Effect of Nucleos(t)ide Analogue Therapy on Risk of Intrahepatic Cholangiocarcinoma in Patients With Chronic Hepatitis B

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This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e71. Learning Objective–Upon completion of this activity, successful learners will be able to identify the high-risk patients for antiviral therapy to prevent hepatitis B virus-related liver cancer.

BACKGROUND & AIMS: **Chronic infection with hepatitis B virus (HBV) increases risk of intrahepatic cholangiocarcinoma (ICC), but it is not clear whether antiviral therapy reduces risk. We investigated the association between nucleos(t)ide analogue therapy and ICC risk.**

METHODS: **We performed a nationwide long-term cohort study using Taiwan's National Health Insurance Research Database to obtain data on 185,843 patients with chronic HBV infection from October 1, 2003 through December 31, 2012. We excluded patients with confounding disorders such as infection with hepatitis C virus, HIV, or other hepatitis-associated viruses; liver flukes; biliary stone diseases; cholangitis; congenital biliary anomalies; biliary tract surgeries; or cancer. We identified 10,062 patients who received nucleos(t)ide analogue therapy (the treated group), and used propensity scores to match them (1:1) with patients who received hepatoprotectants (the untreated group). Cumulative incidences of and hazard ratios (HRs) for ICC development were analyzed.**

RESULTS: **The cumulative incidence of ICC was significantly lower in the treated group after 3 years of therapy (1.28%; 95% CI, 0.56–2.01) than in the untreated group (3.14%; 95% CI, 2.02–4.27) and after 5 years of therapy (1.53%; 95% CI, 0.73–2.33 vs 4.32% in untreated group; 95% CI, 2.96–5.6869). In multivariable regression analysis, nucleos(t)ide analogue therapy was independently associated with a reduced risk of ICC (HR, 0.44; 95% CI, 0.25–0.78; $P = .005$). Older age (HR 1.05 per year; 95% CI, 1.03–1.07) and cirrhosis (HR, 2.80; 95% CI, 1.52–5.1415) were independently associated with an increased risk of ICC. Sensitivity analyses verified the association between nucleos(t)ide analogue therapy and a reduced ICC risk.**

CONCLUSION: **A nationwide long-term cohort study in Taiwan showed that nucleos(t)ide analogue therapy for chronic HBV infection is significantly associated with a reduced ICC risk.**

Keywords: CHB; Antiviral; Biliary; Cancer; Prevention.

Abbreviations used in this paper: **ALT, alanine transaminase; CCA, cholangiocarcinoma; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; ICC, intrahepatic cholangiocarcinoma; ICD, International Classification of Diseases; IQR, interquartile range; NA, nucleos(t)ide analogue; NHI, National Health Insurance; NHIRD, National Health Insurance Research Database.**

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See editorial on page 821.

Primary liver cancer is the second leading cause of cancer death worldwide, and cholangiocarcinoma (CCA) is the second most common type, comprising 10%–25% of all liver cancers.[1,2] In recent years, a rising trend of CCA incidence has been reported in industrialized countries.[3–7] In our previous nationwide population-based study conducted in Taiwan from 1998 to 2008, an increased incidence of CCA was also observed, especially intrahepatic cholangiocarcinoma (ICC).[8] However, only a small proportion of patients diagnosed with CCA can receive a curative treatment, and advanced CCA is usually refractory to any treatments.[9] In view of the rising trends of CCA incidence and the high mortality rate of CCA patients, the prevention of CCA development is a critical public health issue that needs to be addressed urgently.[10]

Several risk factors of CCA, such as liver flukes and biliary stone disease, have been documented, and these risk factors commonly induce chronic inflammation and injury to the biliary epithelium.[2,11] After hepatitis B virus (HBV) infection, the HBV components can be detected in bile duct epithelial cells, and the persistence of HBV may play an important pathogenic role.[12,13] Although studies on the pathology and carcinogenesis in HBV-mediated CCA have been conducted, the pathogenic mechanisms remain largely unknown.[14] However, increasing evidence from epidemiologic studies suggests that chronic HBV infection may increase CCA risk.[5,15] In recent meta-analyses of clinical studies, HBV infection was found to be significantly associated with the development of ICC, and thus HBV infection could be an important risk factor of ICC development that needs to be controlled.[16,17] However, although nucleos(t)ide analogues (NAs) can successfully inhibit HBV replication, the association between NA therapy and the risk of ICC development has not been investigated.

In the recent literature including our previous nationwide cohort study, it has been shown that NA therapy can reduce the risk of hepatocellular carcinoma (HCC).[18,19] However, even though HBV may also play a role in the pathogenesis of ICC, the effect of NA therapy on the prevention of ICC development is still unclear. We hypothesized that NA therapy could effectively reduce ICC risk, and therefore we conducted a nationwide cohort study to evaluate the association between NA therapy and the risk of ICC development.

