Prediction for HBsAg seroconversion in children with chronic hepatitis B

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Abstract

Background: To establish a prediction of HBsAg seroconversion in children with chronic hepatitis B (CHB), so as to help clinicians to choose therapeutic strategy.

Methods: A total of 63 children with HBeAg-positive CHB aged 1 to 17 years, who admitted to the fifth medical center of Chinese PLA general hospital and treated with interferon α (IFNα) 48 weeks were enrolled, the clinical data were measured. Based on the results of HBsAg seroconversion (HBsAg < 0.05 IU/mL and anti-HBsAg > 10 IU/L) at week 48, the patients were divided into HBsAg seroconversion (S) group and non-HBsAg seroconversion (NS) group. Multivariate COX regression was used to identify the impact factors associated with HBsAg seroconversion. A novel prediction index was established and the area under the receiver operating characteristic curve (AUROC) was used to assess the prediction for HBsAg seroconversion.

Results: The 63 patients were divided into S group (20.6%, 13/63) and NS group (79.4%, 50/63). Univariate and multivariate analysis identified age, baseline intrahepatic cccDNA and serum HBsAg levels were independent impact factors for HBsAg seroconversion. Intrahepatic cccDNA was positively correlated with serum HBsAg (r = 0.464, p = 0.000). AUROC of HBV cccDNA was 0.83 (95% CI 0.71 to 0.95) and AUROC of baseline HBsAg was 0.77 (95% CI 0.61 to 0.92). Intrahepatic cccDNA ≤ 0.08 log10 copies/10^6 cell is regarded as cutoff value, the positive predictive value (PPV) and negative predictive value (NPV) for HBsAg seroconversion were 86.8% and 60.0%, respectively, with a sensitivity of 92.0% and specificity of 56.2%. HBsAg ≤ 3.68 log10 IU/mL is used as cut off value, the PPV and NPV for HBsAg seroconversion were 91.2% and 56.3%, respectively; the sensitivity and specificity was 86.0% of 69.2%, respectively. There was no statistical difference between them for predicting HBsAg seroconversion (p = 0.146).

Conclusions: HBsAg seroconversion can be predicted by the baseline serum HBsAg or intrahepatic cccDNA in children with CHB. Using the index, clinicians can choose more reasonable therapeutic strategy and reduce the waste of medical resources.

Keywords: Children, Chronic hepatitis B, HBsAg seroconversion, HBV cccDNA, HBsAg

Background

The patients who acquired hepatitis B virus (HBV) infection in childhood have an enhanced risk for the chronic hepatitis B (CHB), cirrhosis and liver cancer in adulthood [1]. HBsAg loss or seroconversion is the ideal endpoint of antiviral therapy. Effective antiviral treatment is the key to improve the rates of HBsAg loss or seroconversion in patients with CHB [2]. The HBV covalently closed...
circular DNA (cccDNA) is the original template for HBV DNA replication and the basis of HBV persistent infection, which has been used as an important indicator of evaluating antiviral effect in patients with CHB [3, 4]. As an alternative index of HBV cccDNA, hepatitis B surface (HBsAg) has been used as novel serum markers for prediction of prognosis and treatment response in adults [5–7]. However, up until now, the predictive value of HBV cccDNA and serum HBsAg for HBsAg seroconversion in children with CHB remains unknown. Therefore, establishing a prediction index of HBsAg seroconversion become desirable. In this study, we developed a HBsAg seroconversion predictive index of children with CHB can help clinicians to better choose therapeutic strategy. To our knowledge, this is the first prediction index for HBsAg seroconversion in children with CHB.

