were entered into a multivariate analysis. Independent baseline factors associated with clearance of HBsAg were calculated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg while adjusting for on-treatment factors and independent baseline factors. Three covariates of the on-treatment response factors—emergence of rtM204I/V mutants, VBT, and biochemical breakthrough—were set as the time-dependent covariates. Cumulative HBsAg clearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were tested using log-rank tests. We performed Cox regression analysis, Kaplan–Meier curve analysis, and HBsAg kinetics analysis for no more than nine years, as the number of patients with a long-term follow-up of over ten years was too small to permit analysis [30]. Bonferroni adjustments were used to correct for the number of different ways a single predictor variable can be split. Significance was defined as $P < 0.05$ for all two-tailed tests. Data analysis was performed with IBM SPSS version 19.0 software (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics

Thirty-eight (4.8 %) of 791 patients successfully cleared HBsAg. Of these, 24 had received LAM, 7 had switched to ETV treatment, and 7 had been treated with both LAM and ADV (Fig. 1). Of the 38 patients who achieved HBsAg clearance, 18 were HBeAg+, whereas 20 were HBeAg− at baseline. Table 1 provides a comparison of the baseline and on-treatment characteristics between patients who were and were not able to successfully clear HBsAg (all patients, HBeAg+ and − cohorts, respectively). In the HBeAg+ cohort, baseline characteristics that were significantly associated with HBsAg clearance included previous IFN therapy, HBV genotype, HBV DNA, and AST and ALT levels; in the HBeAg− cohort, significant characteristics included HBV genotype and HBsAg levels. Significant on-treatment characteristics in the HBeAg+ cohort included decline in HBsAg, clearance of HBeAg, and decline in HBV DNA to <2.6 log copies/mL at six months;

Table 1 Baseline, demographic, and on-treatment characteristics of patients with and without HBsAg seroclearance

Characteristics	All patients (n = 791)	HBeAg+ at baseline (n = 442)			HBeAg− at baseline (n = 349)		
		Persistently HBsAg+ (n = 424)	HBsAg seroclearance (n = 18)	P	Persistently HBsAg+ (n = 329)	HBsAg seroclearance (n = 20)	P
Baseline							
Age[a] (years) (SD)	43 (11.1)	41 (11.2)	44 (10.5)	0.177	47 (10.3)	46 (10.3)	0.899
Gender (male:female)	627:164	329:95	16:2	0.385	265:64	16:4	1.000
Race				0.446			
Japanese	768 (97)	411 (97)	17 (94)		320 (97)	20 (100)	1.000
Non-Japanese (%) (Asian:Caucasian)	23 (3) (21:2)	13 (3) (20:2)	1 (3) (1:0)		9 (3) (20:2)	0 (3) (1:0)	
Family history of HBV infection	539 (68)	311 (73)	10 (56)	0.107	208 (63)	10 (50)	0.238
Previous IFN therapy	297 (38)	167 (39)	15 (83)	**<0.001**	106 (32)	9 (45)	0.326
IFN duration (weeks)	27 (20–58)	26 (18–53)	52 (21–79)	0.214	32 (22–89)	23 (14–72)	0.457
Duration from the end of IFN to start of lamivudine (weeks)	50 (3–189)	26 (7–124)	37 (2–89)	0.505	119 (3–316)	102 (18–289)	0.746
Previous NA therapy	34 (4)	21 (5)	2 (11)	0.239	10 (3)	1 (5)	0.483
Presence of cirrhosis	169 (21)	76 (18)	2 (11)	0.752	87 (26)	4 (20)	0.610
HBV genotype				**<0.001**			**<0.001**
A	28 (3.5)	14 (3.3)	6 (33)		6 (1.8)	2 (10)	
B	67 (8.5)	16 (3.8)	0 (0)		48 (14.6)	3 (15)	
C	664 (83.9)	374 (88.2)	12 (67)		265 (80.5)	13 (65)	
D	3 (0.4)	2 (0.4)	0 (0)		0 (0)	1 (5)	
F	2 (0.3)	2 (0.4)	0 (0)		0 (0)	0 (0)	
Unclassified/missing	27 (3.4)	16 (3.8)	0 (0)		10 (3.0)	1 (5)	

Table 1 continued

Characteristics	All patients (n = 791)	HBeAg+ at baseline (n = 442)			HBeAg− at baseline (n = 349)		
		Persistently HBsAg+ (n = 424)	HBsAg seroclearance (n = 18)	P	Persistently HBsAg+ (n = 329)	HBsAg seroclearance (n = 20)	P
Baseline HBV DNA (log copies/mL)	7.0 (5.8–8.0)	7.6 (6.7–8.2)	8.0 (7.5–8.4)	**0.027**	6.3 (5.2–7.2)	6.1 (5.0–7.0)	0.652
Baseline HBsAg level (IU/mL)	2530 (907–6590)	3910 (1690–12300)	5280 (943–67600)	0.331	1590 (599–3050)	529 (58–1610)	**0.004**
Baseline AST level (IU/L)	74 (48–135)	81 (52–165)	201 (78–666)	**0.011**	66 (42–113)	57 (39–96)	0.694
Baseline AST level (×ULN)	2.2 (1.5–4.1)	2.5 (1.6–5.0)	6.1 (2.3–20.2)	**0.011**	2.0 (1.3–3.4)	1.7 (1.2–2.9)	0.736
Baseline ALT level (IU/L)	115 (63–252)	130 (72–290)	326 (104–775)	**0.021**	101 (56–194)	101 (55–215)	0.904
Baseline ALT level (×ULN)	3.0 (1.7–6.4)	3.5 (1.9–7.8)	7.8 (2.5–20.3)	**0.040**	2.6 (1.4–5.2)	2.6 (1.4–5.2)	0.955
Baseline total bilirubin level (mg/dL)	0.8 (0.6–1.1)	0.8 (0.5–1.1)	0.9 (0.6–1.9)	0.117	0.7 (0.6–1.0)	0.8 (0.6–0.9)	0.556
Platelet count[a] (10^5/mm^3) (SD)	16.1 (5.7)	16.5 (6.1)	14.7 (3.5)	0.221	15.6 (5.1)	17.7 (6.9)	0.216
On-treatment response							
Decline of HBsAg level (≥0.5 log IU/mL within six months)	97 (1)	67 (16)	13 (72)	**<0.001**	11 (3)	6 (30)	**<0.001**
HBeAg positive → clearance within six months	109 (14)	94 (22)	10 (56)	**0.005**	NA	NA	
Undetectable HBV DNA (<400 copies/mL) at six months	532 (67)	221 (52)	15 (83)	**0.014**	277 (84)	19 (95)	0.330
Emergence of rtM204I/V mutants	439 (55)	251 (59)	9 (50)	0.469	170 (52)	9 (45)	0.646
Viral breakthrough due to mutants	334 (42)	216 (51)	5 (28)	0.055	108 (33)	5 (25)	0.473
Biochemical breakthrough due to mutants	318 (40)	200 (47)	5 (28)	0.146	108 (33)	5 (25)	0.473

Except where marked with a superscript letter a, values are expressed as the median and 25th–75th percentiles (parenthetically), or number and percentage (parenthetically). ULN; AST = 33 IU/L, ALT = 42 IU/L (male), and 27 IU/L (female). *Asterisks* indicate data displayed as mean values and standard deviations. *Bold text* indicates statistically significant P values

the only significant characteristic in the HBeAg− cohort was a decline in HBsAg within six months. ROC curve analysis confirmed a cut-off value of 0.5 log IU/mL for a decline in HBsAg level within six months in the HBeAg+ and − cohorts [area under the curve = 0.810 (95 % CI 0.673–0.947) (HBeAg+ cohort) and 0.760 (95 % CI 0.611–0.909) (HBeAg− cohort)].

LAM-resistant rtM204I/V mutants were detected in 439 (55.5 %) of 791 patients. Of these, 334 (42.2 % of all patients) also developed VBT accompanied by an increase in HBV DNA (≥1 log copies/mL). The rate of VBT was marginally significantly lower in the HBsAg clearance group in the HBeAg+ cohort (Table 1).

