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Association of dynamic changes in serum levels of HBV DNA and risk of hepatocellular carcinoma

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Abstract

Background and aims: We aimed to examine the risk of HCC associated with the long-term change patterns in HBV DNA levels.

Methods: We conducted a longitudinal study of 6,301 participants with chronic HBV infection (CHB) from October 2012 to June 2019 and measured serum levels of HBV DNA at enrollment and during follow-up. The dynamic change patterns of HBV DNA were identified by group-based trajectory models. The associations between change patterns of HBV DNA and HCC were estimated using Cox regression models.

Results: During 35,112 person-years of follow-up, 182 participants developed HCC (518.34 per 10⁵ person-years). Five trajectory groups of repeated measurement of HBV DNA were identified. The risk of HCC was significantly higher for the “high, fast-declined” group whose HBV DNA spontaneously decreased from > 2000 IU/mL at baseline compared with those with persistent undetected HBV DNA (reference group; 963.96 per 10⁵ person-years, HR = 2.62, 95% CI, 1.82 to 3.77, $P < 0.001$). In addition, the “rebound” group whose HBV DNA level increased from undetectable level to > 20,000 IU/mL from baseline to the end of follow-up also showed an obviously higher cumulative HCC incidence rate (1193.29 per 10⁵ person-years, HR = 4.17, 95% CI, 1.87 to 9.31, $P < 0.001$). The positive association remained stable after taking the potential effect of time-dependent antiviral treatment into account.

Conclusions: Significant variability in serum levels of HBV DNA presented during long-term follow-up. Regular monitoring of serum levels of HBV DNA and antiviral treatment are required for the clinical management of CHB patients, as well as those with undetected HBV DNA.

Keywords: Chronic HBV infection, HBV DNA, Dynamic changes, Hepatocellular carcinoma (HCC)

1 Introduction

Hepatocellular carcinoma (HCC), accounting for > 80% of primary liver cancers, was estimated to have been the fourth leading cause of cancer-related death worldwide (Yang et al. 2019). The up-to-date incidences of HCC

vary significantly around the world. In China, although the country contains less than 20% of the world's population, it experiences almost half of all new liver cancer cases (World Health Organization 2018). Hepatitis B virus (HBV) infection is the key determinants of HCC in China, which has affected 90 million people (de Martel et al. 2015; Xiao et al. 2019).

A number of studies have demonstrated a dose-response increase in HCC risk with initial serum HBV DNA levels and recognized increasing serum HBV DNA level as an independent predictor of HCC (Chen et al. 2006; Goedert 2005). Although its prognostic

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implication have been assessed, it is important to note that HBV DNA level is a complex, dynamic variable that changes throughout the natural course of chronic HBV infection. However, most of the previous studies took only one single HBV DNA measurement at enrollment into account, rather than providing a repository of longitudinal quantitative data. A previous study that examined long-term trajectories of HBV DNA and HCC risk showed that spontaneous HBV DNA decrease over time reduces HCC incidence compared with individuals with persistently high viral loads (Chen et al. 2011). However, there was still a lack of antiviral information during long-term follow-up. It should be noticed that time-dependent confounding affected by previous treatment would introduce substantial bias in this longitudinal study. Furthermore, patients with HBV DNA > 20,000 IU/mL (high viral load) are recognized as high-risk group and are recommended to start early antiviral treatment. However, the change patterns of viral load and HCC risk of patients with HBV DNA under limit of detection is not adequately assessed yet, as well as the differing natural histories of each patient.

The aim of this study was to classify infected patients into groups based on their serum HBV DNA trajectories and examine risk for HCC associated with the patterns of long-term changes in HBV DNA levels. HBV DNA trajectories could play a role in realizing the precision medicine and clinical management approach to the infection.

2 Subjects and methods

2.1 Study population

The chronic HBV infection (CHB) cohort was based on a community-based study of National Major S&T Project (Zhu et al. 2018; Zhu et al. 2019). As shown in Fig. 1, from September 2009 to November 2010, 7,250 inhabitants aged > 1 years old residing in Jiangsu province retested positive for hepatitis B surface antigen (HBsAg) and negative for antibody against Hepatitis C virus (anti-HCV) were included in this community-based CHB cohort.

We then excluded participants diagnosed with HCC at baseline, with a history of antiviral treatment or lack of antiviral treatment information in 2010, with lack of information of HBV DNA at baseline or only have one-round data on HBV DNA during follow-up, leaving 6,301 participants for the further analysis (Fig. 1).

2.2 Investigation and testing

All of the 6,301 participants were interviewed with a structured questionnaire administered by trained physicians, nurses, or village doctors at study entry, which collected information on the participants' demographic characteristics, lifestyle habits (history of alcohol drinking and cigarette smoking), chronic disease history,

antiviral treatment (i.e., interferon therapy or oral nucleos(t)ide analogue therapy).

Anthropometric indexes (height and weight) were measured by our trained staff. Five mL of overnight fasting blood sample was collected at study entry and the following four visits. Anti-HCV sero-status was tested at study entry. Alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, albumin, HBsAg, hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe), and HBV DNA were tested at study entry and the following four visits. Detailed measurement platform and diagnostic criteria were described previously (Zhu et al. 2018; Zhu et al. 2019).

