Table 1 Baseline characteristics of HBsAg-positive patients

Parameters	Total patients	HBeAg-positive patients*
Total patients	424	120
Gender (male/female)	239/185	68/52
Age (average ± SD) (years)	40.6 ± 14.0	34.3 ± 13.1
HBeAg status (positive/negative)	183/241	120/0
HBV DNA level (average ± SD) (log copies/mL)	5.5 ± 1.9	7.1 ± 1.2
ALT level (average ± SD) (IU/L)	70.4 ± 79.3	93.2 ± 96.2
PLT number (average ± SD) ($\times 10^4$ number/μL)	20.8 ± 6.6	20.2 ± 6.2
Follow-up (average ± SD) (years)	5.4 ± 5.1	10.0 ± 5.5
Genotype A/B/C/D/not determined	6/30/250/0/138	2/6/110/2
HBsAg level (average ± SD) (log IU/mL)	3.42 ± 1.15	4.02 ± 0.98
Antiviral drugs	48	34
HCC occurrence	18	4

ALT, alanine aminotransferase; PLT, the number of platelets; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; *120 patients of HBeAg-positive patients were followed for more than 5 years.

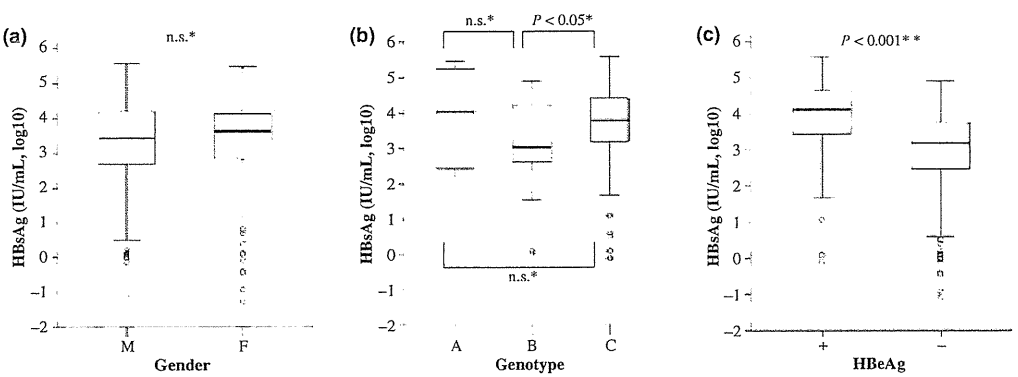

Fig. 1 The association between HBsAg level (log IU/mL) and (a) gender, (b) HBV genotype and (c) HBeAg status. There was no significant difference between HBsAg and gender (P = 0.146, unpaired t-test). In contrast, compared with genotype B, the level of HBsAg in the patients with HBV genotype C was significantly different ($P < 0.05$, unpaired t-test). There was a significant difference according to the positive or negative status of HBeAg ($P < 0.001$, unpaired t-test). HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

raphy, angiography and/or magnetic resonance imaging. For all of the patients who were suspected as HCC by image analysis, the diagnosis of HCC was confirmed by pathological analysis. Univariate analysis revealed that age [compared with young patients: odds ratio (OR) = 1.10, 95% confidence interval (CI) = 1.01–1.11], number of PLTs (compared with patients of low PLTs: OR = 0.98, 95% CI = 0.97–0.99) and HBV DNA level (compared with patients of low HBV DNA levels: OR = 1.32, 95% CI = 1.05–1.67) at baseline were predictive factors for HCC occurrence, not HBsAg titre (compared with patients of low HBsAg levels: OR = 0.79, 95% CI = 0.56–1.10). Multivariate analysis revealed that age (compared with young patients: OR = 1.07, 95% CI = 1.03–1.11) and number of PLTs (compared with patients of low PLTs: OR = 0.99, 95% CI = 0.98–0.99) at baseline were predictive factors for HCC occurrence.

The effect of serial change of HBsAg in HBeAg-positive HBV carriers

The baseline clinical characteristics of 120 HBeAg-positive carriers are shown in Table 1, and the level of HBsAg were

© 2011 Blackwell Publishing Ltd

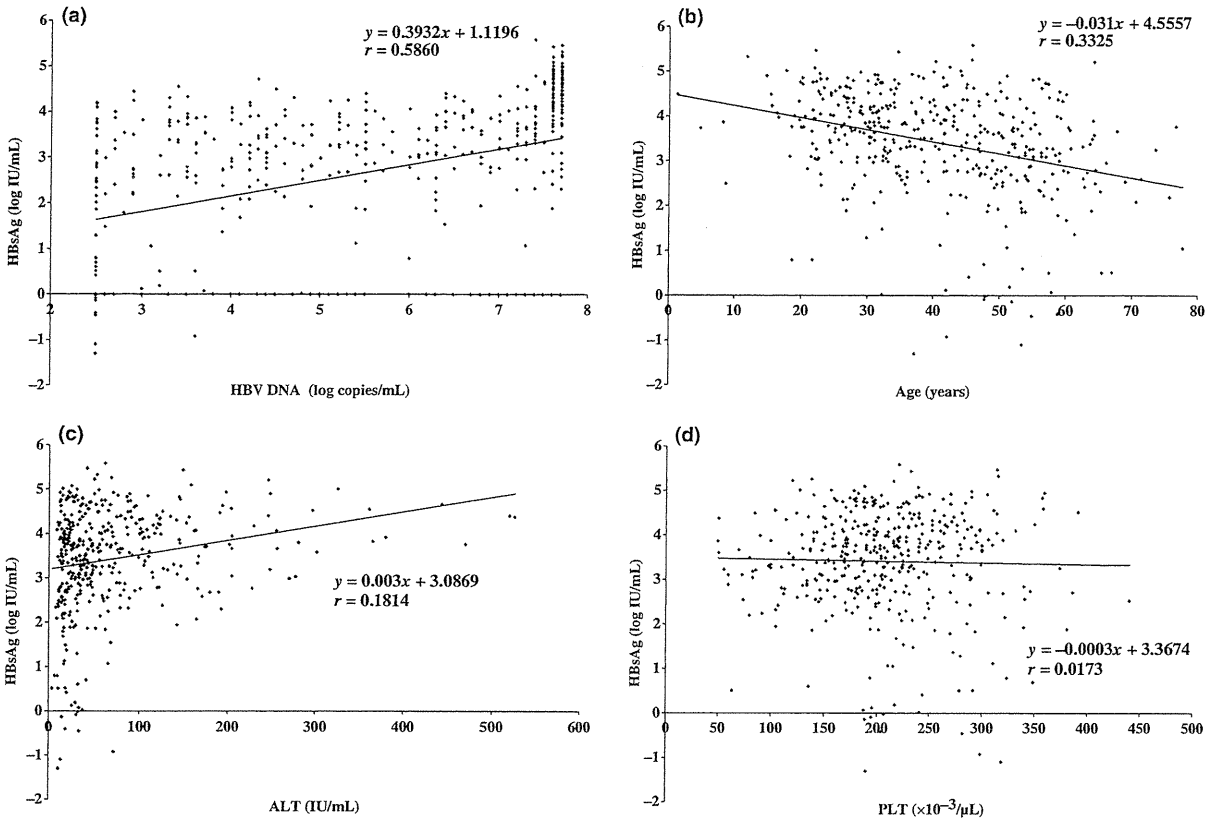

Fig. 2 Correlation between serum HBsAg levels and other clinical markers. (a) HBV DNA levels (Pearson's product moment correlation coefficient analysis; $r = 0.586$, $P < 0.001$), (b) age ($r = 0.333$, $P < 0.001$), (c) serum ALT levels ($r = 0.181$, $P < 0.001$), (d) the number of platelets ($r = 0.017$, $P = 0.347$). HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

evaluated every year. The annualized rate of occurrence of HCC was 0.41% and one patient died of HCC and liver failure, although the death caused from liver failure without HCC was not observed. Seroconversion of HBeAg occurred during the follow-up of 61 patients (average age 32.8 ± 12.5 years). Antiviral drugs were used in 34 patients during follow-up. Of the 120 HBe-positive patients, 34 did not show HBeAg seroconversion and were not given antiviral drugs. Although HBsAg in these patients tended to decrease gradually year-by-year, there was a significant difference only after 5, 9 and 10 years from entry (Mann–Whitney U test, $P < 0.05$) (Fig. 3a). After the start of antiviral drugs, the level of HBsAg showed a significant decrease with statistical difference (paired t-test, $P = 0.035$) (Fig. 3b). Interestingly, in the patients in whom HBeAg seroconversion occurred during the natural course, the changes in HBsAg levels per year showed a significant decrease 2 years before seroconversion compared with the previous year (paired t-test, $P < 0.05$) (Fig. 3c). In addition, the levels of HBsAg showed a significant decrease after HBeAg seroconversion (paired t-test, $P = 0.035$).

The serial change in HBsAg levels before and after HBeAg seroconversion compared with the age- and sex-matched controls

Seroconversion of HBeAg has been reported to be influenced by gender [13], and in addition, from our analysis, the levels of HBsAg showed a gradual decrease. Therefore, we performed a control study, matched with age and sex, between two groups with and without HBeAg seroconversion during follow-up period. We extracted the patients who were matched for age and sex and compared 18 who did not show seroconversion through the course to 21 who showed seroconversion spontaneously, without treatment with a nucleotide analogue or interferon (IFN). A significant difference was not found in clinical background in this control study (Table 2). The changes in HBsAg levels in the groups with and without HBeAg seroconversion are shown in Fig. 4a. The level of HBsAg in the two groups gradually decreased over time, but the decline of HBsAg in the patients without HBeAg seroconversion was not significant over the course of a year. On the contrary, in the patients in whom

© 2011 Blackwell Publishing Ltd

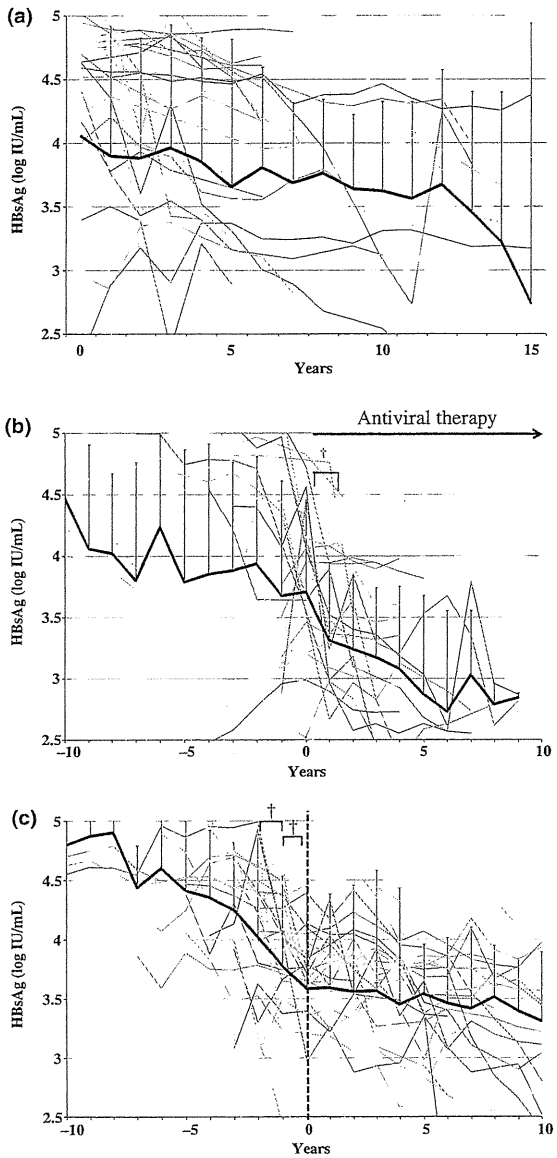

Fig. 3 The serial change of HBsAg level in HBeAg-positive patients with (a) no HBeAg seroconversion and no use of antiviral drugs (n = 34), (b) the use of antiviral drugs (n = 32), (c) HBeAg seroconversion during the follow-up period (n = 35). (a) Compared with the level at entry, a continuous decrease was not observed, although there was a significant difference only after 5, 9 and 10 years from entry ($P < 0.05$, Mann–Whitney U test). (b) The level of HBsAg showed a statistically significant decrease after commencement of antiviral therapy (†$P < 0.05$, paired t-test). (c) HBsAg showed a significant decrease at 2 years before seroconversion (†$P < 0.05$, paired t-test). HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

HBeAg seroconversion occurred during the natural course, the changes in HBsAg levels per year showed a significant decrease 2 years before seroconversion compared with the previous year (paired t-test, $P < 0.05$) (Fig. 4a). Next, we compared the difference in HBsAg levels between the two groups. There was a significant difference between the two groups 1 year before, on and after HBeAg seroconversion (unpaired t-test, $P < 0.05$). The HBeAg titre did not differ significantly between the two groups before seroconversion (Fig. 4b). The levels of HBcrAg showed an obvious decrease after HBeAg seroconversion, but before this, there was no significant decrease in the patients with or without HBeAg seroconversion (Fig. 4c).

