ORIGINAL ARTICLE

Entecavir treatment does not eliminate the risk of hepatocellular carcinoma in chronic hepatitis B: limited role for risk scores in Caucasians

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ABSTRACT

Background Hepatocellular carcinoma (HCC) risk-scores may predict HCC in Asian entecavir (ETV)-treated patients. We aimed to study risk factors and performance of risk scores during ETV treatment in an ethnically diverse Western population.

Methods We studied all HBV monoinfected patients treated with ETV from 11 European referral centres within the VIRGIL Network.

Results A total of 744 patients were included; 42% Caucasian, 29% Asian, 19% other, 10% unknown. At baseline, 164 patients (22%) had cirrhosis. During a median follow-up of 167 (IQR 82–212) weeks, 14 patients developed HCC of whom nine (64%) had cirrhosis at baseline. The 5-year cumulative incidence rate of HCC was 2.1% for non-cirrhotic and 10.9% for cirrhotic patients (p<0.001). HCC incidence was higher in older patients (p<0.001) and patients with lower baseline platelet counts (p=0.02). Twelve patients who developed HCC achieved virologic response (HBV DNA <80 IU/mL) before HCC. At baseline, higher CU-HCC and GAG-HCC, but not REACH-B scores were associated with development of HCC. Discriminatory performance of HCC risk scores was low, with sensitivity ranging from 18% to 73%, and c-statistics from 0.71 to 0.85. Performance was further reduced in Caucasians with c-statistics from 0.54 to 0.74. Predicted risk of HCC based on risk-scores declined during ETV therapy (all p<0.001), but predictive performances after 1 year were comparable to those at baseline.

Conclusions Cumulative incidence of HCC is low in patients treated with ETV, but ETV does not eliminate the risk of HCC. Discriminatory performance of HCC risk scores was limited, particularly in Caucasians, at baseline and during therapy.

Significance of this study

What is already known on this subject?

- Continuous entecavir (ETV) therapy effectively suppresses HBV DNA in the vast majority of patients.
- ETV therapy may reduce risk of hepatocellular carcinoma (HCC) in Asian patients with chronic hepatitis B.
- Previously described risk scores for HCC are associated with development of HCC in Asian chronic hepatitis B patients treated with ETV.

What are the new findings?

- Risk of HCC remains considerable in cirrhotic patients despite ETV therapy.
- Discriminatory performance of HCC risk scores is limited in ETV-treated patients, particularly in Caucasians.
- Application of risk scores during ETV therapy is not clinically useful in Caucasians.

How might it impact on clinical practice in the foreseeable future?

- Since ETV therapy does not eliminate the risk of HCC, careful monitoring remains mandatory, particularly in patients with cirrhosis. Current HCC risk scores developed in untreated Asian patients cannot accurately identify all (Caucasian) patients at high risk of HCC; screening of risk groups, therefore, remains necessary despite successful ETV therapy.

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INTRODUCTION

The goal of treatment of chronic hepatitis B (CHB) infection is to prevent disease progression to (decompensated) cirrhosis, hepatocellular carcinoma (HCC) and death.[1] Current treatment guidelines consider nucleos(t)ide analogues (NA) or peginterferon (PEG-IFN) as first-line treatment for CHB in patients with serum HBV DNA level >2.000 IU/mL in combination with elevated alanine aminotransferase (ALT) levels (>1–2×ULN (upper limit of normal)), or with moderate to severe liver inflammation and/or fibrosis.[2 3] These guidelines are based on the accepted association between HBV DNA levels and progression to cirrhosis, HCC and liver-related mortality in untreated patients.[4] Entecavir (ETV) inhibits HBV

replication in the vast majority of patients and is also able to improve fibrosis scores after continuous therapy in 88% of CHB patients.[5] Furthermore, ETV therapy may reduce the risk of HCC and liver-related events, particularly in patients with cirrhosis.[6–8] Nevertheless, the residual risk of HCC necessitates intensive on-going follow-up of patients with successfully suppressed viral replication.[2] Recently, risk scores based on demographic (age and sex), clinical (cirrhosis, ALT, albumin and bilirubin) and virologic (HBeAg status, HBV DNA) characteristics have been developed in order to predict the risk of HCC in treatment-naive patients. These HCC risk scores were shown to predict HCC in Asian CHB patients treated with ETV as well.[9] However, the performance of these risk-scores in non-Asian patients remains unclear. The aims of the current study were therefore to investigate in this large ethnically diverse European HBV-infected population treated with ETV, (1) the incidence of, and risk factors for, development of liver-related events including HCC and (2) the role of risk scores for prediction of HCC.