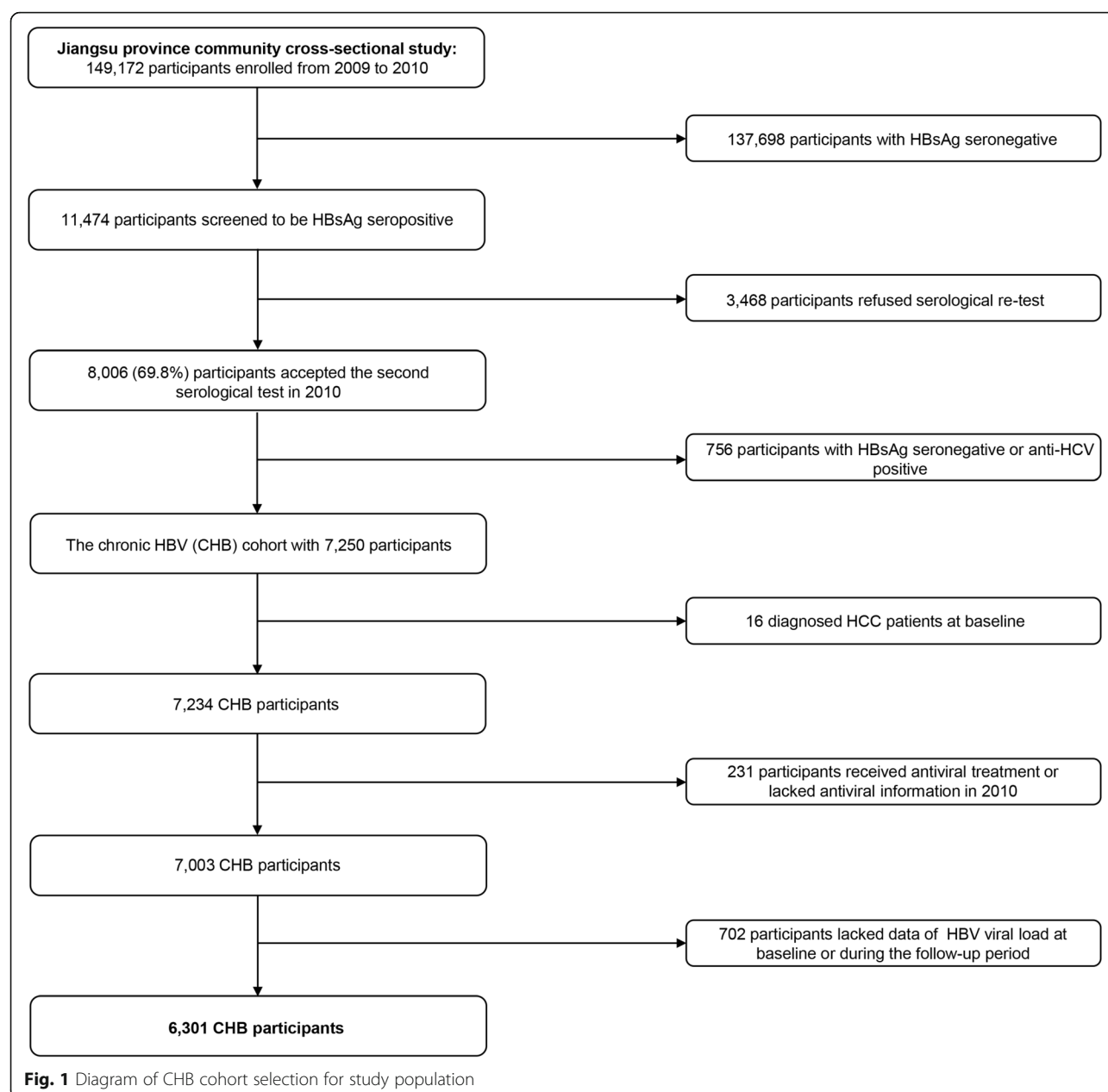
2.3 Outcome follow-up

All cohort participants were followed up in the year of 2013, 2014, 2016 and 2018 in the local health center. The mean years of follow-up is 5.57 (standard deviation = 1.51) years. HCC diagnosis was obtained from the chronic diseases registry system established by the local centers for disease control (CDC). To further verify the diagnosis information, participants themselves or their family members were asked to provide all the hospitalizations records of all kinds of diseases during the follow-up period from 2012 to 2018. We then verified their cancer diagnosis with the admitted hospital according to the International Classification of Diseases for Oncology (ICD-O, ICD-10). HBsAg seroclearance was defined as an individual tested seronegative at the first instance and remained seronegative at the next visit. HBeAg seroconversion was defined as loss of serum HBeAg accompanied by the appearance of anti-HBe. Body mass index (BMI) was classified into four levels in Chinese population: thin (< 18.5 kg/m²), normal weight (18.5–24 kg/m²), overweight (24–28 kg/m²) and obesity (> 28 kg/m²).

2.4 Statistical analysis

Continuous variables were expressed as means ± standard deviation (s), and tested using Welch's t-test, Wilcoxon rank-sum (Mann-Whitney) test and the Kruskal-Wallis H test as appropriate; categorical variables were presented in percentages and tested using Chi-squared test and Fisher's exact test as appropriate. Person-years of follow-up were calculated as the time from the date of enrolment to the date of HCC diagnosis or first date recorded for having been had HCC, whichever was earlier, the date at death or the last date of available data, whichever came first.

To identify subgroups that followed homogenous longitudinal dynamic patterns of HBV DNA levels, group-based trajectory modeling was used to identify subgroups based on HBV DNA trajectories during the follow-up period. Group-based trajectory modeling is a



specialized application of finite mixture modeling and is used to identify groups of individuals following similar trajectories for a particular variable of interest, the HBV DNA level (Nagin 2014; Nagin and Odgers 2010). The Bayesian information criterion (BIC) was used for model selection, which penalizes more complex models to ensure that a well-fitting yet parsimonious model is chosen.

