A, B, and C, respectively [17,18]. The difference among these groups was probably due to the younger age of patients of genotype A and that they were often positive for HBeAg compared to those of genotype B or C. However, the genotype was not a significant predictor of HBV DNA loss after >2 years of entecavir therapy in the present study. There was also no difference in HBeAg seronegativity with entecavir among patients infected with genotype A, B, or C virus. These results were consistent with studies on lamivudine therapy [14,18].

In this study, HBeAg positivity was a significant factor associated with detectable HBV DNA at years 1 through 3, and these results were consistent with those reported by Zoutendijk *et al.* [10]. In addition, lower HBV DNA and HBeAg negativity at baseline were associated with enhanced response to lamivudine therapy [18–20]. We have also previously reported that lamivudine induced a better response in HBeAg-negative patients with higher levels of serum ALT [17]. The most important factor of long-term entecavir therapy therefore was low HBV DNA level.

Low HBV DNA level at baseline correlated significantly with HBeAg seroclearance, but not with seroconversion. One of the reasons was that patients who showed HBeAg seroclearance but no seroconversion had lower HBV DNA (median; 6.7 $\log_{10}$ copies/ml) at baseline compared to patients with seroconversion (median; 7.5 $\log_{10}$ copies/ml, $p = 0.005$).

Univariate analysis showed that age (>40 years), serum albumin level (<3.5 g/dl), and platelet count ($<20 \times 10^4/mm^3$) correlated with HBeAg seroconversion rate. We also investigated the correlation between serum albumin and other factors. Serum albumin level correlated significantly with age ($r = -0.378$, $n = 216$, $p < 0.001$), platelet count ($r = 0.262$, $n = 215$, $p < 0.001$), AFP ($r = -0.372$, $n = 161$, $p < 0.001$), cirrhosis ($P < 0.001$) and male sex ($p = 0.004$). Multivariate analysis identified low serum albumin level (<3.5) as the only significant determinant of HBeAg seroconversion. In this regard, Chien *et al.* [21] reported that pre-treatment ALT was the only significant determinant of HBeAg seroconversion during lamivudine therapy. The reasons for the different findings are probably related to the study design. In our study, the age of patients at baseline was higher (47 *vs.* 32 years) and the duration of treatment was longer (2.4 [median] *vs.* 1 year) than in the study of Chien *et al.* [21]. Furthermore, differences in the pharmacodynamics of lamivudine and entecavir could also contribute to the observed differences between the two studies.

On the other hand, resistant mutants and breakthrough hepatitis seemed to be less frequent during long-term therapy with entecavir than with lamivudine [16–19], indicating that entecavir is better than lamivudine for long-term treatment of CHB and cirrhosis patients. Tenney *et al.* [6] reported that 9 out of 663 (1.4%) patients had baseline lamivudine-resistant mutations, and other studies also found only small numbers of preexisting lamivudine-resistant mutations in treatment-naïve patients [22–24]. It is known that the HBV rtM204V (usually with concomitant rt180M) mutation often acquires one of the entecavir signature mutations at rt184, rt202, or rt250 over long-term treatments and patients develop clinical HBV DNA breakthroughs. Although *in vitro* studies showed that rt204I mutations with or without rt180M conferred 3- to 21-fold decrease in entecavir susceptibility [25], in clinical practice, patients with rt204I mutations, even with the entecavir signature mutations, have lower levels of phenotypic resistance to entecavir and can often achieve undetectable HBV DNA levels [6,9,26]. Interestingly, there were three patients in the present study with VBT who had no HBV DNA mutations at rt184, rt202, or rt250 with rt180M and rt204V (entecavir-resistance). The rtM204V/I mutation, lamivudine's signature mutation, is necessary but not sufficient for entecavir-resistance, causing an 8- to 10-fold decrease in susceptibility to entecavir compared with wild-type HBV. Other mutations at positions rtT184, rtS202, and rtM250 confer additional decreases in entecavir susceptibility [25,27,28]. In the present study, two patients (Patients #3 and 5) with mutations at position rtM204V/I, without rtT184, rtS202, or rtM250 mutations, showed emergence of VBT, as did one patient (Patient #4) with an rtA181T mutation, which was first reported in a LAM-treated patient [29]. Although the rtA181T mutation is related to resistance to adefovir dipivoxil, this mutation has not been linked to additional decreases in entecavir susceptibility. Future *in vitro* analyses using replication-competent HBV clones in patients with rtA181T mutations are therefore necessary.

In conclusion, long-term treatment of treatment-naïve CHB patients with 0.5 mg/day entecavir for 4 years suppressed HBV DNA to undetectable levels in more than 90% of patients, regardless of HBeAg status and genotype. Moreover, the drug was very safe and rarely induced resistance mutations. Further studies exploring the therapeutic efficacy over longer durations may be necessary to confirm these findings.

### Conflict of interest

Hiromitsu Kumada has received speaker's honoraria from Bristol-Myers Squibb. All other authors declare no conflict of interest.

