Table 3 Univariate analysis for factors associated with HBV DNA rebound within 48 weeks after discontinuation of NA treatment

Factors	DNA relapsed ($n = 31$)	DNA non-relapsed ($n = 5$)	Univariate P value
Gender (M:F)	21:10	2:3	0.328[b]
HBV genotype (B:C:ND)	2:27:2	0:4:0	0.523[b]
Before treatment			
Age (years)[a]	41 (25–66)	47 (30–62)	0.749
Platelet ($\times 10^4/\mu L$)[a]	15.6 (9.6–28.0)	17.3 (14.7–18.8)	0.679
ALT (IU/L)[a]	135 (22–780)	192 (94–296)	0.450
HBsAg (IU/mL)[a]	2,983 (66–1,354,400)	4,264 (1,172–10,109)	0.758
HBeAg (+:−)	14:17	2:3	1.000
HBcrAg (log U/mL)[a]	5.4 (3.4–8.8)	6.8 (5.4–7.9)	0.330
HBV DNA (log copies/mL)[a]	7.6 (3.5–10.1)	8.3 (6.7–9.1)	0.766
HBV DNA + RNA titers (log copies/mL)	7.4 (3.4–10.0)	8.0 (6.7–9.0)	0.522
DR ratio	−0.2 (−1.4–0.9)	−0.3 (−0.6 to −0.1)	0.596
After 3 months of treatment			
HBV DNA (log copies/mL)[a]	4.0 (2.2–7.3)	3.7 (3.2–4.2)	0.409
HBV DNA + RNA titers (log copies/mL)	4.8 (2.2–8.2)	4.3 (2.7–4.9)	0.507
DR ratio	0.7 (−0.9–2.7)	0.6 (−0.6–1.4)	0.464
End of treatment			
HBsAg (IU/mL)[a]	2,195 (48–16,301)	533 (<1.1–9,680)	0.105
HBeAg (+:−)	13:18	1:4	0.628[b]
HBcrAg (log U/mL)[a]	4.7 (3.0–8.2)	4.6 (3.6–6.6)	0.657
HBV DNA (log copies/mL)[a]	3.5 (2.1–9.2)	3.0 (2.7–6.1)	0.818
HBV DNA + RNA titers (log copies/mL)	3.7 (2.2–8.7)	4.2 (2.2–5.7)	0.801
DR ratio	0.2 (−1.0–2.7)	0.4 (−0.8–1.2)	0.348
Sequential therapy (+:−)	23:8	3:2	0.603[b]
Duration of treatment (weeks)[a]	36 (24–221)	86 (24–304)	0.278

ND not determined, *DR ratio* HBV DNA + RNA titers/HBV DNA

[a] Median (range) univariate analysis was performed with Mann-Whitney *U* test

[b] Chi-square test

measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio Inc, Tokyo, Japan), as described previously [28, 29].

Evaluation of rebound of HBV DNA and alanine aminotransferase after discontinuation of NA therapy

The rebound of HBV DNA after discontinuation of NA therapy was determined based on two criteria: (1) when the HBV DNA reached >4.0 log copies/mL after discontinuation of NA therapy in patients whose HBV DNA titers became negative (<2.6 log copies/mL) at the end of NA therapy; (2) when the HBV DNA increased to >1.0 log copies/mL after the discontinuation of NA therapy in patients whose HBV DNA titers were still positive (>2.7 log copies/mL) at the end of NA therapy.

Alanine aminotransferase (ALT) rebound after discontinuation of NA therapy was defined using the following criteria: (1) when ALT reached >50 IU/L after discontinuation of NA therapy in those patients whose ALT levels had normalized (≤35 IU/L) at the end of NA therapy; (2) when ALT increased by >80 IU/L (twofold of upper limit of normal) after discontinuation of NA therapy in those patients whose ALT levels were still high (>35 IU/L) at the end of NA therapy.

Statistical analysis

The baseline characteristics of the patients in the two groups were compared, and differences were assessed by the chi-square test with Yate's correction, Fisher's exact probability test, and the Mann–Whitney *U* test. All *P* values of <0.05 by the two-tailed test were considered to be significant. To identify predictors for HBV DNA or ALT rebound, univariate and multivariate logistic regression analyses were performed. Potential predictive factors included the following variables: age, gender, body mass index (BMI), platelet count, prothrombin time, total

