Journal of Clinical and Translational Hepatology **2025**
DOI: 10.14218/JCTH.2025.00205

Original Article

Cumulative Hepatitis B Surface Antigen/Hepatitis B Virus DNA Ratio in Immune-tolerant Hepatitis B Patients: A 10-year Follow-up Study

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Received: May 06, 2025 | **Revised:** June 10, 2025 | **Accepted:** June 17, 2025 | **Published online:** July 04, 2025

Abstract

Background and Aims: Patients with chronic hepatitis B virus (HBV) infection in the immune-tolerant phase may still experience hepatic inflammation and disease progression, and could benefit from early antiviral treatment. This study aimed to investigate changes in the cumulative hepatitis B surface antigen (HBsAg)/HBV DNA ratio in immune-tolerant patients during the transition to the immune-active phase, and to evaluate its potential in predicting the risk of disease progression. ***Methods:*** This longitudinal study included 127 untreated immune-tolerant patients, who were followed for up to 10 years. An independent cohort of 109 subjects was retrospectively enrolled for external validation. The relationship between the cumulative HBsAg/HBV DNA ratio and the duration of immune tolerance or transition to the immune-active phase was examined. The predictive value of the ratio was assessed and validated. ***Results:*** The relationship between the cumulative HBsAg/HBV DNA ratio and disease progression risk showed a non-linear pattern: below a ratio of 1.791, the risk of disease progression decreased rapidly as the ratio increased; above 1.791, the risk plateaued. The area under the curve for predicting disease progression was 0.67, 0.64, and 0.85 for cumulative HBsAg, HBV DNA, and the HBsAg/HBV DNA ratio, respectively. Multivariable Cox regression analysis revealed the cumulative HBsAg/HBV DNA ratio as an independent predictor of disease progression, with higher ratios associated with a lower risk. Prediction models incorporating this ratio were developed and externally validated, demonstrating strong performance and clinical utility. ***Conclusions:*** The cumulative HBsAg/HBV DNA ratio is an independent factor influencing the duration of immune tolerance and shows superior predictive performance. It may serve as a valuable marker for assessing the risk of disease progression in patients with chronic HBV infection.

Citation of this article: Zeng D, Huang Y, Lin S, Kang N, Lin Y, Jiang J, *et al*. Cumulative Hepatitis B Surface Antigen/Hepatitis B Virus DNA Ratio in Immune-tolerant Hepatitis B Patients: A 10-year Follow-up Study. J Clin Transl Hepatol 2025. doi: 10.14218/JCTH.2025.00205.

Introduction

Chronic hepatitis B (CHB) remains a significant health burden worldwide, contributing to the development of cirrhosis and hepatocellular carcinoma (HCC). The hepatitis B surface antigen (HBsAg) has an estimated global seroprevalence of 3% to 4%, with approximately 240 million individuals persistently infected with hepatitis B virus (HBV).[1] Each year, over 880,000 people die from HBV-induced liver disease,[1] with nearly half of these cases occurring in China alone.[2] Therefore, timely antiviral therapy is crucial for improving the prognosis of HBV-associated liver injury, slowing disease progression, and reducing the risk of cirrhosis and HCC.

Currently, the optimal timing for initiating antiviral therapy

Keywords: Hepatitis B virus; Chronic hepatitis B; Hepatitis B surface antigen; HBsAg; Hepatitis B e-antigen; HBeAg; Hepatitis B virus DNA; Immune-tolerant phase; Immune-active phase; Predictive model; Inflammation; Liver fibrosis; Cirrhosis; Hepatocellular carcinoma.

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in hepatitis B has not reached a consensus among major clinical guidelines. Key factors, including disease stage, markers of liver injury, viral load, and patient-specific characteristics, may influence treatment decisions regarding when to start therapy. Consequently, research continues to explore the optimal timing for antiviral initiation to improve patient outcomes. The natural history of chronic HBV infection is divided into distinct phases, with the first phase being the immune-tolerant phase, first proposed by Dudley *et al*. in 1972.[3] This concept suggests that HBV infection, whether self-limiting or persistent, is defined by a T-cell-mediated immune response. During the immune-tolerant phase, high viral loads can result in mild or no hepatic impairment but persistent viral replication; the patient's immune system neither recognizes HBV nor effectively clears it.[4] Later, Yim *et al*.[5] further divided the natural history of chronic HBV infection into four phases: immune-tolerant, immune-clearance, inactive carrier state, and reactivation phases, a classification widely used since then. Patients in the immune-tolerant phase are characterized by high viral replication and low inflammatory activity. Previously, it was believed that patients in this phase had mild liver histological damage, slow disease progression, and poor response to available antiviral drugs.[6,7] Consequently, major global guidelines excluded patients in the immune-tolerant phase from indications for antiviral therapy.

