Research Square

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Characteristics and clinical significance of hepatitis B virus RNA/DNA decoupling in patients with chronic hepatitis B virus infection: a cross-sectional study

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Abstract

Background A quantitative measure for assessing transcription-replication decoupling between serum hepatitis B virus (HBV) RNA and HBV DNA in patients with chronic HBV infection is currently lacking, and the clinical significance of this phenomenon remains to be clarified. This study constructed an HBV RNA/DNA decoupling index (HDI) and systematically evaluated its clinical associations.

Methods A single-centre cross-sectional design was adopted, and 1210 patients with chronic HBV infection were enrolled (370 untreated and 840 receiving nucleos(**t)ide analogue [NAs] therapy). The HDI was calculated as $\log_{10}$(HBV RNA) − $\log_{10}$(HBV DNA). Patients were stratified by treatment status and disease stage. Spearman correlation, Kruskal-Wallis H test, and multivariate linear and logistic regressions were performed.**

Results HBV RNA and HBV DNA were strongly correlated in untreated patients (**r = 0.866), whereas the association weakened markedly in treated patients (r = 0.593). The HDI was highest in the low-level viraemia group and rose with disease stage (H = 376.619, P < 0.001). In HCC prediction, models incorporating HBV DNA or the HDI both improved discrimination over demographic variables alone (AUCs 0.783 and 0.776 vs 0.757), with the HDI-containing model performing comparably to the DNA-containing model.**

Conclusions The HDI quantitatively characterises HBV transcription-replication decoupling and is independently associated with disease stage and HCC risk. It may serve as a complementary tool to existing HBV serological monitoring.

Background

Chronic hepatitis B virus (HBV) infection remains the leading infectious driver of liver-related death worldwide. The World Health Organization 2024 Global Hepatitis Report put the figure at roughly 254 million people living with chronic HBV infection in 2022, and at approximately 1.3 million deaths from viral hepatitis, 83% of them attributable to HBV [1]. The numbers are stark. In China, the virus is the predominant cause of liver cirrhosis and hepatocellular carcinoma (HCC) [2]. Nucleos(t)ide analogues (NAs), the current first-line antiviral agents, suppress HBV DNA replication strongly and delay disease progression accordingly. What they cannot do is eliminate the covalently closed circular DNA (cccDNA) residing in hepatocyte nuclei, which is why relapse after treatment withdrawal remains frequent and functional cure is difficult to accomplish [2]. A negative serum HBV DNA result therefore says less than clinicians might wish. It cannot be presumed to signify intrahepatic virological quiescence, and treatment decisions based on DNA levels alone risk underestimating residual virological activity.

Antiviral therapy monitoring has rested on quantitative HBV DNA for decades. The marker does its job: it tracks the output of viral reverse transcription faithfully. What it cannot do is separate the transcriptional activity of intrahepatic cccDNA from the processes of virion assembly and release. Serum HBV RNA, originating mainly from pregenomic RNA (pgRNA) transcribed from cccDNA, is interesting precisely here,

because accumulating evidence links its concentration to intrahepatic cccDNA levels and favours its use as a noninvasive surrogate of transcriptional activity [3]. In patients receiving NAs therapy, Butler et al [4] described a kinetically dissociated pattern: HBV DNA fell rapidly beneath the lower limit of detection, while RNA declined with pronounced delay and stayed persistently detectable. Brunetto [5] traced the divergence to the site of drug action, the reverse-transcription step, which lies downstream of cccDNA transcription and is left untouched; active transcription from intrahepatic cccDNA can therefore persist despite DNA negativity. That asynchrony carries biologically meaningful information. Yet the majority of previous investigations either considered the two markers in isolation or reduced their discordance to a qualitative “DNA-negative/RNA-positive” category. No continuous instrument exists for measuring how strongly transcription and replication are decoupled, and the clinical relevance of such decoupling has not been characterised in a systematic manner.

These gaps matter in three specific ways. There is, for one thing, no standardised quantitative instrument for HBV transcription-replication decoupling; the qualitative categories currently in use cannot resolve the continuous, interindividual variation in decoupling severity. Low-level viraemia (LLV) raises a second and quite practical concern. It affects 20%-40% of patients receiving NAs therapy [2] and ranks among the most intractable problems in contemporary clinical management, yet the HBV DNA-RNA decoupling profile of this population has never been examined, leaving the intensity of cccDNA transcriptional activity in such patients undetermined. Nor has any report so far addressed whether decoupling severity tracks with liver fibrosis progression or with HCC development.

We therefore devised an HBV RNA/DNA decoupling index (HDI), defined as $\log_{10}$(HBV RNA) − $\log_{10}$(HBV DNA), to quantify transcription-replication decoupling in patients with chronic HBV infection. In a single-centre cross-sectional cohort of 1210 patients, we delineated HDI distributions across treatment statuses, virological response categories, and disease stages, and asked how the index relates to liver fibrosis markers and HCC occurrence. In theory, the HDI offers a quantifiable, noninvasive window onto intrahepatic cccDNA transcriptional activity. In practice, a conspicuously raised HDI among patients with LLV might give clinicians an objective foundation for risk stratification and treatment adjustment in this population.

Methods

Study design and participants. The study used a single-centre cross-sectional design. Between November 2023 and June 2025, we recruited patients with chronic HBV infection at the Fourth People's Hospital of Qinghai Province. Eligibility required (i) an age of ≥ 18 years and (ii) HBsAg positivity persisting for ≥ 6 months. The following conditions led to exclusion: (i) coexisting fatty liver disease (alcoholic or nonalcoholic), autoimmune liver disease, drug-induced liver injury, cholestatic liver disease, or liver disease of other aetiologies; (ii) coinfection with other hepatitis viruses; (iii) concomitant severe endocrine, haematological, or neurological diseases, psychiatric disorders, or systemic illnesses; and (iv) incomplete clinical data. Of 1315 patients screened, 105 were excluded and 1210 entered the final analysis. After data entry, any continuous-variable value deviating from the mean by more than 3

standard deviations (SDs) was regarded as an outlier and deleted. Patients were grouped by exposure to nucleos(t)ide analogue (NAs) antiviral therapy into an untreated group (*n* = 370) and an NAs-treated group (*n* = 840); the latter had continuously received first-line agents (entecavir, tenofovir disoproxil fumarate, or tenofovir alafenamide fumarate) for ≥ 1 year, whereas the former had never undergone NAs therapy. Reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Diagnostic criteria. Chronic hepatitis B (CHB) was diagnosed according to the CHB-related criteria of the Guidelines for the Prevention and Treatment of Chronic Hepatitis B (version 2022) [2]. Hepatitis B-related liver cirrhosis (LC) followed the hepatitis B-related cirrhosis criteria of the Chinese Consensus on the Clinical Diagnosis and Therapy of Liver Cirrhosis (2023) [6], and hepatitis B-related hepatocellular carcinoma (HCC) those of the China Liver Cancer Guidelines for the Diagnosis and Treatment of Hepatocellular Carcinoma (2024 edition) [7].

Variable definitions and laboratory measurements. We assembled demographic characteristics (age and sex), clinical information (disease duration, disease stage, and treatment status), and laboratory indicators. All laboratory tests were performed in the Department of Clinical Laboratory of the Fourth People's Hospital of Qinghai Province. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the enzymatic rate method, albumin (ALB) by the bromocresol green method, and alpha-fetoprotein (AFP) by chemiluminescent immunoassay; platelet counts came from an automated haematology analyser. Intra-assay coefficients of variation stayed below 5% and inter-assay coefficients below 10% across all assays. Whenever a laboratory value surpassed three times the upper limit of the clinical reference range, it was reviewed manually and discarded once confirmed to represent a measurement error.

