Table 1. Patient Characteristics and Demographics

Characteristics	Entire Cohort				Propensity Score Matched Cohort		
	All Patients (n = 1,615)	Entecavir (n = 472)	Control (n = 1,143)	P	Entecavir (n = 316)	Control (n = 316)	P
Age (y)†	42 (13.5)	47 (12.4)	39 (13.1)	<0.001	46 (12.1)	46 (13.5)	0.907
Gender (male:female)	1,035:580	315:157	720: 423	0.171	210:106	210:106	1.000
Alcohol consumption (>200kg)	355 (22)	97 (20.5)	288 (25.1)	0.013	62 (20)	105 (33)	<0.001
Cigarette smoking	443 (27)	157 (33.2)	286 (25.0)	0.005	110 (35)	110 (35)	1.000
Preexisting cirrhosis	311 (19)	116 (25)	195 (17)	0.001	79 (25)	85 (29)	0.324
HBV genotype	–	–	–	<0.001	–	–	0.843
A	53 (3.3)	12 (2.5)	41 (3.6)	–	8 (2.5)	9 (2.8)	–
B	254 (15.7)	66 (14.0)	188 (16.4)	–	49 (15.5)	50 (15.8)	–
C	1,135 (70.3)	344 (72.9)	791 (69.2)	–	225 (71.2)	226 (71.5)	–
D	1 (0.06)	0	1 (0.09)	–	0	0	–
F	1 (0.06)	0	1 (0.09)	–	0	0	–
H	2 (0.1)	2 (0.4)	0	–	0	0	–
Unclassified / missing	169 (10.4)	48 (10.2)	121 (10.5)	–	34 (10.7)	31 (9.8)	–
Baseline HBeAg positive	617 (38)	219 (46)	398 (35)	<0.001	135 (43)	133 (42)	0.936
Baseline HBV DNA (log copies/mL)	6.0 (4.3-7.7)	6.7 (5.3-8.0)	5.8 (4.0-7.5)	<0.001	6.3 (5.2-7.9)	6.6 (4.5-7.8)	0.795
Baseline AST level (IU/L)	35 (22-63)	53 (35-95)	28 (20-50)	<0.001	45 (32-70)	49 (27-98)	0.956
Baseline AST level (x ULN)	1.1 (0.7-1.9)	1.6 (1.1-2.9)	0.8 (0.6-1.5)	<0.001	1.4 (1.0-2.1)	1.5 (0.8-3.0)	0.989
Baseline ALT level (IU/L)	42 (22-88)	70 (42-163)	33 (20-68)	<0.001	61 (39-109)	60 (28-144)	0.110
Baseline ALT level (x ULN)	1.1 (0.7-2.4)	1.9 (1.2-4.3)	0.9 (0.6-1.8)	<0.001	1.7 (1.0-3.3)	1.6 (0.8-3.7)	0.086
Baseline GGTP level (IU/L)	28 (16-59)	39 (24-72)	24 (14-52)	<0.001	34 (23-64)	34 (18-68)	0.088
Baseline total bilirubin level (mg/dL)	0.7 (0.5-0.9)	0.7 (0.5-1.0)	0.6 (0.5-0.9)	<0.001	0.7 (0.5-1.0)	0.7 (0.5-0.9)	0.210
Baseline serum albumin level (g/L)	4.2 (3.9-4.5)	3.9 (3.6-4.1)	4.4 (4.1-4.6)	<0.001	3.9 (3.7-4.2)	4.0 (3.8-4.3)	0.084
†Platelet count (10^5/mm^3) (SD)	19.1 (6.3)	16.9 (5.6)	20.0 (6.4)	<0.001	17.5 (5.2)	17.2 (6.0)	0.349
Follow-up duration (yrs)	5.4 (3.1-13.2)	3.2 (2.1-4.3)	9.5 (4.4-16.1)	<0.001	3.3 (2.3-4.3)	7.6 (3.4-13.7)	<0.001
Person-years of follow-up	13,986	1561	12381	–	1064	2978	–
No. of HCC cases	156	12	144	–	6	72	–
Incidence rates per 1000 person-years	11.15	7.69	11.63	–	5.63	24.1	–
Progression of cirrhosis within 5 year	21 (1.3)	0	21 (1.8)	0.001	0	10 (3.2)	0.001
HBV DNA <400 copies/mL at 1 yearr	–	421 (89)	NA	–	288 (90)	NA	–
Emergence of drug-resistant mutants during ETV treatment	–	4 (0.8)	NA	–	2 (0.6)	NA	–

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; AST, aspartate aminotransferase; GGTP, gamma glutamyltransferase (ULN=33 IU/L); ALT, alanine aminotransferase (ULN=42 IU/L for men and 27 IU/L for women); HCC, hepatocellular carcinoma; ETV, entecavir.

*P <0.05.

**P <0.001, comparison of entecavir-treated group and control group.

†Data displayed as mean ± standard deviation. ‡All other values are expressed as median (25th to 75th percentile) or number (percentage of total, %).

matched control group were 4.0% at year 2, 7.2% at year 3, 10.0% at year 4, and 13.7% at year 5. Log-rank test revealed a statistically significant difference between the incidence of HCC in the ETV group and the control group over time ($P < 0.001$) (Fig. 2). We then used Cox proportional regression analysis to estimate the effects of ETV treatment on HCC risk. Factors that were associated with HCC at year 5 in the propensity score matched cohort were age, gender, alcohol consumption (>200 kg), the presence of cirrhosis, HBeAg positivity, baseline viral load, ALT, γ-GTP, total bilirubin, serum albumin, and platelet counts (Table 2). For ETV treatment effect, we estimated the hazard ratio of HCC development, adjusting for multiple baseline variables (age, gender, alcohol consumption, smoking, preexisting cirrhosis, HBeAg, HBV DNA, ALT, albumin, γ-GTP, total bilirubin, and platelet count) in the propensity matched cohort. Progression of cirrhosis within 5 years was used as a time-dependent covariate in the proportional hazard regression but it did not show a statistically significant hazard to HCC development.

Subanalyses Showing HCC Suppression Effect Between ETV and LAM. PS matching of the LAM-treated patients without rescue therapy (n = 492) with ETV-treated patients resulted in a matched cohort of 182 patients (Supporting Table 3). The rate of non-rescued LAM-treated group having undetectable HBV DNA at 1 year after treatment was lower when compared with the ETV-treated group. The LAM-treated group also had a higher drug-resistant mutation rate. Comparisons of HCC incidence among the ETV-treated group, nonrescued LAM-treated group, and control showed that the HCC suppression effect was greater in ETV-treated ($P < 0.001$) than nonrescued LAM-treated ($P = 0.019$) when compared with the

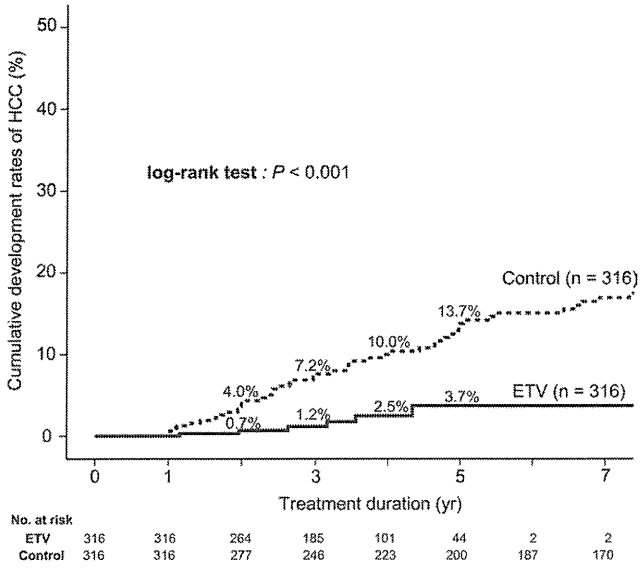


Fig. 2. Comparison of HCC cumulative incidence rates between the entecavir-treated group and the nontreated control group after propensity score matching. The log-rank test revealed a statistically significant difference between the ETV and the control group in the incidence of HCC at 5 years time (log-rank test: $P < 0.001$).

control group (Fig. 3). The difference of effect between ETV and LAM was also significant ($P = 0.043$). The treatment effect was seen in cirrhosis patients but not in noncirrhosis patients. The result showed ETV's superiority to LAM in suppressing HCC.

Effect of ETV on the Reduction of HCC Development by Preexisting Cirrhosis and Risk Scores. To further examine the ETV treatment effect, we compared the ETV and the control groups by preexisting cirrhosis and published risk scores. Viral response rates (HBV DNA < 400 copies/mL) of 1-year post-ETV treatment was 87% in the noncirrhosis patients and 91% in the cirrhosis patients (LC). ALT normalization was 94% and 90% in the chronic hepatitis and cirrhosis patients, respectively. The treatment effect was not inferior by cirrhosis status. Among those who developed HCC, 97 out of 144 patients in the control group and 9 out of 12 patients in the ETV group had cirrhosis. Interactions between preexisting cirrhosis and ETV treatment were not observed ($P = 0.177$).

Cumulative HCC incidence rates by risk scores are compared between the two cohorts in Fig. 4A-G. Figure 4A,B shows the risk scores developed by Yang et al.[10] Figure 4C,D shows the risk scores developed by Yuen et al.[11] Figure 4E-G shows the risk scores developed by Wong et al.[12] All three risk score scales showed that ETV significantly reduced HCC incidence in patients with a higher risk (risk score ≥ 12, $P = 0.006$; risk score ≥ 82, $P = 0.002$; medium risk, $P = 0.062$; high risk, $P < 0.001$). Interactions between risk scores and ETV treatment were not observed (Yang et al.: $P = 0.713$, Yuen et al.: $P = 0.267$, Wong et al.: $P = 0.265$).

Discussion

Our study suggests that long-term ETV therapy would significantly suppress the development of HCC in HBV-infected patients when compared with HBV-infected patients in the control group. The treatment effect was more prominent among patients at high risk of HCC than those at low risk.

Table 2. Factors Associated with HCC Development as Determined by Cox Proportional Hazard Regression Analysis at 5-Year (Propensity Score Matched Cohort)

Variable	Univariate HR (95% CI)	P	Multivariate Adjusted HR (95% CI)	P
Age (per year)	1.05 (1.02-1.07)	<0.001	1.06 (1.03-1.09)	<0.001
Gender (M)	2.81 (1.25-6.32)	0.012		
Alcohol consumption (>200kg)	2.71 (1.49-4.92)	0.001	2.21 (1.18-4.16)	0.013
Cigarette smoking	1.53 (0.84-2.80)	0.164		
Preexisting cirrhosis	12.0 (5.57-25.9)	<0.001	4.28 (1.88-9.73)	0.001
HBV genotype (C)	2.73 (0.98-7.65)	0.056		
HBeAg (positive)	2.64 (1.41-4.94)	0.002	2.26 (1.18-4.34)	0.014
HBV DNA (≥5.0 log copies/mL)	4.66 (1.44-15.1)	0.010		
ALT (≥45 IU/L)	2.29 (1.10-4.77)	0.027		
GGTP (≥50 IU/L)	3.79 (2.02-7.09)	<0.001		
Total bilirubin (≥1.5 mg/dL)	5.51 (2.87-10.6)	<0.001		
Serum albumin (<3.8 g/L)	4.44 (2.42-8.14)	<0.001		
Platelet count ($<1.5\times10^5/mm^3$)	14.8 (5.84-37.7)	<0.001	5.64 (2.13-15.0)	0.001
*Progression of cirrhosis within 5 years	1.80 (0.25-13.2)	0.562		
ETV treatment	0.23 (0.09-0.55)	0.001	0.37 (0.15-0.91)	0.030

Asterisks (*) indicate time-dependent covariates.