However, emerging evidence suggests that the immune-tolerant phase is not without risk of disease progression. Significant liver histologic changes have been observed even in patients with CHB who have normal transaminase levels.[8] For instance, studies have demonstrated that chromosomal HBV DNA integration and clonal hepatocyte expansion can occur even in immune-tolerant patients, indicating ongoing tissue inflammation, HBV-specific immune responses, and potential progression to HCC.[9,10] A Korean cohort study compared 413 untreated hepatitis B e-antigen (HBeAg)-positive patients with high HBV DNA loads and normal alanine aminotransferase (ALT) levels in the immune-tolerant phase with 1,497 immune-clearance patients on antiviral therapy. The study found significantly higher estimated 10-year cumulative HCC morbidity rates (12.7% vs. 6.1%) and death/transplantation rates (9.7% vs. 3.4%) in the immune-tolerant group.[11] This suggests that immune tolerance may confer a greater risk of disease progression to HCC and mortality than previously recognized. Given these findings, the traditional concept of the immune-tolerant phase has been increasingly challenged: the immune-tolerant phase is not a benign period without disease progression. Reflecting these advances, the 2017 European Association for the Study of the Liver guideline reclassified the immune-tolerant phase as "HBeAg-positive chronic HBV infection," de-emphasizing the concept of the immune-tolerant phase.[12] The 2022 China Chronic Hepatitis B Control Guidelines emphasize more aggressive screening and expanded antiviral therapy.[13] Specifically, these guidelines have lowered the thresholds for initiating treatment based on HBV DNA and ALT levels to increase the eligible patient population. The updated guidelines also recommend active management for high-risk patients, including those over 30 years old with a family history of HBV-related cirrhosis or HCC, significant fibrosis or inflammation on liver biopsy or noninvasive evaluation, and those with HBV-related extrahepatic manifestations.[14] As a result, approximately 94% of patients now meet the criteria for treatment, closely aligning with a "treat-all" strategy.

In recent years, dynamic changes in serum HBsAg levels during different stages of chronic HBV infection have attracted much attention. While the ideal goal of current antiviral therapy is HBsAg seroconversion, a decline in serum HBV DNA levels reflects reduced viral replication; however, the correlation between HBsAg and HBV DNA varies across different disease states. Henry Lik-Yuen Chan[15] followed patients at different stages of chronic HBV infection for eight years and found that patients in the immune-tolerant phase had the highest HBsAg levels, approximately 5 log IU/mL. However, a single HBsAg measurement is not an accurate predictor of disease activity or viral clearance. Further studies suggest that the HBsAg/HBV DNA ratio may serve as a potential indicator of the degree of liver inflammation.[16] Notably, a previous study[17] found that the five-year cumulative incidence of patients with chronic HBV infection in the immune-tolerant phase transitioning into the immune-active phase is as high as 38%, with a significantly increased risk of liver-related adverse events. These findings highlight the need for further investigation into factors influencing disease progression in immune-tolerant patients and the identification of early predictive markers.

We conducted a 10-year follow-up study aiming to investigate the factors influencing disease progression in immune-tolerant patients and to explore the predictive value of the cumulative HBsAg/HBV DNA ratio for earlier detection of disease progression during the immune-tolerant phase. The findings may provide evidence to support future research and assist in clinical decision-making.

Methods

Subjects

In this retrospective study, a total of 127 patients with hepatitis B in the immune-tolerant phase were followed up in the Department of Hepatology at the Liver Disease Center, The First Hospital of Fujian Medical University (Fuzhou, Fujian, China) over a 10-year period from 2014 to 2023. Inclusion criteria included: 1) age ≥ 18 years, 2) HBsAg-positive for ≥ six months and HBeAg-positive, 3) no history of antiviral therapy, 4) HBV DNA ≥ 1×10^6 IU/mL, and 5) ALT levels below the upper limit of normal. Exclusion criteria were as follows: 1) coexisting chronic liver diseases, such as autoimmune liver disease, metabolic dysfunction-associated fatty liver disease (previously known as non-alcoholic fatty liver disease), alcoholic liver disease, and drug-induced liver injury; 2) co-infection with human immunodeficiency virus, hepatitis C virus, or hepatitis delta virus; and 3) presence of HCC, or a history of liver resection, immunotherapy, or chemotherapy. None of the study participants received antiviral therapy during the entire follow-up period. The immune-active phase was defined as ALT ≥ 40 U/L with HBV DNA positivity, excluding other causes of ALT elevation. HBsAg, HBeAg, HBV DNA levels, and liver biochemistry were monitored at each visit until either transition to the immune-active phase occurred or the patient was lost to follow-up.

An independent validation cohort of 109 immune-tolerant patients was retrospectively enrolled at Huashan Hospital (Shanghai, China), using the same inclusion and exclusion criteria.

The study was conducted in accordance with the Declarations of Helsinki and Istanbul and was approved by the Institutional Review Board of Fujian Medical University. Due to the retrospective nature of the study, the requirement for written informed consent from participants was waived.

Liver biochemistry and hepatitis B serologic tests

Liver biochemistry parameters, including total bilirubin, albumin, globulin, ALT, aspartate aminotransferase (AST), and

gamma-glutamyltransferase (GGT), were measured using a fully automated biochemical analyzer. HBV serological markers, including HBsAg and HBeAg, were assessed using an enzyme-linked immunosorbent assay kit (Roche Diagnostics, Mannheim, Germany) or the Abbott Architect assay (Abbott Laboratories, Chicago, IL, USA). HBV DNA levels were measured using a real-time quantitative polymerase chain reaction method (PG Company, Shenzhen, China).

Cumulative HBsAg, HBV DNA, and their ratio

Cumulative HBsAg was calculated using the trapezoidal rule, which involves determining the average HBsAg value between two consecutive follow-up visits, multiplying it by the time interval between visits, and summing the results to obtain the total cumulative HBsAg, as previously described.[18] Cumulative HBV DNA and the cumulative HBsAg/HBV DNA ratio were calculated using the same method.