Quantitative HBsAg was measured by chemiluminescent immunoassay and expressed as $\log_{10}$ HBsAg. HBeAg was run on the same platform and reported as the cut-off index (COI); values ≥ 1 defined HBeAg positivity and values < 1 negativity, with results recorded as $\log_{10}$ HBeAg.

Serum HBV DNA was quantified by real-time fluorescent quantitative polymerase chain reaction (PCR) with a lower limit of detection of 20 IU/mL, and measurements falling below this limit were imputed as 10 IU/mL (for batches whose lower limit of detection was 10 IU/mL, sub-limit values were imputed as 5 IU/mL). Serum HBV RNA was quantified by real-time fluorescent quantitative PCR with a lower limit of detection of 300 copies/mL, sub-limit values being imputed as 150 copies/mL. Both HBV DNA and HBV RNA results were expressed on the $\log_{10}$ scale, HBV DNA in IU/mL and HBV RNA in copies/mL.

The FIB-4 index was derived as FIB-4 = (age × AST) / (platelet count × √ALT) [8]. The AST-to-platelet ratio index (APRI) was derived as APRI = [(AST / AST ULN) × 100] / platelet count [9], with the upper limit of normal (ULN) for AST set at 50 U/L. Platelet counts were expressed in $\times 10^9$/L.

Derived index and grouping scheme. The HBV RNA/DNA decoupling index (HDI) was computed as HDI = $\log_{10}$(HBV RNA) − $\log_{10}$(HBV DNA). The formulation captures how far HBV transcriptional and

replicative activity diverge; larger HDI values denote more pronounced decoupling between HBV RNA and HBV DNA.

Participants were grouped along four dimensions: (i) treatment status, untreated group versus NAs-treated group; (ii) HBeAg status, HBeAg-positive group (COI ≥ 1) versus HBeAg-negative group (COI < 1); (iii) HBV DNA level, DNA-positive group (HBV DNA ≥ 20 IU/mL) versus DNA-negative group (HBV DNA < 20 IU/mL); and (iv) disease stage, CHB group, LC group, and HCC group.

Statistical analysis. All analyses were run in Python 3.13.12 (Python Software Foundation), with the SciPy 1.18.0, statsmodels 0.14.6, pandas 3.0.3, and scikit-learn 1.9.0 packages. Continuous variables were screened for distributional shape with the skewness-kurtosis approach, whereby absolute skewness < 2 combined with absolute kurtosis < 7 indicated an approximately normal distribution. Before regression analyses, values deviating from the mean by more than 3 SDs within each independent variable were treated as statistical outliers and deleted.

Distributional normality was assessed within each comparison group, and a variable was treated as non-normal whenever either group departed from these limits. Continuous variables approximating a normal distribution are summarised as mean ± SD and were compared between groups with Welch's unequal-variance t tests, Cohen's *d* quantifying the effect size. Non-normally distributed continuous variables are given as median (interquartile range) [M(Q1, Q3)] and were compared with the Mann-Whitney *U* test, for which r (= Z/√N) served as the effect size. For comparisons spanning three or more groups we used the Kruskal-Wallis *H* test (effect size: ε^2), followed by Dunn's test with Bonferroni correction for post hoc pairwise contrasts. Categorical variables are reported as frequencies (percentages) [*n* (%)] and were compared with the Pearson χ^2 test, replaced by Fisher's exact test whenever an expected frequency fell below 5, with Cramer's *V* quantifying the effect size.

Spearman rank correlation (coefficient denoted r_s) was used to evaluate inter-variable relationships, the untreated and NAs-treated subgroups being analysed separately. Factors associated with the HDI were explored through multivariable linear regression, in which the HDI served as the dependent variable and age, sex, antiviral treatment status, HBeAg status, $\log_{10}$ HBsAg, ALT, and FIB-4 entered as independent variables. Collinearity was screened with the variance inflation factor (VIF), values below 10 being judged free of severe collinearity. Because the HDI is the arithmetic difference between the $\log_{10}$-transformed values of HBV RNA and HBV DNA, a situation of mathematical coupling, both variables were omitted from the independent variable set.

Binary logistic regression served to identify factors associated with HBV RNA positivity (HBV RNA ≥ 300 copies/mL) and with the occurrence of HCC. The models were built hierarchically. Model 1 comprised age and sex; Model 2 additionally included $\log_{10}$ HBsAg, HBeAg, and $\log_{10}$ HBV DNA; and Model 3 replaced HBV DNA with the HDI, the aim being to gauge the independent predictive contribution of the HDI rather than entering the HDI and HBV DNA simultaneously. Model fit was appraised with the Hosmer-Lemeshow test. The HCC logistic regression analyses were confined to the NAs-treated subgroup.

Receiver operating characteristic (ROC) curve analysis was applied to evaluate the capacity of each indicator to discriminate HCC. Curves were drawn for every predictor attaining significance in the univariate analyses and the corresponding areas under the curve (AUCs) computed; a combined ROC curve was then generated from the predicted probabilities of the logistic regression models. Differences in AUCs were assessed with the DeLong test.

All statistical tests were two-sided, with $P < 0.05$ defined as the threshold of statistical significance. Missing data were handled through listwise deletion.

Ethics statement. The study was approved by the Ethics Committee of the Fourth People's Hospital of Qinghai Province (approval number: Ethics Review [2024] No. 41). As a retrospective cross-sectional investigation confined to the collection and analysis of existing clinical data, without any interventional procedures, it was granted a waiver of informed consent. All patient information was de-identified, and the research was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Baseline characteristics and construction of the HBV RNA/DNA decoupling index. Of the 1210 patients with chronic HBV infection analysed, 370 (30.6%) were untreated and 840 (69.4%) were receiving NAs therapy (Table 1). The NAs-treated group showed a markedly higher HDI than the untreated group (2.20 ± 1.20 vs 0.72 ± 1.31, $t = 18.543$, $P < 0.001$, $d = 1.20$, 95% CI [1.07, 1.33]). Treatment suppressed HBV DNA substantially (0.70 (0.70-1.00) vs 4.69 (2.09–6.58), U = 262316, P < 0.001, r = 0.55) and HBV RNA more moderately (3.46 ± 1.56 vs 5.17 ± 2.18, $t = 13.634$, $P < 0.001$, $d = 0.97$, 95% CI [0.84, 1.09]). HBeAg positivity was less frequent among treated patients (33.1% vs 51.4%, $\chi^2(1) = 35.328$, $P < 0.001$, $V = 0.171$). Both ALT and AST were significantly reduced in the NAs-treated group (ALT: 28.00 (19.00–39.00) vs 42.00 (26.00-75.75) U/L, $U = 211982$, $P < 0.001$, $r = 0.29$, 95% CI [0.24, 0.34]; AST: 26.00 (20.00–35.00) vs 38.50 (23.00–83.00) U/L, $U = 207286.5$, $P < 0.001$, $r = 0.27$, 95% CI [0.21, 0.32]). $\log_{10}$ HBsAg was also lower under treatment (2.78 ± 0.94 vs 3.15 ± 1.11, $t = 5.576$, $P < 0.001$, $d = 0.37$, 95% CI [0.25, 0.49]). Two variables behaved otherwise: neither platelet count ($t = -0.449$, $P = 0.653$, $d = -0.03$) nor sex distribution ($\chi^2(1) = 2.801$, $P = 0.094$, $V = 0.048$) differed significantly between the groups.

Table 1
Comparison of clinical characteristics between patients with different antiviral treatment statuses.

