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Temporal trend and risk determinants of hepatocellular carcinoma in chronic hepatitis B patients on entecavir or tenofovir

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Summary

This study aimed to elucidate the temporal change and determinants for the risk of HCC in patients with chronic hepatitis B continuously receiving NUC. Through analysis of the national healthcare database in Taiwan, we screened a total of 65 426 infected patients receiving entecavir or tenofovir for at least 3 months and excluded those with lamivudine, adefovir or telbivudine exposure, malignancy, end-stage renal failure or a diagnosis of HCC within 3 months of starting treatment. Eligible patients (N = 27 820) were followed until HCC occurrence, completion of the allowed 3-year regimen or 31 December 2013. During a median follow-up of 25.1 (12.1-35.6) months, 802 patients developed HCC, with 1-, 2- and 3-year cumulative incidence of 1.82% (95% CI, 1.66-1.99%), 3.05% (95% CI, 2.82-3.28%) and 4.06% (95% CI, 3.77-4.36%), respectively. HCC annual incidence decreased with an adjusted IRR of 0.73 (95% CI, 0.66-0.80) per yearly interval and was associated with cirrhosis (IRR, 10.07; 95% CI, 6.00-16.90 in age <40 years; 4.69; 95% CI, 3.94-5.59 in age ≥40 years), age (IRR, 3.38; 95% CI, 2.10-5.47 for 40-50 years; 6.92; 95% CI, 4.27-11.21 for 50-60 years; 12.50; 95% CI, 7.71-20.25 for ≥60 years; <40 years as reference), male sex (IRR, 1.71; 95% CI, 1.44-2.04), HCV coinfection (IRR, 1.27; 95% CI, 1.02-1.58) and diabetes (IRR, 1.24;

95% CI, 1.05-1.45). In conclusion, the risk of HCC in patients with chronic hepatitis B receiving entecavir or tenofovir declines over time and is determined by cirrhosis, age, male sex, HCV coinfection and diabetes.

KEYWORDS

antiviral treatment, chronic hepatitis B, hepatocellular carcinoma, risk stratification

1 | INTRODUCTION

Chronic infection with HBV is the main aetiology of HCC worldwide, accounting for more than 50% of new HCC cases.[1,2] Vaccination effectively prevents infection among unexposed individuals[3,4] and correlates with substantial reduction in HCC in countries where universal programs were implemented.[5,6] For chronically infected patients, we and others have shown that antiviral therapy with interferon or NUCs is associated with a reduction in the overall risk of occurrence and recurrence of HCC.[7-11] However, antiviral therapy attenuates but cannot eliminate the risk of HBV-related HCC.[12] Despite efficacious antiviral treatment, a residual risk of HCC remained.[13]

HCC risk stratification in NUC-treated CHB has attracted intense research interest.[13-20] Recent studies from Europe found cirrhosis, older age, male sex and low platelet count to be key determinants among treated patients.[14,15] Other studies from Asia reported that liver stiffness, serum alpha-fetoprotein and virological response were also predictive of HCC.[17-20] These findings, however, mainly came from extended follow-up of patients enrolled in interventional trials or observation of selected hospital-based cohorts. Besides, many of these prior studies dealt with older generations of NUCs such as lamivudine that was no longer considered as the first therapy. Population-based data have been lacking. Moreover, it remains elusive how the risk of HCC might change over time among NUC-treated patients, but this knowledge would be essential to estimate the HCC risk on therapy.

Ideally, elucidating the temporal change and risk determinants of HCC in the era of antiviral therapy for CHB requires a large number of participants who are unbiasedly recruited from the general population and continuously treated with an optimal agent. We therefore carried out a nationwide cohort study that fulfils these criteria to examine how the risk of HCC changed over time while on treatment, and what baseline factors determined its occurrence in CHB patients receiving entecavir or tenofovir.

2 | METHODS AND MATERIALS

2.1 | Study design and data source

This cohort study was based on analysis of the Taiwan National Health Insurance Research Database (NHIRD), which has been previously detailed.[21] In brief, the NHIRD comprehensively contains claim data for healthcare services of the entire Taiwanese population, thanks to the universal and compulsory policy of the state-run health insurance. Our prior studies have verified its utility for population-based studies.[8,9] The Registry for Catastrophic Illness Patient Database (RCIPD), which

is a subpart of NHIRD, is reliable for ascertaining serious disease diagnoses.[22] Copayment is waived for patients with a certified RCIPD disease, and therefore, the certification is strictly audited. This study was approved by the Research Ethics Committee of the Taiwan National Health Research Institutes and the Institutional Review Board of the E-Da Hospital (EMRP-102-097), both of which waived obtainment of the informed consent because the analysed data were deidentified.