The log-rank test was used to compare the Kaplan-Meier estimate curves for incidence of HCC, HBsAg seroclearance and HBeAg seroconversion. Multivariable Cox regression models were used to determine the associations between trajectory group membership and

HCC, HBsAg seroclearance and HBeAg seroconversion incidence, when controlling for age at baseline (≤ 39.9 , 40.0–49.9, 50.0–59.9, ≥ 60), gender (female or male), smoking status (yes or no), drinking status (yes or no), HBeAg status at baseline (positive or negative), ALT at baseline (continuous), AST at baseline (continuous), albumin level at baseline (continuous), family history of HBV infection (yes or no), and antiviral treatment at baseline (yes or no).

The medians and quartiles of repeated measurements for ALT and AST were represented in a box and line plot. The multivariable linear mixed model (LMM) by means of maximum likelihood was used to compare

dynamic change feature and concentration for the longitudinal data of ALT and AST among different trajectory groups. The fixed-effect adjustments were same as adjustments for Cox regression model except ALT and AST at baseline, when the random-effect adjustments were individuals and years of time.

2.5 Sensitivity analyses

In the observational longitudinal study, when a time-dependent covariate is not only a risk factor for the outcome of interest and predictor of subsequent exposure but also is affected by previous exposure history, it is called time-dependent confounder (Robins et al. 2000). Exactly, antiviral treatment is such a time-dependent confounder because antiviral treatment has been proven to lower HCC incidence risk and can be affected by the factors relevant to HCC incidence (European Association for the Study of the Liver 2017; Tseng et al. 2019). The other important problem was informative right censoring caused by loss to follow-up. Marginal structural models (MSMs) with inverse probability of treatment weighted (IPTW) was used to eliminate the potential effect of time-dependent antiviral treatment and censoring. The cohort participants were assigned two weight, one proportional to the adjusted probability that treatment for HBV begins and the other one of remaining uncensored. Age was incorporated in the systematic component of logistic regression model to derive the adjusted treatment and censoring probability at each time point. The final pooled weighted logistic regression model weighting the observation in each subject-age and synthetically estimating the effect of treatment and censoring on the casual relationship was obtained by the product of the two conditional probability. Through fitting these models, the confounding effect was controlled to verify the result of causality between trajectory groups and HCC.

Statistical significances of all tests were defined as $P < 0.05$ by 2-tailed tests. All analyses were performed using STATA software (version 15; StataCorp, College Station, TX, USA).

3 Results

3.1 Baseline characteristics of the CHB cohort

During the 35,112 person-years of follow-up, 182 incident HCC cases were ascertained with an incidence rate of 518.34 per 10^5 person-years. The baseline characteristics of the HCC patients are described in Table 1. Compared with those non-HCC CHB participants, the HCC patients were prone to be older (58.3 ± 10.5 vs. 50.9 ± 13.1), males (68.7% vs. 46.8%), smokers (42.3% vs. 27.8%, $P < 0.001$), HBeAg positive (21.4% vs. 10.6%, $P < 0.001$), anti-HBe negative (39.6% vs. 21.4%, $P < 0.001$); the HCC patients had a higher proportion of abnormal elevated

level of ALT (30.2% vs. 14.3%, $P < 0.001$) and AST (43.7% vs. 19.3%, $P < 0.001$), a lower level albumin (45.8 ± 5.1 vs. 48.1 ± 63.1 , $P < 0.001$), and a history of antiviral treatment (29.7% vs. 11.6%, $P < 0.001$) and family history of HBV infection (75.3% vs. 65.9%, $P = 0.008$). Besides, higher serum HBV DNA levels were associated with higher HCC incidence rate, and the participants with HBV DNA > 2000 IU/mL showed the highest incidence rate (963.96 per 10^5 person-years). Notably, the participants with HBV DNA under the limit of detection (LOD, ≤ 100 IU/mL) maintained a considerable HCC incidence at 371.48 per 10^5 person-years, while the participants with HBV DNA at 100–2000 IU/mL presented a lower incidence (261.52 per 10^5 person-years). The results suggested that in addition to the individuals with high viral load, the HCC risks of those with HBV DNA below the LOD are yet matters worthy of attention.

3.2 Group-based HBV DNA trajectory modeling

Analysis of group-based trajectory models based on three classes of baseline HBV DNA levels showed that the five-group linear function model had the optimal fit: group A and group B for the class with baseline HBV DNA levels below the LOD; group C and group D for the class with baseline HBV DNA levels between 100 and 2000 IU/mL; group E for the class with baseline HBV DNA levels over 2000 IU/mL (Fig. 2A). Group A ($n = 2,564$, 40.7%) was characterized with persistent HBV DNA levels below the LOD (hereafter denoted as “sustaining undetected” participants); group B ($n = 108$, 1.7%) was distinguished by a “bounce” trend of HBV DNA levels increasing from undetectable level to $> 20,000$ IU/mL from baseline to the sixth year of follow-up (hereafter denoted as “rebound” participants); group C was composed of 1,031 (16.4%) participants whose HBV DNA levels gradually decreased from 100 to 2000 IU/mL to undetectable level during the follow-up period (hereafter denoted as “medium, slow-declined” participants); group D ($n = 675$, 10.7%) was characterized by gently elevated HBV DNA levels from 100 to 2000 IU/mL at baseline (hereafter denoted as “medium, slow-rise” participants); group E ($n = 1,923$, 30.5%) had the participants with high presenting HBV DNA levels (> 2000 IU/mL) at baseline and decrease at a faster rate during the follow-up period (hereafter denoted as “high, fast-declined” participants).