†Adjusted for age, gender, alcohol, cigarette, cirrhosis, genotype, HBeAg, HBV DNA, ALT, albumin, GGTP, total bilirubin, and platelet counts

Abbreviations: ETV, entecavir; HR, hazard ratio; CI, confidence interval; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; GGTP, gamma glutamyltransferase.

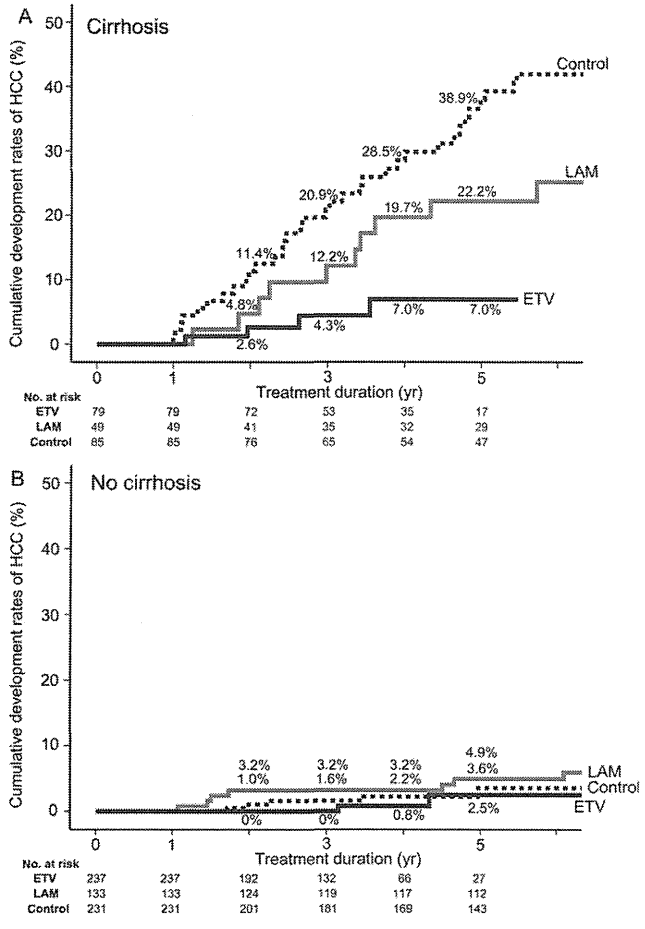


Fig. 3. Comparison of HCC cumulative incidence rates between the entecavir (ETV)-treated group, lamivudine (LAM)-treated, and the nontreated control group after PS matching stratified by cirrhosis. The log-rank test revealed a statistically significant difference in the incidence of HCC at 5 years time in cirrhosis patients: ETV versus control group ($P < 0.001$); LAM versus control ($P = 0.019$); ETV versus LAM ($P = 0.043$). The differences were not seen in the noncirrhosis patients: ETV versus control ($P = 0.440$); LAM versus control ($P = 0.879$); ETV versus LAM ($P = 0.126$).

HBV has been previously shown to influence HCC development. Ikeda et al.[20] reported that the cumulative HCC incidence rates among Japanese HBV patients were 2.1% at 5 years, 4.9% at 10 years, and 18.8% at 15 years among NA-naïve patients. Other studies, both from Japan and other countries, have reported a 5-year cumulative HCC incidence rate of 3.3% among chronic HBV, and 21.2% to 59% among cirrhosis patients.[21,22] The incidence of HCC varies significantly by country and ethnic group,[4] which seems to be attributable to diverse exposure to HCC risk factors.

Carcinogenicity related to HBV infection is somewhat complex and multifactorial when compared with carcinogenicity related to HCV infection. Known HCC risk factors among HBV-infected patients include older age, male gender, cirrhotic status, diabetes mellitus, family history, alcohol consumption, AST, HBsAg, HBeAg, and genotype C.[20,23,25] Chen et al.[5] found a dose-response relationship between pretreatment serum HBV DNA levels and the development of HCC. Baseline ALT is another risk factor for HCC, as elevated ALT levels indicate an active immune response against HBV, resulting in repetitive hepatocyte injury.[5] Our study corroborates these findings on these factors influence on HCC development.

The potential ability of ETV to reduce the risk of HCC is an additional example of a long-term NA treatment effect. Some studies have shown that ETV has low incidence of HCC but these studies did not have a control arm.[9] A meta-analysis and a systematic review showed that NAs can reduce liver complications, including HCC.[26,27] Other studies have begun to show that control of sustained viral loads through drugs such as NAs is important in preventing long-term complications. Chen et al.[28] showed that greater decreases in serum HBV DNA levels ($<10^4$ copies/mL) during follow-up were associated with a lower risk of HCC.

Our comparison among the PS-matched ETV-treated group, nonrescued LAM-treated patients, and the control showed that ETV is superior to LAM in HCC suppression. Kurokawa et al.[29] showed that treatment with lamivudine for an average of 5 years reduced the incidence of HCC in HBV-infected cirrhosis patients, who showed sustained viral response at a median HBV DNA of <4.0 log copies/mL. Unfortunately, only 48% of the patients in this study achieved sustained viral response, while 51% developed lamivudine-resistant tyrosine-methionine-aspartate-aspartate mutation (YMDD mutation) during follow-up.[29] Patients with drug resistance were reported to have a 2.6 times greater chance of developing long-term complications.[26] A systematic review of 21 studies showed that HCC occurred more (2.3% versus 7.5%, $P < 0.001$) in nonresponding patients or in patients with viral breakthrough compared with those who experienced remission.[28] On-treatment drug resistance could subject patients to a variable viral status. Suppression of HCC by NAs requires NAs that do not lead to drug resistance. Compared with other NAs, ETV shows minimal drug resistance. Our results showed that ~90% of the ETV-treated patients had sustained viral suppression at year 1, and that drug resistance was minimal (0.8%) during the median follow-up period of 3.2 years.

We found that the effect of ETV treatment in reducing the risk of HCC was more prominent among high-risk patients. This phenomenon was observed by examining the combination of parameters associated with the recently developed risk scores (Fig. 4). The published risk scores were developed mainly to create

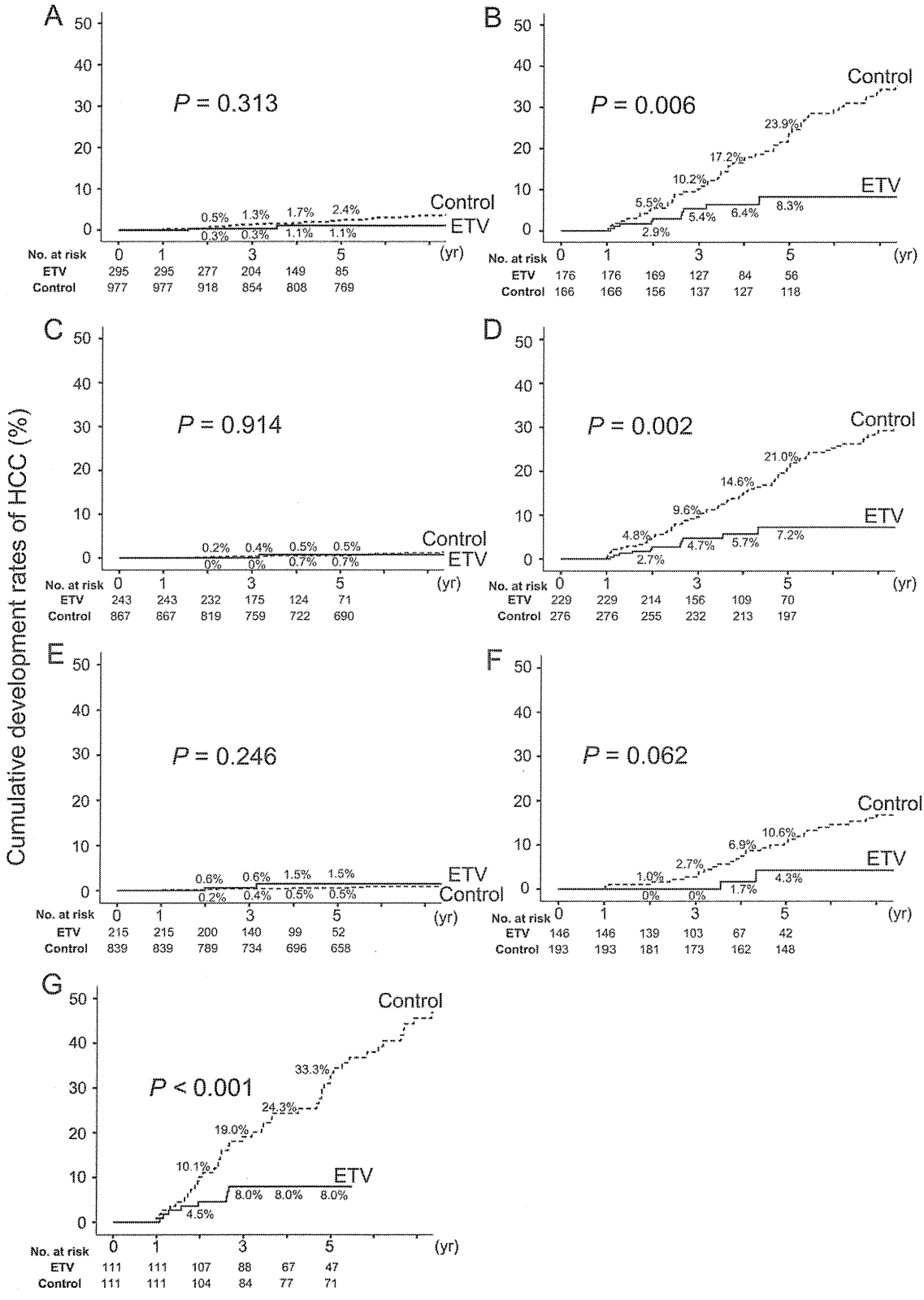


Fig. 4. Cumulative incidence of HCC by risk score scales: comparison between entecavir-treated and nontreated control patients: Risk score cutoff points were based on those presented in articles by the following: A,B (Yang et al.[10]): low-risk score cutoff point < 12; high-risk score cutoff point ≥ 12. C,D (Yuen et al.[11]): low-risk score cutoff point < 82; high-risk score cutoff point ≥ 82. E-G (Wong et al.[12]): low-risk score cutoff point < 4; medium-risk cutoff point 4-19; high-risk score cutoff point ≥ 20. A statistically significant difference in HCC incidence was seen between the ETV group and the control group in the higher-risk groups when observed the incidence of HCC over time (log-rank test $P = 0.006$ for risk score ≥ 12; $P = 0.002$ for risk score ≥ 82; $P = 0.062$ for patients with medium risk; $P < 0.001$ for patients at high risk for HCC).

easy-to-use nomograms based on clinical characteristics to predict the risk of HCC in patients with HBV. These scales have been validated, and can accurately estimate the risk of HCC up to 10 years. The cutoff scores used in these studies were based on their sensitivity to detect HCC derived and validated with non-treated HBV cohorts. The importance of our study using these risk scales in our cohorts was to see the change in risk with the initiation of therapy. We found that the ETV treatment effect to reduce the risk of HCC was more prominent among cirrhosis and high-risk patients despite the lack of interactions between ETV treatment and preexisting cirrhosis or risk factors. The lower treatment effect among lower-risk patients was somewhat not surprising. HCC development among low-risk patients is generally rare, and therefore, the treatment effect may not have occurred in large enough numbers during the treatment period allotted in our study to be able to detect a difference. In addition, HCC development differs greatly by cirrhotic status and risk factors in the control group. The treatment effect of ETV to reduce HCC is probably more likely reflected among cirrhosis or high-risk patients. A study with a longer observation period and higher patient numbers might be necessary to examine this ETV treatment effect among low-risk patients. The development of a scoring system to predict treatment effect of HBV patients with different risk levels will be useful in determining the most appropriate timing of treatment initiation in clinical settings.

