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Original Article

Antiviral Therapy Favors a Lower Risk of Liver Cirrhosis in HBeAg-negative Chronic Hepatitis B with Normal Alanine Transaminase and HBV DNA Positivity

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Abstract

Background and Aims: Direct evidence on the outcomes of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB) patients with normal alanine transaminase after long-term antiviral treatment is lacking. ***Methods:*** HBeAg-negative patients with normal ALT and positive HBV DNA (≥20 IU/mL) were retrospectively enrolled. The endpoints included virological response (HBV DNA<100 IU/mL), changes in aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-4 index (FIB-4), and the incidence of liver nodules, cirrhosis, and hepatocellular carcinoma (HCC). ***Results:*** This cohort (n=194) was divided into three subgroups, untreated (n=67), treatment-continued (n=87), and treatment-discontinued patients (n=40), with a median follow-up of 54 months. The treatment-continued group achieved 100% (95% CI: 94.7–100) virological response, and significantly reduced APRI and FIB-4 scores (both p<0.001). The risk of liver nodules and cirrhosis in that group was reduced by 76% (HR: 0.24, 95% CI: 0.11–0.54, p<0.001) and 89% (HR: 0.11, 95% CI: 0.14–0.91, p=0.041) vs. the untreated group and by 77% (HR: 0.23, 95% CI: 0.10–0.49, p<0.001) and 95% (HR: 0.05, 95% CI: 0.01–0.44, p=0.006) vs. the treatment-discontinued group. For patients with HBV DNA≥2,000 IU/mL, adherence to treatment lowered the risks of liver cirrhosis by 92% (95% CI: 0.01–0.67) and 93% (95% CI: 0.01–0.53) vs. the untreated and treatment-discontinued patients, respectively. No patient adhering to treatment developed HCC, but one in each of the remaining groups did. ***Conclusions:*** Continuous nucleos(t)ide analog (NA) treatment has a satisfactory effectiveness and helps to lower the risk of liver cirrhosis in HBeAg-negative CHB patients with normal alanine transaminase, especially in those with HBV DNA≥2,000 IU/mL.

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Introduction

Chronic hepatitis B virus (CHB) infection continues to be a substantial public health burden accounting for 30% of all deaths from cirrhosis and 40% of all deaths related to hepatocellular carcinoma (HCC) globally.[1] Antiviral treatment in time is effective for reducing the complications associated with chronic hepatitis B virus (HBV) infection.[2] Generally, the necessity of antiviral treatment for patients with CHB is based on comprehensive consideration of the levels of alanine aminotransferase (ALT) and serum HBV DNA, the severity of liver disease, age, and family history of liver cirrhosis/HCC.[3–5] Other factors, such as the effectiveness of antiviral drugs, patient willingness, and compliance, also influence the final decision on antiviral therapy.

For CHB patients with normal ALT levels, especially those with negative hepatitis B e antigen (HBeAg), it has long been considered that treatment can be postponed for no or slight liver injury and a benign prognosis.[6–8] However, new evidence has changed that traditional stereotype. Significant necroinflammation and/or liver fibrosis can exist in CHB patients with normal ALT, indicating the necessity of treatment.[3,4] If untreated, HBeAg-negative CHB patients with normal ALT and a high viral load (≥2,000 IU/mL) are more likely to have an increased risk of HCC and death/transplantation than treated patients with abnormal ALT.[9] A previous study has demonstrated that HBV DNA integration and clonal hepatocyte expansion were present across the disease phases, underscoring that the potential risk of HCC development persists, even in patients with early-stage chronic HBV infection, a stage previously considered benign.[10]

Recently, the indications for antiviral therapy have been further expanded in China with the aim of reducing the risk of HBV-related advanced liver events. Improvement of the effectiveness and accessibility of antiviral drugs, decreased treatment costs and the early completion of the World Health Organization plan to eliminate the global harm of viral hepa-

Keywords: Chronic hepatitis B; Negative HBeAg; Normal ALT; Gray zone; Antiviral therapy.

Abbreviations: ALT, alanine transaminase; APRI, aspartate aminotransferase to platelet ratio index; CHB, chronic hepatitis B; CI, confidence interval; FIB-4, fibrosis-4 index; HCC, hepatocellular carcinoma; HR, hazard ratio; NA, nucleos(t)ide analog; ULN, upper limit of normal.

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titis also promote changes in the management of CHB patients. In the latest Chinese Guidelines for the prevention and treatment of chronic hepatitis B (version 2022), the threshold of ALT for initiating treatment was lowered to 30 U/L for men and 19 U/L for women; meanwhile, patients with normal ALT and positive DNA should be treated if they are over 30 years of age, have a family history of cirrhosis/HCC, or have significant liver necroinflammation/fibrosis by invasive or noninvasive examination.[11]

However, there is still a lack of clinical data on the benefits of antiviral therapy in HBeAg-negative CHB patients with normal ALT levels, as those patients have been discouraged from treatment for quite some time. In this study, we aimed to determine the treatment efficacy and prognostic improvement after a long course of nucleos(t)ide analog (NA) therapy in noncirrhotic CHB patients with normal ALT levels, negative HBeAg, and positive HBV DNA, so as to provide direct and reliable evidence for expanding the indication for antiviral treatment in such a controversial population.

Methods

Patient selection

This was a single-center, retrospective cohort study. CHB patients were enrolled at the hepatitis clinic of West China Hospital of Sichuan University between February 2017 and March 2020. Enrollment criteria were: (1) detectable hepatitis B surface antigen (HBsAg) for at least 24 weeks, with negative HBeAg and positive hepatitis B e antibody (HBeAb); (2) at least three values of ALT below the upper limit of normal (ULN) within 1 year and at least 3 months apart. The upper limit of normal (ULN) was 50 U/L for men and 40 U/L for women; (3) detectable serum HBV DNA (≥20 IU/mL); (4) no signs of cirrhosis or liver nodules; and (5) HBV treatment-naïve patients, without previous NA or interferon treatment. Exclusion criteria were (1) <18 years of age; (2) pregnant; (3) coinfection with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis D virus (HDV); and (4) confirmed liver cirrhosis, HCC or other liver diseases, such as alcoholic, autoimmune, or biliary liver disease. The design of this study was consistent with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines and was approved by the Institutional Review Board of the West China Hospital of Sichuan University. The clinical trial registration number is ChiCTR2100050064.

Treatment and monitoring during follow-up

Grouping was based on whether patients had initiated antiviral treatment. Patients in the treated group self-administered entecavir (ETV) 0.5 mg, tenofovir disoproxil fumarate (TDF) 300 mg, or telbivudine (LDT) 600 mg per day. Patients in the untreated group received no treatment, including NAs or interferon alpha. Abdominal ultrasonography and serum HBV DNA, aspartate aminotransferase (AST) and ALT levels, platelet (PLT) count and alpha-fetoprotein (AFP) were measured at baseline and every 6 months. All data was sourced from the laboratory information management system of West China Hospital of Sichuan University.

Outcomes

The primary outcome was the change in serum HBV DNA, including virological response, breakthrough, and relapse. The virological response was an undetectable serum HBV DNA during therapy. Virological relapse was a serum HBV DNA>2,000 IU/mL after stopping treatment in patients who achieved virological response. Virological breakthrough was >1 $\log_{10}$ (10-fold) increase in serum HBV DNA from the nadir during therapy in patients adherent to treatment and with an initial virological response.[12]

Secondary outcomes included change in aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-4 index (FIB-4) scores, and the incidence of liver nodules, liver cirrhosis, and HCC. Liver cirrhosis was suspected when platelet (PLT) $<100\times10^9$/L (excluding other causes), APRI>2.00, FIB-4>1.45, or ultrasound-suggested signs of cirrhosis and confirmed by liver biopsy, digestive endoscopy, computed tomography (CT), or magnetic resonance imaging (MRI). Patients received regular HCC surveillance by abdominal ultrasonography and serum alpha-fetoprotein (AFP) tests. HCC diagnosis was based on histological examination and/or typical features by dynamic CT and/or MRI.

