Research Article
Viral Hepatitis

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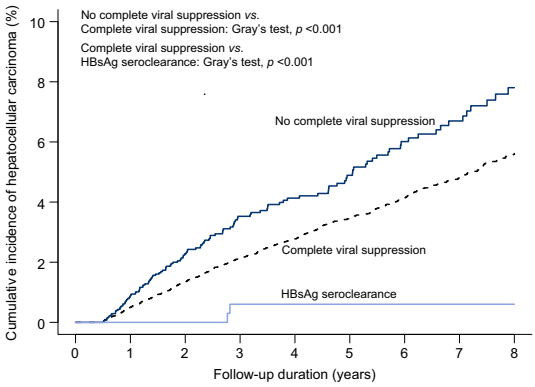
HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues

Graphical abstract



Highlights

- Patients without complete viral suppression have the highest risk of HCC.
- NA-induced HBsAg seroclearance leads to lower HCC risk than complete viral suppression alone.
- Patients with HBsAg seroclearance and complete viral suppression have a similar risk of hepatic events.

Authors

Terry Cheuk-Fung Yip, Grace Lai-Hung Wong, Henry Lik-Yuen Chan, ..., Kelvin Long-Yan Lam, Grace Chung-Yan Lui, Vincent Wai-Sun Wong

Correspondence

wongv@cuhk.edu.hk
(V.W.-S. Wong)

Lay summary

We investigated 20,263 nucleos(t)ide analogue (NA)-treated patients with chronic hepatitis B. Patients with NA-induced hepatitis B surface antigen seroclearance on top of complete viral suppression have a lower risk of hepatocellular carcinoma but not hepatic events than those only achieving complete viral suppression under prolonged NA treatment.

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HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues

Terry Cheuk-Fung Yip[1,2], Grace Lai-Hung Wong[1,2,3], Henry Lik-Yuen Chan[1,2,3], Yee-Kit Tse[1,2], Kelvin Long-Yan Lam[1,2], Grace Chung-Yan Lui[2,4], Vincent Wai-Sun Wong[1,2,3,*]

[1]*Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong Special Administrative Region;* [2]*Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong Special Administrative Region;* [3]*State Key Laboratory of Digestive Disease, The Chinese University of Hong Kong, Hong Kong Special Administrative Region;* [4]*Division of Infectious Disease, The Chinese University of Hong Kong, Hong Kong Special Administrative Region*

Background & Aims: In treated patients with chronic hepatitis B (CHB) who have achieved complete viral suppression, it is unclear if functional cure as indicated by hepatitis B surface antigen (HBsAg) seroclearance confers additional clinical benefit. We compared the risk of hepatocellular carcinoma (HCC) and hepatic events in nucleos(t)ide analogue (NA)-treated patients with and without HBsAg seroclearance.

Methods: We performed a territory-wide retrospective cohort study on all patients with CHB who had received entecavir and/or tenofovir disoproxil fumarate (TDF) for at least 6 months between 2005 and 2016 from Hospital Authority, Hong Kong. Patients' demographics, comorbidities, and laboratory parameters were analyzed. The primary outcome was HCC. The secondary outcomes were hepatic events including cirrhotic complications, liver transplantation, and liver-related mortality.

Results: A total of 20,263 entecavir/TDF-treated patients with CHB were identified; 17,499 (86.4%) patients had complete viral suppression; 376 (2.1%) achieved HBsAg seroclearance. At a median (interquartile range) follow-up of 4.8 (2.8–7.0) years, 603 (3.5%) and 121 (4.4%) patients with and without complete viral suppression developed HCC; 2 (0.5%) patients with HBsAg seroclearance developed HCC. Compared to complete viral suppression, lack of complete viral suppression was associated with a higher risk of HCC (7.8% *vs.* 5.6% at 8 years, Gray's test, p <0.001) (adjusted hazard ratio [aHR] 1.69; 95% CI 1.36–2.09; p <0.001); patients who achieved functional cure had a lower risk of HCC (0.6% *vs.* 5.6% at 8 years, Gray's test, p <0.001) (aHR 0.24; 95% CI 0.06–0.97; p = 0.045) but not hepatic events (aHR 0.99; 95% CI 0.30–3.26; p = 0.991).

Conclusions: Patients who achieved HBsAg seroclearance on top of complete viral suppression with entecavir/TDF treatment may have a lower risk of HCC but not hepatic events.

Lay summary: We investigated 20,263 nucleos(t)ide analogue (NA)-treated patients with chronic hepatitis B. Patients with NA-induced hepatitis B surface antigen seroclearance on top of complete viral suppression have a lower risk of hepatocellular carcinoma but not hepatic events than those only achieving complete viral suppression under prolonged NA treatment.

Introduction

Chronic hepatitis B (CHB) is one of the leading causes of hepatocellular carcinoma (HCC), cirrhotic complications and liver-related death worldwide.[1] Antiviral therapy with potent nucleos(t)ide analogues (NA), namely entecavir and tenofovir disoproxil fumarate (TDF), reduces the risk of HCC and hepatic complications.[2,3] Recent data suggest that complete viral suppression is an important treatment goal because patients with low detectable serum hepatitis B virus (HBV) DNA levels still have a higher risk of developing HCC.[4,5] Complete viral suppression also leads to histological improvement and regression of liver fibrosis and cirrhosis.[6,7]

Hepatitis B surface antigen (HBsAg) seroclearance is currently regarded as the functional cure of CHB.[8–10] Patients who achieve this endpoint often have a favorable clinical course and very low risk of HCC.[11,12] However, HBsAg seroclearance is uncommon in NA-treated patients, especially in Asian patients who acquire HBV infection through perinatal transmission.[6,13] As a result, most NA-treated patients require long-term if not lifelong treatment. Currently, a number of novel therapies have entered clinical development with the aim of achieving functional cure of CHB.[14] The goal is to provide treatment with a finite duration and durable response. There is also hope that functional cure may further reduce the risk of HCC and hepatic events beyond what can be achieved with complete viral suppression alone. Nonetheless, because functional cure is rare among treated patients, its prognostic implication relative to complete viral suppression alone has not been shown. In this territory-wide cohort study, we aimed to compare the risk of HCC and hepatic events in NA-treated patients with CHB who achieved complete viral suppression against those who also developed HBsAg seroclearance.

Keywords: Antiviral therapy; Cohort study; Functional cure; HBsAg seroclearance.
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* Corresponding author. Address: Department of Medicine and Therapeutics, 9/F Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, Hong Kong Special Administrative Region. Tel.: +852 3505 1420; fax: +852 2637 3852.
E-mail address: wongv@cuhk.edu.hk (V.W.-S. Wong).