Statistical analysis

All statistical analyses were carried out using SPSS version 24.0, the R statistical software version 4.4.2, and Empower(R) (http://www.empowerstats.com). Data are presented as means ± standard deviations or as counts with percentages. Differences between continuous and categorical variables were assessed with Student's t-test (or the Mann-Whitney test, when appropriate) and the chi-squared test (or Fisher's exact test, when appropriate). The effects of cumulative HBsAg, cumulative HBV DNA, and the cumulative HBsAg/HBV DNA ratio on the immune tolerance period of hepatitis B were analyzed using the Kaplan-Meier method, restricted cubic spline analysis, and Cox regression. Smoothed curve fitting was used to assess the risk relationship for the transition from the immune-tolerant phase to the immune-active phase and to construct and validate predictive models. A *P*-value of <0.05 was considered statistically significant.

Results

Baseline characteristics

Among 127 individuals included in this study, there were 71 males and 56 females, with a mean follow-up time of 2.23 years. Of these, 52 (40.9%) were confirmed to have transitioned from the immune-tolerant phase to the immune-active phase. Compared with the immune-active group, the immune-tolerant group was younger, and ALT, AST, and GGT levels were lower both at baseline and at the last follow-up, with statistically significant differences (all $P < 0.05$, Table 1). The immune-tolerant group showed significantly higher HBsAg, HBeAg, and HBV DNA levels at baseline. Additionally, HBeAg, HBV DNA, and albumin decreased significantly when patients transitioned from the immune-tolerant phase to the immune-active phase (all $P < 0.05$, Table 1). Furthermore, cumulative HBsAg, cumulative HBeAg, cumulative HBV DNA, and the cumulative HBsAg/HBV DNA ratio were not statistically different between the two groups; however, the immune-tolerant group showed higher levels of these cumulative markers (Table 1).

Association of cumulative HBsAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA with immune-tolerant duration

Cumulative HBsAg, cumulative HBeAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA were stratified into quartiles, and their associations with immune-tolerant duration were assessed using Kaplan-Meier survival analysis (Supplementary Table 1; Fig. 1). Significant differences in survival rates were observed between groups during early follow-up. Higher cumulative HBsAg, cumulative HBeAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA were significantly associated with prolonged median immune-tolerant duration ($P < 0.05$, Supplementary Table 1).

To further evaluate these associations, the indicators were stratified into quartiles and subjected to Cox regression analysis. Quartiles of cumulative HBsAg, HBeAg, and HBV DNA showed no significant association with disease progression (Supplementary Table 2). In contrast, cumulative HBsAg/HBV DNA was identified as a significant factor influencing the progression of immune-tolerant ($P < 0.05$, Supplementary Table 2). The risk of disease progression in Q1 (cumulative HBsAg/HBV DNA < 0.6), Q2 (0.6 ≤ cumulative HBsAg/HBV DNA < 1.1), and Q3 (1.1 ≤ cumulative HBsAg/HBV DNA < 1.9) was 42-fold, 28-fold, and five-fold higher, respectively, than that in Q4 (cumulative HBsAg/HBV DNA ≥ 1.9), indicating a lower risk of disease progression with higher values of the cumulative HBsAg/HBV DNA ratio (Supplementary Table 2).

Non-linear and threshold effect analysis of cumulative HBsAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA in the progression from immune-tolerant to immune-active phase

We performed restricted cubic spline analysis and generated fitting curves for cumulative HBsAg, cumulative HBeAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA, adjusting for sex and age (Fig. 2). Cumulative HBsAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA were associated with immune tolerance disease progression in a non-linear manner. Threshold effect analysis revealed that when cumulative HBsAg and cumulative HBV DNA were below 9.55 and 10.99, respectively, the risk of immune tolerance disease progression decreased rapidly with increasing cumulative HBsAg and cumulative HBV DNA ($P < 0.05$). However, when cumulative HBsAg and cumulative HBV DNA exceeded 9.55 and 10.99, respectively, the risk of disease progression slowed down. Similarly, when the cumulative HBsAg/HBV DNA ratio was below 1.791, the risk of immune tolerance disease progression decreased rapidly with increasing cumulative HBsAg/HBV DNA ratio. However, when the ratio was greater than 1.791, the risk of disease progression stabilized (Fig. 2; Supplementary Table 3).

ROC curve analysis of cumulative HBsAg, cumulative HBV DNA, and the cumulative HBsAg/HBV DNA ratio for predicting progression from the immune-tolerant to immune-active phases

ROC curve analysis was performed to evaluate the predictive performance of cumulative HBsAg, cumulative HBV DNA, and the cumulative HBsAg/HBV DNA ratio in identifying progression from the immune-tolerant phase to the immune-active phase. As presented in Figure 3, cumulative HBsAg demonstrated an area under the curve (AUC) of 0.67, a sensitivity of 0.78, and a specificity of 0.54; cumulative HBV DNA showed an AUC of 0.64, a sensitivity of 0.55, and a specificity of 0.73. Notably, the cumulative HBsAg/HBV DNA ratio exhibited an AUC of 0.85, a sensitivity of 0.92, and a specificity of 0.76, surpassing the predictive performance of cumulative HBsAg or HBV DNA alone in identifying progression from the immune-tolerant to the immune-active phase.