Methods

Study Design

In this retrospective nationwide cohort study, we retrieved medical records from Taiwan's National Health Insurance Research Database (NHIRD) between October 1, 2003 and December 31, 2012. The NHIRD contains healthcare data from more than 99% of Taiwan's entire population of 23.38 million.[20] As outlined in our previous studies,[19,21,22] the NHIRD database comprises comprehensive medical data, and diseases are defined according to the International Classification of Diseases, 9th Revision (ICD-9) codes. The Research Ethics Committee of the National Health Research Institutes in Taiwan approved this study.

Study Population

The process of patient selection is shown in Figure 1. The ICD codes that were used are listed in Supplementary Methods. We screened all patients with chronic hepatitis B who had been diagnosed at least 3 times in outpatient clinics or 1 time in a hospitalization between October 1, 2003 and December 31, 2012. We initially excluded patients who did not use NAs or hepatoprotectants for at least 90 days during the study period. NA therapy for chronic hepatitis B has been reimbursed by the Taiwan National Health Insurance (NHI) program since October 1, 2003, but the NA application requires patients to fulfill certain criteria of active hepatitis B such as serum alanine aminotransferase (ALT) $\geq 2\times$ upper limit of normal and HBV viral load >2000 IU/mL in non-cirrhotic patients and HBV viral load >2000 IU/mL in cirrhotic patients (Supplementary Table 1).[19] Meanwhile, hepatoprotectants (eg, silymarin, liver hydrolysate, and choline bitartrate) are also reimbursed for chronic hepatitis B patients with elevated serum ALT.[19]

We further excluded patients with potential confounding factors including hepatitis C virus infection, other viral hepatitis, human immunodeficiency virus infection, liver flukes, cholangitis (including primary sclerosing cholangitis), congenital anomalies of gallbladder and bile duct, or biliary stone disease during the period of outcome follow-up. Patients who received any surgical or endoscopic operations on the biliary tract 90 days before the date of the study end point were also excluded. In addition, patients with any malignancy before or within 90 days after the first follow-up date were excluded. Finally, patients in the NA-treated cohort were randomly matched 1:1 with patients in the untreated cohort by means of the propensity scores, which were composed of age, sex, cirrhosis, liver decompensation, diabetes mellitus, and hyperlipidemia.

Main Outcome Measurement

The NA-treated and untreated patients were followed up to observe ICC development after the first date of NA therapy initiation and the first date of hepatoprotectant initiation, respectively. Study subjects were followed up until the dates of ICC diagnosis, patient mortality, or the end of the study period (December 31, 2012). All patients who were admitted with a primary diagnosis of ICC were identified, and the validity of ICC diagnosis was confirmed by the inclusion of patients in the Registry for