Patients and methods

Study design and data source

We performed a retrospective cohort study using data from the Clinical Data Analysis and Reporting System (CDARS) of the Hospital Authority, Hong Kong. CDARS facilitates the retrieval of clinical data captured from different operational systems for analysis and reporting and provides good quality information to support retrospective clinical and management decisions by integrating the clinical data resided in Data Warehouse. It covers the electronic health records and laboratory results from all public hospitals and clinics in Hong Kong and represents data of approximately 80% of the local population.[15] All individuals who were dispensed with entecavir and/or TDF for at least 6 months between January 1, 2005 and December 31, 2016 were identified. Patients were excluded if they had incomplete demographic data; were aged below 18 years old; were coinfected with hepatitis C virus and/or hepatitis D virus based on ICD-9-CM codes, viral and/or serological markers; were coinfected with human immunodeficiency virus based on ICD-9-CM codes; had other coexisting autoimmune and metabolic liver diseases based on ICD-9-CM codes; had HCC and/or cirrhotic complications before or within the first 6 months of entecavir and/or TDF treatment by ICD-9-CM codes (Table S3); had undergone liver transplantation before HBsAg seroclearance; were exposed to (pegylated) interferon; were exposed to immunosuppressants and/or chemotherapeutic agents (Table S1). To avoid inadvertent inclusion of patients with acute hepatitis B, we excluded patients with positive immunoglobulin M to hepatitis B core antigen unless it was more than 6 months apart from a positive HBsAg result. Hepatitis C and hepatitis D testing were performed in 23,827 and 4,309 patients, respectively; 368 patients with positive results were excluded. Patients were followed until death, diagnosis of HCC, last follow-up date (31 December, 2017), or 8 years of follow-up, whichever came first. The study protocol was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee.

Data collection

Data were retrieved from the CDARS in January 2018. Baseline date was defined as the date of commencement of entecavir and/or TDF treatment. Demographic data including gender and date of birth were captured. At baseline, liver and renal biochemistries, hematological and virologic parameters were collected. Thereafter, serial liver biochemistries as well as HBV viral markers (HBsAg, hepatitis B e antigen [HBeAg], HBV DNA) were collected until the last follow-up. Other relevant diagnoses, procedures, and laboratory parameters were retrieved and studied.

Drug information concerning prior antiviral treatment before the commencement of entecavir and TDF (*i.e.* lamivudine, adefovir dipivoxil, telbivudine, interferon alpha-2a/2b or peginterferon alpha-2a/2b/lambda-1a) was identified by internal drug codes in Hospital Authority (Table S2).

Virologic response

HBsAg seroclearance

HBsAg seroclearance was defined as loss of HBsAg detectability once or more during the follow-up.

Complete viral suppression

Complete viral suppression was defined as undetectable serum HBV DNA (<20 IU/ml) while on NA treatment maintained till the last clinic visit.

Definitions

The primary outcome was HCC. The secondary outcomes were hepatic events and liver-related mortality.

HCc

HCC was identified based on diagnosis codes (155.0 – hepatocellular carcinoma and 155.2 – carcinoma of liver) or treatment procedure codes for HCC according to International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes retrieved from the CDARS.

Hepatic events

Hepatic events were defined as any cirrhotic complications, liver transplantation (V42.7 – Liver transplant status and 50.59 – Other transplant of liver), and/or liver-related mortality. Cirrhotic complications (excluding HCC) included ascites, spontaneous bacterial peritonitis, variceal bleeding, hepatic encephalopathy, and hepatorenal syndrome according to ICD-9-CM codes retrieved from the CDARS (Table S3). The use of single ICD-9-CM codes for the diagnosis of HCC and cirrhotic complications in CDARS was previously validated to be 99% accurate, when referenced to clinical, laboratory, imaging and endoscopy results from the electronic medical records.[16]

Liver cirrhosis was identified by the ICD-9-CM diagnosis codes for cirrhosis and its related complications (Table S3). Decompensated cirrhosis was defined by the presence of any ICD-9-CM diagnosis codes of cirrhotic complications. Compensated cirrhosis was defined by the presence of any diagnosis codes of cirrhosis, portal hypertension, and varices without any diagnosis of cirrhotic complications. Diabetes mellitus (DM) was identified by the ICD-9-CM diagnosis code for type 1 and type 2 DM (250 – diabetes mellitus), and/or exposure to any anti-diabetic agents, and/or hemoglobin A_{1c} ≥6.5%, and/or fasting glucose ≥7 mmol/L.[17]

Statistical analysis

Data were analyzed using Statistical Product and Service Solutions (SPSS) version 25.0 (SPSS, Inc., Chicago, Illinois), SAS (9.3; SAS Institute Inc., Cary, NC), and R software (3.4.4; R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were expressed in mean ± standard deviation or median (interquartile range [IQR]), as appropriate, while categorical variables were presented as number (percentage). Qualitative and quantitative differences between subgroups were analyzed by chi-square or Fisher's exact tests for categorical parameters and one-way ANOVA or Kruskal-Wallis test for continuous parameters, as appropriate. Serum HBeAg in patients with CHB who achieved HBsAg seroclearance was considered undetectable.[18] Cumulative incidence function of HCC and hepatic events with the adjustment of competing death and liver-related mortality with the adjustment of other competing causes of death were estimated with 95% CIs. Gray's test was used to compare the cumulative incidence functions of different groups in univariate analysis. On multivariable analyses, adjusted hazard ratios (aHR) with 95% CIs was estimated with

Fine-Gray proportional subdistribution hazards regression adjusted for competing risks.[19] We adjusted for the following covariates (percentage of missing data): age, gender, presence of cirrhosis and DM, serum alanine aminotransferase (ALT) (3%), total bilirubin (3%), platelet counts (4%), albumin (3%), and HBeAg status (10%) at baseline; patients with missing data were excluded from the multivariable analysis. Important covariates were selected by backward elimination using likelihood ratio test. Serum ALT, total bilirubin, alpha-fetoprotein and platelet counts at baseline were log-transformed when putting in the model. Schoenfeld's global test was used to test the proportional hazards assumption, which did not detect any significant violations. All statistical tests were 2-sided. Statistical significance was taken as p <0.05. Subgroup analysis was performed in cirrhotic and non-cirrhotic patients. Sensitivity analysis was performed in patients with available APRI or FIB-4 score to identify liver cirrhosis, patients without previous exposure to other NA including lamivudine, adefovir dipivoxil, and telbivudine, and the entire cohort by censoring patients at 1 year after the last available measurement of HBsAg and HBV DNA during follow-up. We also perform another sensitivity analysis by modelling complete viral suppression and HBsAg seroclearance as time-dependent covariates.

