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Hepatocellular carcinoma after treatment cessation in non-cirrhotic HBeAg-negative chronic hepatitis B: A multicentre cohort study

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Abstract

Background and Aims: Scarce data exist on the effect of nucleos(t)ide analogue (NA) discontinuation on hepatocellular carcinoma (HCC) risk in HBeAg-negative chronic hepatitis B (CHBe−). Therefore, we assessed whether HCC risk is increased in non-cirrhotic CHBe− patients who discontinue compared to those remaining on NAs.

Methods: This cohort study included 650 consecutive non-cirrhotic Caucasian or Asian patients with CHBe− without a history of HCC who discontinued NAs after a median of 5 or 3 years (cases, n = 325; Caucasians: 143, Asians: 182) or remained on NA therapy beyond 5 or 3 years respectively (controls, n = 325; Caucasians: 223, Asians: 102). Propensity score (PS) 1:1 matching was applied to adjust for patients' origin, age and sex.

Results: During a median follow-up of 44 months, HCC developed in 7/325 cases and 9/325 controls or 7/245 PS-matched cases and 7/245 PS-matched controls with 5-year cumulative HCC incidence of 5.1% and 4.9% respectively (log-rank, $P = .836$). No difference in 5-year HCC risk was observed between cases and controls of Caucasian (3.0% vs 4.8%; log-rank, $P = .510$) or Asian origin (1.3% vs 2.2%; log-rank, $P = .873$). In

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both cases and controls, HCC incidence was independently associated with age and PAGE-B score. In cases alone, HCC development after NA discontinuation was associated only with pretreatment platelet counts and PAGE-B score, but not with any type of relapse or HBsAg loss.

Conclusions: Our findings suggest that discontinuation of effective long-term NA therapy in non-cirrhotic CHBe− patients are not associated with increased HCC risk, which is not affected by post-NA relapses and/or HBsAg loss.

KEYWORDS
discontinuation, entecavir, liver cancer, nucleoside analogue, tenofovir

1 | INTRODUCTION

Monotherapy with a nucleos(t)ide analogue (NA) of high genetic barrier, entecavir (ETV) and tenofovir either disoproxil fumarate (TDF) or alafenamide currently represents the first-line treatment option for patients with chronic hepatitis B (CHB).[1-3] The major limitation of current NAs is that indefinite treatment is usually required, especially in HBeAg-negative CHB.[1-3] NA discontinuation in non-cirrhotic HBeAg-negative CHB can be considered in patients who have remained in on-therapy virological remission for ≥18 months by the Asian or ≥36 months by the European but not yet by the US recommendations.[1-3]

Several studies show an increased probability of HBsAg loss after NA cessation,[4–10] but virological relapses occur in most patients, of whom some develop biochemical relapses and require retreatment.[5,6,11,12] Cases of jaundice or liver decompensation are exceptionally rare after NA discontinuation in non-cirrhotic CHB patients.[5,6,13] The risk of hepatocellular carcinoma (HCC) is decreased in NA-treated CHB patients, but HCC may develop even after many years of therapy.[14,15] The HCC incidence after NA cessation has not yet been thoroughly examined, although some rational concern has been expressed since even transient virological and/or clinical relapses might promote carcinogenesis.[16,17]

This study aimed to assess the HCC incidence in non-cirrhotic patients with HBeAg-negative CHB who discontinued long-term effective NA therapy, compared to such cases who remained on NA therapy.

Key points

- Recent studies have shown that nucleos(t)ide analogue (NA) discontinuation before HBsAg loss may be a safe option for selected non-cirrhotic chronic hepatitis B (CHB) patients, but it remains uncertain whether such an intervention can affect the hepatocellular carcinoma (HCC) risk.
- In a large cohort of non-cirrhotic HBeAg-negative CHB Caucasian and Asian patients, the 5-year cumulative HCC risk was not increased after NA discontinuation in comparison with matched controls who remain on NA therapy.
- HCC risk after NA discontinuation was not affected by post-NA relapses, retreatment or HBsAg loss.

2 | PATIENTS and METHODS

This retrospective longitudinal cohort study included 650 consecutive adults (≥18 years old) patients with HBeAg-negative CHB without cirrhosis before NA treatment who discontinued (cases, n = 325) or remained on long-term NA therapy (controls, n = 325) during the same period at each participating centre. Of them, 366 were Caucasians followed at the participating centres in Greece (Caucasian cohort) and 284 were Asians followed at the participating centre in Taiwan (Asian cohort). Exclusion criteria were: (a) cirrhosis or unknown severity of liver fibrosis before NA onset, (b) HCC diagnosed before NA discontinuation for cases or before the completion of 5 or 3 years of NA therapy for Caucasian or Asian controls, respectively, (c) coinfection with hepatitis C or D virus or human immunodeficiency virus, (d) history of liver transplantation and (e) poor compliance to follow-up.