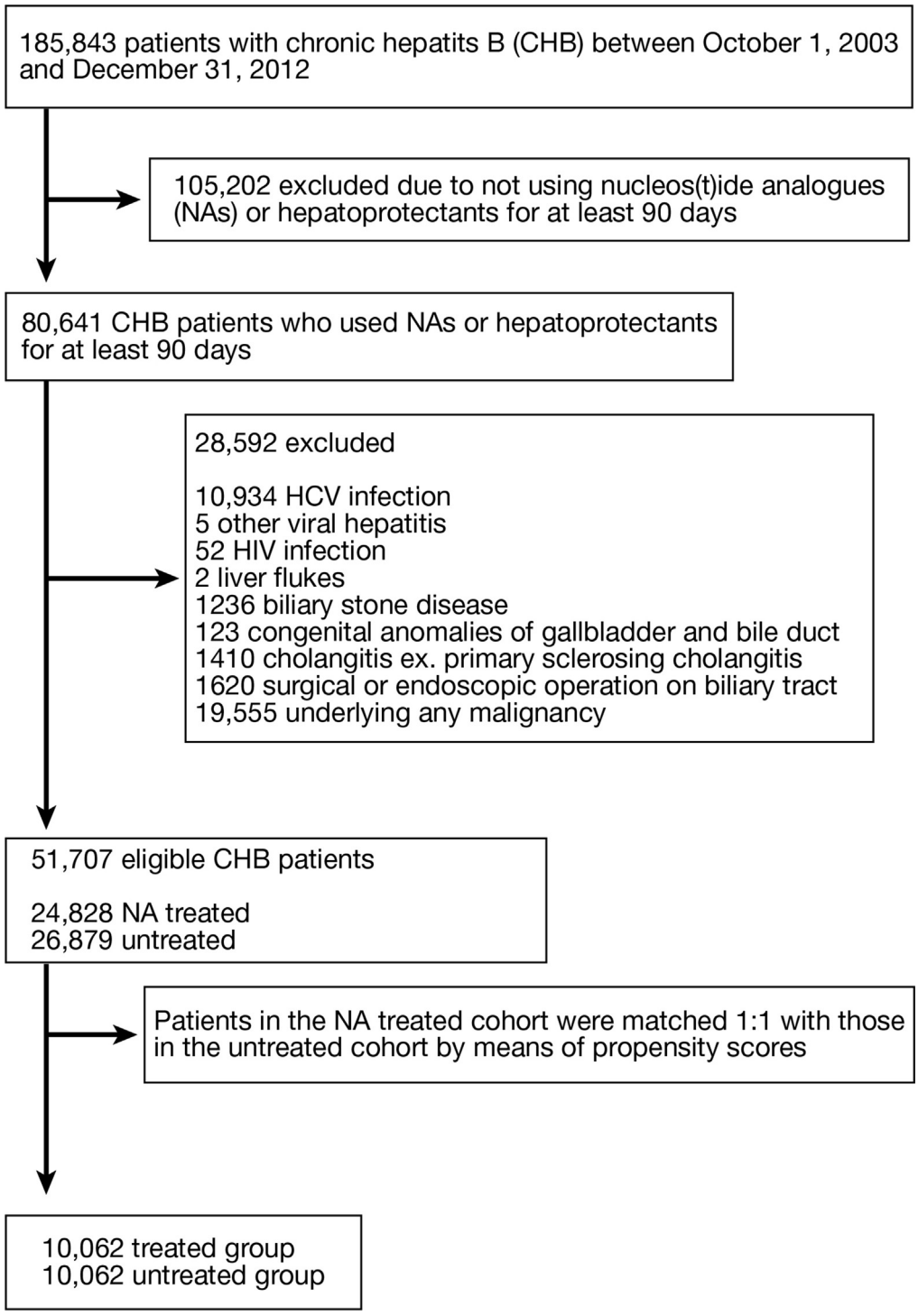


Figure 1. Selection of study subjects.

Catastrophic Illness Patient Database. The Registry for Catastrophic Illness Patient Database is an official NHI sub-system for copayment mitigation, in which histopathologic confirmation or typical imaging characteristics are required for the diagnosis of ICC.[19,21–25]

Prognostic Factor Assessment

In addition to age and sex, major coexisting diseases that might increase the risk of ICC development were evaluated, including alcoholic liver disease, cirrhosis, liver decompensation, diabetes mellitus, and hyperlipidemia. Coexisting diseases had been diagnosed at least 3 times in outpatient clinics or 1 time in a hospitalization.

Sensitivity Analysis

For reassuring the association between NA therapy and the risk of ICC development, we performed a sensitivity analysis by using patients who did not use

NAs or hepatoprotectants as controls. Similar to the process of patient selection in Figure 1, after excluding patients with potential confounding factors, patients in the NA-treated group were randomly matched 1:1 with controls by means of propensity scores. Cumulative incidences of ICC development in the 2 groups were compared.

Statistical Analysis

Continuous and categorical variables of demographic data were compared by using the Student t test and the χ^2 test, respectively. Cumulative incidences of ICC development were presented with 95% confidence intervals (CIs). After adjusting for competing mortality, cumulative incidences were calculated and compared by using a modified Gray method and the Kaplan-Meier method,[23] and differences in the full time-to-event distributions were compared by a modified log-rank test. Multivariable regression analyses were conducted to determine independent risk factors for ICC development. Hazard ratios (HRs) were determined by Cox proportional hazard models. Multivariable stratified analysis of the effects of NA therapy was performed in the patient subgroups. All data were analyzed by using SAS 9.3 software (SAS Institute, Inc, Cary, NC), and Cox models were constructed by using the "cmprsk" package for R (http://cran.r-project.org/web/packages/cmprsk/index.html).

Results

Study Subjects

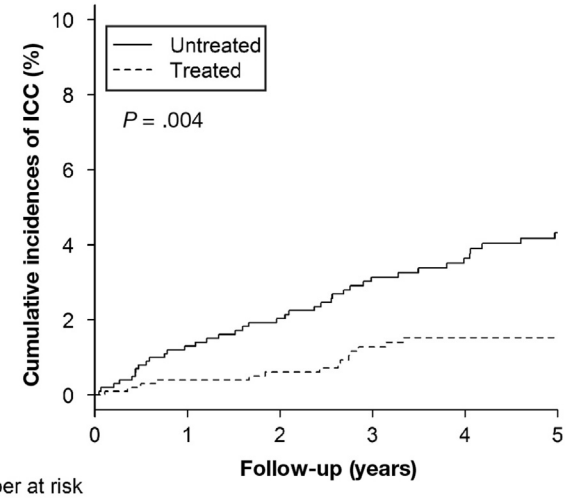
As presented in Figure 1, we screened 185,843 patients who had been diagnosed with chronic hepatitis B between October 1, 2003 and December 31, 2012, and 80,641 patients who used NAs or hepatoprotectants for at least 90 days were identified. After excluding patients with potential confounding factors, 51,707 patients were selected. Furthermore, patients in the NA-treated cohort were matched with patients in the untreated cohort, and a total of 10,062 patients in the treated group and 10,062 in the untreated group were recruited for analysis.

The baseline characteristics of the study subjects are presented in Table 1. Most patients were middle-aged, with a median age of 45.4 years, and 80% of patients were male. The median duration of follow-up was 5.8 years in each group. The frequency of ultrasound surveillance in the treated group was higher than that of the untreated group (median, 0.9 vs 0.7 times per year). The median duration of NA exposure in the treated group was 2.2 years (interquartile range [IQR], 1.5–4.4). In addition, 26.0% of patients were diagnosed with cirrhosis, and 11.5% of patients suffered from liver decompensation. Approximately 7%, 23%, and 27% of the patients had underlying alcoholic liver disease, diabetes mellitus, and hyperlipidemia, respectively.