Results

Patient characteristics

We identified 46,441 individuals who were treated with entecavir and/or TDF; 26,178 were excluded according to the inclusion and exclusion criteria (Fig. 1). Finally, 20,263 patients with CHB receiving entecavir and/or TDF treatment for at least 6 months were included for analysis. Their mean age was 51.8 ± 12.8 years; 13,533 (66.8%) patients were male; 3,521 (17.4%) patients were exposed to other NAs before entecavir and/or TDF treatment (Table 1). Liver cirrhosis was diagnosed in 2,222 (11.0%) patients; 205 (9.2%) patients had decompensated cirrhosis and 2,017 (90.8%) patients had compensated cirrhosis; 379 (18.8%) had esophageal varices among patients with compensated cirrhosis. Serum HBsAg was checked a median (IQR) of 4 (2–6) times including the baseline measurement at an interval of 7.4 (5.0–14.5) months during follow-up; serum HBV DNA was measured of 5 (3–7) times including baseline measurement at an interval of 7.1 (5.5–10.3) months during follow-up. Among patients without complete viral suppression who had a median (IQR) follow-up of 3.5 (1.9–6.2) years, the median (IQR) number of HBV DNA measurement during follow-up was 3 (2–5); the median (IQR) number of detectable HBV DNA was 2 (1–4) in patients without complete viral suppression. A total of 316 patients stopped NA treatment during follow-up after a median of 2.5 (1.5–4.4) years from clearing HBeAg; 199 patients stopped using NA during follow-up at a median of 0.8 (0.5–1.2) years after HBsAg seroclearance.

Among the NA-treated patients analyzed, 17,499 (86.4%) patients achieved complete viral suppression; a further 376 (2.1%) patients had HBsAg seroclearance. Patients with complete viral suppression had undetectable HBV DNA for a median of 2.0 (0.6–4.0) years. They were more likely to be male, had negative HBeAg, lower HBV DNA level, serum total bilirubin and ALT levels at baseline, and were more likely to have received other NA treatment than patients without complete viral suppression (n = 2,764). More patients with complete viral suppression had liver cirrhosis and low platelet counts. Among patients with complete viral suppression, those who achieved HBsAg seroclearance were more likely to be male, had lower alpha-fetoprotein and HBV DNA levels at baseline, had higher platelet counts, serum total bilirubin and ALT levels at baseline, and were more likely to have received other NA treatment compared to the patients without HBsAg seroclearance (Table 1).

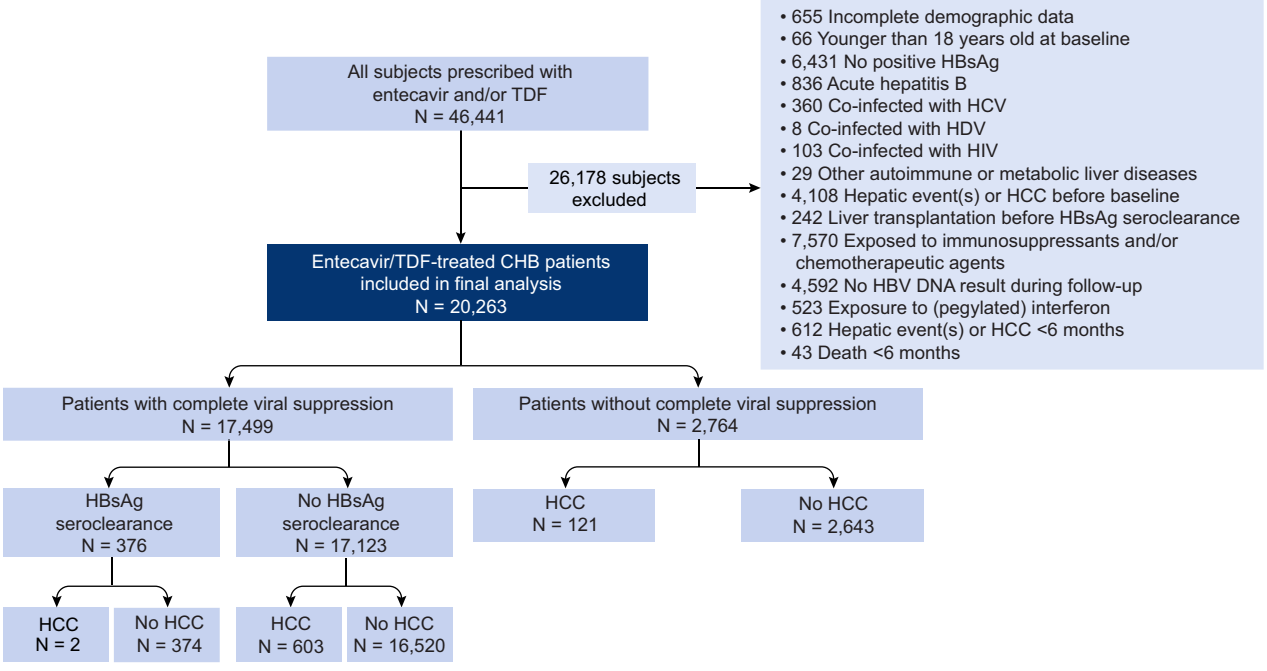

Fig. 1. Patient flowchart. CHB, chronic HBV; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDV, hepatitis D virus; TDF, tenofovir disoproxil fumarate.

Table 1. Clinical characteristics of NA-treated patients with or without complete viral suppression, and HBsAg seroclearance at baseline.