Study Limitations. There were several limitations to our study. First, because our patients were recruited from one hospital, they might not have been representative of the general Japanese HBV population. Second, our control group included historically observed patients who entered the cohort long before the ETV group, resulting in treatment differences during the time gap. However, we used PS matching and a similar follow-up period between the two cohorts to minimize this bias. Third, our study was an observational study with patients having large demographic differences. Although we used a PS to match ETV-treated and control groups, our sample size did not take into account other unobserved confounding factors such as HCC family history, stage of cirrhosis, and comorbidities when determining associating factors for carcinogenesis in HBV. Finally, the observation period of the ETV group was relatively short, and patients in the ETV-treated cohort at 5 years consisted of only less than ~25% of the initial recruited patients. Because of this limitation, we censored patients who were followed for more than 5 years. The observed treatment effect would require confirmation over a longer period and a more complete follow-up.

Conducting a long-term study to examine the effect of antiviral therapy with HCC as the endpoint would be time-consuming and challenging. Such a study would require a large sample size and would, therefore, be costly. In addition, the increases in choices of therapy over time would make it difficult to conduct a long-term study using a single therapy. Owing to ethical issues, it would be difficult to recruit or follow a naïve, untreated cohort over an extended period of time. Because of these challenges, most studies have examined the relationship between antiviral treatment and the risks of HCC involved older drugs, lacked a control group, or were of relatively short duration. Consequently, the association between antiviral treatment and carcinogenesis is inferential and requires additional confirmatory studies.

In conclusion, in our study we observed the effect of HCC risk among HBV-infected patients treated by ETV by comparing them with a group of NA-naïve patients. We followed these Japanese patients for a relatively long period of time and compared them with a large pool of untreated control patients. In this long-term study among Japanese patients, ETV significantly reduced the incidence of HCC among chronic HBV-infected patients, and was more prominent among patients at higher risk for HCC.

Acknowledgment: We thank Fujio Matsuo, MSc, Executive Director, Statistical/Analysis Department, Statcom Company Limited and Yasuo Ohashi, PhD, Professor, Department of Biostatistics, School of Public Health, University of Tokyo for their review and advice on statistical analyses.

References

1. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733-1745.
2. Lai CL, Ratziu V, Yuen MF, Paynard T. Viral hepatitis B. Lancet 2003;362:2089-2094.
3. Merican I, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Chien RN, et al. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol 2000;15:1356-1361.
4. Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. J Gastoenterol Hepatol 1997;12:S294-S298.
5. Chen CJ, Yang HI, Jun S, 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.
6. Liaw YF, Sung JJY, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521-1531.
7. Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, et al. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. Hepatol Res 2005;32:173-184.

8. Yokosuka O, Takaguchi K., Fujioka S, Shindo M, Chayama K, Kobashi H, et al. Long-term use of entecavir in nucleoside-naïve Japanese patients with chronic hepatitis B infection. J Hepatol 2010;52:791-799.
9. Chang TT, Lai CL, Yoon SK, Lee SS, Coelho HSM, Carrilho FJ, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. HEPATOLOGY 2010;51:422-430.
10. Yang HI, Yuen MF, Chan HLY, Han KH, Chen PJ, Kim DY, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol 2011;12:568-574.
11. Yuen MF, Tanaka Y, Fong DYT, Fung J, Wong DKH, Yuen JCH, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. J Hepatol 2009;50:80-88.
12. Wong VWS, Chan SL, Mo F, Chan TC, Loong HH, Wong GL, et al. Clinical scoring system to predict hepatocellular carcinoma in chronic hepatitis B carriers. J Clin Oncol 2010;28:1660-1665.
13. Yang HI, Sherman M, Su J, Chen PJ, Liaw YF, Iloeje UH, et al. Nomograms for risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. J Clin Oncol 2010;28:2437-2444.
14. Rosenbaum PR, Rubin DB. Reducing bias in observational studies using subclassification on the propensity score. J Am Stat Assoc 1984; 79:516-524.
15. Braitman LE, Rosenbaum PR. Rare outcomes, common treatments: analytic strategies using propensity scores. Ann Intern Med 2002;137:693-695.
16. Rosenbaum PR, Rubin DB. Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. J Am Stat Assoc 1985;39:33-38.
17. D'Agostino RB Jr. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. Stat Med 1998;17:2265-2281.
18. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat 1988;16:1141-1154.
19. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496-509.
20. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, et al. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol 1998;28:930-938.
21. Kato Y, Nakata K, Omagari K, Furukawa R, Kusumoto Y, Mori I, et al. Risk of hepatocellular carcinoma in patients with cirrhosis in Japan. Cancer 1994;74:2234-2238.
22. Lo KJ, Tong MJ, Chien MC, Tsai YT, Liaw YF, Yang KC, et al. The natural course of hepatitis B surface antigen-positive chronic active hepatitis in Taiwan. J Infect Dis 1982;146:205-210.
23. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2022;347:168-174.
24. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118:554-559.
25. Chen CJ, Yang HI, Iloeje UH. Hepatitis B virus DNA levels and outcomes in chronic hepatitis B. HEPATOLOGY 2009;49:S72-S84.
26. Zhang Q-Q, An X, Liu YH, Li SY, Zhong Q, Wang J, et al. Long-term nucleos(t)ide analogues therapy for adults with chronic hepatitis B reduces the risk of long-term complications: a meta-analysis. Virol J 2011;8:72.
27. 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: 348-356.
28. 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. Gastoenterology 2011;141: 1240-1248.
29. Kurokawa M, Hiramatsu N, Oze T, Yakushijin T, Miyazaki M, Hosui A, et al. Long-term effect of lamivudine treatment on the incidence of hepatocellular carcinoma in patients with hepatitis B virus infection. J Gastroenterol 2012;47:577-585.

J Gastroenterol (2014) 49:538–546
DOI 10.1007/s00535-013-0821-2

The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Seroclearance rate of hepatitis B surface antigen in 2,112 patients with chronic hepatitis in Japan during long-term follow-up

Mariko Kobayashi · Tetsuya Hosaka · Fumitaka Suzuki · Norio Akuta · Hitomi Sezaki · Yoshiyuki Suzuki · Yusuke Kawamura · Masahiro Kobayashi · Satoshi Saitoh · Yasuji Arase · Kenji Ikeda · Yuzo Miyakawa · Hiromitsu Kumada

Received: 5 February 2013 / Accepted: 18 April 2013 / Published online: 20 June 2013

Abstract
Background Rate of hepatitis B surface antigen (HBsAg) seroclearance was determined in 2,112 Japanese patients with chronic hepatitis B who were followed up for at least 15 years.
Methods Patients had a median age of 37 years and included 1,431 (67.8 %) men. Median values were AST/ALT, 43/62 IU/L; platelet counts, 182 × 10^3/mm^3; HBsAg, 3,400 IU/mL; and hepatitis B virus (HBV) DNA, 6.2 log copies/mL. Factors influencing HBsAg seroclearance were evaluated by the Cox proportional model and annual rate of HBsAg seroclearance by the Kaplan–Meier life table method.
Results The overall annual rate of HBsAg seroclearance was 1.75 % in 2,112 patients; it was 1.65 % in 1,130 untreated and 2.05 % in 982 treated patients ($p = 0.289$). In untreated patients, seroclearance was influenced by age, no HBV infections in third-degree or closer relatives, and HBsAg levels in univariate analysis. Seroclearance was influenced by a median age ≥50 years [relative risk (RR) 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] in multivariate analysis. In treated patients, age, male gender, no HBV infections in third-degree or closer relatives, interferon therapy, chronic hepatitis, high AST and γ-GTP levels, low platelet counts, hepatitis B e antigen (HBeAg)-negative status, low HBsAg levels and the wild-type precore sequence significantly influenced HBsAg seroclearance. In multivariate analysis, no family history [RR 2.22 ($p = 0.006$)], interferon treatment [RR 3.15 ($p < 0.001$)], and HBeAg-negative status [RR 3.75 ($p < 0.001$)] significantly influenced HBsAg seroclearance.
Conclusions In this retrospective cohort study, the annual rate of HBsAg seroclearance was 1.65 % in untreated patients and 2.05 % in treated patients.

Keywords Seroclearance · Hepatitis B surface antigen · Hepatitis B virus · Chronic hepatitis B

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ETV	Entecavir
HBeAg	Hepatitis B e antigen
HBcrAg	Hepatitis B core-related antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus DNA
HBsAg	Hepatitis B surface antigen
IFN	Interferon
LAM	Lamivudine

Introduction

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently. HBV infection is a common disease that can induce a chronic carrier state

Mariko Kobayashi (✉)
Research Institute for Hepatology, Toranomon Hospital, 1-3-1 Kajigaya, Takatsu-ku, Kawasaki 213-8587, Japan
e-mail: vj7m-kbys@asahi-net.or.jp

T. Hosaka · F. Suzuki · N. Akuta · H. Sezaki · Y. Suzuki · Y. Kawamura · Masahiro Kobayashi · S. Saitoh · Y. Arase · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital, 1-3-1 Kajigaya, Takatsu-ku, Kawasaki 213-8587, Japan

Y. Miyakawa
Miyakawa Memorial Research Foundation, Tokyo 107-0062, Japan

and is associated with the risk of developing progressive disease and hepatocellular carcinoma (HCC) [1–5]. In regions highly endemic for HBV, such as Asia and Africa, the persistent carrier state is established by perinatal transmission or early in infancy. Carriers serve as the reservoir of HBV in the community and can spread the infection to susceptible individuals. The incidence of HCC is decreased extremely by eradicating HBV from the circulation that is responsible for liver damage [6–9]. In Japan, interferon (IFN) was introduced for the treatment of persistent HBV infections, and long-term IFN increased seroclearance of hepatitis B surface antigen (HBsAg) [10]. Since 2000, the effect of long-term nucleot(s)ide analogues, such as lamivudine [11, 12] and entecavir [13], on HBsAg seroclearance has been monitored in Japan.

In the current study, we followed untreated or treated patients for at least 15 years. We evaluated the seroclearance of HBsAg, achieved in both groups of patients, by using highly sensitive assays. Our aim was to determine factors that can lead to HBsAg seroclearance and to elucidate the factors associated with its success.