DISCUSSION

The natural history of CHB is typically regarded as consisting of some phases that have been classified mainly by serum ALT levels, HBeAg and HBsAg serostatus, and HBV DNA levels. The understanding of the natural history of CHB has been facilitated by the improved sensitivity of serological and virological makers. HBsAg was the first HBV-encoded protein to be discovered [14]. Detection of HBsAg in serum is the fundamental diagnostic marker of HBV infection. HBsAg is a component of the Dane particle, which contains the viral genome, and subviral particles, but the mechanisms that regulate the production of HBsAg, particularly the subviral particles, are largely unclear [15]. Excess HBsAg may serve as a possible mechanism for evading the host immune responses, in that anti-HBs antibodies provide protective immunity [16]. One of our aims was to determine the change of HBsAg levels during the natural history of infection. Thompson *et al.* [17] reported that the level of HBsAg was related to the HBeAg status, as seen here. Some studies reported that positive correlations have been observed between the level of HBsAg and serum HBV DNA [18,19], again as seen here, but another study reported no such correlation [20]. Regarding the relationship with age, Kohmoto *et al.* [19] reported that the level of HBsAg was negatively correlated with the patient's age. We also found a weak and negative correlation between the levels of HBsAg and age, but in the analysis only of HBeAg-positive patients who did not show HBeAg seroconversion and who were not treated with antiviral drugs during follow-up period, the serial change of HBsAg levels showed no obvious decrease. Thus, the patients' age might have a direct effect on the level of HBsAg, but clinical events such as HBeAg seroconversion or the treatment of antiviral drugs might have a greater impact. Some studies reported that the level of HBsAg showed the difference among HBV genotypes [20,21]. In fact, we showed that the level of HBsAg in the patients with HBV genotype B was less than genotype C, but a limitation of this study was that most HBV carriers in our analysis were infected with genotype C of HBV. Therefore, we could not clarify the difference of HBsAg level among genotypes during HBeAg seroconversion.

In this study, a high HBsAg level was not related to the high incidence of HCC. In contrast, age, PLTs and the HBV

© 2011 Blackwell Publishing Ltd

Table 2 Clinical characteristics of the patients with age- and sex-matched controls

Parameters	Seroconversion (+)	Seroconversion (−)	*P*
Patient numbers	21	18	
Gender (male/female)	8/13	11/7	n.s.
Median Age (years)	30.0 (24–55)	31.0 (18–46)	n.s.
HBV Genotype (A/B/C)	1/3/17	0/0/26	n.s.
HBV DNA level (average ± SD) (log copies/mL)	7.4 ± 0.6	7.4 ± 0.5	n.s.
HBsAg level (average ± SD) (log IU/mL)	4.70 ± 4.79	4.70 ± 4.54	n.s.

HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

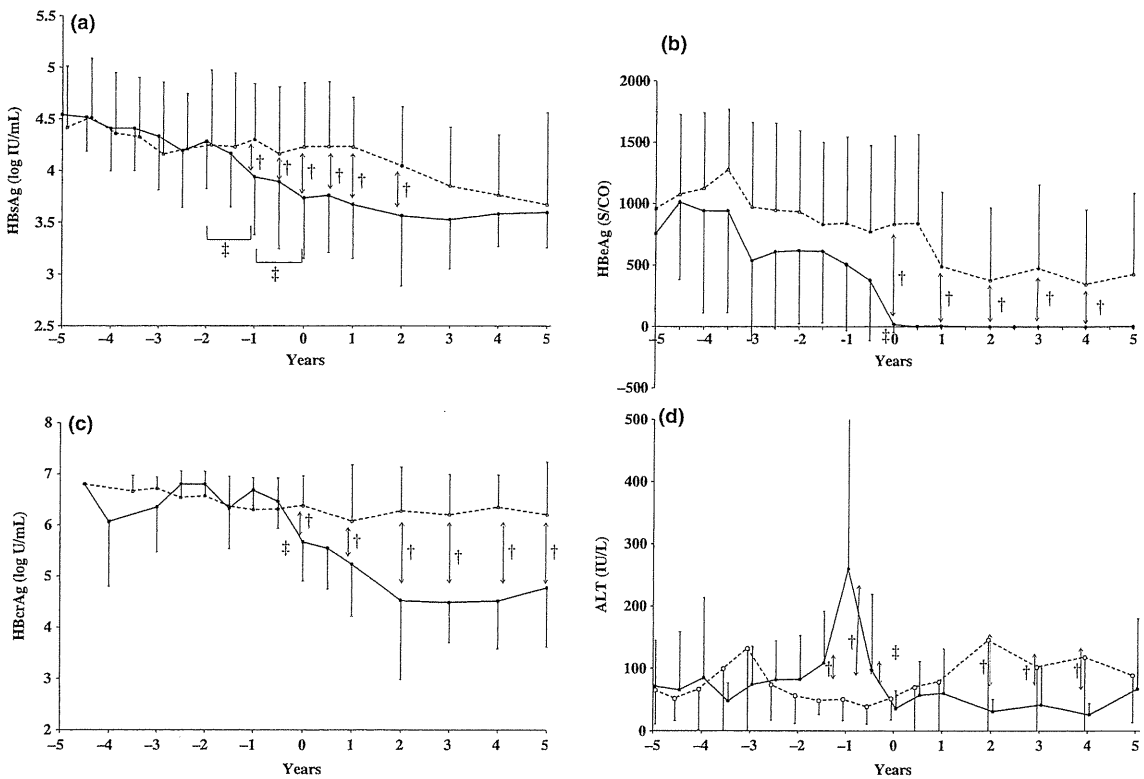

Fig. 4 Comparison of serial changes in (a) HBsAg, (b) HBeAg, (c) HBcrAg level and (d) ALT during 10 years around HBeAg seroconversion with those of age-and sex-matched controls without seroconversion. The group with HBeAg seroconversion (n = 21) is shown with closed circles and that without seroconversion (n = 18) with open circles. The time point of seroconversion was designated as 0 year. (a) There was no significant difference between the two groups until 2 years before seroconversion, but 1 year before, on and after HBeAg seroconversion, there were significant differences between the two groups (†$P < 0.05$, unpaired t-test). In the patients with seroconversion, HBsAg showed a significant decrease 2 years before seroconversion (‡$P < 0.05$, paired t-test). (b) The HBeAg level differed significantly between the two groups after HBeAg seroconversion (†$P < 0.05$, unpaired t-test, ‡$P < 0.05$, paired t-test). (c) The core-related antigen of HBV (HBcrAg) level showed a significant difference between the two groups after seroconversion (†$P < 0.05$, unpaired t-test, ‡$P < 0.05$, paired t-test). (d) The level of ALT in the patients with HBeAg seroconversion showed a significant increase half a year before HBeAg seroconversion. The ALT level differed significantly between the two groups before and after HBeAg seroconversion (†$P < 0.05$, unpaired t-test, ‡$P < 0.05$, paired t-test). HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

© 2011 Blackwell Publishing Ltd

DNA level at baseline showed a strong relation with tumour development, as we have reported previously [22], although HBsAg seroclearance at age <50 years was reported to be associated with a lower risk for the development of HCC [23]; that is, there was a difference in clinical outcome between the low level of HBsAg and its seroclearance. Therefore, the final goal of therapy for HBV carriers might be set as HBsAg seroclearance.

It is well known that seroconversion of HBeAg to its antibody is associated with a decrease in serum HBV DNA to low or undetectable levels, clinical remission and an improvement in hepatic inflammatory activity. Some studies reported that high pretreatment ALT levels and low serum HBV DNA levels were independently associated with an increased rate of seroconversion after treatment with either IFN [24–26] or nucleoside/nucleotide analogues (NA) [27]. Further analysis also showed that factors such as viral genotype [28], quantitative HBeAg [29] and active histological disease [30] also may be important predictors of seroconversion after treatment with IFN or NA [31]. The quantitative monitoring of HBsAg titre also has been suggested as a predictor of treatment response, especially for IFN-based therapies in chronic HBV infection [32]. In our study, HBsAg decreased significantly, compared with a group without seroconversion, for 2 years prior to seroconversion. In the age-and sex-controlled study, we analyzed the relationship between the level of HBsAg at 140 time points and the occurrence within 2 years of HBeAg seroconversion. In 10 of 19 patients (52.6%), when the level of HBsAg showed more than 50% decrease compared with the previous year, HBeAg seroconversion occurred within 2 years, which differed significantly (chi-square test, $P = 0.003$). Thus, this study suggested that quantitative measurement of the HBsAg titre might clinically be useful and that it becomes possible to build a treatment strategy by predicting whether seroconversion will occur. The reason why the level of HBsAg decreased before HBeAg seroconversion with preceding the decrease of HBeAg titre or HBcrAg level remains unclear. The current findings speculated us that it was most likely due to the integration of HBV into the host genome that potentially provides a separate template for the production of HBsAg or the cytokine-dependent modification of viral replication pathways [21].

In a recent report, there was no obvious decline in HBsAg at the time of HBeAg seroconversion, compared with the decline of HBV DNA, from the evaluation of HBsAg only at two points before seroconversion [12]. By the evaluation of serial changes in HBsAg levels before seroconversion, in addition to the difference of HBV genotype or race, our study might show differences from that finding.

HBV covalently closed circular DNA (cccDNA) is important for virus replication and impacts on clinical outcome [33], and HBsAg has been evaluated recently as a surrogate marker of cccDNA [34,35]. Because liver biopsy was not a routine procedure, we did not measure cccDNA directly in liver. Further studies are required to clarify the precise significance of HBsAg levels because a direct association between HBV cccDNA levels in liver and HBsAg levels in serum remains to be shown.

In conclusion, the titre of HBsAg is a new marker related to HBV replication and its serial measurement possibly may be a predictive factor for HBeAg seroconversion.

REFERENCES

1 Lok AS, McMahon BJ. Chronic hepatitis B. AASLD practice guidelines. *Hepatology* 2007; 45: 507–539.

2 Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. *N Engl J Med* 2004; 350: 1118–1129.

3 Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; 45: 1056–1075.

4 Liaw YF. Prevention and surveillance of hepatitis B virus-related hepatocellular carcinoma. *Semin Liver Dis* 2005; 25: 40–47.

5 Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis e antigen to antibody in chronic type B hepatitis. *Ann Intern Med* 1981; 94: 744–748.

6 Fattovich G, Rugge M, Brollo L *et al.* Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. *Hepatology* 1986; 6: 167–172.

7 Yuen MF, Yuan HJ, Wong DK *et al.* Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. *Gut* 2005; 54: 1610–1614.

8 Hui CK, Leung N, Shek TW *et al.* Sustained disease remission reduction in fibrosis progression in chronic hepatitis B Chinese patients. *Hepatology* 2007; 46: 690–698.