### Financial support

This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

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J Gastroenterol (2012) 47:1022–1029
DOI 10.1007/s00535-012-0561-8



# Determinants of the clinical outcome of patients with severe acute exacerbation of chronic hepatitis B virus infection

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Received: 24 August 2011 / Accepted: 7 February 2012 / Published online: 29 February 2012


**Abstract**
*Background* Severe acute exacerbation of chronic hepatitis B can sometimes occur and lead to hepatic failure and death. The objective of this study was to elucidate the predictors of progression to hepatic decompensation during severe acute exacerbation.
*Methods* We prospectively analyzed 37 consecutive patients with acute exacerbation of chronic hepatitis B (accompanied by jaundice and coagulopathy) for clinical outcome and factors that influenced the development of severe acute exacerbation, including viral kinetics.
*Results* Fourteen (37.8%) patients progressed to severe acute exacerbation (accompanied by encephalopathy). Multivariate analysis identified serum bilirubin (>5 mg/dl, $P = 0.002$) as a significant determinant of progression to hepatic failure and prothrombin activity (<45%, $P = 0.028$) and as a determinant of liver-related death. The hepatitis B virus (HBV) DNA level before therapy was measured in 25 patients. HBV DNA levels increased or did not change from before commencement of treatment in all 11 patients who progressed to severe acute exacerbation. On the other hand, HBV DNA levels did not change or increased in 8 of 14 patients (57%) with acute exacerbation ($P = 0.02$).
*Conclusions* Serum bilirubin and prothrombin activities were significant predictors of clinical outcome in patients with severe acute exacerbation of chronic hepatitis B. Viral kinetics until commencement of therapy can predict the severity of acute exacerbation of chronic hepatitis B.

**Keywords** Hepatitis B · Acute exacerbation · HBV DNA · Genotype · Encephalopathy

**Abbreviations**

| | |
|---|---|
| AE | Acute exacerbation |
| ALT | Alanine aminotransferase |
| BCP | Basal core promoter |
| CS | Corticosteroid |
| HBV | Hepatitis B virus |
| IFN | Interferon |
| LMV | Lamivudine |
| NA | Nucleos(t)ide analogue |
| PC | Pre-core |
| PT | Prothrombin activity |
| SAE | Severe acute exacerbation |

## Introduction

More than 3 billion people worldwide and approximately 1.5 million people in Japan are chronically infected with hepatitis B virus (HBV), and chronic HBV infection is one of the most common causes of chronic hepatic failure and hepatocellular carcinoma (HCC) [1, 2]. Other complications of HBV infection include fulminant hepatitis and acute liver failure [3, 4]. Acute exacerbation (AE) in HBV carriers occurs either through a natural course [5, 6] or following intensive chemotherapy or immunosuppressive

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therapy [7, 8]. Some abrupt flares may be so severe that decompensation or even fulminant hepatic failure may occur [9–11]. Previous studies have identified pre-existing cirrhosis, high serum bilirubin levels, prolonged prothrombin time, pre-core/core promoter mutants, and high HBV DNA levels as factors associated with hepatic decompensation during AE in HBV carriers, though little is known about the predictive factors [9, 12, 13].

Liver transplantation is suitable therapy for acute hepatic failure, but the rate of liver transplantation has remained about 20% in Japan, where living donor liver transplantation is dominant [14, 15]. Thus, it is necessary to establish other effective therapies for patients with AE apart from liver transplantation. Steroids can rapidly inhibit excessive immune response and inflammatory reactions, and have been reported to be effective in cases of severe and potentially life-threatening exacerbation of chronic HBV (CHB) infection [16]. With the advent of oral nucleos(t)ide analogues (NAs), most guidelines recommend NAs for patients with AE of CHB infection [17–19], and several observational studies reported the use of NAs [9–11, 20, 21]. Timely use of potent anti-HBV agents, such as NAs, interferon (IFN), and steroids [22], during and/or after the development of hepatic decompensation could be potentially effective against various host- and virus-related factors.

The aim of the present study was to investigate the factor(s) that influence the rapid development of hepatic decompensation during AE of CHB.

## Materials and Methods

### Patients

The study subjects were patients with AE admitted to the Department of Hepatology, Toranomon Hospital, Tokyo, between 1984 and 2010. All patients were either followed up at our hospital with clinicopathologically proven CHB infection or were new patients with sudden-onset hepatic flares who visited our hospital outpatient clinic or were referred to our hospital from other clinics/hospitals. The diagnosis of CHB carrier state was established based on either positivity for hepatitis B surface antigen (HBsAg) for at least 6 months prior to the development of AE, or the presence of a high titer of anti-hepatitis B core antibodies (anti-HBcAb), together with negativity or a low titer of IgM anti-HBcAb. Chronic hepatitis and cirrhosis were confirmed by laparoscopy, needle biopsy, or ultrasonography, or treatment for these conditions for 1 year before the development of AE. AE of CHB infection was diagnosed by the following criteria: (1) an abrupt increase in serum alanine aminotransferase (ALT) levels to >300 IU/l in patients with original ALT levels of less than 5× the upper limit of normal or an abrupt two-fold increase in the serum ALT level to greater than 5× the upper limit of normal, (2) hyperbilirubinemia [serum bilirubin (Bil) >3.0 mg/dl], (3) evidence of coagulopathy with plasma prothrombin activity (PT) of <60% during the clinical course, and (4) lack of encephalopathy at admission. We also applied the following exclusion criteria: (1) the presence of viral markers other than HBV (hepatitis A, C, D, E, Epstein-Barr virus, cytomegalovirus, herpes simplex virus), (2) HBV reactivation induced by immunomodulators or chemo-/immunosuppressive therapy, (3) asymptomatic HBV carriers, (4) recent exposure to drugs and chemical agents as well as recent heavy alcohol intake, (5) breakthrough hepatitis caused by NAs, (6) evidence of decompensated liver disease before the onset of exacerbation as characterized previously, (7) HCC diagnosed by ultrasonography or computed tomography, and (8) coexistence of other serious medical conditions and other liver diseases, or metabolic diseases. Progression to severe acute exacerbation (SAE) was diagnosed by the development of hepatic encephalopathy of more than grade 2 within 8 weeks of onset associated with coagulopathy (PT <40%).