Patients and methods

Patients

During at least 15 years from 1968, 2,112 consecutive patients, chronically mono-infected with HBV (confirmed by HBsAg-positivity for at least 6 months) were followed at the Department of Hepatology, Toranomon Hospital, in Metropolitan Tokyo. Patients met the following inclusion and exclusion criteria: (1) negativity for hepatitis C antibody and/or hepatitis C virus RNA by polymerase chain reaction (PCR) in the serum; (2) no history of HCC; and (3) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis B. Thus, the 2,112 patients were enrolled in this cohort study. A written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved a priori by the institution's human research committee.

Treatment

Nine hundred and eighty-two patients received antiviral treatments. Of them, 156 patients received prednisolone (PSL) 40 mg daily for 1 week, 30 mg daily for 1 week, 20 mg daily for 1 week, and then 10 mg daily for 1 week until it was abruptly withdrawn (total 700 mg). A total of 428 patients received 100 mg lamivudine (LAM) daily as an initial therapy. In total, 333 patients received 3–12 MU of IFN-α or IFN-β. The durations and regimens of treatment were as follows: daily for 2 or 4 weeks and then 2 or 3 times per week for 26–104 weeks. The median duration of treatment was 26 weeks (range 4–981). There were 190 (57 %) patients who received multiple treatments of IFN.

LAM treatment was continued as a rule; median duration of LAM treatment was 75 months (55–102). LAM-resistant rtM204I/V mutants developed in 151 (35 %) of the 428 patients, and they were provided with adefovir dipivoxil (10 mg) added on LAM, as a rescue therapy. The remaining patients continued to receive LAM monotherapy. In addition, 65 patients received 0.5 mg entecavir (ETV) daily as an initial therapy. ETV treatment was continued as a rule, and median duration of ETV treatment was 45 months (1.0–104).

Markers of HBV infection

Serum HBsAg titers were determined annually using ARCHITECT HBsAg QT assay kits (Abbott Laboratories, Tokyo, Japan), which have a lower limit of detection of 0.05 IU/mL and an upper limit of detection of 250 IU/mL. To expand the upper limit from 250 to 125,000 IU/mL, serum samples going off the scale were diluted stepwise to 1:20 and 1:500 with ARCHITECT diluents following instructions from the manufacturer.

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). HBV DNA was quantified using the Amplicor monitor assay (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.6–7.6 log copies/mL, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.1–9.0 log copies/mL. Hepatitis B core-related antigen (HBcrAg) was determined by chemiluminescence enzyme immunoassay (CLEIA) with the HBcrAg assay kit (Fujirebio Inc., Tokyo, Japan). A commercial kit (HBV Genotype EIA; Institute of Immunology, Tokyo, Japan) was used to serologically determine HBV genotypes by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the 7 major genotypes (A–G).

Statistical analysis

Baseline data were obtained on the day of the first visit in untreated patients. In patients who received antivirals, baseline data were obtained at the start of the first day of treatment. Categorical data were compared between groups by chi-squared or Fisher's exact tests. Continuous variables with a nonparametric distribution were analyzed by Mann–Whitney *U* tests, whereas those with a parametric distribution were analyzed by the Student's *t* test. Cox

regression analyses were used to assess variables that were significantly associated with HBsAg seroclearance. All baseline factors that were found to be significantly associated with HBsAg seroclearance by univariate analysis were entered into a multivariate analysis. Independent baseline factors associated with the seroclearance of HBsAg were evaluated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg seroclearance while on-treatment factors and independent baseline factors had been adjusted.

Cumulative HBsAg seroclearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were evaluated using log-rank tests. Significance was defined as $p < 0.05$ for all two-tailed tests. Data analysis was performed with the SPSS software package version 11.0.1 J (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics in the 2,112 patients

The baseline characteristics of studied patients are shown in Table 1. They had a median age of 37 years (range 1–81), included 1,431 (67.8 %) men, and 2,031 (96.2 %) of them had chronic hepatitis. Their baseline values were AST/ALT, 43 (3–2,192)/62 (2–3,020 IU/L); γ-GTP, 27 (4–1,494) IU/L; platelet counts, 182 (40–483) × 10^3/mm^3; and HBV markers were HBsAg, 3,400 (0.06–27,700) IU/mL; and HBV DNA, 6.2 (<2.1 to >9.1) log copies/mL. HBeAg was not detectable in 5.4 % of studied patients, and the distribution of genotypes A/B/C/others was 4.5:15.6:79.6:0.3 %.

The HBsAg seroclearance rate analyzed by the Kaplan–Meier method was 9 % in 5 years, 17 % in 10 years, 27 % in 15 years, 35 % in 20 years, 44 % in 25 years, and 54 % in 30 years. The annual rate of HBsAg seroclearance was 1.75 % during 20 years (Fig. 1).

In the 2,112 patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were cirrhosis [relative risk (RR) 2.40 ($p = 0.014$)]; HBeAg negative [RR 3.01 ($p = 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.13 ($p = 0.004$)]. In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: HBeAg negative [RR 1.81 ($p < 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.60 ($p < 0.001$)] (Table 2).

Untreated patients and treated patients

Differences in the baseline characteristics between 1,130 untreated and 982 treated patients are shown in Table 3: age [31 years vs. 36 ($p < 0.001$)]; male gender [62.4 vs. 71.9 % ($p < 0.001$)]; AST [median 27 vs. 56 IU/L ($p < 0.001$)]; ALT [median 28 vs. 96 IU/L ($p < 0.001$)]; γ-GTP [median 20 vs. 45 IU/L ($p < 0.001$)]; total bilirubin

Table 1 Baseline characteristics 2,112 patients infected with HBV followed for longer than 15 years

Features at the baseline	Patients (n = 2,112)
Demographic data	
Age (years)	37 (1–81)
Men	1,431 (67.8 %)
Liver disease	
Chronic hepatitis	2,031 (96.2 %)
Cirrhosis	81 (3.8 %)
Laboratory data	
AST (IU/L)	43 (3–2,192)
ALT (IU/L)	62 (2–3,020)
γ-GTP (IU/L)	27 (4–1,494)
Total bilirubin (mg/dL)	0.7 (0.1–21.2)
Albumin (g/dL)	4.3 (1.1–5.8)
Platelets (×10^3/mm^3)	182 (40–483)
α-Fetoprotein (μg/L)	4 (1–2,060)
HBV markers	
HBeAg-negative status	1,169 (55.4 %)
HBsAg (IU/mL)	3,400 (0.06–277,000)
HBcrAg (log U/mL)	5.4 (<3.0 to >6.8)
Genotypes (A/B/C/others)	4.5 %/15.6 %/79.6 %/0.3 %
HBV DNA (log copies/mL)	6.2 (<2.1 to >9.1)

Median values with the range in parentheses or numbers with the percentage in parentheses are given

HBV hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

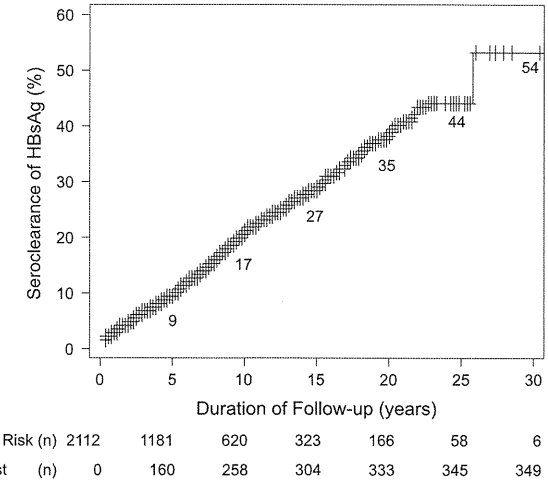


Fig. 1 Seroclearance of HBsAg in the 2,112 patients studied. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

Table 2 Factors influencing the seroclearance of HBsAg in 2,112 patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value
Age ≥50 years	1.06 (0.64–1.76)	0.824		
Male gender	1.15 (0.69–1.90)	0.594		
No HBV infection in family	1.55 (0.93–2.57)	0.092		
Treatment	1.26 (0.72–2.19)	0.413		
Cirrhosis	2.40 (1.20–4.83)	0.014		
AST ≥50 IU/L	1.30 (0.66–2.57)	0.454		
ALT ≥50 IU/L	1.81 (0.89–3.70)	0.104		
γ-GTP ≥20 IU/L	1.26 (0.72–2.23)	0.418		
Total bilirubin ≥1 mg/dL	1.39 (0.69–2.79)	0.358		
Albumin ≥4 g/dL	1.03 (0.58–1.81)	0.927		
Platelets >150 × 10^3/mm^3	1.22 (0.68–2.18)	0.501		
α-Fetoprotein ≤10 μg/L	1.06 (0.59–1.89)	0.845		
Genotype A or B, C	1.55 (0.86–2.76)	0.142		
HBeAg-negative status	3.01 (0.79–2.07)	0.001	1.81 (1.30–2.77)	<0.001
HBV DNA ≥5 log copies/mL	1.17 (0.64–2.15)	0.612		
HBsAg ≤2,000 IU/mL	2.13 (1.27–3.56)	0.004	2.60 (1.94–3.50)	<0.001
HBcrAg ≥4 log U/mL	1.11 (0.61–2.03)	0.731		
Wild-type precore sequence	0.98 (0.59–1.53)	0.964		
Wild-type core promoter sequence	2.74 (0.80–9.30)	0.104		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

[median 0.5 vs. 0.7 mg/dL ($p < 0.001$)]; albumin [median 4.4 vs. 4.3 g/dL ($p < 0.001$)]; platelets [median 202 vs. 181 × 10^3/mm^3 ($p < 0.001$)]; α-fetoprotein [median 4 vs. 4 μg/L ($p < 0.001$)]; HBeAg-negative status [75.8 vs. 31.8 % ($p < 0.001$)]; HBsAg levels [median 2,240 vs. 5,270 IU/mL ($p < 0.001$)]; HBcrAg [median 3.6 vs. >6.8 log U/mL ($p < 0.001$)]; distribution of genotypes A/B/C/others (5.7/20.0/72.6/1.7 vs. 3.4/11.1/84.9/0.5 %, $p < 0.001$); and HBV DNA [median 4.7 vs. 8.0 log copies/mL ($p < 0.001$)].

The rate of HBsAg seroclearance in treated patients was 8 % in 5 years, 20 % in 10 years, 28 % in 15 years, 41 % in 20 years, 49 % in 25 years, and 49 % in 30 years, with an annual HBsAg seroclearance rate of 2.05 % (Fig. 2). The rate in untreated patients was 9 % in 5 years, 18 % in 10 years, 26 % in 15 years, 33 % in 20 years, 42 % in 25 years, and 56 % in 30 years, with an annual HBsAg seroclearance rate of 1.65 %. No differences in the annual HBsAg seroclearance rate were noted between treated and untreated patients ($p = 0.289$).

HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, HBsAg persisted in 930 (82.3 %), whereas HBsAg seroclearance occurred in 200 (17.7 %). In the baseline characteristics, significant differences were found for age ($p < 0.001$), male gender ($p = 0.003$), chronic hepatitis ($p = 0.020$), γ-GTP ($p < 0.001$), albumin ($p = 0.004$), HBV genotypes ($p < 0.001$), HBeAg-negative status ($p < 0.001$), HBV DNA ($p < 0.001$), HBsAg level ($p < 0.001$), HBcrAg ($p < 0.001$), precore wild-type ($p < 0.001$), and core promoter wild-type ($p = 0.001$) (Table 4).