9 Ito K, Arai M, Imazeki F *et al.* Risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Scand J Gastroenterol* 2010; 45: 243–249.

10 Bekku D, Arai M, Imazeki F *et al.* Long-term follow-up in patients with hepatitis B e antigen negative chronic hepatitis B. *J Gastroenterol Hepatol* 2011; 26: 122–128.

11 Chan HL, Wong VW, Chim AM, Chan HY, Wong GL, Sung JJ. Serum HBsAg quantification to predict response to peginterferon therapy of e antigen positive chronic hepatitis B. *Aliment Pharmacol Ther* 2010; 32: 1323–1331.

12 Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. *Hepatology* 2010; 52: 1232–1241.

13 Chu CM, Liaw YF. Predictive factors for reactivation of hepatitis B following hepatitis B e antigen seroconversion in chronic hepatitis B. *Gastroenterology* 2007; 133: 1458–1465.

© 2011 Blackwell Publishing Ltd

14 Blumberg BS, Alter HJ, Visnich S. A "new" antigen in leukemia sera. *JAMA* 1965; 191: 541–546.

15 Nguyen DH, Hu J. Reverse transcriptase- and RNA packaging signal-dependent incorporation of APOBEC3G into hepatitis B virus nucleocapsids. *J Virol* 2008; 82: 6852–6861.

16 Op den Brouw ML, Binda RS, Geijtenbeek TB, Janssen HL, Woltman AM. The mannose receptor acts as hepatitis B virus surface antigen receptor mediating interaction with intrahepatic dendritic cells. *Virology* 2009; 393: 84–90.

17 Thompson AJ, Nguyen T, Iser D *et al.* Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. *Hepatology* 2010; 51: 1933–1944.

18 Deguchi M, Yamashita N, Kagita M *et al.* Quantitation of hepatitis B surface antigen by an automated chemiluminescent microparticle immunoassay. *J Virol Methods* 2004; 115: 217–222.

19 Kohmoto M, Enomoto M, Tamori A *et al.* Quantitative detection of hepatitis B surface antigen by chemiluminescent microparticle immunoassay during lamivudine treatment of chronic hepatitis B virus carriers. *J Med Virol* 2005; 75: 235–239.

20 Nguyen T, Thompson AJ, Bowden S *et al.* Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. *J Hepatol* 2010; 52: 508–513.

21 Jaroszewicz J, Calle Serrano B, Wursthorn K *et al.* Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus. *J Hepatol* 2010; 52: 514–522.

22 Wiegand J, Wedemeyer H, Finger A *et al.* A decline in hepatitis B virus surface antigen (HBsAg) predicts clearance, but does not correlate with quantitative HBeAg or HBV DNA levels. *Antivir Ther* 2008; 13: 547–554.

23 Yuen MF, Wong DK, Fung J *et al.* HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. *Gastroenterology* 2008; 135: 1192–1199.

24 Perrillo RP, Schiff ER, Davis GL *et al.* A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. *N Engl J Med* 1990; 323: 295–301.

25 Lok AS, Wu PC, Lai CL *et al.* A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. *Gastroenterology* 1992; 102: 2091–2097.

26 Hoofnagle JH, Peters M, Mullen KD *et al.* Randomized, controlled trial of recombinant human interferon in patients with chronic hepatitis B. *Gastroenterology* 1988; 95: 1318–1325.

27 Chien RN, Liaw YF, Akins M. Pretherapy alanine transaminase level as a determinant for hepatitis B e seroconversion during lamivudine therapy in patients with chronic hepatitis B. Asian hepatitis lamivudine trial group. *Hepatology* 1999; 30: 770–774.

28 Yuen MF, Fung SK, Tanaka Y *et al.* Longitudinal study of hepatitis activity and viral replication before and after HBeAg seroconversion in chronic hepatitis B patients infected with genotypes B and C. *J Clin Microbiol* 2004; 42: 5036–5040.

29 Fried MW, Piratvisuth T, Lau GK *et al.* HBeAg and hepatitis B virus DNA as outcome predictors during therapy with peginterferon alfa-2a for HBeAg-positive chronic hepatitis B. *Hepatology* 2008; 47: 428–434.

30 Perrillo RP, Lai CL, Liaw YF *et al.* Predictors of HBeAg loss after lamivudine treatment for chronic hepatitis B. *Hepatology* 2002; 36: 186–194.

31 Liu CJ, Chen PJ, Lai MY *et al.* Viral factors correlate with hepatitis B e antigen seroconverson in patients with chronic hepatitis B. *Liver Int* 2006; 26: 949–955.

32 Rijckborst V, Hansen BE, Cakaloglu Y *et al.* Early on-treatment prediction of response to peginterferon alfa-2a for HBeAg-negative chronic hepatitis B using HBsAg and HBV DNA levels. *Hepatology* 2010; 52: 454–461.

33 Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. *J Hepatol* 2005; 42: 302–308.

34 Werle-Lapostolle B, Bowden S, Locarnini S *et al.* Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004; 126: 1750–1758.

35 Chan HL, Wong VW, Tse AM *et al.* Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. *Clin Gastroenterol Hepatol* 2007; 5: 1462–1468.

© 2011 Blackwell Publishing Ltd

Scandinavian Journal of Gastroenterology, 2011; 46: 1111–1117

informa
healthcare

ORIGINAL ARTICLE

Emergence of entecavir-resistant mutations in nucleos(t)ide-naive Japanese patients infected with hepatitis B virus: Virological breakthrough is also dependent on adherence to medication

HIDEHIRO KAMEZAKI, TATSUO KANDA, SHUANG WU, SHINGO NAKAMOTO, MAKOTO ARAI, HITOSHI MARUYAMA, KEIICHI FUJIWARA, FUMIO IMAZEKI & OSAMU YOKOSUKA

Department of Medicine and Clinical Oncology, Chiba University, Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan

Scand J Gastroenterol Downloaded from informahealthcare.com by Chiba University Iohana M Library on 02/27/12
For personal use only.

Abstract

Objective. Currently, five nucleos(t)ide analogues (NUCs) are available for the treatment of chronic hepatitis B in the world. We examined the prevalence of hepatitis B virus (HBV) DNA and alanine aminotransferase normalization in patients receiving entecavir (ETV) and the frequency of ETV-resistant mutations during an approximately 27-month use of ETV in chronic hepatitis B patients in an urban hospital in Japan. ***Materials and methods.*** A retrospective analysis of 81 NUC-naive chronic hepatitis B patients who received 0.5 mg of ETV daily was performed. HBV DNA was measured and sequence analysis of HBV DNA was performed in virological breakthrough patients. ***Results.*** Hepatitis B e antigen (HBeAg)-positive patients with HBV DNA 5.0–7.0 log IU/mL group and all HBeAg-negative patients achieved serum HBV DNA negativity by 12 months. Four patients experienced virological breakthrough during ETV therapy. Two patients had no genotypic mutations, and medical interviews revealed that they had poor adherence to ETV. ***Conclusions.*** We found that some of the HBV virological breakthroughs during ETV treatment were related to poor adherence to medication, highlighting that clinicians should pay attention to the emergence of resistant mutants as well as adherence to ETV.

Key Words: *Adherence, entecavir, HBV, resistant mutants, virological breakthrough*

Introduction

Two billion people have been exposed to hepatitis B virus (HBV), and 350–400 million people remain chronically infected worldwide. In Japan, the prevalence of HBV carriers is estimated at ~1% of the population, but HBV is one of the major health issues because it leads to acute hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1–5].

Recent studies have shown that the serum HBV DNA level is one of the most potent risk factors for the development of cirrhosis or HCC, and it seems that suppressing the serum HBV viral load is essential for improving the prognosis of HBV carriers [6,7]. Currently, there are five approved nucleos(t)ide analogues (NUCs) for the treatment of chronic hepatitis B [8]. At present, the Japanese national health insurance system approves entecavir (ETV) as the first-line therapy for chronic hepatitis B, although some patients are treated with standard interferon-alfa. ETV is an NUC belonging to a new subgroup, cyclopentane [9], and has been shown to be highly effective in suppressing HBV replication to an undetectable level and normalizing alanine aminotransferase (ALT), although NUCs do not eradicate the virus. Most patients therefore require long durations of treatment, but prolonged treatment is associated with increasing rates of drug resistance. There was

Correspondence: Tatsuo Kanda, MD, PhD, Department of Medicine and Clinical Oncology, Chiba University, Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. Tel: +81-43-226-2083. Fax: +81-43-226-2088. E-mail: kandat-cib@umin.ac.jp

(Received 1 March 2011; accepted 25 April 2011)

ISSN 0036-5521 print/ISSN 1502-7708 online © 2011 Informa Healthcare
DOI: 10.3109/00365521.2011.584898

RIGHTSLINK

Scand J Gastroenterol Downloaded from informahealthcare.com by Chiba University Iohana M Library on 02/27/12
For personal use only.

also a report that the 3-year cumulative probability of resistance was 1.7% for 0.5 mg/day ETV therapy in NUC-naive Japanese patients [10].

In the present study, we examined the prevalence of HBV DNA and ALT normalization in patients receiving ETV as well as the frequency of ETV-resistant mutations during an approximately 27-month use of ETV in chronic hepatitis B patients in an urban hospital in Japan. We found a relationship between some of the HBV virological breakthroughs during ETV treatment with poor adherence to medication, and clinicians need to focus on the possible emergence of resistant mutants as well as the adherence to ETV.

Patients and methods

Patients

A retrospective analysis of NUC-naive chronic hepatitis B patients ($n = 81$) receiving 0.5 mg of ETV daily at Chiba University Hospital between May 2003 and December 2009 was performed. The patients were divided into three groups based on their HBV DNA level just before starting ETV according to the Japanese Ministry of Health, Labor and Welfare Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B [11]: HBV DNA <5.0 log IU/mL (n = 14, 17.3%), 5.0–7.0 log IU/mL (n = 30, 37.0%), and >7.0 log IU/mL (n = 37, 45.7%) (Table I). All patients had serum hepatitis B surface antigen (HBsAg) detectable for at least 6 months, regardless of their hepatitis B e antigen (HBeAg) status. They were negative for hepatitis C virus and HIV antibodies. They were followed up at least every 3 months to examine physical status and monitor liver biochemistry and virology. Adherence to ETV was assessed during each visit to the clinic. This study was approved by the Ethics Committee of Chiba University, Graduate School of Medicine.

Serological examination

All clinical laboratory tests including hematological data, biochemical data, and HBV serologies were performed at the Central Laboratory of Chiba University Hospital. HBsAg, HBeAg, and anti-HBe antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [12]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al. [13]. HBV DNA was measured by Roche Amplicor™ PCR assay (detection limits: 2.6 log IU/mL; Roche Diagnostics, Tokyo, Japan). The clinical efficacy of ETV was assessed as the proportion of patients achieving HBV DNA negativity, which is defined as an HBV DNA level of <2.6 log IU/mL and that of patients achieving ALT normalization (normal range: 8–42 IU/L). Using generally available biological parameters, the aspartate aminotransferase (AST) to platelets ratio index (APRI), and serum liver fibrosis score, was calculated according to the following formula: AST/35 × 100/platelet count [14,15].

Sequence analysis of HBV DNA

Sera obtained from patients were stored at -20°C until analysis. HBV polymerase/reverse transcriptase (RT) substitutions were analyzed for all patients who had experienced virological breakthrough (>1 log IU/mL

Table I. Baseline characteristics of patients.