Factors associated with HBsAg clearance

The overall cumulative rates of HBsAg clearance were 0.2 % at one year, 1.2 % at three years, 2.6 % at five years, 4.2 % at seven years, and 6.4 % at nine years in the HBeAg+ cohort; and 0.6 % at one year, 0.9 % at three - years, 2.2 % at five years, 5.2 % at seven years, and 6.9 % at nine years in the HBeAg− cohort. Univariate Cox

Table 2 Baseline and on-treatment response factors associated with HBsAg clearance, as determined by time-dependent univariate and multivariate analyses at year 9 (HBeAg+ cohort)

Variable	Univariate		Multivariate	
	HBsAg clearance rate ratio (95 % CI)	*P*	HBsAg clearance rate ratio (95 % CI)	*P*
Baseline factors				
Age (≥50 years)	1.36 (0.48–3.86)	0.564		
Gender (F)	0.51 (0.12–2.23)	0.371		
Family history of HBV infection	0.42 (0.16–1.09)	0.074		
Previous IFN therapy	**5.60 (1.61–19.5)**	**0.007**	**6.15 (1.69–22.4)**	**0.006**
Previous NA therapy	2.42 (0.55–10.6)	0.242		
Presence of cirrhosis	0.85 (0.52–1.40)	0.527		
HBV genotype (A)	**3.64 (2.21–5.99)**	**<0.001**	**3.18 (1.80–5.62)**	**<0.001**
HBV DNA (≥6.0 log copies/mL)	2.56 (0.34–19.3)	0.362		
HBsAg (<730 IU/mL)	1.57 (0.51–4.81)	0.432		
AST (≥4.5 × ULN)	**4.53 (1.68–12.2)**	**0.003**		
ALT (≥7.2 × ULN)	**3.56 (1.35–9.36)**	**0.010**		
Total bilirubin (≥1.5 mg/dL)	2.63 (0.92–7.46)	0.070		
Platelet count (<1.2 × 10^5/mm^3)	0.58 (0.13–2.59)	0.476		
On-treatment response factors				
Decline of HBsAg level (≥0.5 log IU/mL within six months)	**15.8 (5.14–48.5)**	**<0.001**	**18.6 (5.78–60.0)**	**<0.001**
HBeAg positive → clearance within six months	**4.33 (1.65–11.4)**	**0.003**	**2.95 (1.04–8.39)**	**0.042**
Undetectable HBV DNA (<400 copies/mL) at six months	**3.95 (1.14–13.7)**	**0.031**		
Emergence of rtM204I/V mutants[a]	0.88 (0.32–2.44)	0.802		
Viral breakthrough due to mutants[a]	**0.32 (0.10–1.00)**	**0.050**		
Breakthrough hepatitis due to mutants[a]	0.41 (0.13–1.31)	0.134		

[a] Time-dependent covariates. *Bold text* indicates statically significant *P* valuesVariables analyzed in multivariate analysis: previous IFN therapy, HBV genotype, ALT, decline of HBsAg levels, HBeAg clearance within six months, undetectable HBV DNA at six months, and viral breakthrough due to mutants (time-dependent covariate)

regression analysis identified four baseline characteristics and four on-treatment responses that were associated with HBsAg clearance in the HBeAg+ cohort (Table 2), and two baseline characteristics and two on-treatment responses in the HBeAg− cohort (Table 3). ROC curve analysis provided the optimal cut-off values and indices for the prediction of HBsAg clearance. ROC curve analysis confirmed cut-off indices of 4.5 × ULN for AST and 7.2 × ULN for ALT for HBsAg clearance in the HBeAg+ cohort [area under the curve = 0.677 (95 % CI 0.524–0.830) (AST) and 0.643 (95 % CI 0.503–0.783) (ALT)]. Meanwhile, ROC curve analysis confirmed a cut-off value of 730 IU/mL (2.86 log IU/mL) for HBsAg for HBsAg clearance in the HBeAg− cohort [area under the curve = 0.696 (95 % CI 0.556–0.836)]. Time-dependent multivariate Cox regression analysis identified two significant baseline characteristics and two on-treatment responses related to HBsAg clearance: previous IFN therapy, infection with HBV genotype A, a decline in HBsAg level of ≥0.5 log IU/mL within six months, and HBeAg clearance within six months in the HBeAg+ cohort (Table 2). In the HBeAg− cohort, two baseline characteristics and one on-treatment response were identified in multivariate analysis: infection with HBV genotype A, HBsAg level of <730 IU/mL (2.86 log IU/mL), and a decline in HBsAg level of ≥0.5 log IU/mL within six months (Table 3).

Association between HBV genotype and HBsAg clearance

We performed a detailed analysis of the association between HBV genotype and HBsAg clearance in patients treated with NAs. Median baseline HBsAg levels were 4.7 log IU/mL (25th–75th percentile, 4.4–5.1) among patients with genotype A, 3.8 (3.5–4.2) among patients with genotype B, and 3.5 (3.2–4.0) among patients with genotype C in the HBeAg+ cohort (Fig. 2a); and 3.7 (2.5–4.1) in patients with genotype A, 2.9 (2.6–3.5) in patients with genotype B, and 3.2 (2.8–3.5) in patients with genotype C in the HBeAg− cohort (Fig. 2b). HBeAg+ patients with genotype A had higher baseline HBsAg levels than those with genotypes B or C ($P < 0.001$) (Fig. 2a). There were no significant differences in baseline HBsAg levels between the genotypes in the HBeAg− cohort.

Table 3 Baseline and on-treatment response factors associated with HBsAg clearance, as determined by time-dependent univariate and multivariate analyses at year 9 (HBeAg− cohort)

Variable	Univariate		Multivariate	
	HBsAg clearance rate ratio (95 % CI)	*P*	HBsAg clearance rate ratio (95 % CI)	*P*
Baseline factors				
Age (≥50 years)	1.39 (0.54–3.60)	0.498		
Gender (F)	0.98 (0.28–3.40)	0.971		
Family history of HBV infection	0.49 (0.19–1.27)	0.140		
Previous IFN therapy	0.88 (0.32–2.38)	0.797		
Previous NA therapy	2.41 (0.32–18.2)	0.394		
Presence of cirrhosis	0.71 (0.43–1.16)	0.173		
HBV genotype (A)	**2.79 (1.33–5.85)**	**0.007**	**2.73 (1.29–5.81)**	**0.009**
HBV DNA (≥6.0 log copies/mL)	1.16 (0.43–3.14)	0.772		
HBsAg (<730 IU/mL)	**3.91 (1.59–9.52)**	**0.003**	**4.90 (1.85–10.6)**	**0.001**
AST (≥4.5 × ULN)	1.76 (0.57–5.40)	0.324		
ALT (≥7.2 × ULN)	1.89 (0.62–5.81)	0.265		
Total bilirubin (≥1.5 mg/dL)	1.18 (0.27–5.20)	0.825		
Platelet count (<1.2 × 10^5/mm^3)	0.77 (0.17–3.55)	0.733		
On-treatment response factors				
Decline of HBsAg level (≥0.5 log IU/mL within six months)	**11.5 (4.24–31.0)**	**<0.001**	**16.9 (5.89–48.4)**	**<0.001**
Undetectable HBV DNA (<400 copies/mL) at six months	2.78 (0.37–20.8)	0.322		
Emergence of rtM204I/V mutants[a]	0.64 (0.23–1.79)	0.392		
Viral breakthrough due to mutants[a]	0.72 (0.23–2.29)	0.581		
Breakthrough hepatitis due to mutants[a]	0.65 (0.21–2.06)	0.465		

[a] Time-dependent covariates. *Bold text* indicates statically significant *P* values

Variables analyzed in multivariate analysis: HBV genotype, baseline HBsAg, decline of HBsAg levels

