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Original Article

On-treatment HBV DNA level could predict HBeAg seroclearance in patients with HBeAg-positive chronic hepatitis B with entecavir therapy

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Abstract

Background: Hepatitis B e antigen (HBeAg) status is associated with clinical outcomes, and seroconversion of HBeAg is one of the treatment goals. In this study, we determined the association of on-treatment serum hepatitis B virus (HBV) DNA levels in HBeAg-positive chronic hepatitis B patients who were receiving entecavir (ETV) treatment.

Methods: A retrospective cohort study was conducted involving 135 (78 male and 57 female; mean age, 42.3 ± 13 years) patients with HBeAg-positive chronic hepatitis B (CHB). Between August 2008 and March 2014, each patient was treated with ETV for at least 96 weeks, and their HBV DNA levels were evaluated every 3−6 months. HBeAg seroclearance at the 96th week was defined as an absence of serum HBeAg within 96 weeks after ETV treatment. Univariate and multivariate logistic regression analysis was used to identify the predictors of 96th week HBeAg seroclearance, and a multivariable model was constructed.

Results: Among the 135 ETV-treated HBeAg-positive CHB patients, 37 patients achieved HBeAg seroclearance (Group 1), whereas 98 patients had persistent HBeAg-positive status (Group 2) at the 96th week check-up. The baseline laboratory data was not significantly different between both the two groups. At the 24th and 48th weeks, there were significant differences between Group 1 and Group 2 in the percentage of patients with HBV DNA levels < 20 IU/mL [17/77 (22.1%) and 11/23 (47.8%), $p = 0.032$; 45/89 (50.6%) and 26/35 (74.3%), $p = 0.028$, respectively).

Conclusion: Our study demonstrated that HBV DNA levels < 20 IU/mL at the 24th and 48th weeks could predict serum HBeAg loss in ETV-treated HBeAg-positive patients with CHB.

Keywords: entecavir; hepatitis B e antigen; hepatitis B virus; hepatitis B virus DNA; seroclearance

1. Introduction

Chronic hepatitis B (CHB) is a worldwide public health burden. CHB infection has been associated with an increased risk for developing cirrhosis and hepatocellular carcinoma.[1] In recent decades, there has been a dramatic and rapid progress in the treatment of CHB.[2,3] The current treatment for CHB includes interferon-alfa, lamivudine, adefovir, entecavir (ETV), telbivudine, tenofovir, and pegylated interferon-a2a. There is evidence supporting the concept that antiviral therapy can ameliorate liver damage, progression of cirrhosis, and incidence of hepatocellular carcinoma.[4−6]

In patients with CHB infection, hepatitis B e antigen (HBeAg) is initially positive and accompanied by high levels of hepatitis B virus (HBV) DNA, which may persist for years or even decades.[7] HBeAg-positive immunotolerant patients with significant horizontal and vertical transmission carry a risk of contracting active chronic hepatitis and its complications. The current treatment options are not optimal. Pegylated interferon therapy offers sustained off-treatment responses in

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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only a minority of patients.[8] Nucleoside analogs can suppress the on-treatment HBV DNA, improve liver histological lesions, reverse cirrhosis in the majority of cases, and improve survival rates.[9,10] Antiviral therapies may lead to sustained responses during therapy until the onset of treatment withdrawal. Thus, more commonly, a therapy must be continued to maintain responses achieved during the therapy. The short-term goal of treatment for HBeAg-positive CHB is to achieve an initial response by HBeAg loss or seroconversion and/or HBV DNA suppression, alanine aminotransferase (ALT) normalization, and prevention of hepatic decompensation.[11,12]

HBV DNA levels are associated with clinically significant pathological events, such as cirrhosis; therefore, changes in the serum levels of HBV DNA could determine a risk for hepatocellular carcinoma.[13,14] For on-treatment follow-up, HBeAg loss/seroconversion is important during the nucleoside therapy for CHB patients. In China and other East Asian countries, HBeAg seroconversion was achieved in approximately 44−47% of patients after 4−5 years of lamivudine-based treatment.[15,16] Under lamivudine treatment, studies from several Taiwan reports indicated that the rate of HBeAg seroconversion was associated with the pretreatment ALT level.[17,18]