Factors contributing to HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.63 ($p = 0.002$)]; no family history in third-degree or closer relatives [RR 1.38 ($p = 0.037$)]; and HBsAg ≤2,000 IU/mL [RR 1.87 ($p < 0.006$)].

In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: age ≥50 [RR 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] (Table 5).

HBsAg seroclearance in treated patients

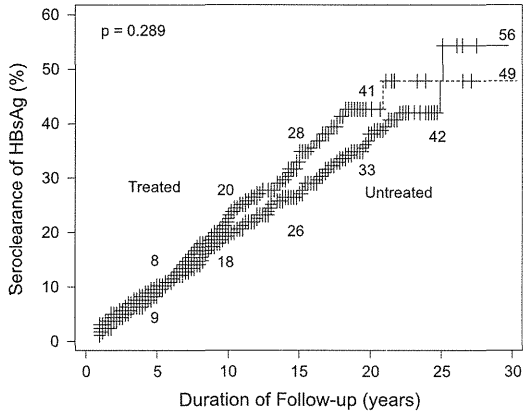
In the 982 treated patients, HBsAg persisted in 833 (84.8 %). HBsAg seroclearance occurred in 149 (15.2 %). In the baseline characteristics, significant difference were found for male gender ($p = 0.004$), no family history in third-degree or closer relatives ($p = 0.010$), chronic hepatitis ($p = 0.001$), AST ($p = 0.010$), γ-GTP ($p = 0.023$), platelet counts ($p < 0.001$), HBeAg-negative status

Table 3 Baseline characteristics in untreated and treated patients

Features at the baseline	Untreated (n = 1,130)	Treated (n = 982)	Differences p value
Age (years)	31 (1–81)	36 (6–75)	<0.001
Men	705 (62.4 %)	726 (71.9 %)	<0.001
Chronic hepatitis	1,094 (96.8 %)	937 (96.4 %)	0.079
Cirrhosis	36 (3.2 %)	45 (3.6 %)	
AST (IU/L)	27 (3–1,776)	56 (6–2,192)	<0.001
ALT (IU/L)	28 (2–3,020)	96 (8–2,740)	<0.001
γ-GTP (IU/L)	20 (4–1,494)	45 (4–1,278)	<0.001
Total bilirubin (mg/dL)	0.5 (0.1–20.1)	0.7 (0.2–21.2)	<0.001
Albumin (g/dL)	4.4 (2.2–5.8)	4.3 (1.1–5.4)	<0.001
Platelets ($\times 10^3$/mm^3)	202 (40–443)	181 (40–483)	<0.001
α-Fetoprotein (μg/L)	4 (1–2,060)	4 (1–1,610)	<0.001
HBeAg-negative status	857 (75.8 %)	312 (31.8 %)	<0.001
HBsAg (IU/mL)	2,240 (0.06–141,000)	5,270 (0.09–277,000)	<0.001
HBcrAg (log U/mL)	3.6 (<3.0 to >6.8)	> 6.8 (<3.0 to >6.8)	<0.001
Genotypes [A/B/C/others (%)]	5.7/20.0/72.6/1.7	3.4/11.1/84.9/0.5	<0.001
HBV DNA (log copies/mL)	4.7 (<2.1 to >9.1)	8.0 (<2.1 to >9.1)	<0.001

Median values with the range in parentheses or numbers with the percentage in parentheses are given

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen



Treated							
Patients at Risk (n)	982	529	221	104	39	8	3
HBsAg Lost (n)	0	66	114	133	145	148	149
Untreated							
Patients at Risk (n)	1130	652	399	219	127	50	3
HBsAg Lost (n)	0	91	142	170	187	197	200

Fig. 2 Comparison of HBsAg seroclearance rates between 982 treated and 1,130 untreated patients. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

($p < 0.001$), HBV DNA ($p = 0.002$), HBsAg ($p < 0.001$), HBcrAg ($p = 0.003$), and precore wild-type ($p = 0.013$) (Table 6).

Factors contributing to HBsAg seroclearance in treated patients

In the 982 treated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.91 ($p = 0.001$)]; male gender [RR 2.14 ($p = 0.001$)], no family history in third-degree or closer relatives [RR 1.58 ($p = 0.005$)]; previous treatment with interferon [RR 2.13 ($p < 0.001$)]; chronic hepatitis [RR 3.12 ($p < 0.001$)]; AST ≥50 IU/L [RR 1.47 ($p = 0.031$)]; γ-GTP ≥20 IU/L [RR 1.87 ($p = 0.001$)]; platelets ≤150 × 10^3/mm^3 [RR 2.10 ($p < 0.001$)]; HBeAg-negative status [RR 2.53 ($p < 0.00$)]; HBV DNA ≤5 log copies/mL [RR 2.07 ($p = 0.001$)]; HBsAg ≤2,000 IU/mL [RR 2.29 ($p < 0.001$)]; HBcrAg ≤4 log U/mL [RR 2.28 ($p = 0.003$)]; and the wild-type precore sequence [RR 2.04 ($p = 0.011$)].

In multivariate analysis, only 3 factors contributed to HBsAg seroclearance: no family history in third-degree or closer relatives [RR 2.22 ($p = 0.006$)]; previous treatments with interferon [RR 3.15 ($p < 0.001$)]; and HBeAg-negative status [RR 3.75 ($p < 0.001$)] (Table 7).

Discussion

In Japan, perinatal materno-fetal transmission was the main route of HBV infection, but this transmission has been prevented since 1986 by the national campaign to prevent it by immunoprophylaxis with combined passive-active immunization of babies born to HBeAg-positive carrier mothers. However, HCC develops in about 10 % of the patients who have established chronic HBV infection by materno-fetal infection or through child-to-child transmission. Hence, HBsAg seroclearance is crucially required for preventing the development of cirrhosis followed by HCC.

In the present study, we analyzed 2,112 patients with persistent HBV infection to establish the factors

Table 4 Differences between the baseline characteristics of 917 untreated patients in whom HBsAg persisted and 213 those who lost HBsAg

Features at the baseline	HBsAg persisted ($n = 917$)	HBsAg lost ($n = 213$)	Differences p value
Age (years)	37 (1–81)	44 (0–80)	<0.001
Men	553 (60.3 %)	152 (71.4 %)	0.003
HBV in family members	349 (38.1 %)	76 (35.7 %)	0.509
Chronic hepatitis	893 (97.4 %)	201 (94.4 %)	0.020
AST (IU/L)	27 (3–1,144)	25 (6–1,776)	0.283
ALT (IU/L)	28 (6–1,960)	27 (6–3,020)	0.389
γ-GTP (IU/L)	22 (1–1,494)	29 (4–1,092)	<0.001
Total bilirubin (mg/dL)	0.6 (0.2–20.1)	0.7 (0.1–4.0)	0.257
Albumin (g/dL)	4.3 (2.0–5.3)	4.4 (1.6–5.7)	0.004
Platelets ($\times 10^3$/mm^3)	203 (40–443)	203 (33–417)	0.473
α-Fetoprotein (μg/L)	3 (1–2,060)	1 (1–478)	0.373
Genotypes [A/B/C/others (%)]	5.7/19.0/73.3/1.9	5.5/24.7/69.2/0.7	<0.001
HBeAg-negative status	663 (72.3 %)	194 (91.1 %)	<0.001
HBV DNA (log copies/mL)	4.9 (<2.1 to >9.1)	3.8 (<2.1 to >9.1)	<0.001
HBsAg (IU/mL)	3,100 (1.94–141,000)	149 (0.06–88,800)	<0.001
HBcrAg (log U/mL)	3.9 (<3.0 to >6.8)	2.9 (<3.0 to >6.8)	<0.001
Wild-type precore sequence	441 (48.1 %)	160 (75.0 %)	<0.001
Wild-type core promoter sequence	320 (34.9 %)	47 (22.0 %)	0.001

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 5 Factors influencing the seroclearance of HBsAg in untreated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.63 (1.19–2.23)	0.002	1.61 (1.09–2.37)	0.018
Male gender	1.08 (0.79–1.48)	0.618		
No HBV infection in family	1.38 (1.02–1.86)	0.037		
Cirrhosis	1.19 (0.73–1.93)	0.484		
AST ≥50 IU/L	1.01 (0.70–1.45)	0.979		
ALT ≥50 IU/L	0.93 (0.68–1.27)	0.633		
γ-GTP ≥20 IU/L	1.17 (0.85–1.61)	0.330		
Total bilirubin ≥1 mg/dL	1.41 (0.80–2.49)	0.239		
Albumin ≥4 g/dL	0.78 (0.51–1.18)	0.239		
Platelets >150 × 10^3/mm^3	0.99 (0.67–1.46)	0.946		
α-Fetoprotein ≤10 μg/L	0.84 (0.48–1.47)	0.543		
Genotype A or B	1.17 (0.81–1.69)	0.410		
HBeAg-negative status	0.78 (0.79–2.07)	0.314		
HBV DNA ≥5 log copies/mL	0.84 (0.58–1.24)	0.383		
HBsAg ≤2,000 IU/mL	1.87 (1.19–2.91)	0.006	1.77 (1.12–2.77)	0.014
HBcrAg ≥4 log U/mL	0.85 (0.50–1.45)	0.555		
Wild-type precore sequence	0.99 (0.60–1.52)	0.967		
Wild-type core promoter sequence	0.78 (0.35–1.73)	0.538		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

contributing to HBsAg seroclearance. The overall rate of HBsAg seroclearance was 1.75 % annually. The annual seroclearance rates of HBsAg are reported to be 1.7 % in Korea [14] and 1.6 % in Taiwan [15–17], as well as 2.5 % in Goto Islands of Japan, where HBV infections are very prevalent [18]. In 1,271 natives in Alaska, the rate of HBsAg seroclearance was 0.7 % annually [19]. These differences could be ascribed, in part, to HBV genotypes distinct among Asian countries and Alaska. Since treatment with IFN and/or nucleot(s)ide analogues has suppressive effects on the development of HCC [6, 20], they may influence HBsAg seroclearance.