HBsAg kinetics over time in the HBeAg+ and − cohorts are shown in Fig. 2c, d, respectively. Among patients with genotype A in the HBeAg+ cohort, the median HBsAg change from baseline was −0.44 log IU/mL at six months, −0.56 at one year, −0.58 at three years, −1.08 at five years, and −1.33 at six years. Among patients with genotype B in the HBeAg+ cohort, median changes were −0.30 log IU/mL at six months, −0.30 at one year, −0.43 at three years, and −0.46 at five years. Kinetics were not calculated for some groups (genotype A at seven years, genotype B at six years) because the number of patients was too small. Finally, among patients with genotype C in the HBeAg+ cohort, median changes were 0.00 log IU/mL at six months, 0.03 at one year, −0.08 at three years, −0.29 at five years, −0.53 at six - years, −0.62 at seven years, −0.70 at eight years, and −0.82 at nine years. Genotype had a significant effect on the slopes between data collection points at six months and six years. In the HBeAg+ cohort, declines were faster in patients with genotype A than in those with genotypes B or C. HBeAg− patients with genotype A displayed a median HBsAg change from baseline of 0.05 log IU/mL at six months, 0.05 at one year, −0.11 at three years, −0.21 at five years, and −0.26 at six years. Among patients with genotype B in the HBeAg− cohort, median changes were −0.03 log IU/mL at six months, −0.06 at one year, −0.25 at three years, −0.31 at five years, −0.51 at six years, −0.62 at seven years, −0.66 at eight years, and −0.61 at nine years. Among patients with genotype C in the HBeAg− cohort, median changes were 0.03 log IU/mL at six months, 0.04 at one year, −0.08 at three years, −0.19 at five years, −0.32 at six years, −0.39 at seven years, −0.46 at eight years, and −0.62 at nine years. The decline was slightly faster in patients with genotype B than in those with genotypes A and C in the HBeAg− cohort.

We investigated whether HBsAg clearance were influenced by genotype or baseline HBeAg. Cumulative HBsAg clearance rates in the HBeAg+ cohort were as follows: 15 % at year 3, and 35 % at year 5 in patients with genotype A; 0 % over all years in patients with genotype B; and 0.6 % at year 3, 1.2 % at year 5, and 5.4 % at year 9 in patients with genotype C (Fig. 2e). In the HBeAg− cohort, clearance rates were 12 % at year 3, and 25 % at year 5 in patients with genotype A; 0 % at year 3, 0 % at year 5, and 11.5 % at year 9 in patients with genotype B; and 0.4 % at year 3, 1.6 % at year 5, and 5.1 % at year 9 in

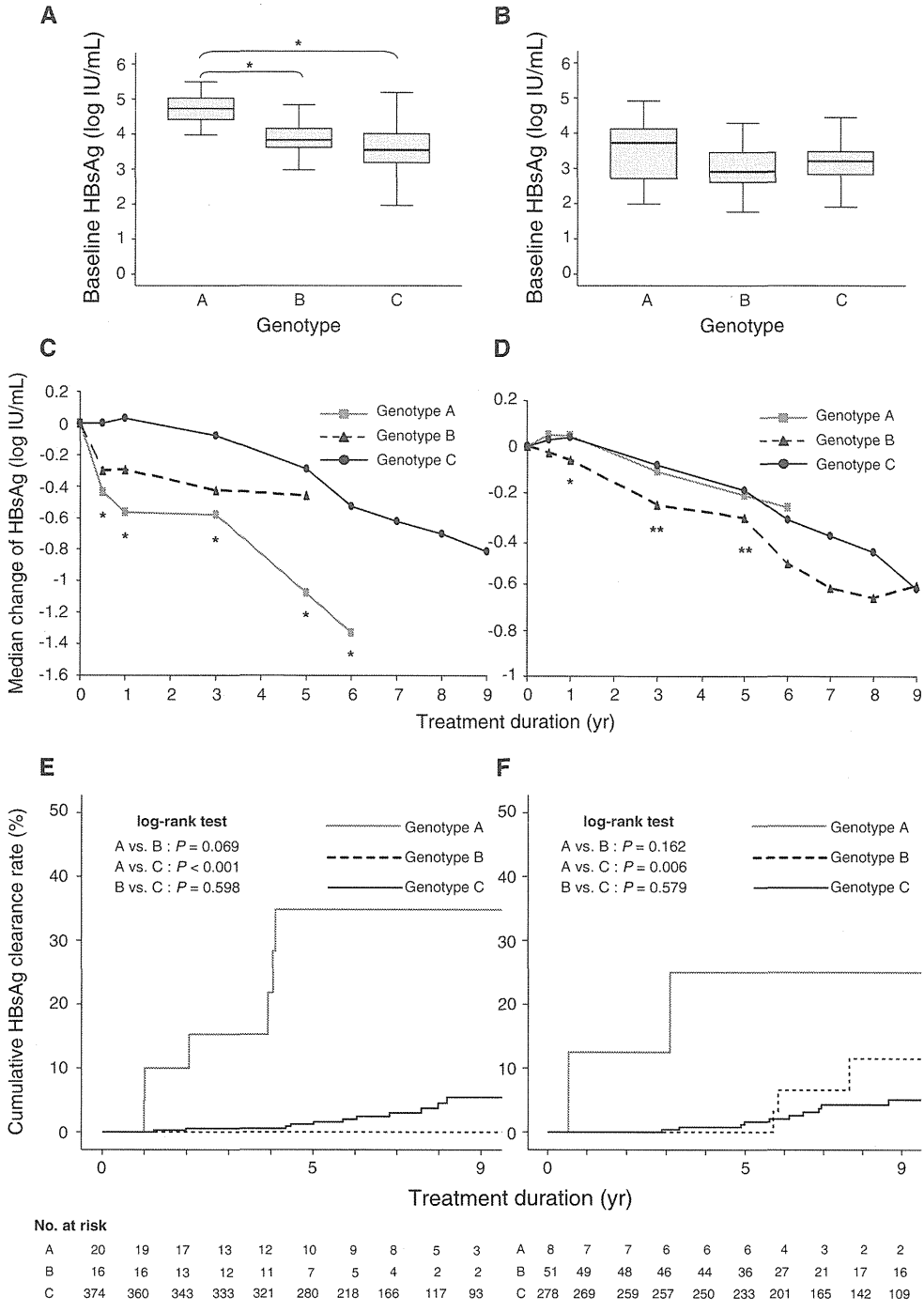

Fig. 2 **a** Box plot of baseline HBsAg levels in patients with different HBV genotypes (HBeAg+ cohort). The *asterisk* (*) indicates a statistical significance of $P < 0.001$, as determined by the Mann–Whitney U test and Bonferroni correction. **b** Box plot of baseline HBsAg levels in patients with different HBV genotypes (HBeAg− cohort). **c** Median change in HBsAg level from baseline in patients with different HBV genotypes (HBeAg+ cohort). A *single asterisk* (*) indicates $P < 0.001$, as determined by the Kruskal–Wallis test. **d** Median change in HBsAg level from baseline in patients with different HBV genotypes (HBeAg− cohort). A *single asterisk* (*) indicates $P < 0.001$ and a *double asterisk* (**) indicates $P < 0.02$, as determined by the Kruskal–Wallis test. **e** Kaplan–Meier life table showing cumulative HBsAg clearance rates in patients with different HBV genotypes (HBeAg+ cohort). Cumulative HBsAg clearance rates were significantly higher among patients with genotype A (log-rank test; A vs. B: $P = 0.069$, A vs. C: $P < 0.001$, B vs. C: $P = 0.598$, after Bonferroni correction). **f** Kaplan–Meier life table showing cumulative HBsAg clearance rates in patients with different HBV genotypes (HBeAg− cohort). Cumulative HBsAg clearance rates were significantly higher among patients with genotype A (log-rank test; A vs. B: $P = 0.169$, A vs. C: $P = 0.006$, B vs. C: $P = 0.579$, after Bonferroni correction)

patients with genotype C (Fig. 2f). Clearance rates were significantly higher in patients with genotype A than in those with genotype C ($P < 0.001$ in the HBeAg+ cohort, $P = 0.006$ in the HBeAg− cohort).