HBV DNA levels were measured serially to investigate the effects of HBV kinetics on the prognosis of patients with severe AE. HBV DNA levels were measured before treatment in 25 patients. "Before treatment" represented 1–8 weeks before commencement of treatment. HBV DNA levels were also measured after treatment in 27 patients. "After treatment" was defined as 2 weeks after commencement of therapy. Viral kinetics was assessed using the same assay in all individuals. The Local Ethics Committee of Toranomon Hospital approved the study, and informed consent was obtained from all patients.

### Virological markers

Serial blood samples were obtained during the clinical course of AE and stored at −80°C until used for HBV molecular analysis. Serological tests for HBsAg, HBsAb, hepatitis e antigen (HBeAg), IgM anti-HBcAb, total anti-HBcAb, and anti-HBeAb were conducted using radioimmunoassay kits (Abbot Diagnostics, Chicago, IL, USA) according to the instructions provided by the manufacturer. Precore (PC) mutations were analyzed by PCR enzyme-linked mini-sequence assay (Roche Diagnostics, Tokyo, Japan), and basal core promoter (BCP) mutations were analyzed by PCR specific probe assay (Roche Diagnostics, Tokyo, Japan). HBV DNA was measured by Amplicor monitor assay (dynamic range 2.6–7.6 log copies/ml, Roche Diagnostics, Tokyo, Japan), COBAS TaqMan v.2.0 (dynamic range 2.1–9.0 log copies/ml, Roche Diagnostics), transcription-mediated amplification and hybridization

protect assay (TMA-HPA) (dynamic range 3.7–8.7 LGE/ml, Chugai Diagnostics Science Co., Tokyo) or sandwich hybridization assay with signal amplification using branched DNA (bDNA, dynamic range 0.7–3800 Meq/ml). The major genotype of HBV was determined using enzyme-linked immunosorbent assay (ELISA, Institute of Immunology, Tokyo, Japan) or PCR-invader assay (BML, Inc, Tokyo, Japan) based on the methods described previously [23, 24]. HBVDNA levels assessed by bDNA were re-measured by TaqMan PCR assay using stored serum samples.

### Statistical analysis

Continuous variables were expressed as median (range), and compared by Mann–Whitney $U$ test. Categorical variables were compared by $\chi^2$ test or Fisher's exact test, as appropriate. Univariate analysis was applied to determine the relationship between SAE and each of the following factors: sex, age, presence of compensated cirrhosis, and various biological and virological markers as measured at baseline (bilirubin, PT, ALT, albumin, HBeAg, HBV DNA, and HBV genotype, PC and BCP mutations). Each continuous variable was transformed into two categories based on the value with the largest capacity to discriminate between patients for univariate and multivariate analyses. Factors that correlated significantly with SAE were entered into multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence intervals (95% CI) were determined. All analyses were performed using The Statistical Package for Social Sciences (SPSS II v. 11.0, Chicago, IL, USA), and statistical significance was taken as a two-sided $P$ value $<0.05$.

## Results

### Clinical features of severe acute exacerbation

A total of 37 patients (30 men and 7 women) fulfilled the criteria of AE and were included in this study. The baseline characteristics at the commencement of therapy of these 37 patients are shown in Table 1. Twenty-two patients were observed at our hospital, and 15 patients were referred from another hospital after the onset of hepatic flares. The majority of patients had genotype C, and 27 patients (72.9%) were HBeAg positive. The PC and BCP mutations were determined in 27 patients; 22 patients had mutations in the PC region, 16 patients had mutations in the BCP region, and 12 patients had mutations in both the PC and BCP regions. During the clinical course, the peak median values were: ALT 713 IU/l (range 307–2857), bilirubin 8.4 mg/dl (3.0–51.4), and PT 47.6% (12.0–60.0).

**Table 1** Baseline characteristics of the 37 patients infected with HBV who developed severe acute exacerbation at the commencement of therapy

| | |
|---|---|
| Number | 37 |
| Sex (male/female) | 30/7 |
| Age (years) | 45 (23–63) |
| Family history (yes/no) | 21/16 |
| Cirrhosis (present/absent) | 7/30 |
| Albumin (g/dl) | 3.4 (2.5–4.6) |
| Bilirubin (mg/dl) | 4.7 (1.0–30.7) |
| AST (IU/l) | 601 (64–2593) |
| ALT (IU/l) | 657 (124–2142) |
| LDH (IU/l) | 297 (106–594) |
| Platelets ($\times 10^4$/mm$^3$) | 12.3 (6.2–32.0) |
| α-Fetoprotein (μg/ml) | 62.0 (3.0–1600) |
| Prothrombin activity (%) | 53 (26–80) |
| Genotype (A/B/C) | 0/5/32 |
| HBeAg (positive/negative) | 27/10 |
| HBV-DNA ($\log_{10}$ copies/ml) | 8.5 (6.8–8.9) |
| PC (wild/mutant/ND) | 5/22/10 |
| BCP (wild/mutant/ND) | 11/16/10 |

Data are median values (range) or number of patients

*AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *HBeAg* hepatitis B envelope antigen, *PC* pre core, *BCP* basal core promoter, *ND* not done

### Treatment

NAs were used in 19 patients, IFN in 8, and corticosteroids (CS) in 20 patients. In addition, 7 patients were treated with a combination of NAs and CS; 2 patients were treated with three drugs (NAs, IFN, and CS). At the time of the study, lamivudine (LMV) was not yet available for the treatment of chronic hepatitis B, and thus IFN was used; 6 patients were treated with both IFN and CS. None of the patients underwent liver transplantation.

