Hepatitis C virus genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously.[16] Inosine triphosphatase (*ITPA*) (rs1127354) and interleukin (*IL28B*) (rs8099917) were genotyped by the Invader assay (Third Wave Technologies, Madison, WI, USA), TaqMan assay or direct sequencing as described.[17–19] The core protein of HCV-1b was determined by the previous report.[20] Clinical evaluation and biochemical and hematological tests were performed at a minimum of 4-week intervals.

Statistical analysis

Non-parametric procedures were employed for the analysis of background features of the patients with and without SVR, including the Mann–Whitney *U*-test, Fisher's exact test and Kruskal–Wallis test. The following variables were evaluated as prognostic factors: sex, age, body mass index, a history of IFN therapy, a HCV RNA level, biochemical factors (AST, alanine aminotransferase, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), platelet count, and HCV RNA 4, 8 and 12 weeks after the initiation of IFN therapy. Changes in hemoglobin, white blood cells and platelets between reduction-dose group and standard-dose group during follow up were analyzed by the Mann–Whitney *U*-test. Significance of trends in SVR based on adherence of IFN and ribavirin was determined with the Cochran–Armitage trend test. The SPSS software package (SPSS, Chicago, IL, USA) was used to perform statistical analysis. $P < 0.05$ was considered a statistically significant difference.

Table 1 Clinical backgrounds before combination therapy of IFN-β and ribavirin in chronic hepatitis c patients

Characteristic	Total	Reduction-dose group	Standard-dose group	*P*-value*
Patients, *n*	45	23	22	
Sex, male (%)	48.9%	30.4%	68.2%	0.017
Age (years)	67.5 ± 2.8	68.1 ± 2.6	66.9 ± 3.0	0.105
Height (cm)	159.4 ± 8.7	155.2 ± 6.6	163.6 ± 8.5	0.008
Weight (kg)	57.1 ± 8.7	54.1 ± 8.6	60.3 ± 7.7	0.017
BMI	22.6 ± 2.5	22.7 ± 2.9	22.5 ± 2.2	0.843
History of IFN (+)	60.0%	52.2%	68.2%	0.365
Diabetes (+/−)	2/43	2/21	0/22	0.489
Hypertension (+/−)	5/40	5/19	0/22	0.049
APRI	1.55 ± 1.22	1.39 ± 1.09	1.71 ± 1.34	0.619
APRI (≥1.5/<1.5)	22/23	10/13	12/10	0.556
HCV RNA (logIU/mL)	6.6 ± 0.6	6.6 ± 0.6	6.5 ± 0.5	0.712
IL28B (TT/TG)	34/11	19/4	15/7	0.314
HCV core 70 (wild/mutant)	31/14	17/6	14/8	0.530
ITPA (CC/CA)	31/14	14/9	17/5	0.337
AST (IU/L)	60 ± 36	58 ± 40	63 ± 33	0.555
ALT (IU/L)	89 ± 87	73 ± 79	109 ± 95	0.804
FPG (mg/dL)	107 ± 30	110 ± 37	105 ± 20	0.121
Triglyceride (mg/dL)	97 ± 41	87 ± 40	108 ± 41	0.073
Total cholesterol (mg/dL)	170 ± 28	164 ± 29	176 ± 27	0.193
HDL cholesterol (mg/dL)	46 ± 10	46 ± 11	46 ± 9	0.864
LDL cholesterol (mg/dL)	88 ± 33	84 ± 32	93 ± 35	0.479
Hemoglobin (g/dL)	13.7 ± 1.3	13.1 ± 1.1	14.4 ± 1.2	<0.001
WBC ($\times 10^3/mm^3$)	4.1 ± 1.1	4.3 ± 1.2	3.9 ± 0.9	0.354
Platelet ($\times 10^4/mm^3$)	15.2 ± 7.7	14.3 ± 5.4	16.2 ± 9.7	0.776

*Non-parametric procedures were employed for the analysis of background features of the patients in the reduction-dose group and the standard-dose group, including the Mann–Whitney *U*-test or Fisher's exact test.

Data are number of patients (percentage) or mean ± standard deviation.

ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon; IL, interleukin; ITPA, inosine triphosphatase; LDL, low density lipoprotein; WBC, white blood cell.

RESULT

Clinical characteristics of the patients

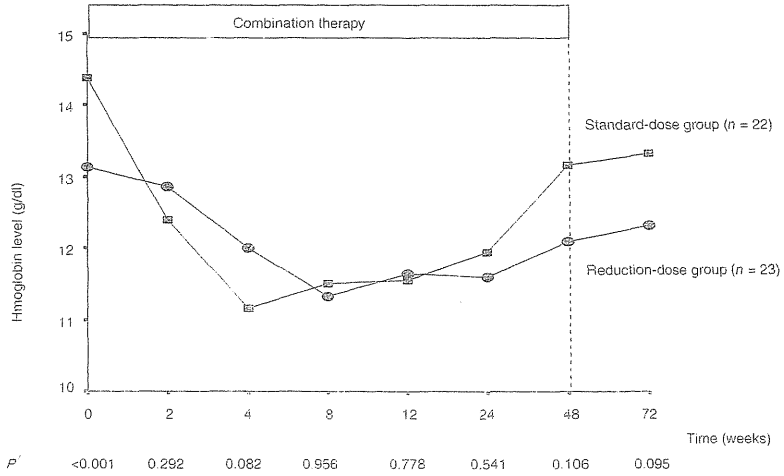
A TOTAL OF 45 patients were enrolled in the present study. Table 1 shows the characteristics before treatment of the elderly patients who received combination therapy. There were no significant differences in clinical backgrounds except for hemoglobin level, sex, height, bodyweight and hypertension between the reduction-dose group and standard-dose group.

Safety and tolerance of IFN

Of the 45 patients included in this study, nine of the patients discontinued combination therapy because of related adverse events (three patients) or poor response (six patients). In the reduction-dose group, one patient discontinued therapy at 8 weeks because of general fatigue and another two discontinued therapy because of poor response at 10 and 20 weeks. In the standard-dose group, two discontinued therapy at 3 and 12 weeks because of bronchitis and skin rash, respectively. Another four discontinued therapy because of poor response at 11, 13, 14 and 21 weeks.

Next, seven patients (four in the reduction-dose group and three in then standard-dose group) had dose reduction of IFN-β from 6 MU to 3 MU because of side-effects (five cases of thrombocytopenia and/or leukopenia, two cases of general fatigue). The onset of dose reduction based on IFN-related side-effects ranged 2–12 weeks after initiation of combination therapy. Moreover, 13 patients (three in the reduction-dose group and 10 in the standard-dose group) had further reduction of ribavirin due to anemia. Further reduction rate of ribavirin during treatment was 13% (3/23) in the reduction-dose group and 45% (10/22) in the standard-dose group. There was a statistically significant difference in further reduction rate of ribavirin between the reduction-dose group and the standard-dose group ($P = 0.008$). One patient of the reduction-dose group and two patients of the standard-dose group received both reduction of IFN-β and ribavirin during treatment.

Figure 1 shows the change of hemoglobin level after the initiation of combination therapy based on the difference between the reduction-dose group and standard-dose group. The hemoglobin level at the initiation of combination therapy in the reduction-dose group was statistically lower than that in the standard-dose group by the use of the Mann–Whitney *U*-test. However, there was no significant difference in the hemoglobin level between the reduction-dose group and the standard-dose group after the initiation of combination therapy. Figures 2 and 3 show the change of white blood cell and platelet levels after the initiation of combination therapy based on the difference between the reduction-dose group and the standard-dose group. There were no significant changes of average white blood cell and

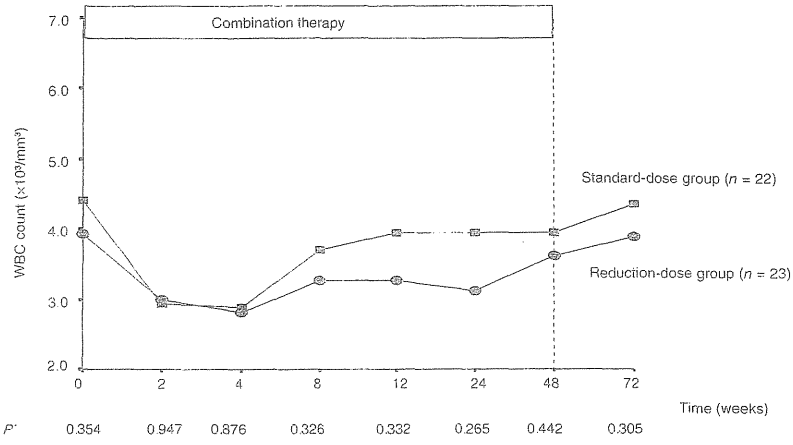


	0	2	4	8	12	24	48	72
P*	<0.001	0.292	0.082	0.956	0.778	0.541	0.106	0.095
Standard group†	0/22	2/22	7/21	8/20	10/18	10/16	10/16	
Reduction group†	0/23	0/23	1/23	2/22	3/21	3/20	3/20	

*Statistical difference in hemoglobin level between reduction group and standard group

†No. of patients who were given new reduction of ribavirin dose during combination therapy/ total no. of patients who were given combination therapy

Figure 1 Change of hemoglobin level after the initiation of the combination therapy of interferon-β and ribavirin in the reduction-dose group and the standard-dose group.