ETV, a new guanosine nucleoside analog with specific activity against HBV DNA polymerase, represents a third agent within the nucleoside/nucleotide HBV polymerase inhibitor class. Compared with lamivudine and adefovir, ETV at 0.5 mg daily reportedly induces greater HBV DNA suppression, with HBV DNA becoming undetectable in 60−71% of HBeAg-positive patients and 88−90% of HBeAg-negative patients at 48−52 weeks.[3,19] The HBeAg seroconversion rate was 31% by 2nd year, and the HBeAg seroconversion rate in 141 HBeAg-positive patients was 23% from 96th week to 240th week.[12] Among patients treated with ETV at 0.5 mg daily, 83−90% patients had undetectable HBV DNA, and 24−44% patients had HBeAg seroconversion at 3rd year of the treatment.[20−22]

ETV could suppress HBV DNA effectively, but the dynamic change of HBV DNA and corresponding HBeAg changes have not yet been clearly elucidated. Whether on-treatment follow-up HBV DNA is capable of predicting HBeAg seroconversion or seroclearance requires further investigation. To address these concerns, we conducted the present study to investigate whether on-treatment serum HBV DNA levels could predict HBeAg seroclearance during ETV-based therapy for HBeAg-positive CHB patients. In this retrospective, study patients were followed-up regularly every 12 weeks. Follow-ups included evaluations of serum HBV DNA levels at the 12th week, 24th week, and 48th week since the ETV treatment initiation. The results of our study provide important information with respect to the prediction of HBeAg seroclearance during ETV therapy among HBeAg-positive CHB patients.

2. Methods

2.1. Patients

We conducted a retrospective cohort study by reviewing medical records and underwent a routine, regular treatment protocol for CHB patients. Between August 2008 and March 2014, a total of 135 (78 male and 57 female; mean age, 42.3 ± 13 years) patients with HBeAg-positive CHB who were treated with ETV for at least 96 weeks were included in this study. Patients were followed-up every 3 months and ALT, HBV DNA, and HBeAg status were routinely assessed every 3−6 months at Taichung Veterans General Hospital. Virological response is defined as a serum level of HBV DNA of < 20 IU/mL.[23]

Patients were excluded in case of the following: (1) they had previously undergone antiviral treatment for hepatitis B; (2) they had HBeAg seroclearance within 24 weeks after ETV treatment; and (3) they were coinfected with the hepatitis C virus, hepatitis D virus, or human immunodeficiency virus (HIV). The study was approved by the Institutional Review Board of our institution (VGHTC CE16037B).

2.2. Outcome measurements

The primary endpoint of our analysis was HBeAg seroclearance, which was defined as negative serum HBeAg levels within 96 weeks of ETV treatment. ALT, bilirubin and HBV DNA levels were also routinely evaluated in our department.

2.3. Laboratory methods

HBV DNA was determined by real-time PCR assay (Roche Cobas TaqMan HBV Test). Hepatitis B surface antigen (HBsAg) and HBeAg and anti-HBe were determined by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany).

2.4. Statistical analysis

Statistical tests were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared using the Chi-square test and Fisher's test, and continuous variables were expressed as mean and range and compared using the Student's t test or Mann−Whitney U test. Logistic regression was performed to analyze factors associated with HBeAg seroclearance at the 96th week, and significant factors ($p < 0.05$) in the univariate analysis were subjected to multivariate analysis to determine independent predictive factors. To avoid co-linearity of both parameters, HBV DNA levels at 24th week and 48th week were analyzed individually in multivariate analysis with age and sex adjustment. Statistical significance was defined as a p value of < 0.05.

3. Results

3.1. Patients' characteristics

Demographic data of ETV-treated patients with HBeAg seroclearance and without HBeAg seroclearance within 96 weeks is shown in Table 1. A total of 135 HBeAg-positive CHB patients received ETV treatment. There were 37

Table 1
Comparison of baseline features.