Development and validation of the predictive model

We used data from the 127 study participants as the model

Table 1. Comparison of baseline characteristics between the immune-tolerant and immune-active groups

	Immune-tolerant group (n = 75)	Immune-active group (n = 52)	Chi-square /Z	*P*
Sex (%)			2.039a	0.153
Male	38 (50.67)	33 (63.46)		
Females	37 (49.33)	19 (36.54)		
Age (years)	28 (24, 29)	31 (30, 33)	−7.427	<0.001
LogHBsAg (IU/mL)	4.62 (4.81, 4.33)	4.52 (4.79, 4.07)	−1.147	0.251
LogHBeAg (IU/mL)	3.17 (3.22, 3.06)	3.12 (3.20, 2.99)	−1.554	0.12
Log DNA (IU/mL)	7.92 (8.23, 7.54)	7.77 (8.16, 7.13)	−1.466	0.143
HBsAg /HBV DNA	0.58 (0.60, 0.54)	0.59 (0.61, 0.55)	−0.848	0.396
TBIL (umol/L)	13.00 (16.80, 9.90)	12.55 (16.93, 9.43)	−0.478	0.633
ALB (g/L)	45.96±3.49	46.06±2.92	0.945	0.864
GLO (U/L)	27.93±3.57	27.88±3.64	0.054	0.936
ALT (U/L)	27.13±11.01	35.25±10.62	0.454	<0.001
AST (U/L)	24.00 (28.00, 19.00)	27.00 (30.00, 24.00)	−3.139	0.002
GGT (U/L)	15.00 (20.00, 11.00)	19.00 (27.00, 14.00)	−3.247	0.001
Last-LogHBsAg (IU/mL)	4.61 (4.75, 4.23)	4.31 (4.69, 3.84)	−1.647	0.099
Last-LogHBeAg (IU/mL)	3.16 (3.22, 2.99)	3.11 (3.19, 2.81)	−2.069	0.039
Last-Log DNA (IU/mL)	7.80 (8.17, 7.12)	7.49 (7.94, 4.50)	−2.415	0.016
Last- HBsAg /HBV DNA	0.58 (0.62, 0.56)	0.62 (0.95, 0.56)	−1.839	0.066
Last-TBIL (umol/L)	12.90 (17.30, 8.20)	14.00 (16.25, 9.45)	−0.944	0.345
Last-ALB (g/L)	46.34±2.79	45.25±2.67	0.465	0.029
Last-GLO (U/L)	27.50 (29.60, 24.90)	28.35 (30.88, 26.30)	−1.846	0.065
Last-ALT (U/L)	23.00 (32.00, 18.00)	82.50 (159.75, 58.50)	−9.558	<0.001
Last-AST (U/L)	21.00 (26.00, 19.00)	49.50 (104.25, 37.25)	−8.779	<0.001
Last-GGT (U/L)	15.00 (21.00, 11.00)	31.50 (53.75, 19.00)	−5.574	<0.001
Cum-HBsAg	8.76 (14.38, 4.72)	7.09 (14.50, 4.08)	−0.946	0.344
Cum-DNA	14.88 (22.82, 7.54)	11.79 (24.95, 7.08)	−0.824	0.41
Cum-HBeAg	6.31 (9.31, 3.06)	4.67 (9.73, 2.44)	−0.897	0.37
Cum-HBsAg/HBV DNA	1.17 (1.97, 0.62)	0.98 (2.07, 0.57)	−0.579	0.563

"Last-" represents values recorded at the last follow-up. "Cum-" represents cumulative values calculated as described in the Methods. HbsAg, Hepatitis B surface antigen; HBeAg, Hepatitis B e-antigen; TBIL, Total bilirubin; ALB, Albumin; GLO, Globulin; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, gamma-glutamyltransferase; Cum-DNA, cumulative DNA; Cum-HBsAg/HBV DNA, cumulative HBsAg/HBV DNA.

group to develop a predictive model incorporating the cumulative HBsAg/HBV DNA ratio (Fig. 4). An independent cohort of 109 patients in the immune-tolerant phase was used as a validation group to evaluate the model's accuracy and performance. Among the 109 individuals in the validation group, there were 65 males and 44 females, with a mean follow-up time of 2.17 years. Of these, 33 (30.3%) progressed from the immune-tolerant to the immune-active phase. ALT, AST, and GGT levels were significantly lower in the immune-tolerant group compared to the immune-active group at both baseline and the last follow-up ($P < 0.05$, Supplementary Table 4). The predictive model achieved a C-index of 0.9350 (95% confidence Interval: 0.912–0.958), indicating excellent predictive performance. The Dxy value was 0.87 ± 0.0235 ($P < 0.0001$), and the mean absolute error of the calibration curve was 0.616, suggesting good model calibration (Supplementary Figs. 1 and 2). In the validation group, the model achieved a C-index of 0.748. The DCA plot demonstrated that the model provides meaningful clinical benefit (Fig. 5).

Subgroup analysis of differences in indicators between age subgroups

A subgroup analysis was conducted to compare clinical indicators among participants stratified by age (Supplementary Table 5). Subjects were divided into two groups: those aged ≥30 years and those aged <30 years. The results indicated that individuals under 30 had lower ALT, AST, and GGT levels, and higher HBeAg and HBV DNA levels, compared to those aged ≥30 years.