Clinical characteristics at baseline	All patients n = 20,263	No complete viral suppression n = 2,764	Complete viral suppression* n = 17,123	HBsAg seroclearance n = 376	*p* values
Male gender (n, %)	13,533 (66.8)	1,734 (62.7)	11,520 (67.3)	279 (74.2)	<0.001
Age (years)	51.8 ± 12.8	48.8 ± 14.0	52.3 ± 12.5	47.8 ± 14.2	<0.001
Cirrhosis (n, %)	2,222 (11.0)	216 (7.8)	1,983 (11.6)	23 (6.1)	<0.001
Diabetes mellitus (n, %)	4,998 (24.7)	692 (25.0)	4,228 (24.7)	78 (20.7)	0.190
Platelet ($x10^9$/L)	182.5 ± 63.8	191.1 ± 65.6	180.7 ± 63.2	198.2 ± 68.7	<0.001
Missing (%)	4.1	3.7	4.1	4.3	
Prothrombin time (s)	12.0 ± 2.1	12.2 ± 2.2	11.9 ± 2.1	12.7 ± 3.6	<0.001
Missing (%)	16.4	16.9	16.5	10.1	
Albumin (g/L)	41.1 ± 4.9	40.3 ± 5.5	41.2 ± 4.7	39.8 ± 5.8	<0.001
Missing (%)	2.7	2.2	2.8	1.1	
Total bilirubin (μmol/L)	19.6 ± 36.6	20.8 ± 47.9	18.2 ± 30.5	71.2 ± 99.6	<0.001
Missing (%)	2.8	2.3	2.9	1.1	
Alanine aminotransferase (IU/L)	60.0 (31.4–136.0)	70.0 (37.0–148.0)	58.0 (31.0–130.0)	232.0 (35.3–1692.3)	<0.001
Missing (%)	2.7	2.2	2.8	1.1	
Alpha fetoprotein (μg/L)	3.8 (2.4–7.1)	3.9 (2.5–7.3)	3.8 (2.4–7.1)	3.4 (2.0–6.1)	0.002
Missing (%)	5.7	6.3	5.2	20.7	
Creatinine (μmol/L)	83.2 ± 58.9	84.3 ± 79.9	82.9 ± 54.8	85.9 ± 58.7	0.377
Missing (%)	5.7	7.2	5.4	5.1	
Positive HBeAg (n, %)Ω	6,093 (33.3)	1,307 (53.1)	4,664 (30.1)	122 (32.4)	<0.001
Missing (%)	9.6	10.9	9.6	0	
HBV DNA level (IU/ml)Ω					<0.001
<2,000 IU/ml	3,176 (25.1)	216 (12.0)	2,887 (27.2)	73 (36.1)	
2,000–20,000 IU/ml	1,054 (8.3)	109 (6.1)	921 (8.7)	24 (11.9)	
20,000–200,000 IU/ml	1,629 (12.9)	181 (10.1)	1,405 (13.2)	43 (21.3)	
≥200,000 IU/ml	6,771 (53.6)	1,289 (71.8)	5,420 (51.0)	62 (30.7)	
Missing (%)	37.7	35.1	37.9	46.3	
Follow-up duration (years)	4.8 (2.8–7.0)	3.5 (1.9–6.2)	4.9 (2.9–7.0)	5.9 (3.8–7.7)	<0.001
Antiviral therapy (n, %)					
Entecavir	17,065 (84.2)	2,314 (83.7)	14,435 (84.3)	316 (84.0)	0.735
TDF	3,198 (15.8)	450 (16.3)	2,688 (15.7)	60 (16.0)	0.735
Entecavir/TDF duration (years)	4.5 (2.5–6.7)	2.6 (1.4–4.9)	4.8 (2.9–6.9)	2.8 (1.2–5.3)	<0.001
Previous antiviral therapy (n, %)					
Other NAs^	3,521 (17.4)	300 (10.9)	3,150 (18.4)	71 (18.9)	<0.001
Antiviral therapy during follow-up (n, %)					
Other NAs^	1,080 (5.3)	143 (5.2)	917 (5.4)	20 (5.3)	0.925

Alanine aminotransferase and duration were expressed in median (interquartile range), whereas other continuous variables were expressed in mean ± standard deviation.
HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate.
*Patients with complete viral suppression but without HBsAg seroclearance.
ΩPercentages were based on non-missing data.
^Other NAs included adefovir dipivoxil, lamivudine, and telbivudine.

Patients without complete viral suppression had a median (IQR) HBV DNA level of 297 (37–9,030) IU/ml during the first year and 180 (32–3,371) IU/ml in the subsequent years of treatment. At the last clinic visit, patients without complete viral suppression had a median (IQR) HBV DNA level of 151 (35–2,280) IU/ml. The median (IQR) serum ALT at the last clinic visit was 28 (19–41) IU/L, 23 (17–32) IU/L and 19 (13–28) IU/L in patients without or with complete viral suppression, and HBsAg seroclearance, respectively. Among 376 patients who achieved HBsAg seroclearance during treatment, their median (IQR) age was 49.2 (38.7–60.9) years old at the time of HBsAg seroclearance. The median (IQR) time from the start of treatment to complete viral suppression was 0.6 (0.3–1.9) years, while it took a median (IQR) of 2.0 (0.7–3.7) years from complete viral suppression to HBsAg seroclearance. Two hundred and eighty-two (75.0%) patients had at least one repeated test to confirm the seronegativity of HBsAg at a median (IQR) of 5.8 (3.6–8.3) months after the first loss of HBsAg detectability; 278 (98.6%) were confirmed to maintain their HBsAg seronegativity. Among 278 patients who had anti-HBs checked after HBsAg seroclearance, 166 (59.7%) patients achieved HBsAg seroconversion to anti-HBs at a median (IQR) of 1.6 (0–6.5) months after HBsAg seroclearance.

Cumulative incidence of HCC, hepatic events, and liver-related mortality

At a median (IQR) follow-up of 4.8 (2.8–7.0) years, 726 (3.6%) patients developed HCC. Their mean age was 60.2 ± 10.0 years at baseline; 568 (78.2%) patients were male; 275 (37.9%) and 274 (37.7%) had liver cirrhosis and DM at baseline, respectively (Table 2). The cumulative incidence rates of HCC at 2, 4, 6 and 8 years after entecavir/TDF treatment were 1.4% (95% CI 1.3%–1.6%), 2.9% (2.7%–3.2%), 4.3% (4.0%–4.6%), and 5.8% (5.3%–6.2%), respectively in the entire cohort (Fig. 2A).

Two hundred and sixty-seven (1.3%) patients had at least one hepatic event: ascites, 146 (0.7%); hepatic encephalopathy, 88 (0.4%); spontaneous bacterial peritonitis, 39 (0.2%); variceal bleeding, 35 (0.2%); hepatorenal syndrome, 23 (0.1%); liver transplantation, 0 (0%); and liver-related death, 134 (0.7%) (Fig. S1, 2). The cumulative incidence rates of hepatic events at 2, 4, 6, and 8 years were 0.7% (0.6%–0.8%), 1.2% (1.0%–1.3%), 1.6% (1.4%–1.8%), and 1.9% (1.7%–2.2%), respectively (Fig. 2B).

Table 2. Clinical characteristics of NA-treated patients who developed HCC.