	Total (n = 135)	HBeAg seroclearance within 96th week		p
		No (n = 98)	Yes(n = 37)	
Sex (Male)	78 (57.8%)	58 (59.2%)	20 (54.1%)	0.732
Age, y	42.3 (18–85)	41.8(18–75)	43.5 (24–85)	0.957
Total bilirubin (mg/dL)	1.6 (0.2–24.1)	1.5(0.2–24.1)	1.7 (0.3–13.6)	0.607
ALT (U/L)	325 (21–2874)	292.2 (21–2309)	409.7 (35–2874)	0.283
ALP (U/L)	126.2 (39–336)	133 (48–336)	108.9 (39–220)	0.067
AFP (ng/mL)	31.5 (1.2–942)	33.8(1.2–942)	25.5 (1.8–246)	0.651
Platelet count (*10^3/CUMM)	182.6 (46–448)	181.8(46–448)	186.7 (52–361)	0.392
Anti-HBe (+) (n = 91 vs. 37)	24 (19.4%)	15(16.5%)	9 (27.3%)	0.277
Spleen size (cm)	10.1 (5.8–20)	10.2(6–20)	9.7 (5.8–16)	0.469
Liver cirrhosis	26 (19.3%)	20 (20.4%)	6 (16.2%)	0.759
Pre-treatment serum HBV DNA (log IU/mL)	7.1 (4–9.1)	7.1(4.2–8.7)	7.2 (3.9–9.1)	0.530

Chi-square test. Mann–Whitney U test. * $p < 0.05$; ** $p < 0.01$.
Continuous data is expressed as median and range.
Categorical data is expressed as n (%).
AFP = alpha-fetoprotein; ALP = alkaline phosphatase; Anti-HBe = Hepatitis B e-antibody; ALT = alanine transaminase; HBV = hepatitits B virus.

(37.4%) ETV-treated patients with HBeAg seroclearance within 96 weeks (Group 1) and 98 (72.6%) ETV-treated patients without HBeAg seroclearance within 96 weeks (Group 2). There were no significant differences in age, sex, spleen size, and liver cirrhosis between the two groups. The baseline laboratory data, including platelet count, ALT, alkaline phosphatase, total bilirubin, anti-HBe, pretreatment serum HBV DNA, and alpha-fetoprotein (AFP) levels were not significantly different in both the groups (Table 1).

3.2. Pretreatment HBV DNA levels could not predict HBeAg seroclearance within 96 weeks

There were no significant differences in pretreatment serum HBV DNA levels between ETV-treated patients with and without HBeAg seroclearance within 96 weeks. Pretreatment serum HBV DNA levels were expressed as log and are shown in Fig. 1.

3.3. On-treatment dynamic change of serum HBV DNA levels and HBeAg seroclearance

There were significant differences where patients achieving virological response within 48 weeks could have a higher rate of HBeAg seroclearance within 96 weeks [48/98 (49%) vs. 27/36 (75%), $p = 0.013$*]. At the 12th week check-up, there were no significant differences between Group 1 and Group 2 in the proportion of patients with HBV DNA levels < 20 IU/mL [4/31 (12.9%) and 1/11 (9.1%), $p = 1.000$, respectively]. Table 2 shows the associations of HBeAg seroclearance within 96 weeks and on-treatment serum HBV DNA levels. At the 24th week check-up, there were significant differences between Group 1 and Group 2 in the proportion of patients with HBV DNA levels < 20 IU/mL [17/77 (22.1%) and 11/23 (47.8%), $p = 0.032$, respectively]. At the 48th week check-up, there were significant differences between the groups in the proportion of patients with HBV DNA levels < 20 IU/mL [45/89 (50.6%) and 26/35 (74.3%), $p = 0.028$, respectively] (Fig. 2).

