

VOLUME 29 NUMBER 1 January 2023

pISSN 2287-2728
eISSN 2387-285X

CLINICAL and MOLECULAR HEPATOLOGY

The forum for latest knowledge of hepatobiliary diseases



HCC incidence is decreasing in Korea
but increasing in elderly

Early changes in biomarkers predict HBsAg response
Baveno-VII predicts decompensation in cACLD

Editorial

Moving toward hepatitis B virus functional cure - the impact of on-treatment kinetics of serum viral markers

Lilian Yan Liang^{1,2}, Vincent Wai-Sun Wong^{1,2}, Grace Lai-Hung Wong^{1,2}, and Terry Cheuk-Fung Yip^{1,2}

¹Medical Data Analytics Center, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong;

²State Key Laboratory of Digestive Disease, Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong

Keywords: Hepatitis B virus; Hepatitis B; Antiviral agents

See Article on Page 146

BACKGROUND

Functional cure of chronic hepatitis B virus (HBV) infection, which is currently set as the treatment goal of new HBV therapies, is serologically defined as the clearance of hepatitis B surface antigen (HBsAg), with or without anti-HBs seroconversion, and undetectable serum HBV DNA.¹ A handful of studies have shown that patients with chronic hepatitis B (CHB) who achieve functional cure generally have a favorable clinical course – namely much reduced risk of hepatic events and hepatocellular carcinoma (HCC).² Nonetheless, there is still a low yet definite risk of HCC occurrence, especially in male patients who achieve functional cure after 50 years of age.³ While the current antiviral treatment with oral nucleos(t)ide analogues (NAs) are potent and safe, they generally lead to very low rates of functional cure; hence, novel HBV therapeutic regimens are eagerly wanted for improving the functional cure rate.^{4,5}

Before achieving functional cure, the holy grail of treatment goals, favourable HBsAg response (FHR) is a reasonable intermediate step towards HBV cure. FHR was defined as HBsAg seroclearance or HBsAg ≤ 100 IU/mL at the end of follow-up (EOFU). Such a low HBsAg cutoff is often adopted for stopping NA therapy in hepatitis B e antigen (HBeAg)-negative patients, as their relapse rate would be low.⁶ End-of-treatment HBsAg < 100 IU/mL is also one of the few virologic predictors of functional cure.¹ Several studies have investigated the functional cure rate after stopping NA in HBeAg-negative patients, with variable rates of success ranging from 2.7–16.7%/year in Caucasian patients and 0–3.8%/year among Asian patients; the most consistent predictor of functional cure is a low HBsAg level at the time of NA withdrawal.¹

KEY FINDINGS

Overview of study methodology

Mak and colleagues⁷ examined the serum hepatitis B core-

Corresponding author: Terry Cheuk-Fung Yip

Department of Medicine and Therapeutics, Prince of Wales Hospital, 5/F, Clinical Sciences Building, 30-32 Ngan Shing Street, Shatin, Hong Kong
Tel: +852-35053125, Fax: +852-26373852, E-mail: tcfyip@cuhk.edu.hk
<https://orcid.org/0000-0002-1819-2464>

Editor: Seung Up Kim, Yonsei University College of Medicine, Korea

Received: Oct. 24, 2022 / **Revised:** Oct. 26, 2022 / **Accepted:** Oct. 26, 2022

related antigen (HBcrAg) and HBV pre-genomic RNA (pgRNA) in the first 48 weeks of NA treatment in 64 CHB patients. Analyses were performed separately in 28 HBeAg-positive and 36 HBeAg-negative patients. These patients had participated in previous phase III trials, during which they received lamivudine, adefovir dipivoxil, telbivudine, clevudine, or entecavir with serum samples collected at weeks 0, 4, 12, 24, 36, and 48 of treatment and paired liver biopsies at weeks 0 and 48. HBcrAg was measured with a lower limit of detection (LLOD) of 2 log U/mL and a reliable quantification at >3 log U/mL. HBV RNA was measured using an investigational assay with a LLOD of 1 log copies/mL. Patients with undetectable serum HBV pgRNA or HBcrAg at week 0 were excluded from the analysis of early on-treatment changes of viral markers. Due to censoring and the difference in follow-up durations between patients with and without FHR at the EOFU, time-dependent area under receiver operating curve was utilized to assess the discriminatory ability of HBcrAg and HBV pgRNA.