Of the 325 cases, 143 were Caucasian patients who discontinued NAs between 2011 and 2017 either for enrolment in 3 prospective studies[4,11,18] or at an individual basis. Caucasian cases had received NAs for a median of 5 (minimum: 4) years before NA discontinuation. All had remained in on-therapy virological remission for ≥36 months, as suggested by EASL guidelines.[1] The remaining 182 cases were Asian patients who had received NA therapy for a median of 3 (minimum: 2) years and discontinued NAs between 2011 and 2019 according to the APASL guidelines.[3] Some of the Asian patients also participated in a previous prospective study.[11] All cases gave informed consent to discontinue NAs and agreed to remain under close monitoring after treatment cessation.

Controls were patients of the Caucasian or Asian cohort receiving NAs during the same periods with cases. Controls fulfilled all the initial criteria and had completed a minimum duration of NA therapy which continued until the end of follow-up. Given that the duration of NA therapy

may affect the HCC risk,[14,15] controls were required to have remained on NAs before enrolment for periods similar to those of cases who discontinued NAs. Since the median duration of NA therapy for Caucasian cases was 5 years, Caucasian controls included only patients followed at the Greek centres who continued NAs for >5 years (n = 223). Similarly, since the median duration of NA therapy for Asian cases was 3 years, Asian controls included only patients followed at the Taiwanese clinics who continued NAs for >3 years (n = 102). All controls have remained in on-therapy virological remission (undetectable HBV DNA) for at least 2 (Asians) or 3 years (Caucasians) before enrolment in this study.

2.1 | Study follow-up

All patients were followed according to the local clinical practice guidelines. During NA therapy, clinical examination and routine laboratory tests were performed at least every 6 months, while serum HBV DNA levels were determined every 6–12 months. Controls continued to be followed in this way during the study follow-up. Cases were followed at least at 1, 2, 3, 6, 9 and 12 months after NA discontinuation and every 6 months thereafter. Retreatment criteria for the Caucasian and Asian cases have been previously described.[4,11,18]

Both cases and controls were followed with ultrasonography, with or without alpha-fetoprotein measurements, every 6 months for HCC surveillance.

2.2 | Definitions

HBeAg-negative CHB was diagnosed made before NA onset based on positive HBsAg and negative HBeAg for ≥6 months and (a) alanine aminotransferase (ALT) higher than twice the upper limit of normal (ULN) and serum HBV DNA > 20 000 IU/mL or (b) elevated ALT but ≤2× ULN on ≥2 monthly determinations and/or HBV DNA 2000–20 000 IU/mL combined with at least moderate histological liver lesions. The criteria used for the exclusion of cirrhosis have been previously described.[4,11,18]

As the starting point of this study was considered the date of NA discontinuation for cases and the date of completion of the 5th or 3rd year of NA therapy for controls of Caucasian or Asian origin respectively. The end of the study period for the analyses regarding HCC development was the date of HCC diagnosis or the last patient visit. The end of the study period for HBsAg loss rates was the date of HBsAg loss or the last patient visit.

The primary endpoint was HCC development, which was diagnosed based on histological findings and/or compatible radiological findings by computed tomography or magnetic resonance imaging.[19] Additional outcomes included HBsAg loss and death or liver transplantation.

PAGE-B risk score was calculated for all patients at NA onset, as it is recommended to assess the HCC risk in Caucasians but can be also useful for HCC prediction in Asian CHB patients treated with NAs.[1,19-21]

2.3 | Propensity score matching analysis

To limit the confounding effect of different ethnic groups and capture the true effect of treatment discontinuation on HCC risk, we performed a 1:1 propensity score (PS) matching analysis adjusting for patient origin as well as age and sex. Each of the 2 PS-matched groups included 245 patients (Figure 1).

2.4 | Statistical analysis

All data were analysed using the statistical package SPSS (SPSS Inc., an IBM Company). Parametric and non-parametric quantitative variables were presented by their mean values ± SD or median values (interquartile range) respectively. Their comparisons between 2 patient groups were performed by the *t* test or non-parametric Mann-Whitney test. The corrected chi-squared or two-sided Fisher's exact test was used to test for association between 2 categorical variables. The PS matching was determined from the fit of a multivariable logistic regression model including the parameters reported above, whereas 1:1 PS matching with the precision of 1 decimal digit was applied. Kaplan-Meier estimates of the cumulative HCC rates were obtained and compared using the log-rank test. Cox proportional hazards regression models were used to estimate the effect of various baseline characteristics on HCC hazard and Cox regression with time-varying covariates was used to assess the association between post-NA relapses or HBsAg loss and HCC development. Multivariable Cox proportional hazards models including all factors with at least trend for significant associations ($P < .10$) in the univariate analyses were used to identify independent predictive factors. Because of a potential effect of retreatment and/or HBsAg loss on the HCC risk, the analyses for cases were also performed after censoring them at the onset of retreatment and/or HBsAg loss. Hazard ratios (HRs) and their 95% confidence intervals (CIs) along with the corresponding *P* values are presented.