MATERIALS AND METHODS

Study population

In this investigator-initiated cohort study within the European network of excellence for Vigilance against Viral Resistance (VIRGIL), all consecutive CHB patients (HBsAg positive for at least 6 months) treated with ETV monotherapy for at least 3 months between 2005 and May 2013 in 11 large European referral centres were included. Patients were excluded if they were coinfected with HIV, HCV or hepatitis delta virus (HDV) or if they had an HCC at baseline. Patients' ethnicity was classified as Caucasian, Asian (including only East Asians from, eg, China, Hong Kong and Thailand) or other (including sub-Saharan Africans). A total of 744 patients were eligible for the current analysis. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Patients gave written informed consent according to standards of the local ethics committees.

Follow-up of participants

All subjects were prospectively monitored every 3–6 months at the discretion of the local treating physician. At every visit, routine examination with biochemical (serum ALT, bilirubin, albumin, international ratio of prothrombin time (INR) and creatinine) and virologic (HBsAg, HBeAg, anti-HBe, HBV DNA level) assessments took place. The diagnosis of cirrhosis at baseline was based on histology or ultrasound examinations with signs of cirrhosis (spleen size >12 cm, portal vein >16 mm, or nodules within the hepatic parenchyma).[6] In cirrhotic patients, screening for HCC was performed at least yearly by ultrasound and/or α-fetoprotein measurement. In non-cirrhotic patients, HCC surveillance varied from centre to centre according to local protocols, and was only performed when other risk factors were present.[3]

Endpoints

HCC was defined by either (1) histological confirmation, or (2) two parallel imaging techniques (ultrasound, computerised tomography, or MRI) showing a focal lesion larger than 2 cm with arterial hypervascularisation, or (3) one imaging technique showing a focal lesion larger than 2 cm with arterial hypervascularisation in the presence of an α fetoprotein level greater than 400 ng/mL.

Clinical events were defined as a composite endpoint of development of HCC, liver decompensation, or death during the study period. Diagnosis of decompensated cirrhosis was based on the presence of ascites confirmed by ultrasound, jaundice with a serum bilirubin level >2.0 mg/dL, bleeding oesophageal varices, or hepatic encephalopathy in cirrhotic patients. Other reported endpoints were virologic response (VR, HBV DNA level<80 IU/mL), HBeAg loss (in HBeAg-positive patients) and HBsAg loss all during the on-treatment follow-up period.

Laboratory tests

Serum ALT, bilirubin, albumin levels and INR were measured locally using standardised automated techniques. Hepatitis B surface antigen (HBsAg), antibody against HBsAg (anti-HBs), hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) were determined using commercially available enzyme immunoassays in all centres. Serum HBV DNA levels were measured using a quantitative real-time PCR assay, the COBAS AmpliPrep-COBAS TaqMan HBV test (CAP-CTM; Roche Molecular Systems, Branchburg, New Jersey, USA), with a lower limit of detection of 12 IU/mL, in 10 of 11 centres. In one centre, serum HBV DNA was measured using Roche Amplicor (linear dynamic range, 400–200 000 copies/mL; Roche Diagnostic Systems, Branchburg, New Jersey, USA). A conversion factor of 5.26 copies/IU was used for conversion of copies/mL to IU/mL. HBV genotypes and detection of HBV polymerase gene mutations was determined by direct sequencing or using the INNO-LiPA assay (Innogenetics, Gent, Belgium).

Data analysis

Data acquisition directly from the patients' charts was performed on site by a single experienced investigator (PA). Data were systematically collected through a standardised clinical record form. HBV DNA levels were logarithmically transformed for analysis. ALT levels are expressed as values representing a ratio to the local ×ULN. Continuous variables are expressed as means±SD or median (IQR) where appropriate. Follow-up times were calculated from the date of ETV treatment initiation to the date of event or end of follow-up. Components of the HCC risk scores included age, cirrhosis, albumin, bilirubin and HBV DNA level for the CU-HCC risk score[10]; age, cirrhosis, sex and HBV DNA for the GAG-HCC risk score[11]; and age, sex, ALT, HBeAg status and HBV DNA for the REACH-B risk score.[12] The cumulative probability of achieving primary or secondary endpoints was estimated by Kaplan–Meier analysis. Cox's regression analysis was used to study which baseline factors were associated with primary or secondary endpoints. The influence of VR was analysed as a time-dependent covariate allowing patients to be at risk in either the VR or non-VR group according to HBV DNA level during follow-up. Therefore, VR was entered in the model as a time-dependent covariate: all patients started (and thus at risk) within the group without VR, and were switched to the group with VR after achieving this endpoint. Sensitivity, negative predictive values (NPV), and c-statistics of the risk scores to predict HCC were estimated and reported, within the entire population as well as in a subgroup of Caucasian patients.