Table 1. Demographic Characteristics of the Study Subjects

Characteristics	Treated n = 10,062	Untreated n = 10,062	*P* value
Age, *y*			
Mean ± SD	45.5 ± 13.1	45.5 ± 13.1	1.00
Median (IQR)	45.4 (35.6–54.5)	45.4 (35.6–54.5)	1.00
Sex, n (%)			1.00
Male	8058 (80.1)	8058 (80.1)	
Female	2004 (19.9)	2004 (19.9)	
Follow-up duration, *y*			
Mean ± SD	5.6 ± 2.5	5.6 ± 2.6	.61
Median (IQR)	5.8 (3.6–7.8)	5.8 (3.6–8.0)	.11
Ultrasound frequency, *n/year*			
Mean ± SD	1.1 ± 1.1	1.0 ± 1.1	<.01
Median (IQR)	0.9 (0.3–1.7)	0.7 (0.3–1.4)	<.01
NA therapy duration, *y*			
Mean ± SD	3.1 ± 2.4	—	
Median (IQR)	2.2 (1.5–4.4)	—	
Hepatoprotectant, *y*			
Mean ± SD	0.8 ± 1.1	1.0 ± 1.0	<.01
Median (IQR)	0.4 (0.1–1.0)	0.7 (0.3–1.4)	<.01
Major coexisting diseases, n (%)			
Alcoholic liver disease	700 (7.0)	700 (7.0)	1.00
Cirrhosis	2618 (26.0)	2618 (26.0)	1.00
Liver decompensation	1161 (11.5)	1161 (11.5)	1.00
Diabetes mellitus	2309 (22.9)	2309 (22.9)	1.00
Hyperlipidemia	2757 (27.4)	2757 (27.4)	1.00

IQR, interquartile range; SD, standard deviation.



Number at risk

Untreated	10062	9669	8948	8136	7088	5934
Treated	10062	9715	9218	8388	7042	5937

Figure 2. Cumulative incidence of ICC in NA-treated or untreated groups. Follow-up from 3 months after drug initiation.

Cumulative Incidence of Intrahepatic Cholangiocarcinoma or Hepatocellular Carcinoma

A total of 56 patients (0.28%) were found to have developed ICC in 5 years, 17 (0.17%) in the treated group and 39 (0.39%) in the untreated group ($P = .005$). As presented in Figure 2, the cumulative incidence of ICC in the treated group was significantly lower than that in the untreated group ($P = .004$). After adjusting for competing mortality, the cumulative incidence of ICC in the treated group was significantly lower than that in the control group in 1 year (0.40%, 95% CI, 0.01%–0.79% vs 1.31%, 95% CI, 0.60%–2.01%), 3 years (1.28%, 95% CI, 0.56%–2.01% vs 3.14%, 95% CI, 2.02%–4.27%), and 5 years (1.53%, 95% CI, 0.73%–2.33% vs 4.32%, 95% CI, 2.96%–5.69%).

As the main primary liver cancer related to HBV, cumulative incidence of HCC development was also calculated during the study period (Supplementary Figure 1). The cumulative incidence of HCC in the treated group was significantly lower than that in the untreated group in 5 years (2.93%, 95% CI, 2.57%–3.28% vs 4.75%, 95% CI, 4.31%–5.20%; $P < .001$). The risk of ICC or HCC development was simultaneously decreased in the treated group.

Multivariable Analysis of Prognostic Factors

In the univariable regression analyses, NA therapy (HR, 0.44; 95% CI, 0.25–0.78) was significantly associated with lower risk of ICC development, but older age (HR, 1.06 per year; 95% CI, 1.05–1.08), cirrhosis (HR, 4.36; 95% CI, 2.55–7.47), and diabetes mellitus (HR, 1.92; 95% CI, 1.11–3.33) were significantly associated with higher risk of ICC development (Table 2). In the multivariable regression analysis, NA therapy remained an independent risk factor that was associated with decreased risk of ICC development (HR, 0.44; 95% CI, 0.25–0.78). Older age (HR, 1.05 per year; 95% CI, 1.03–1.07) and cirrhosis (HR, 2.80; 95% CI, 1.52–5.15) were still significantly associated with higher risk of ICC development.

Multivariable Stratified Analysis for Nucleos(t)ide Analogue Treatment

As presented in Figure 3, multivariable stratified analyses verified the association of NA treatment and decreased ICC development risk in almost all patient subgroups, especially for those aged >45 years (HR, 0.47; 95% CI, 0.26–0.87), men (HR, 0.35; 95% CI, 0.18–0.69), those without coexisting alcoholic liver disease (HR, 0.42; 95% CI, 0.23–0.74), those with underlying cirrhosis (HR, 0.42; 95% CI, 0.20–0.88), those without liver decompensation (HR, 0.48; 95% CI, 0.26–0.87), those without diabetes mellitus (HR, 0.39; 95% CI, 0.19–0.81), and those without hyperlipidemia (HR, 0.30; 95% CI, 0.15–0.60).