Clinical characteristics at baseline	All HCC n = 726	No complete viral suppression n = 121	Complete viral suppression* n = 603	HBsAg seroclearance n = 2#
Male gender (n, %)	568 (78.2)	101 (83.5)	466 (77.3)	1 (50.0)
Age (years)	60.2 ± 10.0	61.8 ± 11.0	59.9 ± 9.7	56.5 (48.9–64.1)
Cirrhosis (n, %)	275 (37.9)	49 (40.5)	225 (37.3)	1 (50.0)
Diabetes mellitus (n, %)	274 (37.7)	59 (48.8)	215 (35.7)	1 (50.0)
Platelet ($x10^9$/L)	141.3 ± 55.4	141.4 ± 52.6	141.4 ± 56.0	91.5 (62.0–121.0)
Missing (%)	1.8	0.8	2.0	0
Prothrombin time (s)	12.4 ± 2.7	13.0 ± 3.6	12.3 ± 2.5	11.1 (10.7–11.4)
Missing (%)	10.3	5.0	11.4	0
Albumin (g/L)	38.8 ± 5.6	37.2 ± 6.3	39.2 ± 5.4	38.5 (33.0–44.0)
Missing (%)	1.2	1.7	1.2	0
Total bilirubin (μmol/L)	20.6 ± 37.2	25.6 ± 62.4	19.6 ± 29.7	15.0 (9.0–21.0)
Missing (%)	1.2	1.7	1.2	0
Alanine aminotransferase (IU/L)	55.0 (35.4–93.5)	58.0 (42.5–85.8)	55.0 (34.0–94.0)	47.0 (16.0–78.0)
Missing (%)	1.2	1.7	1.2	0
Alpha fetoprotein (μg/L)	8.0 (4.3–18.4)	8.0 (5.0–23.0)	8.0 (4.3–17.9)	11.0 (2.5–19.5)
Missing (%)	1.9	2.5	1.8	0
Creatinine (μmol/L)	85.7 ± 55.1	84.1 ± 25.3	86.1 ± 59.4	80.0 (61.0–99.0)
Missing (%)	1.7	1.3	1.7	0
Positive HBeAg (n, %)Ω	178 (26.9)	43 (41.0)	135 (24.4)	0 (0)
Missing (%)	9.0	13.2	8.1	0
HBV DNA level (IU/ml)Ω				
<2,000 IU/ml	96 (22.0)	8 (10.5)	87 (24.3)	1 (50.0)
2,000–20,000 IU/ml	38 (8.7)	6 (7.9)	31 (8.7)	1 (50.0)
20,000–200,000 IU/ml	55 (12.6)	7 (9.2)	48 (13.4)	0 (0)
≥200,000 IU/ml	247 (56.7)	55 (72.4)	192 (53.6)	0 (0)
Missing (%)	39.9	37.2	40.6	0
Follow-up duration (years)	2.5 (1.4–4.5)	2.0 (1.2–4.2)	2.7 (1.4–4.5)	2.8 (2.8–2.8)
Antiviral therapy (n, %)				
Entecavir	652 (89.8)	106 (87.6)	544 (90.2)	2 (100)
TDF	74 (10.2)	15 (12.4)	59 (9.8)	0 (0)
Entecavir/TDF duration (years)	2.5 (1.4–4.4)	2.0 (1.2–3.8)	2.7 (1.4–4.5)	2.3 (1.7–2.8)
Previous antiviral therapy (n, %)				
Other NAs^	106 (14.6)	17 (14.0)	88 (14.6)	1 (50.0)
Antiviral therapy during follow-up (n, %)				
Other NAs^	37 (5.1)	9 (7.4)	28 (4.6)	0 (0)

Alanine aminotransferase was expressed in median (interquartile range), whereas other continuous variables were expressed in mean ± standard deviation.
HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogue; TDF, tenofovir disoproxil fumarate.
* Patients with complete viral suppression but without HBsAg seroclearance.
# Median (range) was used to present the data in patients with HBsAg seroclearance.
Ω Percentages were based on non-missing data.
^ Other NAs included adefovir dipivoxil, lamivudine, and telbivudine.

The cumulative incidence rates of liver-related death at 2, 4, 6, and 8 years were 0.2% (0.2%–0.3%), 0.5% (0.4%–0.6%), 0.8% (0.7%–1.0%), and 1.1% (0.9%–1.3%) (Fig. 2C).

HCC in functional cure *vs.* complete viral suppression

Six hundred and 3 (3.5%) patients who achieved complete viral suppression developed HCC; whereas 121 (4.4%) patients without complete viral suppression developed HCC. The 8-year cumulative incidence rate of HCC was 5.6% (5.1%–6.1%) and 7.8% (6.3%–9.5%) respectively in patients with or without complete viral suppression (Gray's test, p <0.001) (Fig. 3A). Two (0.5%) patients who achieved HBsAg seroclearance developed HCC; the 8-year cumulative incidence rate was 0.6% (0.1%–2.0%). HBsAg seroclearance was associated with a lower risk of HCC development when compared to patients with complete viral suppression (Gray's test, p <0.001). On multivariable analysis, lack of complete viral suppression was an independent risk factor of HCC with an aHR (95% CI) of 1.69 (1.36–2.09; p <0.001). Patients with HBsAg seroclearance had a lower risk of HCC than those with complete viral suppression; the aHR (95% CI) was 0.24 (0.06–0.97; p = 0.045) (Table 3). Compared with 10,999 patients who had achieved complete viral suppression within 2 years of entecavir and/or TDF treatment and sustained until the last clinical visit, HBsAg seroclearance was again associated with a lower risk of HCC (aHR 0.48; 95% CI 0.24–0.97; p = 0.041). The first patient who developed HCC after HBsAg seroclearance was a female patient who started treatment by lamivudine at the age of 62 years old, achieved complete viral suppression after 4.2 months of lamivudine treatment, changed to entecavir treatment after 2.3 years of lamivudine treatment, and cleared HBsAg after additional 10.3 months of entecavir treatment. She was diagnosed with liver cirrhosis and had a platelet counts of 121 × 10^9/L at the time when she switched to entecavir treatment. She developed HCC at 1.9 years after HBsAg seroclearance (Fig. S3). The second patient was a male patient who started entecavir treatment at 49 years old, achieved complete viral suppression after 3.9 months of treatment, lost HBsAg at 2.7 years after entecavir treatment, and was diagnosed with

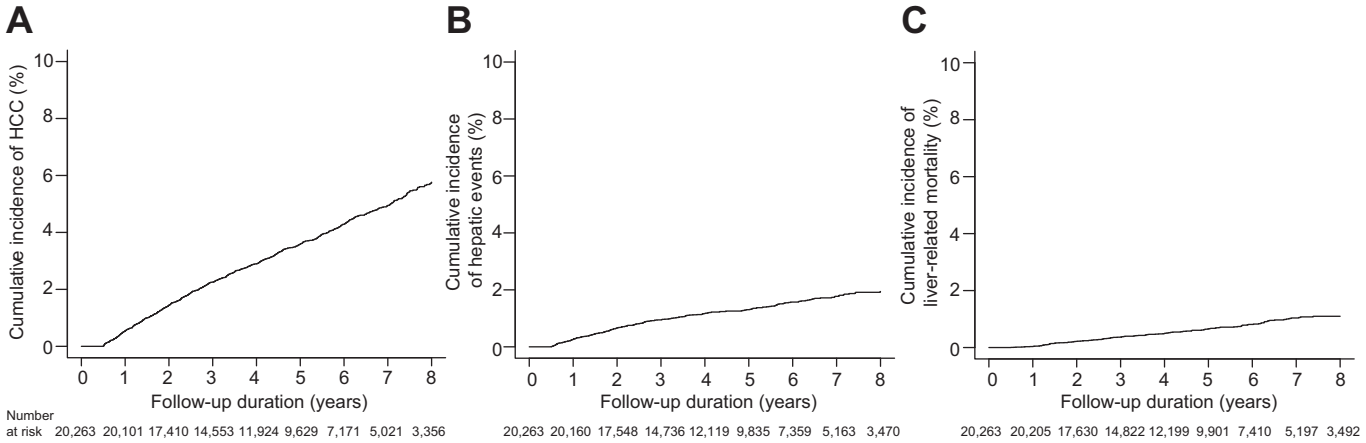

Fig. 2. Cumulative incidence function. (A) HCC; (B) hepatic events; (C) liver-related mortality in all patients. HCC, hepatocellular carcinoma.