All statistical tests were two-sided, and a p value<0.05 was considered to be statistically significant. SPSS V.20.0 (SPSS, Chicago, Illinois, USA) and SAS V.9.2 programme (SAS Institute, Cary, North Carolina, USA) were used for all statistical analyses.

RESULTS

Baseline characteristics

In total, 891 chronic HBV patients treated with ETV were identified. One hundred and forty-seven patients did not fulfil the

entry criteria and were excluded; 70 patients were treated for less than 3 months, 19 patients were coinfected with HCV or HDV, 22 patients had an HCC at baseline, two patients had undergone liver transplantation, two patients were HBsAg negative at baseline, 30 patients received concomitant antiviral therapy and two were non-compliant. A total of 744 CHB patients treated with ETV monotherapy were thus eligible and were included. Baseline characteristics of the study population are shown in table 1 according to the presence of cirrhosis. Forty-two percent of patients were of Caucasian origin, 29% Asian, 19% other and in 10% ethnicity was unknown. At baseline 164 patients (22%) had cirrhosis (by ultrasound or histology), 239 patients (32%) were HBeAg positive, median ALT was 1.4×ULN (IQR 0.8–2.7) and mean HBV DNA 5.3 log IU/mL (6.6 log IU/mL for HBeAg positive and 4.5 log IU/mL for HBeAg-negative patients). Overall median follow-up was 167 weeks (IQR 82–212) and did not differ between cirrhotic and non-cirrhotic patients (p=0.22). Total number of visits was 7160, with a median number of visits per patient of 8 (IQR 5–11), with a median interval of 14 (IQR 12–25)

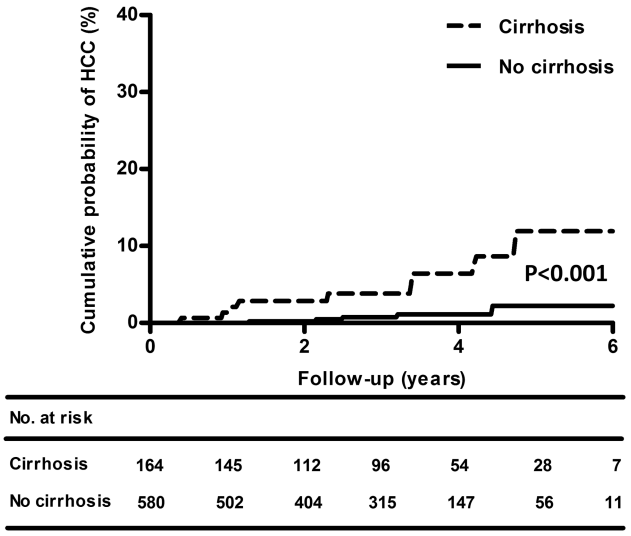

Figure 1 Kaplan-Meier curve for the cumulative probability of developing hepatocellular carcinoma (HCC) according to presence of cirrhosis.

Virologic response during treatment

HBV DNA <80 IU/mL (VR) was achieved in 655 patients. The cumulative probability of VR was 53%, 76%, 90%, 94%, 97% and 99% at 6 months and years 1, 2, 3, 4 and 5, respectively. VR was not influenced by the presence of cirrhosis (p>0.2). HBeAg loss was achieved in 85 (36%) of 239 HBeAg-positive patients. The cumulative probability of HBeAg loss was 11%, 25%, 36%, 45% and 58% at years 1, 2, 3, 4 and 5, and was higher in patients with cirrhosis (p=0.03). Sixteen patients