Table 2. Cox Proportional Hazards Model Analysis for Risk of Cholangiocarcinoma

	Univariate		Multivariable	
Variables	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Treated vs untreated	0.44 (0.25–0.78)	.005	0.44 (0.25–0.78)	.005
Age per year	1.06 (1.05–1.08)	<.001	1.05 (1.03–1.07)	<.001
Cirrhosis	4.36 (2.55–7.47)	<.001	2.80 (1.52–5.15)	<.001
Diabetes mellitus	1.92 (1.11–3.33)	.019	0.96 (0.54–1.70)	.895
Male	0.80 (0.43–1.48)	.478		
Alcoholic liver disease	0.25 (0.03–1.78)	.164		
Liver decompensation	0.90 (0.39–2.10)	.806		
Hyperlipidemia	0.73 (0.39–1.39)	.343		

CI, confidence interval; HR, hazard ratio.

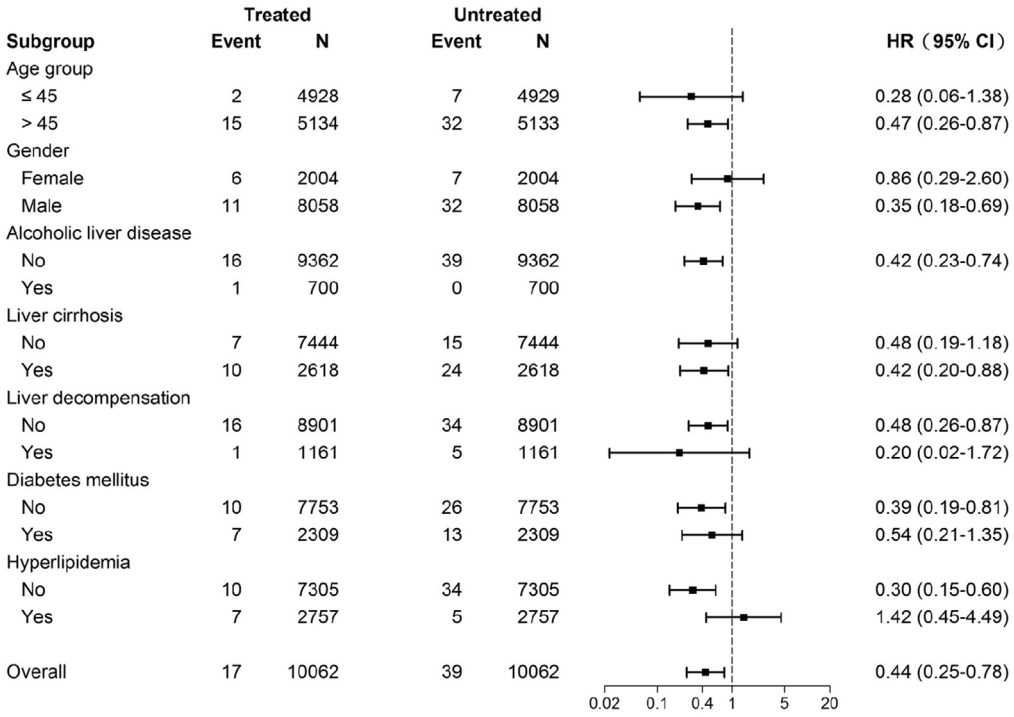


Figure 3. Multivariable stratified analyses of association between NA therapy and ICC development.

Sensitivity Analysis

After the process of patient selection, 9169 patients in the NA-treated group were matched with 9169 patients who did not use NAs or hepatoprotectants (Supplementary Table 2). The cumulative incidence of ICC in the NA-treated group was significantly lower than that in the control group at 1 year (0.44%, 95% CI, 0.01%–0.87% vs 1.44%, 95% CI, 0.66%–2.22%), 3 years (1.28%, 95% CI, 0.52%–2.04% vs 2.17%, 95% CI, 1.19%–3.14%), and 5 years (1.42%, 95% CI, 0.62%–2.23% vs 3.36%, 95% CI, 2.08%–4.63%; $P = .03$) (Supplementary Figure 2). The 5-year cumulative incidence of ICC in this control group was slightly lower compared with that in the hepatoprotectant-treated group (4.32%, 95% CI, 2.96%–5.68%; Figure 2). According to the NHI reimbursement criteria, patients who did not use hepatoprotectants might not suffer from persistent ALT elevation, and the ICC risk in patients with active hepatitis B could thus be underestimated. However, the cumulative incidence of ICC in the NA-treated group remained lower than that in the control group.

Discussion

Although HBV infection has been shown to be a major risk factor for ICC development,[16,17] the protective effect of NA therapy via directly inhibiting HBV replication is poorly understood. This large cohort study demonstrated that ICC development rates were significantly lower in the NA-treated group compared with rates in the untreated group, and NA therapy was an independent risk factor associated with reduced risk of ICC development. This study reports a positive effect of NA therapy on the prevention of ICC development. The findings of this study support the notion that NA therapy could reduce ICC risk, but further prospective studies are required to confirm our findings.