Table 6 Differences in baseline characteristics between the 833 treated patients in whom HBsAg persisted and 149 those who lost HBsAg

Features at the baseline	HBsAg persisted (n = 833)	HBsAg lost (n = 149)	Differences p value
Age (years)	41 (13–88)	43 (17–71)	0.285
Men	601 (72.2 %)	124 (83.2 %)	0.004
HBV in family members	496 (59.6 %)	72 (48.3 %)	0.010
Chronic hepatitis	802 (96.3 %)	134 (89.9 %)	0.001
AST (IU/L)	54 (6–2,192)	78 (7–888)	0.010
ALT (IU/L)	93 (8–2,740)	118 (8–1,700)	0.117
γ-GTP (IU/L)	44 (4–1,278)	46 (4–1,278)	0.023
Total bilirubin (mg/dL)	0.7 (0.2–21.2)	0.7 (0.3–8.4)	0.273
Albumin (g/dL)	4.3 (1.1–5.4)	4.5 (1.4–5.3)	0.281
Platelets ($\times 10^3$/mm^3)	182 (40–483)	171 (50–391)	<0.001
α-Fetoprotein (μg/L)	4 (1–1,610)	4 (1–765)	0.682
Genotypes [A/B/C/others (%)]	3.2/10.7/85.1/1.0	5.1/12.4/81.6/0.9	0.565
HBeAg-negative status	230 (27.6 %)	79 (53.0 %)	<0.001
HBV DNA (log copies/mL)	7.8 (<2.1 to >9.1)	8.3 (<2.1 to >9.1)	0.002
HBsAg (IU/mL)	7,880 (0.04–277,000)	1,380 (0.04–188,000)	<0.001
HBcrAg (log U/mL)	6.9 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	0.003
Wild-type precore sequence	554 (66.6 %)	61 (41.2 %)	0.013
Wild-type core promoter sequence	274 (32.9 %)	67 (45.0 %)	0.836

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 7 Factors influencing the seroclearance of HBsAg in treated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.91 (1.32–2.77)	0.001		
Male gender	2.14 (1.37–3.33)	0.001		
No HBV infection in family	1.58 (1.15–2.19)	0.005	2.22 (2.32–3.94)	0.006
Treatments (interferon vs. others)	2.13 (1.53–2.98)	<0.001	3.15 (1.69–5.87)	<0.001
Chronic hepatitis	3.12 (2.05–4.74)	<0.001		
AST ≥50 IU/L	1.47 (1.04–2.09)	0.031		
ALT ≥50 IU/L	1.29 (0.82–1.92)	0.201		
γ-GTP ≥20 IU/L	1.87 (1.30–2.70)	0.001		
Total bilirubin ≥1 mg/dL	1.35 (0.87–2.08)	0.179		
Albumin ≥4 g/dL	1.11 (0.66–1.86)	0.688		
Platelets ≤150 × 10^3/mm^3	2.10 (1.49–2.96)	<0.001		
α-Fetoprotein ≤10 μg/L	1.33 (0.92–1.92)	0.136		
Genotype A or B vs. others	1.16 (0.74–1.82)	0.529		
HBeAg-negative status	2.53 (1.83–3.50)	<0.001	3.75 (2.09–6.74)	<0.001
HBV DNA ≤5 log copies/mL	2.07 (1.37–3.13)	0.001		
HBsAg ≤2,000 IU/mL	2.29 (1.52–3.47)	<0.001		
HBcrAg ≤4 log U/mL	2.28 (1.31–3.97)	0.003		
Wild-type precore sequence	2.04 (1.18–3.55)	0.011		
Wild-type core promoter sequence	1.18 (0.63–2.21)	0.608		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A176.2/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Therefore, we went on to extend our analysis to untreated patients and those treated with IFN or nucleotide analogues separately. Criteria for upper or lower levels of each parameter were set, taking into consideration the median value or a cutoff value with the lowest p value of the entire 2,112-patient cohort (Table 1), and unified for untreated and treated patients (Tables 5, 7).

Firstly, in the univariate analysis, age, no family history of HBV infection in third-degree or closer relatives, and decreased HBsAg levels lowered the annual rate of HBsAg seroclearance significantly. In multivariate analysis, age $\geq$50 years (RR 1.61, $p = 0.018$) and HBsAg $\leq$2,000 IU/mL (RR 1.77, $p = 0.014$) decreased the annual rate of HBsAg seroclearance significantly. Kato et al. [18] reported high HBsAg seroclearance rates in patients over 40 or over 50 years; in our patients, also, age $\geq$50 years increased RR to 1.61 ($p = 0.018$). As for HBsAg and HBV DNA, low HBsAg and HBV DNA levels increased the HBsAg seroclearance rate to 37.7 %, and therefore, low HBsAg levels are an important factor. In actuality, HBsAg levels $\leq$2,000 IU/mL increased the rate of HBsAg seroclearance with RR 1.77 ($p = 0.014$).

In treated patients, by contrast, age, the male gender, no HBV infections in third-degree or closer relatives, treatment with IFN, chronic hepatitis, high AST levels, high γ-GTP levels, low platelet counts, HBeAg-negative status, low HBsAg levels, low HBcrAg levels and the wild-type precore sequence were significant factors in univariate analysis. In multivariate analysis, no HBV infections in third-degree or closer relatives (RR 2.22, $p = 0.006$), interferon treatments (RR 3.15, $p < 0.001$), and HBeAg-negative status (RR 3.75, $p < 0.001$) were significant factors.

Thus, there were differences in factors predictive of the HBsAg loss between untreated and treated patients. Remarkably, age and HBsAg titer were independent factors in untreated patients, whereas family history and negative HBeAg were independent factors in treated patients. Since this work studied patients who were followed for a long time (>15 years), age and HBsAg titer were factors for clearance of HBsAg in untreated patients. Treated patients, in contrast, would have included more patients with HBeAg, with a good response to antiviral treatment, as well as those without family history who would have been infected with HBV with a sorter duration than those with family history. In other words, most untreated patients were those with favorable clinical course, in whom HBsAg titer gradually decreased and eventually lost it with time. In fact, there would be many such patients, the majority of whom do not visit hospitals and are unaware of HBV infection, who may have unapparent liver disease. Treated patients, on the other hand, would have had higher risks for cirrhosis and HCC, owing to elevated ALT/AST levels; this risk is especially high for patients with a family history of HBV [21]. Therefore, patients with family history would not be able to easily lose HBsAg.

In treated patients, IFN led to HBsAg loss more effectively than other treatments [RR 2.13, $p < 0.001$ (Table 7)]. The immunomodulatory activity of IFN, which is not shared by nucleot(s)ide analogues, would have accelerated the immune response to HBV required for the seroclearance of HBsAg. Of the 333 patients who received IFN, 190 (57 %) were treated with IFN multiply. In them, seroclearance of HBsAg was achieved in 49 of the 190 (26 %) patients with multiple IFN treatments in comparison with 41 of the 143 (29 %) with single IFN treatment. Owing to indications for IFN, patients who received IFN tended to be younger, without previous treatments and higher HBV DNA as well as ALT levels. They might have increased the rate of HBsAg loss that was higher with IFN than other treatments.

Since this is a retrospective cohort study of patients visiting our hospital for more than 15 years, and there has been so much innovation in the treatment of chronic hepatitis B during that period, treated and untreated patients have different backgrounds at the baseline. Hence, treated patients had higher ALT and HBV DNA levels with severer liver disease than untreated patients (Table 3). This might have been responsible, at least in part, for the failure in finding differences in the rate of HBsAg loss between untreated and treated patients (Fig. 2). Future studies will be aimed at analyzing contributing factors in treated and matched controls. This will allow us to analyze factors contributing to HBsAg seroclearance in the treatment of patients with chronic hepatitis B.

Acknowledgments This work was supported in part by grants from the Ministry of Health, Labour and Welfare in Japan.

Conflict of interest These authors disclose the following: Dr. Kumada reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co., MSD KK, Bristol-Myers Squibb, Pharma International, Dentsu Sudler, and Hennessey Inc. Dr. Ikeda reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co. No other potential conflicts of interest relevant to this article were reported.

References

1. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004;350:1118–29.
2. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337:1733–45.
3. Chen CJ, Yang HI, 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.

4. Huo TI, Wu JC, Lee PC, Chau GY, Lui WY, Tsay SH, et al. Seroclearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. Hepatology. 1998;28:231–6.
5. 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.
6. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, et al. Long-term outcome after hepatitis B surface antigen seroclearance in patients with chronic hepatitis B. Am J Med. 2006;119:71.e9–16.
7. Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology. 2002;123:1084–9.
8. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology. 2007;45:1187–92.
9. Yuen MF, Wong DK, Fung J, Ip P, But D, Hung I, et al. HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.
10. Suzuki F, Arase Y, Suzuki Y, Akuta N, Sezaki H, Seko Y, et al. Long-term efficacy of interferon therapy in patients with chronic hepatitis B virus infection in Japan. J Gastroenterol. 2012;47:814–22.
11. Kobayashi M, Suzuki F, Akuta N, Hosaka T, Sezaki H, Yatsuji H, et al. Loss of hepatitis B surface antigen from the serum of patients with chronic hepatitis treated with lamivudine. J Med Virol. 2007;79:1472–7.
12. Akuta N, Suzuki F, Suzuki Y, Sezaki H, Hosaka T, Someya T, et al. Favorable efficacy of long-term lamivudine therapy in patients with chronic hepatitis B: an 8-year follow-up study. J Med Virol. 2005;75:491–8.
13. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Clearance of hepatitis B surface antigen during long-term nucleot(s)ide analog treatment in chronic hepatitis B: results from a nine-year longitudinal study. J Gastroenterol. 2012. doi:10.1007/s00535-012-0688-7.
14. Ahn SH, Park YN, Park JY, Chang HY, Lee JM, Shin JE, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol. 2005;42:188–94.
15. Kim JH, Lee JH, Park SJ, Bae MH, Kim JH, Kim do Y, et al. Factors associated with natural seroclearance of hepatitis B surface antigen and prognosis after seroclearance: a prospective follow-up study. Hepatogastroenterology. 2008;55:578–81.
16. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2:263–83.
17. Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010;139:474–82.
18. Kato Y, Nakao K, Hamasaki K, Kato H, Nakata K, Kusumoto Y, et al. Spontaneous loss of hepatitis B surface antigen in chronic carriers, based on a long-term follow-up study in Goto Islands, Japan. J Gastroenterol. 2000;35:201–5.
19. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska natives chronically infected with hepatitis B virus. Ann Intern Med. 2001;135:759–68.
20. Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010;51:1531–7.
21. Shiraki K, Yoshihara N, Sakurai M, Eto T, Kawana T. Acute hepatitis B in infants born to carrier mothers with the antibody to hepatitis B e antigen. J Pediatr. 1980;97:768–70.

J Gastroenterol (2014) 49:470–480
DOI 10.1007/s00535-013-0779-0

The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Renal dysfunction and hypophosphatemia during long-term lamivudine plus adefovir dipivoxil therapy in patients with chronic hepatitis B

Mio Tanaka · Fumitaka Suzuki · Yuya Seko · Tasuku Hara · Yusuke Kawamura · Hitomi Sezaki · Tetsuya Hosaka · Norio Akuta · Masahiro Kobayashi · Yoshiyuki Suzuki · Satoshi Saitoh · Yasuji Arase · Kenji Ikeda · Mariko Kobayashi · Hiromitsu Kumada

Received: 26 November 2012 / Accepted: 12 February 2013 / Published online: 26 March 2013

Abstract
Background Renal dysfunction and Fanconi's syndrome associated with hypophosphatemia caused by long-term administration of low-dose adefovir dipivoxil (ADV) has been reported in recent years. The aim of this retrospective study was to determine the incidence and factors associated with renal dysfunction and hypophosphatemia in patients with hepatitis B infection on long-term treatment with ADV and lamivudine (LAM).
Methods The study subjects were 292 patients treated with 10 mg/day ADV and 100 mg/day LAM for more than 6 months. We evaluated estimated glomerular filtration rate (eGFR), serum creatinine and serum phosphate level at the start of ADV and every 6 months.
Result During a median treatment duration of 64 months, 28 (9.6 %) patients developed renal impairment (defined as eGFR < 50 ml/min/1.73 m^2), and 73 (27.1 %) developed hypophosphatemia, including 14 with persistent hypophosphatemia. The cumulative incidences of renal impairment at 1, 3, and 5 years were 1.4, 7.5, 10.5 %, respectively, and those of hypophosphatemia were 6.8, 20.6, 26.7 %, respectively. Multivariate analysis identified old age, liver cirrhosis and hypertension as determinants of renal impairment, and male sex, HCC, low baseline serum phosphate as determinants of hypophosphatemia. Three of the 14 patients with persistent hypophosphatemia developed Fanconi's syndrome; their serum creatinine level remained normal, but eGFR was lower than at baseline.
Conclusion Long-term treatment of hepatitis B with low-dose (10 mg/day) ADV and LAM can potentially cause renal impairment and hypophosphatemia. We advocate regular monitoring of serum phosphate and evaluation of eGFR, in addition to serum creatinine, in such patients.