*P**	0.354	0.947	0.876	0.326	0.332	0.265	0.442	0.305
Standard group†	0/22	1/22	1/21	2/20	3/18	3/16	3/16	
Reduction group†	0/23	0/23	2/23	3/22	4/21	4/20	4/20	

*Statistical difference in white blood cell level between reduction-dose group and standard-dose group

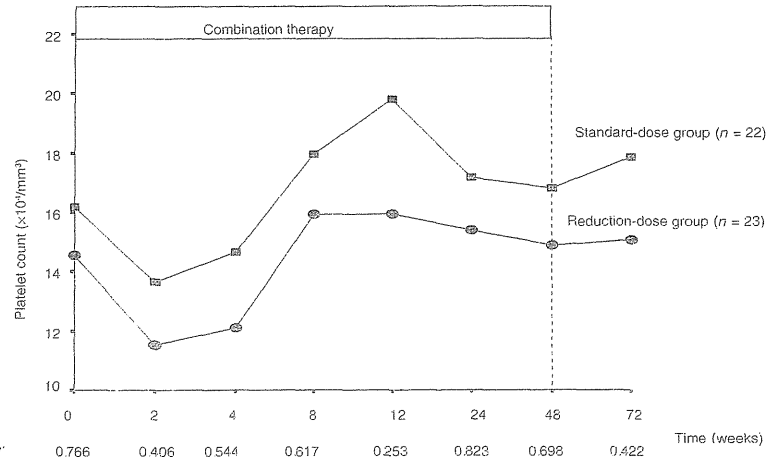
†No. of patients who were given new reduction of IFN-beta dose during combination therapy/ total no. of patients who were given combination therapy

Figure 2 Change of white blood cell count after the initiation of the combination therapy of interferon (IFN)-β and ribavirin in the reduction-dose group and the standard-dose group.

platelet levels during combination therapy between the reduction-dose group and the standard-dose group.

Efficacy of treatment

Out of the 45 patients enrolled in the present study, 15 patients (33.3%) achieved SVR by the intention-to-treat analysis. The SVR rate was 39.1% (9/23) in the reduction-dose group and 27.3% (6/22) in the standard-dose group. There was no significant difference in SVR rate between the reduction-dose group and the standard-dose group ($P = 0.404$). Table 2 shows the difference of clinical backgrounds between patients with and without SVR. On the predictive factor for SVR, the negativity of HCV RNA at 8–24 weeks after the initiation of treatment was an important factor. None of the patients with positive HCV RNA at 24 weeks after the



*P**	0.766	0.406	0.544	0.617	0.253	0.823	0.698	0.422
Standard group†	0/22	1/22	1/21	2/20	3/18	3/16	3/16	
Reduction group†	0/23	0/23	2/23	3/22	4/21	4/20	4/20	

*Statistical difference in platelet level between reduction group and standard group

†No. of patients who were given new reduction of IFN-beta dose during combination therapy/ total no. of patients who were given combination therapy

Figure 3 Change of platelet count after the initiation of the combination therapy of interferon (IFN)-β and ribavirin in the reduction-dose group and the standard-dose group.

Table 2 Difference of clinical backgrounds between patients with SVR and those without SVR

	SVR ($n = 15$)	Non-SVR ($n = 30$)	P-value*
Age (years)	67.6 ± 2.4	67.5 ± 2.9	0.983
Sex (male/female)	5/10	16/14	0.340
Height (cm)	158.9 ± 10.1	159.6 ± 8.2	0.571
Weight (kg)	55.3 ± 5.8	57.8 ± 9.5	0.140
BMI	22.0 ± 2.3	22.9 ± 2.6	0.133
Diabetes (+/−)	0/15	2/28	0.545
Hypertension (+/−)	2/13	3/27	1.000
History of IFN (+/−)	6/9	21/9	0.105
HCV load (logU/mL)	6.5 ± 0.6	6.6 ± 0.5	0.572
APRI	1.15 ± 0.98	1.72 ± 1.29	0.140
IL28B (TT/TG)	15/0	19/11	0.008
HCV core 70 (wild/mutant)	11/4	20/10	0.743
ITPA (CC/CA)	9/6	22/8	0.497
AST (IU/L)	54 ± 28	63 ± 39	0.400
ALT (IU/L)	58 ± 27	73 ± 51	0.293
FPG (mg/dL)	106 ± 43	108 ± 23	0.197
Triglyceride (mg/dL)	99 ± 44	96 ± 41	0.255
Total cholesterol (mg/dL)	177 ± 24	167 ± 29	0.182
HDL cholesterol (mg/dL)	47 ± 9	45 ± 10	0.435
LDL cholesterol (mg/dL)	99 ± 31	84 ± 34	0.071
Hemoglobin (g/dL)	13.7 ± 1.3	13.5 ± 1.4	0.912
WBC ($\times 10^3/mm^3$)	3.9 ± 1.3	4.2 ± 0.9	0.525
Platelet ($\times 10^4/mm^3$)	19.4 ± 11.1	13.4 ± 5.1	0.012
HCV RNA (+/−) 4W	9/6	29/1	0.464
HCV RNA (+/−) 8W	6/9	28/2	0.021
HCV RNA (+/−) 12W	2/13	26/4	<0.001
HCV RNA (+/−) 24W	0/15	24/6	<0.001
Adherence of IFN (%)	89 ± 16	69 ± 31	0.009
Adherence of ribavirin (%)	77 ± 15	61 ± 27	0.064
Reduction group/standard group	9/6	14/16	0.404

*Non-parametric procedures were employed for the analysis of background features of the patients in the reduction-dose group and the standard-dose group, including the Mann–Whitney U-test or Fisher's exact test.
Data are number of patients (percentage) or mean ± standard deviation.
ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon; IL, interleukin; ITPA, inosine triphosphatase; LDL, low density lipoprotein; SVR, sustained virological response; W, weeks; WBC, white blood cell.

initiation of treatment achieved SVR. Based on genetic variations near the *IL28B* gene (rs8099917), SVR was 44.1% (15/34) in patients with TT and 0% (0/11) in patients with TG. SVR rate in patients with TT was significantly higher than that in patients with TG ($P = 0.008$). Regarding HCV core and *ITPA* gene, there was no significant difference between patients with SVR and patients without SVR.

Efficacy based on adherence

Tables 3–5 show the SVR rate based on adherence to combination therapy in the reduction-dose group, the standard-dose group and total patients. Patients with adherence of 2/3 or more for both IFN and ribavirin had an SVR of 40% or more in the reduction-dose group and the standard-dose group.

DISCUSSION

WE HAVE DESCRIBED the efficacy of reduction therapy of IFN-β and ribavirin in elderly patients infected with HCV genotype 1b and high viral load. Several findings from the present study have direct implications for combination therapy for elderly patients with HCV genotype 1b and high viral load in the future.

Table 3 Sustained virological response rate based on adherence of combination therapy in the reduction-dose group

Ribavirin dose	β-Interferon			Total†
	<1/3	≥1/3–<2/3	≥2/3	
<1/3	0% (0/2)	None	None	0% (0/2)
≥1/3–<2/3	None	0% (0/2)	50% (1/2)	25% (1/4)
≥2/3	None	33% (1/3)	50% (7/14)	47% (8/17)
Total*	0% (0/2)	20% (1/5)	50% (8/16)	39% (9/23)

*$P = 0.046$ for comparison of the three interferon groups (Cochran–Armitage trend test).
†$P = 0.075$ for comparison of the three ribavirin groups (Cochran–Armitage trend test).