Baseline characteristics of the five trajectory groups are displayed in Table 2. When compared with other groups, the “high, fast-declined” group was characterized with a worse viral and biochemical profile of which participants were prone to be males (50.6%), had a much higher proportion of HBeAg positive (25.9%) and a lower proportion of anti-HBe positive (64.5%), had a higher proportion of abnormal ALT (28.8%) and AST (32.9%)

Table 1 Baseline characteristics of HCC and non-HCC patients in chronic HBV participants cohort

Characteristic Variable	All subjects (n = 6301)	Incidence rate (per 10 ⁵ PY)	HCC (n = 182)	non-HCC (6119)	P ^a
Age, mean ± s	51.1 ± 13.1	518.34	58.3 ± 10.5	50.9 ± 13.1	< 0.001 ^{b*}
Gender					< 0.001 [*]
Female	3315 (52.6)	304.17	57 (31.3)	3258 (53.2)	
Male	2986 (47.4)	763.46	125 (68.7)	2861 (46.8)	
Smoker					< 0.001 [*]
No	4522 (71.8)	414.96	105 (57.7)	4417 (72.2)	
Yes	1779 (28.2)	785.06	77 (42.3)	1702 (27.8)	
Alcohol drinker					0.283
No	4682 (74.3)	495.66	129 (70.9)	4553 (74.4)	
Yes	1619 (25.7)	583.30	53 (29.1)	1566 (25.6)	
Family history of HBV infection (parents)					0.008 [*]
No	2132 (33.8)	385.74	45 (24.7)	2087 (34.1)	
Yes	4169 (66.2)	584.32	137 (75.3)	4032 (65.9)	
BMI (kg / m²), n = 6044					0.379 ^c
Underweight (< 18.5)	220 (3.6)	516.64	6 (3.4)	214 (3.7)	
Normal weight (18.5–24.9)	3665 (60.6)	554.22	113 (63.8)	3552 (60.5)	
Overweight (25–29.9)	1857 (30.7)	518.33	54 (30.5)	1803 (30.7)	
Obesity (≥30)	302 (5.0)	232.27	4 (2.3)	298 (5.1)	
HBV DNA(IU / mL)					< 0.001 [*]
≤ 100	2672 (42.4)	371.48	56 (30.8)	2616 (42.7)	
100–2000	1706 (27.1)	261.52	25 (13.7)	1681 (27.5)	
> 2000	1923 (30.5)	963.96	101 (55.5)	1822 (29.8)	
HBeAg, n = 6299					< 0.001 [*]
No	5614 (89.1)	454.26	143 (78.6)	5471 (89.4)	
Yes	685 (10.9)	1076.11	39 (21.4)	646 (10.6)	
Anti-HBe, n = 6299					< 0.001 [*]
No	1383 (22.0)	959.97	72 (39.6)	1311 (21.4)	
Yes	4916 (78.0)	398.50	110 (60.4)	4806 (78.6)	
ALT (U / L), n = 6300					< 0.001 [*]
Normal	5370 (85.2)	422.14	127 (69.8)	5243 (85.7)	
≥ 40	930 (14.8)	1095.39	55 (30.2)	875 (14.3)	
AST, n = 6277					< 0.001 [*]
Normal	5024 (80.0)	361.26	102 (56.4)	4922 (80.7)	
≥ 40	1253 (20.0)	1170.62	79 (43.7)	1174 (19.3)	
Albumin, mean ± s, n = 6277	48.0 ± 62.2	518.34	45.8 ± 5.1	48.1 ± 63.1	< 0.001 ^{d*}
Antiviral treatment					< 0.001 [*]
No	5540 (87.9)	412.72	128 (70.3)	5412 (88.5)	
Yes	761 (12.1)	1317.76	54 (29.7)	707 (11.6)	

Abbreviations: ALT Alanine aminotransferase, AST Aspartate aminotransferase, anti-HBe Antibody to hepatitis B e antigen, BMI Body mass index, HBeAg Hepatitis B e antigen, HBV Hepatitis B virus, HCC Hepatocellular carcinoma, s standard deviation

^aChi-square test for categorical variables. ^bWelch's t-test for continuous variables. ^cFisher's exact test for categorical variables. ^dWilcoxon rank-sum (Mann-Whitney) test for continuous variables. *P < 0.05 represents significant difference between HCC and non-HCC