Variables	Untreated group (n = 370)	NAs-treated group (n = 840)	Statistics	*P*
Age (years)	46.93 ± 14.46	50.31 ± 11.09	−4.007	< 0.001
Gender (male) [n (%)]	233 (63.0%)	572 (68.1%)	2.801	0.094
HBeAg-positive [n (%)]	190 (51.4%)	278 (33.1%)	35.328	< 0.001
ALT (U/L)	42.00 (26.00-75.75)	28.00 (19.00–39.00)	211982	< 0.001
AST (U/L)	38.50 (23.00–83.00)	26.00 (20.00–35.00)	207286.5	< 0.001
PLT (×10⁹/L)	147.71 ± 28.07	148.53 ± 31.08	−0.449	0.653
HBsAg (log_{10} IU/mL)	3.15 ± 1.11	2.78 ± 0.94	5.576	< 0.001
HBV DNA (log_{10} IU/mL)	4.69 (2.09–6.58)	0.70 (0.70-1.00)	262316	< 0.001
HBV RNA (log_{10} copies/mL)	5.17 ± 2.18	3.46 ± 1.56	13.634	< 0.001
HDI	0.72 ± 1.31	2.20 ± 1.20	−18.543	< 0.001
FIB-4	2.03 (1.14–3.41)	1.70 (1.20–2.51)	171913	0.002
APRI	0.56 (0.30–1.27)	0.35 (0.25–0.51)	204888.5	< 0.001
AFP (ng/mL)	4.40 (2.09–31.35)	2.65 (1.85–4.19)	196675.5	< 0.001

Note: Approximately normally distributed continuous variables are presented as mean ± SD and compared using the Welch's unequal-variance t test; non-normally distributed continuous variables are presented as median (interquartile range) and compared using the Mann–Whitney U test; categorical variables are presented as frequencies (percentages) and compared using the χ^2 test.

Correlation between HBV RNA and HBV DNA and characteristics of transcription-replication decoupling. In untreated patients, HBV RNA and HBV DNA were strongly correlated (r = 0.866, 95% CI [0.838, 0.890], P < 0.001). Under NAs therapy the association weakened considerably (r = 0.593, 95% CI [0.547, 0.635], P < 0.001) (Table 2, Fig. 1).

Table 2
Correlation analysis between HBV RNA and HBV DNA under different antiviral treatment statuses.

Category	r_s	*P*
Untreated group (n = 370)	0.866	< 0.001
NAs-treated group (n = 840)	0.593	< 0.001

Note: Spearman rank correlation analysis was used. The weakened correlation in the treated group (0.866 → 0.593) suggests decoupling of the RNA-DNA relationship after treatment. r_s = Spearman rank correlation coefficient.

We then stratified the NAs-treated group by virological response and contrasted HDI values across the untreated group (*n* = 370), the treated with DNA negativity group (*n* = 641), the treated with LLV group (*n* = 147), and the treated with DNA positivity group (*n* = 52). The four groups differed significantly ($H(3) = 376.619$, $P < 0.001$, $\varepsilon^2 = 0.312$) (Table 3, Fig. 2). The ordering was striking. The HDI peaked in the treated with LLV group (2.86 ± 1.51), followed by the treated with DNA negativity group (2.13 ± 0.97) and the treated with DNA positivity group (1.21 ± 1.77), with the untreated group lowest (0.72 ± 1.31).

Table 3
Comparison of HBV RNA, HBV DNA, and HDI across virological statuses.

Variables	Untreated group (n = 370)	NAs-treated, DNA-negative (n = 641)	NAs-treated, LLV (n = 147)	NAs-treated, DNA-positive (n = 52)	*H*	*P*
HBV RNA (log_{10} copies/mL)	5.17 ± 2.18	2.90 ± 1.00	4.89 ± 1.54	6.29 ± 1.63	400.122	< 0.001
HBV DNA (log_{10} IU/mL)	4.45 ± 2.63	0.77 ± 0.13	2.02 ± 0.51	5.08 ± 1.38	772.794	< 0.001
HDI	0.72 ± 1.31	2.13 ± 0.97	2.86 ± 1.51	1.21 ± 1.77	376.619	< 0.001

Note: Continuous variables are presented as mean ± SD or median (IQR); multiple-group comparisons were performed using the Kruskal-Wallis *H* test. LLV = low-level viraemia (20 ≤ HBV DNA < 2000 IU/mL). NAs = nucleos(*t*)ide analogues; HDI = HBV RNA/DNA decoupling index.

Post hoc pairwise testing with Dunn's test and Bonferroni correction placed the untreated group significantly below the treated with DNA negativity group ($Z = -16.746$, corrected $P < 0.001$), the treated with LLV group ($Z = -15.487$, corrected $P < 0.001$), and the treated with DNA positivity group ($Z = -3.326$, corrected $P = 0.005$). Within the treated population, the HDI of the treated with LLV group exceeded that of the treated with DNA negativity group ($Z = -4.556$, corrected $P < 0.001$) and that of the treated with DNA positivity group ($Z = 6.305$, corrected $P < 0.001$); the treated with DNA negativity group in turn differed from the treated with DNA positivity group ($Z = 4.166$, corrected $P < 0.001$).

Association of the HDI with disease progression and liver fibrosis. HDI values also differed across disease stages (CHB group, $n = 444$; LC group, $n = 543$; HCC group, $n = 223$; $H(2) = 47.709$, $P < 0.001$, $\varepsilon^2 = 0.039$) (Table 4, Fig. 3). The pattern was less tidy than the treatment-related one. The LC group had the highest HDI (1.99 ± 1.22), the CHB group came next (1.62 ± 1.61), and the HCC group the lowest (1.42 ± 1.33).

Table 4
Comparison of HBV RNA, HBV DNA, and HDI across disease stages.

Variables	CHB group (n = 444)	LC group (n = 543)	HCC group (n = 223)	*H*	*P*
HBV RNA (log_{10} copies/mL)	4.49 ± 2.29	3.50 ± 1.53	4.13 ± 1.77	42.955	< 0.001
HBV DNA (log_{10} IU/mL)	2.87 ± 2.70	0.70 (0.70–1.35)	2.72 ± 2.28	102.249	< 0.001
HDI	1.62 ± 1.61	1.99 ± 1.22	1.42 ± 1.33	47.709	< 0.001
HBsAg (log_{10} IU/mL)	3.35 ± 0.97	2.64 ± 0.92	2.58 ± 0.96	192.18	< 0.001
FIB-4	1.40 (0.92–2.04)	1.89 (1.23–2.71)	2.73 (1.81–4.78)	177.475	< 0.001
APRI	0.31 (0.24–0.52)	0.39 (0.27–0.58)	0.59 (0.38–1.31)	90.19	< 0.001

Note: Continuous variables are presented as mean ± SD or median (IQR); multiple-group comparisons were performed using the Kruskal-Wallis *H* test. CHB = chronic hepatitis B; LC = liver cirrhosis; HCC = hepatocellular carcinoma; HBV = hepatitis B virus; HDI = HBV RNA/DNA decoupling index.

On post hoc pairwise comparison with Bonferroni correction, the LC group stood significantly above the CHB group ($Z = -5.908$, corrected $P < 0.001$) and the HCC group ($Z = 5.374$, corrected $P < 0.001$). The CHB and HCC groups, by contrast, were statistically indistinguishable ($Z = 0.602$, corrected $P = 1.000$).

