

## Entecavir Reduced Serum Hepatitis B Core-Related Antigen in Chronic Hepatitis B Patients with Hepatocellular Carcinoma

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Serum hepatitis B core-related antigen (HBcrAg) was shown to predict the risk of hepatocellular carcinoma (HCC) in chronic hepatitis B (CHB) patients undergoing treatment. We investigated the longitudinal profile of HBcrAg in entecavir (ETV)-treated CHB patients with subsequent HCC development. We identified HCC cases diagnosed at  $\geq 1$  year after ETV initiation. CHB patients without HCC (matched for age, sex, cirrhosis status, baseline hepatitis B virus (HBV) DNA level, and ETV treatment duration) were identified as controls at an HCC:non-HCC ratio of 1:2. Serum samples were retrieved at baseline (ETV initiation) and at 3 and 5 years of ETV therapy for HBcrAg measurement (log IU/mL). In total, 180 patients (60 HCC patients matched with 120 CHB patients without HCC; median age, 56.5 years; 80.6% male; baseline HBV DNA, 5.9 log IU/mL; median follow-up, 6.8 years) were recruited. The median time from ETV initiation to HCC development was 3.2 years. HBcrAg levels were higher in HCC cases than in controls at all three time points: 5.69 log IU/mL versus 5.02 log IU/mL ( $p=0.025$ ), 4.23 log IU/mL versus 3.36 log IU/mL ( $p=0.007$ ), and 3.86 log IU/mL vs 3.36 log IU/mL ( $p=0.009$ ), respectively. ETV led to similar rates of decline in HBcrAg from baseline to 3 years in both groups (0.34 log IU/mL/year vs 0.39 log IU/mL/year,  $p=0.774$ ), although the decline from 3 to 5 years was slower in the non-HCC group (0.05 log IU/mL/year) than in the HCC group (0.09 log IU/mL/year,  $p=0.055$ ). ETV time-dependently reduced HBcrAg in HCC and non-HCC patients. HBcrAg interpretation should consider the antiviral treatment duration. (**Gut Liver, Published online May 29, 2020**)

**Key Words:** Entecavir; Hepatocellular carcinoma; Hepatitis B core-related antigen; Chronic hepatitis B

### INTRODUCTION

In patients with chronic hepatitis B (CHB) infection, antiviral therapy can only reduce, but not eliminate, the risk of hepatocellular carcinoma (HCC) as opposed to untreated patients or the theoretical risk estimated from risk prediction models.<sup>1-4</sup> In the era of widespread availability of effective antiviral therapy, the long-term survival of patients with CHB largely depends on the development of HCC.<sup>5</sup> Predicting the risk of HCC is an essential step which allows the identification of high risk individuals for vigilant surveillance. In treated patients, serum hepatitis B virus (HBV) DNA, being the most important risk factor for HCC development,<sup>6</sup> is already well suppressed, indicating the need of alternative viral markers to predict the risk of HCC. Hepatitis B core-related antigen (HBcrAg) is a novel biomarker of HBV. Its detection is based on the common amino acid sequence shared by hepatitis B core antigen, hepatitis B e antigen (HBeAg) and a 22-kDa precore protein. Serum HBcrAg decreased progressively in patients treated with nucleos(t)ide analogues (NA) for both short-term and long-term treatment.<sup>7,8</sup> This marker was shown to predict risk of HCC in antiviral-treated CHB patients.<sup>9</sup> In a recent study by Hosaka *et al.*,<sup>10</sup> persistently high on-treatment serum HBcrAg at 1 year of antiviral therapy was found to be associated with more than 6-fold (for HBeAg-positive patients) and 2-fold (for HBeAg-negative patients) increase in risks of HCC development compared to those who had lower on-treatment HBcrAg levels. However, it is unclear whether long-term antiviral treatment also reduced serum HBcrAg levels in patients with HCC compared to those without HCC. In this study, we compared the serial profile of serum HBcrAg levels in CHB patients who developed HCC with CHB patients without HCC after long-term ( $\geq 3$  years) antiviral treatment.