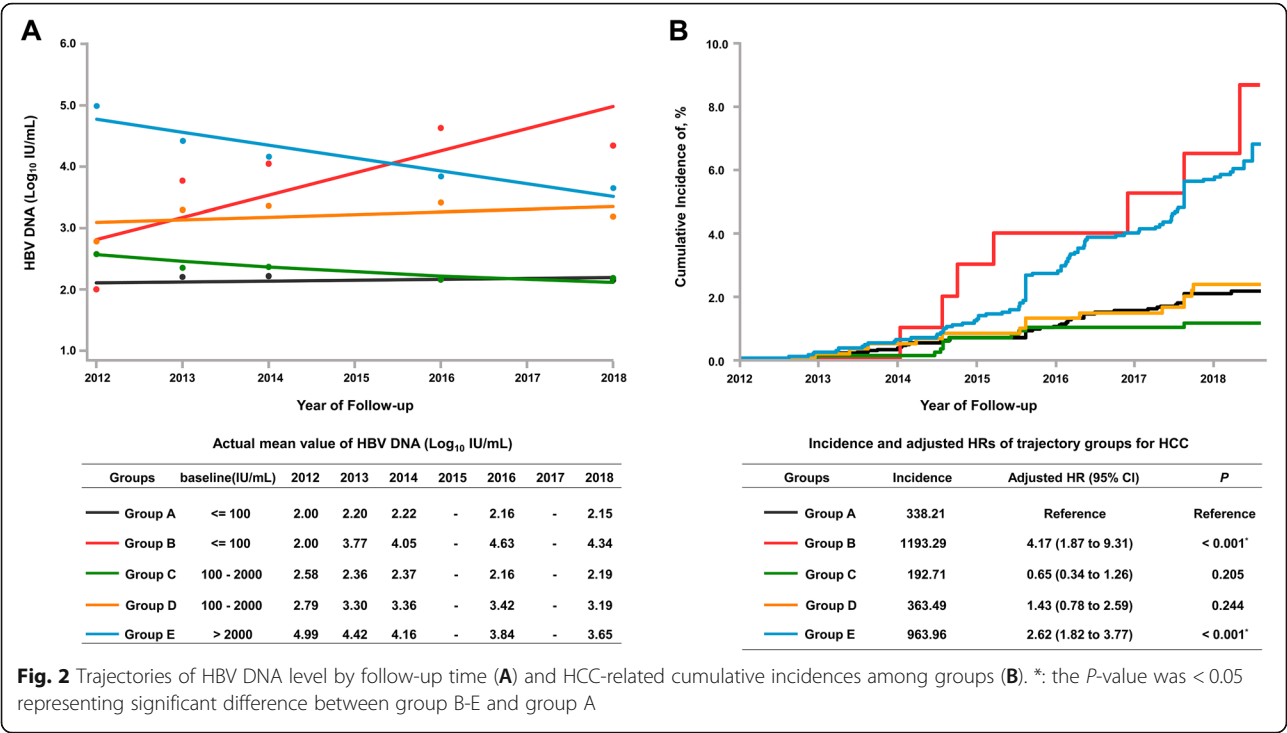


Fig. 2 Trajectories of HBV DNA level by follow-up time (A) and HCC-related cumulative incidences among groups (B). *: the *P*-value was < 0.05 representing significant difference between group B-E and group A

levels, and had a lower level albumin (46.3 ± 3.7 g/L). In addition, we noticed that the “rebound” group was characterized by younger age (49.0 ± 13.1 years old) and low albumin (46.4 ± 3.9 g/L), had a higher proportion of family history of HBV infection (71.3%) and antiviral treatment experience (17.6%), and had a prominent proportion of HBeAg positivity (5.6%) and elevated level of ALT (19.4%).

3.3 Outcome comparison between trajectory groups

Figure 2B shows the cumulative incidence of HCC by trajectory groups of HBV DNA. The “sustaining undetected” group with a cumulative HCC incidence of 338.21 per 10^5 person-years was set as the reference group. The “high, fast-declined” group presented a significantly higher risk of HCC (963.96 per 10^5 person-years, HR = 2.62, 95% CI, 1.82 to 3.77, $P < 0.001$) when compared with the reference group. Interestingly, the “rebound” group also showed an obviously higher cumulative HCC incidence rate (1193.29 per 10^5 person-years, HR = 4.17, 95% CI, 1.87 to 9.31, $P < 0.001$) when compared with the reference group. However, there were no statistical differences in HCC incidence rate between the “medium, slow-declined” and “medium, slow-rose” group and the reference group ($P = 0.205$ and 0.244).

We further analyzed the repeated measurement concentration of ALT and AST, the most commonly used serum-biomarkers of liver cell injury in clinical practice. Figure 3 shows the secular trend for the

mean values of ALT and AST from baseline to 2018. Visual inspection showed a slight ALT and AST fluctuation around the mean value for the “sustaining undetected”, “medium, slow-declined” and “medium, slow-rose” groups. The linear mixed model analysis showed that the “rebound” group had higher ALT and AST levels than the reference group, and a noteworthy increase in the ALT and AST value was observed for the “rebound” group when compared with the reference group (slope for ALT = 4.23, 95% CI, 2.24 to 6.22, $P < 0.001$, $P_{\text{for interaction}} < 0.001$; slope for AST = 1.69, 95% CI, 0.84 to 2.53, $P < 0.001$, $P_{\text{for interaction}} < 0.001$). Besides, although the mean values of ALT and AST in the “high, fast-declined” group were higher than those of the reference group, a declined tendency was presented during the follow-up period (slope for ALT = -0.54, 95% CI, -1.01 to -0.07, $P = 0.024$, $P_{\text{for interaction}} = 0.004$; slope for AST = -1.16, 95% CI, -1.36 to -0.96, $P < 0.001$, $P_{\text{for interaction}} < 0.001$).