### Prognosis of severe acute exacerbation and factors associated with progression to hepatic failure

Of the 37 patients admitted with CHB infection and AE, 23 (62.2%) did not develop SAE. The remaining 14 (37.8%) patients developed SAE; 9 (24.3%) patients died of liver-related death, but 5 (13.5%) survived. Further analysis showed that 8 (36.4%) of 22 patients who were observed in our hospital developed AE, and 6 (27.3%) of these patients died, whereas 6 (40.0%) of 15 patients who were referred from other hospitals after the onset of exacerbation developed AE, and 3 (20.0%) of these patients died. There was no significant difference in prognosis by treatment facility before AE. Ten of 37 patients experienced AE before 2000 when LMV was available in Japan, and 19

**Table 2** Biochemical, virological and histological features of patients with severe acute exacerbation at the commencement of therapy

| Case | Age (years)/ sex | Genotype | HBeAg | HBV-DNA (log copies/ ml) | Preexisting cirrhosis | Serum bilirubin (mg/dl) | ALT (IU/l) | PT (%) | Platelets ($\times 10^4$/ $mm^3$) | Therapy | Outcome (time from treatment to death, weeks) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 63/M | B | − | 8.4 | No | 5.8 | 1680 | 43 | 6.2 | LMV + CS | Death (11) |
| 2 | 32/M | B | − | >8.7 | No | 6.9 | 1340 | 41 | 13.4 | CS | Death (1) |
| 3 | 58/M | B | − | 8.6 | No | 7.4 | 1446 | 36 | 7.7 | CS | Death (2) |
| 4 | 29/M | B | − | >8.7 | No | 15.6 | 307 | 26 | 10.0 | LMV | Recovery (alive) |
| 5 | 54/F | C | + | >8.7 | No | 2.4 | 2077 | 79 | 21.0 | LMV + CS | Recovery (alive) |
| 6 | 37/M | C | + | >8.7 | No | 4.1 | 552 | 53 | 8.9 | CS | Recovery (alive) |
| 7 | 62/M | C | + | 7.0 | No | 12.0 | 220 | 53 | 7.1 | LMV + CS + IFN | Recovery (alive) |
| 8 | 33/F | C | + | >8.7 | No | 14.0 | 632 | 39 | 13.1 | CS | Recovery (alive) |
| 9 | 55/M | C | + | >8.7 | Yes | 4.0 | 1089 | 55 | 10.3 | LMV + CS | Death (1) |
| 10 | 37/F | C | + | 7.1 | Yes | 5.8 | 1444 | 34 | 22.0 | LMV + CS + IFN | Death (10) |
| 11 | 49/M | C | + | 8.0 | Yes | 8.8 | 834 | 58 | 9.9 | CS | Death (10) |
| 12 | 33/M | C | + | 8.5 | No | 9.6 | 657 | 26 | 7.4 | LMV + CS | Death (2) |
| 13 | 54/M | C | + | 7.8 | Yes | 12.1 | 364 | 36 | 15.8 | LMV + CS | Death (2) |
| 14 | 55/M | C | + | >8.7 | No | 24.2 | 520 | 44 | 8.3 | CS | Death (5) |

Abbreviations as in Table 1, *PT* prothrombin activity, *LMV* lamivudine, *CS* corticosteroids, *IFN* interferon-α

patients experienced AE after 2000. The other 8 patients experienced AE before 2000, but received LMV through participation in clinical trials or paid for the drug privately. The clinical features at the commencement of therapy of 14 patients who developed SAE are shown in Table 2 (median age 52 years, range 29–63). The mean time period between admission and death of 9 patients who developed SAE was 2 (range 1–11) weeks. Six patients who were admitted before the availability of LMV were treated with CS alone, 5 patients were treated with the combination of LMV and CS, 1 patient was treated with LMV alone, and 2 other patients were treated with LMV, CS, and IFN. Among 8 patients treated with LMV, of those who developed SAE, 5 died, and 2 patients developed complications caused by bacterial infection. Four patients had genotype B, while 10 patients had genotype C. HBeAg status was positive in 10 patients. The mean HBV DNA level was 8.7 (range 7.0–>8.7) log copies/ml, ALT 746 (220–2077) IU/l, serum bilirubin 8.1 (2.4–24.2) mg/dl, PT 42 (26–79)%, and platelet count was 10.0 (62–220) $\times$ $10^4/mm^3$.

Of the 5 patients who were treated successfully after progression to SAE, one later died of severe breakthrough hepatitis caused by emergence of LMV-resistant virus 3 years after SAE (case 7, Table 2). The other four survived (cases 4–6 and 8, Table 2).

Table 3 shows the results of univariate analysis. The following factors showed significant relationship with the development of SAE at the commencement of treatment: serum bilirubin (>5 mg/dl) and PT (<60%). Multivariate analysis identified serum bilirubin as a significant and independent determinant of the development of SAE (Table 3). On the other hand, two parameters showed significant relationships with liver-related death: serum bilirubin (>7 mg/dl, $P = 0.049$) and PT (<45%, $P = 0.003$). Multivariate analysis identified PT (OR 9.50, 95% CI 1.3–71.0, $P = 0.028$) as a significant determinant of death.