APRI and FIB-4 are noninvasive serological indices to assess liver fibrosis and cirrhosis. APRI=AST (ULN)/PLT (10^9/L)×100. The ULN of AST was 34 IU/L for women and 36 IU/L for men, as previously reported.[13] FIB-4=(Age [years]×AST [U/L])/(PLT [10^9/L]×ALT[U/L]$^{1/2}$). Liver fibrosis staging: the predefined thresholds APRI<1.0 and FIB-4<1.45 were used to reject advanced fibrosis, and APRI>2 and FIB-4>3.25 to rule in cirrhosis. Both indices have indeterminate zones (*i.e*. APRI 1.00–2.00, and FIB-4 1.45–3.25) where the stage of liver fibrosis is unclassifiable.[14]

Serum assays

Serum biochemical indexes were measured by standard procedures (Olympus AU5400, Olympus Corporation, Tokyo, Japan). Serum HBsAg levels were measured with an Elecsys HBsAg II Quant Assay (Roche Diagnostics, Penzberg, Germany). Serum HBeAg status was determined by an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN, USA). Serum HBV DNA concentration was quantitatively determined with Cobas *Taq*Man assay kits (Roche Diagnostics, Branchburg, NJ, USA) with a lower limit of detection of 100 IU/mL.

Statistical analysis

Continuous variables were reported as median and interquartile range (IQR) and categorical variables were reported as absolute and relative frequencies. The frequencies and distributions of categorical variables were compared with chi squared and Fisher's exact tests. Continuous variables were compared with Student's *t*-tests. The Wilcoxon signed-rank test was used to compare paired samples. The cumulative incidence of liver nodules, cirrhosis, and HCC were estimated by the Kaplan-Meier method, and statistical significance was determined using log-rank tests. Univariable Cox proportional hazards models was used to determine the hazard ratio (HR) of liver nodules, liver cirrhosis, and HCC. A two-sided $p<0.05$ was considered statistically significant. The analyses were performed with SPSS for Windows, version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

From February 2017 to December 2020, 529 CHB patients with persistently normal ALT were screened at West China Hospital of Sichuan University. A total of 194 patients were included and divided into a treated group (n=127) and an untreated group (n=67). During follow-up, treatment was interrupted in 40 patients (31.5%) in the treated group, *i.e.* the treatment-discontinued group. The remaining 87 patients were the treatment-continued group. Figure 1 is a

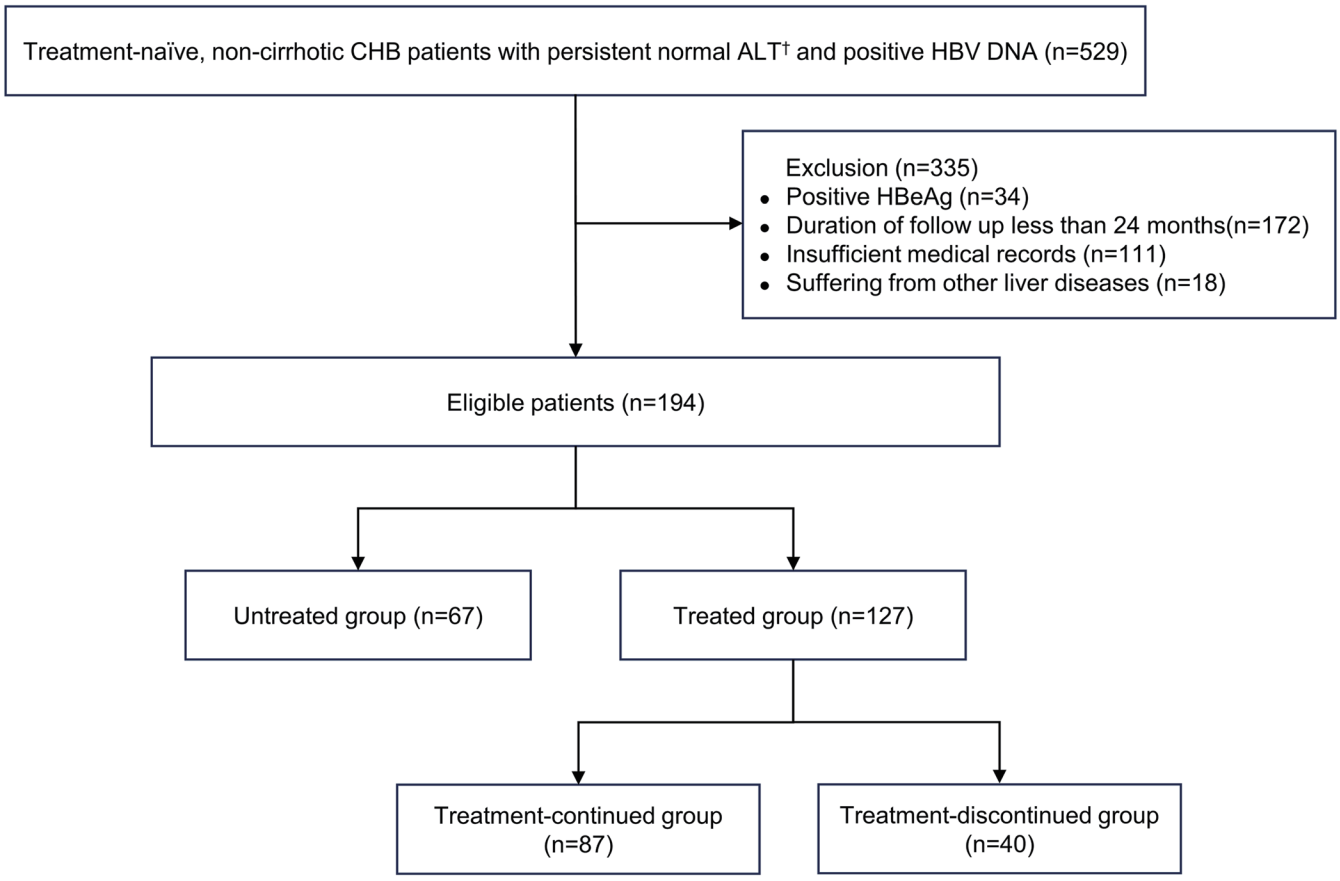

Fig. 1. Patient flow diagram. †Persistent normal ALT was defined as three assays below the ULN, 50 U/L for men and 40 U/L for women at least 3 months apart within 1 year. ALT, alanine aminotransferase; CHB, chronic hepatitis B; ULN, upper limit of normal.

flow diagram of patient selection and grouping. Of the 194 patients, the median duration of follow-up was 54 months. Their median age was 43 years, female patients accounted for a higher proportion (52.6%), and most (80.9%) had HBV DNA concentrations ≥2,000 IU/mL (approximately 3.3 $\log_{10}$ IU/mL). About two-thirds of the patients (67.8%) received ETV treatment. The untreated group was the youngest, with only about 50% of patients ≥40 years of age. The treatment-discontinued group had the lowest AST level and APRI score. The treatment-continued group had a higher median FIB-4 score than either of the other two groups. There were no significant differences in sex, serum HBV DNA load, ALT level, or PLT count among the three subgroups. Table 1 gives the patient baseline characteristics.

Changes in serum HBV DNA level

In the untreated group, serum HBV DNA loads declined from 3.64 $\log_{10}$ IU/mL to 3.27 $\log_{10}$ IU/mL (Fig. 2A). No subject had spontaneously achieved undetectable HBV DNA at the end of the follow-up (Fig. 2D). In the treatment-continued group, the cumulative virologic response rates at 6, 12, and 18 months were 92.0% (80/87, 95% CI: 83.6–96.4), 97.7% (85/87, 95% CI: 91.2–99.6), and 100% (87/87, 95% CI: 94.7–100.0), respectively. No patient experienced virological breakthrough following virological response. Of the 87 patients, 82.8% (72/87, 95% CI: 72.8–89.7) maintained virological response (HBV DNA<100 IU/mL and 16.1% (14/87, 95% CI, 9.4–25.9) had an intermittent fluctuation of low-level of serum HBV DNA (<2,000 IU/mL; Fig. 2B, E). In the treatment-discontinued group, 97.5% of the patients (39/40, 95% CI: 85.3–99.9) achieved virologic response at the end of treatment (EOT). At the end of follow-up (EOF), the median load of HBV DNA rebounded to 3.92 $\log_{10}$ IU/mL, a decrease of 0.25 $\log_{10}$ IU/mL (median) from baseline (p=0.035; Fig. 2C). The cumulative incidence of HBV DNA detectable after virological response within 6, 12, and 24 months were 59.0% (23/39, 95% CI: 42.2–74.0), 87.2% (34/39, 95% CI: 71.8–95.2), and 92.3% (36/39, 95% CI: 78.0–98.0), among whom 84.6% (32/39, 95% CI: 65.9–91.9) had a virological relapse (HBV DNA>2,000 IU/mL). The cumulative incidence of virological relapse at 6, 12, and 24 months were 17.9% (7/39, 95% CI: 8.1–34.1), 48.7% (19/39, 95% CI: 32.7–65.0), and 76.9% (30/39, 95% CI: 60.3–88.3%), respectively (Fig. 2F).