Table 1 Baseline characteristics

	All (n=744)	Cirrhosis (n=164)	No cirrhosis (n=580)	p Value
Male (%)	569 (77)	138 (84)	431 (74)	0.009
Mean age	44±14	51±13	42±13	<0.001
ETV dosage 0.5 mg (%)	640 (86)	123 (75)	517 (89)	<0.001
Race (%)				0.37
Caucasian	316 (42)	74 (45)	242 (42)	
Asian	214 (29)	41 (25)	173 (30)	
Other	139 (19)	26 (16)	113 (19)	
Unknown	75 (10)	23 (14)	52 (9)	
Genotype (%)				0.64
A	100 (13)	23 (14)	77 (13)	
B	48 (7)	7 (4)	41 (8)	
C	78 (11)	17 (10)	61 (11)	
D	186 (25)	40 (24)	146 (25)	
E	52 (7)	8 (5)	44 (8)	
Other	5 (1)	1 (1)	4 (1)	
Unknown	275 (37)	68 (41)	207 (36)	
HBeAg positive (%)	239 (32)	54 (33)	185 (32)	0.92
Mean HBV DNA (log IU/mL)	5.3±2.2	5.4±2.2	5.2±2.2	0.29
Median ALT (×ULN)	1.4 (0.8–2.7)	1.5 (1–3.2)	1.4 (0.8–2.5)	0.57
Platelet count (×10^9/L)*	192±72	138±63	210±66	<0.001
Median bilirubin (μmol/L)	11 (8–15)	14 (10–20)	10 (7–14)	0.001
Albumin (g/dL)**	4.3±0.5	4.1±0.6	4.4±0.4	<0.001
PT INR$^+$	1.1±0.2	1.2±0.2	1.1±0.2	<0.001
Mean CU HCC risk score^{++}	8±9	23±9	4±4	<0.001
Mean GAG HCC risk score	62±18	82±14	56±14	<0.001
Mean REACH-B score	9±3	11±3	9±3	<0.001
NA-naive (%)	569 (77)	108 (66)	461 (80)	<0.001
LAM-naive (%)	617 (83)	122 (74)	495 (85)	0.001
IFN-naive (%)	610 (82)	138 (84)	472 (81)	0.49

Data available for *73%, **75%, $^+$63% and $^{++}$69% of patients, respectively.
ALT, alanine aminotransferase; ETV, entecavir; HCC, hepatocellular carcinoma; HBeAg, Hepatitis B e antigen; IFN, interferon; LAM, lamivudine; NA, nucleos(t)ide analogues; PT INR, international ratio (INR) of prothrombin time; ULN, upper limit of normal.

Table 2 Univariate Cox regression analysis of potential risk factors for developing HCC and clinical events

	Overall population (n=744)						Caucasians (n=316)					
	HCC (n=14)			Overall events (n=34)			HCC (n=7)			Overall events (n=20)		
Risk factor	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value	HR	95% CI	p Value
Age (per year)	1.08	1.04 to 1.13	<0.001	1.06	1.04 to 1.09	<0.001	1.06	1 to 1.13	0.05	1.05	1.02 to 1.09	0.004
Female	0.22	0.03 to 1.68	0.15	0.29	0.09 to 0.96	0.04	0.44	0.05 to 3.67	0.45	0.50	0.15 to 1.71	0.27
Caucasian	1.09	0.55 to 2.14	0.80	1.02	0.80 to 1.30	0.87	NA	NA	NA	NA	NA	NA
Genotype B	0.94	0.54 to 1.61	0.81	1.08	0.78 to 1.49	0.64	0.99	0.47 to 2.06	0.97	1.18	0.72 to 1.91	0.52
HBeAg neg	0.81	0.25 to 2.57	0.72	1.11	0.55 to 2.34	0.78	0.33	0.04 to 2.71	0.30	0.48	0.16 to 1.43	0.19
HBV DNA (log IU/mL)	0.82	0.64 to 1.05	0.12	1.09	0.94 to 1.27	0.26	0.78	0.55 to 1.10	0.15	1.14	0.95 to 1.38	0.17
ALT (×ULN)	0.70	0.41 to 1.18	0.18	1.02	0.98 to 1.06	0.40	0.70	0.35 to 1.41	0.32	1.02	0.98 to 1.06	0.34
Bilirubin (log µmol/L)	2.15	0.36 to 12.9	0.43	5.18	2.14 to 12.6	<0.001	0.24	0.01 to 5.96	0.37	2.44	0.59 to 10.0	0.25
Albumin (g/dL)	0.93	0.83 to 1.04	0.18	0.85	0.81 to 0.89	<0.001	1.01	0.86 to 1.19	0.87	0.83	0.76 to 0.90	<0.001
PT INR (per 0.1)	1.00	0.70 to 1.42	0.99	1.23	1.11 to 1.36	<0.001	0.70	0.29 to 1.68	0.42	1.23	1.05 to 1.45	0.01
Platelet count (per 10×10^9/L)	0.90	0.83 to 0.98	0.02	0.90	0.85 to 0.95	<0.001	0.96	0.86 to 1.08	0.48	0.95	0.89 to 1.01	0.13
MELD score	1.04	0.87 to 1.24	0.70	1.13	1.06 to 1.21	<0.001	0.89	0.53 to 1.49	0.65	1.15	1.05 to 1.26	0.003
Cirrhosis	5.82	1.94 to 17.41	0.002	7.25	3.53 to 14.89	<0.001	3.70	0.82 to 16.6	0.09	4.70	1.92 to 11.52	0.001
Previous NA	0.45	0.16 to 1.31	0.14	0.52	0.26 to 1.05	0.07	0.59	0.13 to 2.63	0.49	1.22	0.44 to 3.36	0.70
Previous LAM	3.21	1.11 to 9.26	0.03	2.45	1.21 to 4.95	0.01	2.60	0.58 to 11.7	0.21	0.93	0.31 to 2.79	0.90
Previous IFN	0.63	0.14 to 2.82	0.55	0.38	0.12 to 1.23	0.11	0.93	0.18 to 4.82	0.93	0.43	0.12 to 1.45	0.17