## Viral kinetics associated with fulminant hepatic failure

To investigate the relationship between viral kinetics and SAE, HBV DNA levels were measured in 25 patients both before and commencement of treatment and also after treatment in 27 patients. Figure 1 shows the viral load of patients who developed and did not develop SAE at commencement of treatment compared with before treatment. Falls in the HBV DNA level occurred naturally. However, in 11 patients who developed SAE, HBV DNA levels increased in 6 patients and did not change in 5 patients. Among the latter 5, HBV DNA levels of 4 patients were >8.7 log copies/ml. In 14 patients who did not develop SAE, HBV DNA levels increased in 4 patients, were unchanged in 4 patients, and decreased in 6 patients. Hence, the HBV DNA level increased/was unchanged in 8 of 14 (57%) patients who did not develop SAE, compared with 11 of 11 (100%) patients who developed SAE. A significantly higher proportion of patients with SAE showed an increase/was unchanged in viral load compared to those who without SAE ($P = 0.02$). We also examined the viral kinetics in 27 patients by comparing HBV DNA levels at the commencement of treatment to after treatment.

**Table 3** Univariate and multivariate analyses of host and viral factors associated with progression of severe acute exacerbation at commencement of treatment

| Parameter | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | OR (95% CI) | *P* | OR (95% CI) | *P* |
| Sex (female) | 1.30 (0.15–4.11) | 0.76 | | |
| Age (>55 years) | 2.64 (0.57–12.3) | 0.22 | | |
| Cirrhosis (present) | 1.90 (0.39–9.26) | 0.43 | | |
| Albumin (<3.5 g/dl) | 1.75 (0.44–6.97) | 0.85 | | |
| Bilirubin (>5 g/dl) | 17.0 (2.92–99.1) | 0.002 | 11.2 (1.71–73.8) | 0.01 |
| ALT (>800 IU/l) | 1.88 (0.48–7.26) | 0.36 | | |
| AST/ALT ratio (>1) | 1.27 (0.31–5.19) | 0.74 | | |
| Prothrombin activity (<60%) | 11.9 (1.33–106.7) | 0.03 | 8.22 (0.73–92.6) | 0.09 |
| Platelets (<15 × $10^4$/$mm^3$) | 0.81 (0.19–3.58) | 0.89 | | |
| Genotype (B) | 8.82 (0.87–89.1) | 0.06 | | |
| HBeAg (positive) | 0.89 (0.20–3.90) | 0.89 | | |
| HBV-DNA (>8.7 log copies/ml) | 2.34 (0.60–9.20) | 0.70 | | |
| PC mutation | 2.29 (0.22–24.1) | 0.49 | | |
| BCP mutation | 0.19 (0.034–1.08) | 0.06 | | |

Abbreviations as in Tables 1 and 2, *OR* odds ratio, *CI* confidence level

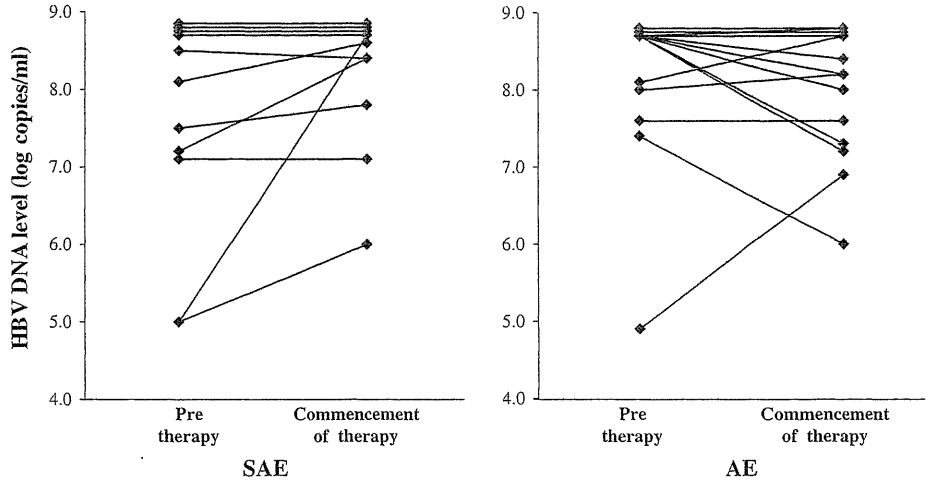


**Fig. 1** Viral kinetics from pre-treatment to commencement of treatment in patients with acute exacerbation. Viral kinetics tended to increase or remained unchanged until treatment in 8 patients with acute exacerbation course ($n = 14$), while the viral load in all patients with severe acute exacerbation ($n = 11$) increased or remained unchanged ($P = 0.02$)

The HBV DNA level decreased more than 1 log copies/ml in 9 of 17 (52.9%) patients who did not develop SAE, compared with 3 of 10 (30.0%) patients who developed SAE, but the difference between the two groups was not significant.

## Discussion

The results of the present study examined the predicting factors of progression to SAE accompanied by coagulopathy and encephalopathy in patients with AE of chronic hepatitis B, as well as the pattern of viral kinetics before and after commencement of therapy. Up to 30% of patients with CHB infection experience reactivation of hepatitis every year [5, 6], while some patients develop acute exacerbation with jaundice and coagulopathy, a severe life-threatening condition with high mortality [9, 12]. It is important to determine the predicting factors of progression to liver decompensation in patients with acute exacerbation. Multivariate analyses in previous studies indicated that pre-existing cirrhosis, a high Child–Pugh score, low albumin level, high serum bilirubin level, prolonged PT, and high HBV DNA levels were associated with the severity or mortality during acute exacerbation [9, 12, 13]. Our results are almost comparable to those of the above studies. Multivariate analysis in the present study identified the serum bilirubin level as a predictor of progression to liver decompensation. Moreover, there were no significant differences in viral load or therapeutic regimen. Genotype B was the predominant HBV strain in patients with SAE compared to patients with variable severity of liver diseases [25]. The frequencies of HBV genotype in patients with chronic hepatitis B admitted to our hospital were 3.0, 12.3, and 84.5%, for genotypes A, B, and C, respectively [26]. In the present study, although patients