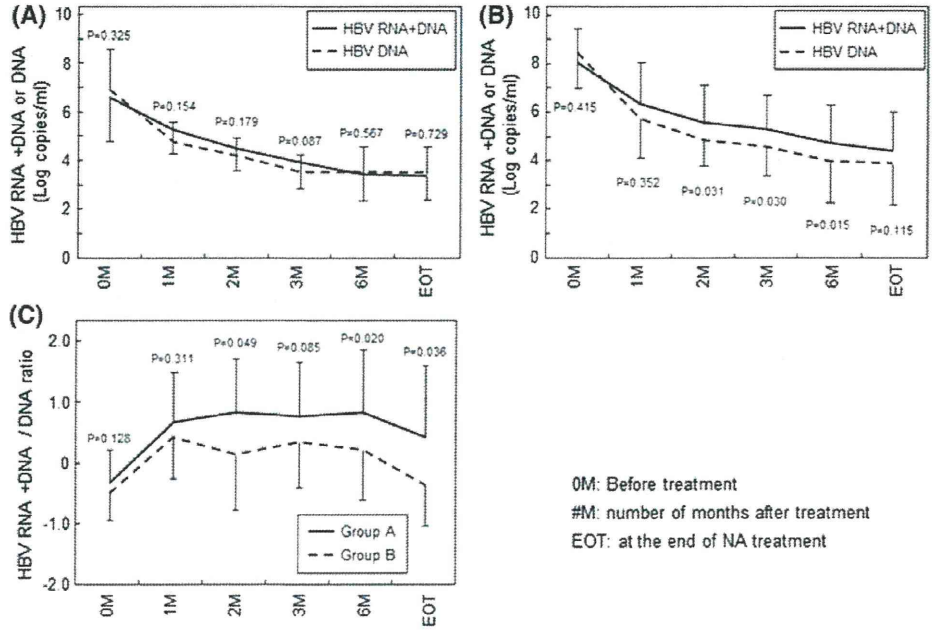

Fig. 2 Change in HBV DNA and HBV DNA + RNA titers during NA therapy. **a**, **b** HBV DNA + RNA titers and HBV DNA titers were compared at each time point for the DNA non-relapse group (**a**) and DNA relapse group (**b**). **c** Changes in the HBV RNA + DNA/HBV DNA ratio were compared with each group. Statistical analyses were performed by the Mann–Whitney *U* test

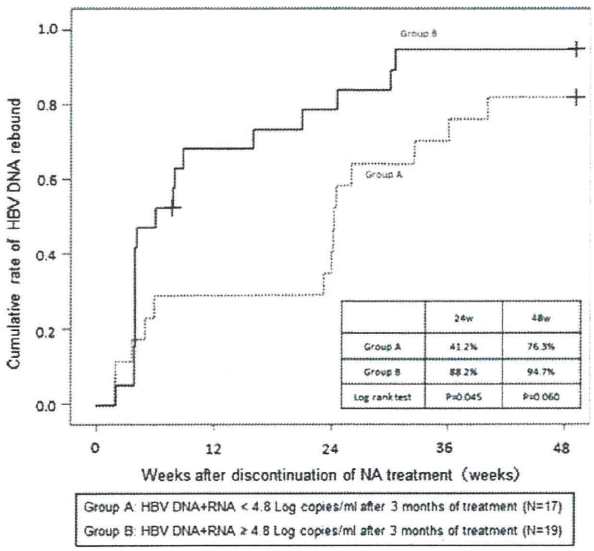

Fig. 3 Cumulative rate of HBV DNA rebound after discontinuation of NA treatment. Seventeen patients whose HBV DNA + RNA titers reached <4.8 log copies/mL after 3 months of treatment, were assigned to group A; the other 19 patients, whose HBV DNA + RNA titers were ≥4.8 log copies/mL after 3 months of treatment, were assigned to group B. The cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients was analyzed using the Kaplan–Meier method

bilirubin, aspartate aminotransferase, ALT, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyltranspeptidase, HBV DNA titer, HBV DNA + RNA titer, and the DR ratio. As shown in a previous study, interferon treatment decreases the production of HBV RNA particles [23]. Thus, HBV RNA + DNA titer at 6 months of treatment was considered to be inappropriate for the statistical analyses in the present study, and these data were not included in these analyses. Odds ratios (OR) and 95 % confidence intervals (95 % CI) were also calculated. Variables with at least marginal significance ($P < 0.10$) in the univariate analysis were entered into the multiple logistic regression analysis to identify significant independent factors. Statistical analyses were performed using SPSS ver. 17.0 (SPSS, Chicago, IL).

Results

Analysis of HBV DNA and ALT rebound rates after discontinuation of NA therapy

Although NA therapy suppressed HBV replication and genomic HBV DNA synthesis, serum HBV DNA and ALT rebound occurred with a high frequency after therapy discontinuation. The cumulative HBV DNA and ALT rebound rates were analyzed to identify associated risk factors. As shown in Fig. 1a, the cumulative HBV DNA rebound rate increased in a time-dependent manner, reaching 58.3 and 91.7 % at 24 and 48 weeks after discontinuation of NA therapy, respectively. The cumulative

Table 4 Univariate analysis for factors associated with HBV DNA rebound within 24 weeks after discontinuation of NA treatment in those patients whose HBV DNA titer became negative at the end of NA treatment