Methods

Study design

A total of 63 children with HBeAg-positive CHB, aged 1–17 years were enrolled, who treated with IFN between February 2016 and February 2019. Based on the results of HBsAg seroconversion (HBsAg < 0.05 IU/mL and anti-HBs > 10 IU/L) at 48 weeks, the patients were divided into HBsAg seroconversion (S) group and non-HBsAg seroconversion (NS) group. Age, sex, baseline HBV DNA, ALT, HBV cccDNA, HBsAg levels, genotype, degree of hepatic inflammation and fibrosis were measured. Univariate and multivariate COX regression analysis was used to identify the impact factors associated with HBsAg seroconversion. A novel prediction index was established. The area under the receiver operating characteristic curve (AUROC) were used to evaluate the predictive value for HBsAg seroconversion of baseline intrahepatic cccDNA and HBsAg in children with CHB receiving 48 weeks antiviral therapy.

Study population

This study was approved by the fifth medical center of Chinese PLA general hospital, Beijing, written informed consent was waived by patients’ parents. Sixty-three pediatric inpatients with HBeAg positive CHB who visited Beijing 302 Hospital from February 2016 to February 2019 were enrolled in the study. All patients were anti-HCV and anti-HIV negative (34 males and 29 females, mean age 5.48 ± 2.64 years; range 0.97–17 years. The main characteristics were patients with persistent elevated ALT levels and high HBV load). The diagnostic and treatment criteria were based on “2012 European Association for the Study of the Liver” and Management Scheme of Diagnostic and Therapy Criteria of Viral Hepatitis, issued by the Chinese Society of Infectious Diseases and Parasitology and the Chinese Society of Hepatology, which have been described in detail in our previous studies [8, 9].

Patients with CHB enrolled in this study met the criteria listed as follows: (1) hepatitis B surface antigen (HBsAg) positive serum of no less than 6 months from the initial onset; (2) serum HBV DNA level more than 10^5 copies/mL; (3) continuous or interval rise of alanine transaminase (ALT)/aspartate transaminase (AST) level; (4) HBeAg positive, anti-HBe negative. The exclusion criteria: (1) previous antiviral treatment for HBV infection; (2) there was no evidence for hepatocellular carcinoma (HCC), or concomitant of HCV, HDV, HIV coinfection and autoimmune liver disease, drug-induced liver injury or Wilson’s disease, liver transplantation. 63 HBV-infected children with persistent elevated ALT levels and high HBV load without jaundice and no evidence of other comorbidities were carried out recombinant human interferon alpha (IFNα), subcutaneous injection (3 MU/m^2 to 5 MU/m^2, qod) and underwent liver biopsy.

Serological markers and quantification of HBV DNA, HBV cccDNA and HBsAg levels

Blood was collected on the day of the liver biopsy. Serum ALT, HBeAg/anti-HBe and other serological markers were routinely measured in the central clinical laboratory of the fifth medical center of Chinese PLA general hospital. Serum HBV DNA levels were determined by real-time quantitative PCR kit (Fosun Pharmaceutical Co., Ltd., Shanghai, China) with a lower detection limit of 100 IU/mL (about 500 copies/mL). Serum HBsAg levels were quantified by Electro-Chemiluminescence Immunoassay using the Roche COBAS 8000 (Roche diagnostics co, Switzerland), according to the manufacturer’s instructions, HBsAg > 0.05 IU/mL was considered to be positive results. Intrahepatic HBV cccDNA levels were measured by plasmid-safe ATP dependent Danes (PSAD) digestion in combination with rolling circle amplification (RCA) and gap-spanning selective real-time PCR assay as described previously [10]. Intrahepatic HBV cccDNA levels were normalized by the amount of human genomic (hg)-beta actin in the samples. Cell numbers were calculated based on an estimation of 6.667 pg/hgDNA per cell. HBV genotype was analyzed as described previously [11]. The stage of fibrosis and the degree of inflammatory activity were evaluated by the Metavir score system [12].

Clinical monitoring

Patients were monitored every 3 months, including blood routine test, thyroid function, autoantibody test, HBsAg quantification, body temperature and mental state.
Statistical methods
When the data present normality or non normality, continuous variables were displayed as means ± standard deviation (SD) or median (interquartile range). The differences between normal data groups were compared using t-test (homogeneity of variance) or adjusted t-test (heterogeneity of variance), and non normal data were analyzed using Wilcoxon test. The calculation of the frequency or proportion of those classified variables for each type of patients was performed, and the differences between the groups was analyzed using x² test. All data were measured bilaterally, and p value was statistically significant under 0.05 [13]. The univariate and multivariate analysis was performed for investigating the independent impact factors of HBsAg seroconversion. Area under the receiver operating characteristic (ROC) curve analysis was performed to predict the likelihood of achieving HBsAg seroconversion.

