doi:10.1111/j.1440-1746.2008.05319.x

HEPATOLOGY

Association between the treatment length and cumulative dose of pegylated interferon alpha-2b plus ribavirin and their effectiveness as a combination treatment for Japanese chronic hepatitis C patients: Project of the Kyushu University Liver Disease Study Group

Norihiro Furusyo,* Eiji Kajiwara,† Kazuhiro Takahashi,‡ Hideyuki Nomura,§ Yuichi Tanabe,¶ Akihide Masumoto,** Toshihiro Maruyama,†† Makoto Nakamuta,‡‡ Munechika Enjoji,§§ Koichi Azuma,¶¶ Junya Shimono,*** Hironori Sakai,††† Shinji Shimoda‡‡‡ and Jun Hayashi* for the Kyushu University Liver Disease Study (KULDS) Group

*Department of General Medicine, Kyushu University Hospital, †Department of Medicine, Hamanomachi Hospital, ¶Department of Medicine, Fukuoka City Hospital, ††Departments of Gastroenterology, National Hospital Organization Kyushu Medical Center, Departments of §§Medicine and Bioregulatory Science, ¶¶Medicine and Clinical Science and ‡‡‡Medicine and Biosystemic Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, ‡Department of Internal Medicine, Nippon Steel Yawata Memorial Hospital, §The Center for Liver Diseases, Shin-Kokura Hospital, **Department of Clinical Research, National Hospital Organization Kokura Hospital, ††Department of Medicine, Kitakyushu Municipal Medical Center, ***Departments of Medicine, Yahata Saiseikai Hospital, Kitakyushu, and †††Department of Gastroenterology, National Hospital Organization Beppu Medical Center, Beppu, Japan

Key words
hepatitis C virus, pegylated interferon alpha-2b, ribavirin.

Accepted for publication 8 November 2007.

Correspondence
Professor Jun Hayashi, Department of General Medicine, Kyushu University Hospital, Higashi-Ku, Fukuoka 812-8582, Japan. Email: hayashij@genmedpr.med.kyushu-u.ac.jp

Abstract

Aim: The aim of the present study was to investigate the association between the length of the treatment period and the cumulative dose of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) and their effectiveness in the treatment of chronic hepatitis C.

Methods: Seven hundred and fifteen patients received peg-IFN alpha-2b plus RBV treatment for 48 weeks and 24 weeks for genotypes 1 ($n = 586$) and 2 ($n = 129$), respectively.

Results: Sustained virological responses (SVR), defined as serum hepatitis C virus (HCV)-RNA undetectable at 24 weeks after the end of treatment, were 42.4% and 74.4% in genotypes 1 and 2, respectively, on an intention-to-treat analysis. SVR significantly increased with treatment length (4.7%, 36.4%, and 51.8% for < 24 weeks, 24–47 weeks, and 48 weeks, respectively, for genotype 1; and 28.6%, 57.1%, 78.3% for < 12 weeks, 12–23 weeks, and 24 weeks, respectively, for genotype 2). SVR significantly increased with total cumulative treatment dose (21.1%, 36.5%, and 52.9% with < 60%, 60–79%, and ≥ 80% in peg-IFN dose; 29.6%, 51.1%, and 59.2% with < 60%, 60–79%, and ≥ 80% in RBV dose) in genotype 1, although it did not differ significantly for genotype 2.

Conclusions: In peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C, it is important to complete the target length of treatment and to continue the target dosage to achieve SVR, especially for genotype 1 patients.

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease, with perhaps 200 million persons infected worldwide. Approximately 1.8 million patients have chronic HCV infection in Japan. The severity of disease varies widely, from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (HCC).[1,2] Eradication of HCV by antiviral treatment improves liver histology and patient survival.[3] A currently popular antiviral treatment regimen for the treatment of chronic HCV infection worldwide is pegylated interferon alpha (peg-IFN alpha) in combination with ribavirin (RBV). The combination treatment has resulted in a higher rate of sustained virological response (SVR), over 50% in Caucasian patients, than standard interferon (IFN) monotherapy.[4,5] However, there are no data concerning the response and safety of the combination treatment for a large number of Japanese patients with chronic HCV infection because this treatment was only approved by the Japanese Ministry of Health, Labor and Welfare in December 2004.

The HCV genotype has been reported to be the most important predictor of IFN treatment response.[6–14] Patients infected with genotypes 2 and 3 achieved approximately 65% SVR in a 24-week

trial of non-peg-IFN alpha in combination with RBV, in contrast to patients with genotype 1 who had < 30% SVR.[13,14] SVR is also achieved consistently more often by patients with a low HCV-RNA level.[4–14] Moreover, host factors affect the chance of SVR, albeit less so than the genotype.[10] These factors include age, race, sex, obesity, and the degree of hepatic fibrosis and steatosis.[15] In a racial analysis, African Americans were shown to have response rates only one-half to one-third those of Caucasians.[15] In addition, Asian patients were more likely to achieve an SVR by treatment with peg-IFN alpha-2a and RBV than Caucasian patients.[16] The reasons for the racial differences in response rates to peg-IFN alpha plus RBV treatment are not well known.

Peg-IFN alpha was a substantive breakthrough in therapy because of the longer effect; the lasting, steady therapeutic blood level is a major pharmacokinetic advance.[4,5] The most frequent adverse effects during peg-IFN alpha plus RBV treatment are depression and hematological disorders such leukopenia, anemia, and thrombocytopenia. Therefore, the peg-IFN alpha plus RBV treatment often results in discontinuation or the need for a reduction of the dosage due to the adverse effects.