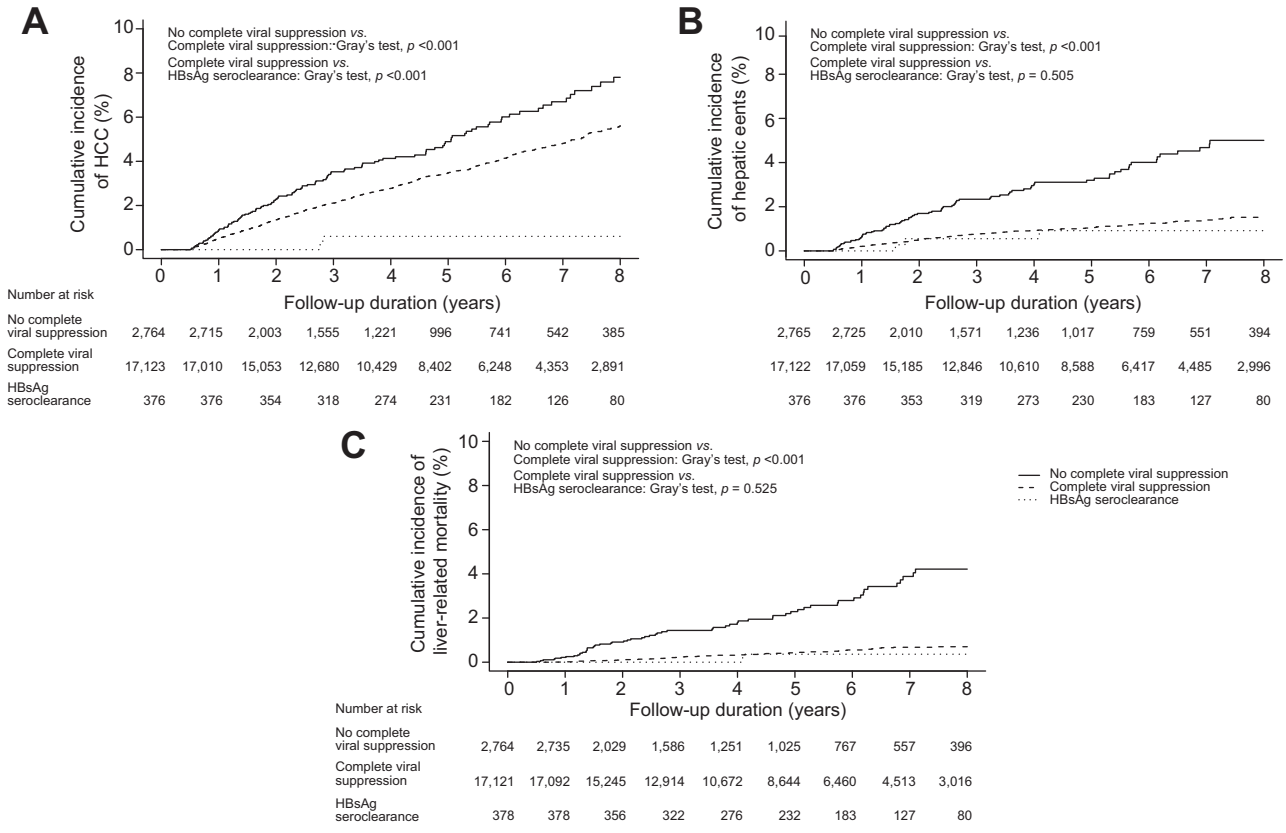

Fig. 3. Cumulative incidence function. (A) HCC (Levels of significance: both p <0.001 [Gray's test]); (B) hepatic events (levels of significance: p <0.001 and p = 0.505 [Gray's test]); (C) liver-related mortality (levels of significance: p <0.001 and p = 0.525 [Gray's test]) in patients with or without complete viral suppression, and patients with complete viral suppression or HBsAg seroclearance. HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma.

HCC at 2 months after clearing HBsAg. He was diagnosed with DM and had a baseline platelet count of 62 $\times$ 10^9/L at baseline (Table 2).

Hepatic events in functional cure *vs.* complete viral suppression

One hundred and eighty-two (1.1%) patients with complete viral suppression developed hepatic events, whereas 82 (3.0%) patients without complete viral suppression had hepatic events (Fig. S1). The 8-year cumulative incidence rate of hepatic events was 1.4% (1.2%–1.7%) and 4.3% (3.2%–5.6%), respectively, in patients with or without complete viral suppression (Gray's test, p <0.001) (Fig. 3B). Meanwhile, 3 (0.8%) patients with HBsAg seroclearance had hepatic events; the 8-year cumulative incidence rate was 1.6% (0.5%–3.8%). HBsAg seroclearance was not associated with a lower risk of hepatic events compared to patients with complete viral suppression (Gray's test, p = 0.581). On multivariable analysis, patients without complete

Table 3. Univariate and multivariable analysis with proportional subdistribution hazards regression model on factors associated with HCC.

	Univariate analysis		Multivariable analysis (n = 17,462)*	
Parameters	**HR (95% CI)**	***p* value**	**aHR (95% CI)**	***p* value**
Viral suppression				
Complete viral suppression	Referent		Referent	
Lack of complete viral suppression	1.47 (1.21–1.79)	<0.001	1.69 (1.36–2.09)	<0.001
HBsAg seroclearance	0.13 (0.03–0.53)	0.004	0.24 (0.06–0.97)	0.045
Age	1.06 (1.06–1.07)	<0.001	1.05 (1.04–1.06)	<0.001
Male gender	1.70 (1.43–2.03)	<0.001	1.90 (1.57–2.31)	0.004
Cirrhosis	4.50 (3.87–5.22)	<0.001	2.15 (1.79–2.59)	<0.001
Diabetes mellitus	1.89 (1.63–2.19)	<0.001		
Platelet (log-transformed)	0.36 (0.30–0.43)	<0.001	0.54 (0.46–0.65)	<0.001
Albumin	0.93 (0.92–0.94)	<0.001		
Total bilirubin (log-transformed)	1.19 (1.09–1.29)	<0.001	0.80 (0.68–0.94)	0.006
Alanine aminotransferase (log-transformed)	0.84 (0.80–0.89)	<0.001	0.86 (0.79–0.94)	<0.001
Alpha-fetoprotein (log-transformed)	1.45 (1.39–1.51)	<0.001	1.41 (1.33–1.49)	<0.001
Positive HBeAg	0.67 (0.56–0.79)	<0.001		
Other NAs prior baseline#	0.84 (0.68–1.03)	0.090		

aHR, adjusted hazard ratio; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogues.
Analysis performed in NA-treated patients with or without complete viral suppression, and HBsAg seroclearance.
*[Baseline platelet, total bilirubin, alanine aminotransferase, alpha-fetoprotein] (log-transformed), albumin, age, gender, cirrhosis, diabetes mellitus, HBeAg status, and prior exposure to other NAs were first included in the proportional subdistribution hazards regression model. Patients with missing data were excluded in the model. Backward elimination by likelihood ratio test was used to select important covariates.
#Other NAs included adefovir dipivoxil, lamivudine, and telbivudine.

viral suppression had an increased risk of hepatic events, with an aHR (95% CI) of 3.74 (2.76–5.07; p <0.001). HBsAg seroclearance was not associated with reduced risk of hepatic events compared to those with complete viral suppression (aHR 0.99; 95% CI 0.30–3.26; p = 0.991) (Table 4). For the 3 patients who had hepatic events after HBsAg seroclearance, they had at least 1 of the coexisting conditions including cirrhosis and DM.