Factors[a]	DNA relapsed ($n = 5$)	DNA non-relapsed ($n = 6$)	Univariate P value[b]
Gender (M:F)	3:2	4:1	0.545 (chi-square test)
HBV genotype (B:C:ND)	0:4:1	0:6:0	0.455 (chi-square test)
Before treatment			
Age (years)[c]	41 (3–52)	54 (32–66)	0.119
Platelet ($\times 10^4/\mu L$)[c]	18.8 (11.7–27.5)	14.8 (10.2–23.6)	0.221
ALT (IU/L)[c]	186 (79–303)	95 (48–270)	0.273
HBsAg (IU/mL)[c]	2,603 (2,064–9,400)	1,984 (406–7,016)	0.180
HBeAg (+:−)	2:3	1:5	0.545 (chi-square test)
HBcrAg (log U/mL)[c]	5.4 (5.0–7.8)	4.1 (3.4–7.9)	0.462
HBV DNA (log copies/mL)[c]	5.7 (3.8–9.2)	7.9 (5.7–9.7)	0.410
HBV DNA + RNA titers (log copies/mL)	5.6 (3.4–9.0)	7.5 (5.0–9.7)	0.583
DR ratio	−0.1 (−0.8–0.1)	−0.4 (−0.7–0.0)	0.527
After 3 months of treatment			
HBV DNA (log copies/mL)[c]	3.8 (2.2–4.8)	3.5 (2.2–4.4)	0.518
HBV DNA + RNA titers (log copies/mL)	4.0 (3.7–6.0)	3.6 (2.2–4.8)	0.313
DR ratio	1.2 (−0.1 to 1.4)	0.4 (−0.9 to 0.7)	0.272
End of treatment			
HBsAg (IU/mL)[c]	5,681 (684–16,301)	1,865 (85–5,711)	0.144
HBeAg (+:−)	1:4	1:5	1.000 (chi-square test)
HBcrAg (log U/mL)[c]	4.5 (3.6–4.9)	3.4 (3.0–5.6)	0.297
HBV DNA (log copies/mL)[c]	2.2 (2.2–2.2)	2.2 (2.2–2.7)	0.562
HBV DNA + RNA titers (log copies/mL)	3.4 (2.2–4.4)	2.6 (2.2–3.7)	0.463
DR ratio	1.3 (0.2–2.1)	0.5 (−0.1 to 1.6)	0.201
Sequential therapy (+:−)	3:2	6:0	0.182 (chi-square test)
Duration of treatment (weeks)[c]	31 (24–175)	24 (24–110)	0.291

[a] Unless indicated otherwise, the values are given as the number (n) of patients

[b] Univariate analysis was performed with Mann-Whitney U test unless indicated otherwise

[c] Median (range)

ALT rebound rate was lower than that of HBV DNA rebound, but the rate also increased in a time-dependent manner. The cumulative ALT rebound rate reached 41.7 and 71.1 % at 24 and 48 weeks after discontinuation of NA therapy, respectively (Fig. 1b). Accordingly, it was difficult to discontinue NA therapy safely over a long period. Therefore, to identify factors associated with the safe discontinuation of NA therapy, we performed a number of analyses.

Predictive factors for HBV DNA rebound

To identify those factors associated with HBV DNA rebound, we divided the patients into two groups, namely, a HBV DNA relapse and a non-relapse group, respectively, based on the timing of HBV DNA rebound. The 22 patients whose HBV DNA titers rebounded within 24 weeks after discontinuation of therapy were included in the relapse group, and the remaining 14 patients were included in the non-relapse group. As shown in Table 2, HBV DNA + RNA titers and the DR ratio after 3 months of treatment were both associated with HBV DNA rebound ($P = 0.015$ and $P = 0.019$, respectively). However, duration of treatment and HBsAg, HBcrAg, and HBV DNA levels at the end of treatment were not significant predictive factors. As shown in Fig. 1a, most HBV DNA rebound occurred within 48 weeks of treatment discontinuation. However, subsequent multivariate

Table 5 Multiple logistic regression for factors associated with HBV DNA rebound within 24 weeks after discontinuation of NA treatment in those patients whose HBV DNA did not become negative at the end of NA treatment

Factors[a]	DNA relapsed ($n = 16$)	DNA non-relapsed ($n = 9$)	Univariate P value[b]	Multiple logistic regression[c]	
				P value	OR (95 % CI)
Gender (M:F)	9:7	3:6	0.691 (chi-square test)		
HBV genotype (B:C:ND)	1:14:1	1:7:1	0.817 (chi-square test)		
Before treatment					
Age (years)[d]	41 (25–59)	39 (30–62)	0.777		
Platelet ($\times 10^4/\mu L$)[d]	17.4 (9.6–28.0)	14.7 (9.6–18.8)	0.183		
ALT (IU/L)[d]	148 (37–780)	118 (22–304)	0.610		
HBsAg (IU/mL)[d]	3,730 (462–1,354,400)	1,384 (66–10,109)	0.267		
HBeAg (+:−)	10:6	3:6	0.226 (chi-square test)		
HBcrAg (log U/mL)[a]	6.4 (4.8–8.8)	6.5 (3.7–7.4)	0.796		
HBV DNA (log copies/mL)[d]	8.4 (3.5–10.1)	7.7 (4.1–9.2)	0.294		
HBV DNA + RNA titers (log copies/mL)	7.9 (3.8–10.0)	7.1 (3.8–9.1)	0.497		
DR ratio	−0.2 (−1.4 to 0.9)	−0.3 (−1.3 to −0.1)	0.359		
After 3 months of treatment					
HBV DNA (log copies/mL)[d]	4.5 (2.4–7.3)	3.8 (3.1– 4.6)	0.118		
HBV DNA + RNA titers (log copies/mL)	5.6 (3.7–8.2)	4.7 (2.4–6.2)	0.089	0.068	2.048 (0.949–4.419)
DR ratio	1.0 (−0.6 to 2.7)	0.0 (−0.7 to 1.4)	0.061	0.320	
End of treatment					
HBsAg (IU/mL)[d]	2,306 (481–11,607)	626 (<1.1–9,680)	0.064	0.839	
HBeAg (+:−)	10:6	2:7	0.097 (chi-square test)	0.490	
HBcrAg (log U/mL)[d]	5.1 (3.0–8.2)	5.1 (3.1–6.6)	1.000		
HBV DNA (log copies/mL)[d]	3.9 (2.8–9.2)	4.1 (2.8–7.1)	0.887		
HBV DNA + RNA titers (log copies/mL)	4.2 (3.1– 8.7)	3.9 (2.2–6.5)	0.411		
DR ratio	0.3 (−1.0 to 2.8)	−0.4 (−0.8 to 1.2)	0.061	0.171	
Sequential therapy (+:−)	10:6	7:2	0.661 (chi-square test)		
Duration of treatment (weeks)[d]	35 (24–221)	86 (24–304)	0.164		