Logistic regression analysis of ALT, AST, GGT, HBeAg, and HBV DNA was performed to further investigate differences across age groups. The analysis revealed a statistically significant difference in ALT levels between participants aged

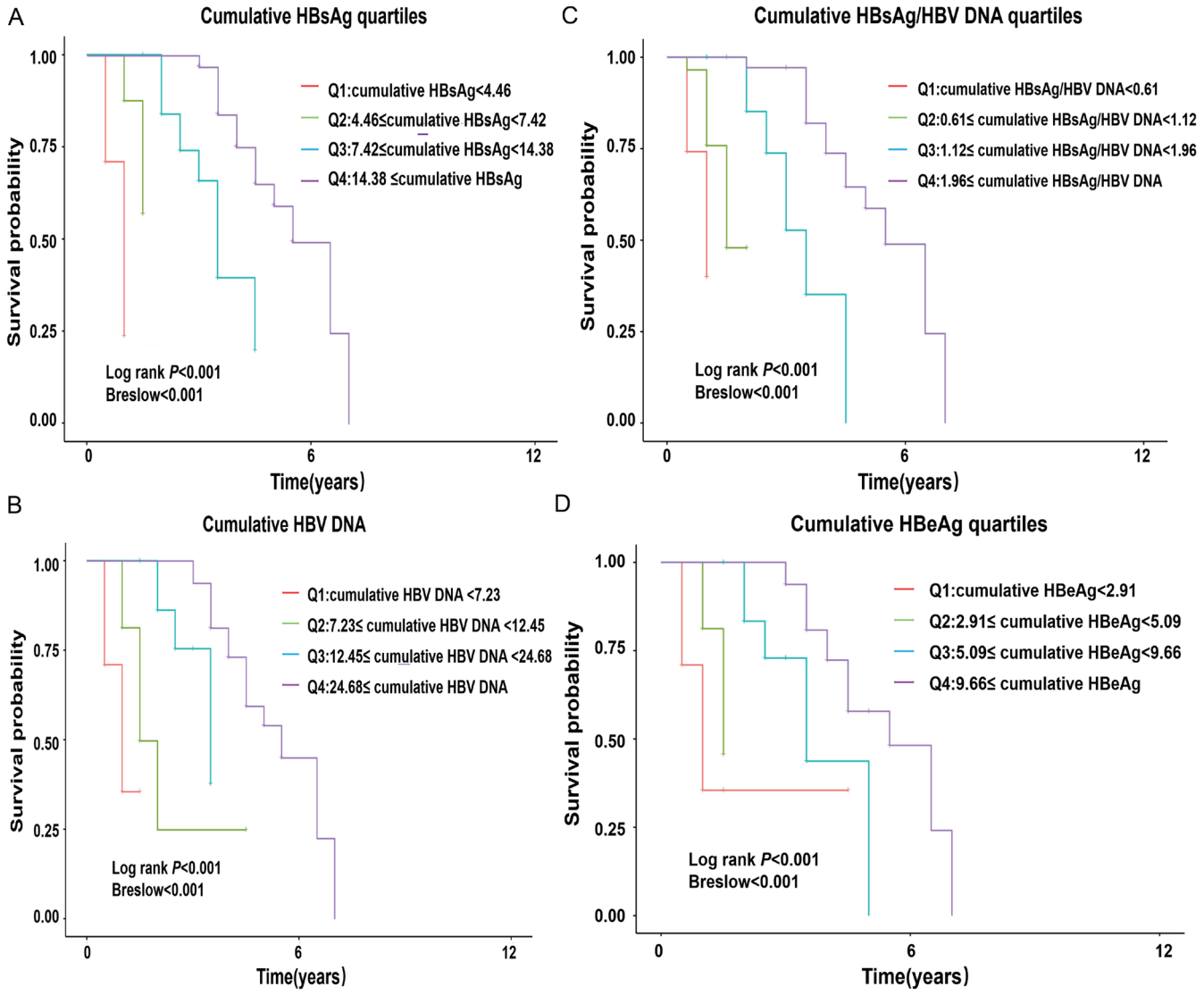

Fig. 1. Kaplan-Meier analysis of immune tolerance duration stratified by quartiles of cumulative HBsAg, cumulative HBV DNA, and their ratio. Kaplan-Meier curves for (A) cumulative HBsAg quartiles; (B) cumulative HBV DNA quartiles; (C) cumulative HBsAg/HBV DNA quartiles; (D) cumulative HBeAg quartiles. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

≥30 years and those aged <30 years (Supplementary Table 6, $P < 0.05$).