	HBV DNA (log IU/mL)				HBeAg	
	Total	<5.0	5.0–7.0	>7.0	Positive	Negative
Number of cases	81	14	30	37	40	41
Age (years)	49.7 ± 12.2	55.7 ± 13.1[a]	50.9 ± 11.3	46.4 ± 11.8[a]	44.7 ± 10.3[e]	54.5 ± 12.0[e]
Gender (male/female)	55/26	7/7	22/8	26/11	28/12	27/14
HBeAg (+/-)	40/41	0/14[b]	11/19[b]	29/8[b]		
Genotype (B/C/N.D.)	2/33/46	0/2/12	2/14/14	0/17/20	1/23/16	1/10/30
ALT (IU/L)	169 ± 186	85.1 ± 115	185 ± 192	189 ± 197	179 ± 190	159 ± 184
AST (IU/L)	108 ± 113	62.6 ± 87.5	128 ± 149	109 ± 81.1	104 ± 83.6	111 ± 136
Platelets ($\times 10^4/mm^3$)	16.2 ± 8.2	21.4 ± 15.5[c]	14.9 ± 5.0[c]	15.5 ± 5.9	15.7 ± 5.5	16.8 ± 10.2
APRI (<0.50/0.50–1.50/>1.50)	14/35/32	7/5/2[d]	4/11/15[d]	3/19/15[d]	2/24/14[f]	12/11/18[f]

Abbreviations: ALT = alanine aminotransferase; APRI = AST to platelets ratio index; AST = aspartate aminotransferase; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; N.D = not determined; SD = standard deviation. APRI, AST/35 × 100/platelet count [15];
Data are expressed as mean ± SD. [a]$p = 0.036$ between <5.0 group and >7.0 group; [b]$p < 0.001$ among three groups; [c]$p = 0.041$ between <5.0 group and 5.0–7.0 group; [d]$p = 0.0049$ among three groups; [e]$p < 0.001$ between HBeAg-positive and HBeAg-negative groups; [f]$p = 0.0020$ between HBeAg-positive and HBeAg-negative groups.

RIGHTSLINK

increase in serum HBV DNA level from nadir) using ETV on-treatment sera. Briefly, HBV DNA was extracted from 100 μL of sera using SepaGene (Sanko Junyaku, Tokyo, Japan). Nested PCR was performed using LA Taq polymerase (Takara Bio, Otsu, Shiga, Japan) under the following conditions: 5-min denaturation at 94°C, 35 cycles with denaturation at 94°C for 40 s, annealing at 58°C for 1 min, and extension at 68°C for 1.5 min [5]. An 862 base-pair fragment (nt 242–1103) containing the polymerase RT domain was amplified on PCR Thermal Cycler Dice Model TP600 (Takara Bio). The primers for the second round of PCR were 5′-CAG AGT CTA GAC TCG TGG-3′ (sense, nt 242–258) and 5′-GGC GAG AAA GTG AAAGCC-3′ (antisense, nt 1103–1086). The PCR product was sequenced using the primers: 5′-TGG CTC AGT TTA CTAGTG CC -3′ (nt 668–687), 5′-GGC ACT AGT AAA CTGAGC CA-3′ (nt 687–668), and the primers for the second round of PCR. To prepare the sequence template, PCR products were treated with ExoSAP-ITR (Affymetrix, Inc., Santa Clara, CA, USA) and then sequenced using a BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Tokyo, Japan). Sequences were analyzed using Applied Biosystems 3730 × 1 (Life Technologies) [16].

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 for Windows™ 7. Continuous variables were expressed as mean ± standard deviation and were compared by one-factor analysis of variance. Categorical variables were compared by Chi-square test. Baseline was taken as the date when the first dose of ETV was taken. Statistical significance was considered at $p < 0.05$.

Results

Baseline characteristics of the patients

Eighty-one patients (67.9% male) were included in this study. The group with HBV DNA >7.0 log IU/mL was younger than that with HBV DNA <5.0 log IU/mL ($p = 0.036$) (Table I). Treatment duration was not significantly different among these three groups (21.4 ± 9.8, 27.8 ± 16.3, and 33.1 ± 24.6 months; $p = 0.16$). The status of HBeAg differed significantly among these three groups ($p < 0.001$). There was a statistically significant difference in platelet counts between the HBV DNA <5.0 log IU/mL and 5.0–7.0 log IU/mL groups ($p = 0.041$). This suggested that patients with an HBV DNA level <5.0 log IU/mL were likely not to progress to liver fibrosis because APRI tended to be lower and HBeAg was negative. To compare HBeAg-positive and HBeAg-negative cases, the patients were divided into two groups based on their HBeAg status just before starting ETV. The HBeAg-positive cases were younger than the HBeAg-negative cases ($p < 0.001$).

Virological response

The numbers (proportions) of patients achieving serum HBV DNA negativity at 3, 6, 12, 18, 24, and 36 months, respectively, in the three groups are shown in Figure 1. At 3 months, HBV DNA negativity of the HBV DNA <5.0 log IU/mL group was higher than that of the HBV DNA 5.0–7.0 log IU/mL group ($p = 0.0040$). At 3 and 6 months, HBV DNA negativity of the HBV DNA <5.0 log IU/mL group was higher than that of the HBV DNA >7.0 log IU/mL group ($p < 0.001$ and $p = 0.018$, respectively) (Figure 1A). At 12 and 24 months, HBV DNA negativity of the HBV DNA 5.0–7.0 log IU/mL group was higher than that of the HBV DNA >7.0 log IU/mL group ($p = 0.034$ and 0.035, respectively).

The patients with HBV DNA <5.0 log IU/mL all achieved serum HBV DNA negativity throughout the duration. The patients with HBV DNA 5.0–7.0 log IU/mL all achieved serum HBV DNA negativity by 12 months after starting treatment. Only one patient in this group experienced an increase in HBV DNA, at 29 months, but the duration of serum HBV DNA detectability was only 4 months, when he had poor adherence. From then, after discussions with his physician, he understood the importance of taking ETV for suppression of HBV replication and strictly adhered to the treatment schedule, and HBV DNA became undetectable again (Patient 3 in Table II). On the other hand, in the HBV DNA >7.0 log IU/mL group, the proportions of patients achieving serum HBV DNA negativity were 84.8%, 88.0%, 76.2%, and 69.2% at 12, 18, 24, and 36 months, respectively. In three patients of this group, HBV DNA increased at 28, 26, and 12 months, respectively (Patients 1, 2, and 4 in Table II). When we investigated the negativity of HBV DNA with or without HBeAg at baseline, HBeAg-positive patients with HBV DNA 5.0–7.0 log IU/mL group (Figure 1B) and all HBeAg-negative patients achieved serum HBV DNA negativity by 12 months (Figure 1C).

The proportions of patients achieving serum HBeAg negativity in HBeAg-positive patients with HBV DNA 5.0–7.0 log IU/mL group were 0%

Scand J Gastroenterol Downloaded from informahealthcare.com by Chiba University Iohana M Library on 02/27/12
For personal use only.

RIGHTSLINK

(0/11), 9.1% (1/11), 9.1% (1/11), 22.2% (2/9), 20% (1/5), and 25% (1/4) at 3, 6, 12, 18, 24, and 36 months, respectively. On the other hand, in the HBV DNA >7.0 log IU/mL HBeAg positive-group, the proportions of patients achieving serum HBeAg negativity were 5.1% (2/28), 11.1% (3/27), 23.1% (6/26), 30.0% (6/20), 38.9% (7/18), and 60.0% (6/10) at 3, 6, 12, 18, 24, and 36 months, respectively.

Biochemical response

At 3 months, the proportion of ALT normalization of the HBV DNA 5.0–7.0 log IU/mL group was higher than that of the HBV DNA >7.0 log IU/mL group (24/28 vs. 20/35, $p = 0.014$). When we investigated the normalization of ALT with or without HBeAg at baseline, HBeAg-positive patients seemed

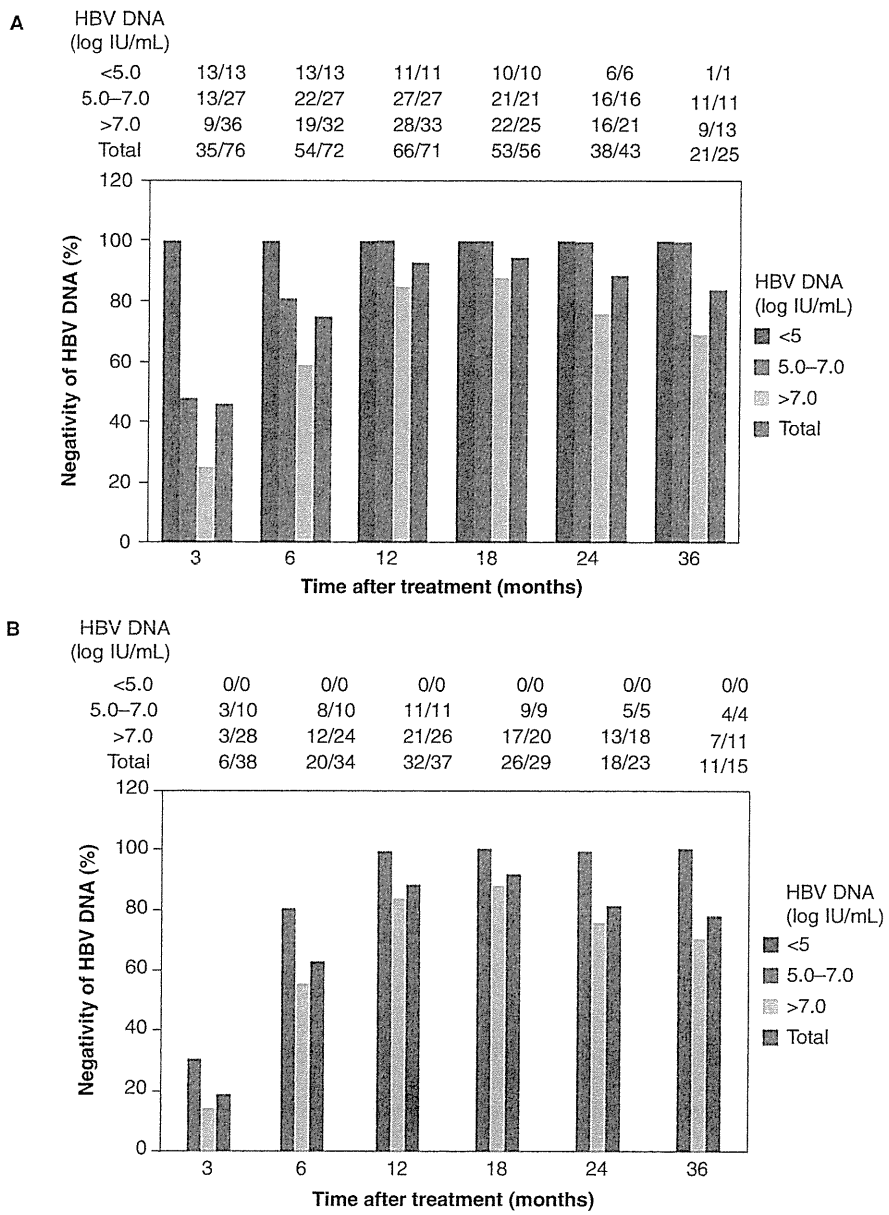

Figure 1. Negativity of HBV DNA (%) during ETV treatment. (A) A total of 81 patients (violet bars) were included in this study. Patients were divided into three groups: HBV DNA <5.0 log IU/mL group ($n = 14$, 17.3%) (blue bars), 5.0–7.0 log IU /mL group ($n = 30$, 37.0%) (red bars), and >7.0 log IU/mL group ($n = 37$, 45.7%) (green bars). (B) HBeAg-positive patients ($n = 40$). (C) HBeAg-negative patients ($n = 41$). ETV = entecavir; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus.

Scand J Gastroenterol Downloaded from informahealthcare.com by Chiba University Iohana M Library on 02/27/12
For personal use only.