[a] Unless indicated otherwise, the values are given as the number (n) of patients

[b] Univariate analysis was performed with Mann-Whitney U test unless indicated otherwise

[c] Multiple logistic regression analysis was performed using variables that were at least marginally significant ($P < 0.10$) in the univariate analysis

[d] Median (range)

analysis aimed at identifying factors associated with HBV DNA rebound within 48 weeks after discontinuation of therapy did not identify any independent factors (Table 3).

Because HBV DNA rebound is assumed to be associated with HBV replication activity, HBV DNA and HBV DNA + RNA titers were compared at several points during treatment (Fig. 2). In the non-relapse group, HBV DNA and HBV DNA + RNA titers decreased rapidly, and no divergence was observed during NA therapy (Fig. 2a). In comparison, while HBV DNA titer also declined rapidly in the relapse group, the reduction in HBV DNA + RNA titers occurred so gradually that the two titers had significantly diverged by 2 months after the start of treatment (Fig. 2b).

Multivariate analysis of HBV DNA rebound was performed using the following candidate factors: HBsAg and HBeAg before nucleotide treatment, HBV DNA, HBV

Table 6 Multiple logistic regression for factors associated with ALT rebound within 24 weeks after discontinuation of NA treatment

Factors[a]	ALT relapsed ($n = 13$)	ALT non-relapsed ($n = 23$)	Univariate P value[b]	Multiple logistic regression[c]	
				P value	OR (95 % CI)
Gender (M:F)	7:6	16:7	0.346 (chi-square test)		
HBV genotype (B:C:ND)	0:12:1	2:19:2	0.540 (chi-square test)		
Before treatment					
Age (years)[d]	40 (25–59)	47 (29–66)	0.149		
Platelet ($\times 10^4/\mu L$)[d]	19.1 (9.6–28.0)	14.8 (9.6–27.5)	0.205		
ALT (IU/L)[d]	35 (37–309)	143 (22–780)	0.795		
HBsAg (IU/mL)[d]	3,730 (462–1,354,400)	2,092 (66–10,109)	0.127		
HBeAg (+:−)	10:3	6:17	0.005 (chi-square test)	0.544	
HBcrAg (log U/mL)[d]	6.4 (5.5–8.8)	5.4 (3.4–7.9)	0.131		
HBV DNA (log copies/mL)[d]	7.7 (5.0–10.1)	7.7 (3.5–9.7)	0.434		
HBV DNA + RNA titers (log copies/mL)	7.8 (5.1–10.0)	7.5 (3.4–9.7)	0.397		
DR ratio	−0.2 (−1.4 to 0.9)	−0.4 (−1.4 to 0.5)	0.336		
After 3 months of treatment					
HBV DNA (log copies/mL)[d]	4.9 (2.4–7.3)	3.7 (2.2–4.8)	0.007	0.228	
HBV DNA + RNA titers (log copies/mL)	5.7 (3.8–8.2)	4.1 (2.2–6.3)	0.004	0.120	
DR ratio	0.9 (−0.2 to 2.7)	0.6 (−0.9 to 1.9)	0.115		
End of treatment					
HBsAg (IU/mL)[d]	2,306 (481–11,607)	824 (<1.1–11,600)	0.019	0.821	
HBeAg (+:−)	10:3	4:19	0.001 (chi-square test)	0.003	13.500 (2.473–73.705)
HBcrAg (log U/mL)[d]	5.4 (3.6–8.2)	4.3 (3.0–6.6)	0.085	0.264	
HBV DNA (log copies/mL)[d]	4.4 (2.2–9.2)	3.3 (2.2–7.1)	0.070	0.380	
HBV DNA + RNA titers (log copies/mL)	4.4 (3.1–8.7)	3.6 (2.2–6.5)	0.004	0.174	
DR ratio	0.4 (−1.0 to 2.8)	0.2 (−0.8 to 1.6)	0.434		
Sequential therapy (+:−)	9:4	17:6	0.527 (chi-square test)		
Duration of treatment (weeks)[d]	29 (24–221)	51 (24–304)	0.169		

[a] Unless indicated otherwise, the values are given as the number (n) of patients

[b] Univariate analysis was performed with Mann-Whitney U test unless indicated otherwise

[c] Multiple logistic regression analysis was performed using variables that were at least marginally significant ($P < 0.10$) in the univariate analysis

[d] Median (range)

DNA + RNA titers, and DR ratio after 3 months of treatment, and HBsAg and HBeAg at the end of treatment. As shown in Table 2, only HBV DNA + RNA titer after 3 months of treatment was identified as an independent predictive factor for the safe discontinuation of NA therapy without HBV DNA rebound ($P = 0.043$, OR 9.474, 95 % CI 1.069–83.957). HBsAg titer at the end of treatment and HBV DNA titer after 3 months of treatment were marginally associated ($P = 0.070$, $P = 0.074$, respectively). These results suggest that HBV rebound is significantly associated with HBV replication activity during NA treatment.