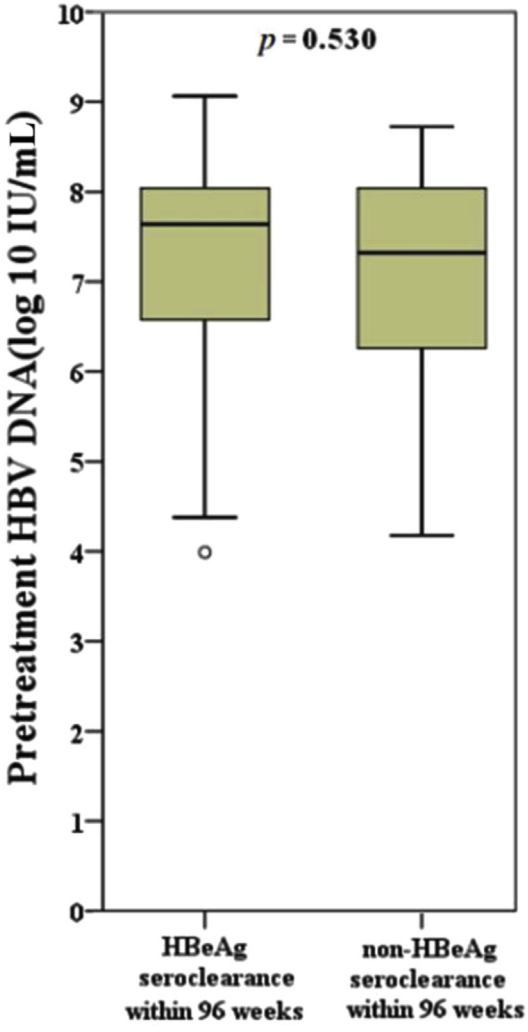

Fig. 1. Pretreatment hepatitis B virus (HBV) DNA could not predict hepatitis B e antigen (HBeAg) seroclearance within 96 weeks.

Table 2
On-treatment hepatitis B virus (HBV) DNA dynamics and hepatitis B e antigen (HBeAg) seroclearance within 96 weeks in entecavir-treated chronic hepatitis B patients.

	Total (n = 135)	HBeAg seroclearance within 96th week		p
		Non (n = 98)	Yes (n = 37)	
Virological response within 48 weeks (n = 98 vs. 36)	75 (56%)	48 (49%)	27 (75%)	0.013*
HBV DNA level < 20 IU/mL at 12th week (n = 31 vs. 1 l)[f]	5 (11.9%)	4(12.9%)	1 (9.1%)	1.000
HBV DNA level < 20 IU/mL at 24th week (n = 77 vs. 23)	28 (28.0%)	17(22.1%)	11 (47.8%)	0.032*
HBV DNA level < 20 IU/mL at 48th week (n = 89 vs. 35)	71 (57.3%)	45 (50.6%)	26 (74.3%)	0.028*

Chi-square test. [f]Fisher's exact test. * $p < 0.05$, ** $p < 0.01$.
Categorical data were expressed as n (%).
Virological response within 48 weeks: serum HBV DNA < 20 IU/ml within 48 weeks.