RIGHTSLINK

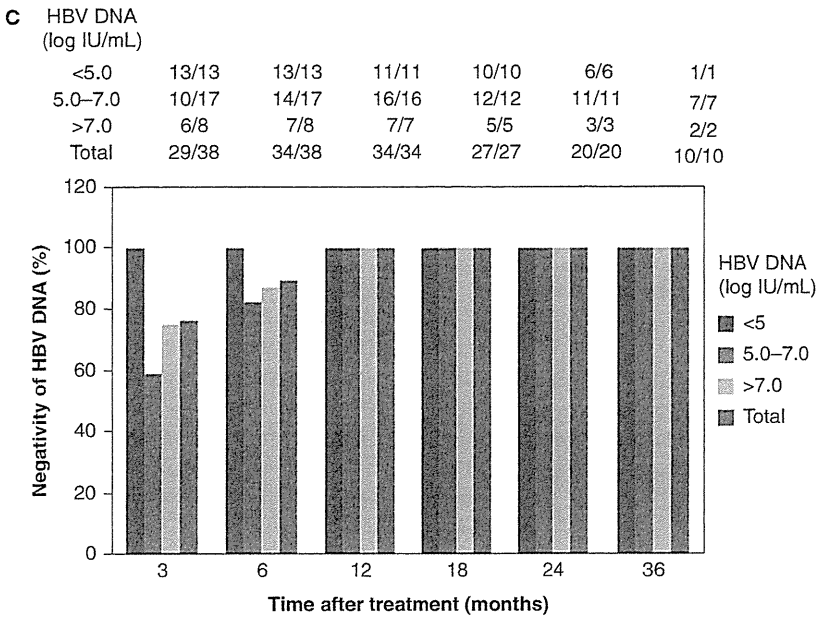

Figure 1. (*Continued*).

to have a slower response to ETV than HBeAg-negative patients (data not shown).

Sequence analysis

Four patients, one with HBV DNA 5.0–7.0 log IU/mL and three with HBV DNA >7.0 log IU/mL, experienced virological breakthrough during ETV therapy and were all analyzed to clarify whether genotypic mutations were acquired or not (Table II). Two of the three patients with HBV DNA >7.0 log IU/mL acquired genotypic mutations resistant to ETV. One had rtS202G (Patient 1 in Table III) and the other rtT184A (Patient 2 in Table III), both accompanied by lamivudine (3TC)-resistant substitutions (rtL180M and rtM204V). The other two patients had no genotypic mutations (Patients 3 and 4 in Table III). The physicians who had seen these patients at the time of virological breakthrough performed medical interviews to ask about their adherence, adding the information to their medical charts, and their poor adherence to ETV was revealed.

Discussion

In the present study, an approximately 27-month ETV treatment for NUC-naive patients resulted in an optimized outcome, in line with previous reports [10,17]. Early on-treatment virological response leads to optimized long-term outcome. We found two HBeAg-positive patients with ETV-resistant mutations. One had rtS202G (Patient 1 in Table III) and the other rtT184A (Patient 2 in Table III), accompanied by 3TC-resistant substitutions (rtL180M and rtM204V) [17–19]. These two patients discontinued ETV and then received a combination therapy of 100 mg 3TC and 10 mg adefovir-dipivoxil daily, as previously reported [20]. As ETV-resistance can be

Table II. Characteristics of patients with HBV virological breakthrough.

Patient	Age (years)/gender	HBV genotype	Baseline HBeAg	Baseline HBV DNA (log IU/mL)	Baseline ALT (IU/L)	Duration of treatment before VB (months)	Adherence to ETV
1	49/M	C	+	7.3	107	28	Good
2	57/M	C	+	>7.6	55	26	Good
3	38/M	C	+	6.9	59	29	Poor
4	46/F	C	+	>7.6	85	12	Poor

Abbreviations: ALT = alanine aminotransferase; ETV = entecavir; HBeAg = hepatitis B e antigen; HBV = hepatitis B virus; VB = virological breakthrough.

Scand J Gastroenterol Downloaded from informahealthcare.com by Chiba University Iohana M Library on 02/27/12
For personal use only.

RIGHTSLINK

Table III. Amino acid mutations of HBV DNA polymerase sequences in patients with virological breakthrough.

Amino acid No	180	184	202	204	250
Wild sequences	L	T	S	M	M
Patient 1	**M**	T	**G**	**V**	M
2	**M**	**A**	S	**V**	M
3	L	T	S	M	M
4	L	T	S	M	M

Abbreviations: HBV = hepatitis B virus; **Bold**, amino acid mutations. Numbers of top line indicate amino acid positions [5].

relatively easily diagnosed, it is important to perform both the HBV DNA and the resistance tests. The mechanism by which ETV-resistant substitutions can induce virological breakthrough during ETV therapy is largely known [21]. We also found two patients with poor adherence to ETV. Multiple drug-resistant strains were also reported, and so monitoring NUC-resistant mutations is essential. In such a case, we have to pay attention to patients with poor adherence as well [19].

In Japan, HBV genotype C is predominant. HBV genotype C is reported to be associated with delayed HBe seroconversion, more advanced liver disease, and increased probability of HCC development [22,23]. No statistical difference was observed in response to ETV among patients with different genotypes [24], although HBV genotype A and B patients were reported to respond to standard interferon-alfa better than HBV genotype C and D patients [23]. In this study, HBeAg-negative patients achieved serum HBV DNA negativity by 12 months. On the other hand, HBeAg-positive patients tended to have poor response to ETV. One HBeAg-negative patient of the total 81 patients became negative for HBsAg (data not shown). These observations might suggest important pathogenic differences in HBV genotypes.

It is well known that non-adherence results in non-control of other common diseases such as diabetes mellitus [25] and hypertension [26]. Poor medication adherence among HIV-infected adults leads to neuropsychological dysfunction [27] as well as increase of HIV RNA [28]. Recently, Ha et al. [29] also reported that medication non-adherence was likely to be a more important contributor to treatment failure than antiviral resistance, especially with new anti-HBV agents such as ETV and tenofovir. Although the number of patients in our study is small, our results highlight the importance of making efforts to ensure medication adherence for HBV-positive patients and providing support to improve poor adherence to control HBV replication.

In the present study, ETV resulted in less ETV-resistant mutations in NUC-naive Japanese patients than in previous reports with other drugs such as 3TC [5,18]. In conclusion, attention should be paid to patients with poor adherence as well as emerging ETV-resistant mutations in HBV during ETV-treatment.

Acknowledgements

This work was supported by grants for Scientific Research 21590829, 21590828, and 21390225 from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (TK, FI, and OY); a grant from the Viral Hepatitis Research Foundation of Japan (TK); and a grant from Chiba University Young Research-Oriented Faculty Member Development Program in Bioscience Areas (TK).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Chang MH, You SL, Chen CJ, Liu CJ, Lee CM, Lin SM, et al. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccines: a 20-year follow-up study. J Natl Cancer Inst 2009;101:1348–55.

[2] Di Bisceglie AM. Hepatitis B and hepatocellular carcinoma. Hepatology 2009;49(5 Suppl):S56–60.

[3] Lavanchy D. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. J Clin Virol 2005; 34(Suppl 1):S1–3.

[4] Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507–39.

[5] Wu S, Fukai K, Imazeki F, Arai M, Kanda T, Yonemitsu Y, Yokosuka O. Initial virological response and viral mutation with adefovir dipivoxil added to ongoing lamivudine therapy in lamivudine-resistant chronic hepatitis B. Dig Dis Sci 2011; 56:1207–14.

[6] Chen CJ, Yang H, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65–73.

[7] Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ, et al. Predicting cirrhosis based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678–86.

[8] Chotiyaputta W, Peterson C, Ditah FA, Goodwin D, Lok AS. Persistence and adherence to nucleos(t)ide analogue treatment for chronic hepatitis B. J Hepatol 2011;54:12–18.

[9] Yuen MF, Lai CL. Treatment of chronic hepatitis B: evolution over two decades. J Gastroenterol Hepatol 2011; 26(Suppl 1):S138–43.

[10] Yokosuka O, Takaguchi K, Fujioka S, Shindo M, Chayama K, Kobayashi H, et al. Long-term use of entecavir in nucleoside-naïve Japanese patients with chronic hepatitis B infection. J Hepatol 2010;52:791–9.

[11] Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, et al. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. Hepatol Res 2010;40:1–7.

Scand J Gastroenterol Downloaded from informahealthcare.com by Chiba University Iohana M Library on 02/27/12
For personal use only.

RIGHTSLINK

[12] Wu S, Kanda T, Imazeki F, Arai M, Yonemitsu Y, Nakamoto S, et al. Hepatitis B virus e antigen downregulates cytokine production in human hepatoma cell lines. Viral Immunol 2010;23:467–76.

[13] Usuda S, Okamoto H, Iwanari H, Baba K, Tsuda F, Miyakawa Y, et al. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. J Virol Methods 1999;80:97–112.

[14] Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38: 518–26.

[15] Ishibashi H, Maruyama H, Takahashi M, Fujiwara K, Imazeki F, Yokosuka O. Assessment of hepatic fibrosis by analysis of the dynamic behavior of microbubbles during contrast ultrasonography. Liver Int 2010;30:1355–63.

[16] Kanda T, Jeong SH, Imazeki F, Fujiwara K, Yokosuka O. Analysis of 5′ nontranslated region of hepatitis A viral RNA genotype I from South Korea: comparison with disease severities. PLoS One 2010;5:e15139.

[17] Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. Hepatology 2009;49:1503–14.

[18] Seta T, Yokosuka O, Imazeki F, Tagawa M, Saisho H. Emergence of YMDD motif mutants of hepatitis B virus during lamivudine treatment of immunocompetent type B hepatitis patients. J Med Virol 2000;60:8–16.

[19] Yim HJ, Hussain M, Liu Y, Wong SN, Fung SK, Lok AS. Evolution of multi-drug resistant hepatitis B virus during sequential therapy. Hepatology 2006;44:703–12.

[20] Kobashi H, Fujioka S, Kawaguchi M, Kumada H, Yokosuka O, Hayashi N, et al. Two cases of development of entecavir resistance during entecavir treatment for nucleotide-naïve chronic hepatitis B. Hepatol Int 2009;3: 403–10.

[21] Mukaide M, Tanaka Y, Shin-I T, Yuen MF, Kurbanov F, Yokosuka O, et al. Mechanism of entecavir resistance of hepatitis B virus with viral breakthrough as determined by long-term clinical assessment and molecular docking simulation. Antimicrob Agents Chemother 2010;54:882–9.

[22] Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology 2003;37:19–26.

[23] Lin CL, Kao JH. The clinical implications of hepatitis B virus genotype: recent advances. J Gastroenterol Hepatol 2011;26 (Suppl 1):123–30.

[24] Luire Y, Manns MP, Gish RG, Chang TT, Yurdaydin C, Lai CL, et al. The efficacy of entecavir is similar regardless of disease-related baseline subgroups in treatment of nucleoside-naïve, HBeAg(+) and HBeAg(-) patients with chronic hepatitis B. J Hepatol 2005;42(Suppl 2):184.

[25] Odegard PS, Gray SL. Barriers to medication adherence in poorly controlled diabetes mellitus. Diabetes Educ 2008;34: 692–7.

[26] Hill MN, Miller NH, Degeest S, American Society of Hypertension Writing Group, Materson BJ, Black HR, et al. Adherence and persistence with taking medication to control high blood pressure. J Am Soc Hypertens 2011;5: 56–63.

[27] Becker BW, Thames AD, Woo E, Castellon SA, Hinkin CH. Longitudinal change in cognitive function and medication adherence in HIV-infected adults. AIDS Behav 2011; Epub ahead of print.

[28] El-Khatib Z, Ekstrom AM, Coovadia A, Abrams EJ, Petzold M, Katzenstein D, et al. Adherence and virologic suppression during the first 24 weeks on antiretroviral therapy among women in Johannesburg, South Africa – a prospective cohort study. BMC Public Health 2011;11:88.