ALT, alanine aminotransferase; HBeAg, Hepatitis B e antigen; HCC, hepatocellular carcinoma; IFN, interferon; LAM, lamivudine; MELD, model for end-stage liver disease; NA, nucleos(t)ide analogues; PT INR, international ratio (INR) of prothrombin time; ULN, upper limit of normal.

(2.2%) achieved HBsAg loss. The cumulative probability of HBsAg loss was 0.1%, 1.2%, 1.6%, 2.3% and 4.1% at years 1, 2, 3, 4 and 5, respectively.

Development of HCC

Fourteen patients developed HCC (7 Caucasians), after a median duration of 125 weeks (IQR 59–188). The cumulative probability of developing HCC was 2.1% for non-cirrhotic versus 10.9% for cirrhotic patients at year 5 of follow-up (p<0.001) (figure 1). Risk of HCC was higher in patients with cirrhosis (p=0.002), older patients (>50 years) (p<0.001), in patients with lower platelet counts (p=0.02), and in patients who were previously treated with lamivudine (p=0.03). When Caucasian patients were studied separately, only age was associated with the occurrence of HCC (HR 1.06, 95% CI 1 to 1.13, p=0.05; table 2).

Occurrence of clinical events

Overall, 34 patients developed a clinical event (including 14 HCC) after a median duration of 87 weeks (IQR 49–169). Twenty-three (68%) had cirrhosis at baseline. Of the 14 patients who developed HCC, 3 patients died. Thirteen patients developed an episode of hepatic decompensation of whom 5 patients died. Overall, 17 patients died during follow-up, 8 liver-related, and 9 of other causes (table 3).

Influence of VR and development of HCC and clinical events

In patients without a clinical event, median time to VR was 23 weeks (IQR 12–47). Of 14 patients who developed HCC, 12 patients already achieved VR before HCC was diagnosed. The other two patients achieved response after the occurrence of HCC. Median time to VR was 24 weeks (IQR 13–41) in patients with HCC. Of the 34 patients with a clinical event, 30 patients achieved VR. Median time to VR in patients who developed a clinical events was 27 weeks (IQR 17–56). In a Cox regression analysis with VR as time-dependent factor, HBV DNA <80 IU/mL was neither significantly associated with the development of HCC (HR 0.87, 95% CI 0.17 to 4.58, p=0.87), nor with the development of a clinical event (HR 0.70, 95% CI 0.28 to 1.77, p=0.46).

Performance of HCC risk scores at baseline

At baseline, mean risk-score was 8 for CU-HCC, 62 for GAG-HCC and 9 for REACH-B. Higher CU-HCC and GAG-HCC, but not REACH-B scores were associated with HCC in the overall population.

When established cut-off values for these risk scores were used (5 for the CU-HCC score, 101 for the GAG-HCC score and 8 for the REACH-B score), only CU-HCC and GAG-HCC risk scores were predictive for HCC development (table 4). C-statistics for the overall population were 0.78 for CU-HCC,

Table 3 Distribution of clinical events

	Cirrhosis (n=164)			No cirrhosis (n=580)	
	Decompensation (n)	HCC (n)	Death (n)	HCC (n)	Death (n)
Overall	11	9	3	5	6
Caucasian	6	4	2	3	5
Asian	1	2	0	2	0
Other	4	3	1	0	1

HCC, hepatocellular carcinoma.