Keywords Adefovir dipivoxil · Hepatitis B virus · Renal dysfunction · Hypophosphatemia · Fanconi's syndrome · Osteomalacia

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CHB	Chronic hepatitis B
CHBI	Chronic hepatitis B infection
CI	Confidence interval
eGFR	Estimated glomerular filtration rate
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
IFN	Interferon
IP	Inorganic phosphate
LC	Liver cirrhosis

Introduction

Hepatitis B virus (HBV) infects more than 350 million people worldwide. Hepatitis B is a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC)

M. Tanaka · F. Suzuki (✉) · Y. Seko · T. Hara · Y. Kawamura · H. Sezaki · T. Hosaka · N. Akuta · M. Kobayashi · Y. Suzuki · S. Saitoh · Y. Arase · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 150-8470, Japan
e-mail: fumitakas@toranomon.gr.jp

M. Kobayashi
Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan

[1]. The goal of therapy is to reduce HBV replication to limit progressive liver disease and improve the natural history of chronic HBV infection (CHBI) [2]. Oral nucleotide analogs are used for antiviral therapy of patients with CHBI. Lamivudine (LAM) is the first nucleotide analogue indicated for CHBI [3]. However, long-term LAM therapy is associated with emergence of drug-resistant HBV mutation, and relapse of hepatitis [4–7]. Subsequent studies indicated that adefovir dipivoxil (ADV) alone or in combination with LAM provides effective antiviral therapy in patients with LAM-resistant HBV [8, 9]. However, ADV monotherapy of LAM-resistant HBV resulted in the appearance of virological breakthrough due to acquisition of ADV-resistant mutation [10]. Therefore, the Japanese guidelines recommend the use of the combination of ADV and LAM for patients with LAM-resistant HBV [11].

Renal impairment is one of the most serious side effects of ADV. Nephrotoxicity associated with ADV is dose-dependent. In CHBI phase III trials, significant renal toxicity was not observed during a median follow-up period of 64 weeks in patient treated with ADV at 10 mg/day [12]. However, renal dysfunction associated with long-term use of low-dose ADV has been documented in a few reports published in recent years [13–15]. Moreover, a few case reports also described hypophosphatemia associated with Fanconi's syndrome in association with the use of ADV at 10 mg/day [14, 16, 17].

On the other hand, there are only a few studies on the incidence of renal dysfunction and hypophosphatemia during long-term combination therapy of ADV and LAM. In the present study, we investigated the incidence of renal impairment and hypophosphatemia associated with long-term use of ADV–LAM combination in patients with CHBI and defined the characteristics of those patients who developed the above side effects.

Patients and methods

Patients

The study group comprised 292 Japanese patients who were treated with the combination therapy of ADV and LAM between November 2002 and December 2011 at Toranomon Hospital, Tokyo, Japan. Patients were included in this study if they met the following criteria: (1) patients with LAM-refractory CHBI who commenced ADV add-on LAM at Toranomon Hospital; (2) the starting dose of ADV was 10 mg/day; (3) normal renal function at the commencement of ADV (serum creatinine < 1.2 mg/dl and estimated glomerular filtration rate (eGFR) of ≥ 50 ml/min/1.73 m^2); (4) patients who received the combination therapy for more than 6 months. Furthermore, we excluded patients who had history of treatment with other nucleotide analogs and co-infection with hepatitis C virus or human immunodeficiency virus (HIV).

Study protocol

Patients visited our hospital every 1–3 months after the initiation of ADV treatment, and blood samples were obtained at every visit. We evaluated virological and biochemical markers at the start of ADV and every 6 months thereafter. The eGFR was calculated by the Japanese GFR equation [$194 \times Cr^{1.094} \times age^{0.287}$ ($\times$ 0.739 for females)]. Renal impairment represented a decrease in eGFR to < 50 ml/min/1.73 m^2, while hypophosphatemia was defined by serum phosphate level of < 2.5 mg/dl. The dosing interval of ADV was modified by the attending physician when serum creatinine level increased to > 1.2 mg/dl. Liver cirrhosis was defined by presence of stage 4 fibrosis on histopathological examination and/or clinical evidence of portal hypertension.

The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the ethics committee of Toranomon Hospital.

Statistical analysis

Descriptive statistics were reported as proportion (%) for categorical variables, and median values (range) for continuous variables. The Mann–Whitney *U* test was used to compare two continuous variables, and Fisher's exact test or Chi square test was used to compare two categorical variables. The cumulative incidences of renal impairment and hypophosphatemia were calculated using the Kaplan–Meier method and group data were evaluated using the log-rank test. The Cox proportional hazard regression model was used to estimate univariate and multivariate risk factors for renal dysfunction and hypophosphatemia. Wilcoxon rank sum test was used to compare changes in the median values of eGFR and serum phosphate. Statistical significance was defined with two-tailed *P* value of < 0.05. Statistical analyses were performed using The Statistical Package for Social Sciences (version11; SPSS, Chicago, IL).

Results

Baseline characteristics

Table 1 lists the baseline clinical and laboratory characteristics at the start of ADV. The total duration of the combination therapy of ADV and LAM was 64.3 months (range: 6–118). The median age of the patient was 47 years

Table 1 Baseline characteristics

n	292
Age (years)	47 (25–75)
Male sex	228 (78.1 %)
Body weight (kg)	63 (39.9–92.5)
Body mass index (kg/m^2)	22.2 (15.8–36.9)
Treatment duration (months)	64.3 (6.0–118)
Current cirrhosis	67 (22.9 %)
Current and/or history of HCC	48 (16.4 %)
History of diabetes mellitus	17 (5.8 %)
History of hypertension	42 (14.4 %)
Genotype (A/B/C/others or unknown)	13/15/240/24 (4.5/5.1/82.2/8.2 %)
HBeAg (positive/negative/unknown)	114/176/2 (39.0/60.3/0.7 %)
Serum HBV-DNA (logIU/ml)	6.9 (< 2.1 to ≤9.0)
Total bilirubin (mg/dl)	0.7 (0.2–6.0)
Alanine aminotransferase (IU/ml)	86 (9–3156)
Albumin (g/dl)	3.9 (2.4–4.7)
Platelet($\times 10^4$/mm^3)	16.1 (3.1–45.2)
Creatinine (mg/dl)	0.8 (0.4–1.1)
eGFR (ml/min/1.73 m^2)	85.2 (51.2–179.9)
Inorganic phosphate (mg/dl)	3.2 (1.6–4.6)

Values are expressed as median (range), or number of patient (%)

eGFR estimated glomerular filtration rate, *HCC* hepatocellular carcinoma

(25–75), and patients were mostly men (78.1 %). Sixty-seven (22.9 %) patients had cirrhosis before starting ADV, and 48 patients (16.4 %) had a history of HCC or had HCC at study entry. Forty-two (14.4 %) patients had diabetes mellitus, and 17 (5.4 %) had arterial hypertension. The median body weight was 63 kg (39.9–92.5), and median BMI was 22.2 kg/m^2 (15.8–36.9). Baseline eGFR was 85.2 ml/min/1.73 m^2 (51.2–179.9), and phosphate was 3.2 mg/dl (1.6–4.6).

ADV-induced nephrotoxicity

Frequency of renal impairment

Twenty-eight (9.6 %) patients developed renal impairment during the combination therapy. The eGFR decreased 20–30 % from baseline in 67 (22.9 %) patients, 30–50 % in 54 (18.5 %) patients, and >50 % in 5 (1.7 %) patients. Figure 1 displays the cumulative incidence of renal impairment. Figure 1a shows the time to eGFR of < 50 ml/min/1.73 m^2 (i.e., renal impairment). The 1-, 3-, and 5-year cumulative incidence of renal impairment was 1.4, 7.5, and 10.5 %, respectively. Figure 1b shows the time to reduction in eGFR of ≥30 % from baseline. The 1-, 3-, 5-year

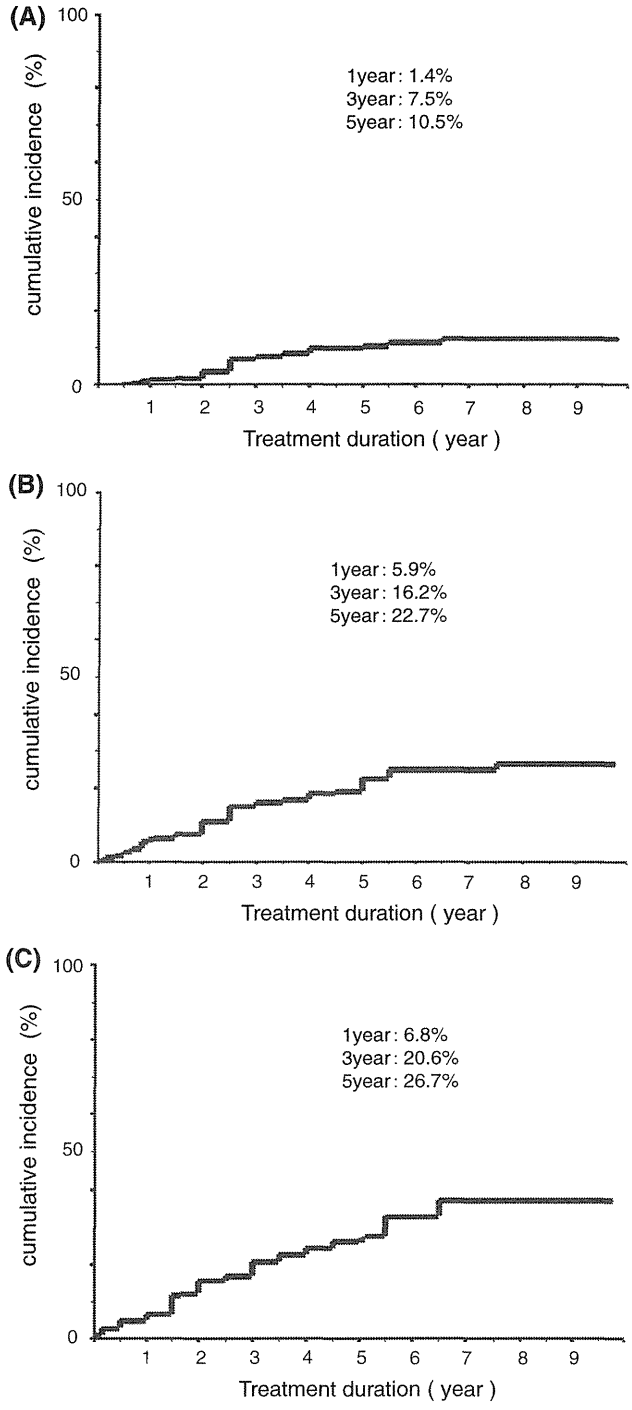


Fig. 1 Cumulative incidence of renal impairment and hypophosphatemia. **a** Cumulative incidence of reduction of eGFR to less than 50 ml/min/1.73 m^2 at 1-, 3-, and 5-years of treatment with ADV and LAM. **b** Cumulative incidence of reduction of eGFR by ≥30 % relative to baseline at 1-, 3-, and 5-years of treatment with ADV and LAM. **c** Cumulative incidence of hypophosphatemia among 269 patients with baseline IP of ≥2.5 mg/dl

cumulative incidence of reduction in eGFR ≥30 % was 5.9, 16.2, 22.7 %, respectively. We also evaluated renal function using serum creatinine. Serum creatinine increased to more

than 1.2 mg/dl in 34 (11.6 %) patients during the study period. The 1-, 3-, and 5-year cumulative incidence of serum creatinine of ≥1.2 mg/dl was 1.4, 6.51, and 11.4 %, respectively. The proportion of patients who developed renal impairment started to increase about 2 years after the commencement of ADV.