Results
Clinical characteristics of patients
The baseline characteristics of the patients were shown in Table 1. As the Table 1 showed that a total of 63 cases were enrolled, including 34 males and 29 females, aged 0.97–17 years, mean age was 5.48 ± 2.64 years. Among them, 20.6% (13/63) patients achieved HBsAg seroconversion at 48 weeks, 34.9% (22/63) achieved HBeAg seroconversion at the week 48, 26.98% (17/63) patients achieved HBsAg seroconversion and 41.27% (26/63) patients experienced HBeAg seroconversion at post-treatment 6 months, all of them had not relapse within 2 years after the end of treatment. Baseline HBV DNA levels were 7.39 ± 1.45 log₁₀ IU/mL, HBsAg levels were 4.01 ± 0.86 log₁₀ IU/mL, HBV cccDNA levels were 6.64 ± 0.74 log₁₀ copies/10⁶ cell, ALT levels were 95.00 (57.00, 170.00). Because ALT values presents a skew distribution, we used the median and quartile to describe it. 16 patients (25.4%) out of the total 63 were infected with genotype B, whereas the rest patients (74.6%) were infected with genotype C. Based on the results of HBsAg seroconversion (HBsAg < 0.05 IU/mL and anti-HBs > 10 IU/L) at 48 weeks antiviral treatment, the patients were divided into HBsAg seroconversion(S) group and non-HBsAg seroconversion (NS) group. There were significant statistical differences between the S and NS group with respect to the age (p = 0.000), cccDNA levels (p = 0.040), HBsAg levels (p = 0.009) at baseline, however, there were no statistical differences were observed regarding the gender (p = 0.208), ALT levels(p = 0.108), HBV DNA (p = 0.063), genotype (p = 1.000), degree of hepatic inflammation (p = 0.082) and fibrosis stage(p = 0.663) at baseline between both groups.

Table 1  Baseline characteristics of the pediatric patients with HBeAg positive CHB

| Variable                        | Overall (n = 63) | HBsAg seroconversion(n = 13) | Non-HBsAg seroconversion(n = 50) | P-value |
|---------------------------------|-----------------|------------------------------|----------------------------------|---------|
| Age(years)                      | 5.48±2.64       | 1.68±0.71                    | 6.47±3.16                        | 0.000   |
| ALT > 2 × ULN, n(%)            | 39(61.9)        | 7(53.8)                      | 32(64.0)                         | 0.108   |
| Serum HBV DNA (log₁₀ IU/mL)    | 7.39±1.45       | 6.72±1.52                    | 7.56±1.34                        | 0.063   |
| HBV cccDNA, (log₁₀ copies/10⁶ cell) | 6.64±0.74      | 6.38±0.79                    | 6.79±0.66                        | 0.040   |
| Serum HBsAg (log₁₀ IU/mL)      | 4.01±0.86       | 3.48±1.22                    | 4.14±0.69                        | 0.009   |
| Gender (n, %)                   |                 |                              |                                  | 0.208   |
| Male                            | 34(54.0%)       | 5(14.7%)                     | 29(85.3%)                        |         |
| Female                          | 29(46.0%)       | 8(27.6%)                     | 21(72.4%)                        |         |
| HBV genotype (n, %)             |                 |                              |                                  | 1.000   |
| B                               | 16(25.4%)       | 3(18.7%)                     | 13(81.3%)                        |         |
| C                               | 47(74.6%)       | 10(21.3%)                    | 37(78.7%)                        |         |
| Grade of inflammation (n, %)    |                 |                              |                                  | 0.082   |
| G0–G1                           | 30(47.6%)       | 9(30.0%)                     | 21(70.0%)                        |         |
| ≥ G2                            | 33(52.4%)       | 4(12.1%)                     | 29(87.9%)                        |         |
| Histological fibrosis stage (n, %)|           |                              |                                  | 0.663   |
| S0–S1                           | 48(76.2%)       | 11(22.9%)                    | 37(77.1%)                        |         |
| ≥ S2                            | 15(23.8%)       | 2(13.3%)                     | 13(86.7%)                        |         |

CHB chronic hepatitis B; HBsAg hepatitis B surface antigen; ALT alanine transaminase, the upper limit of normal for ALT is ALT < 1 × ULN; HBV cccDNA hepatitis B virus covalently closed circular DNA.
interval (CI) 0.036–0.820, p = 0.047); serum HBsAg levels (HR 0.217, 95% CI 0.047–1.006, p = 0.049) and age (HR 0.318, 95% CI 0.129–0.784, p = 0.013) were independent influence factors associated with HBsAg seroconversion for those patients who received 48 weeks antiviral therapy (Table 2). Interestingly, there was a high rate of HBsAg seroconversion among the patients under 5 years old compared to older 5 years (69.2% vs 30.8%, p < 0.05).

The correlations analysis

The relationship between baseline HBV cccDNA and serum HBsAg levels was showed in Fig. 1. The results revealed that baseline intrahepatic cccDNA levels was positively correlated with serum HBsAg levels (r = 0.464, p = 0.000).

Furthermore, Fig. 2 showed HBV cccDNA levels was also positively correlated with HBV DNA levels (r = 0.665, p = 0.000).

As demonstrated in Fig. 3, there was positively correlated between serum HBsAg and HBV DNA levels at baseline (r = 0.512, p = 0.000). However, there was no correlation was observed between ALT and cccDNA levels (r = 0.251, p = 0.057), as well as HBsAg levels (r = 0.106, p = 0.407) at baseline.

### Table 2 Multivariate COX analysis of HBsAg seroconversion

| Factors                  | B     | S.E   | Wald  | P     | HR    | 95% CI |
|--------------------------|-------|-------|-------|-------|-------|--------|
| HBV cccDNA in liver tissues | −1.757 | 0.795 | 4.885 | 0.047 | 0.173 | 0.036–0.820 |
| Serum HBsAg              | −1.528 | 0.783 | 3.811 | 0.049 | 0.217 | 0.047–1.006 |
| Age                      | −1.145 | 0.460 | 6.199 | 0.013 | 0.318 | 0.129–0.784 |

*HBsAg hepatitis B surface antigen; HBV cccDNA hepatitis B virus covalently closed circular DNA*
Construction and assessment of a novel predict index

In order to facilitate clinical use and further assessment, a novel predict index was established. To evaluate the role of HBV cccDNA / HBsAg in predicting HBsAg seroconversion in patients with CHB at week 48, areas under the receiver operating characteristic (AUROC) was performed to predict the HBsAg seroconversion in HBeAg-positive children who received 48 weeks antiviral treatment.

As illustrated in Fig. 4, AUROC of intrahepatic HBV cccDNA and serum HBsAg level was 0.826 (95% CI 0.71–0.95) and 0.768 (95% CI 0.61–0.92), respectively. According to maximum the youden index, the point with the largest area under the ROC curve is the optimal diagnostic point, which is used as the cut off value of cccDNA and HBsAg, respectively. Using intrahepatic cccDNA ≤ 0.08 log10 copies/10^6 cell as cutoff value, the positive predictive value (PPV) and negative predictive value (NPV) for HBsAg seroconversion were 86.8% and 60.0%, respectively, with a sensitivity of 92.0% and specificity of 56.2%. Using HBsAg ≤ 3.68 log10 IU/mL as cut off, the PPV and NPV for HBsAg seroconversion were 91.2% and 56.3%, respectively, with a sensitivity of 86.0% and specificity of 69.2%.

Comparation of the difference between HBV cccDNA and HBsAg for the predicting HBsAg seroconversion

We used paired chi-square test to compared the difference of AUROCs between intrahepatic cccDNA and serum HBsAg for predicting HBsAg seroconversion. There was no significant statistical difference between two parameters in predicting the HBsAg seroconversion for those patients who received 48 weeks antiviral treatment (p = 0.146). These data indicated that HBsAg and cccDNA levels had same important role in predicting HBsAg seroconversion of pediatric patients with CHB.

Safety monitoring

Long-term follow-up results showed that early initiation of antiviral therapy with IFN may led to a rapid and significant serum HBsAg loss. Fever was the most common adverse event, in addition to this issue, no serious adverse events were observed in present study.

Discussion

Antiviral therapy in children with CHB is one of the most controversial clinical problems. Most of children infected with HBV during perinatal period are usually asymptomatic and have normal alanine aminotransferase
(ALT) levels. The guidelines do not recommend treatment. However, some patients may present hepatitis with continuously elevated alanine aminotransferase levels. Previous studies have shown that HBV infection in children under 6 years old is more likely occurred CHB. If patients are infected with HBV in infancy, the chronic rate of HBV infection will increase from 30 to 50% rising to 80% to 90% [14], which greatly increases the risk of adult cirrhosis and hepatocellular carcinoma. However, the current guidelines do not recommend reliable antiviral treatment for this population. In view of the current situation, in order to reduce the consequences of chronicity, experts suggest that active antiviral therapy should be considered to intervene the progress of HBV infection in children. IFN as a widely used antiviral drug had significant antiviral effect for patients with CHB [15]. Our team previously carried out an against HBV treatment for the infants with hepatitis B infection under 1 year old. We found that performing antiviral treatment early can make HBsAg clearance rapidly, moreover, most of them obtained HBsAg seroconversion, but the influence factors of HBsAg seroconversion in antiviral treatment are not clear. Therefore, we designed the present study to explore the predictors of HBsAg seroconversion in children with CHB.

We measured the levels of baseline HBsAg, intrahepatic HBV cccDNA, HBV DNA, ALT, genotype, degree of hepatic inflammation and fibrosis stage of pediatric patients with HBeAg positive CHB, and evaluated the association between the HBsAg seroconversion and above parameters. Our results showed that, compared with non-HBsAg seroconversion group, only age, baseline HBsAg and HBV cccDNA were significant difference between the two groups. Previous studies revealed that baseline higher ALT level and lower HBV DNA load was significantly associated with higher probability of HBsAg seroclearance [16–18]. However, in our study, there was no obvious correlation was observed among the ALT, HBV DNA load and HBsAg seroconversion, which were not consistent with the previous report in adults [16–18]. The immune system of pediatric patients developed incompletely and the immune response to infection might be different compared to that of adults might be the reasons for this difference [19]. Children were more susceptible to immunological tolerance than adults. Therefore, the ALT levels in children may not correctly reflect the degree of liver inflammation. HBV DNA was regarded as an important index of viral replication [16], but its expression levels were affected by many factors. Interestingly, in this study, there was a high rate of HBsAg seroconversion among the patients under 5 years old compared to older 5 years (69.2% vs 30.8%). The major reason may be shorter duration of HBV infection, which might be lead to higher HBsAg seroconversion rate. The previous studies had a similar finding about young age associated with higher HBsAg seroconversion rate after IFN treatment [15].

It has been suggested that achieving HBsAg seroclearance is the ideal endpoint of antiviral therapy [10–12, 14–22]. Effective antiviral therapy is the key to the treatment of CHB; however, some pediatric patients with CHB fail to obtain a virologic response after receiving antiviral therapy. One of the reasons leading to this result is its lack of reliable predictor. The previous study showed that antiviral efficacy was closely related to age, gender, HBV DNA, cccDNA levels, HBsAg, HBV genotypes, host immune status [23]. In the present study, multi-factor analysis showed that the levels of intrahepatic HBV cccDNA, the levels of serum HBsAg and the age of patients were closely related to the HBsAg seroconversion; the lower the baseline serum HBsAg, HBV cccDNA levels and the younger the patients, the better the antiviral treatment effect.

Some studies showed that the levels of serum HBsAg was closely related to the levels of intrahepatic cccDNA [5, 21], serum HBsAg could be considered as a surrogate marker to evaluate HBV cccDNA levels [5]. The results were supported by our study. Our data also revealed that the serum HBsAg levels at baseline was positively correlated with intrahepatic cccDNA levels in children with CHB. Previous reports showed that HBV DNA levels were correlated with HBsAg seroconversion. However, in our study, there was no significant correlation between baseline HBV DNA and HBsAg seroconversion. This might indicate that serum HBV DNA levels may not represent an ideal biomarker for evaluating intrahepatic cccDNA production in the pediatric patients with CHB.

Ruan et al. [6] study showed that intrahepatic cccDNA was predictive for antiviral effect in CHB patients. Takkenberg et al. [18] reported that low baseline level of HBsAg was an effective factor to predict the antiviral effect of interferon in patients with HBeAg positive. However, the cutoff values of HBV cccDNA and HBsAg was still ambiguous. In order to obtain the predict data of HBsAg seroconversion in pediatric patients with CHB, we used the AUROC of HBsAg and HBV cccDNA levels to evaluate the predictive value for HBsAg seroconversion. From the ROC curves, we found that using intrahepatic cccDNA $\leq 0.08 \log_{10}$ copies/$10^6$ cell as cutoff, the PPV for HBsAg seroconversion was 86.8%; using HBsAg $\leq 3.68 \log_{10}$ IU/mL as cut off, the PPV for HBsAg seroconversion was 91.2%. Although the AUROC of cccDNA was higher than that of HBsAg levels (0.826 vs 0.768), the difference was not statistically significant ($p = 0.146$). The detection of HBV cccDNA requires liver
biopsy. The results of this study indicated that detection of HBV cccDNA can be replaced by serum HBsAg quantification.

The primary endpoint of this study was HBsAg seroconversion at 48 weeks of IFN alpha treatment. All predictors were analyzed according to this endpoint. However, it is conventional to determine the rates of HBeAg and HBsAg seroconversion at 6 or 12 months off IFN alpha treatment rather than at the end of treatment, which is a limitation of the present study.

Conclusions
HBsAg seroconversion can be predicted through the baseline serum HBsAg levels. Baseline HBsAg ≤ 3.68 log_{10} IU/mL or intrahepatic cccDNA ≤ 0.08 log_{10} copies/10^6 cell was a strong predictor for HBsAg seroconversion. Clinicians can use reasonably baseline HBsAg levels to perform individualized and optimized treatment for children with CHB.

Abbreviations
HBV: Hepatitis B virus; CHB: Chronic hepatitis B; HBeAg: Hepatitis B surface antigen; HBsAg: Hepatitis B surface antigen; cccDNA: Covalently closed circular DNA; ALT: Alanine transaminase; AST: Aspartate transaminase; HCC: Hepatocellular carcinoma; PSAD: Plasmid-safe ATP dependent Danes; RCA: Rolling circle amplification; ROC: Receiver operating characteristic.

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YWZ had full access to all of the data in the study and takes responsibility for the integrity of the data, the accuracy of the data analysis, the decision to publish, or in the preparation of the manuscript.

Authors’ contributions
YWZ had full access to all of the data in the study and takes responsibility for the integrity of the data, the accuracy of the data analysis, the decision to publish, or in the preparation of the manuscript. YMS, FC, JL, CS, JJX, PL, YJB performed the experiments. YWZ, XHX, XCZ and MZ contributed to analysis and interpretation of data. Statistical analysis was done by YMS and CS. All authors have read and approved the manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The study protocol was approved by the 5th Medical Center of Chinese PLA General Hospital, Xishuang Mid-Road No.100, 100039 Beijing, China. The third author on reasonable request.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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