV DNA was 0.869, which was significantly larger than that in patients with \( 10^{-10} \) IU/mL (0.797) and in patients with \( > 10^3 \) IU/mL.
These results suggest that the predictability of efficacy of HBV antiviral treatment at 96 wk was better among patients with < 10 IU/mL HBV DNA than among patients with 10-10\(^3\) or > 10\(^3\) IU/mL DNA at 24 wk.

**DNA sequence analysis**

Patients with > 200 IU/mL HBV DNA at 96 wk underwent nested PCR for amplification and sequencing (Table 7). Various loci of drug-resistance mutations (mainly rtM204I/V, rtN236T, rtL180M, rtA181V, and rtS202G) were present in 26 patients, of whom three had 10-10\(^3\) IU/mL HBV DNA at 24 and 96 wk. Thus, in a small number of patients, persistence of modest HBVDNA levels may be the reflection of drug-resistant mutations.

**DISCUSSION**

Results in this study revealed a correlation between HBV DNA loads at 24 wk and the HBV DNA and HBeAg responses at 96 wk: Patients with a very low HBV DNA load (<10 IU/mL) at 24 wk had a 100% complete DNA response at 96 wk compared with a complete response rate in about one-third of patients who had a DNA load > 10\(^3\) IU/mL at 24 wk. Similarly, HBeAg negative conversion and seroconversion rates were more favorable in patients with very low HBV DNA loads at 24 wk than in those with higher DNA loads; the conversion rate was approximately 50% at 96 wk in patients with a DNA load < 10 IU/mL, whereas it was approximately 6% in those with a DNA load > 10\(^3\) IU/mL. ALT values also declined in relation to the DNA viral load at 24 wk, but the values did not change significantly between 24 and 96 wk, which may reflect

![Figure 1 Receiver operating characteristic curve analysis. The area under the curve is 0.869 in patients with < 10 IU/mL hepatitis B virus DNA (A), 0.797 in patients with 10-10\(^3\) IU/mL DNA (B), and 0.505 in patients with > 10\(^3\) IU/mL.](image-url)

### Table 6: Predictive value of DNA load for treatment response at 96 wk in the three different groups (according to DNA load at 24 wk)

| Group       | AUC       | 95%CI     | Sensitivity | Specificity | PPV       | NPV       |
|-------------|-----------|-----------|-------------|-------------|-----------|-----------|
| < 10 IU/mL  | 0.869     | 0.778-0.960 | 84.76%     | 87.30%      | 74.09%    | 93.04%    |
| < 1000 IU/mL | 0.797     | 0.684-0.883 | 72.00%     | 82.78%      | 64.18%    | 87.34%    |
| > 1000 IU/mL | 0.505     | 0.344-0.656 | 66.67%     | 45.45%      | 34.37%    | 76.09%    |

### Table 7: Drug-resistant mutations in 26 cases of chronic hepatitis B virus infection

| Number of cases | Resistance mutation                  |
|-----------------|--------------------------------------|
| 1               | rtM204I/V + rtN236T + rtS202G        |
| 1               | rtL180M + rtS202G + rtM204I/V + rtN236T |
| 1               | rtM204I/V + rtN236T                 |
| 1               | rtA181V + rtM204I/V + rtN236T       |
| 1               | rtM204I/V                           |
| 1               | rtA181V + rtN236T + rtS202G         |
| 1               | rtA181V + rtM204I/V                 |
| 1               | rtL180M + rtA181V + rtM204I/V + rtN236T |
| 1               | rtA181V + rtM204I/V + rtN236T       |
| 1               | rtL180M + rtM204I/V                 |
| 1               | rtM204I + rtN236T                   |
| 2               | rtL180M + rtA181V + rtS202G         |
| 3               | rtM204I + rtN236T                   |
| 4               | rtN236T                             |
| 4               | rtA181V + rtM204I/V + rtN236T + rtS202G |

**Figure 1** Receiver operating characteristic curve analysis. The area under the curve is 0.869 in patients with < 10 IU/mL hepatitis B virus DNA (A), 0.797 in patients with 10-10\(^3\) IU/mL DNA (B), and 0.505 in patients with > 10\(^3\) IU/mL.
decreasing hepatic inflammatory activity in the earlier stages of HBV antiviral treatment but not later. ROC curve analysis revealed that the predictability of two-year antiviral treatment efficacy was better in patients with a low initial DNA load than in patients with a higher DNA load at 24 wk. Finally, DNA sequence analysis revealed that some patients who failed to respond to anti-HBV therapy probably had drug-resistant mutations.