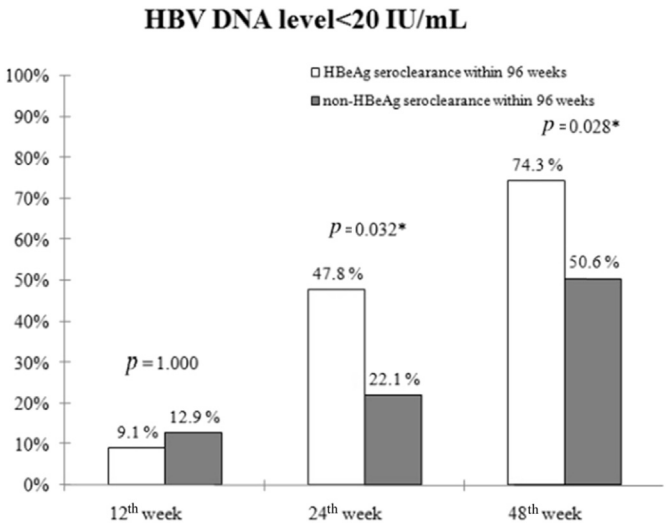

Fig. 2. On-entecavir treatment dynamic change of serum hepatitis B virus (HBV) DNA level and hepatitis B e antigen (HBeAg) seroclearance within 96 weeks. There were no significant differences between the HBeAg seroclearance group and non-HBeAg seroclearance group in the proportion of patients with HBV DNA levels < 20 IU/mL at the 12th week, but significant differences were found at the 24th week and 48th week.

3.4. Factors affecting HBeAg seroclearance within 96 weeks through univariate and multivariate analysis

Table 3 shows univariate and multivariate logistical analyses of clinical factors affecting HBeAg seroclearance within 96 weeks. In univariate analysis, age, sex, comorbidities, ALT level, total bilirubin level, and platelet count were not associated with HBeAg seroclearance within 96 weeks. There were significant differences between Group 1 and Group 2 in time to virological response (weeks), virological response within 48 weeks, and serum HBV DNA levels at the 24th week and 48th week. After adjustment for age and sex, multivariate analysis also demonstrated that HBV DNA levels at the 24th week [odds ratio (OR) = 3.10, 95% confidence interval (CI) = 1.14−8.40, $p = 0.026$) and 48th week (OR = 2.91, 95% CI = 1.22−6.98, $p = 0.026$) were associated with HBeAg seroclearance within 96 weeks under ETV treatment. Patients with HBeAg seroclearance within 96 weeks experienced earlier virological response (OR = 0.97, 95% CI = 0.96−0.99, $p < 0.001$) than those without HBeAg seroclearance within 96 weeks.

Table 3
Logistic analysis of factors for hepatitis B virus seroclearance within 96 weeks.

	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Age	1.01	(0.98−1.04)	0.515			
Spleen size (cm)	0.92	(0.78−1.10)	0.369			
Platelet count (*10³/CUMM)	1.00	(1.00−1.01)	0.711			
Total bilirubin (mg/dL)	1.02	(0.91−1.15)	0.712			
ALT (U/L)	1.00	(1.00−1.00)	0.234			
ALP (U/L)	0.99	(0.98−1.00)	0.089			
AFP (ng/mL)	1.00	(0.99−1.00)	0.684			
Pre-treatment HBV DNA (log10 IU/mL)	1.11	(0.77−1.60)	0.570			
Sex (male)	0.81	(0.38−1.74)	0.591			
Anti-HBe (+)	1.90	(0.74−4.89)	0.183			
Liver cirrhosis	0.75	(0.28−2.06)	0.582			
ALT at 24th week (U/L)	0.98	(0.96−1.00)	0.087			
Time to virological response (weeks)	0.97	(0.96−0.99)	0.001	0.97	0.96−0.99	< 0.001**
Virological response within 48 weeks	3.12	(1.33−7.33)	0.009**	3.24	1.37−7.67	< 0.008**
HBV DNA level < 20 IU/mL at 12th week	0.68	(0.07−6.79)	0.739			
HBV DNA level < 20 IU/mL at 24th week	3.24	(1.21−8.62)	0.019*	3.10	1.14−8.40	0.026*
HBV DNA level < 20 IU/mL at 48th week	2.82	(1.19−6.70)	0.019*	2.91	1.22−6.98	0.016*

Logistic regression. Adjusted for age and sex. * $p < 0.05$; ** $p < 0.01$.
AFP = alpha-fetoprotein; ALP = alkaline phosphatase; Anti-HBe = Hepatitis B e-antibody; ALT = alanine transaminase; CI = confidence interval; OR = odds ratio; virological response (weeks) = time to achieve virological response (weeks).