[29] Ha NB, Ha NB, Garcia RT, Trinh HN, Chaung KT, Nguyen HA, et al. Medication nonadherence with long-term management of patients with hepatitis B e antigen-negative chronic hepatitis B. Dig Dis Sci 2011; [Epub ahead of print].

Scand J Gastroenterol Downloaded from informahealthcare.com by Chiba University Iohana M Library on 02/27/12
For personal use only.

RIGHTSLINK

Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/j.1440-1746.2010.06322.x

HEPATOLOGY

Long-term follow-up of patients with hepatitis B e antigen negative chronic hepatitis B

Dan Bekku,*[1] Makoto Arai,*[1] Fumio Imazeki,* Yutaka Yonemitsu,* Tatsuo Kanda,* Keiichi Fujiwara,* Kenichi Fukai,* Kenichi Sato,† Sakae Itoga,† Fumio Nomura† and Osamu Yokosuka*

Departments of *Medicine and Clinical Oncology and †Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University, Chiba, Japan

Key words
HBe antibody, hepatitis B virus, long-term follow-up.

Accepted for publication 5 March 2010.

Correspondence
Makoto Arai, Department of Medicine and Clinical Oncology (K1), Graduate School of Medicine, Chiba University, Inohana 1-8-1, Chiba 260-8670, Japan. Email: araim-cib@umin.ac.jp

[1]These authors contributed equally to this article.

Abstract

Background and Aim: After hepatitis B virus (HBV) e antigen (HBeAg) seroconversion, HBV-DNA continues to replicate, and HBeAg-negative patients still face the risk of liver disease progression. We investigated the predictive factors for alanine aminotransferase (ALT) elevation, antiviral drug use, and hepatocellular carcinoma (HCC) occurrence in HBeAg-negative patients.

Methods: Age, sex, ALT, platelet counts, HBV-DNA levels, genotype, antidiabetic drug use, body mass index, smoking, and alcohol consumption were analyzed for a total of 244 HBV carriers who were HBeAg-negative.

Results: Of 244 HBeAg-negative patients, 158 (64.8%) showed normal ALT levels at baseline. Multivariate Cox hazard regression analysis identified high HBV-DNA levels and high ALT at baseline as independent risk factors for ALT elevation in the patients with normal ALT at baseline. The threshold ALT and HBV-DNA levels were determined to be 31 IU/L and 5.3 logcopies/mL, respectively. Seventeen (7.0%) patients used antiviral drugs. Multivariate Cox hazard regression analysis identified high HBV-DNA levels (threshold, 5.7 log copies/mL), the use of antidiabetic drugs, and daily alcohol consumption at baseline as an independent risk factor for the use of antiviral drugs in HBeAg-negative patients. In 10 patients (4.1%), HCC was detected, and a low platelet count (threshold, $10.0 \times 10^4/mm^3$) was associated with the occurrence of HCC.

Conclusion: This study identified predictors of future active liver disease in HBeAg-negative patients, i.e. ALT elevation, unavoidable use of antiviral drugs, and occurrence of HCC.

Introduction

Chronic hepatitis caused by hepatitis B virus (HBV) often follows a fluctuating course characterized by periods of active hepatitis interspersed with quiescence. Therefore, close follow-up is necessary to understand the natural history of HBV patients. On the other hand, patients in which HBV is truly inactive have persistently quiescent disease with an excellent prognosis. Determining an accurate prognosis for HBV carriers based on clinical presentation is important for clinical management of the disease. Various studies have been performed to distinguish the positive and negative prognostic factors among HBV carriers.[1–3]

Hepatitis e antigen (HBeAg) seroconversion is an important event in the natural history of HBV infection. HBV-infected patients usually have a very good prognosis after HBeAg seroconversion.[4] Therefore, HBeAg seroconversion has become an important treatment goal during follow-up of HBV carriers.[5] However, it has also been shown that HBV-DNA replication and hepatic inflammation in seroconverted patients continue despite the persistent loss of HBeAg; thus, HBeAg-negative patients are likely to develop liver cirrhosis or hepatocelluar carcinoma.[6] In this study, we focused on the natural history of patients with HBeAg-negative chronic hepatitis B, particularly with respect to alanine aminotransferase (ALT) elevation, antiviral drugs, and hepatocellular carcinoma (HCC).

Recently, prognostic factors for HBeAg-negative patients have been investigated in Taiwan and Canada.[7,8] We expect to identify a unique constellation of prognostic factors for HBeAg-negative chronic hepatitis B in the Japanese population, due to differences in race and HBV genotype.

Methods

Patients

Between January 1985 and April 2007, all patients visiting the Chiba University Hospital with HBV infection were approached for participation in the study. This study was carried out only at

© 2010 Journal of Gastroenterology and Hepatology Foundation and Blackwell Publishing Asia Pty Ltd

one institute, Chiba University Hospital and was approved by ethical the committee of Chiba University. Written informed consent was obtained from all of the patients in accordance with the Declaration of Helsinki. New patients since 1985 and those who were already being followed-up in 1985 were eligible for inclusion in the study. A total of 881 patients were enrolled; of which, 862 were HBsAg positive at enrollment, and 319 were hepatitis B e antibody (HBeAb) positive. Patients who were positive for hepatitis C virus antibody or hepatitis D virus antibody or who had other potential cause of chronic liver diseases (autoimmune hepatitis, primary biliary cirrhosis) were excluded. Patients followed for less than 12 months were also excluded from the analysis. In total, 244 patients were included in the analysis. Serum samples from patients were stored at –20°C and the oldest sample for each patient was used for defining the level of HBV-DNA. The date of evaluation of HBV DNA level by PCR was defined as the baseline. Patient consent was obtained for storage and analysis of serum samples.

Laboratory methods

Serum ALT level was measured using a routine automated method. HBeAg and HBeAb were measured by standard enzyme-linked immunosorbent assays. Patients were screened for hepatitis C virus, hepatitis delta virus, and human immunodeficiency virus antibodies by a third-generation enzyme-linked immunosorbent assay.

HBV-DNA quantitative assay and genotyping

To investigate the level of HBV-DNA in serum, we chose polymerase chain reaction (PCR) assay with an accurate range of 500–200 000 copies/mL (Amplicor HBV monitor test, Roche Diagnostic Systems, Basel, Switzerland). The six major genotypes of HBV (A–F) were determined by enzyme-linked immunosorbent assay (ELISA) (HBV Genotype EIA, Institute of Immunology, Co., Ltd, Tokyo, Japan).

Statistical analysis

ALT elevation was defined as a change from normal ALT (< 42 IU/L) to elevated ALT (≥ 42 IU/L), and normalization was defined as a change from elevated ALT to normal from one visit to the next. Baseline data are presented as mean ± standard deviation (SD). Differences in clinical parameters between groups were analyzed by unpaired *t*-test, Welch *t*-test, and χ^2 tests. The Cox proportional hazards model was used to identify predictive factors for future ALT elevation/normalization, use of antviral drugs, and HCC occurrence using SPSS version 16.1 software (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

To investigate the natural course of HBV carriers with HBeAb, 244 carriers (HBeAg-negative and HBeAb-positive) were enrolled in the study. Follow-up was terminated when the use of antiviral drugs was started or the occurrence of HCC. The baseline clinical and virological characteristics of the 244 HBeAg-negative carriers are shown in Table 1. Because liver biopsy was performed only in 44 (18.0%) out of 244 patients, liver biopsy results could not be analyzed further. Age, sex, ALT, platelet count (PLT), HBV-DNA level, genotype, antidiabetic drug use, body mass index, smoking, and alcohol consumption were analyzed. The average (± SD) period of follow-up was 103.6 ± 74.8 months. Seventeen (7.0%) patients used antiviral drugs (lamivudine in eight and entecavir in nine) and HCC was detected in 10 (4.1%) patients. Two (0.82%) patients died of HCC. In addition, one died of intrahepatic cholangiocarcinoma, one of liver failure due to gastrointestinal bleeding, and one of tongue cancer during the follow-up period. In Japan, the majority of HBV cases are genotype C and B and these genotypes do not cause HBV carrier by way of horizontal infection in adults; therefore, the HBV infection in our HBV carriers mainly occurred by vertical infection or infection during childhood.[9] Thus, the period of HBV infection roughly coincided with the age of HBV carriers in Japan.

ALT and HBV-DNA levels

One hundred and fifty-eight of 244 (64.8%) HBeAg-negative patients had normal ALT levels at baseline. Of these 158 subjects, 85 (53.8%) continued to have normal ALT levels during follow-up, whereas 73 (46.2%) showed fluctuation of ALT levels with intermittently elevated ALT (Fig. 1). A total of 34 (21.5%) patients had ALT ≥ 84 IU/L (more than double the normal limit). Of the 86 patients who had elevated ALT levels at baseline, ALT elevation persisted in 10 (11.6%) and 76 (88.4%) showed ALT fluctuations with intermittently elevated ALT. Although HBV-DNA levels were associated with higher ALT levels in general, correlation was weak ($r^2 = 0.13$).

Platelet count

Patients were sub classified based on PLT as follows: (I) < 100 000 (II) 100 000–149 000 (III) 150 000–199 000 (IV) 200 000–249 000 (V) > 250 000 and more (/mm^3). The numbers of patients in groups I, II, III, IV, and V were 17, 28, 73, 68, and 58, respectively. A total of 84 (34.4%) patients reached a lower platelet count at the end of follow-up.

Risk factors for future ALT elevation in patients with normal ALT levels

Although 158 (64.8%) out of 244 HBeAb-positive patients had normal ALT levels at baseline, 73 patients showed fluctuation of ALT levels with intermittently elevated ALT. We investigated the risk factors for future ALT elevation in these patients. The predictive factors of ALT elevation (ALT > 42 IU/L) in patients with normal ALT levels were HBV-DNA and ALT levels at baseline (Table 2). We carried out an additional univariate analysis changing the threshold of HBV DNA from 3.5 to 7.0 log copies/mL in 0.1 log increments and that of ALT from 15 to 41 IU/L in 1.0 increments. We determined the threshold when the value of probability was smallest; the thresholds for ALT and HBV-DNA levels were 31 IU/L and 5.3 logcopies/mL, respectively. The time

© 2010 Journal of Gastroenterology and Hepatology Foundation and Blackwell Publishing Asia Pty Ltd

Table 1 Baseline characteristics of hepatitis B virus (HBV) e antigen (HBeAg)-negative patients

	Total	Normal ALT	Elevated ALT	*P*
Number	244	158	86	
Age(years) : (mean ± SD)	44.1 ± 12.5	44.1 ± 13.1	44.0 ± 11.4	NS*
<30	35 (14.3%)	24 (15.2%)	11 (12.8%)	
30–39	52 (21.3%)	32 (20.3%)	20 (23.2%)	
40–49	66 (27.0%)	44 (27.8%)	22 (25.6%)	
50–	91 (37.3%)	58 (36.7%)	33 (38.4%)	
Sex				<0.001**
Male	141 (57.8%)	76 (48.1%)	66 (75.9%)	
Female	103 (42.2%)	82 (51.9%)	21 (24.1%)	
Alanine aminotransferase (ALT) (IU/L) (mean ± SD)	58.9 ± 108.1	20.9 ± 8.7	127.9 ± 160	<0.001*
<20	84			
21–30	47			
31–40	27			
42–84	47			
85–	39			
Platelet count ($\times 10^4/mm^3$) (mean ± SD)	205.5 ± 69.6	211.4 ± 60	193.3 ± 81.8	NS*
HBV-DNA (log copies/mL) (mean ± SD)	4.3 ± 1.5	3.8 ± 1.1	5.1 ± 1.7	<0.001*
<4.0	116 (47.5%)	91 (57.6%)	25 (29.1%)	
4.0–4.9	54 (22.1%)	38 (24.1%)	16 (18.6%)	
5.0–5.9	27 (11.1%)	18 (11.4%)	9 (10.5%)	
6.0–6.9	26 (10.7%)	5 (3.2%)	21 (24.4%)	
7.0–	16 (6.6%)	3 (1.9%)	13 (15.1%)	
Genotype				NS**
A	3 (1.2%)	2 (1.3%)	1 (1.2%)	
B	30 (12.3%)	16 (10.1%)	14 (16.3%)	
C	87 (35.7%)	49 (31%)	38 (44.2%)	
Not detected	124 (50.8%)	91 (57.6%)	33 (38.4%)	
Liver Histology ($n = 44$)				
Fibrosis 4/3/2/1	7/8/9/20	0/1/4/13	7/7/5/7	NS**
Activity 3/2/1	7/16/21	1/4/13	6/12/8	NS**
Use of anti-Diabetes drug	20 (8.2%)	3 (1.9%)	6 (7.0%)	NS**
Body mass index (kg/m^2) (mean ± SD)	23.3 ± 3.3	23.1 ± 3.2	24.0 ± 3.5	NS**
Smoker/ ever smoker/ non-smoker	32/15/89	16/5/56	16/10/33	NS**
Daily alcohol consumption	46 (27.1%)	24 (23.1%)	22 (33.3%)	NS**
Follow-up (months) (mean ± SD)	103.6 ± 74.8	109.5 ± 76.1	101.8 ± 74.6	NS*

*Unpaired *t*-test and **χ^2 test. NS, not significant difference.

interval from a visit with a normal ALT to a visit with an elevated ALT was used for Kaplan–Meier and Cox regression analysis. Kaplan–Meier curves were constructed for ALT and HBV-DNA levels (Fig. 2).

