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

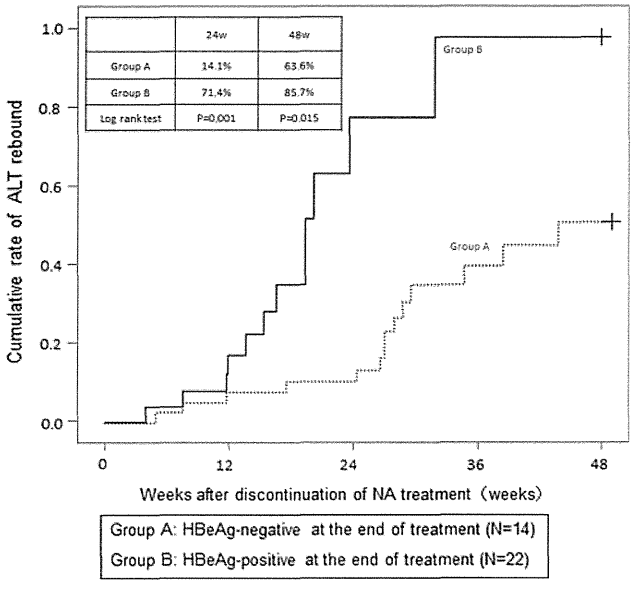

Fig. 4 Cumulative rate of ALT rebound after discontinuation of NA treatment. Fourteen patients who were hepatitis B virus e antigen (*HBeAg*) negative at the end of treatment were assigned to group A; the other 22 patients, who were positive to HBeAg at the end of treatment, were assigned to group B. 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

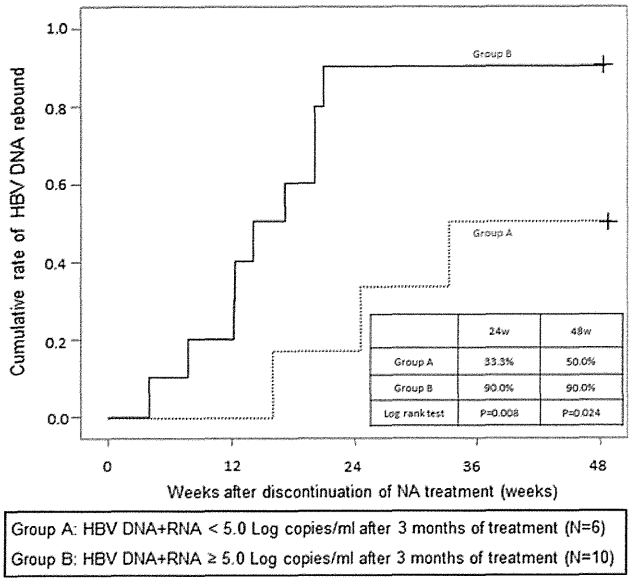

Fig. 5 Cumulative rate of ALT rebound after discontinuation of NA treatment in HBeAg-positive chronic hepatitis B patients. Six patients whose HBV DNA + RNA titers reached <5.0 log copies/mL after 3 months of treatment were assigned to group A; the other ten patients, whose HBV DNA + RNA titers were ≥5.0 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

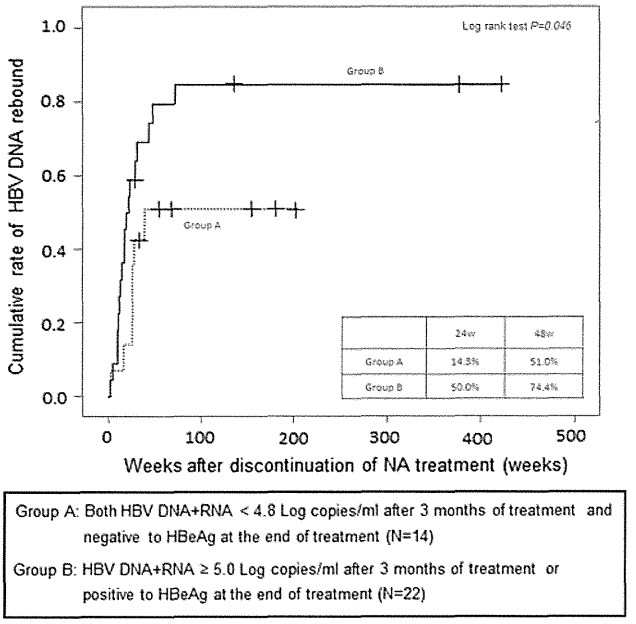

Fig. 6 Cumulative rate of ALT rebound after discontinuation of NA treatment by using combined criteria. The subjects were divided using combined criteria. Fourteen patients whose HBV DNA + RNA titers reached <5.0 log copies/mL after 3 months of treatment and who were HBeAg negative at the end of NA treatment were assigned to group A; the other 22 patients were assigned to group B. The cumulative ALT rebound rate in HBeAg-positive chronic hepatitis B patients was analyzed using the Kaplan–Meier method

DNA + RNA titer after 3 months of treatment was found to be significantly associated with HBV DNA rebound ($P = 0.043$, OR = 9.474; Table 2). Two other factors, HBV DNA titer after 3 months of treatment and HBsAg titer at the end of treatment, were marginally associated with HBV DNA rebound ($P = 0.074$, $P = 0.070$, respectively). After 3 months of NA treatment, HBV DNA titers decreased in both the HBV DNA relapse and non-relapse groups, but HBV DNA + RNA levels in the relapse group remained high. NA therapy suppressed the production of mature HBV particles in both groups, but in the HBV DNA relapse group, high HBV replication activity was likely maintained during the treatment, and immature HBV particles associated with HBV RNA genomes were continuously produced and accumulated in hepatocytes. After discontinuation of the treatment, these accumulated immature HBV particles may have been maturated and been released from the hepatocytes. Thus, rebound of HBV DNA titers occurred rapidly after the discontinuation of NA therapy.