Table 4 Sustained virological response rate based on adherence of combination therapy in the standard-dose group

Ribavirin dose	β-Interferon			Total†
	<1/3	≥1/3–<2/3	≥2/3	
<1/3	0% (0/3)	None	None	0% (0/3)
≥1/3–<2/3	None	0% (0/2)	0% (0/3)	0% (0/5)
≥2/3	None	50% (1/2)	42% (5/12)	43% (6/14)
Total*	0% (0/3)	25% (1/4)	33% (5/15)	27% (6/22)

*$P = 0.130$ for comparison of the three interferon groups (Cochran–Armitage trend test).
†$P = 0.024$ for comparison of the 3 ribavirin groups (Cochran–Armitage trend test).

First, the dropout rate due to side-effects in combination therapy of IFN-β and ribavirin in elderly patients with aged 65 years or older was 4.3% (1/23) in the reduction-dose group and 9.1% (2/22) in the standard-dose group. In the previous study, we reported that 68 of 612 patients treated with peginterferon and ribavirin stopped the treatment due to side-effects and the dropout rate was 14.9% in 1 year.[9] Although the 612 patients treated with peginterferon and ribavirin had a mean age of 53 years, the dropout rate tended to be high compared to combination therapy of IFN-β and ribavirin for elderly patients. This means that combination therapy of IFN-β and ribavirin might be safe compared with combination therapy of peginterferon and ribavirin. However, in the present study, the ratio of patients treated with the scheduled dose was approximately 23% in the standard-dose group. Most patients received reduction of drugs at the initiation of combination therapy or during combination therapy. Thus, physicians in charge should particularly pay attention to onset of treatment-induced side-effects in combination therapy for elderly patients.

Second, 15 out of 45 patients achieved SVR. When patients with genotype 1b and high viral load have been treated with IFN-β monotherapy, it has been reported that the SVR rate ranges 0–11%.[12,21] Thus, the present study indicates that the combination therapy of IFN-β and ribavirin is more effective for elderly patients with HCV genotype 1b and high viral load compared with IFN-β monotherapy.

Table 5 Sustained virological response rate based on adherence of combination therapy in the total patients

Ribavirin dose	β-Interferon			Total†
	<1/3	≥1/3–<2/3	≥2/3	
<1/3	0% (0/5)	None	None	0% (0/5)
≥1/3–<2/3	None	0% (0/4)	20% (1/5)	11% (1/9)
≥2/3	None	40% (2/5)	46% (12/26)	45% (14/31)
Total*	0% (0/5)	22% (2/9)	42% (13/31)	33% (15/45)

*$P = 0.022$ for comparison of the three interferon groups (Cochran–Armitage trend test).
†$P = 0.007$ for comparison of the 3 ribavirin groups (Cochran–Armitage trend test).

Third, the negativity of HCV RNA at 8–24 weeks after the initiation of treatment was an important factor for predicting SVR. None of the patients with positive HCV RNA at 24 weeks after the initiation of treatment achieved SVR. This result shows that negative HCV RNA at 24 weeks after the initiation of treatment could be a predictive marker for eliminating the HCV by combination therapy of IFN-β and ribavirin for 48 weeks.

Fourth, patients with adherence of 2/3 or more for both IFN and ribavirin had SVR of 40% or more in both the reduction-dose group and the standard-dose group. Seventeen of 22 patients in the standard-dose group had dose reduction or discontinuation of treatment. On the other hand, six of 23 patients in the reduction-dose group had dose reduction or discontinuation of treatment. Thus, many patients in the standard-dose group did not receive the dose of IFN and/or ribavirin as scheduled. Our results suggests that adherence of 2/3 or more for both IFN and ribavirin might enhance the elimination of HCV.

Fifth, based on genetic variations near the *IL28B* gene (rs8099917), SVR was approximately 45% in patients with TT. On the other hand, our result shows that SVR was rare in patients with TG. This result suggests that elderly patients with HCV genotype 1b, high viral load and IL28B gene (rs8099917) of TG should avoid combination therapy of IFN-β and ribavirin because of poor clearance of HCV.

Finally, there was no significant difference in the complete blood cell count between the reduction-dose group and the standard-dose group during combination therapy. In the standard-dose group, many patients discontinued the combination therapy or received dose reduction as described above. The further reduction of ribavirin or discontinuation of treatment might produce elevation of the hemoglobin level at 48 weeks after the initiation of combination therapy in the standard-dose group.

The present study was limited to patients with genotype 1b and HCV load of 5.0 logIU/mL or more. Moreover, in 40 of 45 patients histological examination of the liver was not undertaken within 1 year before combination therapy. In the present study, we tried to evaluate liver fibrosis by the APRI.[15] Our results show that SVR was not statistically associated with the APRI. In the present study, unfortunately, we checked HCV mutations in the core region and IFN sensitivity-determining region in only a few patients. Thus, we could not discuss the relationship between HCV mutation and SVR in the present study. Another limitation is that the present study was not a randomized controlled study.

β-Interferon is inconvenient for treatment compared to i.m. or s.c. injection. However, IFN-β-related side-effects are mild and few compared to combination therapy of IFN-α.[8,9] In fact, IFN-β-induced mental disorders are mild compared to those induced by IFN-α.[22] Moreover, IFN-β could be given in elderly patients of 70 years or older because of mild side-effects.[23] Additionally, platelet count recovered to the baseline at 12–48 weeks after the initiation of combination therapy.[24] Thus, combination therapy of IFN-β and ribavirin might be given to patients such as the elderly and/or slightly depressive.

In conclusion, the reduction therapy of IFN-β and ribavirin in elderly HCV patients with genotype 1b, high viral load and IL28B gene (rs8099917) of TT who had complications of anemia, low bodyweight, diabetes mellitus and/or hypertension is one possible selection of treatment.

ACKNOWLEDGMENTS

THE PRESENT WORK was supported in part by Grants-in-Aid from the Japanese Ministry of Health, Labor and Welfare. The authors acknowledge the editorial assistance of Thomas Hughes.

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Hepatology Research 2012; 42: 139–149 doi: 10.1111/j.1872-034X.2011.00910.x

Original Article

Combination of hepatitis B viral antigens and DNA for prediction of relapse after discontinuation of nucleos(t)ide analogs in patients with chronic hepatitis B

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Aim: The factors associated with hepatitis recurrence after discontinuation of nucleos(t)ide analogs (NAs) in patients with chronic hepatitis B were analyzed to predict the risk of relapse more accurately.

Methods: A total of 126 patients who discontinued NA therapy were recruited retrospectively. The clinical conditions of a successful discontinuation were set as alanine aminotransferase (ALT) below 30 IU/L and serum hepatitis B virus (HBV) DNA below 4.0 log copies/mL.

Results: Relapse of hepatitis B were judged to occur when maximal serum ALT became higher than 79 IU/L or when maximal serum HBV DNA surpassed 5.7 log copies/mL following NA discontinuation since these values corresponded with mean values of ALT (30 IU/L) and HBV DNA (4.0 log copies/mL), respectively. At least 90% of patients with either detectable hepatitis B e antigen or serum HBV DNA higher than 3.0 log copies/mL at the time of NA discontinuation relapsed within one year. In the remaining patients, higher levels of both hepatitis B surface and core-related antigens at the time of discontinuation, as well as a shorter course of NA treatment, were significantly associated with relapse by multivariate analysis.

Conclusions: It appears that negative results for hepatitis B e antigen and serum HBV DNA lower than 3.0 log copies/mL are essential for successful NA discontinuation, which may be attained by a longer treatment period. Levels of hepatitis B surface and core-related antigens are also significant factors independently associated with relapse of hepatitis.