Based on treatment indicators at 96 wk, all the therapeutic agents used in this study (lamivudine plus ADV, telbivudine, and entecavir) appeared equally effective in treating chronic hepatitis B (Table 5).

Our findings corroborate and extend the results of few published studies on the time course of response of chronic HBV infection to nucleoside (acidic) analogue therapy. For example, after adefovir treatment for 24 wk, patients with HBV DNA fewer than 1000 copies/mL had 40% HBeAg seroconversion at 52 wk, whereas only 9% of patients whose 24-wk HBV DNA did not reach this value had HBeAg seroconversion[9,10]. Similarly, in the GLOBE study of HBeAg-positive chronic hepatitis B patients treated with telbivudine, patients who achieved complete viral suppression (< 300 copies/mL HBV DNA) at 24 wk had a HBeAg seroconversion rate of 46% at 104 wk[7]. Thus, HBeAg seroconversion was approximately 50% with HBV DNA detection limit of < 300 or < 1000 copies/mL. In this study, we used a more sensitive PCR HBV DNA assay, with the lowest limit of detection of 10 IU/mL, and found that patients with a very low HBV DNA load (<10 IU/mL) at 24 wk had a 100% complete DNA response. Further long-term studies are needed to determine whether these patients will have longer sustained undetectable levels of HBV DNA and HBeAg seroconversion. Such studies should attempt to determine whether viral replication, drug resistance, and risk of recurrence occur as long as detectable HBV DNA remains present.

Functional cure of chronic HBV infection remains elusive and is rarely achieved with currently available antiviral agents[11]. This situation may be partly due to the presence of drug-resistant mutations of the virus and other factors such as intrinsic stability of the nuclear form of viral genome, the covalently closed circular DNA, and dysfunctional anti-HBV immune response of the host[11,15]. Our findings indicate that drug-resistant mutations of the virus are a minor but important reason for failure of virus eradication, a finding that is consistent with a previous report in which approximately 2% of nucleoside/nucleotide analogue-naïve Chinese patients with chronic hepatitis B had drug-resistant HBV[16].

We are aware of reports that drug resistance can lead to HCC in chronic hepatitis B patients, and high HBV DNA load also can increase the risk of HCC[17]. If HBV DNA becomes negative or decreased after treatment, the risk of HCC decreases, and the risk of HCC is lower with lower HBV DNA loads[18]. Thus, failure to convincingly eradicate HBV in all our patients is a concern, but the magnitude of the risk of HCC developing is unknown.

Based on our results, when the HBV DNA load is lower in the early stages of anti-viral treatment, later outcome is better, and the risk of drug resistance is lower. Others have reported that if patients are treated with standard anti-viral medications according to guidelines and treatment is stopped after successful viral response, 44% had virological recurrence[19] and 50% had clinical recurrence[20]. The reason for recurrence is not known, but an important possibility is that, at the end of treatment, HBV DNA is not suppressed to an adequate level. We believe that the lower the HBV DNA load, the better the prognosis. High-sensitivity HBV DNA detection is useful in predicting anti-viral efficacy as well as in monitoring viral replication and recurrence after cessation of treatment. Patients whose HBV DNA is $\geq 10$ IU/mL should be closely monitored, and drug-resistant loci tested when necessary, so that the treatment regimen can be adjusted at an appropriate time.

Our study has some limitations. First, the patient population was from a specific region of China; further studies are needed to determine whether the present results are applicable to broader populations. Second, there may have been a patient-selection bias, as financial consideration may have affected the choice of therapy. Third, patients’ compliance to the prescribed medications was not assessed; thus, it is possible that some patients did not respond to therapy because of noncompliance. Despite the study’s limitations, it expands our knowledge of the therapeutic response to chronic HBV infection in a field still filled with uncertainties about the most efficacious drug regimens and duration of treatment.

The HBV DNA load at 24 wk of antiviral treatment appeared to be a valid predictor of the response rate at 96 wk in patients with chronic hepatitis B. Patients with a lower DNA load at 24 wk had a low DNA load and a higher response rate at 96 wk. Similarly, HBeAg negative conversion and seroconversion rates were more favorable in patients with very low HBV DNA loads at 24 wk than in those with higher DNA loads. ROC curve analysis revealed that the predictability of two-year antiviral treatment efficacy was better in patients with a low initial DNA load than in patients with a higher DNA load at 24 wk. Finally, DNA sequence analysis revealed that some patients who failed to respond to anti-HBV therapy probably had drug-resistant mutations. Results of this study can help in optimizing antiviral therapy in chronic hepatitis B.