Risk factors for future use of antiviral drugs for HBV in HBeAg-negative patients

Seventeen (7.0%) patients used an antiviral drug (lamivudine in 8 and entecavir in 9). We investigated the risk factors for future use of antiviral drugs for HBV. The time interval from baseline to the use of an antiviral drug for HBV was used for Cox regression analysis. HBV-DNA levels, use of antidiabetic drugs, and daily alcohol consumption were predictive of future antiviral drug use for HBV, according to the results of multivariate Cox hazard regression analysis. Hazard ratios for HBV-DNA levels, antidiabetic drug use, and daily alcohol consumption were 1.519 (1.130–2.042, 95% confidence interval [CI]), 3.769 (1.203–11.81), and 3.011 (1.086–8.348), respectively. We repeated the univariate analysis, changing the threshold for HBV DNA from 3.5 to 7.0 log copies/mL in 0.1 log increments. We determined the threshold when the probability value was lowest; the HBV-DNA threshold level was 5.7 log copies/mL. Kaplan–Meier curves were constructed for HBV-DNA levels, antidiabetic drug use, and daily alcohol consumption (Fig. 3).

Risk factors for hepatocellular carcinoma in HBeAg-negative patients

In 10 patients (4.1%), HCC was detected. We investigated the risk factors for HCC in HBeAg-negative patients. The time interval from baseline to occurrence of HCC was used for Cox regression analysis. According to the results of multivariate Cox regression analysis, PLT was predictive of the development of HCC. The hazard ratio for PLT was 0.807 (0.724–0.899, 95% CI). We performed univariate analyses, changing the PLT threshold from 8.0 to $30.0 \times 10^4/mm^3$ in $1.0 \times 10^4/mm^3$ increments. We determined the threshold when the value of probability was smallest; the

© 2010 Journal of Gastroenterology and Hepatology Foundation and Blackwell Publishing Asia Pty Ltd

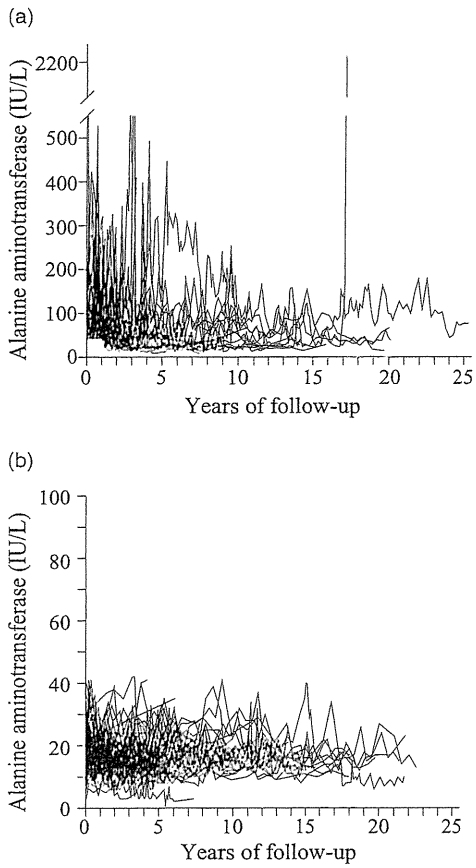

Figure 1 Level of alanine aminotransferase (ALT) in (a) patients with normal ALT at baseline and intermittently elevated ALT during follow-up ($n = 73$) and (b) patients with normal ALT at baseline and during follow-up ($n = 85$).

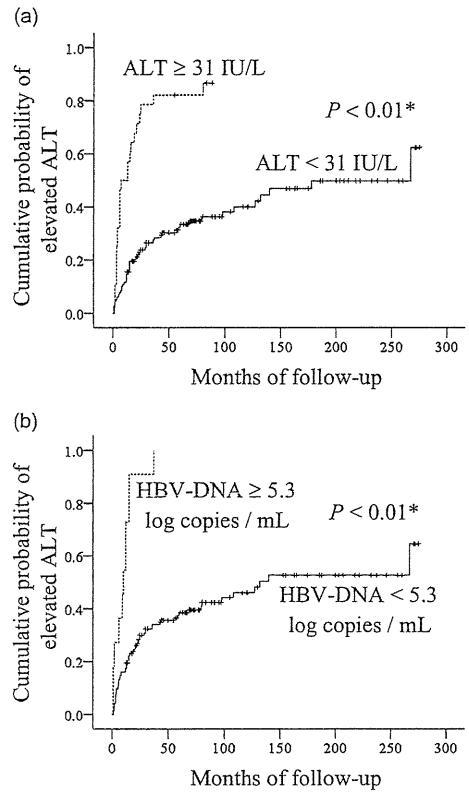

Figure 2 Cumulative occurrence of abnormal alanine aminotransferase (ALT) in HBeAg-negative patients with normal ALT based on (a) ALT and (b) HBV-DNA levels. We determined the threshold for ALT and HBV-DNA levels when the probability value was lowest in the univariate analysis. Kaplan–Meier curves show the time to ALT elevation. Solid lines indicated the control group. *A significant difference was determined by log-rank test.

Table 2 Univariate and multivariate analysis of factors associated with alanine aminotransferase (ALT) elevation in hepatitis B virus (HBV) e antigen (HBeAg)-negative patients with normal ALT levels

	Univariate analysis				Multivariate analysis			
	Standard error	Wald statistic	*P*-value	Hazard ratio (95% confidence interval)	Standard error	Wald statistic	*P*-value	Hazard ratio (95% confidence interval)
Sex (Male)	0.263	0.203	0.652	1.126 (0.673–1.885)				
Age (years)	0.011	5.704	0.017	1.027 (1.005–1.049)	0.252	0.068	0.794	1.015 (0.572–1.534)
HBV-DNA	0.109	17.773	<0.001	1.587 (1.280–1.966)	0.111	10.602	0.001	1.437 (1.155–1.788)
Genotype								
B	0.459	0.22	0.639	0.806 (0.328–1.982)				
C	0.435	0.055	0.815	1.107 (0.472–2.600)				
Alanine aminotransferase	0.014	42.440	<0.001	1.097 (1.067–1.128)	0.015	29.496	<0.001	1.086 (1.054–1.119)
Platelet count	0.019	5.928	0.015	0.955 (0.920–0.991)	0.021	0.754	0.385	0.982 (0.942–1.023)
Use of anti-diabetes drug	0.427	0.470	0.493	1.340 (0.581–3.091)				
Body mass index (kg/m^2)	0.042	0.033	0.855	0.992 (0.913–1.078)				
Smoker and ever smoker	0.374	0.111	0.739	1.133 (0.544–2.359)				
Daily alcohol consumption	0.333	0.512	0.474	1.269 (0.661–2.435)				

© 2010 Journal of Gastroenterology and Hepatology Foundation and Blackwell Publishing Asia Pty Ltd

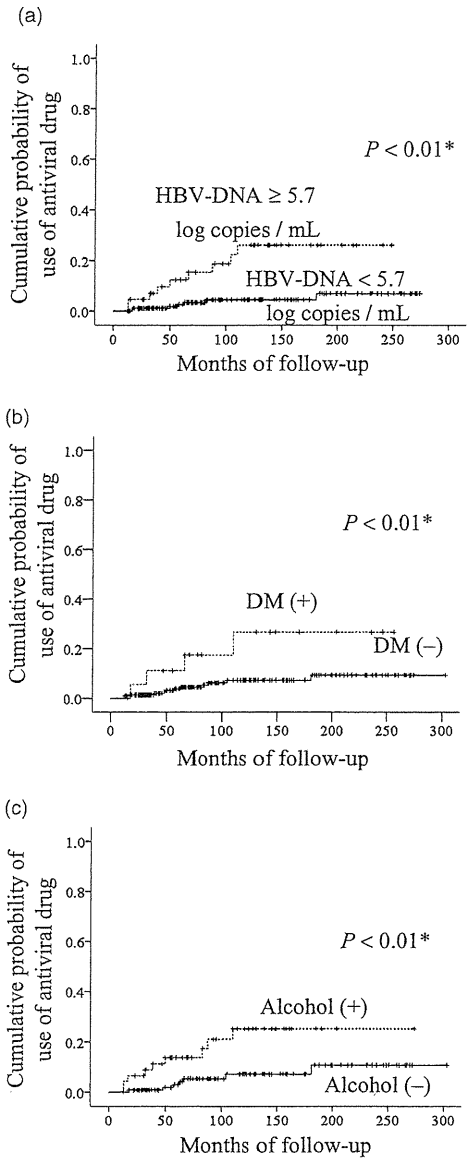

Figure 3 Cumulative occurrence of antiviral drug use for hepatitis B virus (HBV) in HBeAg-negative patients based on (a) HBV-DNA levels (b) use of antidiabetic drug, and (c) daily alcohol consumption. We determined the threshold for HBV-DNA levels when the probability value was lowest in the univariate analysis. Kaplan–Meier curves show the time to use of antiviral drugs for HBV. Solid lines indicated the control group. *A significant difference was determined by log-rank test.

PLT threshold was $10.0 \times 10^4/mm^3$. Kaplan–Meier curves were constructed for PLT (Fig. 4).

Stratification analyses of risk factors for clinical outcomes in HBeAg-negative patients by age, sex, and HBV genotype

The stratification analyses by age, sex, and HBV-genotype were performed to evaluate the risk factors for future ALT elevation in patients with normal ALT levels, future use of antiviral drugs for HBV, and HCC in HBeAg-negative patients (Table 3). The age threshold was 45 years, which was the average age of all the patients. We did not perform stratification analysis for patients infected with HBV genotype B because the number of such cases was very small.

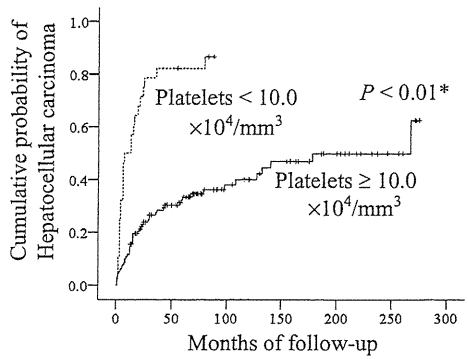

Figure 4 Cumulative occurrence of hepatocellular carcinoma (HCC) based on the platelet counts. We determined the threshold for HBV-DNA levels when the probability value was lowest in the univariate analysis. Kaplan–Meier curves show the time to HCC. Solid lines indicated the control group. *A significant difference was observed by log-rank test.