Changes in APRI and FIB-4

In the untreated group, the APRI decreased from 0.37 at the baseline to 0.35 (p=0.135). The proportion of patients with an APRI<1.0 significantly decreased from 100% to 89.5% (p=0.020). The median score of FIB-4 increased from 1.04 to 1.20 (p<0.001). The proportion of patients with FIB-4<1.45 decreased from 77.6% to 64.2% (p=0.128) and the proportion of those with an FIB-4 of >3.25 increased from 1.5% to 7.5% (Fig. 3). In the treatment-continued group, the APRI decreased significantly from 0.41 to 0.33 (p<0.001). The proportion of patients with an APRI of <1.0 increased to 100% (p=0.244). The median FIB-4 also decreased sig-

Table 1. Baseline characteristics of the enrolled patients

Characteristic	Entire, *n*=194	Treated, *n*=127		Untreated, *n*=67	*p*#
		Continued, *n*=87	Discontinued, *n*=40		
Duration of follow-up*, month	54 (24, 72)	60 (36, 72)	57 (48,72)	48 (24, 72)	0.895
Duration of treatment*, month	–	60 (48, 72)	12 (6, 24)	–	<0.001
Age, years	43 (36, 50)	45 (39, 50)	43 (38, 54)	39 (20, 49)	<0.001
≥40	122 (62.9%)	62 (71.3%)	27 (67.5%)	33 (49.3%)	0.015
Male	92 (47.4%)	44 (50.6%)	20 (50.0%)	28 (41.8%)	0.220
NA(s) regime					0.241
ETV	82 (42.3%)	59 (67.8%)	23 (57.5%)	–	–
TDF	44 (22.7%)	28 (32.2%)	16 (40.0%)	–	–
LDT	1 (0.5%)	0 (0.0%)	1 (2.5%)	–	–
HBV DNA, $\log_{10}$ IU/mL	3.88 (3.41, 4.56)	3.86 (3.43, 4.44)	4.09 (3.61, 4.62)	3.64 (3.08, 4.56)	0.117
≥3.3	157 (80.9%)	74 (85.1%)	34 (85.0%)	49 (73.1%)	0.146
ALT, U/L	22 (17, 27)	21 (17, 25)	22 (18, 28)	24 (17, 30)	0.101
ALT ≤20	78 (40.2%)	39 (44.8%)	13 (32.5%)	26 (38.8%)	0.402
AST, U/L	24 (20, 28)	25 (22, 28)	21 (17, 25)	24 (20, 28)	<0.001
PLT, $\times10^9$/L	183±51	177±55	186±53	195±50	0.118
APRI	0.39(0.29, 0.51)	0.41 (0.32, 0.57)	0.31 (0.24, 0.46)	0.37 (0.27, 0.44)	<0.001
<1.00	191 (98.5%)	84 (96.6%)	40 (100%)	67 (100%)	0.311
1.00–2.00	3 (1.5%)	3 (3.4%)	0 (0.0%)	0 (0.0%)	0.311
>2.00	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–
FIB-4	1.22 (0.87, 1.67)	1.47 (1.04, 1.99)	0.98 (0.81, 1.57)	1.04 (0.71, 1.44)	<0.001
<1.45	124 (63.9%)	43 (49.4%)	29 (72.5%)	52 (77.6%)	0.001
1.45–3.25	64 (33.0%)	39 (44.8%)	11 (27.5%)	14 (20.9%)	0.005
>3.25	6 (3.1%)	5 (5.7%)	0 (0.0%)	1 (1.5%)	0.186

Data are *n* (%), mean±standard deviation, or median (IQR), unless otherwise specified. *Median (range); #*p*-values are compared results in the three groups, except the *p*-values of the duration of treatment and the NA (s) regimen (continued vs. discontinued). ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; ETV, entecavir; FIB-4, fibrosis-4 index; IQR, interquartile range; LDT, telbivudine; NA, nucleos(t)ide analog; PLT, platelet count; TDF, tenofovir disoproxil fumarate.

nificantly from 1.47 to 1.10 (p<0.001), with the proportion of FIB-4<1.45 increasing significantly to 70.1% (p=0.005), and that with an FIB-4>3.25 decreased to 3.5% (Fig. 3). In the treatment-discontinued group, the median duration of treatment was 12 (6–18) months. At the EOT, the ALT level had increased significantly vs. baseline (22 vs. 25 U/L, p=0.019), but the APRI score (0.31 vs. 0.31, p=0.627) and FIB-4 score (0.98 vs. 1.11, p=0.572) had not significantly changed. At the EOF, the APRI and FIB-4 scores had significantly increased from those at the EOT (APRI, 0.31 vs. 0.67, p<0.001; FIB-4, 1.11 vs. 2.34, p<0.001) and baseline (APRI, 0.31 vs. 0.67, p<0.001; FIB-4, 0.98 vs. 2.34, p<0.001; Supplementary Table 1 and Supplementary Fig. 1). The proportion of patients with an APRI of <1.0 decreased significantly from 100% to 85.0% (p=0.034). The proportion of patients with an FIB-4 of <1.45 decreased significantly from 72.5% to 27.5% (p<0.001), and that of patients with an FIB-4 of >3.25 increased significantly from 0.0% to 10.0% (Fig. 3). The changes in scores of APRI and FIB-4 in relapsed and nonrelapsed patients had the same tendencies (Supplementary Table 1 and Supplementary Fig. 1). There were no significant differences in the increase in APRI and FIB-4 at EOF compared with the scores at EOT in the two subgroups. The $APRI_{EOF-EOT}$ scores in relapsed vs. nonrelapsed patients were 0.35 vs. 0.30, p=0.453. The $FIB\text{-}4_{EOF-EOT}$ scores in relapsed vs. nonrelapsed patients were 1.21 vs. 1.53, p=0.798 (Supplementary Table 2 and Supplementary Fig. 2).

Liver nodules, liver cirrhosis, and HCC

Patients at all HBV DNA levels: Adverse event of liver nodules was reported in 53 patients (27.3%, 95% CI: 21.3–34.3) of the entire cohort (n=194), with a mean follow-up of 55.5 months (95% CI: 51.4–59.5), 26 in the untreated, 10 in the treatment-continued, and 17 in the treatment-discontinued groups. Of the 53 patients with liver nodules, 16 (30.2%, 95% CI: 18.7–44.5) progressed to cirrhosis, with a mean follow-up of 60.8 months (95% CI: 53.2–68.3), one, eight, and seven in the three subgroups. Of the 16 patients with liver cirrhosis, two (12.5%, 95% CI: 2.2–39.6) developed HCC, with a mean follow-up of 72 months, one in the untreated group and the other in the treatment-discontinued group. The incidence of adverse events reported in each subgroup during follow-up are shown in Supplementary Figure 3A, B.