Discussion

It is estimated that approximately 59.4 million people globally and 15.84 million in China have the immune-tolerant phase.[19] Early studies have shown no or mild liver inflammation during the immune-tolerant phase, in which the disease progresses slowly, and antiviral therapy is generally not recommended. Jeon *et al*.[20] followed immune-tolerant patients with a FIB-4 index < 1.45 who did not receive antiviral therapy for five years and reported a 0% HCC incidence. Virologic rebound commonly occurs after antiviral therapy in patients with immune tolerance. For instance, an observational study on the efficacy of discontinuing tenofovir disoproxil fumarate (TDF) in immune-tolerant patients[21] included 21 patients who completed 192 weeks of TDF ± emtricitabine treatment. After 192 weeks, TDF was continued in one patient and discontinued in the remaining 20. It was found that all patients experienced virologic relapse within four weeks. Therefore, antiviral therapy is not routinely recommended during the immune-tolerant phase; instead, dynamic monitoring is preferred. Some studies have indicated that 22.5% to 49.4% of the immune-tolerant phase patients had significant liver inflammation and fibrosis.[22,23] Chu *et al*.[23] followed immune-tolerant patients for up to 17 years and found that the cumulative incidence of cirrhosis was as high as 12.6%. Chen *et al*. followed 251 immune-tolerant patients for 13 years and reported a cumulative HCC incidence of 5.1%. Currently, debate over antiviral therapy during the immune-tolerant phase persists. It is important to recognize the transition from the immune-tolerant to the immune-active phase early to allow timely intervention. However, significant necroinflammation and fibrosis may occur even with normal ALT levels, suggesting a need for antiviral therapy.[24,25] In

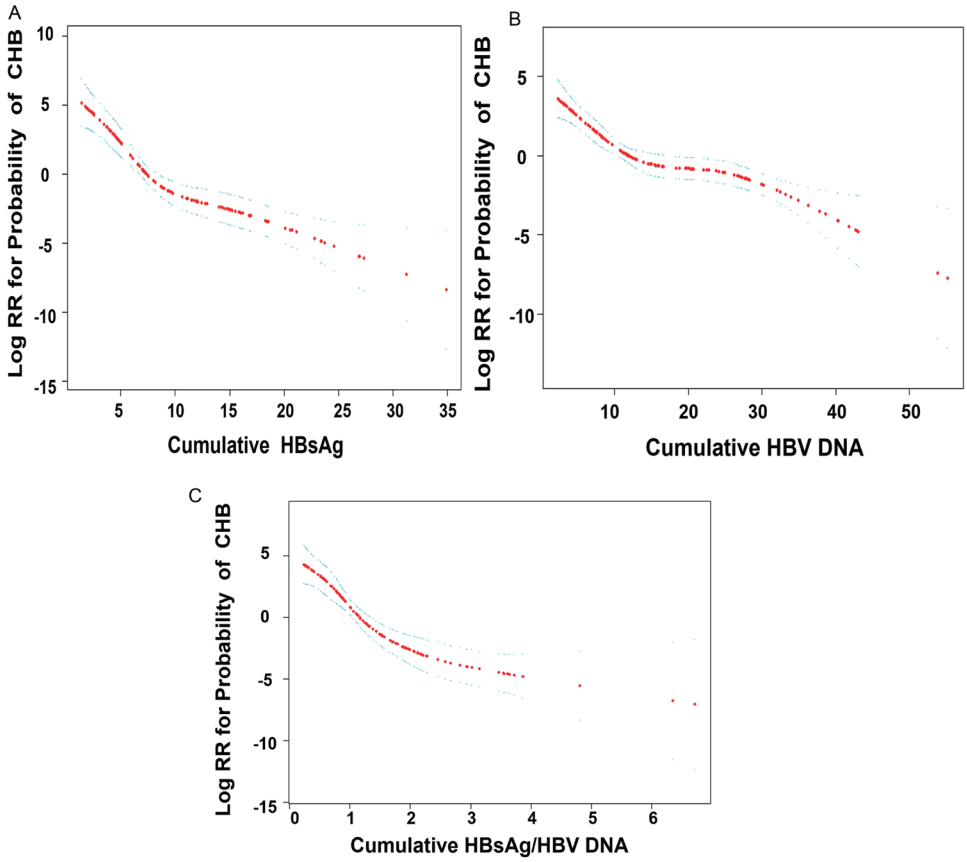

Fig. 2. Restricted cubic spline fitting curves for cumulative HBsAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA ratio in relation to the transition from the immune-tolerant phase to the immune-active phase. (A) Cumulative HBsAg; (B) Cumulative HBV DNA; (C) Cumulative HBsAg/HBV DNA ratio. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; CHB, chronic hepatitis B.

response, the 2022 Chinese guidelines for the prevention and treatment of CHB recommend expanding antiviral treatment to include chronically HBV-infected patients aged ≥30 years, even if their aminotransferase levels are within the normal range. Thus, indications for antiviral therapy have been further expanded to include immune-tolerant patients over 30 years of age.[13]

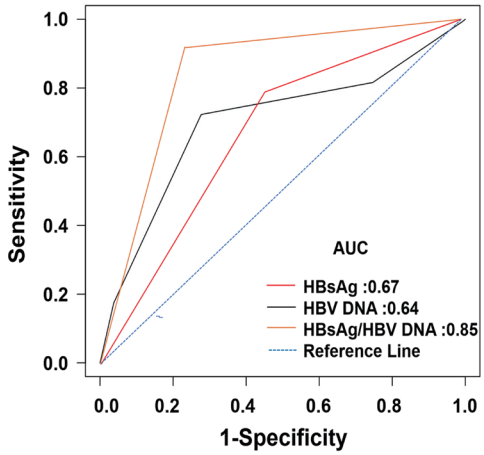

Fig. 3. ROC curves for cumulative HBsAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA ratio. The ROC curves illustrate the performance of cumulative HBsAg, cumulative HBV DNA, and the cumulative HBsAg/HBV DNA ratio in predicting the progression from the immune-tolerant phase to the immune-active phase. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; ROC, Receiver operating characteristic; AUC, area under the curve.