To analyze the cumulative HBV DNA rebound rate, we divided the 36 subjects into two groups. Cut-off values for assigning patients to the groups were determined by inspection of the receiver operating characteristic (ROC) curve. According to this curve, the best cut-off value of HBV DNA + RNA after 3 months of treatment was

Table 7 Multiple logistic regression for factors associated with ALT rebound within 48 weeks after discontinuation of NA treatment

Factors[a]	ALT relapsed ($n = 25$)	ALT non-relapsed ($n = 11$)	Univariate P value[b]	Multiple logistic regression[c]	
				P value	OR (95 % CI)
Gender (M:F)	17:8	6:5	0.475 (chi-square test)		
HBV genotype (B:C:ND)	2:21:2	0:10:1	0.627 (chi-square test)		
Before treatment					
Age (years)[d]	41 (25–64)	45 (29–66)	0.877		
Platelet ($\times 10^4/\mu L$)[d]	15.6 (9.6–28.0)	16.5 (9.6–27.5)	0.768		
ALT (IU/L)[d]	143 (22–402)	118 (48–780)	0.945		
HBsAg (IU/mL)[d]	2,878 (66–1,354,400)	4,908 (1,172–10,109)	0.490		
HBeAg (+:−)	12:13	4:7	0.718 (chi-square test)		
HBcrAg (log U/mL)[d]	6.3 (4.0–8.8)	5.8 (3.4–7.9)	0.518		
HBV DNA (log copies/mL)[d]	7.7 (3.5–10.1)	7.7 (3.8–9.6)	0.353		
HBV DNA + RNA titers (log copies/mL)	7.8 (3.8–10.0)	7.4 (3.4–9.0)	0.429		
DR ratio	−0.2 (−1.4 to 0.9)	−0.4 (−1.3 to 0.5)	0.201		
After 3 months of treatment					
HBV DNA (log copies/mL)[d]	4.2 (2.2–7.3)	3.6 (2.2–4.6)	0.082	0.106	
HBV DNA + RNA titers (log copies/mL)	4.8 (2.2–8.2)	4.2 (2.2–6.3)	0.271		
DR ratio	0.7 (−0.9 to 2.7)	0.6 (−0.7 to 1.9)	0.757		
End of treatment					
HBsAg (IU/mL)[d]	2,387 (48–16,301)	812 (<1.1–11,600)	0.183		
HBeAg (+:−)	13:12	2:9	0.142 (chi-square test)		
HBcrAg (log U/mL)[d]	5.1 (3.0–8.2)	3.9 (3.0–6.6)	0.291		
HBV DNA (log copies/mL)[d]	3.6 (2.1–9.2)	3.3 (2.2–7.1)	0.782		
HBV DNA + RNA titers (log copies/mL)	3.7 (2.2–8.7)	3.6 (2.2–6.5)	0.655		
DR ratio	0.3 (−1.0 to 2.8)	−0.1 (−0.8 to 1.3)	0.135		
Sequential therapy (+:−)	20:5	6:5	0.224 (chi-square test)		
Duration of treatment (weeks)[d]	31 (24–221)	91 (24–304)	0.028	0.034	1.014 (1.001–1.027)

[a] Unless indicated otherwise, the values are given as the number (n) of patients

[b] Univariate analysis was performed with Mann-Whitney U test unless indicated otherwise

[c] Multiple logistic regression analysis was performed using variables that were at least marginally significant ($P < 0.10$) in the univariate analysis

[d] Median (range)

4.8 log copies/mL (sensitivity 0.733, specificity 0.619, positive predictive value 0.578, negative predictive value 0.765). Seventeen subjects who achieved a titer of <4.8 log copies/mL of HBV DNA + RNA after 3 months of treatment were assigned to group A; the remaining 19 subjects were assigned to group B. The cumulative HBV DNA rebound rate of group A was significantly lower than that of group B at 24 weeks after discontinuation ($P = 0.045$, Fig. 3).

To address potential bias in the study criteria, we analyzed subjects separately depending on whether HBV DNA titer became negative or not at the end of treatment to identify factors associated with HBV DNA rebound. No significant factors for HBV DNA rebound were identified in patients whose HBV DNA titer became negative at the end of NA treatment ($n = 11$) (Table 4). In patients whose HBV DNA did not become negative at the end of NA treatment ($n = 25$), HBV DNA + RNA titer after

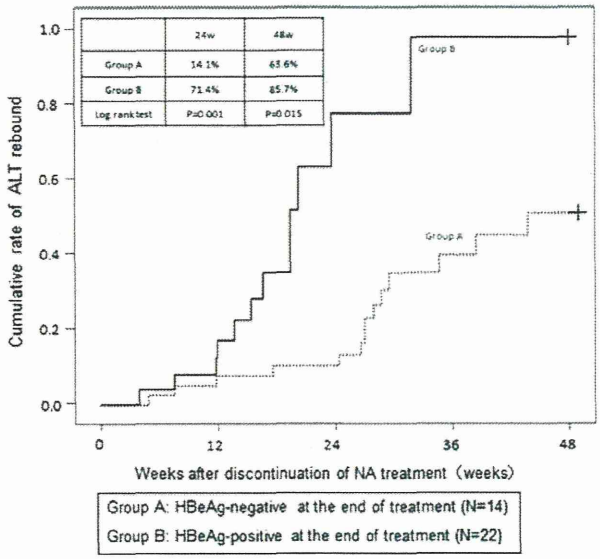

Fig. 4 Cumulative rate of ALT rebound after discontinuation of NA treatment. Fourteen patients who were hepatitis B virus e antigen (*HBeAg*) negative at the end of treatment were assigned to group A; the other 22 patients, who were positive to HBeAg at the end of treatment, were assigned to group B. The cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients was analyzed using the Kaplan–Meier method

3 months of treatment was identified as a marginally significant predictive factor for safe discontinuation of NA therapy without HBV DNA rebound ($P = 0.068$, OR 2.048, 95 % CI 0.949–4.419) (Table 5).