Although HBV can be found in bile duct epithelial cells and tumor tissues of CCA,[12,13,24] the precise pathogenic mechanisms of HBV-mediated CCA remain largely unclear.[14] In previous pathologic studies for HBV-related CCA, the detection rate of hepatitis B virus X protein could be as high as 60%–70%.[24,25] In a zebrafish model, liver fibrosis and ICC were induced by dual expression of hepatitis B virus X and hepatitis C virus core protein, and the signaling pathway of transforming growth factor beta 1 was involved.[26] Although other lines are needed to elucidate all of the relationships involved in this mechanism, the current evidence suggests that HBV may play a role in the pathogenesis of CCA development. By directly inhibiting HBV replication, NA therapy may effectively reduce HBV burden, liver inflammation, and fibrosis in liver; the role of NA therapy in the prevention of HCC development or recurrence has been established in previous studies.[19,22,27] However, the potential mechanisms of CCA prevention by NA treatment have rarely been investigated.

A growing body of clinical studies supports the relationship between HBV infection and ICC development. In a meta-analysis of case-control studies, HBV significantly increased ICC risk (odds ratio, 3.97).[16] HBV was also shown to be related to ICC risk in a large cohort study in Taiwan.[15] Moreover, HBV appears to be a potential risk factor for extrahepatic CCA.[28] In a large case-control study conducted in Taiwan, the odds ratio of HBV

infection related to intrahepatic and extrahepatic CCA was 3.5 and 2.6, respectively.[29] In a recent meta-analysis study of CCA development, the relative risk of HBV infection was 3.42 and 1.68 for ICC and extrahepatic CCA, respectively.[17] However, the relationship of HBV infection with ICC development is stronger and more consistent than that with extrahepatic CCA development, so the hypothesis that NA therapy may prevent ICC development should be examined first.

As revealed in previous reports,[29] older age and cirrhosis were revealed to be independent risk factors related to ICC development in our study. ICC is usually caused by exposure to risk factors for many years,[30] and it is conceivable that old or cirrhotic patients are therefore more susceptible to the development of ICC. In our previous nationwide population-based study for the period 1998–2008, a rising trend of ICC incidence was observed among patients older than 65 years of age, and the incidence remained low and stable among younger patients (<55 years).[8] Although a universal vaccination program against HBV has been conducted in Taiwan since 1984, the effects of HBV vaccination on ICC prevention may be expected several decades later. However, for patients who have been suffering from chronic hepatitis B, NA therapy may provide a useful way to prevent ICC development. In our subgroup analyses, NA therapy could effectively prevent ICC development in older and cirrhotic patients.

Several limitations should be acknowledged. First, although we carried out a quasi-experimental design that took potential confounders into account, a causal relationship between NA therapy and ICC risk could not be ascertained owing to the observational nature of this study. Nonetheless, it is probably neither ethical nor practical to conduct a randomized trial to resolve this issue. Second, although our database does not contain detailed laboratory data, the hepatitis status of the study subjects could be defined according to the above-mentioned NA reimbursement criteria in Taiwan. Patients receiving NA therapy must fulfill certain criteria of active hepatitis B such as HBV viral load >2000 IU/mL in cirrhotic patients. Thus, it was expected that hepatitis would be more severe in the NA-treated group than that in the untreated counterpart, and the protective effect of NA therapy might have been underestimated. The conclusion of this study was not affected. Even though few patients with active hepatitis may leave untreated with NA, the ICC risk in the untreated group should not be higher than that in the NA-treated group. In the sensitivity analysis that used patients who did not use NAs or hepatoprotectants as controls, the ICC risk in the NA-treated group remained lower than that in the control group. Third, cirrhosis was defined according to the ICD codes. For validating the diagnosis, patients with cirrhosis had been diagnosed at least 3 times in outpatient clinics or 1 time in a hospitalization, and we further identified patients with liver decompensation, which was a specific condition and must be confirmed by medical records. Patients with cirrhosis or liver decompensation were matched in both groups, and the differences on the diagnosis of cirrhosis might have been minimized. Fourth, HBV infection might be completely eradicated after 1 episode of acute infection or exacerbation. However, NA therapy could be reimbursed only for chronic hepatitis B, and we also excluded patients who did not use NAs for at least 90 days. This concern was probably very unlikely in NA-treated patients. For patients in the untreated group, even though HBV infection might have disappeared, the ICC risk would thus be reduced. The difference in ICC risk between the 2 groups could have been underestimated, but the conclusion of this study was not changed. Fifth, patients in the untreated group had a higher risk of HCC development (Supplementary Figure 1), and they might have suffered from HCC and died before ICC occurrence. Patients in the untreated group had a lower chance of developing ICC, and the difference in ICC development between the 2 groups could have been underestimated. However, the conclusion of this study remains unchanged. Last, it is possible that some patients in the untreated cohort used self-financed NAs, but nevertheless, this limitation of potential misclassification would have biased the result toward a null difference between the study cohorts.

