Invited Editorial

Towards HBV functional cure – do we have a crystal ball for that?

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

\textsuperscript{1}Medical Data Analytics Center, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong

\textsuperscript{2}State Key Laboratory of Digestive Disease, Institute of Digestive Disease, The Chinese University of Hong Kong

Word count: 1,416

Correspondence:

Professor Terry Yip, PhD.

Department of Medicine and Therapeutics, 5/F, Clinical Sciences Building, Prince of Wales Hospital, 30-32 Ngan Shing Street, Shatin, Hong Kong

Tel: +852 35053125 Fax: +852 26373852

Email: tcfyip@cuhk.edu.hk
Background

Functional cure of chronic hepatitis B virus (HBV) infection is currently set as the treatment goal of new HBV therapies, which is serologically defined as clearance of hepatitis B surface antigen (HBsAg), with or without anti-HBs seroconversion, and undetectable serum HBV DNA.[1] A handful of studies showed 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 HCC.[2] Nonetheless, there is still a low yet definite risk of HCC occurrence, especially male patients who achieve functional cure after 50 years old.[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 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. [Wong J Hep 22] Several studies 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 low HBsAg level at the time of NA withdrawal.[1]

Key findings

Overview of study methodology
Mak and colleagues examined the serum hepatitis B core-related antigen (HBcrAg) and HBV pre-genomic RNA (pgRNA) in the first 48 weeks of NA treatment in 64 chronic hepatitis B CHB patients.[7] Analyses were performed separately in 28 hepatitis B e antigen (HBeAg)-positive and 36 HBeAg-negative patients. These patients had participated in previous phase III trials receiving 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. Among 64 patients, 14 patients with undetectable HBV pgRNA or HBcrAg at week 0 were excluded from the analysis of temporal level change. Due to censoring and the difference in follow-up duration between patients with and without FHR at the end of follow-up, time-dependent area under receiver operating curve were utilized to assess the discriminatory ability of HBcrAg and HBV pgRNA.

**Clinical meaning of HBV pgRNA decline**

At a median follow-up of 17 years, 22/64 patients achieved FHR including 8 HBsAg seroclearance. At the start of NA therapy, HBeAg-positive patients had a higher HBV pgRNA than 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 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 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 the clinical utility.[11]

Clinical meaning of HBcrAg decline

Mak and colleagues 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.[7] HBcrAg is a novel biomarker in CHB patients and consists of hepatitis B core antigen, HBeAg, and the 22-kDa precore protein.[12] It gradually decreased following 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.\cite{14} While the HBeAg expression outnumbers 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.\cite{15} (Table 1) It may be appropriate to monitor HBcrAg to assess clinical outcomes and treatment effects. HBcrAg would be a substitute marker for predicting the 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 to predict the development of hepatocellular carcinoma (HCC) in CHB patients. For treatment-naïve CHB patients, high serum HBcrAg level was associated with the development of HCC.\cite{16} Patients with decreased HBcrAg had lower HCC risk than those with persistently high HBcrAg levels.\cite{17} Serum HBcrAg also had a superior prediction value for predicting the HCC risk than other HBV markers like 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.\cite{4} It also works in cirrhotic CHB patients. Consistently high on-treatment serum HBcrAg level is associated with a higher HCC incidence despite prolonged antiviral treatment in both HBeAg-positive and -negative CHB patients (Table 1).\cite{17} The predictive value for post-
treatment HCC recurrence of HBcrAg was also demonstrated. Further studies were needed to explore the underline mechanism of correlation between high HBcrAg and HCC.

**Unanswered questions**

Like all important studies, the study by Mak and colleagues raises a number of interesting questions.[7] 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.

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

Importantly, the field is currently working towards functional cure of CHB (i.e., HBsAg seroclearance and sustained off-treatment HBV DNA suppression).[1, 19] Because HBsAg seroclearance is rare with the current oral NAs, this 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 is one of the hottest research areas in hepatology.
In summary, the study by Mak and colleagues is 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 of CHB will be within reach.
References


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

<table>
<thead>
<tr>
<th>Clinical outcomes</th>
<th>Findings</th>
<th>HBcrAg levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBeAg loss</strong></td>
<td>HBcrAg decline correlated with HBeAg loss</td>
<td>2.3 log U/mL reduction</td>
</tr>
<tr>
<td><strong>HBsAg loss</strong></td>
<td>Lower HBcrAg correlated with higher incidence of HBsAg loss</td>
<td>&lt; 2 log U/mL</td>
</tr>
<tr>
<td><strong>HCC</strong></td>
<td>Higher HBcrAg correlated with higher HCC risk in treatment-naïve patients</td>
<td>&gt; 2.9 log U/mL</td>
</tr>
</tbody>
</table>
|                   | 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.