3 | RESULTS

The main characteristics of all study patients and of the PS matched groups at the onset of NA therapy are depicted in Table 1. The median follow-up was 44 (35), 47 (34) months after NA discontinuation for cases and 40 (32) months after completion of 5 or 3 years of NA therapy for Caucasian or Asian controls. The patient characteristics in relation to their origin are presented in Table S1.

3.1 | Incidence and predictors of HCC in all study patients

HCC developed in 16/650 (2.5%) patients; 5 cases and 8 controls of the Caucasian cohort as well as 2 cases and 1 control of the

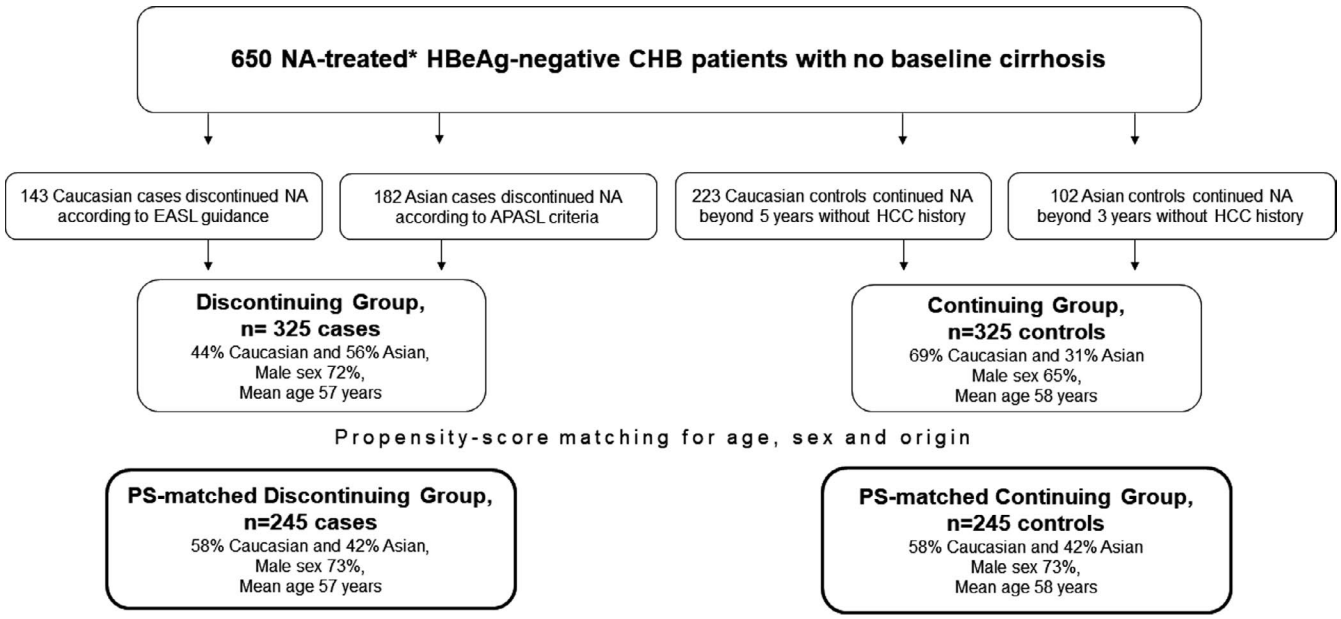

FIGURE 1 Flow chart of study patients. CHB, chronic hepatitis B; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogue. *Median NA therapy duration: 5 and 3 y for Caucasian and Asian patients, respectively

Asian cohort. The 1-, 3- and 5-year cumulative HCC incidence rates were 0.7%, 1.4% and 2.0% for cases and 1.0%, 2.2% and 4.2% for controls respectively (log-rank, $P = .494$) (Figure 2A). The 1-, 3- and 5-year cumulative HCC rates were not significantly different between Caucasian cases and controls (1.5%, 1.5% and 3.0% vs 1.4%, 3.5% and 4.8%; log-rank, $P = .510$) (Figure 2B) or between Asian cases and controls (0.7%, 1.3% and 1.3% vs 0.0%, 0.0% and 2.2%; log-rank, $P = .873$) (Figure 2C). Amongst pretreatment characteristics, older age, Caucasian origin and higher PAGE-B scores were significantly associated with increased HCC risk (Table 2).

Amongst the 490 PS matched patients, HCC developed in 7/245 PS matched cases and 7/245 PS matched controls. The 1-, 3- and 5-year cumulative HCC incidence rates were similar between PS matched cases (0.9%, 1.9% and 5.1%) and PS matched controls (0.4%, 2.5% and 4.9%) (log-rank, $P = .836$) (Figure 2D). Similarly, there was no significant difference in the HCC incidence rates between cases and controls after censoring cases for onset of retreatment (log-rank, $P = .985$) or HBsAg loss (log-rank, $P = .520$) or both (log-rank, $P = .985$). The associations of HCC development with age and PAGE-B score remained significant in the PS-matched groups, whereas no other HCC predictor emerged (Table 2).