4. Discussion

ETV is one of the major treatments for patients with hepatitis B infection owing to its superior ability to effectively suppress HBV DNA. However, HBeAg seroconversion or seroclearance is one of the major goals of nucleoside treatment; therefore, a clinical indicator for predicting the absence of HBeAg would be useful during the on-treatment follow-up. In this study, one-third of the patients who achieved HBeAg seroclearance under ETV treatment did so within 96 weeks. Our results demonstrated that HBV DNA levels < 20 IU/mL at the 24th and 48th week could predict HBeAg loss within 96 weeks in ETV-treated CHB patients.

A Chinese study involving lamivudine treatment for CHB demonstrated that CHB patients showed enhanced seroconversion rates, and up to two-thirds of patients with moderately elevated pretreatment ALT levels achieved HBeAg seroconversion after 3 years of therapy.[24]

Wang et al[25] demonstrated that both baseline serum ALT levels > 5 times the upper normal limit and HBV DNA levels could predict HBeAg seroconversion.[25] However, the baseline serum ALT level was not a predictor for HBeAg seroconversion during ETV treatment in our study as well as in another study.[26] The reasons are possibly due to the variation of the baseline serum ALT level and the number of cases. A 4-year study reported that the baseline HBV DNA level was a significant factor associated with undetectable HBV DNA and HBeAg seroclearance at 1−3 years.[27] Thus, undetectable HBV DNA could be considered as another predicting factor in HBeAg serological change. Our data indicated that HBV DNA levels < 20 IU/mL at both 24th week and 48th week were positively associated with favorable serological change in CHB patients with ETV treatment. This finding is consistent with previous studies.[26,28] Additional predictors for HBeAg seroconversion during treatment have been studied, and serum HBV RNA levels may serve as a novel tool for the prediction of serological response during polymerase inhibitor treatment in HBeAg-positive patients.[29] However, currently it is not commonly used in routine follow-up for CHB treatment. Among HBeAg-positive CHB patients, nucleoside could significantly improve the rates of histologic, virological, and biochemical profiles. Our results are similar to those of previous reports. Without significant and rapid HBV DNA suppression, ETV-treated CHB patients possibly need a longer treatment duration in order to obtain histological and serological benefits.[12]

HBeAg-positive patients older than 40 years with persistently high or high to normal ALT levels may be diagnosed with significant hepatic necroinflammation or fibrosis.[30] During treatment, the clinical significance of timing of HBeAg loss or seroconversion remains unclear. However, a longer duration of a HBeAg-positive status may indicate ongoing hepatic inflammation. Quantitative HBsAg (qHBsAg) levels have the potential for a follow-up treatment response in CHB for the prediction of a response to interferon-alpha. In HBeAg-positive patients with ETV therapy, patients with HBeAg seroclearance or seroconversion showed an early decline in their HBsAg levels.[31−34] However, the baseline HBsAg level and decline at 12th week or 24th week could not predict HBeAg seroconversion at 2 years.[35] In the present study, we did not routinely check qHBsAg during ETV treatment for CHB. Thus, we could not compare qHBsAg or HBV DNA dynamics for any prediction of HBeAg seroconversion.

The ideal treatment end-point is sustained HBsAg loss with or without anti-HBs seroconversion in HBeAg-positive and HBeAg-negative patients. Actually, the duration of ETV treatment for HBeAg positive CHB may be up to 240 weeks.[12] A duration of consolidation therapy after HBeAg seroconversion is indicated.[36] Our results showed that HBV DNA levels < 20 IU/mL at the 24th week could early predict the likelihood of HBeAg loss.

Our study had some limitations. First, in this single-institution study, the number of patients enrolled was limited. Second, HBeAg-positive hepatitis B patients under treatment are regularly followed-up by protocol. However, as a retrospective study, there still remained portions of missing data from our patients. Third, compliance of medication prescription schedules is important in a pharmacological study and follow-up. There are some difficulties encountered when attempting to confirm compliance of our patients by strictly reviewing medical records alone. We had 18 patients who developed virological breakthrough under follow-up, which is mostly due to poor compliance; therefore, we assumed that these patients in our study could achieve 90% compliance. Fourth, we considered that HBeAg seroclearance within 6 months of ETV was possibly due to the efficacy of antiviral therapy or spontaneous HBeAg seroclearance. We excluded patients with HBeAg seroclearance within 6 months, which might introduce a selection bias due to including only later HBeAg seroclearance patients. This could be the reason why this study failed to investigate the HBV DNA at 12th week when predicting HBeAg seroclearance within 96 weeks. Besides, further studies will be warranted to understand the association of the early dynamic change of HBV DNA and serologic response in HBeAg-positive patients.

