

Serum HBV RNA is a Potential Predictor of Hepatitis B Surface Antigen Reversion

Jie Wang,^{1*} Xiangmei Chen,^{1*} Yali Wu,^{2*} Zhenhuan Cao,² Leijie Wang,¹ Hongxin Huang,¹ Xinyue Chen,² and Fengmin Lu^{1,*}

Because sustained hepatitis B surface antigen (HBsAg) seroclearance with or without anti-HB appearance represents a functional cure of chronic hepatitis B (CHB), HBsAg loss has been recommended as an optimal endpoint of antiviral treatment.⁽¹⁾ However, composite reversion including HBsAg reversion and/or hepatitis B virus (HBV) DNA reappearance may still occur after drug withdrawal, even after achieving HBsAg loss.⁽²⁾ Therefore, a novel marker predicting the risk of composite reversion is needed. Because serum HBV RNA has been suggested as a potential predictor of nucleos(t)ide analogue (NA) off-therapy viral rebound in our previous study,⁽³⁾ we wondered whether it could be used to predict the risk of composite reversion. (*Hepatology Communications* 2018;2:1168-1171).

Methods and Results

In this study, 32 CHB patients who achieved seroclearance of both HBsAg and HBV DNA after receiving pegylated interferon (Peg-IFN) treatment alone or in combination with NA therapy were enrolled, including 12 patients with composite reversion and 20 patients without. Posttherapy monitoring interval was 12-24 weeks. The levels of HBV serum markers and liver function examination were tested during the monitoring process. Among the 12 patients with composite reversion, 2 patients occurred at 4 weeks following drug withdrawal, 8 patients at 24-48 weeks, and the last 2 patients at more than 48 weeks. The clinical characteristics of the patients are given in Table 1 and the Supporting Information. Serum HBV RNA levels at the end of treatment were compared between the composite reversion and the noncomposite reversion groups. As indicated in Table 1, 7 of 12 patients were positive for serum HBV RNA at the end of treatment in the composite reversion group, and 0 of

20 patients were positive in the noncomposite reversion group. In other words, all 7 patients who were positive for HBV RNA at the end of treatment had composite reversion occur after drug withdrawal, whereas composite reversion occurred in only 5 of the 25 patients who were negative for HBV RNA (Table 1). The positive predictive value and negative predictive value of serum HBV RNA levels at the end of treatment for the prediction of composite reversion was 100% and 80%, respectively. This indicates that being serum HBV RNA-positive at the end of treatment could predict the risk of composite reversion after drug withdrawal.

Next, the association between serum HBV RNA and the type of HBV composite reversion was analyzed in the composite reversion group. Among the 12 composite reversion patients, 6 patients showed HBsAg reversion, 3 showed HBV DNA reappearance, and 3 showed both HBsAg reversion and HBV DNA reappearance. As given in Table 2 and Supporting Table S1, all 6 patients who experienced HBsAg reversion were serum HBV RNA-positive at the end of treatment,

Abbreviations: cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; Peg-IFN, pegylated interferon.

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*These authors contributed equally to this work.

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TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE ENROLLED PATIENTS AT THE END OF TREATMENT

Characteristics	Composite Reversion (n = 12)	No Composite Reversion (n = 20)	P Value*
Age, median (years)	37 (26-56)	37 (21-61)	1.000
Male sex, n (%)	8 (66.67)	13 (65)	1.000
HBV DNA < 20 IU/mL, n (%)	12 (100)	20 (100)	1.000
HBsAg < 0.05 IU/mL, n (%)	12 (100)	20 (100)	1.000
Anti-HBs level (IU/mL), median (range)	403.4 (38.53-1000)	478.15 (2-1000)	0.709
Anti-HBs level < 100 IU/L, n (%) [†]	3 (25)	2 (10)	0.338
HBeAg < 1.0 (S/CO), n (%)	12 (100)	20 (100)	1.000
Anti-HBe-positive (S/CO), n (%) [‡]	10 (83.33)	20 (100)	0.133
ALT < 40 IU/mL, n (%)	10 (83.33)	15 (75)	0.683
AST < 40 IU/mL, n (%)	10 (83.33)	15 (75)	0.683
Antiviral drugs:	9 (75)	13 (65)	0.703
Peg-IFN combined with NAs, n (%)	3 (25)	7 (35)	0.703
Peg-IFN alone, n (%)			
Duration of HBsAg loss before drug withdrawal (weeks), [§] median (range)	42.5 (24-84)	43 (0-100)	0.585
Follow-up time after drug withdrawal (weeks), [§] median (range)	48 (20-92)	171 (48-273)	< 0.001
HBV RNA positive, n (%)	7 (58.33)	0 (0)	< 0.001

*Mann-Whitney U test or Fisher's exact test.

[†]Because it has been reported that anti-HBs \geq 100 IU/mL is important to prevent relapse,⁽⁴⁾ the number of patients with anti-HBs \geq 100 IU/mL or < 100 IU/mL in both groups was also analyzed.