2.2 | Eligibility criteria

We screened for eligibility all Taiwanese residents who received entecavir or tenofovir for CHB from 2008 to 2013. Inclusion required fulfilment of all the following criteria: age >18 years, HBV infection defined by a specific ICD-9-CM code (070.2, 070.3, V02.61) combined with a test for HBsAg and using (defined as filled prescription) entecavir or tenofovir continuously (defined as gaps between prescription fills <7 days) for a minimum of 3 months. We excluded patients with a diagnosis of any malignant disease or end-stage renal failure preceding the index date of starting entecavir or tenofovir, occurrence of HCC or death within 3 months of starting therapy or exposure to NUCs with a low genetic barrier to drug resistance (defined as filled prescription of lamivudine, adefovir or telbivudine for 3 months or longer) prior to the current antiviral regimen.

2.3 | Antiviral therapy

The Taiwan national health insurance started to reimburse CHB patients for entecavir and tenofovir since 1 August 2008 and 1 June 2011, respectively. The reimbursement regulation has been described.[9] Briefly, serum HBV DNA >2000 IU/mL is required, unless there is hepatic decompensation, organ transplantation or malignancy that necessitates cytotoxic agents. In addition, patients need to have biochemical hepatitis with serum alanine aminotransferase (ALT) higher than at least 2 folds the upper limit of normal levels if the liver is not cirrhotic. Furthermore, serum ALT should be elevated for at least 3 months among CHB patients who are HBeAg negative. Treatment duration is restricted to a maximum of 3 years in patients without cirrhosis, malignancy or organ transplant.[9]

2.4 | Definition of the study outcome and comorbidity

The primary outcome was occurrence of HCC, defined by ICD-9-CM code (155.0 or 155.2) and certified registration in the RCIPD.

Observation for HCC occurrence commenced after 3 months of starting CHB treatment and continued until the end of the allowed 3-year treatment period, death, discontinuation of antiviral therapy (interruption of treatment for 3 months or longer) or 31 December 2013. The incidence of HCC was adjusted for competing morality because death would lead to informative censoring.[23]

Baseline comorbidity was defined by the ICD-9-CM code in conjunction with the corresponding pharmacotherapy (Table S1). For instance, we defined diabetes mellitus (DM), dyslipidemia and hypertension by disease-specific codes combined with filled prescriptions of disease-defining medication for a minimum of 3 months. We subclassified DM according to insulin use. The definition of cirrhosis was based on the ICD-9-CM codes. Decompensated cirrhosis denoted encephalopathy, variceal bleeding or refractory ascites.[23] Exposure to metformin and statin was separately analysed, in view of their potential effect on HCC risk.[24-26] A user was defined by cumulative filled prescription for at least 3 months.

2.5 | Data analysis statistical tests

We managed the data set using the SAS software (9.2 version; SAS Institute, Cary, NC, USA) and the R software with the "cmprsk_2.1-4" package for the competing risk analyses. Continuous variables were expressed by median and IQR, and categorical variables by percentage and exact number. Calculated estimates were reported along with their 95% CIs. All statistical tests were two-sided, and a P value <.05 defined the statistical significance.