Key words: discontinuation, hepatitis B core-related antigen, hepatitis B surface antigen, nucleos(t)ide analogs, relapse of hepatitis

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major health concern that has an estimated 350 to 400 million carriers worldwide. Chronic infection with HBV can cause chronic hepatitis, and may eventually develop into liver cirrhosis and hepatocellular carcinoma.[1–3] Over the last decade, major advances in the treatment of chronic hepatitis B have been made with nucleos(t)ide

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Financial support
This research was supported in part by a research grant from the Ministry of Health, Labor and Welfare of Japan.
Received 7 August 2011; revision 31 August 2011; accepted 5 September 2011.

analogs (NAs) such as lamivudine (LVD), adefovir dipivoxil (ADV), and entecavir (ETV).[4] NAs are orally administered and are associated with low rates of adverse effects. Treatment with NAs shows strong suppression of HBV replication and consequently rapid improvement of elevated ALT levels. Furthermore, these drugs have been reported to lower the risk of complicating cirrhosis and hepatocellular carcinoma,[5–7] and so NAs are becoming widely used to treat patients with chronic hepatitis B. On the other hand, NAs carry the risk of developing drug-resistance;[8] drug-resistant viruses emerging during treatment may be associated with hepatitis flare-ups. Hepatitis B patients are also required to undergo prolonged treatment with NAs because early discontinuance often leads to relapse of hepatitis and ensuing hepatic failure following rises in alanine aminotransferase (ALT) level.[9,10]

Serum HBV DNA is normally used to monitor the antiviral effect of NAs. HBV DNA decreases rapidly and becomes undetectable in the majority of patients who are treated with NAs,[11–13] but relapse after discontinuation is not rare.[14–17] Since it is also true that favorable virological and biochemical responses to NAs may continue indefinitely in some patients,[9,15] reliable markers that can predict relapse of hepatitis after NA discontinuation are needed. Such markers would benefit not only patients who are considering discontinuation of NA treatment, but also clinicians, hospitals, and the medical economy.

In the present study, we assessed several factors associated with relapse of hepatitis after discontinuation of NAs in patients with chronic hepatitis B, including hepatitis B viral antigens, which have been reported as new and promising markers for monitoring the effect of antiviral agents, such as interferon and NAs.

METHODS

Patients

A TOTAL OF 126 patients with chronic hepatitis B who underwent and completed NA treatment between 2000 and 2010 were enrolled in this study. Patients were recruited retrospectively from 11 hospitals across Japan (Toranomon Hospital, Hokkaido University Hospital, Nagoya City University Hospital, Shinshu University Hospital, Hiroshima University Hospital, National Hospital Organization Nagasaki Medical Center, Chiba University Hospital, The Hospital of Hyogo College of Medicine, Japanese Red Cross Nagoya Daini Hospital, and Tokyo Women's Medical University Hospital, Sapporo Kosei General Hospital) and met the following conditions: (i) serum ALT higher than 30 IU/L and serum HBV DNA higher than 4.0 log copies/mL were observed at least twice within the 6 months prior to administration of NAs; (ii) stored serum samples at initiation and discontinuation of NAs were available for measurements of viral markers; (iii) clinical outcomes were followed for at least 6 months after the discontinuation of NAs; and (iv) tests for hepatitis C and human immunodeficiency virus antibodies were negative. Hepatitis B surface antigen (HBsAg) was confirmed to be positive on at least two occasions at least 6 months apart in all patients before treatment. Patients complicated with hepatocellular carcinoma or signs of hepatic failure at treatment discontinuation were excluded from the study. Our cohort consisted of 83 men and 43 women with a median age of 46 (range, 19 to 79) years when NA administration was discontinued. Hepatitis B e antigen (HBeAg) was positive in 64 patients (51%) at the initiation of treatment and in 24 patients (19%) at its discontinuation. HBV genotype was A in two (2%) patients, B in five (4%), C in 102 (81%), and undetermined in 17 (13%). Thirty-five of the 126 patients in this study were younger than 35 years old. Although not recommended as the first line treatment for this group by Japanese guidelines,[18] NA treatment was commenced since chronic active hepatitis had been persisting in all cases irrespective of their HBeAg status (26 positive and nine negative) at the initiation of treatment.

The decision to discontinue NAs was made by individual physicians using similar, but not uniform, conditions. Four patients who halted NAs for financial reasons were included. No patient underwent interferon treatment during or after NA treatment. The decision to recommence NA administration was also made by individual physicians, essentially when relapse of hepatitis became obvious. With few exceptions, patients were seen at least once a month during the first year after discontinuation of NAs, and at least once every several months afterwards. Stored serum samples were kept frozen at −20°C or below until assayed. This study was approved by the Ethics Committees of all participating institutions.

Hepatitis B viral markers

Serological markers for HBV, including HBsAg, HBeAg, and antibody to HBe (anti-HBe) were tested using commercially available enzyme immunoassay kits (Abbott Japan Co., Ltd, Tokyo, Japan; Fujirebio Inc., Tokyo, Japan; and/or Sysmex Co., Kobe, Japan) at each hospital. Quantitative measurement of HBsAg[19] was done using a chemiluminescence enzyme immunoassay

(CLEIA)-based HISCL HBsAg assay manufactured by Sysmex Corporation (Kobe, Japan). The assay had a quantitative range of −1.5 to 3.3 log IU/mL. End titer was determined by diluting samples with normal human serum when initial results exceeded the upper limit of the assay range.

Serum concentration of HBV DNA was determined using an Amplicor HBV monitor kit (Roche, Tokyo, Japan),[20] which had a quantitative range of 2.6 to 7.6 log copies/mL. Serum HBV DNA was also determined using a COBAS TaqMan HBV kit (Roche, Tokyo, Japan)[21] with a quantitative range of 2.1 to 9.0 log copies/mL in 43 patients whose serum samples were available at the time of NA discontinuation. According to the manufacturer's instructions, detection of a positive signal below the quantitative range was described as a positive signal, and no signal detection was described as a negative signal. Six HBV genotypes (A–F) were evaluated according to the restriction patterns of DNA fragments from the method reported by Mizokami *et al.*[22]

Serum hepatitis B core-related antigen (HBcrAg) levels were measured using a CLEIA HBcrAg assay kit with a fully automated Lumipulse System analyzer (Fujirebio Inc., Tokyo, Japan) as described previously.[23,24] Briefly, 150 μL of serum was incubated with pretreatment solution and then added to a ferrite microparticle suspension in an assay cartridge. Ferrite particles were coated with a monoclonal antibody mixture against denatured HBcAg, HBeAg, and the 22 kDa precore protein. After incubation and washing, further incubation was carried out with alkaline phosphatase conjugated with two kinds of monoclonal antibodies against denatured HBcAg, HBeAg, and the 22 kDa precore protein. Following washing, a substrate solution was added to the test cartridge and then incubated. The relative chemiluminescence intensity was measured, and HBcrAg concentration was calculated by a standard curve generated using recombinant pro-HBeAg. The immunoreactivity of pro-HBeAg at 10 fg/mL was defined as 1 U/mL. We expressed HBcrAg in terms of log U/mL, with a quantitative range set at 3.0 to 6.8 log U/mL.

Statistical analyses

A linear regression model was used to examine for associations between mean and maximal values of both ALT and HBV DNA. Correlations between variables were calculated using the Spearman's rank correction correlation coefficient test. Each cut-off value was decided using receiver operating characteristic curve (ROC) analysis and results were evaluated by measuring the area under the curve (AUC). The Fisher's exact and Pearson's χ^2 tests were adopted to test for differences between subgroups of patients. To compare continuous data, the Mann–Whitney *U*-test was used. The Kaplan–Meier method was used to estimate rates of non-relapse observations, and the log-rank test was used to test hypotheses concerning differences in non-relapse observations between selected groups. Multivariate analyses were performed using the Cox regression model. Variables associated with a *P*-value < 0.2 in univariate analyses were included in a stepwise Cox regression analysis to identify independent factors associated with relapse of hepatitis after discontinuation of NAs. All tests were performed using the IBM SPSS Statistics Desktop for Japan ver. 19.0 (IBM Japan Inc., Tokyo, Japan). *P*-values of less than 0.05 were considered to be statistically significant.

RESULTS

Definition of hepatitis relapse after discontinuation of NAs

THE CLINICAL CONDITIONS of a successful discontinuation of NAs were set at serum HBV DNA below 4.0 log copies/mL and ALT below 30 IU/L according to the Japanese guidelines for the treatment of hepatitis B.[18] However, these criteria could not be directly applied to our cohort as post-therapy fluctuations in ALT and HBV DNA were difficult to evaluate consistently. In total, 26 (76%) of 34 patients with successful discontinuation of NAs showed transient abnormal levels of ALT and/or HBV DNA, especially during the early phase after cessation. We therefore used mean and maximal values of these markers to evaluate relapse of hepatitis B in this study; mean values were used to evaluate relapse of hepatitis as a whole, and maximal values were used to dynamically assess relapse during the follow-up period after NA discontinuation. Both ALT and HBV DNA were measured 11.0 times per year on average during the first year and 4.1 times per year on average thereafter.