with genotype B were only 5 of the total 37 (13.5%), 4 of 14 (28.6%) patients with SAE and 3 of 9 (33.3%) patients who died of liver failure were infected with genotype B. The different HBV genotypes also cause different clinical and epidemiological features. In a study from Japan, a high prevalence of genotype B HBV was found among patients with acute fulminant hepatitis [27]. In two case control studies conducted in Hong Kong, genotype B was the predominant HBV strain among patients with SAE compared to control patients with various severities of liver diseases [25, 28]. In this regard, another study indicated that genotype Bj was associated with high extracellular expression of HBV DNA in vitro [29]. The tendency of genotype Bj to produce high extracellular virion levels would be associated with a more vigorous immune response, leading to a higher risk of hepatic decompensation during the hepatitis flare. Several studies examined the association between specific mutations in the HBV genome and fulminant hepatitis or acute-on-chronic liver failure, especially in the PC (nt 1896) and BCP (nt 1762 and 1764) regions [30–32]. The PC and BCP regions are crucial replications of HBV [33], so alteration of the phenotype by the emergence of mutations in the PC and BCP regions might causes changes in the relationship between the virus and hepatocytes [30], and lead to fulminant hepatitis and acute exacerbation of chronic hepatitis. In the present study, genotype B and PC/BCP mutations were not significant predictors associated with the development of SAE or liver-related death, which is probably related to the small number of cases.

Jeng et al. [13] reported that HBV DNA levels greater than $1.55 \times 10^9$ copies/ml in patients with AE may predict subsequent occurrence of hepatic decompensation. While the overall viral load in our subjects was high (8.5 log copies/ml, Table 1), there was no relationship between viral load and the severity of AE or mortality. In addition, the HBV DNA level could not be estimated correctly when it was above the upper limit. Interestingly, the level of HBV DNA re-measured by TaqMan PCR in stored blood samples was higher than the upper limit (>9.1 log copies/ml) in one-third of the patients. The extremely high HBV DNA levels in patients with AE suggest that the vigorous immune attack on HBV and resultant liver injury will continue and may progress into hepatic decompensation. The present results showed that the decrease of viral load was significantly lower in patients with fulminant hepatic failure than in those with AE. These findings suggest that viral kinetics before the commencement of therapy are an important predictor of hepatic decompensation in patients with CHB infection complicated with AE. Interestingly, there was no significant difference in viral kinetics after the commencement of therapy between the two groups. To our knowledge, this is the first report that identifies viral kinetics before the commencement of therapy as a predictor of prognosis of patients with AE of chronic hepatitis B.

LMV monotherapy does not seem to improve short-term mortality in patients with AE [9], although other studies showed a possible decrease in the mortality rate with earlier administration [21]. In a recent randomized trial designed for the treatment of acute-on-chronic liver failure due to severe reactivation of hepatitis B, the use of tenofovir significantly reduced the mortality rate compared with placebo [11], and the results suggested that rapid suppression of HBV DNA replication with potent antiviral therapy could inhibit the ongoing necroinflammation and permitted hepatic regeneration. Although 8 of 14 patients were treated with LMV in the present study, two patients had to start LMV after the development of SAE because of the rapid exacerbation soon after admission. Five patients developed SAE within a median period of 8 days (range 1–17 days) after the commencement of LMV. The other one patient developed complications caused by bacterial infection and gradually progressed to liver failure over 2 months. Thus, it is thought that most of these patients developed SAE earlier than the available effect of LMV.

The prevailing idea is that AE is the result of a robust quantitative recovery of HBV specific T cells, which directly cause liver injury [34]. Other mechanisms of the effects of CS in AE may be related to the prevention of endotoxin-induced secondary liver injury [35], prevention of cytolysis of ballooned hepatocytes by stabilization of the lysosomal membrane [36], and improvement of the functional activity of the remaining hepatocytes [37]. Other studies showed that the preferential increase in the number of HBV-specific CD8 T and CD4 T cells is associated with viral control rather than liver damage [38, 39]. Whatever the mechanism of AE, a few weeks are needed for sufficient suppression of the production of HBV-related proteins by preventing HBV replication even when NAs are used [40]. Thus, earlier introduction of CS in combination with potent antiviral therapy is a reasonable approach for the initial treatment of AE to prevent excessive immunological reactions and progression of liver cell injury [22, 41]. NA or CS used on its own has limits in the resolution of the serious conditions. Considered together, it is necessary to establish effective standardized strategies, such as the combination of NA and CS. Moreover, to provide cover for NA, especially for the time until NA starts to exert its potent antiviral effect, IFN could be added with NA and CS.

In conclusion, the results of this study suggest that viral kinetics before therapy may influence the clinical course and fate of patients with SAE complicating chronic hepatitis B. Antiviral therapies, including NA and/or IFN with CS, should be started as soon as possible in cases with high serum bilirubin and/or low PT levels, genotype B, and viral

load to prevent progression into hepatic decompensation. Although ethical issues could be an obstacle to randomized trials in such severe cases, more effective strategies are necessary for the treatment of AE associated with chronic hepatitis B.

**Acknowledgment** This study was supported in part by a Grant-in-aid from the Ministry of Health, Labor and Welfare of Japan.