Time-course of renal impairment

Figure 2a shows serial changes in the median value of eGFR after the addition of ADV to LAM. We excluded from this analysis those patients in whom the dose of ADV was reduced at the point of modification. The eGFR of 264 patients without renal impairment remained stable throughout the study. On the other hand, the eGFR of 28 patients with renal impairment decreased rapidly within about 2 years after the addition of ADV.

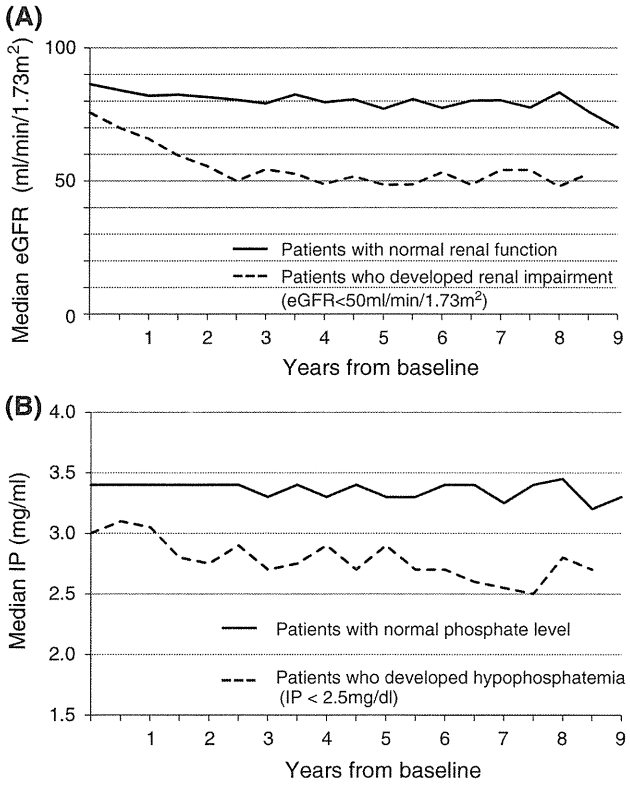


Fig. 2 Clinical course after the addition of ADV to LAM for treatment of chronic hepatitis B infection. **a** Changes in median eGFR level after the addition of ADV to LAM. *Solid line* patients with normal renal function, *broken line* patients who developed renal impairment (excluding patients who required reduction of the dose of ADV at the point). **b** Changes in the median level of serum phosphate after the addition of ADV to LAM. *Solid line* patients with normal phosphate level, broken line: patients who developed hypophosphatemia (excluding patients required reduction of the dose of ADV at the point)

Predictive factors for renal impairment

The results of univariate and multivariate analyses, including the hazard ratio for eGFR to < 50 ml/min/1.73 m^2, are shown in Table 2. Univariate analysis showed that old age (≥ 50 years, $P < 0.0001$), current cirrhosis ($P < 0.0001$), current and/or history of HCC ($P = 0.001$), history of hypertension ($P < 0.0001$), mild renal dysfunction at baseline (eGFR < 80 ml/min/1.73 m^2, $P = 0.001$), and thrombocytopenia (platelet count < $15 \times 10^4/mm^3$, $P = 0.003$) were associated with the development of nephrotoxicity. Multivariate analysis indicated that old age ($P = 0.006$), cirrhosis ($P = 0.011$), and history of hypertension ($P = 0.005$) were significant predictors of renal impairment.

Univariate and multivariate analyses were also performed for a fall in eGFR of ≥30 % relative to baseline. The results of univariate analysis showed that old age ($P < 0.0001$), female sex ($P = 0.007$), small body weight (< 60 kg, $P = 0.002$), history of diabetes mellitus ($P < 0.0001$), mild renal dysfunction at baseline ($P = 0.018$), hypo-albuminemia ($P = 0.010$), and thrombocytopenia ($P = 0.007$) were associated with decrease in eGFR of ≥30 % relative to baseline. On the other hand, multivariate analysis identified old age ($P < 0.001$), small body weight ($P = 0.015$), history of diabetes mellitus ($P = 0.020$), and mild renal dysfunction at baseline ($P < 0.0001$) as significant predictors of fall in eGFR of ≥30 % relative to baseline.

In either case, old age was a significant contributing factor of ADV-induced renal impairment. History of diabetes mellitus and arterial hypertension were also significant predictors.

Effect of modification of ADV dosing interval on renal impairment

Seventeen (5.8 %) patients required modification of the ADV dosing interval because of renal impairment. The ADV dosing interval was changed from 10 mg every day to 10 mg every other day when creatinine increased to ≥1.2 mg/dl. The clinical characteristics of the 17 patients could be summarized as follows: all were men with a median age of 54 years (35–63), 8 (47.1 %) patients had cirrhosis, 4 (23.5 %) patients had a history of HCC, baseline eGFR was 69.3 ml/min/1.73 m^2 (58.2–89.3), phosphate was 3.3 mg/dl (2.1–3.9), and the median time to modification of ADV dose was 48.5 months (20.7–70.0). Figure 3a shows changes in eGFR and Fig. 3b shows changes in serum phosphate after modification of the ADV dosing interval. The dose modification significantly improved eGFR and serum phosphate as measured at 6 months and 1 year after the modification. Analysis of the

Table 2 Determinants of renal impairment (eGFR less than 50 ml/min/1.73 m^2)

	Univariate analysis		Multivariate analysis	
	HR (95 % CI)	*P* value	HR (95 % CI)	*P* value
Age ≥50 years	7.661 (2.898–20.252)	<0.0001	4.280 (1.505–12.169)	0.006
Male sex	1.227 (0.464–3.236)	0.680		
Body weight < 60 (kg)	1.470 (0.687–3.145)	0.320		
Current cirrhosis	5.344 (2.479–11.518)	<0.0001	2.861 (1.279–6.401)	0.011
Current and/or history of HCC	3.855 (1.788–8.311)	0.001		
History of diabetes mellitus	2.841 (0.982–8.149)	0.054		
History of hypertension	5.116 (2.393–10.938)	<0.0001	3.087 (1.403–6.791)	0.005
Baseline eGFR < 80 (eGFR ≥50)	4.219 (1.786–10.00)	0.001		
Baseline IP < 3.2 mg/dl	1.634 (0.766–3.497)	0.204		
Platelet count < 15 × 10^4/mm^3	3.448 (1.511–7.874)	0.003		

CI confidence interval, *IP* inorganic phosphate, *HCC* hepatocellular carcinoma, *HR* hazard ratio

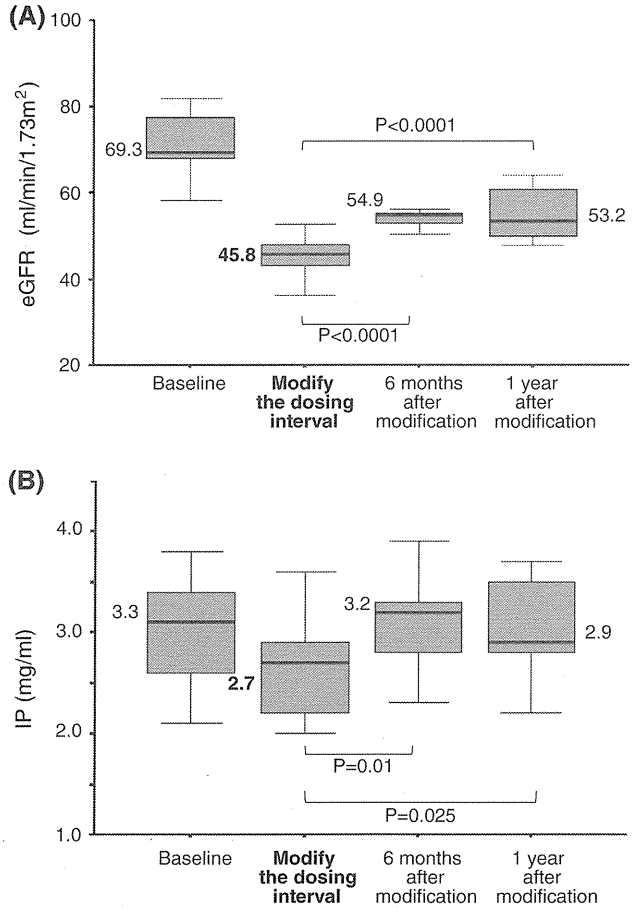


Fig. 3 Changes in eGFR and serum phosphate after modification of the ADV dosing interval. **a** Changes in eGFR. **b** Changes in serum phosphate level

long-term courses of eGFR and phosphate in these 17 patients after modification of ADV showed that the median eGFR after 1-, 2-, and 3- years of modification was 53.2, 56.7, 53.9 ml/min/1.73 m^2, respectively. eGFR remained > 50 ml/min/1.73 m^2 after modification, but never recovered to baseline level. None of the patients required discontinuation of ADV due to renal impairment.

ADV-induced hypophosphatemia

Frequency of hypophosphatemia

Seventy-three (27.1 %) of 269 patients who had normal phosphate at baseline developed hypophosphatemia during the course of the study. Fourteen (19.1 %) of the 73 patients who developed hypophosphatemia continued to show hypophosphatemia until the end of the study. On the other hand, the remaining 59 patients developed transient hypophosphatemia only. The cumulative incidence of hypophosphatemia is shown in Fig. 1c. The 1-, 3-, and 5-year cumulative incidence of hypophosphatemia was 6.8, 20.6, and 26.7 %, respectively. On the other hand, 23 patients had hypophosphatemia at baseline. Seven (30.4 %) of these 23 patients had chronic hypophosphatemia. The phosphate level of 4 (17.4 %) patients reverted spontaneously to normal, while serum phosphate level of the other 12 (52.2 %) patients fluctuated during the study.

Time-course of hypophosphatemia

Figure 2b shows changes in the median serum level of phosphate after the addition of ADV to LAM. We excluded from this analysis those patients in whom the dose of ADV was reduced at the point of modification. The median phosphate level decreased gradually after the addition of ADV in patients who subsequently developed hypophosphatemia.

Predictive factors for hypophosphatemia

Table 3 shows the results of univariate and multivariate analyses, including hazard ratio, of the factors associated