3.2 | Predictors of HCC in cases who discontinued NA therapy

In the 325 patients who discontinued NAs, HCC developed in similar proportions of cases with and without post-NAs virological relapses defined as HBV DNA > 2000 IU/mL (5/228 [2.2%] vs 2/97 [2.1%]; $P = .651$) or HBV DNA > 20 000 IU/mL (4/172 [2.3%] vs 3/153 [2.0%]; $P = .564$). HCC development was not associated with ALT flares defined as ALT > 5× ULN (2/97 [2.1%] vs 5/222 [2.3%]; $P = .638$). Regarding combined relapses defined as HBV DNA > 2000 IU/mL and ALT > 2× ULN, none of the 118 patients who presented with such a relapse-developed HCC, which was diagnosed only in patients without combined relapse (0/118 vs 7/206 [3.4%]; $P = .040$).

Of the 7 patients who developed HCC after NA discontinuation, none had achieved HBsAg loss before HCC diagnosis, but the overall proportion of patients with HCC did not differ significantly between cases with and without HBsAg clearance (0/60 vs 7/265 [2.6%]; $P = .236$). Three patients with HCC after NA discontinuation cleared HBsAg during the remaining follow-up after HCC diagnosis. Finally, 2 HCC cases were amongst the 125 retreated patients, whereas the other 5 HCC cases were amongst the 200 non-retreated patients (2/125 [1.6%] vs 5/200 [2.5%]; $P = .453$).

Pretreatment characteristics, factors at NA discontinuation and post-NAs outcomes were also examined as possible predictors of HCC development by Cox regression analyses (Table 3). Using a time-dependent Cox regression model, post-NA virological relapses and/or combined relapses were not associated with increased HCC incidence. Platelet count and PAGE-B score at NA onset were associated with HCC development (HR/10^3/mm^3: 0.98, 95% CI: 0.97–1.00, $P = .011$ and HR/PAGE-B point: 1.29, 95% CI: 1.06–1.59, $P = .016$). All 7 patients who developed HCC were males and had pretreatment platelet counts between 100 000 and 200 000/mm^3. The only patient who developed HCC before the age of 50 (42 years old at baseline) had pretreatment platelet counts of 135 000/mm^3; he remained in sustained post-NA remission for 48 months when he cleared HBsAg, while HCC was diagnosed at 46 months after NA discontinuation when he was 46 years old.

TABLE 1 Main characteristics at the onset of nucleos(t)ide analogue (NA) therapy for all study patients (N = 650), Caucasian or Asian cases (patients who discontinued NA therapy) vs controls (patients who continued NA therapy), before and after applying 1:1 propensity score (PS) matching based on age, sex and origin

		Before PS matching			After PS matching		
Patient characteristics at the onset of NA therapy	**All patients N = 650**	**Cases, n = 325**	**Controls, n = 325**	***P***	**Cases, n = 245**	**Controls, n = 245**	***P***
Age, y	57 ± 12	56 ± 12	58 ± 12	.125	57 ± 12	58 ± 12	.273
Male sex, n (%)	444 (68)	233 (72)	211 (65)	**.038**	178 (73)	178 (73)	.540
Origin, n (%)				**<.001**			.500
Caucasians	366 (56)	143 (44)	223 (69)		143 (58)	143 (58)	
Asians	284 (44)	182 (56)	102 (31)		102 (42)	102 (42)	
Body mass index, kg/m^2	25 ± 4	25 ± 4	26 ± 4	.102	25 ± 4	25 ± 4	.523
Alcohol use, n (%)				.500			.500
None	611 (94)	305 (94)	306 (94)		225 (92)	226 (92)	
Social use[a]	39 (6)	20 (6)	19 (6)		20 (8)	19 (8)	
Diabetes, n (%)	78 (12)	40 (12)	38 (12)	.904	31 (13)	34 (14)	.790
ALT levels, IU/L	146 (242)	150 [223]	140 [404]	.753	111 [67]	144 [415]	**.002**
Albumin, g/dL	4.4 ± 0.6	4.3 ± 0.6	4.4 ± 0.5	.984	4.4 ± 0.6	4.5 ± 0.4	.894
Platelet count, ×10^3/mL	195 [78]	195 [82]	195 [73]	.558	196 [84]	196 [70]	.830
HBV DNA levels, log$_{10}$ IU/mL	5.7 [2.6]	5.7 [1.8]	5.6 [2.8]	.286	5.8 [2.2]	5.6 [2.7]	.538
PAGE-B score	14 [8]	14 [8]	14 [8]	.434	14 [8]	14 [8]	.905
Prior treatment, n (%)	246 (38)	115 (35)	131 (40)	.225	88 (36)	96 (39)	.433
Type of last NA, n (%)				.430			.234
Entecavir	327 (50.3)	159 (48.9)	168 (51.7)		111 (45.3)	133 (54.3)	
TDF	265 (40.8)	133 (40.9)	132 (40.6)		108 (44.1)	92 (37.6)	
Lamivudine	46 (7.1)	28 (8.6)	18 (5.5)		21 (8.6)	15 (6.1)	
Other	12 (1.8)	5 (1.5)	7 (2.2)		5 (2.0)	5 (2.0)	

Note: Quantitative variables are presented as mean ± SD or median values [interquartile range].