The incidence of liver nodules was 1.82 per 100 patient-

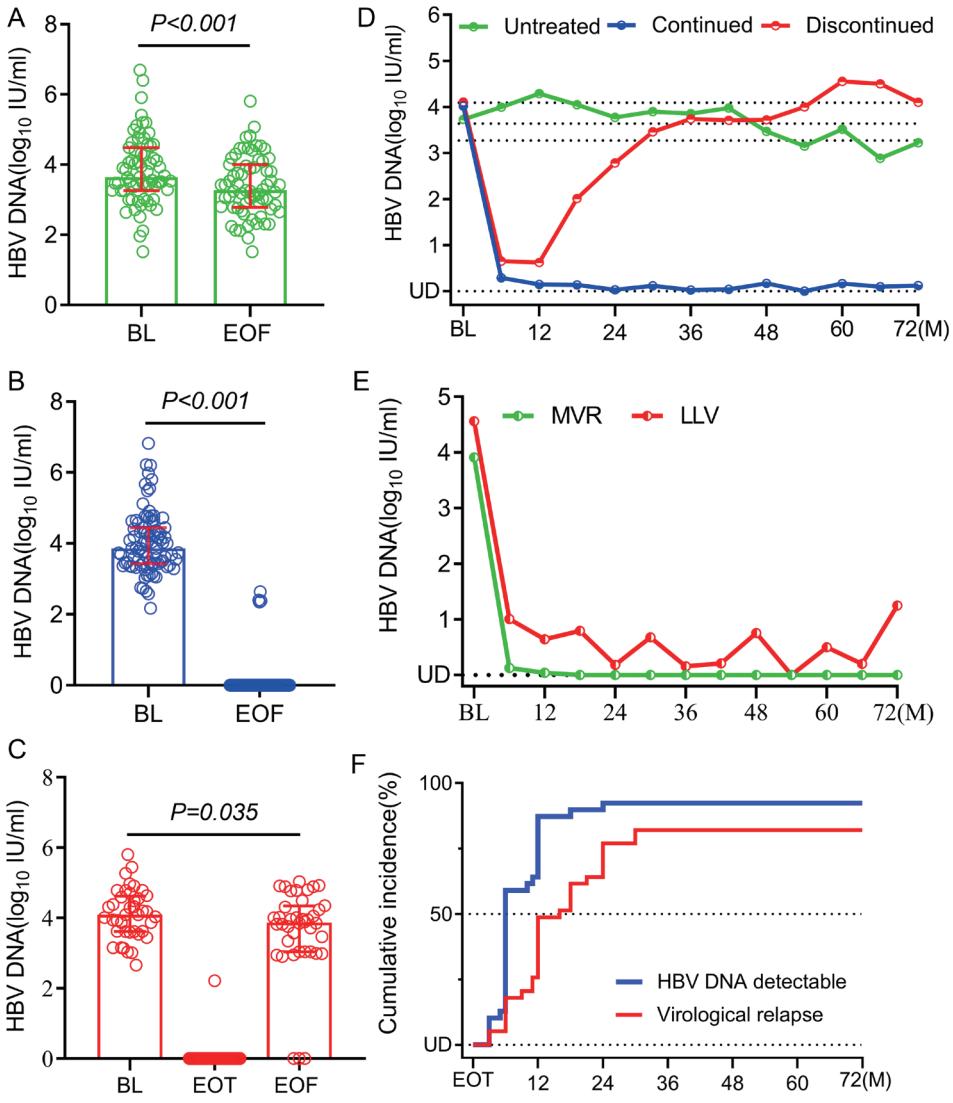

Fig. 2. Change in serum HBV DNA levels. (A, B, C) Change in serum HBV DNA levels in the three subgroups from baseline to the end of the follow-up. (D) Change in HBV DNA levels of the three subgroups over time. (E) MVR and intermittent LLV of the treatment-continued group. (F) Percentages of patients with detectable serum HBV DNA and virologic relapse after stopping antiviral therapy in the treatment-discontinued group. BL, baseline; EOF, end of follow-up; EOT, end of treatment; LLV, low-level viremia; MVR, maintained virological response; UD, undetectable.

years (PYs) in the treatment-continued group, significantly lower than that of the untreated group (10.4 per 100 PYs, $p<0.001$, hazard ratio (HR) 0.24, 95% CI: 0.11–0.49, $p<0.001$) and the treatment-discontinued group (1.82 vs. 3.11 per 100 PYs, HR: 0.23, 95% CI: 0.10–0.49, $p<0.001$). Although the estimated incidence of liver nodules in the untreated group was higher than that in the treatment-discontinued group, the difference was not significant (10.4 vs. 3.11 per 100 PYs, HR: 1.06, 95% CI: 0.57–1.98, $p=0.845$; Fig. 4A and Table 2). The cumulative incidence of liver cirrhosis was also significantly reduced in the treatment-continued group compared with the untreated group (0.17 vs. 2.49 per 100 PYs, HR: 0.11, 95% CI: 0.01–0.91, $p=0.013$) and treatment-discontinued group (0.17 vs. 1.37 per 100 PYs, HR: 0.05, 95% CI: 0.01–0.44, $p=0.006$). Difference in the incidence of liver cirrhosis in the untreated and treatment-discontinued groups was not significant ($p=0.121$; Fig. 4B and Table 2). Differences in the incidence of HCC among the three subgroups were not significant ($p=0.228$; Fig. 4C and Table 2).

Patients with HBV DNA≥2,000 IU/mL: Of the 194 patients, about four-fifths ($n=157$) had HBV DNA≥2,000 IU/mL, 49 in the untreated group, 74 in the treatment-continued group, and 34 in the treatment-discontinued group. Except for the shortest follow-up duration in the untreated group, the lowest AST and APRI levels in the treatment-discontinued group, and the highest FIB-4 score in the treatment-continued group, there were no significant differences in the baseline characteristics in the three new subgroups (Table 3).

Liver nodules developed in 45 (28.6%, 95% CI: 21.9–36.5%) of the 157 patients, with a median follow-up of 55.3 months (95% CI: 50.9–59.8), 20 in the untreated, nine in the treatment-continued, and 16 in the treatment-discontinued groups. Of the 45 patients with liver nodules, 15 (33.3%, 95% CI: 20.4–49.1) progressed to cirrhosis, with a mean follow-up of 60.0 months (95% CI: 52.1–67.9), seven, one, and seven in the three subgroups, respectively. Of the 15