A decrease in serum HBsAg reflects reduced transcriptional activity of covalently closed circular DNA or mRNA from integrated sequences, and a decline in serum HBV DNA levels reflects lower viral replication.[26] Since the discovery of HBsAg, its significance in selecting antiviral drugs and predicting antiviral efficacy has been established.[27,28] However, the correlation between HBsAg and HBV DNA varies during the course of CHB. Song *et al*.[29] found that the HBsAg/HBV DNA ratio better predicted virologic response during entecavir treatment than HBsAg or HBV DNA alone in a 26-month study of 52 patients with CHB. This ratio is also closely related to liver inflammation and fibrosis. Among HBeAg-positive patients with mild to moderate or severe hepatic inflammatory activity, HBsAg titers and the HBsAg/HBV DNA ratio gradually decreased.[30] Similarly, Zhang *et al*.[31] noted that HBsAg and the HBsAg/HBV DNA ratio could be used as indicators of liver inflammation, with the specificity of the HBsAg/HBV DNA ratio significantly higher than that of ALT. However, other studies have suggested that the HBsAg/HBV DNA ratio varies in different stages of chronic HBV infection, and that an elevated ratio might indicate disease progression toward cirrhosis or death. Differences in the HBsAg/HBV DNA ratio suggest that changes in HBsAg and HBV DNA do not always occur in parallel. This dissociation may arise

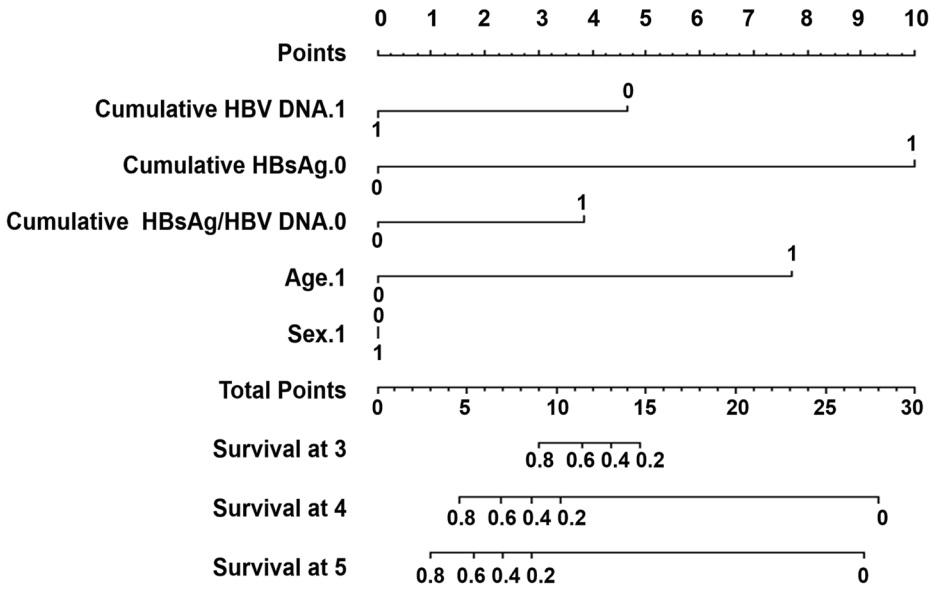

Fig. 4. Column line plot for predicting the duration of immune tolerance in hepatitis B patients in the immune-tolerant phase. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

from: (1) HBsAg production from an integrated viral genome during low-level HBV replication, or (2) preferential control of HBV replication through cytokine effects. In either scenario, a high HBsAg/HBV DNA ratio may indicate enhanced host immunity that suppresses HBV replication (specifically, the transcription of pregenomic RNA) while relatively sparing HBsAg transcription.[26,32] Based on this hypothesis, we examined the correlation between the HBsAg/HBV DNA ratio and hepatic inflammatory activity.

In our study, the two-year cumulative incidence of transition from the immune-tolerant phase to the immune-active phase was 40%, which is higher than the 38% observed in a retrospective Canadian study over five years.[18] This difference may be attributed to two factors: (1) the Canadian study had a female predominance and uniformly high HBV DNA levels (HBV DNA ≥ 6 $\log_{10}$ IU/mL), and (2) the inclusion criteria for the active phase in the Canadian study required ALT levels to exceed 80 U/mL. Consequently, the cumulative incidence of transition in the Canadian study was lower than in ours. We noted that the immune-tolerant group exhibited higher levels of HBsAg, HBeAg, and HBV DNA. As immune-tolerant patients transitioned to the immune-active phase, these levels decreased, particularly for HBeAg and HBV DNA. This suggests that a reduction in HBV replication is more pronounced during the transition from the immune-tolerant to the immune-active phase. Cumulative HBsAg, cumulative HBeAg, cumulative HBV DNA, and cumulative HBsAg/HBV DNA showed significant differences in the duration of immune tolerance at an early stage. Notably, greater cumulative levels of HBsAg, HBeAg, HBV DNA, and the HBsAg/HBV DNA ratio were associated with a longer duration before transitioning from the immune-tolerant to the active phase. Additionally, our study found a non-linear relationship between cumulative HBsAg, cumulative HBV DNA, and the cumulative HBsAg/HBV DNA ratio with disease progression during immune tolerance. Threshold effect analysis revealed that the risk of disease progression decreased rapidly with increasing cumulative HBsAg and cumulative HBV DNA when these values were below 9.55 and 10.99, respectively, but slowed when cumulative HBsAg and cumulative HBV DNA exceeded these thresholds. Similarly, when the cumulative HBsAg/HBV DNA ratio was below 1.791, the risk of disease progression decreased rapidly as the ratio increased; however, the risk stabilized when the ratio was above this threshold. The AUC for predicting progression from immune tolerance to the immunologically active stage reached 0.85 for the cumulative HBsAg/HBV DNA ratio, which was greater than the AUCs for cumulative HBsAg (0.67) and cumulative HBV DNA (0.64). Multivariate Cox regression analysis identified cumulative HBsAg/HBV DNA as an independent factor affecting the duration of immune tolerance. The risk of disease progression was 42-, 28-, and five-fold higher for cumulative HBsAg/HBV DNA ratios of <0.6, between 0.6 and