**Conflict of interest** The authors declare no conflict of interest.

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Hepatology Research

Hepatology Research 2012; 42: 949–957 doi: 10.1111/j.1872-034X.2012.01008.x

*Original Article*

# Efficacy of reduction therapy of natural human β-interferon and ribavirin in elderly patients with chronic hepatitis C, genotype 1b and high viral load

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*Aim:* To evaluate the efficacy of reduction therapy of natural human interferon (IFN)-β and ribavirin in elderly patients with hepatitis C virus (HCV) genotype 1b and high viral load who had complications of anemia, low bodyweight (<50 kg), diabetes mellitus and/or hypertension.

*Methods:* Inclusion criteria were age of 65 years or older, HCV genotype 1b, and serum HCV RNA level of 5.0 logIU/mL or higher. A total of 23 subjects with hemoglobin level of less than 13 g/dL, low bodyweight, diabetes mellitus and/or hypertension were enrolled in this study (reduction-dose group). IFN-β was administrated i.v. at a dose of 6 million units daily for 4 weeks initially, followed by three times a week for 44 weeks. Ribavirin was given daily for 48 weeks at a decreased dose of one tablet per day compared to the ordinary dose described based on bodyweight. As a control, another 22 patients without anemia, low bodyweight and/or complications treated with the standard dose of ribavirin (standard-dose group) were enrolled.

*Results:* Patients' rates with further dose reduction or discontinuation of treatment was 26.1% (6/23) in the reduction-dose group and 77.3% (17/22) in the standard-dose group. The sustained virological response (SVR) was 39.1% (9/23) in the reduction-dose group and 27.3% (6/22) in the standard-dose group ($P = 0.404$). 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 ($P = 0.008$).

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

**Key words:** β-interferon, chronic hepatitis C, hepatitis C virus genotype 1b, natural ribavirin

## INTRODUCTION

COMBINATION THERAPY OF peginterferon and ribavirin has been widely recommended as a first choice for chronic hepatitis C patients with high viral load.[1–7] In addition, recent study suggests that combination therapy of peginterferon, ribavirin and protease inhibitor is more effective compared to combination therapy of peginterferon and ribavirin against hepatitis C virus (HCV) of genotype 1 and high viral load.[8,9] The sustained virological response (SVR) rate was approximately 75% in naïve cases with genotype 1 and high viral load treated with three-drug combination therapy of peginterferon, ribavirin and protease inhibitor for 24 weeks. Thus, combination therapy of peginterferon, ribavirin and protease inhibitor might be recommended as a first choice for chronic hepatitis C patients with genotype 1 and high viral load in future.

However, the big problem in combination therapy of peginterferon and ribavirin or combination therapy based on three drugs of peginterferon, ribavirin, and protease inhibitor is the side-effects due to treatment.[9–11] Combination therapy of peginterferon, ribavirin and protease inhibitor might cause severe dermatitis and anemia compared to conventional treatments. The adverse events due to combination therapy of

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*Received 4 February 2012; revision 5 March 2012; accepted 18 March 2012.*

peginterferon and ribavirin or combination therapy of peginterferon, ribavirin and protease inhibitor have a tendency to occur in elderly patients compared to young patients. Several authors have reported that interferon (IFN)-β plus ribavirin therapy might seem to have a strong effect and mild side-effects from reports of treatment to date.[12–14] This indicates the possibility that IFN-β plus ribavirin therapy could be given to elderly patients for eradication of HCV. In particular, dose reduction might enhance the tolerability of IFN-β plus ribavirin therapy.

However, there is little information regarding efficacy of dose reduction in IFN-β plus ribavirin for elderly patients with chronic hepatitis C. Thus, in the present study, we performed a retrospective study to examine the efficacy of reduction therapy of IFN-β and ribavirin in elderly patients of 65 years or older with HCV genotype 1b and high viral load who had complications of anemia, low bodyweight (<50 kg), diabetes mellitus and/or hypertension.

## METHODS

### Patients

ELIGIBILITY CRITERIA FOR entry into the study included the following: (i) age of 65 years or older; (ii) HCV genotype 1b; (iii) serum level of HCV RNA of 5.0 logIU/mL or higher before treatment; (iv) no corticosteroid, immunosuppressive agents or antiviral agents used within 6 months; (v) no hepatitis B surface antigens, antinuclear antibodies or anti-mitochondrial antibodies detectable in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; (vi) leukocytes of more than 2000/mm$^3$, platelet count of more than 80 000/mm$^3$ and bilirubin of less than 2.0 mg/dL; (vii) follow up for more than 6 months before treatment; (viii) complication of anemia (hemoglobin <13 g/dL), low bodyweight (<50 kg), diabetes mellitus and/or hypertension. We excluded from the study all of the patients with the following: (i) a history of alcohol abuse; (ii) complication of malignancy; (iii) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices or ascites. From December 2007 to October 2010, a total of 23 HCV patients were enrolled in this retrospective cohort study at the study hospital. In these 23 patients, combination therapy was started with dose reduction of ribavirin. As control, another 22 patients without complications anemia, low bodyweight, and/or diabetes mellitus and/or hypertension treated with the standard dose of IFN-β and ribavirin were enrolled (standard-dose group). All collection and analysis of patient data for the dose-reduction group and standard-dose group was performed retrospectively from the patient records. This study had been approved by Institutional Review Board of our hospital.

### Combination therapy of IFN-β and ribavirin

Treatment was provided for 48 weeks. IFN-β (Feron; Toray Industries, Tokyo, Japan) was administrated i.v. at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 44 weeks. Ribavirin (Rebetol; MSD, Whitehouse Station, NJ, USA) were given at the dose described based on bodyweight. In the standard-dose group, the ribavirin dose was adjusted according to bodyweight (600 mg for ≤60 kg, 800 mg for >60 kg and ≤80 kg, and 1000 mg for >80 kg). Twenty-two patients were given the standard dose of ribavirin as described above at the initiation of combination therapy (standard-dose group). On the other hand, 23 patients were given a reduced dose of ribavirin that decreased by one tablet per day compared to the standard group due to complications of having a hemoglobin level of less than 13 g/dL, bodyweight of less than 50 kg, diabetes and/or hypertension (reduction-dose group).