The mean values of HBV DNA were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.853. Similarly, the mean values of ALT were significantly ($P < 0.001$) correlated with maximal values with a correlation coefficient of 0.940 (Fig. 1). The mean HBV DNA value of 4.0 log copies/mL corresponded to a maximal HBV DNA value of 5.7 by ROC analysis (AUC = 0.930, $P < 0.001$), and the mean ALT value of 30 IU/L corresponded to a maximal ALT value of 79 IU/L (AUC = 0.988, $P < 0.001$). These results suggested that patients having serum HBV DNA higher

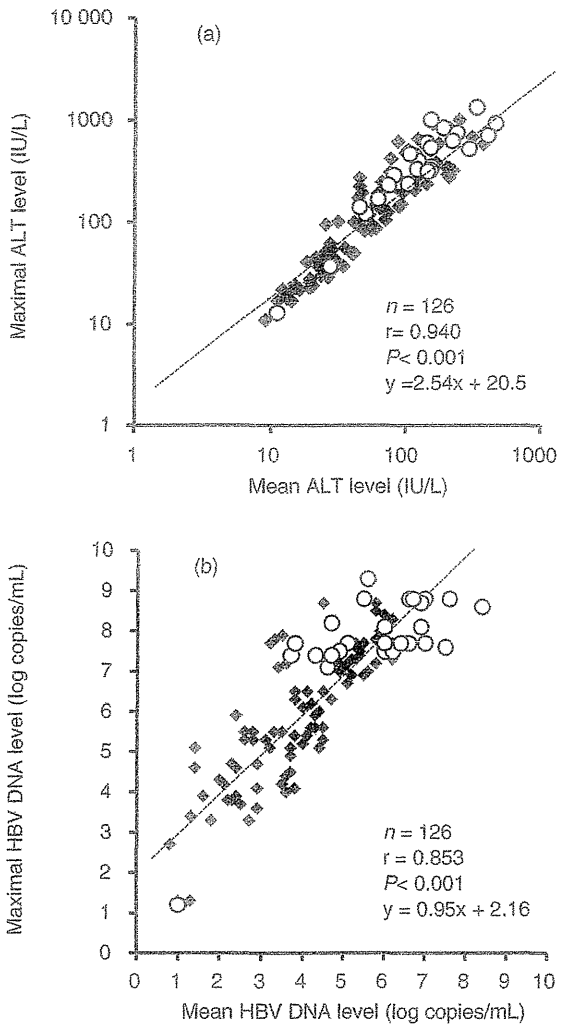

Figure 1 Correlation between maximal and mean levels of alanine aminotransferase (ALT) (a) and hepatitis B virus (HBV) DNA (b) after discontinuation of nucleos(t)ide analogs (NAs). Open circles indicate patients with detectable hepatitis B e antigen (HBeAg) and closed squares indicate patients without detectable HBeAg.

than 5.7 log copies/mL during the follow-up period after NA discontinuation were not likely to achieve the HBV DNA criterion of a successful discontinuation of below 4.0 log copies/mL. Similarly, it could be inferred that patients reaching ALT levels higher than 79 IU/L would also not likely achieve the ALT criterion of a successful discontinuation of below 30 IU/L.

Based on our findings, we judged that a relapse of hepatitis B occurred when serum ALT exceeded 79 IU/L or when serum HBV DNA exceeded 5.7 log copies/mL following NA discontinuation. Accordingly, 92 (73%) of the 126 patients enrolled in the present study showed a relapse. We set the follow-up period as discontinuation to relapse for relapse patients and as discontinuation to the last recorded examination for patients without relapse. Whereas re-administration of NAs due to relapse was commenced in 70% of relapse patients in the follow-up period, none was performed in non-relapse patients during that time.

Elimination of cases likely to show relapse of hepatitis

As it is generally believed that patients who are positive for HBeAg and/or have a higher level of HBV DNA at discontinuation of NAs are likely to relapse, these factors were assessed first. The progression of analyses in the present study and the population structure of each analysis are shown in Figure 2.

The non-relapse rate was compared using the Kaplan–Meier method between 31 patients with HBV DNA equal to or higher than 3.0 log copies/mL and 95 patients with levels lower than 3.0 log copies/mL when NAs were discontinued (Fig. 3). The revised cut-off value of 3.0 log copies/mL was determined by ROC analysis (AUC = 0.709, $P < 0.001$). Thirty (97%) of 31 patients with HBV DNA equal to or higher than 3.0 log copies/mL relapsed within one year of discontinuation. On the other hand, approximately 30% of patients with levels lower than 3.0 log copies/mL showed prolonged non-relapse. Thus, the 31 patients with high HBV DNA at the time of discontinuation were eliminated from the following analyses.

In the remaining 95 patients, the non-relapse rate was compared using the Kaplan–Meier method between 10 patients with detectable HBeAg and 85 patients without HBeAg when NAs were discontinued (Fig. 4). Ninety percent of patients with HBeAg experienced relapse within one year, which was significantly ($P = 0.005$) higher than in cases without HBeAg. In patients without HBeAg, the non-relapse rate decreased rapidly during the first year to approximately 45%, and then decreased relatively slowly over the following 3 years to nearly 30%. It is noteworthy that this subgroup did not relapse afterwards. Since the relapse rate was high among patients with detectable HBeAg, they were excluded from the following analyses as well.

Factors associated with relapse of hepatitis after discontinuation of NAs

Additional factors associated with relapse of hepatitis were analyzed in the remaining 85 patients who were

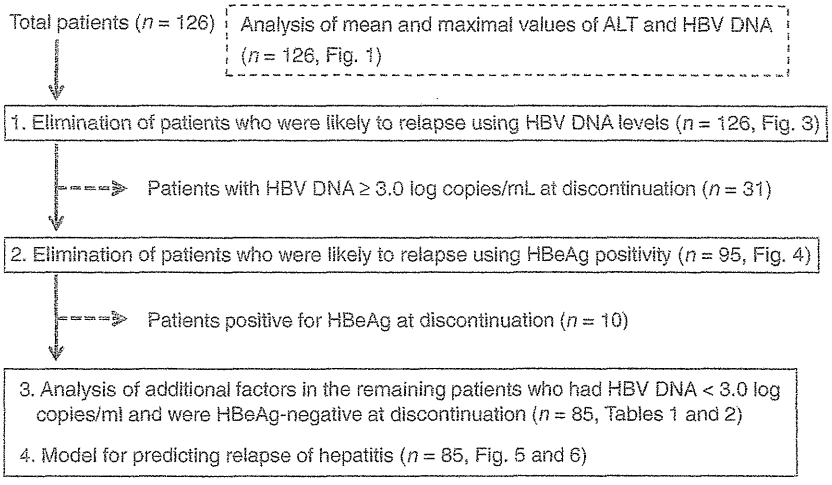

Figure 2 The progression of analyses in the present study and population structure of each analysis.

both negative for HBeAg and whose serum HBV DNA was lower than 3.0 log copies/mL at NA cessation. Table 1 shows the comparison of clinical and virological backgrounds between the 53 relapse and 32 non-relapse patients using univariate analysis. Age and gender distributions were similar between the groups. Approximately 75% of the 85 patients had HBV genotype C, but the distribution of genotypes did not differ between the groups. Approximately 90% of patients were being treated with LVD alone at the time of discontinuation, compared with 6% of patients being given ETV. The median duration of NA treatment was about two times longer in patients without relapse. Levels of both HBsAg and HBcrAg were significantly lower in non-relapse patients than in relapse patients at the time of NA discontinuation. The difference between serum HBsAg was also significant at the initiation of NAs, but not that of HBcrAg. As only patients with HBV DNA lower than 3.0 log copies/mL were analyzed, the majority of these cases showed levels below the 2.6 log copies/mL lower detection limit of the Amplicor assay at NA discontinuation. We therefore also tested HBV DNA with a TaqMan assay, which had a higher sensitivity than the Amplicor assay, in 43 patients whose serum samples were available. The prevalence of patients having a negative detection signal did not differ between the two groups. The number of

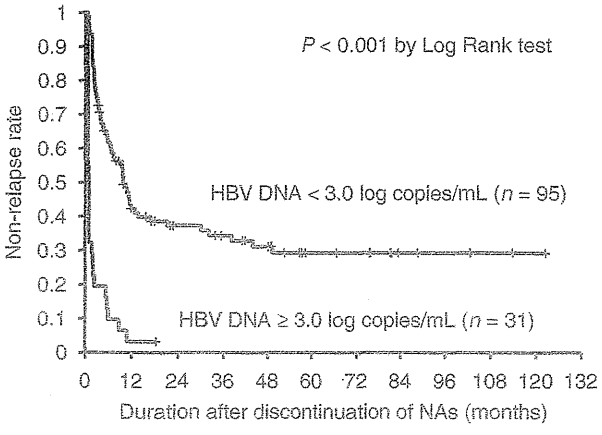

Figure 3 Comparison of non-relapse rates using the Kaplan–Meier method between 31 patients with serum hepatitis B virus (HBV) DNA equal to or higher than 3.0 log copies/mL and 95 patients with serum HBV DNA lower than 3.0 log copies/mL at the time of nucleos(t)ide analog (NA) discontinuation.