Association between on-treatment response and subsequent HBsAg clearance

We stratified patients into three groups according to the amount of HBsAg decline within the first six months of treatment; this allowed us to evaluate the impact of on-treatment response factors on the clearance of HBsAg. The stratifications were as follows: rapid decline (≥1.0 log IU/mL), intermediate decline (0.5–1.0 log IU/mL), and slow decline or steady (<0.5 log IU/mL). Cumulative HBsAg clearance rates in the HBeAg+ cohort were 11 % at year 3, and 40 % at year 5 in the rapid decline group; 0 % at year 3, 2.2 % at year 5, and 13 % at year 9 in the intermediate decline group; and 0 % at year 3, 0 % at year 5, and 2.9 % at year 9 in the slow decline or steady group (Fig. 3a).

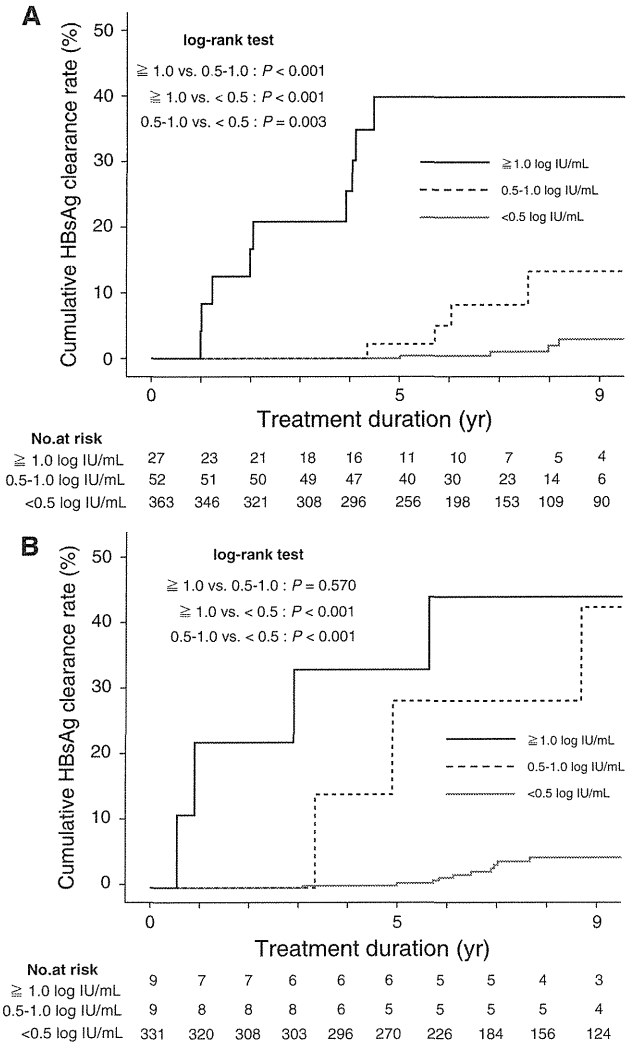

Fig. 3 **a** Kaplan–Meier life table showing cumulative HBsAg clearance rates in patients with varying rates of HBsAg decline within the first six months (HBeAg+ cohort). Clearance rates were highest in the rapid decline group, followed by the intermediate decline group and the slow or steady group (log-rank test; rapid vs. intermediate: $P < 0.001$, rapid vs. slow: $P < 0.001$, intermediate vs. slow: $P = 0.003$, after Bonferroni correction). **b** Kaplan–Meier life table showing cumulative HBsAg clearance rates in patients with varying rates of HBsAg decline within the first six months (HBeAg− cohort). Clearance rates were highest in the rapid decline group, followed by the intermediate decline group and the slow or steady group (log-rank test; rapid vs. intermediate: $P = 0.570$, rapid vs. slow: $P < 0.001$, intermediate vs. slow: $P < 0.001$, after Bonferroni correction)

Cumulative HBsAg clearance rates in the HBeAg− cohort were 33 % at year 5, and 44 % at year 7 in the rapid decline group; 0 % at year 3, 29 % at year 5, and 43 % at year 9 in the intermediate decline group; and 0.3 % at year 3, 0.7 % at year 5, and 4.6 % at year 9 in the slow decline or steady group (Fig. 3b). Clearance rates were highest in the rapid decline group, followed by the intermediate decline group and the slow or steady group in both the HBeAg+ and HBeAg− cohorts. The decline of HBsAg within the first six months was a strong predictor of HBsAg clearance.

Viral breakthrough and subsequent HBsAg clearance

Although VBT was not associated with HBsAg clearance in the multivariate model, as described above, HBsAg clearance was observed in ten patients who experienced VBT (five patients in the HBeAg+ cohort and five in the HBeAg− cohort). All ten patients achieved clearance of HBsAg after VBT occurred. Six of these patients received ADV added on to LAM for VBT, and subsequently achieved clearance of HBsAg (five patients in the HBeAg+ cohort and one in the HBeAg− cohort). The other four patients spontaneously recovered from VBT while continuing to receive LAM monotherapy, and subsequently achieved clearance of HBsAg (one patient in the HBeAg+ cohort and three in the HBeAg− cohort). LAM-resistant mutant strains (M204I/V mutants) were detected in nine patients in whom VBT occurred. HBV DNA negativity continued for the follow-up period after HBsAg clearance in these ten patients. The typical clinical and virological courses of two representative patients who achieved HBsAg clearance after VBT are shown in Fig. 4a, b.

Virological courses after discontinuation of NAs

Sixteen (42.1 %) of 38 patients with HBsAg clearance discontinued NA treatment due to HBsAg clearance. Median interval between HBsAg clearance and discontinuation of NAs was nine months (range 2–29 months). Median follow-up period after discontinuation of NAs was 24 months (range 7–171) in these patients. No relapses of serum HBsAg or HBV DNA were observed during the follow-up period. Serum anti-HBs appeared in 12 (75 %) of the 16 patients who discontinued NAs. Median time to the appearance of anti-HBs after HBsAg clearance was 16 months (range 2–92) in patients who discontinued NAs. Two of 22 patients who continued NAs with HBsAg clearance had the appearance of anti-HBs, and median time to the appearance of anti-HBs after HBsAg clearance was two and seven months in these two patients, respectively.

Discussion

We found that three baseline factors and two on-treatment response factors are associated with HBsAg clearance in patients who begin treatment with LAM and continue with long-term NA therapy. HBV genotype and the decline in HBsAg over the first six months were associated with

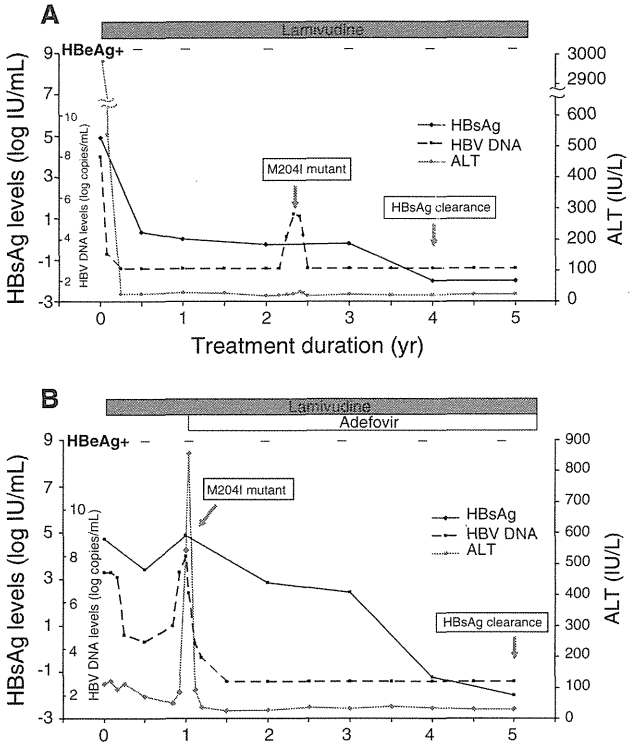

Fig. 4 Case presentation of the typical clinical and virological courses of two representative patients who achieved HBsAg clearance after VBT occurred. **a** Patient 1, a 45-year-old man who was HBeAg+ at baseline and had genotype A. **b** Patient 2, a 38-year-old man who was HBeAg+ at baseline and had genotype A. *VBT* virological breakthrough

HBsAg clearance in both the HBeAg+ and − cohorts, whereas the clearance of HBsAg was associated with previous IFN therapy and the clearance of HBeAg over the first six months only in the HBeAg+ cohort, and baseline HBsAg levels only in the HBeAg− cohort.