Clinical meaning of HBV pgRNA decline

At a median follow-up duration of 17 years, 22/64 patients achieved FHR, including eight cases of HBsAg seroclearance. At the start of NA therapy, HBeAg-positive patients had higher HBV pgRNA compared to HBeAg-negative patients (4.9 vs. 3.3 log copies/mL). The median HBV pgRNA reduced to the LLOD at week 48 and week 12 for HBeAg-positive and HBeAg-negative patients, respectively. The difference in median reduction of HBV pgRNA between FHR and non-FHR patients was prominent among HBeAg-positive patients, with over 1 log difference sustained between week 4 and week 48. Patients with FHR had a higher HBV pgRNA in the first 12 weeks than those without FHR, while the level was comparable after week 24. The decline of HBV pgRNA at weeks 4 and 48 were the most discriminative of FHR. In contrast, among HBeAg-negative patients, the HBV pgRNA level and its reduction did not significantly differ throughout the first 48 weeks between patients with and without FHR.

Serum HBV RNA is a novel biomarker that can measure the

circulating HBV pgRNA present in virus-like particles.^{8,9} Serum HBV RNA is a mixture of intact, pre-genomic and subgenomic, spliced, truncated, and polyA-free species,¹⁰ which reflects the transcriptional activity of intrahepatic covalently closed circular DNA (cccDNA).¹¹ HBV RNA level at NA treatment cessation also correlates with post-treatment viral relapse.¹¹ Notably, while there was a significant reduction of HBV pgRNA, no significant differences in the reduction of intrahepatic total HBV DNA and cccDNA level were observed at week 48 between HBeAg-positive patients with and without FHR.⁷ One may speculate that cccDNA transcriptional activity is more predictive of FHR than its absolute amount. Nevertheless, the difference in the role of pgRNA in HBeAg-positive and HBeAg-negative patients remain unclear. Also, assays for serum HBV RNA should be standardized and validated to better define its clinical utility.¹¹

Clinical meaning of HBcrAg decline

Mak and colleagues⁷ also demonstrated the importance of early decline in serum HBcrAg at week 4 during antiviral treatment, which was associated with FHR in HBeAg-negative patients. HBcrAg is a novel biomarker in CHB patients and consists of the hepatitis B core antigen, HBeAg, and the 22-kDa precore protein.¹² It gradually decreased after antiviral therapy. Patients with severe alanine transaminase flares had increased HBcrAg levels after antiviral therapy cessation, and the concentration declined after recommencing antiviral therapy.¹³

The decline of HBcrAg during antiviral therapy reflects intrahepatic cccDNA reduction and suppression of viral replication activity. HBcrAg decline was positively correlated with HBsAg reduction or HBeAg seroconversion.¹⁴ While the HBeAg expression outnumbered the HBcrAg expression in HBeAg-positive patients, the predictive performance of HBcrAg on FHR may be affected. After antiviral treatment cessation, patients with lower HBcrAg levels are more likely to have HBsAg loss (Table 1).¹⁵ It may be appropriate to monitor HBcrAg to assess clinical outcomes and treatment effects. HBcrAg would be a substitute marker for predicting the

Abbreviations:

cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; EOFU, end of follow-up; FHR, favourable HBsAg response; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LLOD, lower limit of detection; NAs, nucleos(t)ide analogues; pgRNA, pre-genomic RNA

Table 1. Correlation between HBcrAg and important clinical outcomes in CHB patients

Clinical outcome	Finding	HBcrAg level
HBeAg loss	HBcrAg decline correlated with HBeAg loss	2.3 log U/mL reduction
HBsAg loss	Lower HBcrAg correlated with higher incidence of HBsAg loss	<2 log U/mL
HCC	Higher HBcrAg correlated with higher HCC risk in treatment-naïve patients	>2.9 log U/mL
	Higher HBcrAg correlated with higher HCC risk in antiviral-treated patients	≥4.9 log U/mL for HBeAg-positive patients ≥4.4 log U/mL for HBeAg-negative patients
	Higher HBcrAg correlated with higher post-treatment HCC recurrence	≥4.8 log U/mL

HBcrAg, hepatitis B core-related antigen; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma.

course of the disease.