**COMMENTS**

**Background**

Chronic hepatitis B is a human viral liver disease that is especially challenging...
because of its resistance to treatment and proclivity for progression to chronic liver disease with serious manifestations. Although effective and safe immunization to prevent hepatitis B virus infection is available, hepatitis B virus (HBV) continues to pose a major threat to human health worldwide. An estimated 350 million people have had chronic HBV infection. Universal agreement for the treatment of chronic hepatitis B has not been achieved. Newer medications, i.e., orally administered nucleoside (acidic) analogues, strongly inhibit HBV and have been extensively used in clinical practice. However, the optimal course of nucleoside analogue therapy remains uncertain. Moreover, drug resistance may develop after long-term administration of nucleoside analogues.

Research frontiers
Complete eradication of the virus is a goal in treatment of HBV infection. However, this goal is rarely achieved with available antiviral agents. Therapeutic regimens to reach optimal outcomes are being explored.

Innovations and breakthroughs
In our research, the authors aimed to determine if the amount of hepatitis B viral DNA present in patients’ blood after 24 wk of treatment with nucleoside analogues would accurately predict the amount of virus present at 96 wk. This information would help determine the patients’ need for continued treatment and their possible long-term outcomes. The authors evaluated 172 Chinese patients who were newly diagnosed with chronic hepatitis B and were treated with nucleoside analogues. They found that indeed the hepatitis B viral DNA load at 24 wk of antiviral treatment was a valid predictor of the response rate at 96 wk in patients with chronic hepatitis B. The findings are consistent with but more extensive than results of few published studies on the time course of response to treatment of chronic hepatitis B virus infection treated with nucleoside (acidic) analogues.

Applications
The new information derived from our study will help in optimizing antiviral therapy in chronic hepatitis B. It will help in determining if medical treatment for 24 wk is adequate or whether treatment for longer than 96 wk will be needed.

Terminology
Most readers will understand the terms used in this study. They may wish to know that nucleoside analogues are an important class of antiviral agents now commonly used in the therapy of human immunodeficiency virus infection, HBV, cytomegalovirus, and herpes simplex virus infection.

Peer-review
A very good article, suitable for publication. The research methodology is nice, the article is well written and clear, and the conclusions accord with the results.

REFERENCES
1. Pan CQ, Zhang JX. Natural History and Clinical Consequences of Hepatitis B Virus Infection. Int J Med Sci 2005; 2: 36-40 [PMID: 15968338]
2. Dienstag JL. Hepatitis B virus infection. N Engl J Med 2008; 359: 1486-1500 [PMID: 18832247 DOI: 10.1056/NEJMra0801644]
3. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med 2004; 350: 1118-1129 [PMID: 15014185 DOI: 10.1056/NEJMra031087]
4. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B virus. Gastroenterology 2006; 130: 678-686 [PMID: 16530509 DOI: 10.1053/j.gastro.2005.11.016]
5. European Association For The Study Of The Liver. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012; 57: 167-185 [PMID: 22436854 DOI: 10.1016/j.jhep.2012.02.010]
6. Liaw YF, Kao JH, Praratvishit T, Chan HL, Chien RN, Liu CJ, Gane E, Locarnini S, Lim SG, Han KH, Amarpurkar D, Cooksley G, Jafri W, Mohamed R, Hou JL, Chuang WL, Lesmana LA, Sollano JD, Suh DJ, Omata M. Asian-Pacific consensus statement on the management of chronic hepatitis B virus infection. J Hepatol 2012; 56: 531-561 [PMID: 22601469 DOI: 10.1007/s00262-012-2365-4]
7. Liaw YF, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, Heathcoe IJ, Manns M, Bzowej N, Niu J, Han SH, Hwang SG, Kulkarni SY, Tong MJ, Papatheodoridis G, Chen Y, Brown NA, Alarcon A, Gali K, Naoumov NV. 2-Year GLOBE trial results: telbivudine is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology 2009; 136: 486-495 [PMID: 19027013 DOI: 10.1053/j.gastro.2008.10.026]
8. Zeuzem S, Gane E, Liaw YF, Lim SG, DiBiaseGaglioti D, Bucet M, Chutaputtia A, Rasanakul J, Hou J, O'Brien C, Nguyen TT, Jia J, Poynard T, Belanger B, Bao W, Naoumov NV. Baseline characteristics and early on-treatment response prediction of the outcomes of 2 years of telbivudine treatment of chronic hepatitis B. J Hepatol 2009; 51: 11-20 [PMID: 19345349 DOI: 10.1016/j.jhep.2008.12.019]
9. Zoulim F, Biakowska-Warzecha J, Diiculescu MM, Goldis AE, Heyne R, Mach T, Marcellin P, Petersen J, Simon K, Bendahmane S, Klauck L, Wasiak W, Janssen HL. Entecavir plus tenofovir combination therapy for chronic hepatitis B in patients with previous nucleos(t)ide treatment failure. Hepatol Int 2016; 10: 779-788 [PMID: 27206517 DOI: 10.1007/s12072-016-9737-2]
10. Liang X, Fan R, Sun J, Shaikh J, Taneja A, Gupta S, Hamed K. Effect of Telbivudine Versus Other Nucleos(t)ide Analogs on HBV/Ag Seroconversion and Other Outcomes in Patients with Chronic Hepatitis B: A Network Meta-Analysis. Adv Ther 2016; 33: 519-531 [PMID: 26921204 DOI: 10.1007/s12325-016-0305-x]
11. Chang J, Guo F, Zhao X, Guo JT. Therapeutic strategies for a functional cure of chronic hepatitis B virus infection. Acta Pharm Sin B 2014; 4: 248-257 [PMID: 26579392 DOI: 10.1016/j.apsb.2014.05.002]
12. Zhu H, Wang C, Zhang Y, Wei S, Li X, Zhang Z. Prediction model for sustained hepatitis B e antigen sercoreversion to peginterferon alfa-2a in chronic hepatitis B. J Gastroenterol Hepatol 2016; 31: 1963-1970 [PMID: 27075693 DOI: 10.1111/jgh.13444]
13. Martino-Perignon M, Lapalus M, Maylin S, Boyer N, Castelnaud C, Giuly N, Pouteau M, Moucari R, Asselah T, Marcellin P. Baseline HBsAg and HBeAg titres allow peginterferon-based ‘precision medicine’ in HBeAg-negative chronic hepatitis B patients. J Viral Hepat 2016; 23: 905-911 [PMID: 27375231 DOI: 10.1111/jvhe.12565]
14. van Bonnel F, Bartens A, Myrickova A, Hofmann J, Krüger DH, Berg T, Edelmann A. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen sercoreversion during treatment with polymerase inhibitors. Hepatology 2015; 61: 66-76 [PMID: 25132147 DOI: 10.1002/hep.27381]
15. Lin CL, Kao JH. Review article: novel therapies for hepatitis B virus cure - advances and perspectives. Aliment Pharmacol Ther 2016; 44: 213-222 [PMID: 27302653 DOI: 10.1111/apt.13694]
16. Li X, Liu Y, Zhao P, Wang Y, Chen L, Xin S, Zhang XG, Xu D. Investigation into drug-resistant mutations of HBV from 845 nucleoside/nucleotide analogue-naive Chinese patients with chronic HBV infection. Antivir Ther 2015; 20: 141-147 [PMID: 24992206 DOI: 10.3851/IPMP2813]
17. Chan HL, Tse CH, Mo F, Koh J, Wong VW, Wong GL, Lam Chan S, Yeo W, Sung JJ, Mok TS. High viral load and hepatitis B virus subgenotype ce are associated with increased risk of hepatocellular carcinoma. J Clin Oncol 2008; 26: 177-182 [PMID: 18182659 DOI: 10.1200/JCO.2007.13.2043]
18. Ikeda K, Arase Y, Kobayashi M, Someya T, Hosaka T, Saitoh S, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Kumada H. Hepatitis B virus-related hepatocellular carcinogenesis and its prevention. Intervirology 2005; 48: 29-38 [PMID: 15758087 DOI: 10.1159/000082092]
19. Fung J, Lai CL, Chan SC, But D, Seto WK, Cheng C, Wong DK, Lo CM, Fan ST, Yuen MF. Correlation of liver stiffness and histological features in healthy persons and in patients with occult
hepatitis B, chronic active hepatitis B, or hepatitis B cirrhosis. *Am J Gastroenterol* 2010; **105**: 1116-1122 [PMID: 19920809 DOI: 10.1038/ajg.2009.665]

Liang Y, Jiang J, Su M, Liu Z, Guo W, Huang X, Xie R, Ge S, Hu J, Jiang Z, Zhu M, Wong VW, Chan HL. Predictors of relapse in chronic hepatitis B after discontinuation of anti-viral therapy. *Aliment Pharmacol Ther* 2011; **34**: 344-352 [PMID: 21671967 DOI: 10.1111/j.1365-2036.2011.04738.x]

**P- Reviewer:** Jeng WJ, Rakhshan V  
**S- Editor:** Qi Y  
**L- Editor:** Wang TQ  
**E- Editor:** Wang CH