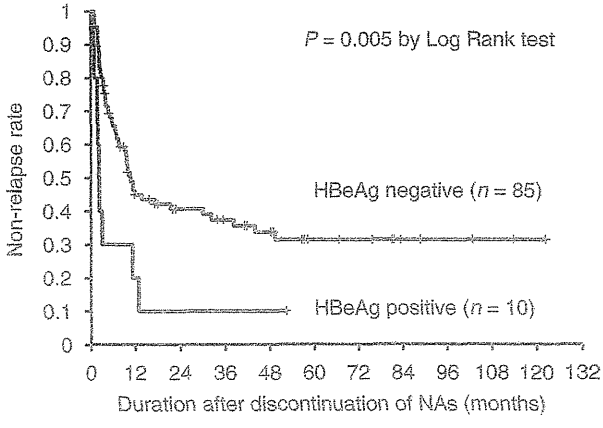

Figure 4 Comparison of non-relapse rates using the Kaplan–Meier method between 10 patients with detectable hepatitis B e antigen (HBeAg) and 85 patients without detectable HBeAg at the time of nucleos(t)ide analog (NA) discontinuation.

Table 1 Comparison of clinical and virological backgrounds between patients with and without relapse of hepatitis at initiation and discontinuation of nucleos(t)ide analogs (NAs)

Background	Non-relapse patients ($n = 32$)	Relapse patients ($n = 53$)	*P*-value
At initiation of NAs			
Age (years)†	47 (17–75)	48 (26–74)	>0.2
Gender (M : F)	23:9	32:21	>0.2
ALT (IU/L)†	183 (9–1182)	187 (20–2052)	>0.2
Genotype (A : B : C : UD)	1:2:21:8	0:3:44:6	0.193
HBeAg (positive)‡	11 (34%)	16 (30%)	>0.2
HBV DNA			
Amplicor assay (log copies/mL)†	6.2 (<2.6–>7.6)	6.5 (<2.6–>7.6)	0.099
HBsAg (log IU/mL)†	2.7 (0.1–4.3)	3.3 (1.6–3.9)	0.018
HBcrAg (log U/mL)†	5.2 (<3.0–>6.8)	5.6 (<3.0–>6.8)	>0.2
At discontinuation of NAs			
Age (years)†	50 (21–78)	49 (26–79)	>0.2
NAs (LVD : LVD+ADV : ETV : ADV)	28:1:3:0	50:0:2:1	>0.2
Duration of NA treatment (months)†	36 (4–129)	17 (4–84)	0.007
Follow-up period after discontinuation of NAs (months)†	45 (6–123)	12 (1–111)	0.002
ALT (IU/L)†	16 (7–38)	20 (9–65)	0.002
HBV DNA			
Amplicor assay (log copies/mL)†	<2.6 (<2.6–2.9)	<2.6 (<2.6–2.9)	>0.2
TaqMan assay (negative signal)‡	5 (23%) ($n = 22$)	3 (14%) ($n = 21$)	>0.2
TaqMan assay (negative or positive signal)‡	13 (59%) ($n = 22$)	13 (62%) ($n = 21$)	>0.2
HBsAg (log IU/ml)†	2.0 (<–1.5–4.3)	3.1 (0.6–4.0)	0.001
HBcrAg (log IU/mL)†	3.4 (<3.0–4.9)	4.3 (<3.0–>6.8)	0.003

†Data are expressed as the median (range)
‡Data are expressed as a positive number (%)
ADV; adefovir dipivoxil; ALT, alanine aminotransferase; ETV, entecavir; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; LVD, lamivudine; UD, undetermined.

patients with a negative detection signal or a positive signal also did not vary significantly. The follow-up period after discontinuation of NAs was significantly shorter in patients with relapse than in those without because formal follow-up ended once patients relapsed. The median period of follow-up was 45 months in patients without relapse.

Multivariate analyses revealed that a shorter duration of NA treatment and higher levels of HBsAg and HBcrAg at discontinuation were significantly associated with the occurrence of hepatitis relapse (Table 2). The cut-off values that showed the highest significance by ROC analysis were 1.9 log IU/mL for HBsAg (AUC = 0.707, $P = 0.001$), 4.0 log U/mL for HBcrAg (AUC = 0.692, $P = 0.003$), and 16 months (AUC = 0.674, $P = 0.007$) for treatment duration.

Model for predicting relapse of hepatitis using levels of HBsAg and HBcrAg

The existence of a second cut-off value was suggested by ROC analysis for both of HBsAg (2.9 log IU/mL) and HBcrAg (3.0 log IU/mL) to discriminate between

Table 2 Multivariate analysis of factors associated with relapse of hepatitis after discontinuation of nucleos(t)ide analogs (NAs)

Factor	Hazard ratio	95%CI	*P*-value
HBsAg at discontinuation ≥ 1.9 log IU/mL	5.21	1.87–14.55	0.002
HBcrAg at discontinuation ≥ 4.0 log U/mL	2.20	1.25–3.87	0.006
Duration of NA treatment ≥ 16 months	0.54	0.31–0.93	0.027

CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen.

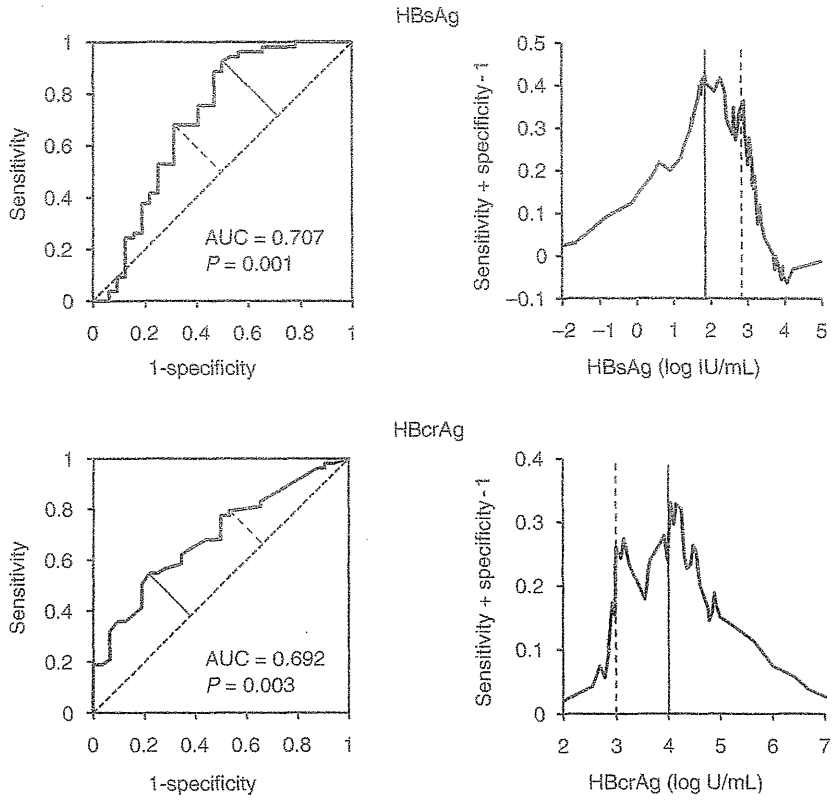

Figure 5 Receiver operating characteristic curve (ROC) analysis of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg. Short diagonal lines indicate main inflection points and short broken diagonal lines indicate second inflection points. Vertical lines indicate actual values of antigens that correspond to the main inflection points and vertical broken lines indicate actual values of antigens that correspond to the second inflection points.

patients with and without relapse (Fig. 5). Thus, we set cut-off values as 1.9 and 2.9 log IU/mL for HBsAg and 3.0 and 4.0 log U/mL for HBcrAg in our model for predicting hepatitis relapse.