Our study demonstrated that HBV DNA levels < 20 IU/mL at the 24th week and 48th week could predict serum HBeAg loss in ETV-treated HBeAg-positive CHB patients. This finding indicates that ETV-treated HBeAg-positive CHB patients are less likely to achieve HBeAg seroclearance within 96 weeks if they did not have an HBV DNA level of < 20 IU/mL at the 24th or 48th week.

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References

1. Dienstag JL. Hepatitis B virus infection. *N Engl J Med* 2008;**359**:1486−500.
2. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009;**50**:661−2.

3. Liaw YF, Kao JH, Piratvisuth T, Chan HL, Chien RN, Liu CJ, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. *Hepatol Int* 2012;**6**:531−61.
4. Papatheodoridis GV, Lampertico P, Manolakopoulos S, Lok A. Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: a systematic review. *J Hepatol* 2010;**53**:348−56.
5. Wang JP, Kao FY, Wu CY, Hung YP, Chao Y, Chou YJ, et al. Nucleos(t) ide analogues associated with a reduced risk of hepatocellular carcinoma in hepatitis B patients: a population-based cohort study. *Cancer* 2015;**121**: 1446−55.
6. Yuen MF, Lai CL. Treatment of chronic hepatitis B: evolution over two decades. *J Gastroenterol Hepatol* 2011;**26**:138−43.
7. McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology* 2009;**49**:S45−55.
8. Kao JH. Hbeag-positive chronic hepatitis B: why do I treat my patients with pegylated interferon? *Liver Int* 2014;**34**:112−9.
9. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for Hbeag-positive chronic hepatitis B. *N Engl J Med* 2006;**354**:1001−10.
10. Dienstag JL, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, et al. Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003;**124**:105−17.
11. Zoulim F, Perrillo R. Hepatitis B: reflections on the current approach to antiviral therapy. *J Hepatol* 2008;**48**:S2−19.
12. Chang TT, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2010;**51**:422−30.
13. Chen CF, Lee WC, Yang HI, Chang HC, Jen CL, Iloeje UH, et al. Changes in serum levels of Hbv DNA and alanine aminotransferase determine risk for hepatocellular carcinoma. *Gastroenterology* 2011;**141**:1240−8. 48.e1−2.
14. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006;**130**:678−86.
15. Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2004;**19**:1276−82.
16. Yao GB, Zhu M, Cui ZY, Wang BE, Yao JL, Zeng MD. A 7-year study of lamivudine therapy for hepatitis B virus e antigen-positive chronic hepatitis B patients in China. *J Dig Dis* 2009;**10**:131−7.
17. Chien RN, Liaw YF, Atkins M. Pretherapy alanine transaminase level as a determinant for hepatitis B e antigen seroconversion during lamivudine therapy in patients with chronic hepatitis B. Asian Hepatitis Lamivudine Trial Group. *Hepatology* 1999;**30**:770−4.
18. Tseng TC, Liu CJ, Wang CC, Chen PJ, Lai MY, Kao JH, et al. A higher alanine aminotransferase level correlates with earlier hepatitis B e antigen seroconversion in lamivudine-treated chronic hepatitis B patients. *Liver Int* 2008;**28**:1034−41.
19. Gish RG, Lok AS, Chang TT, de Man RA, Gadano A, Sollano J, et al. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology* 2007;**133**:1437−44.
20. Yokosuka O, Takaguchi K, Fujioka S, Shindo M, Chayama K, Kobashi H, et al. Long-term use of entecavir in nucleoside-naive Japanese patients with chronic hepatitis B infection. *J Hepatol* 2010;**52**:791−9.