Liver-related mortality in functional cure *vs.* complete viral suppression

Seventy-four (0.4%) patients with complete viral suppression and 59 (2.1%) patients without complete viral suppression died from liver-related cause during follow-up (Fig. S2); the 8-year cumulative incidence rates were 0.7% (0.5%–0.9%) and 4.0% (2.9%–5.4%), respectively, in patients with or without complete viral suppression (Gray's test, p <0.001) (Fig. 3C). Meanwhile, 1 (0.3%) patient who cleared HBsAg under NA treatment died from liver-related causes; the 8-year cumulative incidence rate was 0.4% (0.04%–2.3%). HBsAg seroclearance had a comparable risk of liver-related death to patients with complete viral suppression (Gray's test, p = 0.708). On multivariable analysis, lack of complete viral suppression was associated with a higher risk of liver-related death after the adjustment of age, gender, presence of cirrhosis, alcohol misuse, and liver biochemistries; the aHR (95% CI) was 6.85 (4.59–10.23; p <0.001). HBsAg seroclearance did not further reduce liver-related deaths compared to complete viral suppression (aHR 0.93; 95% CI 0.13–6.93; p = 0.943) (Table 4). The patient who died after HBsAg seroclearance had DM and developed HCC and intrahepatic bile duct carcinoma.

Sensitivity analysis

All analyses on HCC were repeated under cirrhotic and non-cirrhotic patients (Fig. S4A-4B), patients with available APRI or FIB-4 score (Fig. S5), patients without previous exposure to lamivudine, adefovir dipivoxil, and/or telbivudine (Fig. S6), and the entire cohort by censored patients at 1 year after the last available measurement of HBsAg and HBV DNA during follow-up (Fig. S7). In another sensitivity analysis, complete viral suppression and HBsAg seroclearance were modelled as time-dependent covariates (Fig. S8). All the findings showed a similar trend to those in the main analysis (Fig. S9). Among 11,651 patients with available APRI or FIB-4 score, 2,843 (24.4%) patients were classified as cirrhotic patients by APRI score >2.0 and/or ICD-9-CM diagnosis codes for cirrhosis and its related complications; 3,317 (28.5%) patients were classified as cirrhotic patients by FIB-4 score >3.25 and/or ICD-9-CM diagnosis codes for cirrhosis and its related complications.

Discussion

This territory-wide cohort study aims to compare the risk of HCC and hepatic events in NA-treated patients with CHB who achieve functional cure or complete viral suppression alone. Complete viral suppression was associated with a lower risk of HCC and hepatic events than those without complete viral suppression. However, there was a further HCC risk reduction in patients who achieved functional cure of CHB compared to those who achieved complete viral suppression alone. HBsAg seroclearance was not associated with a lower risk of hepatic events and liver-related mortality over complete viral suppression.

Entecavir and TDF are the current first-line antiviral treatments for patients with CHB, which has high antiviral potency and a high genetic barrier to drug-resistance mutations.[20] Overall, 70% to 80% of patients with CHB can achieve complete viral suppression with 3-year therapy of entecavir or TDF; the proportion of complete viral suppression continues to increase after 5 years of treatment.[2,6–7,21] This results in an improvement of liver histology, regression in fibrosis, and a reduced risk of liver-related complications and mortality.[2,8–10] In our study, over 80% of patients achieved complete viral suppression in long-term NA treatment. This was associated with a lower risk of HCC, hepatic events, and liver-related mortality than in patients without complete viral suppression. Thus, complete viral suppression is an important goal of antiviral treatment. It

Table 4. Univariate and multivariable analysis with proportional subdistribution hazards regression model on factors associated with hepatic decompensation and liver-related mortality.

Parameters	Hepatic events				Liver-related mortality			
	Univariate analysis		Multivariable analysis (n = 17,462)*		Univariate analysis		Multivariable analysis (n = 17,462)*	
	HR (95% CI)	p value	aHR (95% CI)	p value	HR (95% CI)	p value	aHR (95% CI)	p value
Viral Suppression								
Complete viral suppression	Referent		Referent		Referent		Referent	
Lack of complete viral suppression	3.26 (2.51–4.23)	<0.001	3.74 (2.76–5.07)	<0.001	6.01 (4.28–8.44)	<0.001	6.85 (4.59–10.23)	<0.001
HBsAg seroclearance	0.68 (0.22–2.13)	0.508	0.99 (0.30–3.26)	0.991	0.54 (0.08–3.86)	0.536	0.93 (0.13–6.93)	0.943
Age	1.07 (1.06–1.07)	<0.001	1.02 (1.01–1.03)	<0.001	1.10 (1.08–1.11)	<0.001	1.06 (1.04–1.08)	<0.001
Male gender	1.34 (1.02–1.76)	0.036			1.23 (0.84–1.79)	0.291	1.69 (1.11–2.59)	0.015
Cirrhosis	10.16 (7.97–12.96)	<0.001	3.16 (2.32–4.33)	<0.001	13.97 (9.75–20.01)	<0.001	4.74 (3.02–7.46)	<0.001
Diabetes mellitus	2.97 (2.34–3.78)	<0.001	1.75 (1.33–2.31)	<0.001	2.59 (1.84–3.63)	<0.001		
Platelet (log-transformed)	0.27 (0.21–0.35)	<0.001	0.40 (0.32–0.49)	<0.001	0.29 (0.23–0.37)	<0.001	0.50 (0.36–0.70)	<0.001
Albumin	0.85 (0.84–0.86)	<0.001	0.92 (0.90–0.94)	<0.001	0.86 (0.85–0.88)	<0.001	0.95 (0.91–0.98)	0.002
Total bilirubin (log-transformed)	2.08 (1.92–2.25)	<0.001	1.50 (1.26–1.79)	<0.001	1.81 (1.58–2.07)	<0.001		
Alanine aminotransferase (log-transformed)	0.89 (0.80–0.98)	0.018	0.82 (0.71–0.95)	0.009	0.77 (0.66–0.88)	<0.001	0.79 (0.64–0.97)	0.023
Alpha-fetoprotein (log-transformed)	1.36 (1.26–1.45)	<0.001			1.44 (1.33–1.55)	<0.001	1.16 (1.01–1.34)	0.033
Positive HBeAg	0.49 (0.36–0.67)	<0.001	0.67 (0.48–0.95)	0.023	0.54 (0.36–0.82)	0.004		
Other NAs prior baseline#	0.76 (0.53–1.07)	0.118			0.67 (0.40–1.14)	0.140		

aHR, adjusted hazard ratio; CI, confidence interval; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogues.
Analysis performed in NA-treated patients with or without complete viral suppression, and HBsAg seroclearance.
*[Baseline platelet, total bilirubin, alanine aminotransferase, alpha-fetoprotein] (log-transformed), albumin, age, gender, cirrhosis, diabetes mellitus, HBeAg status, and prior exposure to other NAs were first included in the proportional subdistribution hazards regression model. Patients with missing data were excluded in the model. Backward elimination by likelihood ratio test was used to select important covariates.
#Other NAs included adefovir dipivoxil, lamivudine, and telbivudine.