The cumulative incidence of HCC was estimated by the competing risk analysis according to the modified Kaplan–Meier method. Predictors of HCC were explored by the modified proportional hazard model
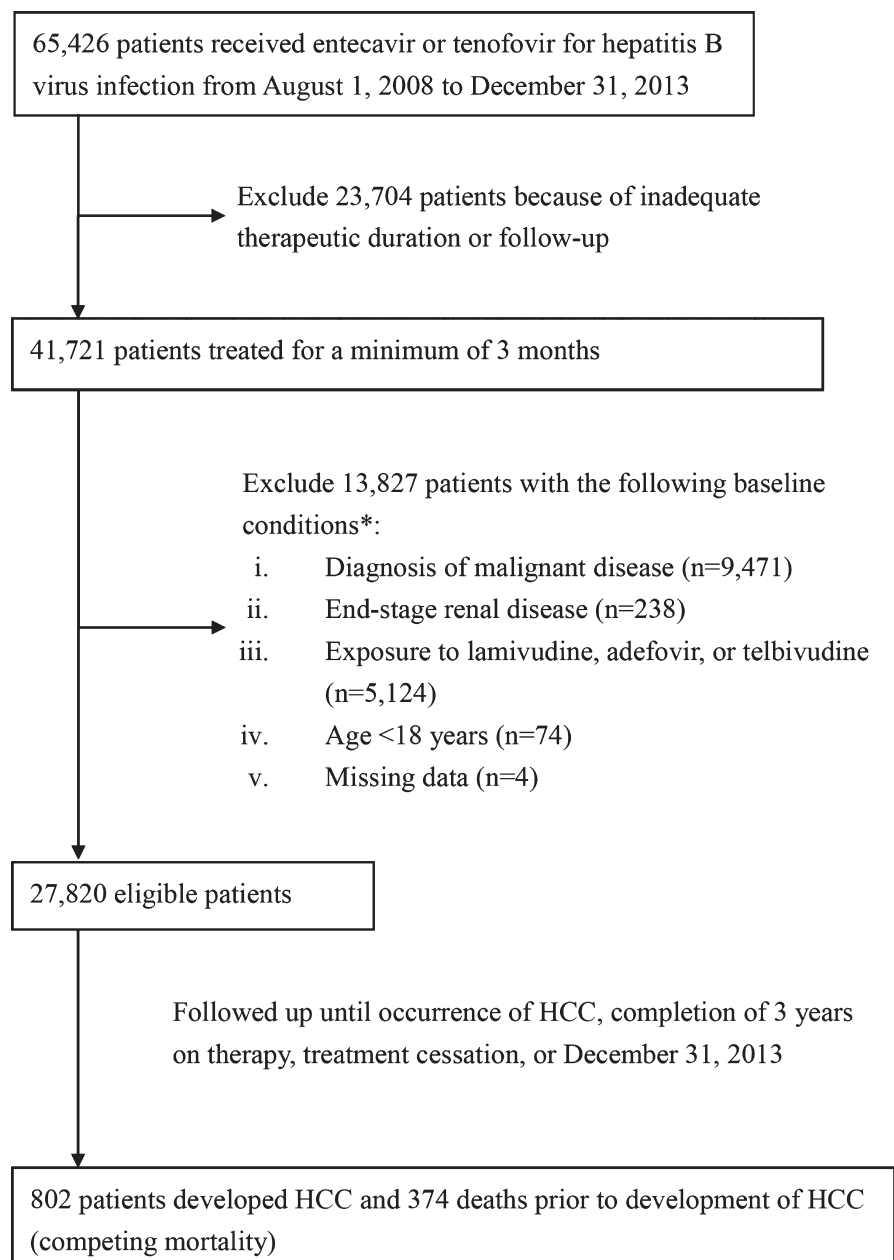
FIGURE 1 The flow chart for patient identification and enrolment

adjusted for competing mortality.[27] All baseline characteristics including demographics, comorbidities and medications were examined. The model started with all variables and was developed by backward elimination. Model selection was carried out according to the Akaike information criterion. The final model also took into account significant variables in the Poisson regression analysis. Adjusted HRs were reported. The study cohort was then stratified by the identified predictors into subgroups to illustrate their capability of risk stratification. The between-group difference in the HCC incidence was examined by the Gray's method.[28] To appraise how the risk of HCC might change over time, we performed the Poisson regression to calculate the annual incidence rate. Multivariate-adjusted IRRs were computed in the final model.

3 | RESULTS

3.1 | Characteristics of the study population

From 1 August 2008 through 31 December 2013, a total of 65 426 Taiwanese residents received entecavir or tenofovir for CHB and 27 820 patients were eligible (Figure 1). Their baseline characteristics were summarized (Table 1). Approximately one-third of the study cohort were diagnosed with liver cirrhosis. The vast majority used entecavir because it was reimbursed for since 1 August 2008, while tenofovir was not reimbursed for until 1 June 2011. Among the 1,946 patients coinfected with hepatitis C virus (HCV), only a small proportion (n = 242, 12.4%) ever received interferon-based treatment.