In summary, the results of this nationwide long-term cohort study demonstrated that NA therapy was significantly associated with reduced risk of ICC development in patients with chronic hepatitis B.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2017.09.031.

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Conflicts of interest
The authors disclose no conflicts.

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Supplementary Methods

International Classification of Diseases, 9th Revision Codes Used for Identifying Patients With Underlying Diseases

Chronic hepatitis B (ICD codes 070.2, 070.3, and V02.61)

Hepatitis C virus infection (ICD codes 070.41, 070.44, 070.51, 070.54, 070.7, and V02.62)

Other viral hepatitis (ICD code 573.2)

Human immunodeficiency virus infection (ICD code 042)

Liver flukes (ICD codes 121.0, 121.1, and 121.3)

Cholangitis (including primary sclerosing cholangitis) (ICD code 576.1)

Congenital anomalies of gallbladder and bile duct (ICD code 751.69)

Biliary stone disease (ICD codes 574.3–574.9)

Surgical or endoscopic operations on the biliary tract (ICD procedure codes 51.36, 51.37, 51.39, and 51.4–51.9)

Malignancy (ICD codes 140–208)

ICC (ICD codes 155.1 and 156.9)

HCC (ICD code 155.0)

Alcoholic liver disease (ICD codes 571.0–571.3, 303.9, V11.3, and V79.1)

Cirrhosis (ICD codes 571.2, 571.5, and 571.6)

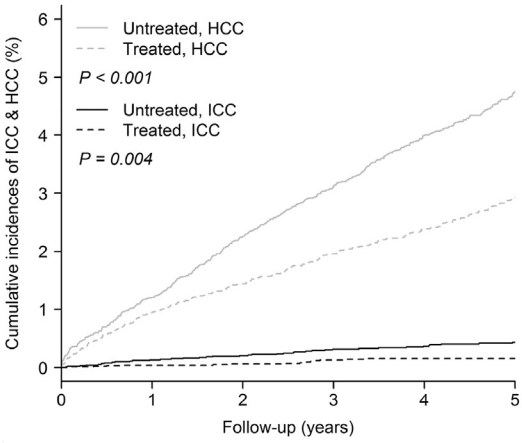
Liver decompensation (ICD codes 789.5, 572.2, and 572.4)

Diabetes mellitus (ICD codes 250 and 648.0)

Hyperlipidemia (ICD codes 272.0–272.4)

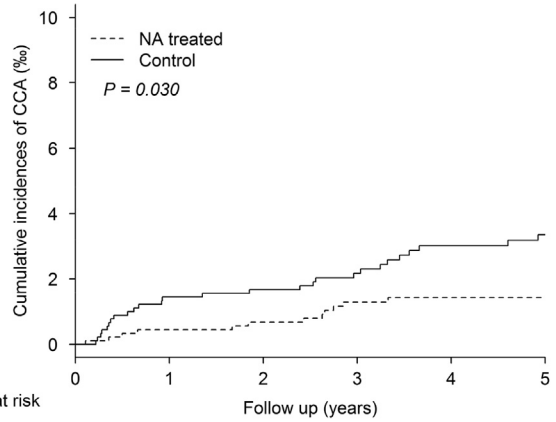
Excluded Patients With Potential Confounding Factors

1. Patients with hepatitis C virus infection, other viral hepatitis, human immunodeficiency virus infection, liver flukes, cholangitis (including primary sclerosing cholangitis), congenital anomalies of gallbladder and bile duct, or biliary stone disease during the period of outcome follow-up.
2. Patients who received any surgical or endoscopic operations on the biliary tract 90 days before the date of the study end point.
3. Patients with any malignancy before or within 90 days after the first follow-up date.



Number at risk						
Untreated, HCC	10062	9592	8823	7989	6928	5786
Treated, HCC	10062	9657	9135	8299	6966	5865
Untreated, ICC	10062	9669	8948	8136	7088	5934
Treated, ICC	10062	9715	9218	8388	7042	5937

Supplementary Figure 1. Cumulative incidence of HCC or ICC in NA-treated or untreated groups. Follow-up from 3 months after drug initiation.



Number at risk						
NA treated	9147	8875	8464	7770	6551	5561
Control	9147	8426	7786	7001	5790	4831

Supplementary Figure 2. Cumulative incidence of ICC development in NA-treated or control groups. Follow-up from 3 months after NA initiation.