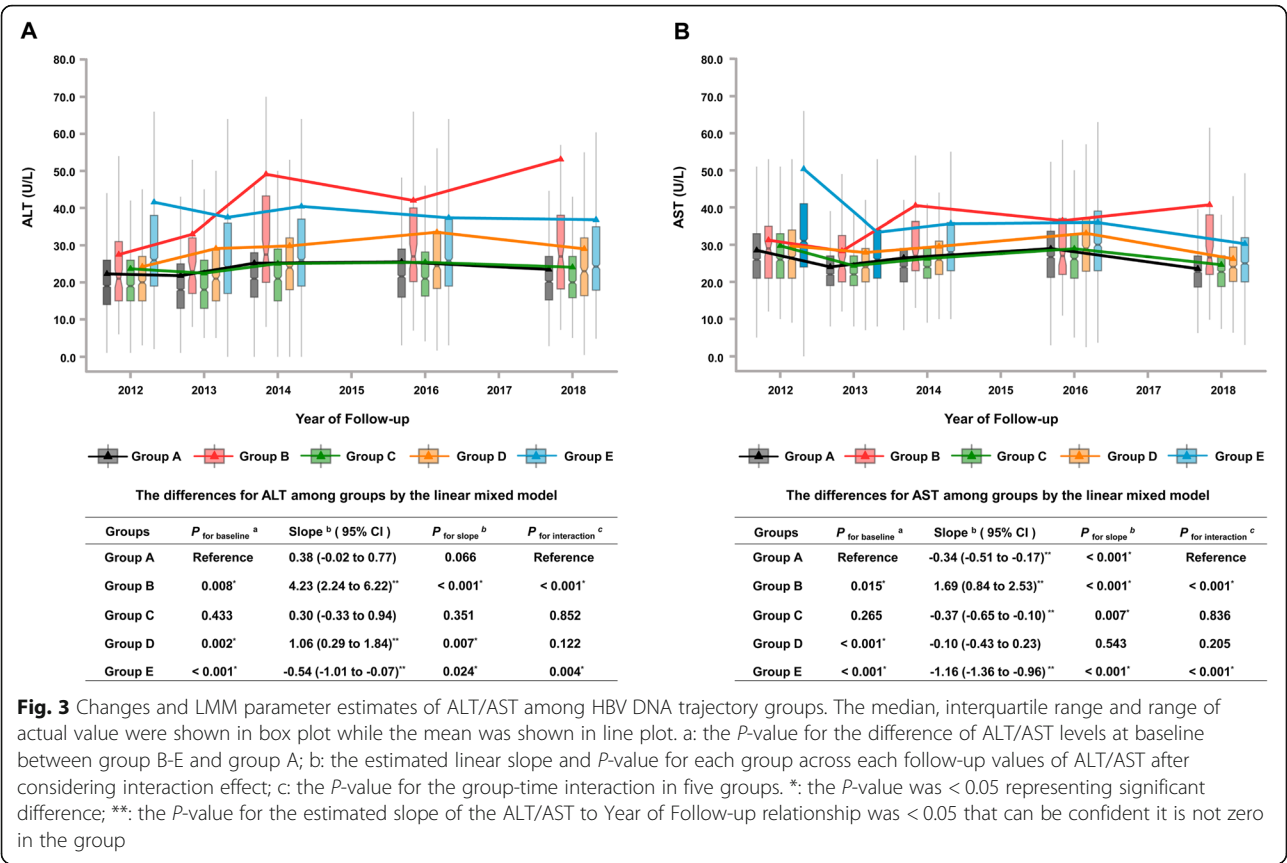
HBsAg seroclearance and HBeAg seroconversion have been shown to confer favorable outcomes and are strong predictors of prolonged survival. As shown in Figure S1, the “medium, slow-declined” and “high, fast-declined” group presented a lower incidence of HBsAg seroclearance when compared with the reference group. The “high, fast-declined” group also showed a lower incidence of HBeAg seroconversion (HR = 0.37, 95% CI, 0.28 to 0.48, $P < 0.001$). Although no statistically significant difference, we observed that

Table 2 Demographic characteristics of participants according to HBV DNA trajectory groups