Bold indicates significant value $P < .05$.

Abbreviation: TDF, tenofovir disoproxil fumarate.

[a]Social use of alcohol: daily alcohol use of <40 g for males and <20 g for females during the last 5 y.

3.3 | Other outcomes

Sixty-three of the 325 cases cleared HBsAg after NA discontinuation with 1-, 3- and 5-year cumulative rates of 11%, 16% and 22%. Specifically, 50/143 (35.0%) Caucasian cases cleared HBsAg with 1-, 3- and 5-year cumulative rates of 15.5%, 29.7% and 40.1%, whereas only 13/182 (7.1%) Asian cases cleared HBsAg after NA discontinuation with 1-, 3- and 5--year cumulative rates of 4.4%, 5.2% and 8.2% (log-rank, $P < .001$). Of the 325 cases, 125 were retreated with 1-, 3- and 5-year cumulative rates of 24%, 40% and 42%. Retreatment was initiated in 40/143 (28.0%) Caucasian cases with 1-, 3- and 5-year cumulative incidence of 8.4%, 28.2% and 29.3% and in 85/182 (55.9%) Asian cases with 1-, 3- and 5-year cumulative rates of 23.2%, 48.5% and 51.5% (log-rank, $P = .001$).

None of the 325 controls achieved HBsAg loss during the study follow-up. Eleven patients died during the follow-up, whereas none underwent liver transplantation. Five patients who developed HCC died from liver-related death (3 cases and 2 controls; all from the Caucasian cohort), whereas 6 patients (2 Caucasian controls and 4 Asian cases) died from non-liver-related causes.

4 | DISCUSSION

This is the largest longitudinal cohort study, to date, assessing the HCC risk in Caucasian and Asian non-cirrhotic HBeAg-negative CHB patients who discontinued NA therapy. Our findings suggest that the HCC incidence during a median follow-up of 4 years does not differ between either Caucasian or Asian patients who discontinue and those who continue long-term NA therapy.

Few previous studies have reported the HCC incidence in CHB patients who discontinued NAs.[7,22-28] All such data come from cohort studies and in particular Asian centres, which usually applied the APASL criteria for NA discontinuation[3] and often included not only non-cirrhotic but cirrhotic patients too. In contrast, there are no data on Caucasian patient populations regarding HCC incidence after NA discontinuation. Nonetheless, HCC rates in our study