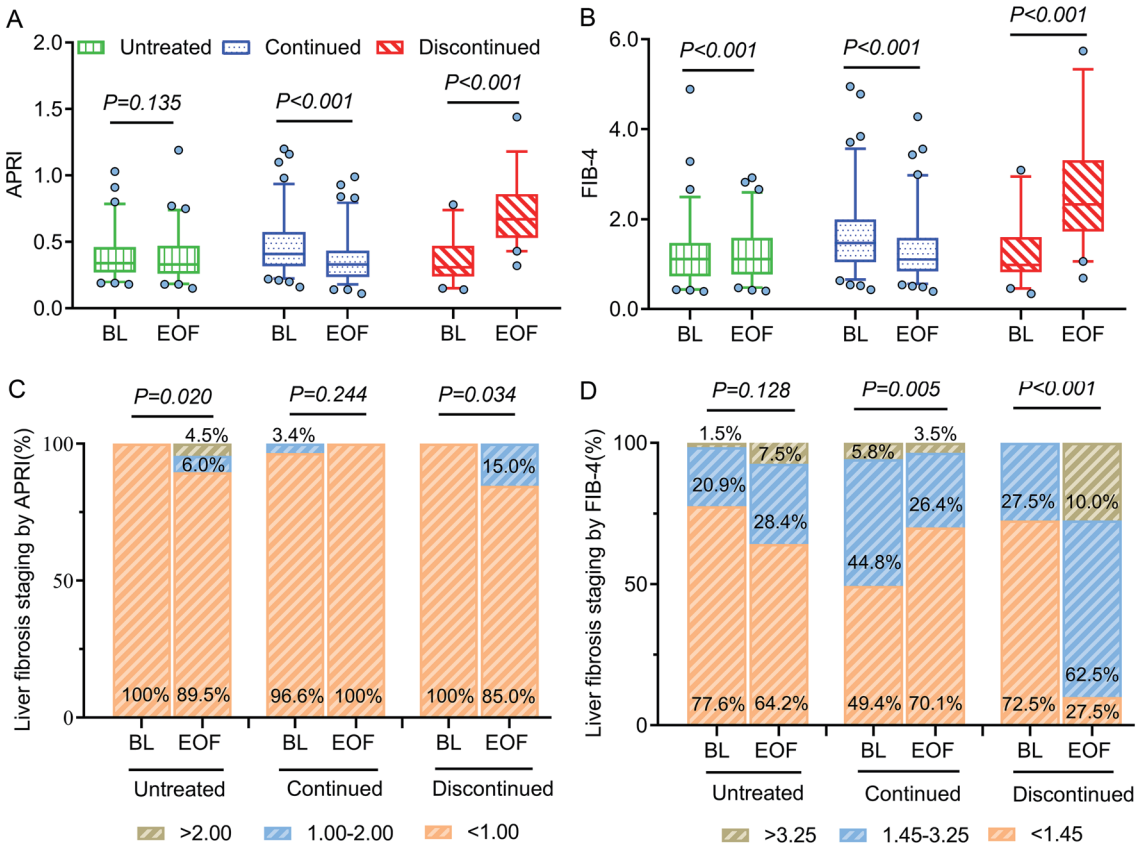

Fig. 3. Changes in APRI and FIB-4 in the three subgroups. (A, B) APRI and FIB-4 scores at baseline vs. EOF. (C, D) Stage of liver fibrosis by APRI and FIB-4 at baseline vs. EOF. Predefined thresholds of <1.0 for APRI and <1.45 for FIB-4 were used to reject advanced fibrosis. APRI>2.00 and FIB-4>3.25 were used to rule in cirrhosis. Both indices had indeterminate zones (*i.e.* APRI 1.00–2.00 and FIB-4 1.45–3.25) where liver fibrosis was unclassifiable. *p*-values in C and D are analysed results of proportions of APRI<1.0 and FIB-4<1.45 at baseline vs. EOF. APRI, aspartate aminotransferase to platelet ratio index; BL, baseline; EOF, end of follow-up; FIB-4, fibrosis-4 index.

patients with liver cirrhosis, 1 (6.7%, 95% CI: 0.35–34.0%) in the untreated group developed HCC at a follow-up of 72 months. The cumulative incidences of adverse events are shown in Supplementary Figure 3C, D.

Patients with HBV DNA≥2,000 IU/mL in the treatment-continued group had a significantly lower incidence of liver nodules (1.65 vs. 2.96 vs. 8.13 per 100 PYs) and liver cirrhosis (0.17 vs. 2.51 vs. 1.19 per 100 PYs) than those who were untreated and discontinued treatment (Fig. 4D, E). There were no significant differences in the incidence of liver nodules (p=0.887) and liver cirrhosis (p=0.551) in the untreated and the treatment-discontinued groups (Fig. 4D, E). Compared with untreated patients, the risks of liver nodules and liver cirrhosis in patients remaining on antiviral therapy decreased by 78% (HR: 0.22, 95% CI: 0.10–0.48) and 92% (HR: 0.08, 95% CI: 0.01–0.67), respectively (Table 2). Compared with those interrupting therapy, the risks of liver nodules and liver cirrhosis decreased by 79% (HR: 0.21, 95% CI: 0.09–0.47) and 93% (HR: 0.07, 95% CI: 0.01–0.53), respectively (Table 2). No patients in the treatment-continued or the treatment-discontinued groups developed HCC, but one in the untreated group did. Differences in the HCC incidence among the three subgroups were not significant (p=0.462; Table 2 and Fig. 4F).

Patients with HBV DNA of <2,000 IU/mL: There were 37 patients with HBV DNA<2,000 IU/mL, 18 in the untreated, 13 in the treatment-continued, and six in the treatment-discontinued groups. Eight patients developed liver nodules, six in the untreated group and one each in the other two groups. Patients of the treatment-continued group had a lower incidence of liver nodules than those untreated (0.20 vs. 2.47 per 100 PYs, p=0.105), and the treatment-discontinued group (0.20 vs. 0.26 per 100 PYs, p=0.619), but the differences were not significant. Compared with the untreated group, patients with interrupted therapy had a significantly higher incidence of liver cirrhosis (0.25 vs. 0.0 per 100 PYs, p=0.019) and HCC (0.25 vs. 0.0 per 100 PYs, p=0.003), but neither incidence was significantly higher than those in the treatment-continued group (0.25 vs. 0.0 per 100 PYs, p=0.061 for liver cirrhosis; 0.25 vs. 0.0 per 100 PYs, p=0.317 for HCC) (Table 4).

Discussion

In this single-center retrospective study, we analyzed the efficacy of NAs treatment and the improvement of long-term outcomes in HBeAg-negative patients with persistent normal ALT and detectable HBV DNA. The results showed those patients had an excellent virological response to NAs therapy but also a high rate of virological relapse if treatment was interrupted. Compared with those untreated and those stopping treatment, we observed significant declines of APRI and FIB-4 scores, significantly reduced risk of liver nodules and liver cirrhosis, and a low incidence of HCC in patients who

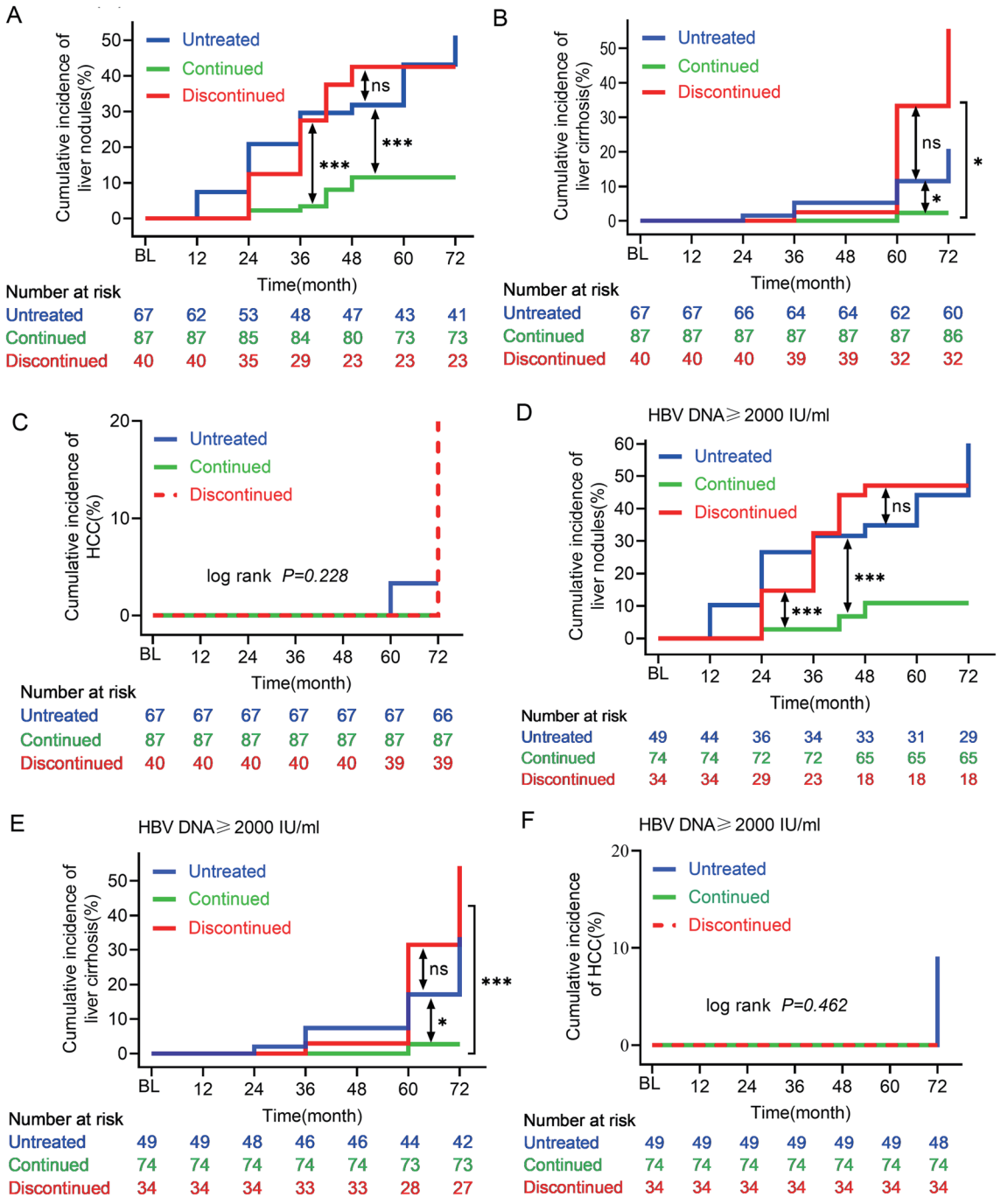

Fig. 4. Cumulative incidence of liver nodules, liver cirrhosis, and HCC in the untreated, treatment-continued, and treatment-discontinued groups. (A, B, C) Comparison of the cumulative incidence of the three events in patients with HBV DNA at all levels. (D, E, F) Comparison of the cumulative incidence of the three events in patients with HBV DNA≥2,000 IU/mL of the three subgroups. $^{***}p<0.001$; $^{*}p<0.0167$; ns, $p>0.05$. BL, baseline; HCC, hepatocellular carcinoma.

continued antiviral therapy, especially in those with HBV DNA≥2,000 IU/mL, who accounted for 80.9% of the whole cohort. To our knowledge, this is the first study to investigate the long-term prognosis of antiviral therapy in HBeAg-negative CHB patients with persistently normal ALT and positive HBV DNA, providing direct evidence for expanding the indications of antiviral treatment in such a population.