Predictive factors for ALT rebound

To identify predictive factors for ALT rebound, patients were divided into two groups based on the timing of ALT elevation. The 13 patients whose ALT levels rebounded within 24 weeks after discontinuation of therapy were assigned to the ALT relapse group, and the remaining 23 patients were assigned to the ALT non-relapse group. As shown in Table 6, HBeAg presence before treatment, HBV DNA and HBV DNA + RNA titers after 3 months of treatment, and HBeAg presence, HBV DNA + RNA levels, and HBsAg titer at the end of treatment were significantly associated with ALT relapse in the univariate analysis. However, ALT, duration of treatment, and DR ratio at the end of treatment were not significant.

As shown in Table 6, multivariate analysis of ALT rebound was performed using the following candidate factors: HBeAg presence before treatment, HBV DNA and HBV DNA + RNA levels after 3 months of treatment, and HBeAg presence, HBV DNA and DNA + RNA levels, HBcrAg titer, and HBsAg titer at the end of treatment. Only the presence of HBeAg at the end of treatment was identified as an independent predictive factor for safe discontinuation of NA therapy without ALT rebound ($P = 0.003$, OR 13.500, 95 % CI 2.473–73.705). These results suggest that ALT rebound is also significantly associated with HBV replication activity during NA therapy.

As shown in Fig. 1b, most ALT rebound also occurred within 48 weeks. We performed further analysis to identify factors associated with ALT rebound within 48 weeks after discontinuation of NA therapy. In the univariate analysis, duration of NA treatment was significantly associated with ALT relapse, and HBV DNA level after 3 months of treatment was marginally associated with ALT relapse. Only duration of NA treatment was identified as an independent predictive factor for safe discontinuation of NA therapy without ALT rebound by multivariate analysis ($P = 0.034$, OR 1.014, 95 % CI 1.001–1.027) (Table 7).

To analyze the cumulative ALT rebound rate, the 36 subjects were divided into two groups based on HBeAg presence. Twenty-two subjects who were HBeAg-negative at the end of treatment were assigned to group A, and the remaining 14 subjects were assigned to group B. The cumulative ALT rebound rate of group A was significantly lower than that of group B at 24 and 48 weeks after discontinuation of therapy ($P = 0.001$, $P = 0.015$, respectively; Fig. 4).

To account for potential bias in the study criteria, we analyzed subjects separately based on whether ALT was normalized or not at the end of treatment, with the aim of identifying factors for ALT rebound. In patients whose ALT was normalized at the end of NA treatment ($n = 25$), HBeAg presence before treatment, HBV DNA and HBV DNA + RNA titers after 3 months of treatment, and HBeAg presence at the end of treatment were significantly associated with ALT relapse in the univariate analysis. HBeAg presence at the end of treatment was identified as an independent predictive factor for safe discontinuation of NA therapy without ALT relapse (Table 8). In patients whose ALT was not normalized at the end of NA treatment ($n = 11$), only HBV DNA titer after 3 months of treatment was marginally associated with ALT relapse in the univariate analysis ($P = 0.052$; Table 9).

Predictive factors for ALT rebound in HBeAg-positive patients

Because the cumulative rate of ALT rebound in HBeAg-positive CHB patients was significantly higher thanthat in HBeAg-negative patients, we focused on the 16 HBeAg-positive patients to identify factors associated with ALT rebound in these patients. As shown in Table 10, only the HBV DNA + RNA titer after 3 months of treatment was significant in the univariate analysis. However, in multivariate analysis, the HBV DNA + RNA titer after

Table 8 Multiple logistic regression for factors associated with HBV DNA rebound within 24 weeks after discontinuation of NA treatment in those patients whose ALT levels had normalized at the end of NA treatment