HBcrAg and HCC

In addition to being a useful biomarker for monitoring viral replication activity and cccDNA, HBcrAg is of high value in predicting the development of HCC in CHB patients. For treatment-naïve CHB patients, high serum HBcrAg level was associated with the development of HCC.¹⁶ Patients with decreased HBcrAg had lower HCC risk than those with persistently high HBcrAg levels.¹⁷ Serum HBcrAg also had a superior prediction value for predicting the HCC risk than other HBV markers, such as HBV DNA level (Table 1).

HCC risk was not eliminated in antiviral-treated HBeAg-negative patients with high HBcrAg levels. For antiviral-treated CHB patients, among whom HBV DNA and HBsAg levels may not perform well in HCC risk prediction, HBcrAg can predict the incidence of HCC accurately in HBeAg-negative patients with high sensitivity and negative predictive value.⁴ It also works in cirrhotic CHB patients. Consistently high on-treatment serum HBcrAg level was associated with a higher HCC incidence, despite prolonged antiviral treatment, in both HBeAg-positive and HBeAg-negative CHB patients (Table 1).¹⁷ The predictive value for post-treatment HCC recurrence of HBcrAg was also demonstrated. Further studies are needed to explore the underlying mechanism of correlation between high HBcrAg and HCC.

UNANSWERED QUESTIONS

As with all important studies, the study by Mak and colleagues⁷ raises a number of interesting questions. For historical reasons, the majority of patients in this study received older generations of NAs, namely lamivudine, adefovir dipivoxil, and telbivudine. The development of drug-resistant mutants and changes in antiviral drugs during follow-up would have affected the association between early changes in viral markers and long-term disease control. Future studies in patients receiving current first-line treatments (entecavir and tenofovir) are needed.

Moreover, we also need to understand the meaning of FHR in this study. A low serum HBsAg level was associated with a lower risk of HCC in patients with low HBV DNA,¹⁸ and the risk was even lower after HBsAg seroclearance.² Although a serum HBsAg level of <100 IU/mL correlated with a lower risk of virological relapse after NA cessation, the prediction was imperfect.⁶ It would be interesting to determine the role of early pgRNA and HBcrAg response in predicting the off-treatment response and prognostication.

Notably, the field of hepatology is currently working towards functional cure of CHB (i.e., HBsAg seroclearance and sustained off-treatment HBV DNA suppression).^{1,19} Since HBsAg seroclearance is rare with the current oral NAs, the current oral NA treatment is unlikely the area where the new virological markers will be applied. Rather, the roles of HBV RNA, HBcrAg, and HBsAg levels in predicting the response to novel direct-acting antivirals and immunological treatments in HBV cure programs are some of the hottest research areas in hepatology.

In summary, the study by Mak and colleagues was exceptional in exploring the meaning of early changes in novel virological markers in a cohort with very long follow-up. With concerted effort using novel treatments and biomarkers, we are hopeful that the “holy grail” of functional cure for CHB will be achievable in the future.

Authors' contribution

All the authors were responsible for the interpretation of findings, the drafting, and critical revision of the editorial for important intellectual content. All authors approved the final version of the article.

Conflicts of Interest

Lilian Liang declared that she has no competing interests.

Vincent Wong has served as a consultant or advisory committee member for AbbVie, Boehringer Ingelheim, Echosens, Intercept, Inventiva, Novo Nordisk, Pfizer, and TARGET PharmaSolutions; and a speaker for Abbott, AbbVie, Gilead Sciences, and Novo Nordisk. He has received a research grant from Gilead Sciences, and is a cofounder of Illuminatio Medical Technology Limited.

Grace Wong has served as an advisory committee member for Gilead Sciences and Janssen, and as a speaker for Abbott, AbbVie, Ascletris, Bristol-Myers Squibb, Echosens, Gilead Sciences, Janssen, and Roche. She has also received a research grant from Gilead Sciences.

Terry Yip has served as an advisory committee member and a speaker for Gilead Sciences.