Table 4 Performance of HCC risk scores at baseline and after 1 year of ETV treatment

	Overall (N=744)							Caucasian (N=316)						
Baseline	**HR**	**95% CI**	**p Value**	**c**	**95% CI of c**	**NPV at 4 years, %**	**Sensitivity at 4 years, %**	**HR**	**95% CI**	**p Value**	**c**	**95% CI of c**	**NPV at 4 years, %**	**Sensitivity at 4 years, %**
CU-HCC	1.07	1.03 to 1.11	0.001	0.78	0.65 to 0.91	–	–	1.04	0.98 to 1.11	0.23	0.66	0.44 to 0.88	–	–
GAG-HCC	1.08	1.04 to 1.12	<0.001	0.85	0.78 to 0.91	–	–	1.06	1.01 to 1.11	0.03	0.74	0.60 to 0.89	–	–
REACH-B	1.18	0.99 to 1.39	0.06	0.71	0.58 to 0.85	–	–	1.01	0.78 to 1.31	0.92	0.54	0.32 to 0.75	–	–
Cirrhosis	1.04	0.79 to 1.36	0.80	0.63	0.44 to 0.81	–	–	0.90	0.62 to 1.32	0.60	0.46	0.20 to 0.72	–	–
No cirrhosis	1.25	0.91 to 1.72	0.16	0.69	0.45 to 0.94	–	–	0.99	0.67 to 1.46	0.96	0.58	0.37 to 0.78		–
CU-HCC >5	4.67	1.26 to 17.30	0.02	0.70	0.58 to 0.83	98	78	2.44	0.45 to 13.34	0.30	0.63	0.44 to 0.82	98	67
GAG-HCC >101	15.95	3.4 to 74.79	<0.001	0.57	0.47 to 0.68	95	18	28.15	2.81 to 281.8	0.005	0.61	0.42 to 0.80	97	25
REACH-B >8	1.09	0.30 to 3.90	0.90	0.55	0.47 to 0.63	95	82	0.68	0.13 to 3.51	0.65	0.52	0.36 to 0.68	96	75
Cirrhosis	0.50	0.10 to 2.44	0.39	0.50	0.41 to 0.60	–	–	0.28	0.03 to 2.77	0.28	0.55	0.38 to 0.71		
No cirrhosis	1.43	0.16 to 12.82	0.75	0.55	0.39 to 0.71	–	–	0.71	0.06 to 7.81	0.78	0.55	0.27 to 0.84		
	Overall (N=744)							**Caucasian (N=316)**						
Year 1	**HR**	**95% CI**	**p Value**	**c**	**95% CI of c**	**NPV at 4 years, %**	**Sensitivity at 4 years, %**	**HR**	**95% CI**	**p Value**	**c**	**95% CI of c**	**NPV at 4 years, %**	**Sensitivity at 4 years, %**
CU-HCC	1.07	0.98 to 1.17	0.13	0.73	0.60 to 0.87	–	–	1.06	0.97 to 1.15	0.18	0.71	0.59 to 0.84	–	–
GAG-HCC	1.07	1.03 to 1.12	0.004	0.84	0.76 to 0.92	–	–	1.06	1.01 to 1.11	0.02	0.77	0.64 to 0.90	–	–
REACH-B	1.27	1.07 to 1.52	0.008	0.79	0.69 to 0.89	–	–	1.13	0.87 to 1.46	0.37	0.65	0.52 to 0.79	–	–
Cirrhosis	1.07	0.72 to 1.60	0.74	0.54	0.40 to 0.68	–	–	0.93	0.57 to 1.52	0.78	0.58	0.36 to 0.81	–	–
No cirrhosis	1.37	1.10 to 1.72	0.005	0.91	0.83 to 0.98	–	–	1.21	0.87 to 1.67	0.26	0.79	0.66 to 0.93	–	–
CU-HCC >5	4.20	0.53 to 33.20	0.17	0.61	0.51 to 0.70	98	89	3.14	0.38 to 26.17	0.29	0.59	0.45 to 0.73	97	75
GAG-HCC >101	13.80	1.65 to 115.7	0.02	0.58	0.43 to 0.72	95	11	24.76	2.48 to 247.8	0.006	0.61	0.42 to 0.80	97	25
REACH-B >8	4.34	1.16 to 16.23	0.03	0.63	0.45 to 0.82	97	50	1.85	0.31 to 11.12	0.50	0.49	0.30 to 0.68	97	0
Cirrhosis	1.30	0.22 to 7.92	0.77	0.46	0.25 to 0.67	–	–	0.74	0.07 to 8.25	0.81	0.60	0.37 to 0.83	–	–
No cirrhosis	13.4	1.38 to 129.7	0.025	0.79	0.59 to 0.99	–	–	4.08	0.25 to 65.75	0.32	0.56	0.24 to 0.89	–	–

ETV, entecavir HCC, hepatocellular carcinoma; NPV, negative predictive value.