Although the presence of HBeAg before treatment, HBV DNA and DNA + RNA titers after 3 months of treatment, and the presence of HBeAg, HBsAg titer, and HBV DNA + RNA titer at the end of treatment were all significantly associated with ALT rebound in the univariate analysis, only the presence of HBeAg at the end of treatment was identified as an independent predictive factor for ALT rebound following multivariate analysis (Table 4). HBeAg is commonly strongly associated with the activity of HBV replication, and HBV DNA levels are high in HBeAg-positive HBV carriers. Thus, HBe seroconversion usually indicates suppression of HBV activity, and the absence of HBeAg is thought to indicate the inactivation of HBV replication.

ALT rebound following the discontinuation of NA therapy was not observed in six of the 16 patients (37.5 %) who were HBeAg-positive at the end of treatment. After examining predictive factors for ALT rebound in these HBeAg-positive patients, only the HBV DNA + RNA titer after 3 months of treatment was identified as an independent predictive factor for ALT rebound in HBeAg-positive patients (Table 6). Although the presence of HBeAg indicates high activities of HBV replication and hepatitis, it is expected to be difficult to discontinue NA therapy without ALT rebound in these patients. However, these results indicate that HBV replication activities vary greatly among individuals and suggest that it might be possible to predict future replication activity based on HBV DNA + RNA titers after 3 months of treatment.

A limitation of this study is the small sample size; as such, selection bias might have affected the internal validity of the study. As it is not common to discontinue

NA therapy in Japan, we were only able to examine 36 subjects in our study. Because HBV-related markers such as HBsAg, HBcrAg, and HBV DNA + RNA titers varied widely among individuals, HBeAg an HBV DNA + RNA titers were only marginally associated with HBV DNA or ALT rebound after the discontinuation of NA therapy. In a previous study, Matsumoto et al. [34] analyzed predictive factors for the safe discontinuation of NA therapy in 126 clinical HBeAg-negative subjects from 12 clinical centers. These authors reported that HBsAg and HBcrAg titers at the end of treatment were predictive factors for the safe discontinuation of therapy. In our study, we also found that the absence of HBeAg at the end of treatment was important for the safe discontinuation of NA therapy, but we found no association between safety and HBsAg or HBcrAg titers. However, while HBsAg and HBcrAg are known to be associated with HBV replication activity, our results involving HBeAg and HBV DNA + RNA titers as important factors for safe discontinuation appear to be consistent.

In our study, the duration of NA therapy was quite short (mean duration was 36 weeks). Similar results might be observed if the NA therapy was extended, but it might be difficult to depress the potential of infected HBV replication with long-term NA therapy. HBsAg titers represent HBV replication in human hepatocytes, and it is difficult to decrease HBsAg levels by NA therapy. Thus, HBV DNA + RNA levels might be an important factor for predicting the HBV DNA or ALT rebounds.

As it may be difficult to discontinue therapy in patients with advanced liver fibrosis, our study subjects were selected based on liver spare capacities. As shown in Fig. 1, ALT rebound is likely to occur in most patients following the discontinuation of NA therapy, and severe hepatitis could occur in some patients. Thus, if the liver spare capacity were low, NA therapy would not be discontinued; the patients in this study were selected solely based on clinical aspects, which may have influenced our interpretation of the results.

In conclusion, HBV replication activity was found to be an important predictor of safe discontinuation of NA therapy. These findings suggest that monitoring of serum HBV DNA + RNA levels would be a useful method for predicting the re-activation of chronic hepatitis B following discontinuation of NA therapy.

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Conflict of interest None to declare.

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ORIGINAL ARTICLE

Factors correlating with acoustic radiation force impulse elastography in chronic hepatitis C

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Abstract

AIM: To investigate the factors other than fibrosis stage correlating with acoustic radiation force impulse (ARFI) elastograpy in chronic hepatitis C.

METHODS: ARFI elastograpy was performed in 108 consecutive patients with chronic hepatitis C who underwent a liver biopsy. The proportion of fibrosis area in the biopsy specimens was measured by computer-assisted morphometric image analysis.

RESULTS: ARFI correlated significantly with fibrosis stage (β = 0.1865, P < 0.0001) and hyaluronic acid levels (β = 0.0008, P = 0.0039) in all patients by multiple regression analysis. Fibrosis area correlated significantly with ARFI by Spearman's rank correlation test but not by multiple regression analysis. ARFI correlated significantly with body mass index (BMI) (β = -0.0334, P = 0.0001) in $F0$ or $F1$, with γ-glutamyltranspeptidase levels (β = 0.0048, P = 0.0012) in $F2$, and with fibrosis stage (β = 0.2921, P = 0.0044) and hyaluronic acid levels (β = 0.0012, P = 0.0025) in $F3$ or $F4$. The ARFI cutoff value was 1.28 m/s for $F \geqslant 2$, 1.44 m/s for $F \geqslant 3$, and 1.73 m/s for $F4$.

CONCLUSION: ARFI correlated with fibrosis stage and hyaluronic acid but not with inflammation. ARFI was affected by BMI, γ-glutamyltranspeptidase, and hyaluronic acid in each fibrosis stage.

Key words: Acoustic radiation force impulse; Body mass index; Chronic hepatitis C; Computer-assisted morphometric image analysis; Fibrosis stage; Hyaluronic acid; Liver stiffness measurement; Transient elastography; Velocity of shear wave

Core tip: The assessment of liver fibrosis stage is important to estimate prognosis and to identify the patients requiring antiviral treatment in chronic hepatitis C. Liver biopsy is a gold standard for assessing fibrosis, but is invasive. Thus methods for noninvasively assessing fibrosis have been developed. Liver stiffness measurement (LSM) by Fibroscan and acoustic radiation force impulse correlate with fibrosis stage. However, LSM may be affected by factors other than fibrosis, such as edema, steatosis, and inflammation.