3.2 | Cumulative incidence of HCC

The study cohort was placed on treatment for a median duration of 28.9 (IQR, 15.9-36.4) months. Observation commenced after the first 3 months of "washout period" and lasted for a median period of 25.1 (IQR, 12.1-35.6) months. During the 53 188 person-years of observation, a total of 802 patients developed HCC. The cumulative incidence after 1, 2 and 3 years on therapy was 1.82% (95% CI, 1.66-1.99%), 3.05% (95% CI, 2.82-3.28%) and 4.06% (95% CI, 3.77-4.36%), respectively (Figure 2). The annual incidence (per 1000 person-years) was 18.72‰ (95% CI, 17.00-20.44‰) in the first (0-1) year, 12.81‰ (95% CI, 11.14-14.48‰) in the second[1,2] year and 10.69‰ (95% CI, 8.77-12.61‰) in the third [2,3] year (P < .001). The annual incidence rate of HCC declined over time with a crude IRR of 0.739 (95% CI, 0.671-0.813) per yearly interval.

### 3.3 | Pretreatment factors associated with risk of HBV-related HCC on therapy

The multivariate Cox proportional hazard analysis revealed that liver cirrhosis (adjusted HR, 4.91; 95% CI, 4.10-5.87; P < .001), older age (HR, 1.65 per decade; 95% CI, 1.56-1.74; P < .001), male sex (1.73; 95% CI, 1.46-2.07; P < .001), HCV coinfection (1.23; 95% CI, 0.98-1.53; P = .068) and DM (1.25; 95% CI, 1.06-1.47; P = .008) were associated with the risk of HCC (Table 2). The incidences of HCC were distinctly separated among patients according to each and

every of these factors (Figure 3A-F). For example, HCC occurred in 625 of 9235 (6.8%) patients with cirrhosis (Figure 3A), translating to a cumulative incidence of 9.52% (95% CI, 8.76-10.27%) at 3 years, whereas 177 of 18 585 (0.95%) patients without cirrhosis

TABLE 1 Characteristics of the study cohort of CHB patients on treatment with entecavir or tenofovir

Characteristics	Total (27 820)
Age, years	48.1 (38.5-57.1)
Male sex, n (%)	20 598 (74.04)
Cirrhosis, n (%)	9235 (33.20)
Compensated, n (%)	6953 (24.99)
Decompensated, n (%)	2282 (8.20)
Entecavir user, n (%)	26 593 (95.59)
Tenofovir user, n (%)	1227 (4.41)
Hepatitis C virus coinfection, n (%)	1946 (6.99)
Diabetes mellitus, n (%)	3777 (13.58)
Insulin independent, n (%)	2012 (7.23)
Insulin dependent, n (%)	1765 (6.34)
Hyperlipidemia, n (%)	2246 (8.07)
Hypertension, n (%)	7501 (26.96)
Interferon exposure, n (%)	953 (3.43)
Metformin exposure, n (%)	3275 (11.77)
Statin exposure, n (%)	2845 (10.23)
Hepatocellular carcinoma occurrence, n (%)	802 (2.88
Competing mortality, n (%)	374 (1.34)
Therapeutic duration, months	28.9 (15.9-36.4)
Follow-up[a], months	25.1 (12.1-35.6)

[a]Observation for outcomes commenced after the "washout period" (no HCC within the first 3 months of therapy in the study cohort) and continued until interruption of antiviral therapy (no filled prescription >3 months), end of the 3-year treatment, death or 31 December 2013.

Number at risk, Number of event in parentheses
27820 **(457)** 20958 **(226)** 14453 **(119)** 6160
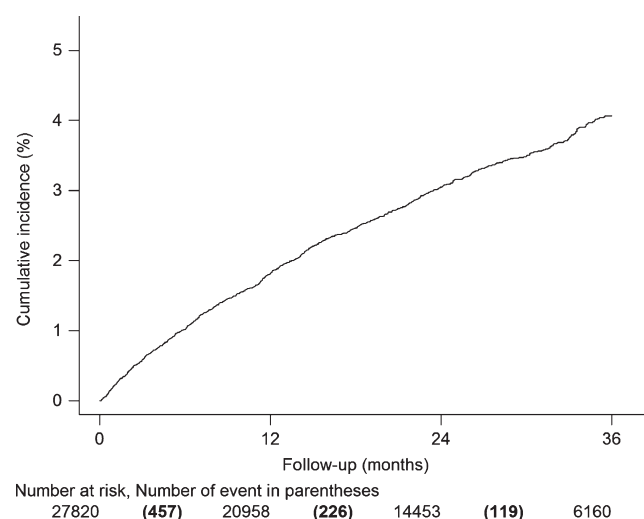
FIGURE 2 The cumulative incidence of hepatocellular carcinoma in the study cohort