0.85 for GAG-HCC and 0.71 for REACH-B risk score. In Caucasians, the scores were 0.66, 0.74 and 0.54, for CU-HCC, GAG-HCC and REACH-B, respectively. NPVs at 4 years of therapy for all risk scores at baseline were more than 95% (CU-HCC 84/86, GAG-HCC 184/193 and REACH-B 39/41) with a sensitivity ranging from 18% (2/11) for GAG-HCC, 78% (7/9) for CU-HCC and 82% (9/11) for REACH-B. Comparable NPVs were found in Caucasians, and also at years 3 and 5 (see online supplementary table S1). Additionally, when cirrhotic and non-cirrhotic patients were studied separately, only GAG-HCC score remained predictive for the occurrence of an HCC (see online supplementary table S2).

Influence of ETV treatment on HCC risk scores

Overall, predicted HCC risk based on CU-HCC, GAG-HCC and REACH-B declined after 1 year of ETV therapy in the overall population, as well as in cirrhotic, non-cirrhotic and Caucasian patients (all p values <0.001 for the change during follow-up with baseline). The decline in HCC risk scores from baseline to year 1 was comparable in patients who developed HCC versus those who did not (figure 2). Furthermore, HRs of the dynamic risks for development of an HCC by an HCC risk score measurement at a random visit were comparable with those at baseline (table 4 vs figures 2A–C) Despite the observation that the mean calculated risk scores were consistently higher in patients who developed HCC, diagnostic performance remained suboptimal during treatment. NPVs in all patients with a minimum of 4 years of follow-up for all risk scores at year 1 of therapy were more than 95% (CU-HCC 51/52, GAG-HCC 157/165 and REACH-B 124/128) with a sensitivity of 11% (1/9) for GAG-HCC, 89% (8/9) for CU-HCC and 50% (4/8) for REACH-B. Comparable values were found in the Caucasian subpopulation (table 4) and also when using a single HCC risk score measurement at a random visit (see online supplementary table S3).

DISCUSSION

In this European multicenter real-life cohort study, we showed that CHB patients treated with ETV remain at considerable risk for developing HCC. The risk of HCC cannot be confidently predicted using HCC risk scores at baseline nor during therapy, particularly not in Caucasians. Careful follow-up, therefore, remains necessary even if HBV DNA is adequately suppressed.

ETV therapy effectively suppresses viral replication, and in the current study virtually all patients achieved an undetectable HBV DNA during therapy. Recent studies have shown that a reduction of HBV DNA to low or undetectable levels reduces the risk of liver-related events and HCC.[6–8] However, in the current study, we were unable to confirm the association between time to, and duration of, viral suppression and a reduction in the incidence of HCC or clinical events. Our findings are in line with another large European study which also found considerable rates of HCC despite long-term viral suppression.[13] The reason for the differences between the Asian studies and those conducted in Europe are currently unclear, but may be accounted for by differences in HBV genotype distribution, time since infection, and previous treatment exposure in the Western cohorts.

Considering the residual risk of HCC even in patients with undetectable HBV DNA, careful monitoring remains of vital importance. A recent study from Hong Kong suggests that previously identified risk scores for HCC in untreated patients may also be applied effectively in ETV-treated subjects.[9] We were unable to confirm these findings in our ethnically diverse cohort. While baseline GAG-HCC and CU-HCC risk scores were higher in patients that developed HCC, REACH-B scores offered little prognostic help. Furthermore, the discriminatory performance of the risk scores was limited by the low sensitivity observed in the overall population and mainly in the