HBV genotype was recently reported to influence declines in and the clearance of HBsAg among patients who underwent PEG-IFN therapy [31]. In one study where negativity for serum HBV DNA and seroconversion of HBeAg represented the study end point, genotype was not found to influence response to NA therapy [31]. However, other reports have indicated that genotype does impact on declines in and the clearance of HBsAg [20, 29]. Heathcote et al. [20] reported that 20 HBeAg+ patients (8 %) who were treated with tenofovir achieved HBsAg clearance in three years. Twelve (60 %) of 20 patients were infected with genotype A and the others with genotype D. In this study, cumulative HBsAg clearance rates were 15 % at year 3 in HBeAg+ patients with genotype A. This result seems to be similar regardless of the antiviral potential. Previous studies with more ethnically diverse study populations than ours found that HBsAg clearance rates were highest in patients with genotype A. The similarity between those results and ours implies that the HBV genotype is more influential than ethnicity on HBsAg clearance during NA therapy. Of 28 genotype A patients in our population, the majority (79 %) did not have a family history of infection. Recent work has shown that sexual transmission of acute HBV genotype A infections is increasing in Japan, resulting in chronic HBV infection, especially in young adult patients [32, 33]. Cumulatively, these findings imply that HBsAg clearance is more likely in genotype A patients because they have been infected with HBV for a shorter period of time. Furthermore, Hou et al. [34] demonstrated that genotype A responded better than other HBV genotypes to IFN therapy. They revealed that a lower number of amino acid substitutions at baseline were associated with a better response to IFN therapy, and that this variable was linked with HBV genotype A, which had the lowest number of amino acid substitutions in the core gene among genotypes B, C, or D. Although amino acid substitutions in the core gene were not analyzed in this study, the relation between the core gene and treatment responses of NAs is necessary to be investigated in the future.

Although Gish et al. [19] reported that previous IFN therapy is not associated with HBsAg clearance in patients who are HBeAg+, the opposite was true in our HBeAg+ cohort. These contradictory findings may result from the fact that their patients received NA therapy over a much shorter time period (median duration 23 vs. 75 months, a 3.2-fold difference). We believe that there are two main reasons why HBsAg clearance rates were higher in patients who had previously received IFN therapy: the influence of AST/ALT flares after IFN therapy and changes in host immune response to HBV as a result of the immune-modulating activity of IFN. It has previously been shown that in patients with high baseline ALT levels, HBV DNA and HBeAg are likely to rapidly decrease during NA therapy [35, 36]. In this study, HBsAg clearance was likely to occur in patients who had high ALT levels at baseline, and in patients with previous IFN therapy (Table 2) in the HBeAg+ cohort. High virological responses have been reported in response to robust ALT flares induced by IFN therapy [37, 38]. Moreover, Wursthorn et al. [29] recently indicated that the antiviral potential of NAs and antiviral T cell reactivity are associated with HBsAg clearance in response to telbivudine treatment. These findings may be also associated with the achievement of HBsAg clearance after VBT occurs. Taken together, these results imply that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance, especially in HBeAg+ patients.

We found that the initial HBsAg reduction was a strong predictor of subsequent HBsAg clearance during NA therapy, which supports a similar previous finding [29]. HBsAg reduction over the initial six months is important

for predicting the subsequent HBsAg kinetics in both HBeAg+ and HBeAg− patients. The novel finding in this study was that HBeAg− individuals achieved HBsAg clearance. We found that the median duration to HBsAg clearance was longer in patients with HBeAg− than in those who were HBeAg+ in this study (6.0 vs. 4.4 years). Manesis et al. [28] used modeling to determine that HBeAg− patients receiving LAM treatment would likely require >10 years to achieve HBsAg loss. Furthermore, baseline HBsAg titers were <730 IU/mL in 60 % (12/20) of HBeAg− patients who achieved HBsAg clearance. The only baseline predictive factor of HBsAg clearance was baseline HBsAg levels in HBeAg− patients, except for genotype. There was no difference in HBsAg clearance rates in HBeAg− patients with high- and low-baseline HBV DNA or ALT levels. We hypothesize that HBsAg clearance in these patients may result from long treatment duration and low HBsAg titers.

Our study was limited by the fact that it was a hospital-based retrospective analysis, which means there may be some bias associated with patient type and treatment selection. We were unable to compare HBsAg clearance rates obtained in our study with those of controls untreated with NA. Because all subjects in the study received LAM as an initial NA, and then received rescue therapy when drug-resistant mutations emerged, NA therapy regimens were not uniform across all patients, and there were variations in both treatment dose and duration of previous IFN therapy. We were not able to collect immunological data on our subjects. Finally, our results need to be validated by further studies investigating a large study population receiving long-term ETV or tenofovir with high antiviral potential and a high genetic barrier.

Despite these drawbacks, we were able to determine several factors associated with HBsAg clearance, including HBV genotype and a decline in HBsAg over the initial six months of treatment (HBeAg+ and − cohorts); previous IFN therapy and clearance of HBeAg over the initial six months of treatment (HBeAg+ cohort only); and HBsAg levels (HBeAg− cohort only). It seems that both direct antiviral potential and host immune response are needed to achieve HBsAg clearance by NA therapy. Future studies are needed to validate these findings and to develop treatment regimens for HBsAg clearance in patients with chronic hepatitis B.

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Conflict of interest Dr. Kumada reports having received investigator, lecture, and consulting fees from Bristol-Myers Squibb, Dainippon Sumitomo Pharma Co., MSD K.K., and Toray Co. 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. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med. 2006;354:1001–10.
4. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med. 1999;341:1256–63.
5. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med. 2003;348:800–7.
6. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med. 1998;339:61–8.
7. Lai CL, Gane E, Liaw YF, Hsu CW, Thongsawat S, Wang Y, et al. Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med. 2007;357:2576–88.
8. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2006;354:1011–20.
9. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med. 2003;348:808–16.
10. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med. 2008;359:2442–55.
11. Di Marco V, Marzano A, Lampertico P, Andreone P, Santantonio T, Almasio PL, et al. Clinical outcome of HBeAg-negative chronic hepatitis B in relation to virological response to lamivudine. Hepatology. 2004;40:883–91.
12. Suzuki Y, Arase Y, Ikeda K, Saitoh S, Tsubota A, Suzuki F, et al. Histological improvements after a three-year lamivudine therapy in patients with chronic hepatitis B in whom YMDD mutants did not or did develop. Intervirology. 2003;46:164–70.
13. Newbold JE, Xin H, Tencza M, Sherman G, Dean J, Bowden S, et al. The covalently closed duplex form of the hepadnavirus genome exists in situ as a heterogeneous population of viral minichromosomes. J Virol. 1995;69:3350–7.
14. Wu TT, Coates L, Aldrich CE, Summers J, Mason WS. In hepatocytes infected with duck hepatitis B virus, the template for viral RNA synthesis is amplified by an intracellular pathway. Virology. 1990;175:255–61.
15. Zoulim F. New insight on hepatitis B virus persistence from the study of intrahepatic viral cccDNA. J Hepatol. 2005;42:302–8.
16. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004;126:1750–8.
17. Wursthorn K, Lutgehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, et al. Peginterferon alpha-2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. Hepatology. 2006;44:675–84.