Factors[a]	ALT relapsed ($n = 6$)	ALT non-relapsed ($n = 19$)	Univariate P value[b]	Multiple logistic regression[c]	
				P value	OR (95 % CI)
Gender (M:F)	5:1	12:7	0.073 (chi-square test)	0.073	
HBV genotype (B:C:ND)	0:6:0	2:16:1	0.584 (chi-square test)		
Before treatment					
Age (years)[d]	41 (31–59)	46 (29–66)	0.545		
Platelet ($\times 10^4/\mu L$)[d]	20.3 (9.6–28.0)	14.7 (9.6–27.5)	0.484		
ALT (IU/L)[d]	161 (62–309)	118 (22–780)	0.750		
HBsAg (IU/mL)[d]	3,573 (462–1,354,400)	2,485 (66–0.109)	0.201		
HBeAg (+:−)	5:1	5:14	0.023 (chi-square test)	0.707	
HBcrAg (log U/mL)[d]	7.1 (6.5–7.8)	5.3 (3.4–7.9)	0.264		
HBV DNA (log copies/mL)[d]	9.1 (6.8–10.0)	8.1 (3.5–9.6)	0.252		
HBV DNA + RNA titers (log copies/mL)	8.3 (6.1–9.7)	7.5 (3.4–9.2)	0.477		
DR ratio	−0.5 (−1.4 to 0.0)	−0.4 (−1.4 to 0.5)	0.503		
After 3 months of treatment					
HBV DNA (log copies/mL)[d]	3.7 (2.4–6.9)	3.7 (2.2–4.8)	0.503		
HBV DNA + RNA titers (log copies/mL)	3.7 (2.4–6.9)	4.2 (2.2–6.3)	0.041	0.413	
DR ratio	1.4 (−0.2 to 1.9)	0.7 (−0.9 to 1.9)	0.111		
End of treatment					
HBsAg (IU/mL)[d]	2,978 (481–16,301)	812 (<1.1–11,600)	0.127		
HBeAg (+:−)	5:1	3:16	0.006 (chi-square test)	0.009	26.667 (2.242–317.147)
HBcrAg (log U/mL)[d]	4.1 (3.6–5.8)	3.7 (3.0–6.6)	0.406		
HBV DNA (log copies/mL)[d]	3.3 (2.2–6.3)	3.4 (2.2–6.1)	0.632		
HBV DNA + RNA titers (log copies/mL)	4.1 (3.2–7.1)	3.6 (2.2–5.7)	0.064	0.444	
DR ratio	0.6 (−1.0 to 2.8)	0.2 (−0.8 to 1.5)	0.340		
Sequential therapy (+:−)	3:3	13:6	0.630 (chi-square test)		
Duration of treatment (weeks)[d]	59 (25–221)	51 (24–304)	0.702		

[a] Unless indicated otherwise, the values are given as the number (n) of patients

[b] Univariate analysis was performed with Mann-Whitney U test unless indicated otherwise

[c] Multiple logistic regression analysis was performed using variables that were at least marginally significant ($P < 0.10$) in the univariate analysis

[d] Median (range)

3 months of treatment was only marginally associated with the safe discontinuation of NA therapy without ALT rebound ($P = 0.050$, OR 8.032, 95 % CI 0.997–64.683). These results suggest that ALT rebound in HBeAg-positive patients might be associated with HBV replication activity during the NA treatment.

To analyze the cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients, the 16 subjects were divided into two groups based on HBV DNA + RNA levels. The cut-off value of HBV DNA + RNA after 3 months of treatment (4.8 log copies/mL) was determined by inspection of the ROC curve (sensitivity 0.833, specificity: 0.889, positive predictive value 0.833, negative predictive value 0.889). Six subjects who achieved <5.0 log copies/mL of HBV DNA + RNA levels after 3 months of treatment were assigned to group A and the remaining

Table 9 Univariate analysis for factors associated with HBV DNA rebound within 24 weeks after discontinuation of NA treatment in the patients in whom ALT levels did not normalize at the end of NA treatment

Factors	ALT relapsed ($n = 7$)	ALT non-relapsed ($n = 4$)	Univariate P value
Gender (M:F)	6:1	4:0	1.000[b]
HBV genotype (B:C:ND)	0:6:1	0:3:1	1.000[b]
Before treatment			
Age (years)[a]	36 (25–56)	50 (30–64)	0.218
Platelet ($\times 10^4/\mu L$)[a]	17.0 (13.1–27.5)	16.1 (15.6–16.5)	0.770
ALT (IU/L)[a]	101 (37–303)	148 (114–270)	0.571
HBsAg (IU/mL)[a]	11,113 (1,180–40,967)	1,384 (406–7,016)	0.197
HBeAg (+: −)	5:2	1:3	0.242[b]
HBcrAg (log U/mL)[a]	5.9 (5.5–8.8)	6.7 (5.0–7.7)	1.000
HBV DNA (log copies/mL)[a]	7.1 (5.0–10.1)	6.7 (5.7–9.7)	0.635
HBV DNA + RNA titers (log copies/mL)	6.9 (5.1–10.0)	6.3 (5.0–9.7)	0.571
DR ratio	−0.1 (−0.2–0.9)	−0.4 (−0.7–0.0)	0.279
After 3 months of treatment			
HBV DNA (log copies/mL)[a]	5.1 (3.8–7.3)	4.2 (2.2–4.4)	0.052
HBV DNA + RNA titers (log copies/mL)	5.7 (3.9–8.2)	4.4 (2.9–6.2)	0.185
DR ratio	0.6 (−0.2–2.7)	0.1 (−0.1–0.6)	0.255
End of treatment			
HBsAg (IU/mL)[a]	4,317 (2,306–11,607)	5,209 (85–5,711)	0.915
HBeAg (+: −)	5:2	1:3	0.242[b]
HBcrAg (log U/mL)[a]	5.4 (3.6–8.2)	5.6 (4.9–5.9)	1.000
HBV DNA (log copies/mL)[a]	4.4 (2.2–9.2)	2.2 (2.2–7.1)	0.178
HBV DNA + RNA titers (log copies/mL)	4.9 (3.1–8.7)	3.0 (2.2–6.5)	0.131
DR ratio	−0.1 (−0.5–2.7)	0.1 (−0.6–1.6)	0.850
Sequential therapy (+: −)	6;1	4:0	1.000[b]
Duration of treatment (weeks)[a]	24 (24–36)	44 (24–110)	0.091

ND not determined, *DR ratio* HBV DNA + RNA titers/HBV DNA

[a] Median (range) univariate analysis was performed with Mann-Whitney *U* test

[b] Chi-square test

ten subjects were assigned to group B. The cumulative ALT rebound rate of group A was significantly lower than that of group B at 24 and 48 weeks after the discontinuation of therapy ($P = 0.008$, $P = 0.024$, respectively, Fig. 5).