REFERENCES

1. Wong GLH, Gane E, Lok ASF. How to achieve functional cure of HBV: stopping NUCs, adding interferon or new drug development? *J Hepatol* 2022;76:1249-1262.
2. Yip TCF, Wong GLH, Chan HLY, Tse YK, Lam KLY, Lui GCY, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. *J Hepatol* 2019;70:361-370.
3. Yip TC, Wong VW, Tse YK, Chan HL, Wong GL. Hepatitis B surface antigen seroclearance in a cohort of 154,740 patients with chronic hepatitis B: a 15-year follow-up study. *Hepatol Int* 2017;11(Suppl 1):S75.
4. Liang LY, Wong VWS, Toyoda H, Tse YK, Yip TCF, Yuen BWY, et al. Serum hepatitis B core-related antigen predicts hepatocellular carcinoma in hepatitis B e antigen-negative patients. *J Gastroenterol* 2020;55:899-908.
5. Kim SW, Yoon JS, Lee M, Cho Y. Toward a complete cure for chronic hepatitis B: novel therapeutic targets for hepatitis B virus. *Clin Mol Hepatol* 2022;28:17-30.
6. Chan HLY, Wong GLH, Chim AML, Chan HY, Chu SHT, Wong VWS. Prediction of off-treatment response to lamivudine by serum hepatitis B surface antigen quantification in hepatitis B e antigen-negative patients. *Antivir Ther* 2011;16:1249-1257.
7. Mak LY, Wong D, Kuchta A, Hilfiker M, Hamilton A, Chow N, et al. Moving toward hepatitis B virus functional cure - the impact of on-treatment kinetics of serum viral markers. *Clin Mol Hepatol* 2023;29:146-162.
8. Wang J, Shen T, Huang X, Kumar GR, Chen X, Zeng Z, et al. Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. *J Hepatol* 2016;65:700-710.
9. Inoue T, Tanaka Y. Novel biomarkers for the management of chronic hepatitis B. *Clin Mol Hepatol* 2020;26:261-279.
10. Liu Y, Jiang M, Xue J, Yan H, Liang X. Serum HBV RNA quantification: useful for monitoring natural history of chronic hepatitis B infection. *BMC Gastroenterol* 2019;19:53.
11. Cornberg M, Lok AS, Terrault NA, Zoulim F; 2019 EASL-AASLD HBV Treatment Endpoints Conference Faculty. Guidance for design and endpoints of clinical trials in chronic hepatitis B - Report from the 2019 EASL-AASLD HBV Treatment Endpoints Conference. *J Hepatol* 2020;72:539-557.
12. Wu JW, Kao JH, Tseng TC. Three heads are better than two: hepatitis B core-related antigen as a new predictor of hepatitis B virus-related hepatocellular carcinoma. *Clin Mol Hepatol* 2021;27:524-534.
13. Carey I, Gersch J, Wang B, Moigboi C, Kuhns M, Cloherty G, et al. Pregenomic HBV RNA and hepatitis B core-related antigen predict outcomes in hepatitis B e antigen-negative chronic hepatitis B patients suppressed on nucleos(t)ide analogue therapy. *Hepatology* 2020;72:42-57.
14. Li J, Wu Z, Wang GQ, Zhao H. Hepatitis B core-related antigen reflects viral replication and protein production in chronic hepatitis B patients. *Chin Med J (Engl)* 2021;134:1160-1167.
15. Sonneveld MJ, Chiu SM, Park JY, Brakenhoff SM, Kaewdech A, Seto WK, et al. Probability of HBsAg loss after nucleos(t)ide analogue withdrawal depends on HBV genotype and viral antigen levels. *J Hepatol* 2022;76:1042-1050.
16. Tada T, Kumada T, Toyoda H, Kiriya S, Tanikawa M, Hisanaga Y,

- et al. HBcrAg predicts hepatocellular carcinoma development: an analysis using time-dependent receiver operating characteristics. *J Hepatol* 2016;65:48-56.
17. Hosaka T, Suzuki F, Kobayashi M, Fujiyama S, Kawamura Y, Sezaki H, et al. Impact of hepatitis B core-related antigen on the incidence of hepatocellular carcinoma in patients treated with nucleos(t)ide analogues. *Aliment Pharmacol Ther* 2019;49:457-471.
 18. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. *Gastroenterology* 2012;142:1140-1149.
 19. Yip TCF, Lok ASF. How do we determine whether a functional cure for HBV infection has been achieved? *Clin Gastroenterol Hepatol* 2020;18:548-550.