Discussion

Most patients who have undergone HBeAg seroconversion have normal serum ALT levels, which is indicative of a good clinical outcome.[10] Therefore, various therapies for early seroconversion have been used.[5] Recently, HBeAg-negative viral mutants have been shown to be responsible for continuous HBV-DNA replication.[7] That is, there exists the possibility that liver disease will get worse after HBeAg seroconversion. In fact, previous reports revealed that HBeAg status is not a predictive factor for HCC,[11,12] and fulminant hepatitis can occur by the infection of HBV with HBeAg-negative.[13] HBeAg-negative patients should be monitored closely, even though most of these patients show normal ALT levels and no progressive liver disease.[14] Therefore, predictive factors for active liver disease in HBeAg-negative patients need to be identified in order to facilitate optimal disease management. This study provides data regarding the prediction of future active liver disease, i.e. ALT elevation, unavoidable use of antiviral drugs, and occurrence of HCC.

Many previous reports have attempted to define a threshold HBV-DNA level that corresponds to the presence of active liver disease.[15] A National Institute of Health workshop demonstrated that an HBV-DNA level of 10^5 copies/mL could be used to distinguish active HBV infection from inactive HBV infection.[16] Other studies also suggested that the threshold HBV-DNA level lies somewhere between 10^4 and 10^6 copies/mL.[8] In this study, in order to clarify the natural course of HBeAg-negative patients with normal ALT levels, we used a HBV-DNA threshold of $10^{5.3}$ copies/mL. By log rank analysis, the ALT levels in patients with $>10^{5.3}$ copies/mL HBV-DNA level were significantly higher than in patients with HBV-DNA below this level. In HCV patients, ALT is

© 2010 Journal of Gastroenterology and Hepatology Foundation and Blackwell Publishing Asia Pty Ltd

Table 3 Stratification analysis multivariate analysis of factors associated with alanine aminotransferase (ALT) elevation in hepatitis B virus (HBV) e antigen (HBeAg)-negative patients with normal ALT levels, future use of antiviral drugs for HBV, and occurrence of hepatocellular carcinoma

		Age (years)						Sex						Genotype					
		≥45 years			<45 years			Male			Female			C					
		$n = 126$			$n = 118$			$n = 141$			$n = 103$			$n = 87$					
	Factors	Hazard ratio (95% CI)	*P*-value	Factors	Hazard ratio (95% CI)	*P*-value	Factors	Hazard ratio (95% CI)	*P*-value	Factors	Hazard ratio (95% CI)	*P*-value	Factors	Hazard ratio (95% CI)	*P*-value	Factors	Hazard ratio (95% CI)	*P*-value
Future ALT elevation in the patients with normal ALT level	HBV-DNA	1.535 (1.146–2.057)	0.004	ALT	1.106 (1.059–1.156)	<0.001	ALT	1.194 (1.075–1.325)	<0.001	ALT	1.060 (1.015–1.108)	0.008	ALT	1.149 (1.075–1.228)	<0.001			
	ALT	1.077 (1.035–1.122)	<0.001							HBV-DNA	1.739 (1.213–2.492)	0.003	HBV-DNA	1.902 (1.223–2.956)	0.004			
Future use of anti-viral drugs for HBV	Alcohol	4.744 (1.362–16.52)	0.014	HBV-DNA	2.238 (1.107–4.526)	0.025	HBV-DNA	1.486 (1.053–2.098)	0.024	DM†	86.14 (1.842–4027.3)	0.023	Alcohol	5.617 (1.431–22.05)	0.013			
										BMI†	0.408 (0.187–0.890)	0.024						
Occurrence of hepatocellular carcinoma	PLT	0.772 (0.659–0.905)	0.001	(–)†			PLT	0.832 (0.731–0.948)	0.006	PLT†	0.775 (0.635–0.947)	0.013	PLT	0.833 (0.732–0.948)	0.006			

†Three patients were used in subgroup for HBV antiviral drugs and HCC occurrence.
Alcohol, Daily alcohol consumption; BMI, Body mass index; CI, confidence interval; DM, use of antidiabetic medication; PLT, platelet count.

a poor surrogate marker for inflammation and fibrosis.[17] Therefore, even if the patient's ALT level was within normal limits, they should still be monitored closely, and HCV eradication therapy is recommended under certain circumstances. Similarly, even if the ALT levels are within normal limits in HBV-infected patients who are HBeAg-negative, the higher their ALT levels were, the more frequently their ALT levels would be high in the future, which might cause progressive liver disease.[18]

Some of the patients with progressive liver disease caused by HBV infection were treated with the antiviral drugs lamivudine and entecavir. The use of lamivudine or entecavir might result in mutant HBV resistance to antiviral drugs[19,20] and the associated costs are not trivial. The baseline levels of HBeAg, ALT, and HBV-DNA, and the presence of either chronic hepatitis or cirrhosis have been established as determinants for eligibility for antiviral treatment.[21] According to treatment guidelines in the Unites States (National Guideline Clearinghouse, http://www.guideline.gov), patients with HBeAg-negative chronic hepatitis B should be considered for antiviral treatment based on their HBV-DNA and ALT levels (serum HBV-DNA >20 000 IU/mL and elevated ALT >2 times normal). In this study, only four out of 17 patients treated with an antiviral drug showed normal ALT levels at baseline, and all four patients showed elevated ALT levels in 8–57 months later. Therefore, this study revealed that patients with high HBV-DNA levels tended to have high ALT levels at baseline or in the future; as a result, such patients have a tendency for future treatment with and antiviral drug.

Hepatocellular carcinoma occurrence was noted in only 10 cases (4.1%). The only predictive factor for HCC occurrence was PLT, which meant that patients with advanced liver disease tended to develop HCC later, because the decrease in PLT corresponded to the extent of liver fibrosis. Four patients (1.6%) died of liver-related diseases and one (0.4%) died of cancer in another organ. The number of deaths was too small to determine the predictive factors for death of HBeAg-negative HBV carriers. Further analysis is needed to properly address this factor.

Stratification analyses of risk factors for clinical outcomes by age, sex, and HBV-genotype were performed. Because the numbers of female patients with future use of antiviral drugs for HBV ($n = 3$), HCC occurrence ($n = 3$), or who were under 45 years old with HCC occurrence ($n = 3$) were very small, it was not possible to properly evaluate these subgroups. The risk factors among subgroups for future ALT elevation in patients with normal ALT levels, and for HCC were almost equal to those of the entire patient population. However, daily alcohol consumption, not HBV-DNA level, was predictive of future use of antiviral drugs for HBV in patients ≥45 years old or in patients infected with HBV genotype C. In these subgroups, alcohol consumption was an important factor for predicting the clinical course of HBV carriers; i.e. advising patients to abstain from drinking might reduce the need for antiviral drugs in the future.

Coffee or caffeine consumption is reported to be strongly related to ALT levels and HCC occurrence.[22–24] In our study, we did not survey caffeine consumption; therefore, further analysis is needed to determine the importance of coffee or caffeine consumption as a predictive factor of the clinical course in HBeAg-negative HBV carriers.

In conclusion, we established that low HBV-DNA levels and ALT levels at baseline were good predictors for future ALT eleva-

© 2010 Journal of Gastroenterology and Hepatology Foundation and Blackwell Publishing Asia Pty Ltd

tion in HBeAg-negative HBV carriers with normal ALT levels. In addition, this study provides data on the prediction of unavoidable antiviral drug use and HCC occurrence.

References

1 Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J. Hepatol.* 2008; **48**: 335–52.
2 Di Marco V, Lo Iacono O, Camma C *et al.* The long-term course of chronic hepatitis B. *Hepatology* 1999; **30**: 257–64.
3 Fattovich G, Olivari N, Pasino M *et al.* Long-term outcome of chronic hepatitis B in Caucasian patients: mortality after 25 years. *Gut* 2008; **57**: 84–90.
4 Hoofnagle JH, Dusheiko GM, Seeff LB *et al.* Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann. Intern. Med.* 1981; **94**: 744–8.
5 Korenman J, Baker B, Waggoner J *et al.* Long-term remission of chronic hepatitis B after alpha-interferon therapy. *Ann. Intern. Med.* 1991; **114**: 629–34.
6 Sumi H, Yokosuka O, Seki N *et al.* Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; **37**: 19–26.
7 Lin CL, Liao LY, Liu CJ *et al.* Hepatitis B viral factors in HBeAg-negative carriers with persistently normal serum alanine aminotransferase levels. *Hepatology* 2007; **45**: 1193–8.
8 Feld JJ, Ayers M, El-Ashry D *et al.* Hepatitis B virus DNA prediction rules for hepatitis B e antigen-negative chronic hepatitis B. *Hepatology* 2007; **46**: 1057–70.
9 Orito E, Ichida T, Sakugawa H *et al.* Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* 2001; **34**: 590–4.
10 Lai CL, Yuen MF. The natural history of chronic hepatitis B. *J. Viral. Hepat.* 2007; **14** (Suppl. 1): 6–10.
11 Murata K, Sugimoto K, Shiraki K *et al.* Relative predictive factors for hepatocellular carcinoma after HBeAg seroconversion in HBV infection. *World J. Gastroenterol.* 2005; **11**: 6848–52.
12 Pokorski RJ, Ohlmer U. Long-term morbidity and mortality in Chinese insurance applicants infected with the hepatitis B virus. *J. Insur. Med.* 2001; **33**: 143–64.
13 Fujiwara K, Yokosuka O, Ehata T *et al.* The two different states of hepatitis B virus DNA in asymptomatic carriers: HBe-antigen-positive versus anti-HBe-positive asymptomatic carriers. *Dig. Dis. Sci.* 1998; **43**: 368–76.
14 Kumar M, Sarin SK, Hissar S *et al.* Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. *Gastroenterology* 2008; **134**: 1376–84.
15 Mels GC, Bellati G, Leandro G *et al.* Fluctuations in viremia, aminotransferases and IgM antibody to hepatitis B core antigen in chronic hepatitis B patients with disease exacerbations. *Liver* 1994; **14**: 175–81.
16 Hoofnagle JH, Doo E, Liang TJ *et al.* Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; **45**: 1056–75.
17 Sanai FM, Benmousa A, Al-Hussaini H *et al.* Is serum alanine transaminase level a reliable marker of histological disease in chronic hepatitis C infection? *Liver Int.* 2008; **28**: 1011–18.
18 Lai M, Hyatt BJ, Nasser I *et al.* The clinical significance of persistently normal ALT in chronic hepatitis B infection. *J. Hepatol.* 2007; **47**: 760–7.
19 Villet S, Ollivet A, Pichoud C *et al.* Stepwise process for the development of entecavir resistance in a chronic hepatitis B virus infected patient. *J. Hepatol.* 2007; **46**: 531–8.
20 Sa-nguanmoo P, Tangkijvanich P, Payungporn S *et al.* Dynamics of HBV DNA levels, HBV mutations and biochemical parameters during antiviral therapy in a patient with HBeAg-negative chronic hepatitis B. *Asian Pac. J. Allergy Immunol.* 2007; **25**: 183–8.
21 Buster EH, van Erpecum KJ, Schalm SW *et al.* Treatment of chronic hepatitis B virus infection—Dutch national guidelines. *Neth. J. Med.* 2008; **66**: 292–306.
22 Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2005; **128**: 24–32.
23 Ruhl CE, Everhart JE. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterology* 2005; **129**: 1928–36.
24 Larsson SC, Wolk A. Coffee consumption and risk of liver cancer: a metaanalysis. *Gastroenterology* 2007; **132**: 1740–5.

© 2010 Journal of Gastroenterology and Hepatology Foundation and Blackwell Publishing Asia Pty Ltd