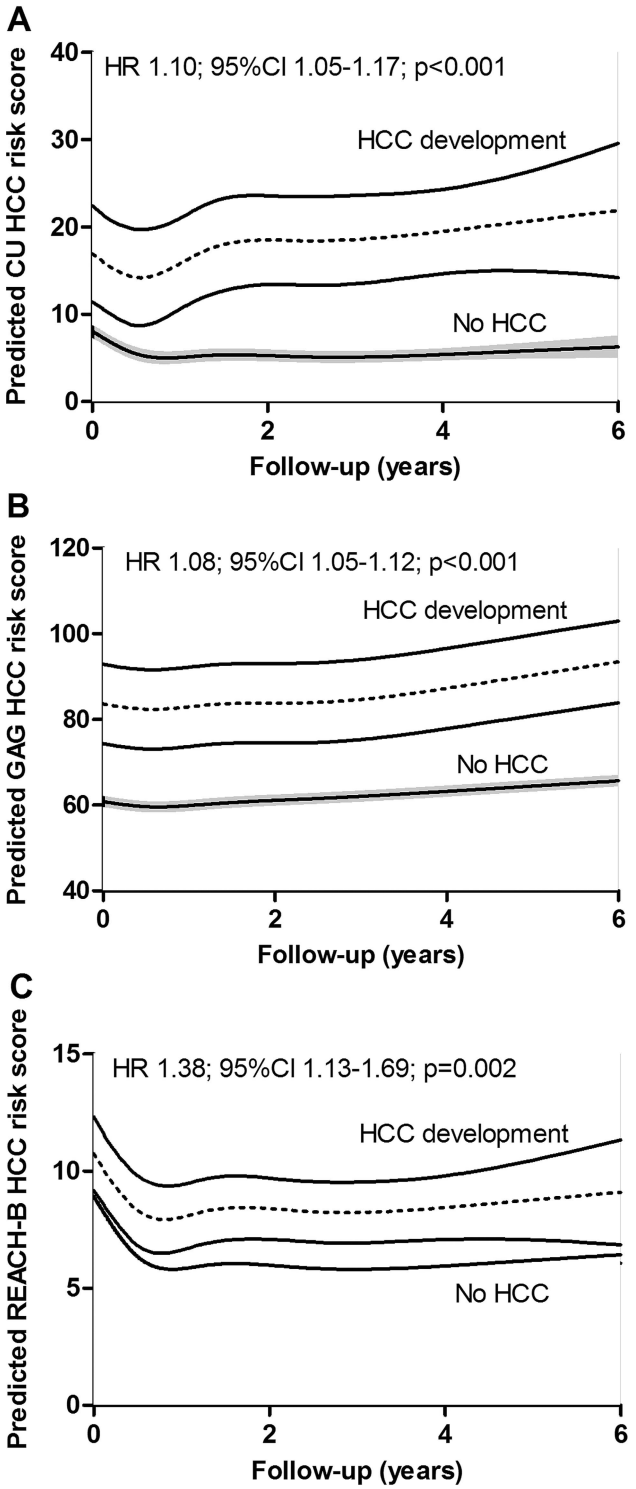

Figure 2 (A) CU hepatocellular carcinoma (HCC) (B) GAG-HCC and (C) REACH-B risk scores over time with 95% CI by development of HCC during entecavir (ETV) treatment. HR represents the dynamic risks for development of an HCC by an HCC risk score measurement at a random visit. HR was corrected for duration of therapy and multiple visits per patient.

Caucasians. These findings are of major clinical importance because they show that a considerable proportion of patients who will develop HCC is not identified using previously defined risk score cut-offs. Moreover, this implies that there is little to no additional value of those HCC risk scores to the pre-existing lifetime risk of HCC in CHB patients, and the clinical relevance for daily practice of these risk scores remains disputable, particularly in Caucasian patients.

Given the fact that ETV effectively suppresses HBV DNA in the majority of patients after a single year of therapy, we considered applying the risk scores at various on-treatment time-points. While we observed a decline in the predicted risk of HCC over time, the patterns were comparable for patients who developed HCC compared to those who did not, and predictive performance after 1 year was therefore comparable to that at baseline. Furthermore, HR did not alter over time when looking at the dynamic risks for development of an HCC by an HCC risk score measurement at a random visit. These findings suggest that there is little reason to continue calculating the risk scores during therapy. However, studies with longer follow-up may be required to estimate the risk of HCC during therapy beyond 5 years. Despite our large cohort of CHB patients, our study was limited by the fact that we observed a limited number of HCCs. Since the availability of ETV limits our duration of follow-up, future long-term follow-up may help us to understand the long-term effect of ETV on HCC risk. Furthermore, the risk of HCC in non-cirrhotic patients might be underestimated since screening may be less frequent or suboptimal when compared to the cirrhotic population.

In conclusion, in this European multicentre real-life cohort study, we showed that continuous ETV therapy effectively suppresses HBV DNA in the vast majority of patients. While the risk of HCC in ETV-treated patients is low through up to 5 years of treatment, ETV therapy does not eliminate the risk of HCC. Previously described risk scores for HCC have limited sensitivity for HCC in Caucasian patients and do not appear to be clinically useful either at baseline nor during therapy. Screening of risk groups, therefore, remains necessary despite successful ETV therapy, at least during the first years of treatment.

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Patient consent Obtained.

Ethics approval The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. Patients gave written informed consent according to standards of the local ethics committees.

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