We tentatively defined three groups using the sum of the scores for HBsAg and HBcrAg levels at the time of NA discontinuation for our model. Conversions were made by assigning a score of 0 for an HBsAg level lower than 1.9 log IU/mL, 1 for a level from 1.9 to 2.8 log IU/mL, and 2 for a level equal to or higher than 2.9 log IU/mL. HBcrAg was scored as 0 for a level lower than 3.0 log U/mL, 1 for a level from 3.0 to 3.9 log U/mL, and 2 for a level equal to or higher than 4.0 log U/mL. Overall, group 1 consisted of patients with a total score of 0, group 2 of patients with a total score of 1 or 2, and group 3 of patients with a total score of 3 or 4.

Patients whose HBV DNA was lower than 3.0 log copies/mL and in whom HBeAg was negative at the time of NA discontinuation were assigned to one of the three groups. Figure 6 shows the comparison of non-relapse rates among the three groups using Kaplan–Meier analysis, which differed significantly. The non-relapse rate was approximately 90% in group 1, as low as 10% in group 3, and intermediate in group 2. When factors associated with relapse were analyzed in group 3 patients, an age of over 40 years at the time of discontinuation was calculated as a significant factor (hazard

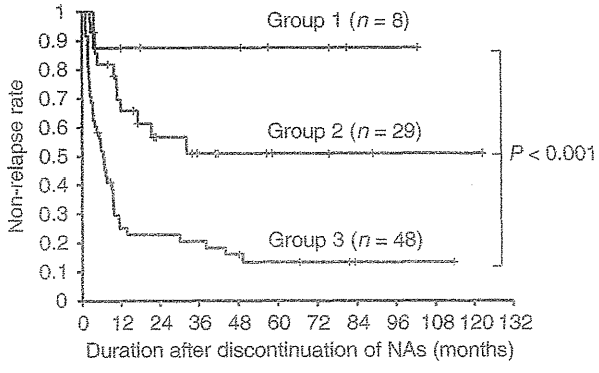

Figure 6 Comparison of non-relapse rates using the Kaplan–Meier method among three groups classified by the sum of the scores of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) levels at the time of nucleo-s(t)ide analog (NA) discontinuation.

ratio = 5.25, range 2.37–11.65, $P < 0.001$). No significant factors were associated with relapse in group 2 patients.

DISCUSSION

THE EUROPEAN ASSOCIATION for the Study of the Liver recommends continuation of NA treatment until HBsAg is cleared.[25] Liu *et al.* came to a similar conclusion in their study of chronic hepatitis B patients treated with LVD.[14] Indeed, the clearance of HBsAg is a reliable marker for the safe discontinuation of NAs, but the rate of patients who can clear HBsAg is relatively low (1–3%/year).[26–28] Thus, additional factors associated with relapse of hepatitis B after discontinuation of NAs were analyzed in the present study to better identify candidates who could achieve drug-free status. Such studies are relatively few, possibly because patients who discontinue NAs prematurely often experience severe complicating relapse and hepatic failure.[9] Although prospective studies are desirable to obtain accurate results, retrospective studies, such as ours, are also necessary to minimize the risk of adverse complications.

Since HBV cannot be completely eradicated in hosts, the primary goal in treating chronic hepatitis B is to convert symptomatic patients into inactive carriers in whom HBeAg is negative (usually anti-HBe-positive), serum HBV DNA is low, and serum ALT is normal.[1,2,18,29] Thus, we set the clinical conditions of a successful discontinuation of NAs as serum HBV DNA level below 4.0 log copies/mL and ALT below 30 IU/L following NA cessation. Patients who satisfy these conditions are not recommended for treatment by the Japanese guidelines for hepatitis B,[18] and it is also widely accepted that the risk of developing cirrhosis or complicating hepatocellular carcinoma is very low in such patients.[30,31] We used our cohort's mean and maximal values of HBV DNA and ALT for relapse analyses. Mean values were useful for evaluating relapse of hepatitis as a whole since parameter levels often fluctuated after discontinuation, and maximal values were used to evaluate relapse in a real-time fashion during the follow-up period. It is noteworthy that the mean and maximal values correlated very closely for both HBV DNA and ALT. The mean HBV DNA value of 4.0 log copies/mL corresponded to the maximal HBV DNA value of 5.7 by ROC analysis, and similarly the mean ALT value of 30 IU/L corresponded to the maximal ALT value of 79 IU/L. Thus, relapse of hepatitis B was judged to occur when serum ALT became higher than 79 IU/L or when serum HBV DNA surpassed 5.7 log copies/mL after the time of NA discontinuation. Such criteria may also be useful for physicians to detect relapse at an early phase and avoid the occurrence of severe reactivation or unnecessary discontinuation of NAs.

It is generally understood that patients with a higher level of HBV DNA at the time of NA discontinuation are likely to relapse, but this cut-off value has not been analyzed sufficiently. Our findings using ROC analysis showed that patients with levels lower than 3.0 log copies/mL have a good possibility to achieve successful discontinuation. The presence of HBeAg is also generally accepted as a reliable factor to predict relapse of hepatitis. Our study showed that patients with detectable HBeAg at the time of NA discontinuation were likely to relapse, even if their HBV DNA levels were lower than 3.0 log copies/mL. Therefore, we next analyzed additional factors associated with a relapse of hepatitis after discontinuation of NAs by selecting patients who met both of these criteria.

Nucleos(t)ide analog treatment produces a rapid decrease in serum HBV DNA by suppressing reverse transcription of pregenomic HBV RNA. However, the key intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA), tends to remain and is capable of reinitiating replication once NAs are ceased.[32] Measurement of HBV cccDNA has been reported to be useful for monitoring and predicting responses to antiviral treatments.[33] However, its measurement is difficult in the clinical setting as it requires a liver biopsy. Due to the mechanism of action of NAs mentioned above, serum HBV DNA does not reflect intrahepatic HBV cccDNA in patients undergoing NA treatment.[34] To address this, quantitative measurement of HBV antigens has been reported to be useful for predicting the effect of antiviral treatment in patients with chronic hepatitis B. Although HBsAg is usually used as a serum marker for the diagnosis of HBV infection, several groups have shown that HBsAg levels can also be reflective of the response to peg-interferon in chronic hepatitis B.[28,35,36] The HBcrAg assay measures serum levels of HB core and e antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these two denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related.[37] Serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during NA treatment,[24,34,38] and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs.[39,40] It is possible that levels of HBsAg and HBcrAg have different roles in

monitoring antiviral effects because the transcription of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome.[3] Therefore, we analyzed both of these antigens to elucidate their ability to predict relapse of hepatitis after discontinuation of NAs.

Multivariate analysis demonstrated that levels of HBsAg and HBcrAg at the time of NA discontinuation were independent factors significantly associated with relapse of hepatitis. Thus, we believe these factors can also be applied for predicting relapse in patients whose HBV DNA is lower than 3.0 log copies/mL and whose HBeAg is negative at NA discontinuation. HBV DNA levels were further analyzed using a highly sensitive assay based on real-time polymerase chain reaction (PCR). However, even the level of a negative signal did not ensure successful discontinuation of NAs. The results obtained here indicate that the combined use of HBV-related antigens are useful makers for monitoring the effect of anti-viral treatment in ways different from HBV DNA. Finally, since prolonged NA administration was also a significant factor associated with safe discontinuation, physicians are advised to continue patient treatment for at least 16 months for the best possible outcome.

From our data, a tentative model for predicting relapse of hepatitis after discontinuation of NAs was constructed using levels of HBsAg and HBcrAg at discontinuation. A negative result for HBeAg and HBV DNA lower than 3.0 log copies/mL at the time of NA discontinuation are the essential conditions in this system. Levels of HBsAg and HBcrAg were each converted into scores from 0 to 2 partly because two cut-off values were needed for each antigen and partly because a scoring system may be more convenient for clinical use. The sum of the two scores, which ranged from 0 to 4, was used to prospect relapse. We found that group 1 patients who had a low score (0) could be recommended to discontinue NAs because nearly 90% of this group achieved successful discontinuation. Further analysis of factors associated with relapse are needed for group 2 patients who had middle range scores (1 or 2), since the odds of achieving successful discontinuation were approximately 50%. Continuation of NA treatment is recommended for group 3 patients having high scores (3 or 4) because nearly 90% of this group relapsed. However, this recommendation may be reconsidered in patients younger than 40 years; such cases tended to have a lower relapse rate in group 3. It is also noteworthy that relapse occurred mainly during the first and second years following NA discontinuation in all groups, similarly to a report by Liu *et al.*[14] Thus, clinicians should be vigilant in the early phase after discontinuation.