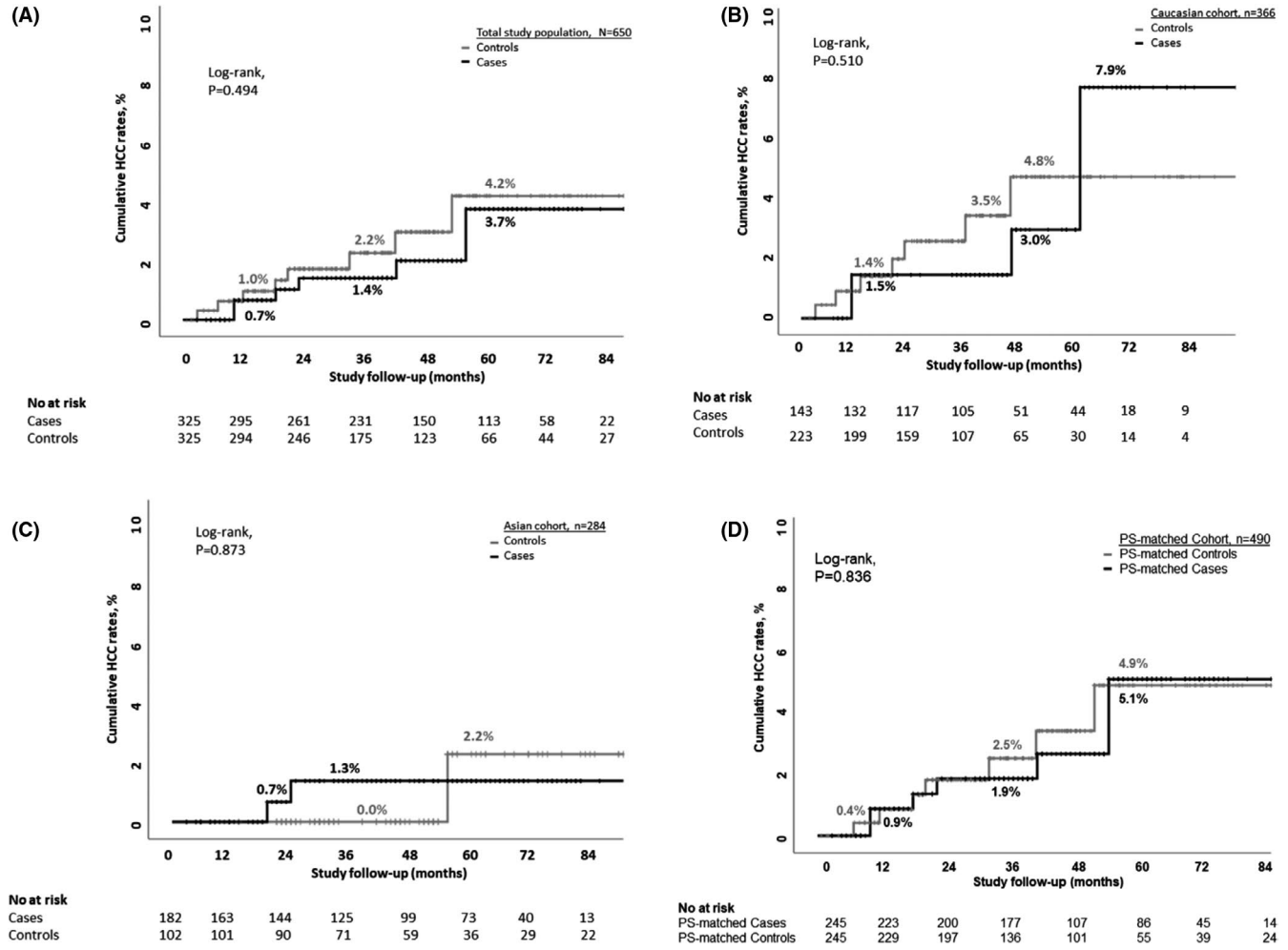

FIGURE 2 Kaplan-Meier estimates of hepatocellular carcinoma (HCC) risk in non-cirrhotic HBeAg-negative chronic hepatitis B patients from (A) the total study cohort, (B) the Caucasian cohort only, (C) the Asian cohort only and (D) the propensity score matched cohort. Comparisons in each graph between patients who discontinued nucleos(t)ide analogues (NAs) (cases) and those who remained on NA therapy (controls) are made by the log-rank test

(5-year cumulative HCC incidence of 2% in the 325 patients who discontinued NAs) were within the range of the results of those previous Asian studies, in which the 5-year cumulative HCC rates ranged between 0% and 5.2%.[7,22-29] Accordingly, there is limited data comparing the HCC incidence between HBeAg-negative CHB patients who continued or discontinued NAs before HBsAg loss. In fact, there are 2 Asian studies that included HBeAg-negative CHB patients with compensated cirrhosis (after HBsAg loss in 1 study) and showed no significant difference in HCC development between patients who discontinued and those who remained on NA therapy.[8,30] Both studies used PS matching analysis to reduce confounder bias, but the numbers of their PS matched patients were much smaller (n = 152, n = 104)[8,30] compared to our study (n = 490). Given that the latter 2 studies included cirrhotic patients who are at high risk for HCC development,[8,30] their findings further support the absence of an effect of NA discontinuation on HCC development.

In our entire cohort, HCC incidence seemed higher in Caucasian than Asian patients, either cases or controls, while Caucasian origin, alongside older age and higher PAGE-B score, was found to be associated with higher HCC risk. However, Caucasian and Asian patients differed significantly in the distribution of several HCC risk factors including age (Table S1) and therefore origin was not maintained as a significant HCC predictor after PS matching for origin, age and sex. Similarly, cumulative HCC incidence rates were numerically higher in Caucasian cases than controls as well as in Asian cases than controls. Again, the differences became numerically lower after PS matching, highlighting the importance of the distribution of HCC risk factors on the HCC incidence rates reported in each cohort. In both adjusted and unadjusted cohorts, PAGE-B score was an independent predictor of HCC suggesting that it can represent a useful tool for HCC risk stratification even in patients who discontinue NAs.

One of the most alarming issues regarding the decision of stopping NAs is the possible consequences of post-NA HBV reactivations on hepatocyte carcinogenesis.[16,17] In the quest for a reliable answer to this research question, we scrutinized HCC development in our cases against any definition of post-NA relapses as well as retreatment. However, no relationship was discovered between post-NA virological or combined relapses and HCC risk.