18. Chang TT, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatology. 2010;51:422–30.
19. Gish RG, Chang TT, Lai CL, de Man R, Gadano A, Poordad F, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naive HBeAg-positive patients with chronic hepatitis B. J Viral Hepat. 2010;17:16–22.
20. Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, et al. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. Gastroenterology. 2011;140:132–43.
21. 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.
22. Liaw YF, Gane E, Leung N, Zeuzem S, Wang Y, Lai CL, et al. 2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology. 2009;136:486–95.
23. Reijnders JG, Rijckborst V, Sonneveld MJ, Scherbeijn SM, Boucher CA, Hansen BE, et al. Kinetics of hepatitis B surface antigen differ between treatment with peginterferon and entecavir. J Hepatol. 2011;54:449–54.
24. Buster EH, Flink HJ, Cakaloglu Y, Simon K, Trojan J, Tabak F, et al. Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. Gastroenterology. 2008;135:459–67.
25. Buster EH, Flink HJ, Simsek H, Heathcote EJ, Sharmila S, Kitis GE, et al. Early HBeAg loss during peginterferon alpha-2b therapy predicts HBsAg loss: results of a long-term follow-up study in chronic hepatitis B patients. Am J Gastroenterol. 2009;104:2449–57.
26. Marcellin P, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterology. 2009;136:2169–79, e2161–64.
27. Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology. 2009;49:1151–7.
28. Manesis EK, Hadziyannis ES, Angelopoulou OP, Hadziyannis SJ. Prediction of treatment-related HBsAg loss in HBeAg-negative chronic hepatitis B: a clue from serum HBsAg levels. Antivir Ther. 2007;12:73–82.
29. Wursthorn K, Jung M, Riva A, Goodman ZD, Lopez P, Bao W, et al. Kinetics of hepatitis B surface antigen decline during 3 years of telbivudine treatment in hepatitis B e antigen-positive patients. Hepatology. 2010;52:1611–20.
30. Pocock SJ, Clayton TC, Altman DG. Survival plots of time-to-event outcomes in clinical trials: good practice and pitfalls. Lancet. 2002;359:1686–9.
31. Raimondi S, Maisonneuve P, Bruno S, Mondelli MU. Is response to antiviral treatment influenced by hepatitis B virus genotype? J Hepatol. 2010;52:441–9.
32. Suzuki Y, Kobayashi M, Ikeda K, Suzuki F, Arfase Y, Akuta N, et al. Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. J Med Virol. 2005;76:33–9.
33. Sugauchi F, Orito E, Ohno T, Tanaka Y, Ozasa A, Kang JH, et al. Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. Hepatol Res. 2006;36:107–14.
34. Hou J, Schilling R, Janssen HLA, Hansen BE, Heijtink R, Sablon E, et al. Genetic characteristics of hepatitis B virus genotypes as a factor for interferon-induced HBeAg clearance. J Med Virol. 2007;79:1055–63.
35. Akuta N, Tsubota A, Suzuki F, Suzuki Y, Hosaka T, Someya T, et al. Long-term prognosis by lamivudine monotherapy for severe acute exacerbation in chronic hepatitis B infection: emergence of YMDD motif mutant and risk of breakthrough hepatitis—an open-cohort study. J Hepatol. 2003;38:91–7.
36. Liaw YF, Tsai SL, Chien RN, Yeh CT, Chu CM. Prednisolone priming enhances Th1 response and efficacy of subsequent lamivudine therapy in patients with chronic hepatitis B. Hepatology. 2000;32:604–9.
37. Flink HJ, Sprengers D, Hansen BE, van Zonneveld M, de Man RA, Schalm SW, et al. Flares in chronic hepatitis B patients induced by the host or the virus? Relation to treatment response during Peg-interferon {alpha}-2b therapy. Gut. 2005;54:1604–9.
38. Nair S, Perrillo RP. Serum alanine aminotransferase flares during interferon treatment of chronic hepatitis B: is sustained clearance of HBV DNA dependent on levels of pretreatment viremia? Hepatology. 2001;34:1021–6.

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Title:

Strategy for preventing hepatitis B reactivation in patients with resolved HBV infection following rituximab-containing chemotherapy

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To the editor:

In a recent article in Hepatology, Hsu et al.[1] reported a prospective study (NCT00931299) to determine the incidence of hepatitis B virus (HBV) reactivation in 150 patients with resolved HBV infection receiving rituximab-CHOP chemotherapy. The authors indicated that HBV reactivation is not uncommon and can be managed with regular monitoring of HBV DNA in serum. However, there are some concerns regarding the management of HBV DNA monitoring as described in this report.

First, Hsu et al.[1] reported that no HBV-related death occurred during the study period, but HBV-related severe hepatitis and chemotherapy delay occurred in 7 (4.6%) and 2 (1.3%) patients, respectively. Furthermore, patients with HBV reactivation may have a poorer prognosis than those without reactivation, suggesting that HBV DNA monitoring could not enable the successful management of HBV reactivation in this setting. In fact, the authors have already described the usefulness of a more sensitive HBV DNA assay and they should show whether a second PCR assay (detection limit 300 copies/mL, assay #2) could prevent severe hepatitis flare due to HBV reactivation by estimating in their retrospective analysis the exact time between early HBV DNA detection and the onset of hepatitis.

Second, Hsu et al.[1] concluded that re-appearance of HBsAg was the most important

predictor of HBV-related hepatitis flare, but there is no information regarding the sensitivity and specificity of the HBsAg assay, and these might influence clinical outcome. The authors should provide information regarding the HBsAg assay in the methods section and specify the time between the re-appearance of HBsAg and the onset of HBV-related hepatitis. In addition, they should specify the incidence of re-appearance of HBsAg with persistence for more than 6 months in patients with HBV reactivation, because the chronic HBV carrier state might negatively influence long-term outcomes, regardless of fulminant hepatitis and HBV-related death.

Third, Hsu et al.[1] discussed the importance of host factors associated with HBV reactivation, but several papers have reported that the development of fulminant hepatitis was associated with viral factors, which especially included high levels of replication associated with mutations in the precore region[2,3]. The authors should specify whether the kinetics of HBV DNA and severe hepatitis were associated with precore and/or basal core promoter mutations in the patients with HBV reactivation, because general readers need to be aware of such important viral factors to perform safe monitoring of HBV DNA.

Preemptive antiviral therapy guided by regular monitoring of HBV DNA is a reasonable strategy to prevent HBV reactivation in patients with resolved HBV

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infection[1, 4, 5], but a standard management according to the risk of HBV reactivation has not been established yet. We hope that the additional information can help the readers regarding the optimal interval and the sensitivity of the HBV DNA monitoring assay. In addition, if the re-appearance of HBsAg and the viral mutations related to viral replication are good predictive markers for severe hepatitis due to HBV reactivation, we can recommend that antiviral treatment should be started immediately for those patients with HBV reactivation.

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REFERENCES

1. Hsu C, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL, et al: Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: A prospective study. HEPATOLOGY 2013 Sep 3.

2. Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M.: Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med 1991;324:1699-1704.

3. Ozasa A, Tanaka Y, Orito E, Sugiyama M, Kang JH, Hige S, et al: Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. HEPATOLOGY 2006;44:326-334.

4. Oketani M, Ido A, Uto H, Tsubouchi H: Prevention of hepatitis B virus reactivation in patients receiving immunosuppressive therapy or chemotherapy. Hepatol Res 2012;42:627-636.

5. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 2012;57:167-185.

Is Antiviral Prophylaxis Necessary to Prevent Hepatitis B Virus (HBV) Reactivation in Patients With HBV-Resolved Infection Receiving Rituximab-Containing Chemotherapy?

TO THE EDITOR: In a recent article in *Journal of Clinical Oncology*, Huang et al[1] reported a randomized controlled trial (NCT00926757) of entecavir prophylaxis to prevent hepatitis B virus (HBV) reactivation in 80 patients with HBV-resolved infection receiving rituximab-containing chemotherapy, in which interesting and important data were included. However, some concerns regarding study design and results in this report are worth considering.