Continuous inhibition of virological replication and favorable compliance are foundations of reduced risk of liver cirrhosis. In this study, a high rate of virological response was achieved in both the treated and treatment-discontinued groups. However, poor off-treatment sustainability of NAs effectiveness was observed, with more than 80% of patients experiencing virological relapse (HBV DNA>2,000 IU/mL) in the treatment-discontinued group. Worse still, those with virological relapse had a significantly higher incidence of liver

Table 2. Incidence of liver nodules, liver cirrhosis, and HCC in patients with HBV DNA at all levels and with HBV DNA≥2,000 IU/mL of the three subgroups[¶]

Subgroup	HBV DNA at all levels, *n*=194				HBV DNA≥2,000 IU/mL, *n*=157			
	Patient-years	No. of events	No./100 patient-years (95% CI)	HR (95% CI)	Patient-years	No. of events	No./100 patient-years (95% CI)	HR (95% CI)
Liver nodules								
Untreated	249	26	10.4 (7.06–15.01)	0.24 (0.11–0.49)	246	20	8.13 (5.16–12.46)	0.22 (0.10–0.48)
Discontinued	547	17	3.11 (1.88–5.03)	0.23 (0.10–0.49)	541	16	2.96 (1.76–4.87)	0.21 (0.09–0.47)
Continued	550	10	1.82 (0.93–3.43)	–	545	9	1.65 (0.81–3.22)	–
Liver cirrhosis								
Untreated	281	7	2.49 (1.10–5.28)	0.11 (0.14–0.91)	279	7	2.51 (1.10–5.32)	0.08 (0.01–0.67)
Discontinued	586	8	1.37 (0.64–2.79)	0.05 (0.01–0.44)	586	7	1.19 (0.52–2.55)	0.07 (0.01–0.53)
Continued	594	1	0.17 (0.01–1.09)	–	589	1	0.17 (0.01–1.10)	–
HCC[§]								
Untreated	283	1	0.35 (0.02–1.09)	–	281	1	0.36 (0.02–2.92)	–
Discontinued	591	1	0.17 (0.01–1.09)	–	591	0	0.0 (0.0–0.81)	–
Continued	600	0	0.0 (0.0–0.79)	–	595	0	0.0 (0.0–0.80)	–

[¶]The three subgroups include untreated, treatment-continued, and treatment-discontinued patients. [§]The HR of HCC are not calculated for the small sample. The HR of liver nodules and liver cirrhosis are the results of the treatment-continued group vs. the untreated group, and the treatment-discontinued group, respectively. CI, confidence interval; HCC, hepatocellular carcinoma; HR, hazard ratio.

nodules (2.77 vs. 1.82 per 100 PYs, $p<0.001$) and cirrhosis (1.19 vs. 0.17 per 100 PYs, $p<0.001$) than those adherent to treatment. Additionally, the median APRI (0.28 vs. 0.66, $p<0.001$) and FIB-4 scores (0.94 vs. 2.24, $p<0.001$) increased significantly from the baseline in patients with virological relapse. This is consistent with the results of a 10-year longitudinal observational study that included 894 treatment-naïve CHB patients who received ETV therapy and revealed poor medication adherence was associated with increased mortality and risk of HCC and cirrhotic complications.[15] Moreover, inappropriate discontinuation or intermittent treatment failed to effectively inhibit virological replication and led to virological resistance and low-level viremia.[13,15]

Several reasons were responsible for treatment interruption. By making phone calls and reviewing electronic medical records, we identified some reasons for stopping medication, including lack of awareness of long-term treatment, conflict between the time for oral medicines and daily dietary habits, inability to adhere to long-term therapy, mistaken belief that continuously undetectable serum HBV DNA and normal ALT are signs of medication withdrawal, fear of drug side effects and virological resistance during long-term medication, and concerns of drug interactions because of treatment for other diseases. Data from a previous study revealed that adherence to NAs treatment by CHB patients was about 75% in low-, middle-, and high-income countries.[16] A Chinese study based on a questionnaire survey (Morisky Medication Adherence Scale-8) reported that over 50% of patients had low medication compliance (a score of <6 points) and only 16.5% patients, those with a score of eight points, had high adherence, much lower than the proportions reported by similar studies conducted in other countries such as Australia (74.1%), the USA (66%), and New England (52%).[17] Therefore, increased attention should be paid to patient compliance management. Given the limited effectiveness and increased risk of adverse liver events after drug withdrawal, sustained treatment is recommended.

Continuous antiviral therapy helps delay the progression of liver fibrosis. Liver biopsy is the most accurate method to evaluate liver inflammation and fibrosis, but is challenging to carry out in clinical practice. Therefore, this study used noninvasive methods, APRI and FIB-4, which are recommended by major international guidelines, to assess liver fibrosis.[12,18–20] APRI and FIB-4 have good specificity in excluding significant liver fibrosis and cirrhosis.[14] In this study, the APRI and FIB-4 scores in the continuous treatment group were significantly decreased from baseline, and the proportion of APRI<1.00 ($p=0.244$) and FIB-4<1.45 ($p=0.005$) increased, but changes in the untreated and treatment-discontinued groups were almost opposite. That suggests that the process of liver fibrosis did not stop in patients with normal ALT, and long-term antiviral therapy helped to slow down hidden changes in the liver even in patients with normal ALT. Although the mechanism of the reversal of liver fibrosis at an early stage and delay of cirrhosis by antiviral treatment has not been fully explained, published results based on liver pathology also support the use of long-term standardized antiviral therapy to reverse liver fibrosis and early-stage cirrhosis in some patients.[21–23]

Serum HBV DNA has an important role in the antiviral treatment decision. In a study by Lim *et al*.,[9] the untreated replicative phase group (ALT<ULN, HBV DNA≥2,000 IU/mL) had significantly increased risks of HCC (HR: 1.76; 95% CI: 1.00–3.10, $p=0.05$) and death/transplantation (HR: 2.14; 95% CI: 1.09–4.21, $p=0.03$) compared with the treated active-phase group (ALT≥2ULN, HBV DNA≥2,000 IU/mL). Therefore, early antiviral intervention before ALT elevation

Table 3. Baseline characteristics of patients with HBV DNA≥2,000 IU/mL in the three subgroups[¶]

Characteristic	Entire, *n*=157	Treatment group, *n*=108		Untreated, *n*=49	*p*
		Continued, *n*=74	Discontinued, *n*=34		
Duration of follow-up[*], months	54 (24, 72)	57 (48, 72)	57 (48, 72)	48 (24, 72)	0.006
Duration of treatment[*], months	–	60 (36, 72)	12 (6, 24)	–	<0.001
Age, years	43±12.5	44±8.6	44±9.6	41±11.1	0.194
≥40	96 (61.1%)	50 (67.6%)	21 (61.8%)	25 (51.0%)	0.186
Male	75 (47.8%)	38 (48.6%)	16 (47.1%)	30 (61.2%)	0.311
NA (s) regimen					0.061[#]
ETV	68 (63.0%)	51 (68.9%)	17 (50.0%)	–	
TDF	39 (36.1%)	23 (31.1%)	16 (47.1%)	–	
LDT	1 (0.9%)	0 (0.0%)	1 (2.9%)	–	
HBV DNA, $\log_{10}$ IU/mL	4.11 (3.61, 4.71)	4.08 (3.59, 4.64)	4.25 (3.87, 4.72)	4.11 (3.58, 4.74)	0.368
ALT, U/L	23±7	22±6	22±6	24±9	0.178
ALT≤20	62 (39.5%)	32 (43.2%)	12 (35.3%)	18 (36.7%)	0.687
AST, U/L	24±6	26±6	21±6	24±5	<0.001
PLT, $\times10^9$/L	187±53	179±57	187±51	193±47	0.361
APRI	0.38 (0.29, 0.51)	0.41 (0.32, 0.58)	0.31 (0.23, 0.44)	0.37 (0.27, 0.46)	0.004
<1.00	154 (98.1%)	71 (95.9%)	34 (100.0%)	49 (100.0%)	0.313
1.00–2.00	3 (1.9%)	3 (4.1%)	0 (0.0%)	0 (0.0%)	0.313
>2.00	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	–
FIB-4	1.18 (0.87, 1.70)	1.38 (1.00, 1.99)	0.98 (0.68, 1.52)	1.04 (0.75, 1.52)	0.003
<1.45	99 (63.1%)	38 (51.4%)	25 (73.5%)	36 (73.5%)	0.016
1.45–3.25	52 (33.1%)	31 (41.9%)	9 (26.5%)	12 (24.5%)	0.089
>3.25	6 (3.8%)	5 (56.8%)	0 (0.0%)	1 (2.0%)	0.306