is observed that the proportion of patients with positive HBeAg at baseline was higher in patients who did not have complete viral suppression; positive serum HBeAg can reflect active viral replication in hepatocytes and act as a surrogate marker for the presence of HBV DNA. Positive HBeAg is one of the risk factors for HCC in patients without complete viral suppression,[22] yet HBeAg positivity was not selected in the multivariable model on HCC development.

HBsAg seroclearance is considered as the functional cure of CHB, and widely accepted as a treatment goal of current antiviral treatment as well as new HBV therapies under clinical development.[14] Previous studies showed that patients who achieved HBsAg seroclearance have a very low risk of HCC and hepatic events.[11–12,15,17] While complete viral suppression *per se* is associated with lower risk of hepatic complications, it is important to examine the impact of HBsAg seroclearance on top of complete viral suppression. In our study, the risk of HCC development was lower in patients who cleared HBsAg than patients with complete viral suppression alone. None of the patients with CHB who achieved HBsAg seroclearance developed HCC after the first 3 years. Only 2 patients who had some of the established risk factors including age older than 50 years old at HBsAg seroclearance, male gender, cirrhosis, and DM at baseline, as suggested in previous studies, developed HCC after HBsAg seroclearance.[15,17] Thus, HBsAg seroclearance is an important endpoint for antiviral treatment that aims to further reduce the risk of HCC development on top of complete viral suppression.

In contrast, the risk of hepatic events and liver-related mortality was similar in patients with complete viral suppression and HBsAg seroclearance. This may suggest that hepatic necroinflammation and injury is the main driver of hepatic events. HBsAg seroclearance may not have additional effect on halting liver injury and promoting fibrosis regression over long-term inhibition of HBV replication which reduces liver inflammation, regresses liver fibrosis and prevents progression to cirrhosis.[6–7] The presence of cirrhosis remains as a major risk factor for hepatic events and liver-related death in patients under long-term NA treatment.

The strength of our study includes a large sample size and the provision of clinically important data on the impact of HBsAg seroclearance on top of complete viral suppression in NA treatment on the risk of HCC. To our knowledge, no previous studies have identified this respectable number of patients with antiviral treatment-induced HBsAg seroclearance for a similar comparison. Data from real-life cohorts represent a wider spectrum of patients than those in randomized controlled trials, in which patients at multiple comorbidities are often excluded. Also, stringent exclusion criteria are adopted to minimize bias. However, our study has a few limitations. Firstly, missing data and irregular intervals of laboratory measurement may lead to delayed detection of HBsAg seroclearance, missing HBV DNA measurement at baseline, and other biases as in other retrospective studies, though these biases can be partially compensated by our large cohort size. Secondly, the presence of cirrhosis was defined by ICD-9-CM codes. In real-life clinical practice, physicians may use different criteria to diagnose liver cirrhosis, which may affect the diagnosis coding in the computer system. Liver biopsy is not a routine clinical practice, and liver stiffness measurement is not widely available across public hospitals in Hong Kong. Some patients who developed HCC might have undiagnosed cirrhosis, but this can partly be

reflected by their age, male gender, and platelet counts at baseline. We also examined more definable ICD-9-CM codes for liver-related complications which do not rely on a more accurate diagnosis of cirrhosis for the identification of presence of cirrhosis. Thirdly, drug-resistance testing is not available in the public healthcare system. As some of our patients received NA with low genetic barrier of resistance (lamivudine, adefovir dipivoxil and telbivudine) prior to entecavir and/or TDF treatment, we are not certain if drug resistance was associated with incomplete viral suppression, and played a role on the development of HCC.[23] Lastly, other unmeasured factors might have confounded the results. The difference of clinical characteristics between patient groups may not be completely adjusted by the multivariable analyses using available baseline clinical characteristics. HBsAg quantification is not a routine clinical test in Hong Kong. Thus, the information of HBsAg levels is not available. We do not have the information on the adherence of clinicians and patients to the HCC surveillance guideline. We do not have the information of patients' family history of HCC. We do not have the information on drug adherence and HBV genotype, yet the proportion of patients who achieved complete viral suppression in our study was similar to that shown in phase III drug trials.[6,24] Previous studies have shown that the majority of patients with CHB in Hong Kong have either genotype B or C HBV, and genotype C HBV is associated with more severe liver fibrosis and an increased risk of HCC.[25–27]

In conclusion, this territory-wide cohort study confirms that complete viral suppression is associated with a lower risk of HCC and hepatic events in patients with CHB receiving antiviral treatment. HBsAg seroclearance may further reduce the risk of HCC but not the risk of hepatic events and liver-related mortality on top of complete viral suppression. These findings confirm HBsAg seroclearance as a treatment endpoint for international treatment guidelines and the development of novel therapies for CHB in patients with complete viral suppression.

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Conflict of interest

Grace Wong has served as an advisory committee member for Gilead. She has also served as a speaker for Abbott, Abbvie, Bristol-Myers Squibb, Echosens, Furui, Gilead, Janssen, and Roche. Henry Chan is a consultant for AbbVie, Bristol-Myers Squibb, Gilead, and Roche, has received honorarium for lecture for Abbvie, Bristol-Myers Squibb, Echosens, Gilead, Glaxo-Smith-Kline, Merck, Novartis and Roche, and has received an unrestricted grant from Roche for hepatitis B research.

Grace Lui has served as an advisory committee member for Gilead, speaker for Merck and Gilead, and received research grant from Gilead. Vincent Wong has served as an advisory committee member for Abbvie, Roche, Novartis, Gilead and Otsuka. He has also served as a speaker for Abbvie, Bristol-Myers Squibb, Roche, Novartis, Abbott Diagnostics and Echosens. The other authors declare that they have no competing interests.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Terry Yip, Yee-Kit Tse, Kelvin Lam and Grace Wong had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors were responsible for the study concept and design. Terry Yip, Yee-Kit Tse and Grace Wong were responsible for the acquisition and analysis of data. All authors were responsible for the interpretation of data, the drafting, and critical revision of the manuscript for important intellectual content.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2018.10.014.

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