Characteristic Variable	Group A (n = 2564, 40.7%)	Group B (n = 108, 1.7%)	Group C (n = 1031, 16.4%)	Group D (n = 675, 10.7%)	Group E (n = 1923, 30.5%)	P ^a
Age at baseline, mean ± s	52.5 ± 13.1	49.0 ± 13.1	51.8 ± 12.8	49.5 ± 11.7	49.7 ± 13.5	< 0.001 ^{b*}
Age, yrs						< 0.001 [*]
≤ 39.9	459 (17.9)	25 (23.2)	191 (18.5)	144 (21.3)	460 (23.9)	
40–49.9	707 (27.6)	34 (31.5)	317 (30.8)	243 (36.0)	561 (29.2)	
50–59.9	625 (24.4)	28 (25.9)	243 (23.6)	150 (22.2)	450 (23.4)	
≥ 60	773 (30.2)	21 (19.4)	280 (27.2)	138 (20.4)	452 (23.5)	
Gender						0.016 [*]
Female	1380 (53.8)	56 (51.9)	568 (55.1)	361 (53.5)	950 (49.4)	
Male	1184 (46.2)	52 (48.2)	463 (44.9)	314 (46.5)	973 (50.6)	
Smoker						0.846
No	1836 (71.6)	73 (67.6)	748 (72.5)	488 (72.3)	1377 (71.6)	
Yes	728 (28.4)	35 (32.4)	283 (27.5)	187 (27.7)	546 (28.4)	
Alcohol drinker						0.520
No	1894 (73.9)	85 (78.7)	774 (75.1)	489 (72.4)	1440 (74.9)	
Yes	670 (26.1)	23 (21.3)	257 (24.9)	186 (27.6)	483 (25.1)	
Family history of HBV infection (parents)						0.038 [*]
No	843 (32.9)	31 (28.7)	339 (32.9)	216 (32.0)	703 (36.6)	
Yes	1721 (67.1)	77 (71.3)	692 (67.1)	459 (68.0)	1220 (63.4)	
Baseline BMI (kg / m ²), n = 6044						0.219
< 18.5	90 (3.6)	8 (7.6)	30 (3.1)	15 (2.3)	77 (4.2)	
18.5–25	1497 (60.5)	58 (55.2)	594 (60.9)	397 (60.7)	1119 (61.0)	
25–30	747 (30.2)	34 (32.4)	306 (31.4)	209 (32.0)	561 (30.6)	
≥ 30	139 (5.6)	5 (4.8)	46 (4.7)	33 (5.1)	79 (4.3)	
Baseline HBeAg, n = 6299						< 0.001 [*]
No	2451 (95.7)	102 (94.4)	985 (95.5)	650 (96.3)	1426 (74.2)	
Yes	111 (4.3)	6 (5.6)	46 (4.5)	25 (3.7)	497 (25.9)	
Baseline anti-HBe, n = 6299						< 0.001 [*]
No	434 (16.9)	25 (23.2)	132 (12.8)	110 (16.3)	682 (35.5)	
Yes	2128 (83.1)	83 (76.8)	899 (87.2)	565 (83.7)	1241 (64.5)	
Baseline ALT (U / L), n = 6300						< 0.001 [*]
Normal	2368 (92.4)	87 (80.6)	932 (90.4)	614 (91.0)	1369 (71.2)	
≥ 40	196 (7.6)	21 (19.4)	99 (9.6)	61 (9.0)	553 (28.8)	
Baseline AST (U / L), n = 6277						< 0.001 [*]
Normal	2213 (86.6)	90 (83.3)	872 (85.2)	562 (83.6)	1287 (67.1)	
≥ 40	343 (13.4)	18 (16.7)	151 (14.8)	110 (16.4)	631 (32.9)	
Baseline albumin (g / L), mean ± s, n = 6277	49.9 ± 97.4	46.4 ± 3.9	47.0 ± 3.2	47.3 ± 2.9	46.3 ± 3.7	< 0.001 ^{b*}
Baseline antiviral treatment						< 0.001 [*]
No	2283 (89.0)	89 (82.4)	943 (91.5)	596 (88.3)	1629 (84.7)	
Yes	281 (11.0)	19 (17.6)	88 (8.5)	79 (11.7)	294 (15.3)	

Abbreviations: ALT Alanine aminotransferase, AST Aspartate aminotransferase, anti-HBe Antibody to hepatitis B e antigen, BMI Body mass index, HBeAg hepatitis B e antigen, HBV Hepatitis B virus, HCC Hepatocellular carcinoma, s standard deviation

^aTwo-sided chi-square test for categorical variables. ^bthe Kruskal-Wallis H test for continuous variables. *P < 0.05 represents significant difference among groups



the “rebound” group was yet at a low incidence of HBsAg seroclearance and HBeAg seroconversion.

3.4 Sensitivity analysis

To avoid the bias caused by the time-dependent confounders including antiviral treatment and right informative censoring, the Cox proportional hazards (Cox-PH) Marginal Structural Model with the inverse probability of treatment and censoring weighted (IPTCW) was further used. The higher risk of HCC was yet observed in the “rebound” and “high, fast-declined” group when compared to the reference group (HR, 6.41, 95% CI, 1.17 to 35.23, P = 0.033; HR, 3.09, 95% CI, 1.50 to 6.34, P = 0.002, respectively) (Supplementary Table 1).

4 Discussion

In this community-based cohort study of CHB carriers, we intended to use detailed information on individual characteristics and extensive follow-up data to delineate the change patterns of HBV DNA levels and its association with HCC incidence. Participants with rebounded viral load during follow-up had a significant elevated risk of HCC compared to those with persistent undetected HBV DNA levels. In other word, participants with similar HBV DNA levels at baseline had varying degrees of HCC risk depending on their HBV DNA levels during follow-up. Our findings suggest the necessity of regular monitoring of serum HBV DNA levels for the clinical management of CHB carriers.

Indeed, CHB patients with viral loads > 2000 IU/mL at enrollment had a high risk of future HCC, even after adjustment for age, gender, smoking and drinking status, HBeAg status, ALT and AST, albumin, family history of HBV infection and history of antiviral treatment. This is consistent with the previous studies which found that baseline HBV DNA level was the independent predictor of HCC (Chan et al. 2008; Wong et al. 2010; Yu et al. 2005). In our study, the “high, fast-declined” participants were characterized by a decreased viral load and unstable ALT and AST level. Possibly, a large proportion of the “high, fast-declined” participants was at the immune active phase. Their high HCC risk may be attributable to the liver cells damage resulting from immune system responding to HBV infected cells and the integration that HBV DNA into the host genome.

Most of the participants enrolled in our study were grouped in the “sustaining undetected” group (2,564, 40.7%). The “sustaining undetected” participants were characterized with persistent undetectable viral load and HBeAg seroconversion. The “sustaining undetected” participants were at a low level of HCC risk and therefore set as the reference group in our study.

As explained, virologic response is regarded as a crucial endpoint for measuring antiviral efficacy in CHB patients. Sustained suppression of virus replication has been associated with improvements in clinical outcomes, including development of cirrhosis and cancer (Chen et al. 2006; Yu et al. 2017).