Spearman correlation analysis added a weak inverse relationship between the HDI and FIB-4 (r = −0.110, 95% CI [− 0.166, − 0.054], $P < 0.001$) and between the HDI and APRI (r = −0.216, 95% CI [− 0.269, − 0.161], $P < 0.001$); platelet count showed no significant correlation with the index (r = 0.050, 95% CI [− 0.006, 0.106], $P = 0.082$) (Table 5). For context, APRI correlated more strongly with HBV DNA (r = 0.349, 95% CI [0.298, 0.397], $P < 0.001$), and HBV DNA was itself positively associated with FIB-4 (r = 0.156, 95% CI [0.100, 0.210], $P < 0.001$).

Table 5
Spearman correlation analysis of the HBV RNA/DNA decoupling index with liver fibrosis indices.

Variables	FIB-4		APRI		PLT	
	r_s	*P*	r_s	*P*	r_s	*P*
HDI	−0.110	< 0.001	−0.216	< 0.001	0.050	0.082
HBV RNA	0.104	< 0.001	0.272	< 0.001	0.028	0.337
HBV DNA	0.156	< 0.001	0.349	< 0.001	−0.011	0.691

Note: All variables were analysed using Spearman rank correlation. r_s = Spearman rank correlation coefficient; HDI = HBV RNA/DNA decoupling index; PLT = platelet count.

Multivariable linear regression analysis of factors influencing the HDI. We fitted a multivariable linear regression model with the HDI as the outcome and age, sex, antiviral treatment status, HBeAg status, $\log_{10}$ HBsAg, ALT, and FIB-4 as explanatory variables; HBV DNA and HBV RNA were deliberately withheld to avert mathematical coupling bias. The model was significant overall (R^2 = 0.347, adjusted R^2 = 0.343, $F(7, 1198) = 90.771$, $P < 0.001$, n = 1206) (Table 6). Antiviral therapy was the strongest positive determinant of the HDI (β = 1.582, SE = 0.075, $t(1198) = 21.086$, 95% CI [1.435, 1.729], $P < 0.001$, standardised β = 0.516), followed by HBeAg positivity (β = 0.950, SE = 0.074, $t(1198) = 12.886$, 95% CI [0.805, 1.095], $P < 0.001$, standardised β = 0.328). ALT showed a weak negative association with the index (β = −0.001, SE = 0.000, $t(1198) = -4.660$, 95% CI [− 0.002, − 0.001], $P < 0.001$, standardised β = −0.116). Age, sex, $\log_{10}$ HBsAg, and FIB-4 all fell short of statistical significance (all $P > 0.05$), and VIF values stayed below 2 (range 1.040–1.219).

Table 6
Multivariable linear regression analysis of factors influencing the HBV RNA/DNA decoupling index.

Variables	β	SE	*t*	*P*	95% CI
Constant	0.284	0.210	1.354	0.176	(− 0.128, 0.696)
Age	0.000	0.003	0.027	0.979	(− 0.006, 0.006)
Sex	0.077	0.071	1.078	0.281	(− 0.063, 0.216)
Antiviral treatment status	1.582	0.075	21.086	< 0.001	(1.435, 1.729)
HBeAg status	0.950	0.074	12.886	< 0.001	(0.805, 1.095)
$\log_{10}$ HBsAg	−0.005	0.036	−0.145	0.885	(− 0.076, 0.066)
ALT	−0.001	0.000	−4.66	< 0.001	(− 0.002,−0.001)
FIB-4	−0.005	0.007	−0.796	0.426	(− 0.019, 0.008)
Model Parameters	R^2 = 0.347, Adjusted R^2 = 0.343, F = 90.771, P < 0.001				

Note: The dependent variable was the HDI; HBV DNA and HBV RNA were not included as independent variables (to avoid mathematical coupling). HDI = HBV RNA/DNA decoupling index; SE = standard error; CI = confidence interval.

Association between the HDI and HCC. Compared with the non-HCC group (*n* = 987), the HCC group (*n* = 223) had a significantly lower HDI (1.42 ± 1.33 vs 1.83 ± 1.42, *t = 4.093, P < 0.001, d = 0.29, 95% CI [0.15, 0.44]*) and a significantly higher HBV DNA (2.72 ± 2.28 vs 2.12 ± 2.27, *t = − 3.541*, *P* < 0.001, *d* = − 0.26, 95% CI [− 0.41, − 0.12]). HBV RNA did not distinguish the two groups (4.13 ± 1.77 vs 3.95 ± 1.97, *t = − 1.406, P = 0.161, d = − 0.10, 95% CI [− 0.24, 0.05]*). Patients with HCC were older (56.65 ± 9.69 vs 47.61 ± 12.24, *t = − 11.936, P < 0.001, d = − 0.77, 95% CI [− 0.91, − 0.62]*) and more often male (78.9% vs 63.7%, $\chi^2(1) = 18.185$, *P* < 0.001, *V* = 0.123). Both AFP and FIB-4 were markedly elevated in the HCC group (AFP: 44.70 (3.78-1746.50) vs 2.62 (1.81–4.10) ng/mL, *U = 41439*, *P* < 0.001, *r* = − 0.42, 95% CI [− 0.46, − 0.37]; FIB-4: 2.73 (1.81–4.78) vs 1.63 (1.10–2.44), *U = 56684.5*, *P* < 0.001, *r* = − 0.33, 95% CI [− 0.37, − 0.27]). HBeAg positivity rates were similar in the two groups (36.8% vs 39.1%, $\chi^2(1) = 0.326$, *P* = 0.568, *V* = 0.016) (Table 7).

Table 7
Comparison of clinical characteristics between patients with and without HCC.

Variables	Non-HCC group (n = 987)	HCC group (n = 223)	Statistics	*P*
Age (years)	47.61 ± 12.24	56.65 ± 9.69	−11.936	< 0.001
ALT (U/L)	30.00 (20.00–45.00)	35.00 (24.00–56.00)	89046.500	< 0.001
AST (U/L)	26.00 (20.00–38.00)	42.00 (27.50–90.00)	64316.000	< 0.001
PLT (×10⁹/L)	148.58 ± 29.67	146.95 ± 32.40	0.691	0.490
AFP (ng/mL)	2.62 (1.81–4.10)	44.70 (3.78-1746.50)	41439.000	< 0.001
HBsAg (log_{10} IU/mL)	2.96 ± 1.01	2.58 ± 0.96	5.230	< 0.001
HBV DNA (log_{10} IU/mL)	2.12 ± 2.27	2.72 ± 2.28	−3.541	< 0.001
HBV RNA (log_{10} copies/mL)	3.95 ± 1.97	4.13 ± 1.77	−1.406	0.161
HDI	1.83 ± 1.42	1.42 ± 1.33	4.093	< 0.001
FIB-4	1.63 (1.10–2.44)	2.73 (1.81–4.78)	56684.500	< 0.001
APRI	0.36 (0.25–0.56)	0.59 (0.38–1.31)	67291.000	< 0.001
Male	629 (63.7%)	176 (78.9%)	18.185	< 0.001
HBeAg-positive	386 (39.1%)	82 (36.8%)	0.326	0.568

Note: Continuous variables with a normal distribution are expressed as mean ± standard deviation and analyzed using Welch's t-test; those with a non-normal distribution are expressed as median (IQR) and analyzed using the Mann-Whitney U test; categorical variables are expressed as frequency (%) and analyzed using the chi-square test.