Nishikawa T, Hashimoto S, Kawabe N, Harata M, Nitta Y, Murao M, Nakano T, Mizuno Y, Shimazaki H, Kan T, Nakaoka K, Takagawa Y, Ohki M, Ichino N, Osakabe K, Yoshioka K.

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INTRODUCTION

The assessment of liver fibrosis stage is important to estimate prognosis and to identify the patients requiring antiviral treatment in chronic hepatitis C.

Methods for noninvasively assessing liver fibrosis have been developed. Liver stiffness measurement (LSM) by transient elastography (TE) with Fibroscan[1-3] and velocity of shear wave (Vs) measured by acoustic radiation force impulse (ARFI)[4-6] correlate with liver fibrosis stage in various liver diseases. However, LSM is affected by factors other than liver fibrosis, such as edema, steatosis, inflammation and necrosis. In particular, inflammation affects LSM; acute or chronic inflammation can result in a high LSM, indicating the presence of falsely higher fibrosis stage than the actual fibrosis stage by both TE[7-9] and ARFI[10-12]. However, Rizzo *et al*[13] reported that ARFI is not correlated with alanine aminotransferase (ALT) levels[13].

Liver fibrosis is usually semi-quantitatively assessed by the numerical systems of Scheuer[14], the Metavir group[15] or Ishak *et al*[16]. Direct measurements of the amount of fibrosis in a biopsy specimen by computer-assisted morphometric image analysis has been reported, in which morphometric collagen content is measured quantitatively; it has been shown to correlate well with liver biopsy assessment numerical systems scores[17-19]. Isgro *et al*[20] reported that fibrosis area has a better relationship with TE than Ishak stage[20], whereas our previous study demonstrated a better correlation of TE with fibrosis stage than with fibrosis area in patients with chronic hepatitis C[21].

In the present study, factors other than fibrosis stage that affect ARFI were investigated in patients with chronic hepatitis C. The proportion of fibrosis area was quantitatively measured by image analysis software in liver biopsy specimens and the correlation with ARFI was assessed.

MATERIALS AND METHODS

Ethical statement

This study was performed in strict accordance with the ethical guidelines of the Declaration of Helsinki and was approved by the Fujita Health University ethics committee. All study participants provided written informed consent.

Patients

A total of 108 consecutive patients with chronic hepatitis C virus infection who underwent a liver biopsy before treatment with interferon at Fujita Health University Hospital from October 2009 to October 2012 were included (Table 1). Liver biopsy was performed using a 14G disposable true-cut needle under ultrasonographic guidance. Sections were stained with hematoxylin-eosin and azan stain. Liver specimens of at least 1.5 cm length with more than 8 portal tracts were assessed. Liver biopsy specimens were assessed by two hepatologists (Yoshioka K and Nakaoka K) blinded to the clinical data according to Metavir score[15]. Fibrosis was staged as follows: *F*0, no fibrosis; *F*1, portal fibrosis without septa; *F*2, portal fibrosis and few septa; *F*3, numerous septa without cirrhosis; and *F*4, cirrhosis. Activity was graded as follows: A0, none; A1, mild; A2, moderate; and A3, severe activity. Steatosis was graded according to the nonalcoholic fatty liver disease activity score as follows: grade 0; < 5% of hepatocytes involved, grade 1, 5%-33%; grade 2, >33%-66%; and grade 3, > 66%[22]. When fibrosis stage, activity grade, or steatosis grade evaluated by the two hepatologists differed, the higher fibrosis stage, activity grade, or steatosis grade was adopted.

ARFI measurement

Vs measurement by ARFI was performed with a Siemens ACUSON S2000 (Mochida Siemens Medical Systems Co., Ltd., Tokyo, Japan) within 1 wk of liver biopsy[4]. A region in liver to be examined for elastic properties is targeted with a region-of-interest (ROI) cursor while performing B-mode imaging. Tissue at the ROI is mechanically excited using acoustic push pulses to generate localized tissue displacements. The displacements result in propagation of shear-wave away from the region of excitation which is tracked using ultrasonic correlation-based methods. The maximal displacement is estimated for many ultrasound tracking beams laterally adjacent to the single push-beam. By measuring the time to peak displacement at each lateral location, the shear wave propagation velocity can be reconstructed. The examination was performed on the right lobe of the liver. A measurement depth of 2-3 cm below the liver capsule was chosen. Ten successful acquisitions at different locations were performed on each patient, and the results are expressed in meters/second (m/s), and the median value was calculated. The shear wave propagation velocity is considered to be proportional to the square root of tissue elasticity.

The procedures were performed by two investigators (Nishikawa T and Hashimoto S) who were blind to clinical, serological and histological data. The correlation in Vs measurement between two operators was good (r = 0.934).