More importantly, we observed that some participants with undetectable HBV DNA at enrollment had a rebounded viral load during follow-up. That means the “rebound” participants were probably at the reactivate phase and they presented an absolute high risk of HCC. HBV reactivation starts with viral replication, followed by liver injury that results from a delayed immune reconstitution. The severity of liver injury varies greatly among individuals, ranging from an asymptomatic rise in ALT levels to severe hepatitis, or even liver failure (Hoofnagle 2009). Considering that antiviral treatment and right informative censoring as time-dependent covariates, we used MSMs with IPTW to simulate a pseudo scenario of no treatment and no loss to follow-up where the causal effect of the exposure factors (change patterns of HBV DNA) on the event of interest (incidence of HCC) could be reliably verified. The sensitivity analysis further validated positive relationship between the “rebound” virus load trend with HCC incidence. In a word, our study suggested that low-level viremia still remained a noteworthy issue (Kim et al. 2017). The recent American Association for the Study of Liver Diseases guidance suggests that patients with low-level viremia (< 2000 IU/mL) with antiviral treatment should also continue monotherapy (Terrault et al. 2018). Our study further evidenced that patients with low-level viremia should also be closely monitored.

We also found a similar overall growth tendency of ALT and AST in the “rebound” group. Liver cell damages caused by immune system responding to HBV infected cells and the integration that HBV DNA into the host genome can be reflected by ALT and AST, intracellular enzymes that are released after cell injury or death (World Health Organization 2015). The indicative function of elevated ALT and AST for HBV-related HCC has been well recognized (Hernaiz et al. 2013; Wu et al. 2008). Therefore, it is reasonable to speculate that virus reactivation happens primarily by promoting hepatocytic inflammation, which indirectly leads to malignant transformation.

Our study has a few limitations. First, the limitation of detection was set as 100 IU/ml in our study. A more sensitive detection platform is needed to identify the participants with “undetected” HBV DNA in the further follow-up. Second, because of the single-center nature, our study has limitations for generalising the results. Further studies in patients with a different ethnicity and mode of transmission are warranted.

In conclusion, a dynamic change in serum levels of HBV DNA is an independent risk predictor of HCC. Re-activation may occur among patients with undetected baseline HBV DNA, along with an elevated HCC risk. Regular monitoring of serum levels of HBV DNA and antiviral treatment are required for the clinical management of CHB patients, as well as those with undetected HBV DNA.

Abbreviations

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; anti-HBe: Antibody to hepatitis B e antigen; anti-HCV: Antibody against Hepatitis C virus; BIC: Bayesian information criterion; BMI: Body mass index; CHB cohort: Chronic hepatitis B virus cohort; HBeAg: Hepatitis B e antigen; HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; ICD: The International Classification of Diseases; LMM: Linear mixed model; LOD: The limit of detection; PY: Person-years; s: Standard deviation

5 Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44194-022-00008-9>.

Additional file 1: Supplemental Table 1. The causal effect of dynamic HBV DNA on HCC by Cox-PH marginal structural model with IPTW. ^a: Covariates for calculating the numerator of inverse probability of treatment weighted included age at baseline (<39.9, 40.0–49.9, 50.0–59.9, ≥60), gender (female or male), smoking status (yes or no), drinking status (yes or no), and HBV DNA trajectories (group A-E); covariates for the denominator of inverse probability of treatment weighted were same as the numerator plus HBeAg status (positive or negative), ALT (continuous), and AST (continuous). Covariates for calculating the numerator of inverse probability of censoring weighted included age at baseline (<39.9, 40.0–49.9, 50.0–59.9, ≥60), gender (female or male), smoking status (yes or no), drinking status (yes or no), family history of HBV infection (yes or no), and HBV DNA trajectories (group A-E); covariates for the denominator of inverse probability of censoring weighted were same as the numerator plus HBsAg status (positive or negative), HBeAg status (positive or negative), ALT (continuous), AST (continuous), and albumin level (continuous). ^b: The overall stabilized weight shown in median (interquartile range) for each group was the summary of the final (exposure and censoring) stabilized inverse probability weights for each participants that the product of weights for all time points from the baseline to the last visit for that person. **Supplemental Figure 1.** Cumulative incidence of HBsAg seroconversion (A) and HBeAg seroconversion (B) among five trajectory groups. (A) Cox regression model adjusting for age at baseline, gender, smoking status, drinking status, HBeAg status at baseline, ALT at baseline, AST at baseline, albumin level at baseline, family history of HBV infection, and antiviral treatment at baseline. (B) Cox regression model adjusting for age at baseline, gender, smoking status, drinking status, ALT at baseline, AST at baseline, albumin level at baseline, family history of HBV infection, and antiviral treatment at baseline. *: the P-value was < 0.05 representing significant difference between group B-E and group A.

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Authors' contributions

XX analyzed and interpreted the patient data regarding HBV DNA levels and hepatocellular carcinoma and was a major contributor in writing the manuscript and applied for funding. JJ was a major contributor for conducting field epidemiological investigation and collecting biological samples and laboratory testing. CS supervised the analysis and interpretation of the patient data and was responsible for revising the manuscript and applied for funding. CY helped analyze and interpret the data and applied

for funding. LZ helped conduct field epidemiological investigation and collect biological samples. JQ helped laboratory testing. TT and YD helped collect the epidemiological data. FZ supervised the field epidemiological investigation. ZH supervised the process of the study. XZ was responsible for proposing a hypothesis and supervising the process of the study and applying for approval and funding. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analytic methods used during and/or analyzed during the current study are not publicly available due to data security and privacy but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Jiangsu Province Center for Disease Control and Prevention. All participants provided written informed consent for an interview, as well as follow-up interviews and blood sample collection.

Competing interests

The authors declare that they have no competing interests.

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