Supplementary Table 1. NA Reimbursement Criteria of Taiwan's National Health Insurance for Patients With Chronic Hepatitis B During Study Period

Date	Medication	NHI criteria (HBsAg+ > 6 months)
October 1, 2003	Lamivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation (jaundice or prothrombin time ≥ 3 seconds) 3. Hepatitis after organ transplantation (prophylaxis before liver transplantation)
February 1, 2004	Lamivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation (bilirubin ≥ 3.0 mg/dL or prothrombin time ≥ 3 seconds) 3. Organ transplantation 4. Hepatitis after chemotherapy
August 1, 2004	Lamivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation (bilirubin ≥ 3.0 mg/dL or prothrombin time ≥ 3 seconds) 3. Organ transplantation 4. Hepatitis after chemotherapy 5. ALT ≥ 2× ULN, liver Bx: HBcAg+
October 1, 2005	Lamivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation (bilirubin ≥ 2.0 mg/dL or prothrombin time ≥ 3 seconds) 3. Organ transplantation 4. Hepatitis during chemotherapy 5. ALT ≥ 2× ULN, liver Bx: HBcAg+
October 1, 2006	Lamivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation (bilirubin ≥ 2.0 mg/dL or prothrombin time ≥ 3 seconds) 3. Organ transplantation (long-term use) 4. Hepatitis during chemotherapy 5. ALT ≥ 2× ULN, liver Bx: HBcAg+
	Adefovir	ALT ≥ 2× ULN, YMDD mutation (shift to adefovir)
August 1, 2008	Lamivudine Entecavir (0.5 mg) Telbivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation 3. Organ transplantation (long-term use) 4. Hepatitis after chemotherapy 5. ALT ≥ 2× ULN, liver Bx: HBcAg+
	Adefovir	ALT ≥ 2× ULN, YMDD mutation
November 1, 2009	Lamivudine Entecavir Telbivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation 3. Organ transplantation (long-term use) 4. Prophylaxis before chemotherapy or hepatitis during chemotherapy 5. ALT ≥ 2× ULN, liver Bx: HBcAg+ or blood HBV DNA ≥ 20,000 for HBeAg+ (HBV DNA ≥ 2000 IU/mL for HbeAg–)
	Adefovir Entecavir (1 mg)	HBV DNA increase > 1 log IU/mL: add-on adefovir or shift to entecavir (only for lamivudine user)
July 1, 2010	Lamivudine Entecavir Telbivudine	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation 3. Organ transplantation (long-term use) 4. Prophylaxis or hepatitis after chemotherapy 5. ALT ≥ 2× ULN, liver Bx: HBcAg+ or blood HBV DNA ≥ 20,000 for HBeAg+ (HBV DNA ≥ 2000 IU/mL for HbeAg–) 6. Cirrhosis, HBV DNA ≥ 2000 IU/mL (long-term use)
	Adefovir Entecavir 1 mg	HBV DNA increase > 1 log IU/mL: add-on adefovir or shift to entecavir (only for lamivudine user)
June 1, 2011 The end of study period (December 31, 2012)	Lamivudine Entecavir Telbivudine Tenofovir	1. HBeAg+, ALT ≥ 5× ULN 2. Liver decompensation 3. Organ transplantation (long-term use) 4. Prophylaxis or hepatitis after chemotherapy 5. ALT ≥ 2× ULN, liver Bx: HBcAg+ or blood HBV DNA ≥ 20,000 for HBeAg+ (HBV DNA ≥ 2000 IU/mL for HbeAg–) 6. Cirrhosis, HBV DNA ≥ 2000 IU/mL (long-term use)
	Adefovir Entecavir 1 mg	HBV DNA increase > 1 log IU/mL: add-on adefovir or shift to entecavir (only for lamivudine user)

ALT, alanine aminotransferase; Bx, biopsy; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ULN, upper limit of normal.

Supplementary Table 2. Demographic Characteristics of the Study Subjects

Characteristics	NA treated n = 9147	Control n = 9147	*P* value
Age, *y*			
Mean ± SD	44.7 ± 12.9	44.7 ± 12.9	.98
Median (IQR)	44.6 (34.9–53.8)	44.6 (34.9–53.8)	.98
Sex, n (%)			1.00
Male	7303 (79.8)	7303 (79.8)	
Female	1844 (20.2)	1844 (20.2)	
Follow-up duration, *y*			
Mean ± SD	5.7 ± 2.4	5.2 ± 2.6	<.01
Median (IQR)	6.0 (3.7–7.8)	5.3 (3.1–7.5)	<.01
Ultrasound frequency, *n/y*			
Mean ± SD	1.1 ± 1.0	1.4 ± 7.4	<.01
Median (IQR)	0.9 (0.3–1.6)	0.4 (0.1–0.9)	<.01
NA therapy duration, *y*			
Mean ± SD	3.1 ± 2.4	—	
Median (IQR)	2.1 (1.5–4.3)	—	
Hepatoprotectant, *y*			
Mean ± SD	0.7 ± 1.0	0.1 ± 0.2	<.01
Median (IQR)	0.4 (0.1–0.9)	0 (0–0.1)	<.01
Major coexisting diseases, n (%)			
Alcoholic liver disease	526 (5.8)	548 (6.0)	.51
Cirrhosis	1921 (21.0)	1937 (21.2)	.79
Liver decompensation	755 (8.3)	774 (8.5)	.63
Diabetes mellitus	1986 (21.7)	1996 (21.8)	.87
Hyperlipidemia	2641 (28.9)	2655 (29.0)	.83

IQR, interquartile range; SD, standard deviation.