developed HCC with a 3-year cumulative incidence of 1.38% (95% CI, 1.17-1.60%).

The cumulative incidence of HCC did not differ among diabetic patients according to insulin use (Figure S1A). It was also similarly high in patients with liver cirrhosis, irrespective of hepatic decompensation (Figure S1B).

3.4 | Multivariate-adjusted analysis for HCC risk during time on therapy

In the process of model development, we observed a strong interaction between cirrhosis and age (Table S2). The association between cirrhosis and HCC was consistent in direction regardless of age, but the magnitude was more pronounced in younger patients: cirrhosis was associated with an adjusted IRR of 10.07 (95% CI, 6.00-16.90) in patients younger than 40 years, but it was 4.69 (95% CI, 3.94-5.59) in those aged 40 years or older. The final Poisson regression model showed the years on therapy, cirrhosis, age, sex, HCV coinfection and DM were significant factors that affected the risk of HCC (Table 3). In the subgroup with cirrhosis, the years on therapy, age and sex were still significant factors, whereas HCV coinfection and DM became insignificant (Table S3).

4 | DISCUSSION

This study revealed a declining incidence rate of HCC in a nationwide cohort of 27 820 CHB patients who were continuously treated with entecavir or tenofovir. With more than 53 000 person-years of observation, we were able to demonstrate that the chronological decrease in HCC was independent to adjustment for other risk factors and remained significant even in the high-risk subgroup with liver cirrhosis. We also uncovered 5 pretreatment predictors including cirrhosis, age, male sex, HCV coinfection and DM. The two most influential factors, liver cirrhosis and age, interacted with each other with cirrhosis being more impactful in younger than in older patients. These findings

implicate a longer duration of treatment for even greater reduction in HCC risk and are essential for accurate risk stratification to accomplish the goal of personalized management.

Our study corroborates the efficacy of NUC in lowering HCC risks by showing that the incidence of HCC decreases over the years on therapy. Despite the lack of untreated controls for comparison, it was neither rational to believe a spontaneous decrease in HCC without the effect of antiviral therapy nor ethical to withhold HBV treatment in the patients with clear indications. This finding implicates that treatment may modify risk factors for HCC. In fact, it has been shown that cirrhosis, which is the major risk determinant, can regress on NUC therapy.[29,30] Therefore, the duration of therapy may be viewed as a surrogate to reflect the sum of the modifying effects. Our findings call for further investigation to explore the identified pretreatment factors as time-varying parameters and to examine whether their changes (e.g regression of cirrhosis, remission of hyperglycaemia, cure of HCV infection) can modify the HCC risk.
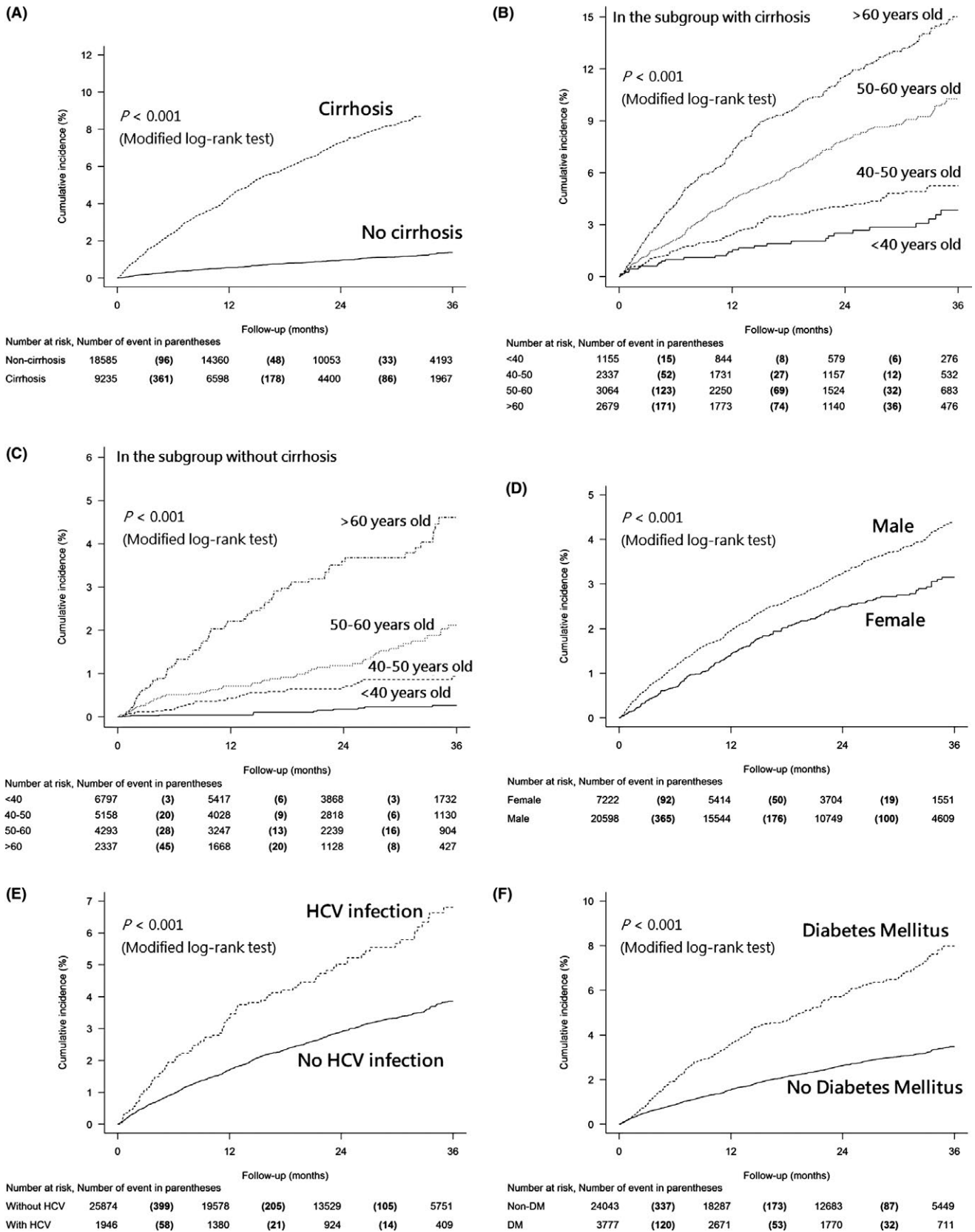
Prolonged inhibition of HBV replication is likely essential to keep preventing the occurrence of HCC because a large body of evidence has revealed that viral activity is the driving force of hepatocellular carcinogenesis in CHB patients.[31-33] The 3-year maximum follow-up was probably too short to observe a plateau in the cumulative incidence of HCC, and we could not exclude that further benefit might have been seen if the treatment had been prolonged. Therefore, the rationale of limiting NUC treatment to a certain duration, 3 years in Taiwan for example, is questionable. This warrants further research to evaluate whether and when the cumulative incidence may eventually stabilize, and how the risk may change if NUC is discontinued or interrupted.

Liver cirrhosis, older age and male sex have previously been identified as predictors of HCC in CHB patients on NUCs.[14-16,18,19] Papatheodoridis et al[15] reported in a multicentre hospital-based cohort that these 3 pretreatment factors as well as thrombocytopenia were predictive of HCC among European Caucasians. Our study went further to reveal a significant interaction between the two most influential risk factors: liver cirrhosis and age. Among patients 40 years and younger, cirrhosis is associated with a striking 10-fold higher incidence

TABLE 2 Cox proportional hazard model to predict HCC risk among CHB patients on NUC treatment based on pretreatment factors

	Patient number	Person-year	HCC event	Crude HR (95% CI)	P	Adjusted HR (95% CI)	P
Cirrhosis	9235	167 401	625	7.43 (6.29-8.78)	<.001	4.91 (4.10-5.87)	<.001
Male sex	20 598	39 485	641	1.38 (1.16-1.64)	<.001	1.73 (1.46-2.07)	<.001
Age, per decade				1.89 (1.81-1.98)	<.001	1.65 (1.56-1.74)	<.001
HCV infection	1946	3528	93	1.80 (1.45-2.24)	<.001	1.23 (0.98-1.53)	.068
Diabetes mellitus	3777	6749	205	2.30 (1.97-2.70)	<.001	1.25 (1.06-1.47)	.008
Hyperlipidemia	2246	6749	83	1.39 (1.10-1.74)	.005		
Hypertension	7501	13 620	343	2.13 (1.85-2.45)	<.001		
Interferon use	953	1648	13	0.51 (0.29-0.88)	.016		
Metformin use	3275	5813	174	2.20 (1.86-2.60)	<.001		
Statin use	2845	5111	104	1.39 (1.13-1.70)	.002		

HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HR, hazard ratio; NUC, nucleos(t)ide analogues.

(A)

Number at risk, Number of event in parentheses

Non-cirrhosis	18585	(96)	14360	(48)	10053	(33)	4193
Cirrhosis	9235	(361)	6598	(178)	4400	(86)	1967

(B) In the subgroup with cirrhosis

Number at risk, Number of event in parentheses

<40	1155	(15)	844	(8)	579	(6)	276
40-50	2337	(52)	1731	(27)	1157	(12)	532
50-60	3064	(123)	2250	(69)	1524	(32)	683
>60	2679	(171)	1773	(74)	1140	(36)	476

(C) In the subgroup without cirrhosis

Number at risk, Number of event in parentheses

<40	6797	(3)	5417	(6)	3868	(3)	1732
40-50	5158	(20)	4028	(9)	2818	(6)	1130
50-60	4293	(28)	3247	(13)	2239	(16)	904
>60	2337	(45)	1668	(20)	1128	(8)	427

(D)

Number at risk, Number of event in parentheses

Female	7222	(92)	5414	(50)	3704	(19)	1551
Male	20598	(365)	15544	(176)	10749	(100)	4609

(E)

Number at risk, Number of event in parentheses

Without HCV	25874	(399)	19578	(205)	13529	(105)	5751
With HCV	1946	(58)	1380	(21)	924	(14)	409

(F)

Number at risk, Number of event in parentheses

Non-DM	24043	(337)	18287	(173)	12683	(87)	5449
DM	3777	(120)	2671	(53)	1770	(32)	711

FIGURE 3 The risk of hepatocellular carcinoma according to pretreatment factors: liver cirrhosis (panel A), age (panels B and C), sex (panel D), hepatitis C virus coinfection (panel E) and diabetes mellitus (panel F). Because of the interaction between age and cirrhosis, how patient age stratified the risk of hepatocellular carcinoma was illustrated in patients with (B) and those without cirrhosis (C), respectively. DM, diabetes mellitus; HCV, hepatitis C virus

TABLE 3 Risk determinants for HCC in the final Poisson regression model

Variables	Adjusted incidence rate ratio
Years on therapy, per annual interval	0.73 (0.66-0.80)
Age: 40-50 years	3.38 (2.10-5.47)
Age: 50-60 years	6.92 (4.27-11.21)
Age: 60 years or older	12.50 (7.71-20.25)
Cirrhosis in age <40 years	10.07 (6.00-16.90)
Cirrhosis in age ≥40 years	4.69 (3.94-5.59)
Male sex	1.71 (1.44-2.04)
Hepatitis C virus coinfection	1.27 (1.02-1.58)
Diabetes mellitus	1.24 (1.05-1.45)

HCC, hepatocellular carcinoma.

rate of HCC, whereas in patients older than 40 years, its associated excessive risk was <5 folds. This finding reminds physicians not to overlook the risk of HCC in the youth and argues for a central role of cirrhosis in risk stratification among treated patients. In the context of daily clinical practice, cirrhosis is usually diagnosed based on ultrasound in conjunction with other imaging, laboratory or clinical data. Although there could be misclassification compared to histological definition, the elevated HCC risk in the patients with cirrhosis defined in this study confers additional convergent validity to the clinical definition.

While HCV is an independent major aetiology of HCC,[34] it has not been reported as risk factor among CHB patients on NUCs. We chose not to analyse the effect of HCV treatment because only a small (12.4%) proportion (242 of 1946) of HCV-infected patients received interferon-based regimens; direct acting antiviral agent (DAA) was unavailable during the study period. Furthermore, how many of them achieved viral eradication could not be ascertained. As a result, HCV infection in our analysis generally represented the untreated status and the results many not be extrapolated to answer whether HCV clearance would affect the risk prediction of HCC in dually infected patients. Liu and colleagues have reported the efficacy of peginterferon plus ribavirin in lowering the risk of HCC in patients with dual infections.[35] However, interferon-based regimen has become obsolete for HCV treatment because of the low community effectiveness.[36] The optimal antiviral strategy for the dual infection remains to be defined in the current era of DAAs. It has been reported that acute exacerbation of HBV may follow HCV eradication in DAA-treated patients.[37,38] Moreover, there is a complex interaction between the two viral infections on the risk of HCC.[39]

This study strengthens current evidence for DM as a risk factor for HCC.[40] We have previously shown in a hospital-based cohort with cirrhosis that DM was associated with HCC despite NUC treatment.[19] The carcinogenic mechanism is incompletely understood but may involve steatohepatitis that is common in diabetic patients.[41,42] We did not find a significant association with metformin or insulin in the present study, unlike our previous report.[19,26] This discrepancy may

result from differences in study populations, definition of exposure or unmeasured confounding. Our findings forecast a bigger role of metabolic disorder as the aetiology of HCC after chronic viral hepatitis is controlled,[43] and urge more research to elucidate the underlying mechanism and to develop strategies to attenuate the excessive risk attributable to DM.

A growing body of the literature has shown that the baseline virological parameters such as viral load, HBeAg and viral genotype were not associated with HCC occurrence in treated CHB patients,[12,15,19] although our present study could not directly examine these factors. This probably results from the potent and sustained viral inhibition achieved by continuous NUC therapy.[44] After all, these biochemical, serological and viral factors are indicators that reflect viral activity.

The large sample size from a population-based setting is a major strength of our analysis. With more than 27 000 at-risk patients and 800 incident cases, the analysis was powered to evaluate uncommon risk factors (e.g., HCV coinfection), interaction between age and cirrhosis and the nonlinear relationship with age. The national registry minimizes attrition bias. Furthermore, eligibility was restricted to uninterrupted treatment using entecavir or tenofovir, which has been shown to achieve potent and sustained viral inhibition in continuously treated patients. Lastly, our analysis was adjusted for death as a competing risk event, which would have otherwise overestimated HCC risk.

We recognize the following limitations. First, the database does not contain all information that may be relevant to the risk of HCC. For instance, family history and lifestyle (diet, smoking and alcohol) were unavailable. Second, observation was confined to 3 years because of the healthcare policy in Taiwan. Analysing the period beyond 3 years would have introduced a selection bias because only the patients who fulfilled stringent criteria of severe diseases would remain on treatment longer than 3 years. Whether the risk of HCC will further decrease on NUC therapy beyond the first 3 years is certainly important but cannot be addressed in the present study. Third, virological response or viral breakthrough could not be investigated. To overcome this limitation, we deliberately restricted enrolment to patients without exposure to other NUCs, so that the concern of drug resistance was mitigated. Besides, eligible patients needed to fill and refill the prescription without gaps longer than 1 week. Viral resurgence from poor medication adherence, accordingly, should not be a concern. In fact, data have been mounting to indicate that the risk of HCC was unrelated to virological response in patients on potent NUCs.[14,45] Finally, external validation to other populations and countries (particularly the West) is necessary to generalize our findings.

In summary, this population-based cohort study reveals the incidence and risk of HCC decline over time in CHB patients on 3-year NUC treatment and is determined by 5 pretreatment risk factors (cirrhosis, age, male sex, HCV coinfection and DM). Notably, the two most influential determinants, i.e., age and cirrhosis, interacted with each other on the association with HCC. These findings argue for a longer duration of NUC, implicate potentially modifiable risk factors and may inform the development of a predictive model for HCC in CHB patients on NUC therapy.

COMPETING INTERESTS

Yao-Chun Hsu reports having received lecture fees from Bristol-Myers Squibb, Roche, and Gilead Science. No competing interests were reported by other authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.