TABLE 2 Factors associated with hepatocellular carcinoma (HCC) development in HBeAg-negative chronic hepatitis B patients with (cases) and without (controls) discontinuation of nucleos(t)ide analogue (NA) therapy before and after 1:1 propensity score (PS) matching for age, sex and origin. Hazard ratios (HR) (95% confidence intervals [CI]) for univariate analyses are presented

Factors at the onset of NAs	Before PS matching (n = 650)		After PS matching (n = 490)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, per year	**1.09 (1.03, 1.14)**	**.001**	**1.13 (1.05, 1.20)**	**<.001**
Origin, Caucasian vs Asian	**4.48 (1.32, 16.6)**	**.017**	2.96 (0.79, 11.29)	.111
Gender, male vs female	Not estimated[a]		Not estimated[a]	
Body mass index, per kg/m^2	1.05 (0.96, 1.16)	.258	1.07 (0.92, 1.26)	.370
Alcohol use, no vs social[b]	1.37 (0.18, 10.6)	.763	4.04 (0.85, 19.01)	.078
Diabetes, yes vs no	1.01 (0.23, 4.45)	.990	0.50 (0.07, 3.82)	.504
ALT levels, per IU/L	1.00 (0.99, 1.00)	.477	0.99 (0.98, 1.00)	.544
Albumin levels, per g/dL	1.04 (0.57, 1.91)	.896	1.03 (0.55, 1.92)	.919
Platelets, per $10^3/mm^3$	1.00 (1.00, 1.00)	.108	1.00 (1.00, 1.00)	.229
HBV DNA, per $\log_{10}$ IU/mL	1.11 (0.80, 1.55)	.514	0.96 (0.63, 1.46)	.834
PAGE-B score, per unit	**1.19 (1.05, 1.33)**	**.005**	**1.24 (1.06, 1.44)**	**.008**
Prior treatment, yes vs no	0.85 (0.32, 2.28)	.741	1.38 (0.36, 5.22)	.632
Type of last NA[c], ETV vs TDF	1.34 (0.45, 4.02)	.602	1.59 (0.48, 5.29)	.449
Cessation of NA, yes vs no	1.41 (0.52, 3.84)	.496	1.49 (0.44, 5.12)	.523

Abbreviations: ETV, entecavir; TDF, tenofovir disoproxil fumarate.

Bold indicates significant value $P < .05$.

[a]HR (95% CI) were not estimated as there was no patient with HCC amongst the females.

[b]Social use of alcohol: daily alcohol use of <40 g for males and <20 g for females.

[c]Only in patients treated with ETV or TDF.

HCC events and probabilities were numerically similar between our cases who discontinued NAs with and without post-NA virological relapses, while HCC did not develop in any patient with combined virological and biochemical relapses. Despite concerns regarding post-NA flares and HCC risk,[16,17] no relative evidence had been previously published and our data are the first to explore this association in a large real-world cohort. Additionally, we showed that neither was HCC development associated with the need for retreatment since only 2 of the 125 retreated patients developed HCC.

Among the patients who discontinued NAs, no other factor before or at discontinuation of NA therapy was associated with HCC development, except for pretreatment lower platelet counts and higher PAGE-B score probably supporting an association of the HCC risk with more advanced liver fibrosis even in non-cirrhotic patients. The type of NA and particularly the use of TDF compared to ETV has been recently suggested to be associated with earlier relapses after NA discontinuation,[31] which might affect the HCC incidence, as well as with lower HCC incidence rates for CHB patients remaining on NA therapy,[32] although such observations remain controversial.[33] In our study, there was no association between the use of TDF or ETV and the HCC incidence in all patients (Table 2) or in cases who discontinued NAs (Table 3), despite that virological relapse defined as HBV DNA >2000 IU/mL occurred earlier on cases who discontinued TDF than ETV (data not shown). It should be noted, however, that there was no difference in the incidence of any other type of relapse or retreatment between our cases who discontinued TDF or ETV (data not shown), while no type of post-NA relapse was associated with HCC incidence.

Our results confirmed that HBsAg loss occurs significantly more frequently after cessation than a continuation of NA therapy in both Caucasian and Asian patients with HBeAg-negative CHB (5-year cumulative rates: 22% vs 0%, $P < .001$).[6–8,12,13,16,17] In agreement with previous reports,[8,12] HBsAg loss rates in our study were higher in Caucasian than Asian patients who stopped NAs (5-year cumulative rates: 40% vs 8%, $P < .001$). The higher rates of HBsAg seroclearance after discontinuation, compared to continuation of NA therapy, may result in further reduction of the HCC incidence after stopping NAs.[34] In our study, HBsAg loss was not significantly associated with HCC development, but many large numbers of patients will be required to reliably assess the effect of HBsAg loss on the relatively low HCC incidence of non-cirrhotic CHB patients. It should be noted that HCC did not develop after HBsAg seroclearance in any of our 60 patients without

TABLE 3 Factors associated with hepatocellular carcinoma (HCC) after discontinuation of nucleos(t)ide analogue (NA) therapy in 325 non-cirrhotic patients with HBeAg-negative chronic hepatitis B. Hazard ratios (HR) (95% confidence intervals [CI]) for univariate analyses are presented