Prediction of ALT rebound after discontinuation of therapy using two extracted factors

To predict successful discontinuation of therapy, we analyzed cumulative ALT rebound by using HBV DNA plus RNA levels at 3 months of NA treatment and existence of HBeAg at the end of treatment. Fourteen subjects who achieved both <4.8 log copies/mL of HBV DNA + RNA levels after 3 months of treatment and negative HBeAg at the end of treatment were assigned to group A and the remaining 22 subjects were assigned to group B. The cumulative ALT rebound rate of group A was significantly lower than that of group B among all observation periods ($P = 0.046$, Fig. 6).

Discussion

Since the introduction of NAs, chronic hepatitis B progression has been drastically suppressed. NAs strongly suppress HBV replication in human hepatocytes and rapidly decrease serum HBV DNA titers to undetectable levels [30–33]. However, even if HBV DNA is continuously maintained at undetectable levels, it is difficult to

Table 10 Multiple logistic regression for factors associated with ALT rebound within 24 weeks after discontinuation of NA therapy in HBeAg-positive patients ($n = 16$)

Factors[a]	ALT relapsed ($N = 10$)	ALT non-relapsed ($N = 6$)	Univariate P value[b]	Multiple logistic regression[c]	
				P value	OR (95 % CI)
Gender (M:F)	5:5	3:3	0.696 (chi-square test)		
HBV genotype (B:C)	0:10	0:6	1.000 (chi-square test)		
Before treatment					
Age (years)[d]	35 (25–56)	38 (29–47)	0.957		
Platelets ($\times 10^4/\mu L$)[d]	20.3 (9.6–28.0)	17.3 (14.5–27.5)	0.768		
ALT (IU/L)[d]	148 (37–309)	155 (46–270)	0.958		
HBsAg (IU/mL)[d]	11,113 (462–1,354,400)	6,283 (66–10,109)	0.662		
HBcrAg (log U/mL)[d]	7.1 (5.5–8.8)	7.4 (5.2–7.7)	0.714		
HBV DNA (log copies/mL)[d]	9.1 (6.5–10.1)	8.8 (3.8–9.7)	0.792		
HBV DNA + RNA titers (log copies/mL)	8.3 (6.1–10.0)	8.6 (3.4–9.7)	0.958		
DR ratio	−0.2 (−1.4 to 0.9)	−0.3 (−0.7 to 0.0)	0.776		
After 3 months of treatment					
HBV DNA (log copies/mL)[d]	5.0 (3.5–7.3)	4.1 (2.2–4.4)	0.056	0.897	
HBV DNA + RNA titers (log copies/mL)	5.8 (4.8–8.2)	4.7 (3.7–6.3)	0.011	0.050	8.032 (0.997–64.683)
DR ratio	1.1 (−0.2 to 2.7)	1.1 (−0.6 to 1.9)	0.792		
End of treatment					
HBsAg (IU/mL)[d]	4,736 (823–16,301)	3,523 (48–11,600)	0.529		
HBeAg (+:−)	10:0	4:2	0.125 (chi-square test)		
HBcrAg (log U/mL)[d]	5.6 (4.1–8.2)	5.3 (4.0–6.6)	0.310		
HBV DNA (log copies/mL)[d]	4.4 (2.2–9.2)	3.7 (2.1–6.1)	0.220		
HBV DNA + RNA titers (log copies/mL)	4.9 (3.7–8.7)	3.9 (3.4–5.7)	0.093	0.543	
DR ratio	0.5 (−1.0 to 2.8)	0.2 (−0.8 to 1.6)	0.635		
Sequential therapy (+:−)	7:3	4:2	0.654 (chi-square test)		
Duration of treatment (weeks)[d]	29 (24– 221)	119 (24–175)	0.169		

[a] Unless indicated otherwise, the values are given as the number (n) of patients

[b] Univariate analysis was performed with Mann-Whitney U test unless indicated otherwise

[c] Multiple logistic regression analysis was performed using variables that were at least marginally significant ($P < 0.10$) in the univariate analysis

[d] Median (range)

completely eliminate HBV from the liver. The goal of NA therapy is therefore to reduce the HBV DNA titer and to induce an inactive state of hepatitis, but, as a result, it is necessary that NA therapy should be continued for a long period of time. As it is well known that long-term treatment with NAs increases the incidence of HBV drug resistance [14], we propose that patients who maintain an inactive state of hepatitis with NA therapy may be able to discontinue the NA therapy to prevent the appearance of drug-resistant strains. However, as shown in Fig. 1, in our patient cohort, hepatitis was re-activated after discontinuation of the therapy in more than 70 % of the patients who discontinued the NA therapy. Therefore, in this study, we analyzed predictive factors for the safe discontinuation of NA therapy.

After discontinuation of NA therapy, serum HBV DNA titers increased in 91.7 % of our patients within 48 weeks (Fig. 1a). In the multivariate logistic regression, the HBV