This study has several limitations. The patients who discontinued NAs were recruited retrospectively, and thus the decision to halt NA treatment was made by individual physicians without uniformly established criteria. Based on this, prospective studies are required to confirm our results. Furthermore, as over 90% of the patients we enrolled had genotype C and over 90% of cases were treated with LVD until discontinuation, the results obtained here can not be applied directly to other HBV genotypes or other types of NAs.

In conclusion, the present study showed that maximal levels of serum ALT and HBV DNA were useful for defining relapse patients after discontinuation of NAs. Along with serum HBV DNA of less than 3.0 log copies/mL and negative serum HBeAg, serum levels of HBsAg and HBcrAg at the time of NA discontinuation were able to predict relapse of hepatitis B and should therefore be considered when establishing uniform guidelines regarding the safe withdrawal of NA treatment. To this end, NA administration of more than 16 months is advisable to achieve successful discontinuation.

ACKNOWLEDGMENTS

THIS RESEARCH WAS supported in part by a research grant from the Ministry of Health, Labor and Welfare of Japan.

We thank Ms. Hiroe Banno for her secretarial assistance and thank Ms. Nozomi Kamijo and Ms. Etsuko Iigahama for their technical assistance. We also thank Mr Trevor Ralph for his English editorial assistance.

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Journal of Medical Virology 84:1593–1599 (2012)

Association of *IL28B* Genotype and Viral Response of Hepatitis C Virus Genotype 2 to Interferon Plus Ribavirin Combination Therapy

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The impacts of IL28B genotype to treatment response of hepatitis C virus (HCV) genotype 2 are still not clear. A total of 381 consecutive Japanese patients infected with HCV genotype 2, who could complete combination therapy with interferon (IFN) plus ribavirin for 24 weeks, were evaluated to investigate pretreatment predictors. Patients, who could not achieve sustained virological response at the first course of 24-week IFN plus ribavirin, were recruited into the study protocol of total 48-week IFN plus ribavirin. In 24-week regimen, rates of sustained virological response and rapid virological response were 82% and 50%, respectively. There were no significant differences in rates of sustained virological response and rapid virological response, according to IL28B genotype. Multivariate analysis identified younger age, higher level of albumin, absence of past history of IFN, and lower level of viremia as significant determinants of sustained virological response. As significant or marginal significant determinants of non-sustained virological response regardless of rapid virological response, multivariate analysis identified IL28B rs8099917 genotype TG + GG and lower level of albumin. In 48-week regimen to 10 patients of non-sustained virological response at the first course of 24-week regimen, sustained virological response rates were 70%. All of six patients, with IL28B TT and relapse at the first course of 24-week regimen, could achieve sustained virological response, but two patients with IL28B TG could not achieve sustained virological response. In conclusion, the present results suggest that IL28B genotype might partly affect viral response of HCV genotype 2 to combination therapy. ***J. Med. Virol. 84:1593–1599, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: HCV; IL28B; genotype 2; interferon; ribavirin; sustained virological response

INTRODUCTION

The response to interferon (IFN)-based therapy varies according to hepatitis C virus (HCV) genotype [Simmonds, 1997; Haydon et al., 1998]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b (HCV-1b), and about 30% are HCV genotype 2a or 2b (HCV-2a/2b) [Akuta et al., 2002]. Sustained virological response to 48-week IFN plus ribavirin combination therapy is about 50% in HCV-1b infection, and sustained virological response to 24-week combination therapy is more than 80% in HCV-2 infection [Manns et al., 2001; Fried et al., 2002; Mangia et al., 2005, 2009; von Wagner et al., 2005; Fujiwara et al., 2006].

IFN plus ribavirin combination therapy carries potential serious side effects and is costly especially when used long enough to achieve a high sustained virological response. For these reasons, especially in HCV-2 infection, it is needed to identify those patients who could achieve sustained virological response with shorter treatment course (16 weeks or less) to free them of unnecessary side effects and reduce costs,

Grant sponsor: Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

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Accepted 14 June 2012
DOI 10.1002/jmv.23368
Published online in Wiley Online Library (wileyonlinelibrary.com).

preferably as early as possible [Mangia et al., 2005, 2009; von Wagner et al., 2005]. However, the suitable treatment duration, based on the consideration of risk/benefit and cost/benefit, is still unclear in patients infected with HCV-2.

Furthermore, IL28B genotype is a pretreatment predictor of virological response to PEG-IFN/ribavirin dual therapy or telaprevir/PEG-IFN/ribavirin triple therapy in patients infected with HCV-1 [Ge et al., 2009; Tanaka et al., 2009; Suppiah et al., 2009; Akuta et al., 2010a]. Recent studies have investigated the effect of IL28B genotype on treatment efficacy to PEG-IFN/ribavirin combination therapy in cohort including HCV-2 patients [Rauch et al., 2010; Mangia et al., 2010; Kawaoka et al., 2011; Sakamoto et al., 2011], but it is not clear at this stage whether IL28B genotype can be used to predict the virological response to HCV-2.

The present study included 381 Japanese patients with infected HCV-2, who could complete a total of 24 weeks of IFN plus ribavirin combination therapy. The aims of the study were to investigate pretreatment predictive factors including IL28B genotype and the extending combination therapy with IFN plus ribavirin for HCV-2.

PATIENTS AND METHODS

Patients and Study Design

A total of 517 HCV genotype 2 (HCV-2)-infected Japanese patients were consecutively recruited into the study protocol of the combination therapy with IFN (PEG-IFNα-2b, IFNα-2b, or IFNβ) plus ribavirin for 24 weeks between March 2002 and February 2011 at Toranomon Hospital, Tokyo, Japan. Among these, 381 patients, who could complete a total of 24 weeks of combination therapy, were enrolled in this retrospective study and fulfilled the following criteria: (1) They were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan). (2) They were naive to ribavirin therapy. (3) They were infected with HCV-2a or HCV-2b alone, confirmed by sequence analysis. (4) Absence of decompensated liver cirrhosis and hepatocellular carcinoma. (5) All were free of coinfection with human immunodeficiency virus. (6) None had been treated with antiviral or immunosuppressive agents within the preceding 3 months of enrolment. (7) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (8) None had other forms of hepatitis, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) They consented to the study, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected by approval by the human ethics review committee. They were evaluated the rates of sustained virological response (HCV-RNA undetectable at 24 weeks after the completion of therapy), rapid virological response (HCV-RNA undetectable at 4 weeks after the commencement of therapy), and non-response (HCV-RNA detectable during or at the end of therapy), based on the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). Furthermore, pretreatment predictors of treatment efficacy were investigated in 24-week regimen with IFN plus ribavirin combination therapy. Furthermore, patients, who could not achieve sustained virological response at the first course of 24-week regimen, were recruited into the study protocol of total 48-week combination therapy with IFN plus ribavirin. The decision to receive 48-week regimen was made by the patient, and they were evaluated treatment efficacy of extending combination therapy with IFN plus ribavirin.

Table I summarizes the profiles and data of the 381 patients at the commencement of 24-week combination therapy with IFN plus ribavirin. They included 188 men and 193 women, aged 15–76 years (median, 55 years). In all patients, the total duration of treatment was 24 weeks. In 107 of the 381 (28.1%) patients, the dose of ribavirin was reduced during treatment due to a fall in Hb concentration. With regard to the treatment protocol, 266 (69.8%) patients received PEG-IFNα-2b plus ribavirin for 24 weeks, and the remaining 115 (30.2%) patients received IFNα-2b or IFNβ plus ribavirin for 24 weeks. They received PEG-IFNα-2b at a median dose of 1.5 μg/kg (range, 0.6–1.9 μg/kg) subcutaneously each week, or IFNα-2b or IFNβ at a median dose of 6 million units (range, 3–6 million units) intramuscularly each day (seven times per week for initial 2 or 4 weeks, followed by three times per week for 24 weeks). They also received oral ribavirin at a median dose of 11.3 mg/kg (range, 3.1–15.3 mg/kg) daily.

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment and were analyzed for levels of alanine aminotransferase and HCV-RNA. The serum samples were frozen at –80°C within 4 hr of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of NS5 region [Chayama et al., 1993]. HCV-RNA levels were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml.

Determination of IL28B and ITPA Genotype

IL28B (rs8099917) and ITPA (rs1127354) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003, 2011].

Statistical Analysis

Non-parametric tests (Chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate