Serum HBV RNA as a predictor of virological response in treatment-naive chronic HBeAg-positive HBV-infected patients with normal alanine aminotransferase

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According to current guidelines, patients with chronic hepatitis B virus (HBV) infection whose HBV DNA is positive but whose alanine aminotransferase (ALT) levels are normal do not currently need antiviral treatment.[1] However, studies have found that even chronic HBV infection patients with normal ALT may progress to cirrhosis or hepatocellular carcinoma (HCC). Furthermore, almost half of patients with normal ALT levels were found to have moderate to severe inflammation and/or significant fibrosis.[2] Currently there were only a few studies on the treatment of chronic HBV patients with normal ALT levels, and the factors that affect their virological response (VR) are still unclear. An increasing number of studies have proven that serum HBV RNA can be used as a non-invasive indicator to predict a VR.[3] In this study, we explored the predictive value of HBV RNA in anticipating a VR in normal ALT chronic HBV patients.

A total of 63 treatment-naive adult chronic HBV patients with normal ALT who had undergone liver biopsy and received entecavir therapy were recruited from multi-centers. Patients with other types of viral hepatitis coinfection, and other chronic liver diseases, such as genetic or autoimmune liver disease, alcoholic liver disease, non-alcoholic fatty liver disease, and HCC, were excluded. All 63 patients were followed up to 78 weeks with a second liver biopsy. Serum HBV RNA titer was detected by the RNA simultaneous amplification testing method (HBV-SAT) based on real-time fluorescence detection of DNA transcription-mediated nucleic acid amplification using a HBV-SAT kit (Rendu Biotechnology, Shanghai, China) for all 63 patients both at baseline and after 78 weeks of treatment. The complete protocol for this clinical trial has been registered at clinicaltrials.gov (NCT01679769).

Hepatic inflammation was graded using the histology activity index (HAI), and fibrosis was staged using the Ishak fibrosis score (F). HAI <5 and F < 3 were considered as mild or minimal hepatic inflammation and fibrosis.[2] All statistical analyses were performed using SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA). The figure was created with GraphPad Prism version 6.0 (GraphPad, San Diego, CA, USA).

At baseline, among the 63 patients, the male/female ratio was 1.4:1, and the median age was 41 years. Fifty-three (84%) patients were older than 30 years. Thirty (48%) patients were hepatitis B e antigen (HBeAg)-positive and another 33 (52%) patients were HBeAg-negative. The median serum HBV RNA level (range) was 4.28 (1.70–7.87) log10 copies/mL before treatment. The median HAI and F scores were 5.0 (1.0–11.0) and 3.0 (0–6.0), respectively (Table 1).

With follow-up to 78 weeks, 42 (67%) patients achieved a VR, shown as serum HBV DNA <1.30 log10 IU/mL, and 24 patients achieved both serum HBV DNA and HBV RNA undetectable. Details of the efficacy evaluation after 78 weeks of therapy are shown in Supplementary Table 1, http://links.lww.com/CM9/B361. Among the 42 patients who achieved a VR, 15 (36%) were HBeAg positive and 27 (64%) were HBeAg negative. Compared with patients in the non-virological response (NVR) group, patients who achieved a VR had lower levels of HBV RNA (3.72[1.70–7.42] log10 copies/mL vs. 5.15[1.86–8.78] log10 copies/mL, P = 0.001), HBV DNA (5.48[1.30–8.26] log10 IU/mL vs. 6.17[3.05–8.66] log10 IU/mL, P = 0.013), and quantitative of hepatitis B surface antigen (qHBsAg) (3.37[1.20–4.52] log10 IU/mL vs. 3.76[2.50–4.92] log10 IU/mL, P = 0.002), as shown in Supplementary Figure 1, http://links.lww.com/CM9/B361. After further dividing the VR.

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Table 1: Baseline characteristics of treatment-naive chronic HBV patients with normal ALT.

| Characteristics                  | HBeAg-positive (n = 30) | HBeAg-negative (n = 33) | Total (n = 63) | P value |
|----------------------------------|-------------------------|-------------------------|----------------|---------|
| Male/female                      | 22/8                    | 15/18                   | 37/26          | 0.026   |
| Age, years                       | 39.5 (22–57)            | 46.0 (22–62)            | 41.0 (22–62)   | 0.469   |
| Age > 30 years old, n (%)        | 24 (80)                 | 29 (88)                 | 53 (84)        | 0.397   |
| BMI, kg/m²                       | 23.33 (18.42–30.12)     | 23.43 (14.82–31.14)     | 23.43 (14.82–31.14) | 0.912   |
| PLT, ×10⁹/L                      | 152.0 (65–296)          | 162.0 (62–268)          | 155.5 (62–296) | 0.860   |
| ALT/ULN                          | 0.85 (0.26–0.98)        | 0.64 (0.28–0.95)        | 0.76 (0.26–0.98) | 0.014   |
| AST/ULN                          | 0.82 (0.44–2.97)        | 0.72 (0.43–1.46)        | 0.75 (0.43–2.97) | 0.063   |
| TBIL, μmol/L                     | 13.6 (7.18–49.80)       | 13.2 (4.31–58.5)        | 13.5 (4.31–58.5) | 0.831   |
| AFP, ng/mL                       | 4.37 (1.00–77.37)       | 2.79 (0.60–113.30)      | 3.26 (0.60–113.30) | 0.172   |
| HBV RNA, log₁₀ copies/mL         | 5.46 (3.00–7.87)        | 3.63 (1.70–6.14)        | 4.28 (1.70–7.87) | <0.0001 |
| HBV RNA undetectable, n (%)      | 0                       | 4 (12)                  | 4 (6)          | 0.051   |
| HBV DNA, log₁₀ IU/mL             | 6.34 (3.98–8.66)        | 4.18 (1.30–7.53)        | 5.70 (1.30–8.66) | <0.0001 |
| qHBsAg, log₁₀ IU/mL              | 1.43 (−3.44–3.90)       | 1.26 (−2.10–3.44)       | 1.39 (−3.44–3.90) | 0.731   |
| qAnti-Hbc, log₁₀ IU/mL           | 3.57 (1.20–4.92)        | 3.40 (2.08–4.27)        | 3.48 (1.20–4.92) | 0.106   |
| APRI                             | 3.39 (2.42–4.44)        | 3.88 (2.86–5.00)        | 3.64 (2.42–5.00) | 0.0001  |
| FIB-4                            | 0.51 (0.22–1.98)        | 0.47 (0.16–1.65)        | 0.48 (0.16–1.98) | 0.297   |
| Fibroscan, kPa                   | 1.63 (0.29–5.93)        | 1.38 (0.34–4.54)        | 1.45 (0.29–5.93) | 0.683   |
| HAI 0–4/5–6/7–9/10–18, n         | 13/12/3/2               | 16/13/3/1               | 29/25/6/3      | 0.594   |
| HAI score                        | 5.0 (3.0–11.0)          | 5.0 (1.0–10.0)          | 5.0 (1.0–11.0)  | 0.449   |
| F 0–2/3/4/5–6, n                 | 13/4/8/5               | 11/9/11/2               | 24/13/19/7     | 0.971   |
| F score                          | 3.0 (1.0–6.0)           | 3.0 (0–5.0)             | 3.0 (0–6.0)    | 0.810   |

Values were shown as n, n (%), or median (range). ALT: Alanine aminotransferase; AFP: Alpha-fetoprotein; APRI: Aspartate aminotransferase-to-platelet ratio index; AST: Aspartate aminotransferase; BMI: Body mass index; F: Ishak F score; FIB-4: Fibrosis 4 score; HAI: Histology activity index; HBeAg: Hepatitis B e antigen; HBV: Hepatitis B virus; PLT: Platelet; qAnti-Hbc: Quantitative of anti-hepatitis B virus core antibody; qHBsAg: Quantitative of hepatitis B surface antigen; TBIL: Total bilirubin; ULN: Upper limit of normal.

and NVR patients by HBeAg status, both the HBeAg-positive and HBeAg-negative groups had the same trends. Furthermore, we also observed that patients older than 30 years were more likely to achieve a VR (P = 0.008). After 78 weeks of therapy, serum HBV RNA [1.70 (1.70–5.12) log₁₀ copies/mL vs. 4.31 [1.70–6.90] log₁₀ copies/mL, P = 0.002] and qHBsAg [3.26 [0.82–4.12] log₁₀ IU/mL vs. 3.62 [2.37–4.59] log₁₀ IU/mL, P = 0.006] levels were also lower in the VR group than in the NVR group. The proportion of serum HBV RNA below the lower limit of detection after 78 weeks of treatment in the VR and NVR groups was 57% (24/42) and 29% (6/21), respectively, P = 0.034.

Univariate and multivariate analyses were applied to investigate the independent variables associated with the VR to antiviral therapy in the HBeAg-positive and HBeAg-negative patients, respectively [Supplementary Table 2, http://links.lww.com/CMJ9/B361]. In the HBeAg-positive group, HBV RNA, HBV DNA, and the HAI score were significantly correlated to the VR at 78 weeks based on the univariate analysis, whereas only the HAI score (P = 0.023) and HBV RNA (P = 0.018) were independent predictors of a VR at 78 weeks based on multivariate analysis. Serum HBV RNA at baseline was not related to the 78-week VR in HBeAg-negative patients.

The area under the receiver operating characteristic (AUROC) curves of serum HBV RNA at baseline for predicting a VR at 78 weeks in HBeAg-positive patients was 0.751 (0.573–0.929), P = 0.019, while the AUROC of the HAI score was 0.756 (0.580–0.931), P = 0.017. The cutoff values of serum HBV RNA and the HAI score at baseline were 4.47 log₁₀ copies/mL (sensitivity = 0.533, specificity = 0.933) and 5.5 (sensitivity = 0.60, specificity = 0.867), respectively.

To our knowledge, this is a unique study since it evaluated the predictive value of HBV RNA in anticipating a VR in normal ALT chronic HBV patients. A considerable number of patients with chronic HBV infection with normal ALT are not currently being treated with antivirals based on the current guidelines. Our results demonstrated that the baseline serum HBV RNA level was an independent non-invasive predictor of VR in HBeAg-positive patients. Unfortunately, no correlation between serum HBV RNA and VR in HBeAg-negative patients was found, which is consistent with Liu et al.’s report,[4] which revealed that lower initial serum HBV RNA was independently associated with a rapid VR in HBeAg-positive patients. Ji et al.[5] found that the serum HBV RNA level at week 12 could predict a VR at 96 weeks in HBeAg-positive patients.

The receiver operating characteristic curve analyses and the optimal threshold cutoff value of the serum HBV RNA and HAI score indicated that in chronic hepatitis B (CHB) patients, serum HBV RNA levels below 4.47 log₁₀ copies/mL and HAI scores > 5.5 before treatment indicated they were more likely to achieve a VR after 78 weeks of therapy. However, it is challenging to identify patients’ HAI scores, as the application of liver biopsy is restricted because of its invasiveness, sampling error, and non-repeatability in the
short term. The application prospects of serum HBV RNA as a non-invasive indicator to predict a VR is more extensive.

In conclusion, this study showed that serum HBV RNA was an independent non-invasive indicator to predict a VR in HBeAg-positive patients with normal ALT. Although the number of patients was small and the follow-up time was too short to evaluate HBV RNA drug withdrawal predictor values of long-term nucleos(t)ide analogues therapy, these results will provide a reference for antiviral treatment. Because most normal ALT patients (67%) achieved a VR during therapy, patients who have high levels of HBV RNA (over 4.47 log_{10} copies/mL) before treatment should be given more attention during treatment.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and anonymity cannot be guaranteed. This study was approved by The Ethical Committees of Peking University First Hospital (No. 2012-455).

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Conflicts of interest
None.

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