Proportion of fibrosis area in the liver biopsy specimens

The proportion of fibrosis area in the biopsy specimens was measured by computer-assisted morphometric image analysis. Liver biopsy specimens were stained with azan stain. Microscopic images of the entire biopsy specimen were obtained with a digital microscope (BZ-9000, Keyence, Tokyo, Japan). Fibrosis area, which was stained blue with azan, was marked and measured with Image Pro

Table 1 Characteristics of 108 patients with chronic hepatitis C virus infection

	All patients (n = 108)	F0 (n = 14)	P values of Mann-Whitney U test between F0 and F1	F1 (n = 17)	P values of Mann-Whitney U test between F0-1 and F2	F2 (n = 32)	P values of Mann-Whitney U test between F2 and F3	F3 (n = 31)	P values of Mann-Whitney U test between F3 and F4	F4 (n = 14)
Age (yr)[1]	59.5 (49.0-66.0)	48.0 (41.0-60.0)	NS	51.0 (41.8-65.5)	NS	61.5 (51.5-66.5)	NS	61.0 (52.0-67.0)	NS	60.5 (54.0-66.0)
Gender (female/male)[2]	52/56	8/6	NS	8/9	NS	15/17	NS	13/18	NS	8/6
BMI	22.5 (20.5-24.6)	22.0 (20.0-23.2)	NS	23.5 (19.8-25.4)	NS	23.0 (20.7-24.4)	NS	23.2 (21.1-26.1)	NS	21.9 (19.0-23.4)
Fibrosis stage (F0/F1/F2/F3/F4)	14/17/32/31/14	-		-		-		-		-
Inflammatory grade (A0/A1/A2/A3)	12/32/53/11	9/5/0/0	0.0261	2/15/0/0	0.0001	1/9/22/0	0.0060	0/2/22/7	NS	0/1/9/4
Steatosis grade (S0/S1/S/2/S3)	42/42/14/10	8/5/0/1	NS	7/9/0/1	NS	10/11/6/5	NS	9/14/5/3	NS	8/3/3/0
AST (IU/L)[1]	44.0 (31.5-82.0)	28.5 (24.0-38.0)	NS	36.0 (23.0-41.3)	0.0033	48.5 (36.5-101.5)	NS	48.0 (42.5-85.3)	NS	65.5 (37.0-88.0)
ALT (IU/L)[1]	55.0 (35.0-91.5)	37.5 (22.0-59.0)	NS	39.0 (24.8-52.0)	0.0095	65.0 (41.0-153.0)	NS	70.0 (41.3-109.0)	NS	64.0 (36.0-91.0)
γ-GTP (IU/L)[1]	33.0 (23.5-75.0)	23.0 (14.0-27.0)	0.0802	28.0 (19.5-71.3)	NS	39.5 (24.5-89.5)	NS	41.0 (28.0-96.8)	0.0329	30.5 (27.0-38.0)
Platelet count ($\times 10^4/\mu L$)[1]	14.3 (11.3-17.6)	14.6 (11.7-20.2)	NS	18.2 (16.6-21.2)	0.0107	16.1 (14.0-17.4)	0.0080	12.2 (11.3-14.3)	0.0078	10.1 (7.1-11.6)
Prothrombin time (INR)[1]	1.00 (0.96-1.06)	0.95 (0.90-0.99)	NS	0.96 (0.93-1.02)	NS	1.00 (0.95-1.03)	0.0144	1.03 (1.00-1.08)	0.0229	1.10 (1.03-1.12)
Albumin (g/dL)[1]	4.2 (4.0-4.5)	4.4 (4.1-4.6)	NS	4.4 (4.2-4.5)	NS	4.3 (4.0-4.5)	0.0524	4.1 (3.8-4.3)	NS	4.0 (3.8-4.2)
Total cholesterol (mg/dL)[1]	170 (150-188)	193 (177-207)	0.0619	169 (155-193)	NS	172 (156-189)	0.0615	159 (141-177)	NS	160 (144-183)
γ-globulin (g/dL)[1]	1.51 (1.33-1.79)	1.28 (1.14-1.40)	0.0262	1.40 (1.28-1.70)	NS	1.44 (1.34-1.64)	0.0067	1.63 (1.51-2.11)	NS	1.66 (1.43-1.97)
Hyaluronic acid (ng/mL)[1]	89 (49-206)	39 (30-64)	NS	49 (26-77)	0.0041	89 (66-185)	0.0601	184 (82-245)	0.0291	232 (191-338)
HCV genotype (1/2)	81/26	10/4	NS	12/5	NS	22/9	NS	5/26	NS	11/3
HCV RNA (logIU/mL)[1]	6.6 (5.8-7.0)	6.5 (6.0-6.9)	NS	6.6 (5.4-7.0)	NS	6.6 (5.8-7.1)	NS	6.7 (5.9-7.1)	NS	6.6 (6.3-6.8)
Fibrosis area (%)[1]	2.63% (1.35-4.95)	0.85% (0.41-1.04)	0.0111	1.37% (0.73-1.85)	0.0022	2.20% (1.62-2.74)	< 0.0001	4.83% (4.03-6.24)	< 0.0001	8.87% (8.04-10.52)
Velocity of shear wave (m/s)[1]	1.38 (1.19-1.71)	1.2 (1.0-1.3)	NS	1.1 (1.0 -1.2)	0.0010	1.3 (1.2-1.6)	0.0014	1.6 (1.5-1.8)	0.0008	2.1 (1.9-2.2)

[1]Data are shown as median (interquartile range); [2]Difference of frequency of gender was assessed by χ^2 test. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GTP: γ-glutamyltranspeptidase.

Plus 4.0 imaging software (Nippon Roper Co., Ltd., Tokyo, Japan).

Statistical analysis

Patients were categorized according to fibrosis stage. The groups were compared with the χ^2 test and Mann-Whitney U test. Factors correlated with ARFI were estimated by Spearman's rank correlation test. Factors independently correlated with ARFI were assessed by multiple regression analysis. The diagnostic performance of ARFI and fibrosis area was determined in terms of sensitivity, specificity, positive and negative predictive value, positive likelihood ratio, diagnostic accuracy, and area under the receiver operating characteristics (ROC) curve. Optimal cutoff values between fibrosis categories were determined at maximum sum of sensitivity and specificity. Data were analyzed using StatFlex version 5.0 for Windows (StatFlex, Osaka, Japan). A two-sided P value of < 0.05 indicated statistical significance.