Data are *n* (%), mean±standard deviation, or median (interquartile range), unless otherwise specified. [*]Median (range). [¶]Three subgroups include untreated, treatment-continued, and treatment-discontinued groups. [#]p-value of the treatment-continued vs. -discontinued subgroups. ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; ETV, entecavir; FIB-4, fibrosis-4 score; LDT, telbivudine; NAs, nucleos(t)ide analogues; PLT, platelet; TDF, tenofovir disoproxil fumarate.

Table 4. Incidence of liver nodules, liver cirrhosis, and HCC in patients with HBV DNA<2,000 IU/mL of the three subgroups[¶]

Subgroup	Patient-years	No. of events	No./100 patient-years (95% CI)	*p*
Liver nodules				
Untreated (*n*=18)	243	6	2.47 (1.01–5.56)	0.105[*]
Continued (*n*=13)	494	1	0.20 (0.001–1.30)	–
Discontinued (*n*=6)	380	1	0.26 (0.01–1.69)	0.619[*]
Liver cirrhosis				
Untreated (*n*=18)	273	0	0.0 (0.0–1.73)	0.019[#]
Continued (*n*=13)	534	0	0.0 (0.0–0.89)	0.061[#]
Discontinued (*n*=6)	402	1	0.25 (0.01–1.60)	–
HCC				
Untreated (*n*=18)	274	0	0.0 (0.0–1.72)	0.003[#]
Continued (*n*=13)	540	0	0.0 (0.0–0.88)	0.317[#]
Discontinued (*n*=6)	406	1	0.25 (0.01–1.59)	–

[¶]The three subgroups include untreated, treatment-continued, and treatment-discontinued patients. [*]Treatment-continued group vs. untreated, and treatment-discontinued groups. [#]Treatment-discontinued group vs. untreated, and treatment-continued groups. ALT, alanine aminotransferase; CI, confidence interval; HCC, hepatocellular carcinoma.

was recommended for selected HBeAg-negative CHB patients with high viral loads. Limitations of that study were that active-phase patients were the control group and replicative phase patients were not treated. Hence, the concrete benefit of antiviral therapy is not known if replicative phase patients receive therapy. Our study results fill the gap, finding that for a median follow-up of 60 months, cirrhosis in patients adhering to antiviral treatment was reduced by 91%, with a 92% reduction in patients with an HBV DNA≥2,000 IU/mL compared with their counterparts and no patients developed HCC.

Significant necroinflammation and fibrosis may exist even with a normal ALT, indicating the necessity of antiviral treatment. Zhuang *et al*.[4] assessed changes of liver histology in 327 HBeAg-negative CHB patients with normal ALT levels, finding that an ALT>20 U/L and a HBV DNA≥2,000 IU/mL were independently predictive of significant histopathology. Therefore, we used 20 U/L as the critical stratification value. However, there were no significant difference in the incidence of liver nodules (3.76 vs. 6.91 per 100 PYs, p=0.815) or liver cirrhosis (0.36 vs. 1.43 per 100 PYs, p=0.330) in patients with an ALT<20 U/L (n=26) vs. ALT≥20 U/L (n=43) in the untreated group, indicating the limitation in predicting disease progression by ALT level. The results are not consistent with a previous study that found patients with ALT levels of 0.5–1×ULN had an increased risk of liver-related complications and HCC than those with ALT levels of <0.5×ULN (both p<0.001).[24] Therefore, the results need validation by future studies.

However, patients in the untreated group with HBV DNA≥2,000 IU/mL had a higher incidence of liver cirrhosis than those with HBV DNA<2,000 IU/mL (2.51 vs. 0.0 per 100 PYs, p=0.036), indicating a certain correlation underlying the risk of liver cirrhosis and serum viral load. The correlation between HBV DNA level and disease outcomes has been reported in previous studies.[25,26] Later studies have also shown a higher risk of HCC in patients with higher serum HBV DNA levels.[1,25,27,28] In this study, antiviral treatment, significantly lowered the risks of liver nodules and liver cirrhosis in treated patients with HBV DNA levels ≥2,000 IU/mL compared with untreated patients.

We suggest increasing the weight of serum HBV DNA in antiviral treatment decisions rather than rigidly adhering to ALT level, age, or family history of cirrhosis/HCC, considering HBeAg-negative patients with high viral loads are common in clinical practice, and antiviral therapy decreases the long-term risk of adverse liver events.[5,29,30] Recent Chinese hepatitis B guidelines (version 2022) have lowered the ALT level for initiating treatment to 30 U/L for men and 19 U/L for women.[31] That will enable more patients to meet treatment standards but still fails to include some patients with a high risk of disease progression, such as those with ALT levels below the ULN but with high viral loads ≥2,000 IU/mL.

This study has some limitations. (1) It was a retrospective study and the sample size was relatively small if HCC is taken as endpoint event, thus, the benefits of antiviral therapy may be underestimated. However, when we started this study, only a small number of patients had received long-term (at least 5 years) antiviral therapy following the recommended regular examination and no treatment in most clinical settings. In the beginning, 529 patients with persistent normal ALT were screened, but only 194 cases met the inclusion criteria. Thus, multicenter, prospective studies with a larger sample size and prolonged follow-up time are urgently needed. (2) APRI and FIB-4 replaced liver biopsy to estimate liver fibrosis because many patients were reluctant to accept this invasive examination. In the future, hepatitis B treatment indications will be less dependent on liver biopsy, with more emphasis on high-risk factors, such as viral load. (3) The number of patients with HBV DNA loads <2,000 IU/mL and younger than 30 years of age is small and the benefits of antiviral therapy for those individuals need further confirmation. (4) Our subjects were all Chinese, consequently the results need to be verified in non-Chinese patients.

Conclusions

HBeAg-negative CHB patients with normal ALT levels and detectable HBV DNA can achieve excellent virological response with continuous NA treatment. Sustained antiviral therapy delays the progression of liver fibrosis and significantly decreases the risk of liver nodules and cirrhosis. Therefore, the indications of antiviral therapy should be expanded in that population, especially for those with HBV DNA levels ≥2,000 IU/mL. After treatment is initiated, patient medication compliance needs to be monitored continuously.

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Conflict of interest

EQC has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2018. The other authors declare no conflicts of interest related to this publication.

Author contributions

Study concept and design, data acquisition, analysis, interpretation, and manuscript drafting (JZ, EQC), assistance in data acquisition, analysis, and interpretation (FDW, LQL, YJL, SYW). All authors made significant contributions to this study and approved the final manuscript.

Ethical statement

The design of this study was consistent with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines and was approved by the Institutional Review Board of the West China Hospital of Sichuan University (ChiCTR2100050064).

Data sharing statement

The data used in support of the findings of this study have not been made available because of privacy.