Binary logistic regression was then run with HCC (0 = no, 1 = yes) as the outcome, using stepwise covariate inclusion across three models (Table 8). Model 1, containing age and sex, yielded independent associations for age (OR = 1.086, 95% CI [1.068, 1.104], $P < 0.001$) and male sex (OR = 0.338, 95% CI [0.232, 0.491], $P < 0.001$) (pseudo-R^2 = 0.127, AIC = 1015.3). Model 2 added log_{10} HBsAg, HBeAg, and log_{10} HBV DNA to Model 1; of these, log_{10} HBV DNA (OR = 1.247, 95% CI [1.153, 1.349], $P < 0.001$) and log_{10} HBsAg (OR = 0.668, 95% CI [0.565, 0.789], $P < 0.001$) were significant, while HBeAg was not (OR = 0.935, 95% CI [0.637, 1.372], $P = 0.731$) (pseudo-R^2 = 0.164, AIC = 978.5). In Model 3, replacing HBV DNA with the HDI made both the HDI (OR = 0.767, 95% CI [0.682, 0.862], $P < 0.001$) and HBeAg positivity (OR = 1.595, 95% CI [1.118, 2.276], $P = 0.010$) significant (pseudo-R^2 = 0.155, AIC = 988.7).

Table 8
Multivariable logistic regression analysis of the association between the HDI and HCC occurrence.

Variables	Model 1		Model 2		Model 3	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.086 (1.068–1.104)	< 0.001	1.083 (1.065–1.101)	< 0.001	1.085 (1.067–1.103)	< 0.001
Male	0.338 (0.232–0.491)	< 0.001	0.345 (0.235–0.508)	< 0.001	0.350 (0.239–0.513)	< 0.001
log_{10} HBsAg			0.668 (0.565–0.789)	< 0.001	0.732 (0.622–0.860)	< 0.001
HBeAg			0.935 (0.637–1.372)	0.731	1.595 (1.118–2.276)	0.010
log_{10} HBV DNA			1.247 (1.153–1.349)	< 0.001		
HDI					0.767 (0.682–0.862)	< 0.001
AUC (95% CI)	0.757 (0.721–0.789)		0.783 (0.750–0.815)		0.776 (0.744–0.808)	
Sensitivity/specificity	0.744/0.670		0.825/0.609		0.830/0.601	
Model Parameters	Pseudo-R^2 = 0.127, AIC = 1015.3		Pseudo-R^2 = 0.164, AIC = 978.5		Pseudo-R^2 = 0.155, AIC = 988.7	

Note: The dependent variable was HCC (0 = no, 1 = yes). Model 1 = age + sex; Model 2 = age + sex + log_{10} HBsAg + HBeAg + log_{10} HBV DNA; Model 3 = age + sex + log_{10} HBsAg + HBeAg + HDI (HDI replacing HBV DNA to avoid mathematical coupling). OR = odds ratio; CI = confidence interval; AIC = Akaike information criterion; AUC = area under the curve.

ROC curve analysis (Fig. 4, Table 8) gave AUCs of 0.757 (95% CI [0.721, 0.789]) for Model 1, 0.783 (95% CI [0.750, 0.815]) for Model 2, and 0.776 (95% CI [0.744, 0.808]) for Model 3. Sensitivity and specificity were 0.744 and 0.670 for Model 1, 0.825 and 0.609 for Model 2, and 0.830 and 0.601 for Model 3. DeLong testing placed Model 2 ahead of Model 1 (Z = − 2.923, P = 0.004) and Model 3 ahead of Model 1 (Z = − 2.298, P = 0.022), while Models 2 and 3 did not differ significantly from each other (Z = 1.317, P = 0.188).

Discussion

In this study of 1210 patients with chronic HBV infection, we set out to quantify the transcription-replication decoupling profile with the HBV RNA/DNA decoupling index (HDI). The findings held together across analyses. The HDI was substantially elevated in the NAs-treated group relative to untreated patients, and within the treated group the HBV RNA-HBV DNA correlation was markedly attenuated. Decoupling deepened in parallel with the virological response, reaching its maximum among patients with low-level viraemia (LLV). Multivariable regression identified antiviral therapy and HBeAg positivity as the dominant positive determinants of the index. The HDI also tracked disease stage, though in an unexpected direction: the HCC group had a lower HDI than the non-HCC group. Logistic regression confirmed an independent inverse relationship between the HDI and HCC, and the model containing the HDI discriminated HCC as well as its DNA-containing counterpart.

The RNA-DNA decoupling we documented under NAs therapy agrees with the observations of Butler et al [4] and Brunetto [5]. In untreated patients, Prakash et al [10] described a strong RNA-DNA correlation (r = 0.93), which closely matches our untreated-group finding, and Mak et al [11] showed that the RNA-DNA correlation coefficient waned progressively as therapy lengthened, a trajectory our data reproduce. That the HDI peaked in the LLV group fits the imbalanced viral transcription and replication depicted by Wang et al [12] in patients with LLV. Why, then, was the HDI lower in the HCC group? The earlier literature would predict the opposite, at least superficially. Kumada et al [13] and Liu et al [14] linked persistently high RNA transcription to a heightened risk of HCC, yet their cohorts had already attained DNA negativity, whereas the modest HDI of our HCC group was driven chiefly by elevated DNA levels. Lin et al [15] found pgRNA levels to be lower in the HCC group than in the CHB group, in line with our observations, and studies conducted in China [16] support the utility of HBV RNA in HCC evaluation.

The mechanism is not mysterious. By inhibiting the reverse transcription of pgRNA, NAs curtail DNA synthesis while leaving cccDNA transcription untouched [5,17], so HBV DNA falls rapidly whereas HBV RNA lingers. Lythgoe et al [18] estimated the half-life of cccDNA at 26–61 days, implying a long-lived hepatocyte subpopulation that maintains the cccDNA reservoir. We see three candidate explanations for the relatively low HDI of the HCC group. Attrition of the cccDNA pool secondary to destruction of liver parenchyma [17–18] is one. Predominance of integrated HBV DNA in HCC [19–21], which gives rise mainly to HBsAg transcripts rather than pgRNA, is another. A third is the longer treatment exposure typical of HCC patients, which permits progressive exhaustion of cccDNA. Hiraoka et al [22] demonstrated that the RNA/DNA ratio varies with HBeAg status and drug type, which accords with our view that decoupling is governed by multiple factors.

The practical value of the index lies in what it adds beyond existing categories. Converting HBV RNA/DNA decoupling from a qualitative category into a quantitative continuous index extends the current framework in a direct way. During NAs therapy, a persistently raised HDI despite DNA negativity may signal inadequate suppression of cccDNA transcription and herald a heightened relapse risk after treatment cessation [5]. The capacity of HBV RNA to predict HBeAg clearance during NAs therapy, reported by Qu et al [23], supports the value of RNA-centred markers in gauging treatment response. Among patients with LLV, the HDI could help identify subgroups with brisk transcriptional activity when

risk stratification is attempted [12]. For HCC assessment, our model incorporating the HDI performed comparably with its DNA-containing counterpart, positioning the index as a potential complement to established markers [13–14].

This study has limitations, and several of them matter. The cross-sectional design rules out inference of causal direction; the HDI-HCC relationship captured here is a point-in-time association and cannot show that HDI changes antedate HCC development. All patients came from a single centre, which may constrain the generalisability of the findings. Intrahepatic cccDNA and integrated HBV DNA went unmeasured, so the mechanistic discussion lacks direct histological corroboration. We did not stratify NAs agents by drug; given that entecavir and tenofovir alafenamide exert divergent effects on RNA kinetics, as Hiraoka et al [22] demonstrated, drug-specific subgroup analyses were precluded. The HCC group sample size (*n* = 223) is another concern, and may have undermined the stability of parameter estimation within the multivariable logistic regression model.

Several questions remain open. Longitudinal cohorts tracking HDI dynamics are needed to verify its predictive capacity for post-discontinuation relapse and for HCC development, and multicentre efforts could delineate standard reference ranges for the index. Integration of quantitative intrahepatic cccDNA measurement with integrated HBV DNA detection [19–20,24] may illuminate the molecular underpinnings of decoupling.

Conclusions

On the present evidence, the HDI quantifies HBV transcription-replication decoupling under NAs therapy reliably and is independently associated with disease stage and HCC risk, supporting its potential role as a supplementary marker within the existing HBV serological monitoring framework.

Abbreviations

ALT
alanine aminotransferase
APRI
AST-to-platelet ratio index
AST
aspartate aminotransferase
AUC
area under the curve
cccDNA
covalently closed circular DNA
CHB
chronic hepatitis B
CI

confidence interval
COI
cut-off index
FIB-4
fibrosis-4 index
HBsAg
hepatitis B surface antigen
HBeAg
hepatitis B e antigen
HBV
hepatitis B virus
HCC
hepatocellular carcinoma
HDI
HBV RNA/DNA decoupling index
IQR
interquartile range
LC
liver cirrhosis
LLV
low-level viraemia
NAs
nucleos(t)ide analogues
OR
odds ratio
PCR
polymerase chain reaction
pgRNA
pregenomic RNA
PLT
platelet count
RNA
ribonucleic acid
ROC
receiver operating characteristic
SD
standard deviation
ULN
upper limit of normal.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Fourth People's Hospital of Qinghai Province (approval number: Ethics Review [2024] No. 41). As a retrospective cross-sectional investigation confined to the collection and analysis of existing clinical data, the requirement for informed consent was waived, and all patient information was de-identified. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

Long Zhou: Conceived and designed the study; conducted the research; collected data; analyzed or interpreted the data; drafted the manuscript; and completed the statistical analysis; Hongmei Zu: Guided the study design; provided administrative, technical, or material support; secured research funding; and reviewed key scientific content of the manuscript; Pengfei Zhao: Participated in the review of the manuscript's scientific content and provided research guidance and supportive contributions; Yajing Li: Participated in reviewing the manuscript, provided guidance on refining statistical methods and writing approaches, and made supportive contributions. Xing Chen: Assisted in organizing some of the data, was responsible for typesetting the manuscript, and handled the submission process.

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Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. World Health Organization. Global hepatitis report 2024. Geneva: World Health Organization; 2024.
2. You H, Wang F, Li T, et al. Guidelines for the prevention and treatment of chronic hepatitis B (version 2022). J Clin Transl Hepatol. 2023;11:1425-42.
3. Liu S, Zhou B, Valdes JD, Sun J, Guo H. Serum hepatitis B virus RNA: a new potential biomarker for chronic hepatitis B virus infection. Hepatology. 2019;69:1816-27.
4. Butler EK, Gersch J, McNamara A, et al. Hepatitis B virus serum DNA and RNA levels in nucleos(t)ide analog-treated or untreated patients during chronic and acute infection. Hepatology. 2018;68:2106-17.
5. Brunetto MR. Hepatitis B virus RNA detection and a mindful use of serum hepatitis B virus DNA and hepatitis B surface antigen measures in clinical practice. Hepatol Commun. 2020;4:949-52.
6. Chinese Society of Gastroenterology, Chinese Medical Association. Chinese consensus on the diagnosis and treatment of liver cirrhosis (2023 edition). Chin J Dig. 2023;43:505-12.
7. Zhou J, Sun H, Wang Z, et al. China liver cancer guidelines for the diagnosis and treatment of hepatocellular carcinoma (2024 edition). Liver Cancer. 2025;14:779-835.
8. Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology. 2006;43:1317-25.
9. Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518-26.
10. Prakash K, Rydell GE, Larsson SB, et al. High serum levels of pregenomic RNA reflect frequently failing reverse transcription in hepatitis B virus particles. Virol J. 2018;15:86.
11. Mak LY, Anderson M, Stec M, et al. Longitudinal profile of plasma pregenomic RNA in patients with chronic hepatitis B infection on long-term nucleoside analogues and its interaction with clinical parameters. Clin Mol Hepatol. 2025;31:460-73.
12. Wang B, Zeng T, Yin W, et al. Unveiling low level viremia in chronic hepatitis B: challenges and new horizons. Front Cell Infect Microbiol. 2025;15:1663858.
13. Kumada T, Toyoda H, Yasuda S, et al. Hepatitis B virus RNA predicts hepatocellular carcinoma despite viral suppression. Aliment Pharmacol Ther. 2026;63:1089-98.
14. Liu S, Wong GL, Fan R, et al. Role of early on-treatment serum HBV RNA declines in predicting hepatocellular carcinoma risk in patients with chronic hepatitis B. Clin Gastroenterol Hepatol. 2025;23:291-9.
15. Lin N, Ye A, Lin J, et al. Diagnostic value of detection of pregenomic RNA in sera of hepatitis B virus-infected patients with different clinical outcomes. J Clin Microbiol. 2020;58:e01275-19.

16. Liu S, Deng R, Zhou B, et al. Association of serum hepatitis B virus RNA with hepatocellular carcinoma risk in chronic hepatitis B patients under nucleos(t)ide analogues therapy. J Infect Dis. 2022;226:881-90.
17. Hong X, Hu J. Serum HBV RNA composition dynamics as a marker for intrahepatic HBV cccDNA turnover. J Med Virol. 2020;92:935-7.
18. Lythgoe KA, Lumley SF, Pellis L, McKeating JA, Matthews PC. Estimating hepatitis B virus cccDNA persistence in chronic infection. Virus Evol. 2021;7:veaa063.
19. Tu T, Budzinska MA, Shackel NA, Urban S. HBV DNA integration: molecular mechanisms and clinical implications. Viruses. 2017;9:75.
20. Li W, Wang S, Jin Y, et al. The role of the hepatitis B virus genome and its integration in the hepatocellular carcinoma. Front Microbiol. 2024;15:1469016.
21. Chu YD, Ho PH, Hsu CW, Chiou CY, Lai MW, Yeh CT. Exploratory analysis of lamivudine legacy and clonal selection of HBs truncation mutants in HBV-related hepatocellular carcinoma. Infect Agent Cancer. 2025;20:78.
22. Hiraoka K, Tsuge M, Kawahara M, et al. Utility of serum HBV RNA measurement during nucleoside/nucleotide analog therapy in chronic hepatitis B patients. Int J Mol Sci. 2025;26:10141.
23. Qu Z, Chen X, Liu Y, et al. Relationship between serum HBV RNA levels and HBeAg clearance/seroconversion in patients with chronic hepatitis B receiving nucleoside analogues. Chin J Infect Chemother. 2024;24:285-91.
24. Wang Y, Yu D, Mei Y, et al. Long-read sequencing reveals HBV integration patterns and oncogenic impact on early-onset hepatocellular carcinoma. Genome Res. 2025;35:2389-405.

Figures

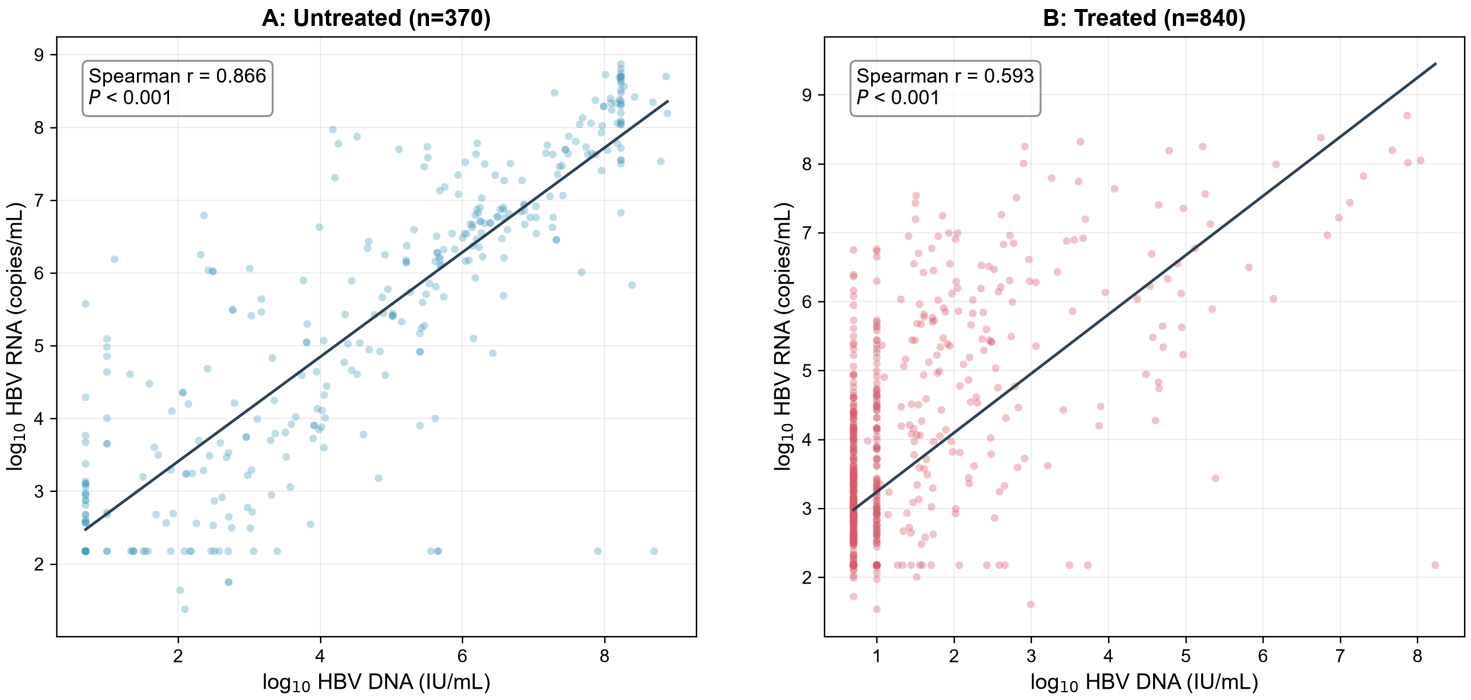


Figure 1

Correlation between HBV RNA and HBV DNA under different antiviral treatment statuses.

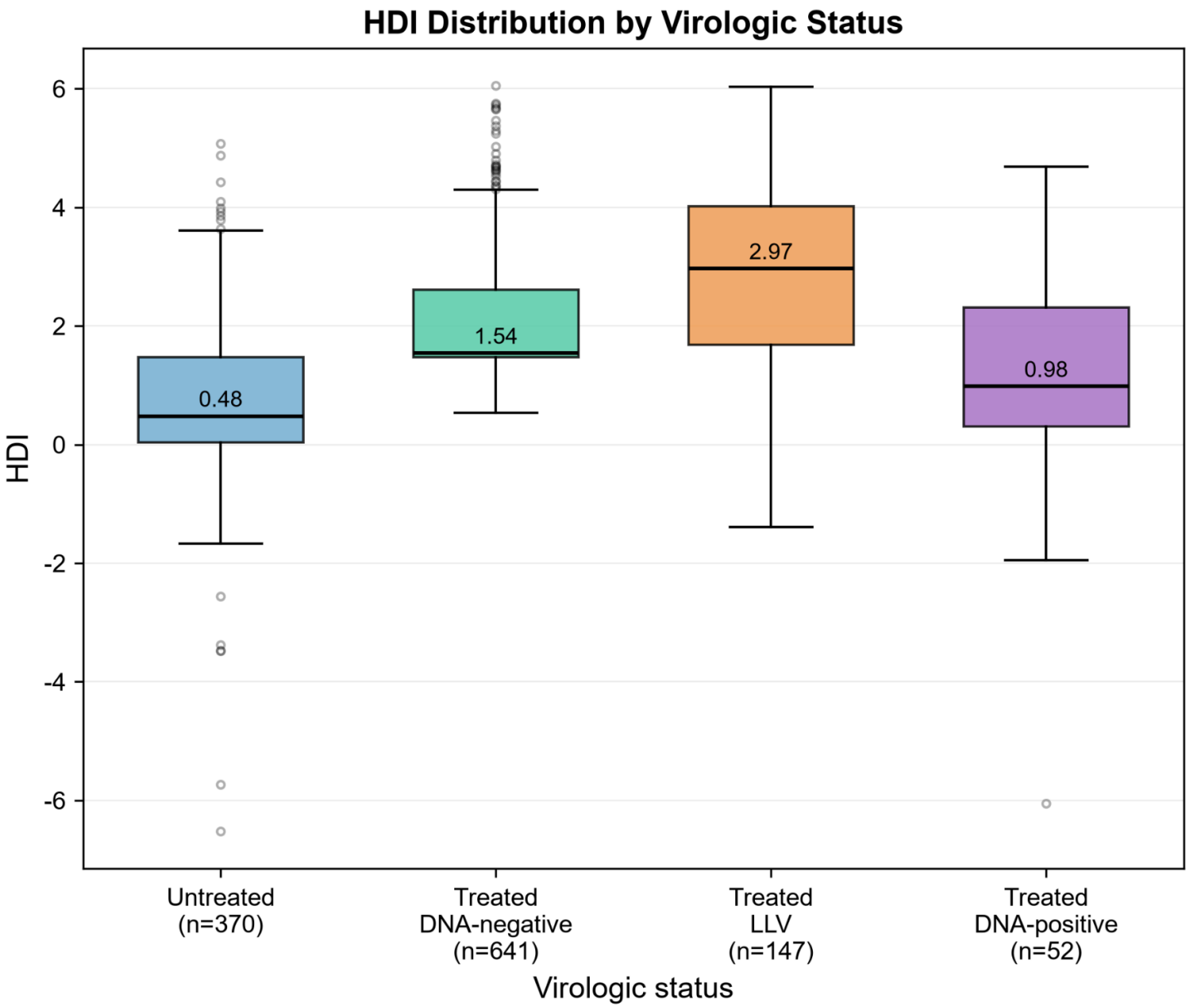


Figure 2

Comparison of the HBV RNA/DNA decoupling index (HDI) among patients with different virological statuses.

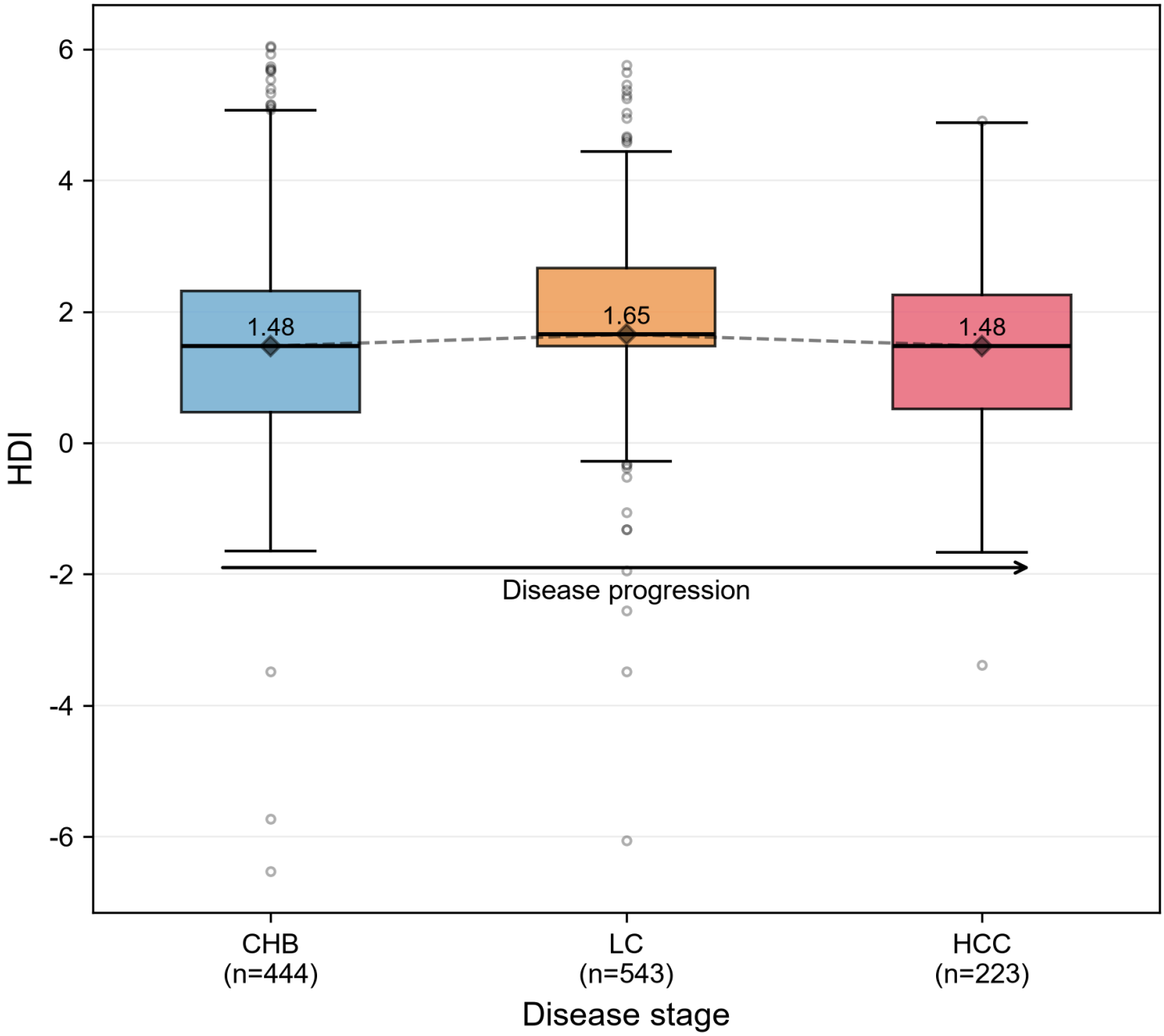


Figure 3

Trends of the HBV RNA/DNA decoupling index across disease stages. HBV, hepatitis B virus; HDI, HBV RNA/DNA decoupling index; CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

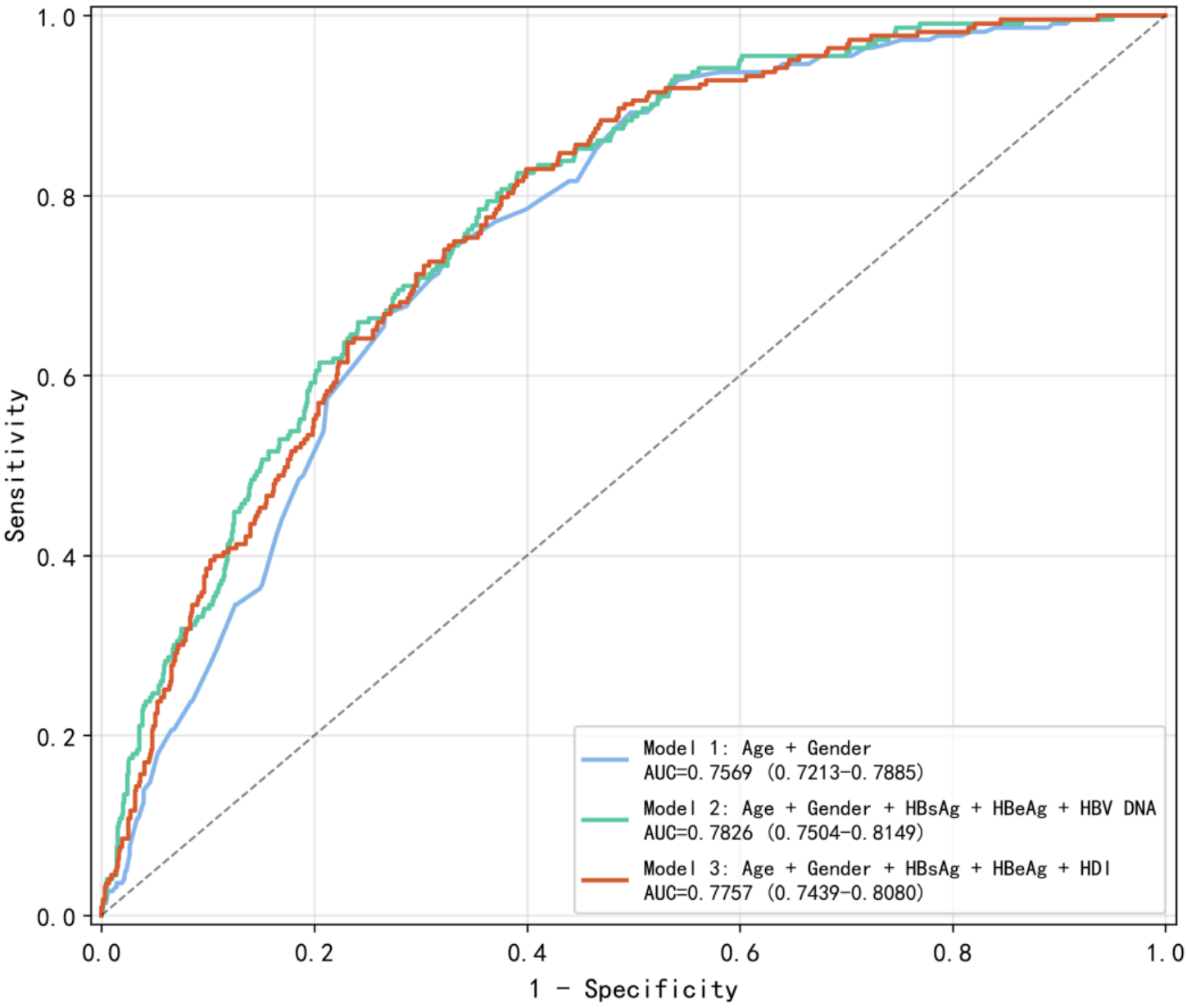


Figure 4

ROC curves of three multivariable logistic regression models for HCC prediction. Model 1 included age and sex; Model 2 added $\log_{10}$ HBsAg, HBeAg and HBV DNA, yielding the highest AUC (0.783); Model 3 substituted HBV DNA with the HBV RNA/DNA decoupling index (HDI; AUC 0.776). The DeLong test indicated no significant difference between Model 2 and Model 3 (P = 0.188), suggesting that the HDI captures complementary information comparable to conventional virological markers. ROC, receiver operating characteristic; AUC, area under the curve; HCC, hepatocellular carcinoma; NAs, nucleos(t)ide analogues; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDI, HBV RNA/DNA decoupling index.