RESULTS

Semiquantitative histological assessment using the Metavir system

The liver biopsies of the 108 patients were assessed by the Metavir system. Fibrosis stage was $F0$ in 14 patients, $F1$ in 17, $F2$ in 32, $F3$ in 31 and $F4$ in 14 (Table 1).

ARFI measurement

ARFI was significantly correlated with fibrosis stage ($\rho = 0.732$, $P < 0.0001$) (Figure 1A). ARFI values differed significantly between stages $F1$ and $F2$ ($P = 0.0010$), between $F2$

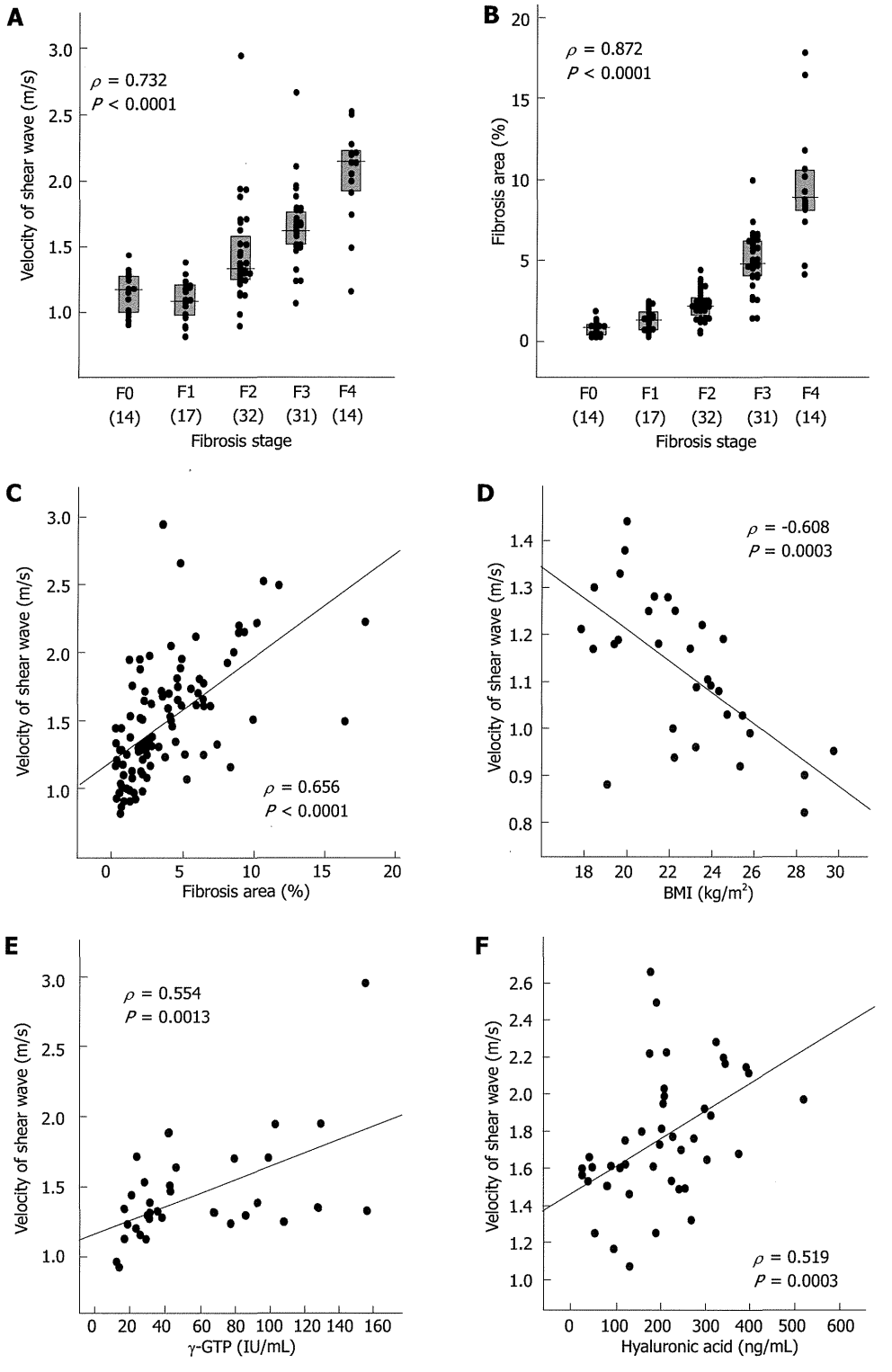

Figure 1 Correlation. A: Between acoustic radiation force impulse (ARFI) and fibrosis stages. The velocity of the sheer wave measured by ARFI was significantly correlated with fibrosis stage in all 108 patients as assessed by the Metavir system ($\rho = 0.732$, $P < 0.0001$). Vertical lines and boxes indicate median values and interquartile ranges, respectively; B: Between proportion of fibrosis area and fibrosis stage. The proportion of fibrosis area was significantly correlated with fibrosis stage in all 108 patients as assessed by the Metavir system ($\rho = 0.872$, $P < 0.0001$). Vertical lines and boxes indicate median values and interquartile ranges, respectively; C: Between ARFI and proportion of fibrosis area. The velocity of the sheer wave measured by ARFI significantly correlated with the proportion of fibrosis area in all 108 patients ($\rho = 0.656$, $P < 0.0001$); D: Between ARFI and body mass index (BMI). The velocity of the sheer wave measured by ARFI significantly negatively correlated with BMI in patients with stage F0 or F1 ($\rho = -0.608$, $P = 0.0003$); E: Between ARFI and γ-glutamyltranspeptidase (γ-GTP) levels. The velocity of the sheer wave measured by ARFI significantly correlated with γ-GTP levels in patients with stage F2 ($\rho = 0.544$, $P = 0.0013$); F: Between ARFI and hyaluronic acid levels. The velocity of the sheer wave as measured by ARFI significantly correlated with hyaluronic acid levels in patients with stage F3 or F4 ($\rho = 0.519$, $P = 0.0003$).

and *F*3 ($P = 0.0014$), and between *F*3 and *F*4 ($P = 0.0008$) (Table 1).