First, Huang et al[1] reported that the incidence of HBV reactivation, the primary end point in this study, was defined as elevation of HBV viral load to 2,000 IU/mL with two consecutive determinations (> 2 weeks apart). However, the ClinicalTrials.gov archive[2] indicated that the primary end point had been changed, whereby HBV reactivation was defined as greater than 10-fold increase, compared with previous nadir levels of HBV DNA in the serum as of June 2009 at the beginning of the study. Previous secondary end points were defined as hepatitis and hepatic failure attributed to HBV reactivation. It is important for the reader to be aware of the reason why the authors changed the definition of the primary end point, and to be able to assess the incidence of HBV reactivation according to the previous original definition. Because some patients had HBV reactivation with high viral loads, readers need to know the kinetics of HBV viral load development as well as the clinical outcomes attributed to HBV reactivation during follow-up.

Second, Huang et al[1] reported that the HBV viral load was determined using a Cobas Amplicor HBV monitor (Roche Molecular Systems, Pleasanton, CA), with a detection limit of 12 IU/mL. However, it has been reported by others that the detection limit with this Cobas Amplicor HBV monitor is 60 IU/mL.[3] It may be necessary to amend the description regarding HBV viral load measurement for evaluation of the primary end point.

Third, Huang et al[1] reported that seven of 39 patients (17.9%) developed HBV reactivation (2,000 IU/mL), but only one (2.6%) had hepatitis attributed to HBV reactivation in the control group shown in Table 2. Furthermore, they also reported that no patients developed HBV-related liver decompensation or mortality in this study. These data might be unrepresentative, but if they are confirmed, the low incidence of hepatitis and no mortality associated with HBV reactivation is interesting in this prospective study, which would strongly suggest that antiviral prophylaxis is not cost effective for all patients with resolved hepatitis B receiving rituximab-containing chemotherapy. As Huang et al[1] suggested, regular monitoring of HBV viral load is more reasonable and cost effective, and some guidelines have already recommended this strategy to prevent HBV reactivation.[4,5] Recently, we presented data showing that monthly monitoring of HBV DNA could prevent hepatitis associated with HBV reactivation, even in HBV-resolved patients with highly replicative viral clones (interim analysis of a prospective study).[6]

The identification of risk factors associated with HBV reactivation is an important research question in patients with HBV-resolved infection following systemic chemotherapy, especially when the latter contains molecularly targeted drugs. If high-risk patients can be accurately predicted, we will be able to prevent HBV reactivation more effectively.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Huang YH, Hsiao LY, Hong YC, et al: Randomized controlled trial of entecavir prophylaxis for rituximab-associated hepatitis B virus reactivation in patients with lymphoma and resolved hepatitis B. J Clin Oncol 31:2765-2772, 2013
2. US National Institutes of Health: ClinicalTrials.gov archive. http://clinicaltrials.gov/archive/NCT00926757/2013_05_28/changes
3. Roche Diagnostics: Package insert, COBAS AMPLICOR HBV MONITOR Test. Revision 6.0, 2009
4. Oketani M, Ido A, Uto H, et al: Prevention of hepatitis B virus reactivation in patients receiving immunosuppressive therapy or chemotherapy. Hepatol Res 42:627-636, 2012
5. European Association for the Study of the Liver: EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol 57:167-185, 2012
6. Kusumoto S, Tanaka Y, Suzuki R, et al: Prospective nationwide observational study of hepatitis B virus (HBV) DNA monitoring and preemptive antiviral therapy for HBV reactivation in patients with B-cell non-Hodgkin lymphoma following rituximab containing chemotherapy: Results of interim analysis. Blood 120, 2012 (abstr 2641)

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Hepatology Research

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Case Report

Reactivation of hepatitis B virus in a patient with adult T-cell leukemia–lymphoma receiving the anti-CC chemokine receptor 4 antibody mogamulizumab

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The introduction of molecularly targeted drugs has increased the risk of reactivation of hepatitis B virus (HBV), which is a potentially fatal complication following anticancer chemotherapy even in patients who have previously resolved their HBV infection. CC chemokine receptor 4 (CCR4) has been identified as a novel molecular target in antibody therapy for patients with adult T-cell leukemia–lymphoma (ATL) and peripheral T-cell lymphoma, and the humanized anti-CCR4 monoclonal antibody mogamulizumab has been developed. We reported HBV reactivation of an ATL patient with previously resolved HBV infection after mogamulizumab treatment in a dose-finding study for this antibody. Our retrospective analysis using preserved samples also revealed the detailed kinetics of HBV DNA levels before and just after HBV reactivation.

Key words: CC chemokine receptor 4, hepatitis B virus, mogamulizumab, reactivation

INTRODUCTION

REACTIVATION OF HEPATITIS B virus (HBV) following anticancer chemotherapy and immunosuppressive therapy is a potentially fatal complication that needs to be followed up carefully.[1] The advent of molecularly targeted drugs, which have immunosuppressive or immunomodulating actions, has increased the risk of HBV reactivation. The anti-CD20 monoclonal antibody rituximab, which forms part of the standard regimen for B-cell non-Hodgkin's lymphoma, has the potential to cause HBV reactivation, even in patients who have previously resolved their HBV infection and are hepatitis B surface antigen (HBsAg) negative at baseline.[2–6] CC chemokine receptor 4 (CCR4) has been identified as a novel molecular target in antibody therapy for patients with adult T-cell leukemia–lymphoma (ATL) and peripheral T-cell lymphoma, and the humanized anti-CCR4 monoclonal antibody mogamulizumab, the Fc region of which is de-fucosylated to enhance antibody-dependent cellular cytotoxicity, has been developed.[7–10] We herein report HBV reactivation of an ATL patient with previously resolved HBV infection after mogamulizumab treatment in a dose-finding study for this antibody.

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CASE REPORT

A 65-YEAR-OLD JAPANESE woman complained of persistent fatigue and weight loss of 8 kg in 2 weeks. The laboratory findings showed that her white blood cell count was elevated to 16 800/μL, of which abnormal lymphocytes accounted for 18%, and seropositivity for human T-cell leukemia virus type-1 (HTLV-1). Monoclonal integration of HTLV-1 was revealed by Southern blotting of DNA from peripheral blood. She was diagnosed as ATL, chronic type, in April 2004. Since then, she had experienced repeating infectious episodes and systemic lymph node swelling. On April 2005, she began to receive systemic chemotherapy composed of sobuzoxane (400 mg/day), etoposide (25 mg/day) and prednisolone (10 mg/day) p.o. twice a week because of disease progression to acute type which was accompanied by new ATL involvement in her right breast region and right axilla lymphadenopathy. As her disease was refractory to this regimen, she received four cycles of THP-COP regimen (cyclophosphamide, pirarubicin, vincristine and prednisolone) from August 2005 through October 2005 (Fig. 1a). She achieved a partial response and was followed up without subsequent chemotherapy including steroids for 1.4 years, but her disease progressed with markedly increased ATL cells