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**CASE REPORTS**

We identified adult CHB patients who were started on entecavir (ETV) during the period of January 2002 to December 2015 in the Liver Clinics in Queen Mary Hospital, Hong Kong. Patients with HCC diagnosis at  $\geq 1$  year of ETV treatment were identified as cases. A control group in a 1:2 ratio was recruited, which comprised CHB patients who did not have HCC that were matched for age, gender, baseline HBV DNA, baseline cirrhosis status and treatment duration. Serum samples were analyzed at three time points: baseline (date of ETV initiation), 3-year from ETV treatment and 5-year from ETV treatment. Serum HBV DNA levels were measured by Cobas Taqman (Roche Diagnostics, Branchburg, NJ, USA) with lower limit of detection of 20 IU/mL. Serum HBcrAg levels were measured by the Lumipulse G HBcrAg assay in a Lumipulse G1200 analyzer (Fujirebio Inc, Tokyo, Japan) with a lower detection limit of 100 international units per milliliter (IU/mL). The values of HBcrAg were log transformed and were expressed in log IU/mL. Cirrhosis was diagnosed ultrasonographically by the presence of small nodular liver, splenomegaly or ascites. The study protocol was approved by the Institutional Review Board/Ethics Committee of the University of Hong Kong and the Hong Kong West Cluster of Hospital Authority (IRB number: UW 17-539). All study subjects provided informed consent.

Data were presented as median (with interquartile ranges), or as percentages. Comparison between continuous variables was performed using the Mann-Whitney or the Kruskal Wallis non-parametric tests when appropriate. A two-sided  $p$ -value  $< 0.05$  was considered to be statistically significant. Statistical analysis

**Table 1.** Baseline Characteristics of Patients

Variable	HCC (n=60)	Non-HCC (n=120)	p-value
Age at ETV, yr*	56.9 (53.1–63.9)	56.4 (50.6–61.1)	0.141
FU duration, yr*	6.3 (4.2–8.3)	6.8 (5.6–8.3)	0.199
Male sex*	49/60 (81.7)	96/120 (80.0)	0.844
Log HBV DNA, IU/mL*	5.8 (2.1–6.9)	5.9 (2.0–6.9)	0.918
Cirrhosis*	24/59 (40.7)	33/114 (28.9)	0.128
HBeAg+ at ETV	12/60 (20.0)	14/120 (11.7)	0.176
Albumin, g/L	41 (37–42)	43 (40–45)	$< 0.001$
Bilirubin, $\mu$ mol/L	12 (9–15)	11 (8–15)	0.143
ALT, U/L	56 (36–82)	61 (43–113)	0.260
AST, U/L	47 (38–78)	50 (36–72)	0.979
Platelet, $\times 10^9/L$	135 (133–183)	158 (109–191)	0.071

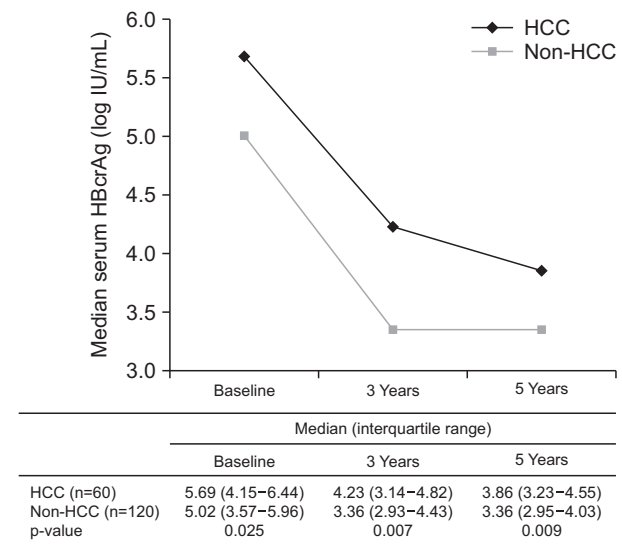
Data are presented as median (interquartile range) or number/number (%).