### Aspartate aminotransferase to platelet ratio index (APRI) calculation method and prevalence of significant fibrosis

The hepatic fibrosis was evaluated by the APRI, which was calculated according to the following formula: APRI = (AST level / ULN) × 100 / platelet count ($10^9$/L), where ULN was the aspartate aminotransferase (AST) upper limit of normal (33 IU/L).

As previously reported, an APRI of more than 1.50 is predictive of significant fibrosis (positive predictive value, 88%; negative predictive value, 64%).[15]

### Laboratory investigation

In this study, HCV RNA levels were evaluated at least once every month before, during and after therapy. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The linear dynamic range of the assay was 1.2–7.8 logIU/mL, and the undetectable samples were defined as negative. An SVR was defined as clearance of HCV RNA by COBAS TaqMan HCV test (Roche Diagnostics) at 6 months after the cessation of combination therapy.

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

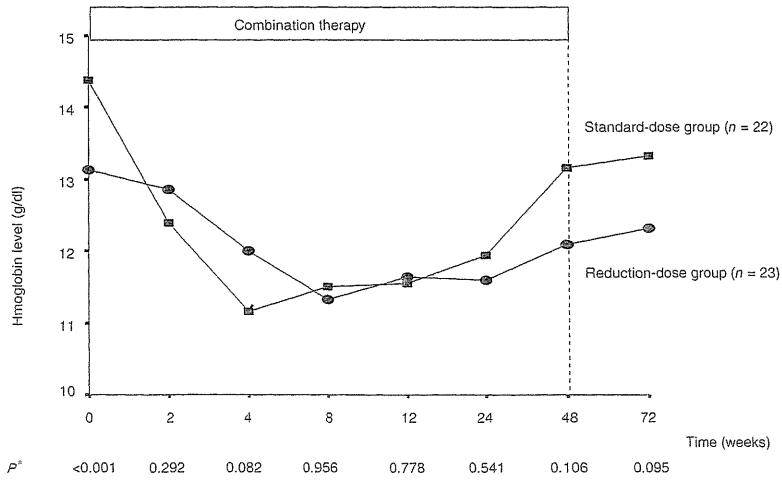
A TOTAL OF 45 patients were enrolled in the present study. Table 1 shows the characteristics before treatment of the elderly patients who received combination therapy. There were no significant differences in clinical backgrounds except for hemoglobin level, sex, height, bodyweight and hypertension between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

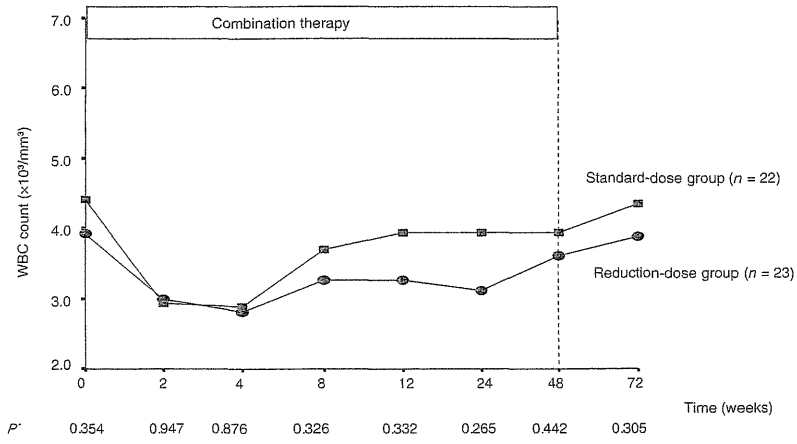



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| P* | <0.001 | 0.292 | 0.082 | 0.956 | 0.778 | 0.541 | 0.106 | 0.095 |
| Standard group† | 0/22 | 2/22 | 7/21 | 8/20 | 10/18 | 10/16 | 10/16 | |
| Reduction droup† | 0/23 | 0/23 | 1/23 | 2/22 | 3/21 | 3/20 | 3/20 | |

*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

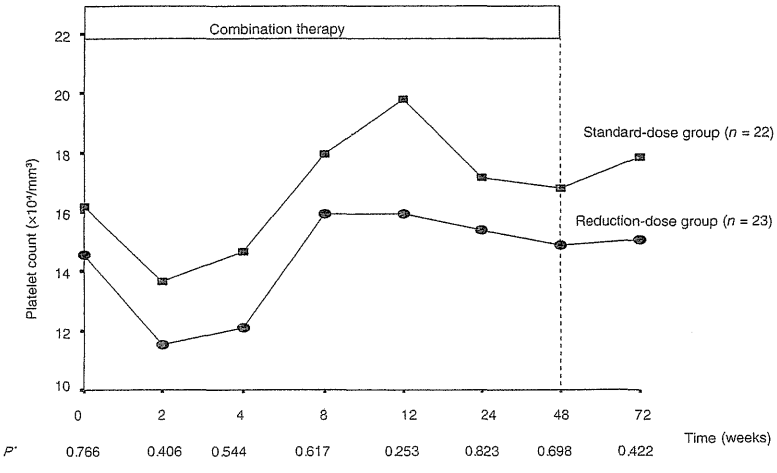
†No. of patients who were given new reduction of IFN-beta dose during combination therapy/ total no. of patients who were given combination therapy

**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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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).