References

[1] GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. GDaIIaP. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392(10159):1789–1858. doi:10.1016/s0140-6736(18)32279-7, PMID:30496104.
[2] Hoang JK, Yang HI, Le A, Nguyen NH, Lin D, Vu VD, *et al*. Lower liver cancer risk with antiviral therapy in chronic hepatitis B patients with normal to minimally elevated ALT and no cirrhosis. Medicine 2016;95(31):e4433. doi:10.1097/md.0000000000004433, PMID:27495067.
[3] Ormeci A, Aydın Y, Sumnu A, Baran B, Soyer OM, Pınarbasi B, *et al*. Predictors of treatment requirement in HBeAg-negative chronic hepatitis B patients with persistently normal alanine aminotransferase and high serum HBV DNA levels. Int J Infect Dis 2016;52:68–73. doi:10.1016/j.ijid.2016.09.007, PMID:27619844.
[4] Duan M, Chi X, Xiao H, Liu X, Zhuang H. High-normal alanine aminotrans-

ferase is an indicator for liver histopathology in HBeAg-negative chronic hepatitis B. Hepatol Int 2021;15(2):318–327. doi:10.1007/s12072-021-10153-2, PMID:33638049.
[5] Wang J, Yan X, Zhu L, Liu J, Qiu Y, Li Y, *et al*. Significant histological disease of patients with chronic hepatitis B virus infection in the grey zone. Aliment Pharmacol Ther 2023;57(5):464–474. doi:10.1111/apt.17272, PMID:36324235.
[6] Oliveri F, Surace L, Cavallone D, Colombatto P, Ricco G, Salvati N, *et al*. Long-term outcome of inactive and active, low viraemic HBeAg-negative-hepatitis B virus infection: Benign course towards HBsAg clearance. Liver Int 2017;37(11):1622–1631. doi:10.1111/liv.13416, PMID:28296013.
[7] Bonacci M, Lens S, Mariño Z, Londoño MC, Rodríguez-Tajes S, Mas A, *et al*. Anti-viral therapy can be delayed or avoided in a significant proportion of HBeAg-negative Caucasian patients in the Grey Zone. Aliment Pharmacol Ther 2018;47(10):1397–1408. doi:10.1111/apt.14613, PMID:29577350.
[8] Koffas A, Kumar M, Gill US, Jindal A, Kennedy PTF, Sarin SK. Chronic hepatitis B: the demise of the 'inactive carrier' phase. Hepatol Int 2021;15(2):290–300. doi:10.1007/s12072-021-10137-2, PMID:33638770.
[9] Choi GH, Kim GA, Choi J, Han S, Lim YS. High risk of clinical events in untreated HBeAg-negative chronic hepatitis B patients with high viral load and no significant ALT elevation. Aliment Pharmacol Ther 2019;50(2):215–226. doi:10.1111/apt.15311, PMID:31135074.
[10] Mason WS, Gill US, Litwin S, Zhou Y, Peri S, Pop O, *et al*. HBV DNA Integration and Clonal Hepatocyte Expansion in Chronic Hepatitis B Patients Considered Immune Tolerant. Gastroenterology 2016;151(5):986–998.e4. doi:10.1053/j.gastro.2016.07.012, PMID:27453547.
[11] Chinese Society of Infectious Diseases CMACSoH, Chinese Medical Association. Guidelines for the prevention and treatment of chronic hepatitis B (version 2022). Zhonghua gan zang bing za zhi 2022;30(12):1309–1331. doi:10.3760/cma.j.cn501113-20221204-00607, PMID:36891718.
[12] Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH. AASLD guidelines for treatment of chronic hepatitis B. Hepatology 2016;63(1):261–283. doi:10.1002/hep.28156, PMID:26566064.
[13] Kim WR, Berg T, Asselah T, Flisiak R, Fung S, Gordon SC, *et al*. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. J Hepatol 2016;64(4):773–780. doi:10.1016/j.jhep.2015.11.012, PMID:26626497.
[14] EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol 2015;63(1):237–264. doi:10.1016/j.jhep.2015.04.006, PMID:25911335.
[15] Shin JW, Jung SW, Lee SB, Lee BU, Park BR, Park EJ, *et al*. Medication Nonadherence Increases Hepatocellular Carcinoma, Cirrhotic Complications, and Mortality in Chronic Hepatitis B Patients Treated With Entecavir. Am J Gastroenterol 2018;113(7):998–1008. doi:10.1038/s41395-018-0093-9, PMID:29880971.
[16] Ford N, Scourse R, Lemoine M, Hutin Y, Bulterys M, Shubber Z, *et al*. Adherence to Nucleos(t)ide Analogue Therapies for Chronic Hepatitis B Infection: A Systematic Review and Meta-Analysis. Hepatol Commun 2018;2(10):1160–1167. doi:10.1002/hep4.1247, PMID:30288470.
[17] Xu K, Liu LM, Farazi PA, Wang H, Rochling FA, Watanabe-Galloway S, *et al*. Adherence and perceived barriers to oral antiviral therapy for chronic hepatitis B. Global health action 2018;11(1):1433987. doi:10.1080/16549716.2018.1433987, PMID:29447614.
[18] Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, *et al*. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10(1):1–98. doi:10.1007/s12072-015-9675-4, PMID:26563120.
[19] European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67(2):370–398. doi:10.1016/j.jhep.2017.03.021, PMID:28427875.
[20] Chinese Society of Infectious Diseases CMACSoH, Chinese Medical Association. The guidelines of prevention and treatment for chronic hepatitis B (2019 version). Zhonghua gan zang bing za zhi 2019;27(12):938–961. doi:10.3760/cma.j.issn.1007-3418.2019.12.007, PMID:31941257.
[21] Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, *et al*. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010;52(3):886–893. doi:10.1002/hep.23785, PMID:20683932.
[22] Li Q, Chen L, Zhou Y. Changes of FibroScan, APRI, and FIB-4 in chronic hepatitis B patients with significant liver histological changes receiving 3-year entecavir therapy. Clin Exp Med 2018;18(2):273–282. doi:10.1007/s10238-018-0486-5, PMID:29350286.
[23] Liu R, Guo J, Lu Y, Zhang L, Shen G, Wu S, *et al*. Changes in APRI and FIB-4 in HBeAg-negative treatment-naive chronic hepatitis B patients with significant liver histological lesions receiving 5-year entecavir therapy. Clin Exp Med 2019;19(3):309–320. doi:10.1007/s10238-019-00560-z, PMID:31111345.
[24] Yuen MF, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, *et al*. Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. Gut 2005;54(11):1610–1614. doi:10.1136/gut.2005.065136, PMID:15871997.
[25] Mendy ME, Welzel T, Lesi OA, Hainaut P, Hall AJ, Kuniholm MH, *et al*. Hepatitis B viral load and risk for liver cirrhosis and hepatocellular carcinoma in The Gambia, West Africa. J Viral Hepat 2010;17(2):115–122. doi:10.1111/j.1365-2893.2009.01168.x, PMID:19874478.
[26] Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130(3):678–686. doi:10.1053/j.gastro.2005.11.016, PMID:16530509.
[27] Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, *et al*. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. Jama 2006;295(1):65–73. doi:10.1001/jama.295.1.65, PMID:16391218.
[28] Kim GA, Han S, Choi GH, Choi J, Lim YS. Moderate levels of serum hepatitis B virus DNA are associated with the highest risk of hepatocellular carcinoma in chronic hepatitis B patients. Aliment Pharmacol Ther 2020;51(11):1169–1179. doi:10.1111/apt.15725, PMID:32291781.
[29] Zhou J, Wang F, Li L, Chen E. Expanding antiviral therapy indications for HBeAg-negative chronic hepatitis B patients with normal ALT and positive HBV DNA. Precis Clin Med 2022;5(4):pbac030. doi:10.1093/pcmedi/pbac030, PMID:36519139.
[30] Huang DQ, Li X, Le MH, Le AK, Yeo YH, Trinh HN, *et al*. Natural History and Hepatocellular Carcinoma Risk in Untreated Chronic Hepatitis B Patients With Indeterminate Phase. Clin Gastroenterol Hepatol 2021;20(8):1803–1812.e5. doi:10.1016/j.cgh.2021.01.019, PMID:33465482.
[31] Martin P, Nguyen MH, Dieterich DT, Lau DT, Janssen HLA, Peters MG, *et al*. Treatment Algorithm for Managing Chronic Hepatitis B Virus Infection in the United States: 2021 Update. Clin Gastroenterol Hepatol 2022;20(8):1766–1775. doi:10.1016/j.cgh.2021.07.036, PMID:34329775.