The ARFI cutoff values for different fibrosis stages determined by ROC analysis were 1.28 m/s for $F \geqslant 1$,

Table 2 Optimal cutoff value of velocity of shear wave for each fibrosis stage was determined at maximum sum of sensitivity and specificity

	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F4$
Cutoff value (m/s)	1.28	1.28	1.44	1.73
Positive predictive value	97.0%	94.0%	78.4%	48.0%
Negative predictive value	29.3%	65.9%	91.2%	97.6%
Sensitivity	69.1%	81.8%	88.9%	85.7%
Specificity	85.7%	87.1%	82.5%	86.2%
Positive likelihood ratio	4.8	6.3	5.1	6.2
Diagnostic accuracy	71.3%	83.3%	85.2%	86.1%
AUROC	0.810	0.909	0.869	0.885
Standard error of AUROC	0.046	0.027	0.036	0.058

AUROC: Area under receiver operating characteristic curve

Table 3 Optimal cutoff value of fibrosis area for each fibrosis stage was determined at maximum sum of sensitivity and specificity

	$F \geq 1$	$F \geq 2$	$F \geq 3$	$F4$
Cutoff value	1.17%	1.8%	3.71%	7.32%
Positive predictive value	97.7%	94.3%	93.0%	92.3%
Negative predictive value	60.0%	71.1%	92.3%	97.9%
Sensitivity	91.5%	85.7%	88.9%	85.7%
Specificity	85.7%	87.1%	95.2%	98.9%
Positive likelihood ratio	6.4	6.6	18.7	80.6
Diagnostic accuracy	90.7%	86.1%	92.6%	97.20%
AUROC	0.935	0.927	0.963	0.962
Standard error of AUROC	0.025	0.024	0.018	0.023

AUROC: Area under receiver operating characteristic curve

1.28 m/s for $F \geq 2$, 1.44 m/s for $F \geq 3$, and 1.73 m/s for *F*4 (Table 2).

Fibrosis area in liver biopsy specimens

The proportion of fibrosis area was significantly correlated with fibrosis stage as assessed by the Metavir system ($\rho = 0.872$, $P < 0.0001$) (Figure 1B). The fibrosis area values differed significantly between stages *F*0 and *F*1 ($P = 0.0111$), *F*1 and *F*2 ($P = 0.0022$), *F*2 and *F*3 ($P < 0.0001$), and between *F*3 and *F*4 ($P < 0.0001$) (Table 1).

The fibrosis area cutoff values for the different fibrosis stages determined by ROC analysis were 1.17% for $F \geq 1$, 1.80% for $F \geq 2$, 3.71% for $F \geq 3$, and 7.32% for *F*4 (Table 3).

Factors correlating with ARFI in all 108 patients

ARFI was significantly correlated with fibrosis stage ($P < 0.0001$) (Figure 1A), inflammatory grade ($P < 0.0001$), aspartate aminotransferase (AST) levels ($P < 0.0001$), ALT levels ($P = 0.0008$), γ-glutamyltranspeptidase (γ-GTP) levels ($P < 0.0001$), platelet count ($P < 0.0001$), prothrombin time (INR) ($P = 0.0003$), albumin levels ($P = 0.0002$), total cholesterol levels ($P = 0.0004$), γ-globulin levels ($P = 0.0087$), hyaluronic acid levels ($P < 0.0001$), and fibrosis area ($P < 0.0001$) (Figure 1) by Spearman's rank correlation test (Table 4). ARFI tended to be higher in genotype 1 [median, 1.49 (interquartile range, 1.22-1.75) m/s] than in genotype 2 [1.30 (1.17-1.46)] ($P = 0.0728$). The multiple regression analysis selected fibrosis stage ($\beta = 0.1865$, $P < 0.0001$) and hyaluronic acid levels ($\beta = 0.0008$, $P = 0.0039$) as factors that independently correlated with ARFI, whereas inflammatory grade, AST, ALT and fibrosis area were not selected (Table 4).

Factors correlating with ARFI in stage F0 or F1 patients

To elucidate the factors affecting ARFI other than fibrosis stage, patients with stage *F*0 or *F*1, those with *F*2, and those with *F*3 or *F*4 were analyzed separately.

Body mass index (BMI) was significantly correlated with ARFI ($P = 0.0003$) (Figure 1D) and ALT levels ($P = 0.0593$) and γ-GTP levels ($P = 0.0614$) tended to be correlated with ARFI by Spearman's rank correlation test in the 31 patients with stage *F*0 or *F*1 (Table 4). Only BMI was correlated with ARFI by multiple regression analysis ($\beta = -0.0334$, $P = 0.0001$).

Factors correlating with ARFI in the stage F2 patients

γ-GTP levels were significantly correlated with ARFI ($P = 0.0013$) (Figure 1E) and γ-globulin levels ($P = 0.0581$) tended to be correlated with ARFI in the 32 patients with stage *F*2 by Spearman's rank correlation test (Table 4). The multiple regression analysis only selected γ-GTP levels as a factor correlating with ARFI ($\beta = 0.0048$, $P = 0.0012$).