HCC, hepatocellular carcinoma; ETV, entecavir; FU, follow-up; HBV, hepatitis B virus; HBeAg+, hepatitis B e antigen positive; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

\*Matched between HCC and non-HCC group.

was carried out using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

A total of 180 patients (80.6% male, 85.6% HBeAg negative) were recruited: 60 cases were matched with 120 controls. The median age at baseline (i.e., initiation of ETV) was 56.5 years (interquartile range [IQR], 51.5 to 62.6 years) and the median follow-up duration was 6.8 years (IQR, 5.4 to 8.3 years). The median HBV DNA was 5.9 log IU/mL (IQR, 2.0 to 6.9 log IU/mL) at baseline and all patients had undetectable HBV DNA ( $< 20$  IU/mL) at 3-year and 5-year of ETV therapy. Although there was higher proportion of cirrhosis in the HCC group compared to non-HCC group without statistical significance (40.7% vs 28.9%,  $p=0.128$ ), the baseline parameters including age, treatment duration, gender and baseline HBV DNA were well matched between the two groups. The median time from ETV initiation to HCC diagnosis was 3.2 years (IQR, 2.0 to 4.8 years) (Table 1). Compared to patients without HCC, patients with HCC had lower median albumin (43 g/L vs 41 g/L,  $p<0.001$ ), higher median HBcrAg at baseline (5.69 log IU/mL vs 5.02 log IU/mL,  $p=0.025$ ), 3-year (4.23 log IU/mL vs 3.36 log IU/mL,  $p=0.007$ ) and 5-year (3.86 log IU/mL vs 3.36 log IU/mL,  $p=0.009$ ) (Fig. 1). Subgroup analysis was performed for patients with and without cirrhosis and the results are shown in Fig. 1. The median serum HBcrAg was higher in HCC patients compared to non-HCC pa-



**Subgroup analysis of the median serum HBcrAg in HCC and non-HCC patients with or without cirrhosis**

HBcrAg	Cirrhotic			Non-cirrhotic		
	HCC	Non-HCC	p-value	HCC	Non-HCC	p-value
<b>Baseline</b>	5.93	5.37	0.093	5.36	4.98	0.136
<b>3 Years</b>	4.67	3.90	0.075	3.78	3.28	0.200
<b>5 Years</b>	4.19	3.46	0.019	3.7	3.34	0.216

**Fig. 1.** Serum hepatitis B core-related antigen (HBcrAg) level in hepatocellular carcinoma (HCC) and non-HCC patients at baseline and after 3 and 5 years of entecavir treatment.

tients at all time points for patients with cirrhosis. In contrast, no statistical significance was observed between the median serum HBcrAg in HCC cases and control for patients without cirrhosis (Fig. 1).

ETV treatment led to progressive decline of serum HBcrAg in both HCC and non-HCC group. Over a 5-year treatment period, the rate of decline was similar in both groups (0.27 log IU/mL/year vs 0.27 log IU/mL/year,  $p=0.903$ ). The treatment period was divided into initial treatment phase (baseline to 3-year) and late treatment phase (3-year to 5-year) for further evaluation. For both groups, the decline of serum HBcrAg was faster in the initial treatment phase (HCC: 0.34 log IU/mL/year vs non-HCC: 0.39 log IU/mL/year,  $p=0.774$ ) which subsequently slowed down in the late treatment phase, and was even slower in the control group compared to the HCC group (0.05 log IU/mL/year vs 0.09 log IU/mL/year,  $p=0.055$ ).

## DISCUSSION

In this cohort of ETV-treated CHB patients, serum HBcrAg levels were persistently higher in HCC group compared to matched controls at baseline, 3-year and 5-year of ETV treatment, after adjustment for other known important risk factors of HCC including age, gender, treatment duration, baseline cirrhosis status and baseline HBV DNA. This implies that HBcrAg levels are associated with the development of HCC in patients receiving antiviral treatment, similar to what we had previously shown in a study with untreated patients,<sup>11</sup> and these findings provided further evidence in line with Hosaka *et al.*<sup>10</sup> which suggested that persistently higher on-treatment HBcrAg levels are associated with higher risk of HCC.

In this study, long-term ETV treatment led to gradual decline in serum HBcrAg regardless of HCC development. This finding is consistent with a recent report showing the gradual decline of serum HBcrAg in CHB patients without HCC over 7 years of ETV treatment.<sup>7</sup> This is the first study reporting the longitudinal profile of serum HBcrAg in HCC patients who were under long-term ETV treatment. In this study, the rate of decline was similar between two groups, although there was a trend for slower decline in the late treatment phase for non-HCC patients compared to HCC patients. This could be explained by the fact that HCC patients had higher baseline serum HBcrAg, allowing room for bigger drop upon antiviral treatment. However, the reason why the rate of decline was similar in the initial phase for both groups was not certain. Nevertheless, the serum median HBcrAg levels were always higher in HCC group compared to non-HCC group at all three time points even after ETV treatment. Nucleoside analogues act on a single step in the viral replication cycle—inhibition of the reverse transcriptase—and is expected to affect viral DNA synthesis only, leaving the upstream steps of viral protein and genomic material synthesis untouched. However, recent evidence showed that nucleoside analogues led to a reduc-

tion in the intrahepatic covalently closed circular DNA (cccDNA) by 1.03 logs at 1 year, and 2.94 logs at a median interval of 126 months. Since cccDNA is replenished and amplified from newly formed HBV relaxed circular DNA (rcDNA), inhibition of reverse transcriptase by nucleoside analogues would in turn inhibit formation of rcDNA-containing capsids and thus lead to depletion of cccDNA.<sup>12</sup> The mechanisms of inadequate suppression of HBcrAg production in NA-treated patients with HCC might be related to other intracellular signals at the transcriptional level. In a study by Honda *et al.*,<sup>13</sup> the amount of HBV DNA and pregenomic RNA in the liver were significantly higher in 16 HBcrAg-positive patients, compared to 12 HBcrAg-negative patients. Hepatic gene profiling showed that HBV-promoting transcriptional factors, including hepatocyte nuclear factor-4 $\alpha$ , peroxisome proliferator-activated receptor  $\alpha$ , and liver receptor homolog-1 were upregulated in HBcrAg-positive livers. The enhanced HBV-promoting transcriptional factors thereby allows ongoing hepatic carcinogenesis and is reflected by a higher on-treatment HBcrAg in NA-treated patients who developed HCC.

There are some limitations in the current study. Firstly, we did not perform genotyping for the CHB patients in this study. Nevertheless, the predominant genotype in our locality is genotype C, which is known to be associated with higher risk of HCC compared to other genotypes.<sup>14,15</sup> Ideally, the effect of HBV genotype on HCC risk should also be investigated. Secondly, cirrhosis was defined by sonographic features, which are qualitative and are prone to interobserver variability. Despite case-control matching, the median serum albumin and platelet were lower in HCC patients compared to non-HCC patients (Table 1). More accurate markers for cirrhosis, preferably noninvasive tests (e.g., transient elastography, serum biomarkers), should be adopted to better define the cirrhosis status. Lastly, the retrospective nature of the current study would lead to selection bias from patients lost to follow-up and unavailability of serum samples.

In conclusion, serum HBcrAg progressively declined during long-term ETV treatment in CHB patients with or without HCC. However, HBcrAg levels were consistently higher in ETV-treated patients with HCC. Interpretation of serum HBcrAg should take into account the duration of antiviral treatment.

## CONFLICTS OF INTEREST

Fujirebio Inc, Tokyo, Japan provided reagent kits for HBcrAg measurements which were done in our own laboratory. No other potential conflict of interest relevant to this article was reported.

## AUTHOR CONTRIBUTIONS

Conceptualization: M.F.Y. Methodology: W.K.S., J.F. Formal analysis: L.Y.M., D.K.H.W., K.L.K., W.P.T. Project administration: M.F.Y. Visualization: M.F.Y. Writing - original draft: L.Y.M.

Writing - review and editing: L.Y.M., W.K. S., J.F., M.F.Y. Approval of final manuscript: all authors.

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