21. Yuen MF, Seto WK, Fung J, Wong DK, Yuen JC, Lai CL. Three years of continuous entecavir therapy in treatment-naive chronic hepatitis B patients: viral suppression, viral resistance, and clinical safety. *Am J Gastroenterol* 2011;**106**:1264−71.
22. Zoutendijk R, Reijnders JG, Brown A, Zoulim F, Mutimer D, Deterding K, et al. Entecavir treatment for chronic hepatitis B: adaptation is not needed for the majority of naive patients with a partial virological response. *Hepatology* 2011;**54**:443−51.
23. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016;**10**:1−98.
24. Leung NW, Lai CL, Chang TT, Guan R, Lee CM, Ng KY, et al. Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology* 2001;**33**:1527−32.
25. Wang CC, Tseng KC, Peng CY, Hsieh TY, Lin CL, Su TH, et al. Viral load and alanine aminotransferase correlate with serologic response in chronic hepatitis B patients treated with entecavir. *J Gastroenterol Hepatol* 2013; **28**:46−50.
26. Peng CY, Hsieh TC, Hsieh TY, Tseng KC, Lin CL, Su TH, et al. Hbv-DNA level at 6 months of entecavir treatment predicts Hbeag loss in Hbeag-positive chronic hepatitis B patients. *J Formos Med Assoc* 2015; **114**:308−13.
27. Ono A, Suzuki F, Kawamura Y, Sezaki H, Hosaka T, Akuta N, et al. Long-term continuous entecavir therapy in nucleos(t)ide-naive chronic hepatitis B patients. *J Hepatol* 2012;**57**:508−14.
28. Wong GL, Wong VW, Chan HY, Tse PC, Wong J, Chim AM, et al. Undetectable Hbv DNA at month 12 of entecavir treatment predicts maintained viral suppression and Hbeag-seroconversion in chronic hepatitis B patients at 3 years. *Aliment Pharmacol Ther* 2012;**35**:1326−35.
29. van Bommel F, Bartens A, Mysickova A, Hofmann J, Kruger DH, Berg T, et al. Serum hepatitis B virus RNA levels as an early predictor of hepatitis B envelope antigen seroconversion during treatment with polymerase inhibitors. *Hepatology* 2015;**61**:66−76.
30. Lai M, Hyatt BJ, Nasser I, Curry M, Afdhal NH. The clinical significance of persistently normal ALT in chronic hepatitis B infection. *J Hepatol* 2007;**47**:760−7.
31. Fung J, Wong DK, Seto WK, Kopaniszen M, Lai CL, Yuen MF. Hepatitis B surface antigen seroclearance: relationship to hepatitis B e-antigen seroclearance and hepatitis B e-antigen-negative hepatitis. *Am J Gastroenterol* 2014;**109**:1764−70.
32. Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, et al. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. *Gastroenterology* 2011;**140**:132−43.
33. Reijnders JG, Rijckborst V, Sonneveld MJ, Scherbeijn SM, Boucher CA, Hansen BE, et al. Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir. *J Hepatol* 2011;**54**:449−54.
34. Gish RG, Chang TT, Lai CL, de Man RA, Gadano A, Llamoso C, et al. Quantitative hepatitis B surface antigen analysis in hepatitis B e antigen-positive nucleoside-naive patients treated with entecavir. *Antivir Ther* 2013;**18**:691−8.
35. Fung J, Lai CL, Young J, Wong DK, Yuen J, Seto WK, et al. Quantitative hepatitis B surface antigen levels in patients with chronic hepatitis B after 2 years of entecavir treatment. *Am J Gastroenterol* 2011;**106**:1766−73.
36. Terrault NA, Bzowej NH, Chang KM, Hwang JP, Jonas MM, Murad MH. AASLD guidelines for treatment of chronic hepatitis B. *Hepatology* 2016; **63**:261−83.