	HR (95% CI)	*P*
Factors at NAs onset		
Age, per year	1.06 (0.99, 1.13)	.119
Origin, Caucasian vs Asian	3.63 (0.70, 18.8)	.125
Gender, male vs female	Not estimated[a]	
Body mass index, per kg/m^2	0.99 (0.80, 1.22)	.899
Alcohol consumption, no vs social use[b]	Not estimated[a]	
Diabetes, yes vs no	Not estimated[a]	
ALT, per IU/L	1.00 (0.99, 1.00)	.804
Albumin, per g/dL	1.04 (0.56, 1.96)	.893
Platelets, per 10^3/mm^3	0.98 (0.97, 1.00)	.011
Serum HBV DNA, per $\log_{10}$ IU/mL	0.91 (0.56, 1.46)	.681
PAGE-B score, per unit	1.29 (1.05, 1.59)	.016
Therapy before NAs, yes vs no	0.53 (0.12, 2.37)	.404
Factors at NAs discontinuation		
Duration of NA therapy, per month	1.01 (0.99, 1.03)	.440
Duration of on-NA virological remission, per month	1.02 (0.99, 1.04)	.167
ALT, per IU/L	0.99 (0.90, 1.11)	.978
HBsAg levels, per $\log_{10}$ IU/mL	0.77 (0.47, 1.26)	.304
Type of last NA[c], ETV vs TDF	3.96 (0.46, 34.01)	*.210*
Outcomes after NAs discontinuation		
Post-NA virological relapse[d], yes vs no		
HBV DNA levels > 2000 IU/mL	1.64 (0.18, 14.78)	.659
HBV DNA levels > 20 000 IU/mL	1.45 (0.24, 8.71)	.688
Post-NA ALT flare[d], yes vs no		
ALT > 2× upper limit of normal (ULN)	0.71 (0.12, 4.29)	.712
ALT > 5× ULN	0.80 (0.09, 7.17)	.843
Post-NA combined relapse[d], yes vs no		
HBV DNA > 2000 IU/mL & ALT > ULN	0.27 (0.00, 73)	.371
HBV DNA > 20 000 IU/mL & ALT > ULN	0.74 (0.08, 6.59)	.784
HBV DNA > 2000 IU/mL & ALT > 2× ULN	0.34 (0.03, 3.08)	.340
HBsAg loss, yes vs no[d]	2.61 (0.43, 15.63)	.295

Abbreviations: ETV, entecavir; TDF, tenofovir disoproxil fumarate; ULN, upper limit of normal.

[a]HR (95% CI) were not estimated as there was no patient with HCC amongst the females or patients with the social use of alcohol or patients with diabetes.

[b]Social use of alcohol: daily alcohol use of <40 g for males and <20 g for females.

[c]Only in patients treated with ETV or TDF.

[d]This covariate is time dependent and was examined as a time-varying covariate of the Cox regression model.

HCC before that event, while 3 of the patients who developed HCC cleared HBsAg after HCC diagnosis.

Another interesting observation was that there was no death because of liver failure and all deaths in Asian or Caucasian patients were owing to HCC or liver unrelated causes.

Our study has some limitations. First, the retrospective design of our multicentre real-world cohort study inevitably confines us to potential selection bias owing to possible hidden confounding factors. Undoubtedly, a randomized controlled trial would offer a more optimal design and usually avoidance of selection bias, but it may not always be feasible for several reasons. Despite the absence of randomization, our patients fulfilled similar criteria for treatment initiation and were followed by experts who applied similar follow-up, while NA discontinuation was offered as an option to patients fulfilling the same criteria over time either in the context of clinical studies or in daily clinical practice. In addition, PAGE-B score did not differ between patients who discontinued or remained on NA therapy supporting that the baseline HCC risk was similar between cases and controls and that NA discontinuation was not biased for patients with lower HCC risk. Second, our sample size may have lacked in power to detect an existing difference in rather low HCC incidence rates between the 2 groups. We retrospectively estimated that 325 patients per group could offer approximately 50% power for detection of 2.2% difference in 5-year cumulative HCC rates, whereas almost 1000 patients per group would be needed to offer study power of 80%. Yet, in this large real-world cohort, HCC events did not differ statistically between the 2 groups and were numerically lower or equal in cases who discontinued NAs, providing hints that the strategy of NA discontinuation is a reasonable option for non-cirrhotic HBeAg-negative CHB without increasing the risk of carcinogenesis.

In conclusion, we showed that the HCC incidence was numerically similar after NAs discontinuation in non-cirrhotic HBeAg-negative CHB patients, compared to patients who did not discontinue NA therapy, adjusted for their ethnic group, age and sex. HCC risk was not more frequent in patients with any type of post-NA relapses or retreatment, whereas PAGE-B risk score could predict HCC risk and might provide useful guidance in such settings of post-NA HCC risk assessment. Future studies with longer follow-up periods and preferably randomized study design are warranted to validate our findings that discontinuation of NAs in non-cirrhotic HBeAg-negative CHB patients who have remained in long-term virological remission does not jeopardize long-term patient outcomes regarding liver carcinogenesis.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author (GVP) upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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