Factors correlating with ARFI in the stage F3 or F4 patients

In the patients with stage *F*3 or *F*4, fibrosis stage ($P = 0.0004$), platelet count ($P = 0.0036$), prothrombin time (INR) ($P = 0.0080$), albumin levels ($P = 0.0015$), hyaluronic acid levels ($P = 0.0003$) (Figure 1F), and fibrosis area ($P = 0.0481$) were significantly correlated with ARFI by Spearman's rank correlation test (Table 4). The multiple regression analysis selected fibrosis stage ($\beta = 0.2921$, $P = 0.0044$) and hyaluronic acid levels ($\beta = 0.0012$, $P = 0.0025$) as factors correlating with ARFI.

DISCUSSION

The assessment of fibrosis stage is important to estimate prognosis and to identify the patients requiring antiviral treatment in chronic hepatitis C. A lot of noninvasive methods to assess liver fibrosis stage other than liver biopsy are available, for example, ARFI, TE, real-time elastography[23], and algorithm of serum fibrosis markers such as FibroTest[24] and APRI[25]. They provide good performances in estimation of fibrosis stage, while there are problems such as influence of inflammation. In the present study, factors other than fibrosis stage that affect ARFI were investigated in patients with chronic hepatitis C.

The present study confirmed findings reported previously that ARFI correlates with fibrosis stage[10-13,26,27]. The ARFI cutoff values for different fibrosis stages were 1.28 m/s for $F \geq 1$, 1.28 m/s for $F \geq 2$, 1.44 m/s for $F \geq 3$ and 1.73 m/s for *F*4. This result suggests that distinguishing between *F*0 and *F*1 is impossible, as the cutoff

Table 4 Factors correlating with velocity of shear wave in 108 patients with chronic hepatitis C virus infection

	All patients (n = 108)				Patients with $F0$ or $F1$ (n = 31)				Patients with $F2$ (n = 32)				Patients with $F3$ or $F4$ (n = 45)			
	Spearman's rank correlation test		Multiple regression analysis		Spearman's rank correlation test		Multiple regression analysis		Spearman's rank correlation test		Multiple regression analysis		Spearman's rank correlation test		Multiple regression analysis	
	ρ	*P* value	β	*P* value	ρ	*P* value	β	*P* value	ρ	*P* value	β	*P* value	ρ	*P* value	β	*P* value
Age (yr)		NS				NS				NS				NS		
Gender (female/male)[1]		NS				NS				NS				NS		
BMI		NS			-0.608	0.0003	-0.033	0.0001		NS				NS		
Fibrosis stage	0.732	< 0.0001	0.187	0.0001		NS							0.505	0.0004	0.292	0.0044
Inflammatory grade	0.612	< 0.0001		NS		NS				NS				NS		
Steatosis grade		NS				NS				NS				NS		
AST (IU/L)	0.430	< 0.0001		NS		NS				NS				NS		
ALT (IU/L)	0.318	0.0008		NS	0.343	0.0593		NS		NS				NS		
γ-GTP (IU/L)	0.407	< 0.0001		NS	0.340	0.0614		NS	0.544	0.0013	0.005	0.0012		NS		
Platelet count (× 10^4/μL)	-0.441	< 0.0001		NS		NS				NS			-0.425	0.0036		NS
Prothrombin time (INR)	0.344	0.0003		NS		NS				NS			0.390	0.0080		NS
Albumin (g/dL)	-0.347	0.0002		NS		NS				NS			-0.459	0.0015		NS
Total cholesterol (mg/mL)	-0.337	0.0004		NS		NS				NS				NS		
γ-globulin (g/dL)	0.252	0.0087		NS		NS			-0.344	0.0581		NS		NS		
Hyaluronic acid (ng/mL)	0.576	< 0.0001	8.00E-4	0.0039		NS				NS			0.519	0.0003	0.001	0.0025
HCV genotype (1/2)[1]		0.0728		NS		NS				NS				NS		
HCV RNA (logIU/mL)		NS				NS				NS				NS		
Fibrosis area (%)	0.656	< 0.0001		NS		NS				NS			0.296	0.0481		NS
R			0.707				0.645				0.546				0.634	
Adjusted R			0.490				0.396				0.275				0.373	
F			51.800				20.700				12.700				14.100	
P value			< 0.0001				0.0001				0.0012				< 0.0001	

[1]Difference of frequency of gender or genotype was assessed by Mann-Whitney *U* test. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γ-GTP: γ-glutamyltranspeptidase.

value for $F \geq 1$ and that for $F \geq 2$ are the same. However, Sporea *et al*[26] reported that the cutoff value is 1.19 m/s for $F \geq 1$, 1.33 m/s for $F \geq 2$, 1.43 m/s for $F \geq 3$, and 1.55 m/s for $F4$[26]. Rizzo *et al*[13] reported that the cutoff value is 1.3 m/s for $F \geq 2$, 1.7 m/s for $F \geq 3$ and 2.0 m/s for $F4$[13]. Thus, discrepancies are apparent among the cutoff values reported in different studies. The discrepancies are probably attributed to the difference in the population studied. Further studies should be conducted to establish standard ARFI cutoff values for staging fibrosis.