[‡]Positive anti-HBe is defined as anti-HBe < 1.0 S/CO according to the manufacturer's instructions.

[§]Drug withdrawal in each case was decided by the attending physician with use of similar but nonuniform criteria.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; and S/CO, signal-to-cutoff ratio.

whereas only 1 of 3 patients with both HBsAg reversion and HBV DNA reappearance, and none of the 3 patients with HBV DNA reappearance, were serum HBV RNA-positive. This result indicates that being serum HBV RNA-positive at the end of treatment could predict a high risk of HBsAg reversion after drug

withdrawal. Moreover, HBV *S* gene mutation was analyzed in a patient who had an HBV DNA level (7,020 IU/mL) high enough for the polymerase chain reaction (PCR)-based sequence analysis. The result reveals that multiple point mutations, including I126T/S, T140I, and D144A in the *S* gene, were found in this patient.

ARTICLE INFORMATION:

From the ¹State Key Laboratory of Natural and Biomimetic Drugs, Department of Microbiology and Infectious Disease Center, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China; ²International Medical Department, Beijing Youan Hospital, Capital Medical University, Beijing, China

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Fengmin Lu, M.D.
State Key Laboratory of Natural and Biomimetic Drugs
Department of Microbiology and Infectious Disease Center
School of Basic Medical Sciences, Peking University Health
Science Center
38 Xueyuan Road
Beijing 100191, China
E-mail: lu.fengmin@hsc.pku.edu.cn
Tel: +86-10-82805136

Or
Xinyue Chen, MS
International Medical Department
Beijing Youan Hospital
Capital Medical University
Beijing 100069, China
E-mail: chenxydoc@163.com

TABLE 2. ASSOCIATION BETWEEN SERUM HBV RNA AND THE TYPE OF HBV COMPOSITE REVERSION AFTER DRUG WITHDRAWAL

Group	HBsAg Reversion	HBsAg Reversion and HBV DNA Reappearance	HBV DNA Reappearance	Total (n)	P Value*
HBV RNA-positive [†]	6	1	0	7	0.006
HBV RNA-undetectable	0	2	3	5	
Total (n)	6	3	3	12	

*Fisher's exact test.

[†]Positive HBV RNA is defined as the presence of positive amplification signal (appeared amplification curve).

Discussion

It has been reported that longer durations of HBsAg loss before NA withdrawal may result in more durable HBsAg seroclearance.⁽⁵⁾ However, perhaps because of the small cohort in this study, the association between the duration of HBsAg loss and the risk of HBsAg reversion was not observed. In this study, all 7 CHB patients with positive HBV RNA at the end of treatment developed composite reversion after drug withdrawal, indicating that the intrahepatic covalently closed circular DNA (cccDNA) in these patients were present and still transcriptionally active. In other words, at least in some CHB patients who appeared to achieve "functional cure," the template of HBV replication-cccDNA may still be transcriptionally active.

Based on previous reports, negative serum HBsAg might be caused by the *S* gene mutation-mediated poor detectability of HBsAg rather than the real HBsAg seroclearance.⁽⁶⁾ In this study, the *S* gene mutation was found in a patient with HBV DNA reappearance and HBV DNA level high enough for the PCR-based sequence analysis. Furthermore, the positive serum HBV RNA with negative HBV DNA indicated that intrahepatic cccDNA might be in a transcriptional active state, but with the decreased reverse transcriptional activity of HBV DNA polymerase (p-protein) mediated by either NAs treatment⁽³⁾ and/or the *P* gene mutation.⁽⁷⁾ Accordingly, for the diagnosis of occult HBV infection (OBI), we suggest that the persistence of HBV RNA should also be taken into consideration. Because serum HBV RNA as a virological marker showed certain superiority in reflecting cccDNA activity, especially among those patients under NA treatment, the OBI should be redefined as the persistence of HBV DNA and/or HBV RNA (HBV nucleic acid positive) in the serum or liver of individuals with undetectable HBsAg. Moreover, because HBV DNA was reverse transcribed from pregenomic RNA (pgRNA) by p-protein, and the reverse transcriptional activity of

p-protein might decrease for those patients with positive serum HBV RNA at the end of treatment, it might lead to the phenomenon that patients with HBV DNA reappearance have less HBV RNA positivity at the end of treatment.

In summary, the current study suggested that serum HBV RNA might be a potential viral marker in predicting composite reversion, especially HBsAg reversion, after antiviral drug withdrawal. Like serum HBV RNA, serum hepatitis B core-related antigen, another viral marker associated with intrahepatic cccDNA, may complement HBV RNA in predicting composite reversion after drug withdrawal. However, multicenter, large-scale, and prospective studies should be conducted to validate these findings and presumptions in the future.

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Author names in bold designate shared co-first authorship.

Supporting Information

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