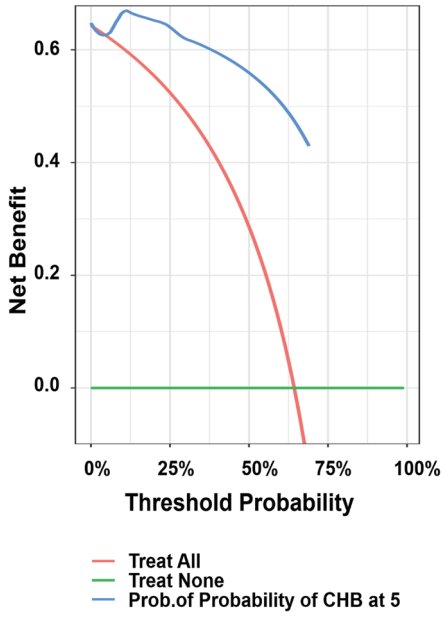

Fig. 5. Decision curve analysis of the model. CHB, chronic hepatitis B.

1.1, and between 1.1 and 1.9, respectively, compared to a ratio ≥ 1.9. This suggests that the lower the cumulative HBsAg/HBV DNA ratio, the greater the risk of disease progression. After HBV infection, humoral and cellular immune responses lead to significant hepatocyte damage and necrosis. Due to differences in the synthesis pathways of HBsAg and HBV DNA, the HBsAg/HBV DNA ratio gradually decreases as the disease progresses. Cumulative HBsAg/HBV DNA can provide a better assessment of the risk of progression from the immune-tolerant to the immune-active phase, thus aiding in understanding disease progression in CHB patients. Finally, our model construction and validation suggest that this predictive model may hold clinical significance. In clinical practice, the probability of hepatitis B immune tolerance can be roughly determined, allowing for regular monitoring, timely antiviral treatment, and reduced unnecessary costs and risks.

It is worth noting that subgroup analysis found individuals under 30 years of age had lower levels of ALT, AST, and GGT, and higher levels of HBeAg and HBV DNA. This suggests that HBV carriers younger than 30 have lower hepatic inflammation and higher hepatic anabolic and metabolic functions. Results in Supplementary Table 6 indicate that ALT levels were significantly different between those aged ≥30 and those <30 years, suggesting that patients aged ≥30 have a higher risk of disease progression. These findings support age-based risk stratification in managing HBV infection. Specifically, closer monitoring may be warranted for patients aged 30 and older, who may require more frequent assessments and potentially more aggressive therapeutic interventions compared to younger patients, to mitigate the risk of liver disease progression.

Our study has limitations. First, it is a single-center retrospective study. Although the cumulative HBsAg/HBV DNA ratio suggests a strong correlation with hepatitis B immune tolerance duration in this study, further research in other regions or prospective studies is needed to validate these findings. Second, there may be potential selection bias in recruiting study subjects in the immune-tolerant phase, which could impact the results and needs further verification. Third, given the decade-long follow-up, systematic re-evaluation of alternative causes of elevated ALT (e.g., alcohol use) would strengthen the attribution of causality. However, we were unable to identify alternative causes due to unavailable data.

Conclusions

This study identifies the cumulative HBsAg/HBV DNA ratio as an independent predictor of immune tolerance duration in patients with chronic HBV infection. A lower ratio correlates with an increased risk of transition from the immune-tolerant phase to the immune-active phase, particularly when it falls below 1.791, at which point the risk of disease progression increases sharply. Additionally, patients aged ≥30 years face a notably higher risk of disease progression, underscoring the need for vigilant monitoring in this population. These findings have important implications for the proactive management of immune-tolerant patients. Early detection of disease progression and timely antiviral treatment may ultimately improve clinical outcomes and societal benefits for individuals suffering from chronic HBV infection.

Funding

This study was supported by the Fujian Provincial Health Technology Project (No. 2023CXA023) and the Fujian Province Science and Technology Plan Project of China (No. 2023Y0015).

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Material preparation, data collection and analysis (DZ, YH, SL, QZ, JZ), and drafting of the manuscript (DZ). All authors contributed to the study conception and design, and commented on previous versions of the manuscript. All authors read and approved the final version and publication of the manuscript.

Ethical statement

The study was conducted in accordance with the Declarations of Helsinki (as revised in 2024) and Istanbul and was approved by the Institutional Review Board of Fujian Medical University (approval number: MTCA, ECFAH of FMU [2015] 084-3). The written informed consent from participants was waived for the retrospective study.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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