In the present study, AST, ALT and inflammatory grade were correlated with ARFI in the univariate analysis that included all patients, but were not selected as factors independently correlating with ARFI in the multiple regression analysis. In addition, inflammatory factors did not correlate with ARFI when patients with different fibrosis stages were analyzed separately. These results suggest that inflammatory activity does not affect ARFI in patients with chronic hepatitis C. Rizzo *et al*[13] also reported that ARFI is not associated with ALT, BMI, Metavir grade, or liver steatosis, whereas TE is significantly correlated with ALT[13]. Bota *et al*[10] reported that discordance of at least two fibrosis stages between ARFI and histologic assessment were associated with female sex, interquartile range interval (IQR) ≥ 30%, high AST and high ALT in univariate analysis, while, in multivariate analysis, the female gender and IQR ≥ 30% ($P = 0.004$) were associated with the discordances. In contrast, Yoon *et al*[12] reported that the optimum ARFI cutoff values are 1.13 m/s for $F \geq 2$ and 1.98 m/s for $F4$, whereas these values decreased to 1.09 m/s for $F \geq 2$ and 1.81 m/s for $F4$ when patients with normal ALT levels were selected. Chen *et al*[11] reported that ALT, ActiTest A score, Metavir activity (A) grade, Metavir F stage, BMI, and platelet count are independently associated with ARFI and suggested that a 100 IU/L increase in serum ALT levels augmented ARFI by approximately 0.155 m/s. In the present study, only 25 patients had ALT levels of 100 IU/L

or higher. The low ALT levels among the patients studied may be a reason why ALT was not correlated with ARFI.

A multiple linear regression analysis in our previous study on TE selected fibrosis area, ALT levels, γ-GTP levels, prothrombin time, and hyaluronic acid levels as factors correlating with TE[21]. Many studies on TE have reported that LSM is affected by ALT levels. Franquelli *et al*[28] reported that TE fibrosis staging is overestimated by necroinflammatory activity and steatosis. Coco *et al*[7] found that LSM is higher in patients with an elevated ALT than in those with either spontaneous biochemical remission or after antiviral therapy. Thus, it is probable that ALT or inflammatory activity affects TE. However, it is still unclear whether they also affect ARFI. Further studies are needed to clarify factors that affect ARFI other than fibrosis stage.

ARFI was significantly correlated with BMI in the 31 patients with stage *F*0 or *F*1; the higher the BMI, the lower the ARFI. However, ARFI was not associated with steatosis grade. Motosugi *et al*[29] reported that fat deposition in the liver does not affect ARFI. Thus, the negative correlation between BMI and ARFI could not be attributed to steatosis, which accompanies higher BMI[30]. Actually, BMI and steatosis grade were not correlated in patients with stage *F*0 or *F*1 in the present study (data not shown). The mechanism of the association between higher BMI and lower ARFI is unclear. Because a higher BMI is associated with lower ARFI, and may cause an underestimation of fibrosis staging, careful attention should be paid to BMI during ARFI staging of fibrosis in patients with stage *F*0 or *F*1 disease.

ARFI significantly correlated with γ-GTP levels in patients with *F*2 and with fibrosis stage and hyaluronic acid levels in patients with stage *F*3 or *F*4. γ-GTP[24,31] and hyaluronic acid[32,33] levels have been regarded as the most informative fibrosis markers. Thus, it is reasonable that γ-GTP and hyaluronic acid levels independently correlated with ARFI.

Isgro *et al*[20] showed that the collagen proportional area has a better relationship with TE and with hepatic venous pressure gradient compared with Ishak stage. In the present study, fibrosis area was correlated significantly with fibrosis stage, but only fibrosis stage and hyaluronic acid levels were selected as factors independently correlating with ARFI. Our previous study demonstrated a better correlation of TE with fibrosis stage than with fibrosis area in patients with chronic hepatitis C[21]. The Metavir stages represent categories of increasing fibrosis severity based on a combination of location and quantity of scarring as well as whether the fibrous tissue forms septa, bridges, or nodules. Fibrosis area represents only the quantity of fibrosis in liver tissues. Our results indicate that not only the quantity of fibrosis but also other histological factors such as patterns of fibrosis also affect ARFI.

The present study demonstrated that ARFI correlated with fibrosis stage but was not associated with inflammation. BMI negatively correlated with ARFI in the patients with stage *F*0 or *F*1. γ-GTP and hyaluronic acid levels were positively correlated in those with stage *F*2 and in those with *F*3 or *F*4, respectively. Thus, careful attention should be paid to BMI, γ-GTP levels, and hyaluronic acid levels when estimating fibrosis stage by ARFI. Fibrosis stage showed a better correlation with ARFI than fibrosis area, indicating that not only the quantity of fibrosis but also other factors such as patterns of fibrosis also affect ARFI. Since the number of the patients studied is small, further studies are needed to confirm the conclusion of the present study.

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COMMENTS

Background

Most studies reported that liver stiffness measurement by Fibroscan was affected by inflammation. There have been both of the reports which demonstrated the correlation of inflammation and acoustic radiation force impulse (ARFI) and those which denied their correlation. The present study confirmed findings reported previously that ARFI correlates with fibrosis stage, and demonstrated that aspartate aminotransferase, alanine aminotransferase and inflammatory grade did not independently correlate with ARFI in the multiple regression analysis. The present study also demonstrated the correlation of body mass index (BMI) and ARFI for the first time.

Innovations and breakthroughs

The new findings of this study are the correlation of BMI and ARFI, and the denial of the correlation between ARFI and inflammation.

Applications

The results showed that ARFI correlated significantly with liver fibrosis stage and hyaluronic acid in all patients. ARFI correlated significantly with BMI in fibrosis stage F0-1, with γ-glutamyltranspeptidase (GTP) in F2, and with fibrosis stage and hyaluronic acid in F3-4. In conclusion, ARFI correlated with fibrosis stage and hyaluronic acid but not with inflammation. ARFI was affected by BMI, γ-GTP, and hyaluronic acid in each fibrosis stage.

Peer review

The authors reported the utilities of ARFI elastography for evaluation of hepatic fibrosis in patients with chronic hepatitis C. This paper looks very important and has a novelty in this study field.

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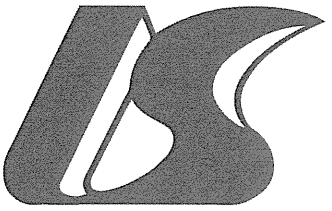

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