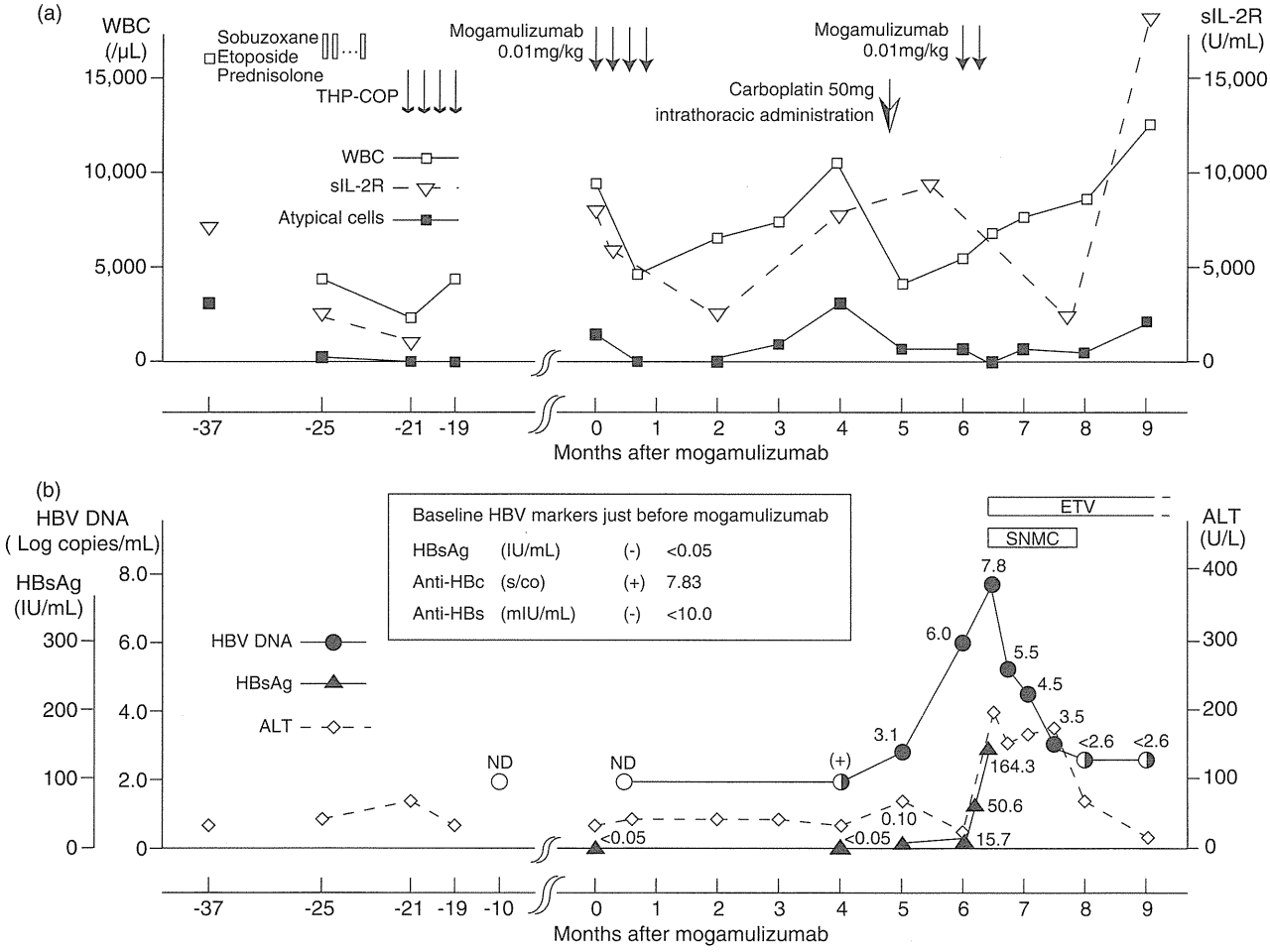

Figure 1 Clinical course and kinetics of HBV markers in a patient with adult T-cell leukemia–lymphoma before and after the anti-CC chemokine receptor 4 monoclonal antibody mogamulizumab treatment. ALT, alanine aminotransferase; anti-HBc, antibody against hepatitis core antigen; anti-HBs, antibody against hepatitis surface antigen; ETV, entecavir; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ND, not detectable; sIL-2R, soluble interleukin-2 receptor; SNMC, Stronger Neo-Minophagen C; THP-COP, cyclophosphamide, pirarubicin, vincristine and prednisolone; WBC, white blood cells.

and an elevated lactate dehydrogenase value in peripheral blood in March 2007. She was enrolled into a phase 1 study for dose-finding of the anti-CCR4 antibody, mogamulizumab,[9] and received this antibody at 0.01 mg/kg by i.v. infusion once a week for 4 weeks (Fig. 1a, thin arrows). No combination of other anticancer chemotherapy was performed and no steroids were given, except for allergic prophylaxis. She was HBsAg negative at baseline on enrollment in the phase 1 study. Retrospective analysis using preserved samples revealed that she was anti-hepatitis B core positive, anti-hepatitis B surface negative, and HBV DNA was undetectable at baseline, attributed to previously resolved HBV infection (Fig. 1b). After mogamulizumab, ATL cells disappeared immediately from the peripheral blood, the nodal disease partially improved and no severe adverse event was observed. However, at 9 weeks after the end of mogamulizumab, the ATL cells reappeared in the peripheral blood. Furthermore, she received intrathoracic administration of carboplatin for involvement of ATL (right pleural effusion) in August 2007. During the next month, her cervical lymph nodes enlarged rapidly and we decided to re-treat with mogamulizumab because of the previous efficacy and safety of this antibody. After two doses of mogamulizumab, she was hospitalized in emergency due to ALT flare on October 2007 (Fig. 1b, 6.5 months after mogamulizumab). The laboratory findings showed that HBsAg had become positive and her HBV DNA levels increased to 7.8 log copies/mL, suggesting that the liver damage was caused by HBV reactivation. Entecavir (0.5 mg/day) and Stronger Neo-Minophagen C (40 mg/day) were given immediately and hepatitis B improved gradually (with ALT peaking at 205 U/L) for approximately 2 months. Entecavir was effective in controlling hepatitis B, and was continued for 1.5 years without any severe adverse events.

DISCUSSION

THE PRESENTED CASE is the first report of HBV reactivation in a HBsAg negative patient receiving mogamulizumab. We analyzed preserved samples retrospectively and showed that her liver damage was attributable to HBV reactivation. Also, those analyses showed the following important findings regarding the kinetics of HBV DNA during reactivation: First, HBV DNA was undetectable at baseline, before administration of mogamulizumab. Elevated HBV DNA levels were detectable, in which polymerase chain reaction (PCR) signals were only detected 10 weeks prior to the development of hepatitis and 13 weeks after the end of this antibody treatment. HBV DNA levels, measured by PCR-based assay, increased rapidly from 3.1 to 6.0 log copies/mL for 1 month and, finally, up to 7.8 log copies/mL. Second, the elevated HBV DNA levels preceded the detection of HBsAg (Architect Assay; Abbott Laboratories, North Chicago, IL, USA) by 1 month. Third, the patient was infected with HBV genotype C with a point mutation in the precore regions (G1896A) which might have been associated with the rapidly increasing kinetics of HBV DNA levels in this case.

How was the anti-CCR4 antibody mogamulizumab involved in the HBV reactivation? CCR4 is a chemokine receptor expressed on T-helper type 2 and regulatory T cells, and is thought to carry an important role in maintaining the balance of the human immune system.[7–9] It is difficult to demonstrate how mogamulizumab caused HBV reactivation in this case; the reduction of CCR4-expressing cells following this antibody treatment might have been associated with imbalance of antiviral immunity, resulting in the development of hepatitis due to HBV reactivation. Other than mogamulizumab, the intrathoracic administration of carboplatin and the ATL disease progression are considered to be factors potentially influencing HBV reactivation. However, retrospective analysis showed that HBV DNA levels were detectable in the peripheral blood before administration of carboplatin, suggesting that carboplatin is unlikely to have been mainly involved in the HBV reactivation. ATL is often diagnosed with a compromised immune system, and the disease progression might have been associated with reactivation of the virus. Interestingly, the timing of the rapid increase in ATL cells in the peripheral blood coincided with that of HBV replication in this case. However, disease progression of ATL alone is very unlikely to have caused the HBV reactivation because reactivation did not occur during the previous ATL progression.

To prevent hepatitis due to HBV reactivation, what lesson can we learn from this case? HBV reactivation following immunosuppressive therapy may lead to acute liver failure or fulminant hepatitis, and the patients have poor prognosis regardless of intensive antiviral treatment.[11,12] For preventing HBV reactivation in patients with previously resolved HBV infection, monitoring of HBV DNA-guided preemptive antiviral therapy is recommended in some guidelines,[13,14] however, the evidence of optimal interval of HBV DNA monitoring is limited. Most recently, monthly monitoring of HBV DNA was shown to effectively prevent HBV reactivation in patients with previously resolved HBV