To investigate the efficacy and safety of antiviral treatments for Japanese chronic hepatitis B and C patients, a multicenter study, the Kyushu University Liver Disease Study (KULDS), was launched in 2003. Our group has previously reported several clinical studies.[17–21] The present report is a prospective, multicenter study carried out to analyze the association between the treatment length and the cumulative dose and effectiveness of peg-IFN alpha-2b plus RBV treatment for a large number of Japanese patients with chronic hepatitis C.

Methods

Patients

Treatment of chronic hepatitis C with a combination of peg-IFN alpha-2b and RBV was accepted by the Japanese Ministry of Health in October, 2004. A prospective study of 715 Japanese patients aged 18 years or older (586 and 129 patients with genotypes 1b and 2, respectively) treated with peg-IFN alpha-2b plus RBV between December 2004 and February 2007 who were all positive for antibody to HCV and HCV-RNA for over 6 months was carried out. The respective distribution rates were 82.0% and 18.0% for genotypes 1b and 2, similar to the reported epidemiological distribution.[22]

Criteria for exclusion were: (i) clinical or biochemical evidence of hepatic decompensation, advanced cirrhosis identified by large esophageal varices (F2 or F3), history of gastrointestinal bleeding, ascites, encephalopathy, or hepatocellular carcinoma; (ii) hemoglobin level < 115 g/L, white blood cell count $< 3 \times 10^9$/L, and platelet count $< 50 \times 10^9$/L; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen positive or HIV positive); (iv) excessive active alcohol consumption > 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within 12 months prior to the enrollment. Patients who fulfilled the above criteria were recruited at Kyushu University Hospital and 32 affiliated hospitals in the northern Kyushu area of Japan.

Within the 3 months before the start of the treatment and every 3 months during the treatment period, each patient was tested for alpha-fetoprotein (AFP) and had abdominal ultrasonographic examination. If an abnormal AFP level of ≥ 40 ng/mL and/or an appearance of focal lesions at ultrasonographic examination was found at any testing, further testing for hepatocellular carcinoma (HCC) was done, which included dynamic computed tomography (CT), angiography, and/or tumor biopsy. Patients so confirmed to have HCC within 3 months after the start of the treatment were excluded from this study.

Table 1 shows the baseline characteristics of the enrolled patients. The median age was 58.0 years. Of the 715 patients, 198 (27.6%) were aged 65 years or over. In Japan, many older patients with chronic hepatitis C are candidates for antiviral treatment, different from many other countries. The rates of prior non-peg-IFN monotherapy significantly differed among the genotype-classified patients (genotype 1, 40.8% and genotype 2, 28.7%).

Table 1 Characteristics of 715 chronic hepatitis C patients treated with a combination of pegylated IFN alpha-2b and ribavirin, classified by HCV genotype

Characteristics	Total $n = 715$	Genotype 1 $n = 586$	Genotype 2 $n = 129$	*P*-value
Male *n* (%)	388 (54.3)	321 (54.8)	67 (51.9)	0.6250
Age (years)	56.8 ± 11.7	57.8 ± 10.3	52.6 ± 14.1	0.0004
Body mass index (kg/m^2)	23.4 ± 3.2	23.5 ± 3.1	23.5 ± 3.3	0.4999
Prior IFN monotherapy *n* (%)	276 (38.6)	239 (40.8)	37 (28.7)	0.0140
Prior combined IFN plus RBV treatment *n* (%)	69 (9.7)	60 (10.9)	5 (3.9)	0.0221
Alanine aminotransferase (IU/L)	77.1 ± 55.4	77.5 ± 52.8	70.9 ± 55.3	0.0594
γ-Glutamyltranspeptidase (IU/L)	60.6 ± 60.3	61.8 ± 58.6	50.8 ± 45.2	0.0241
Albumin (g/dL)	4.1 ± 0.4	4.1 ± 0.3	4.1 ± 0.3	0.1305
White blood cell (/mm^3)	5030.8 ± 1439.2	4993.0 ± 140.8	5260.6 ± 1658.2	0.3005
Hemoglobin (g/dL)	13.9 ± 1.4	13.9 ± 1.4	13.9 ± 1.5	0.7092
Platelet count (10^9/L)	165 ± 56	161 ± 52	185 ± 69	0.0013
Creatinine (mg/dL)	0.70 ± 0.16	0.70 ± 0.17	0.71 ± 0.16	0.1230
Creatinine clearance (mL/min)	97.9 ± 29.9	97.1 ± 29.8	101.3 ± 31.3	0.3621

Data are shown as the mean ± standard deviation.
HCV, hepatitis C virus; IFN, interferon; RBV, rivabirin.

Also, the rates of prior non-peg-IFN alpha plus RBV treatment significantly differed (genotype 1, 10.9% and genotype 2, 3.9%). These differences are explained by the necessity of re-treatment of patients with genotype 1 who had lower SVR by the standard IFN monotherapy than did non-genotype 1 patients, and because the RBV combination treatment with peg-IFN alpha-2b was approved in stages, first for patients with genotype 1 in October 2004, then for those with non-genotype 1 in January 2006. The means for age, platelet count, and γ-glutamyltranspeptidase (γ-GTP) in genotype 1 patients were significantly different than those of genotype 2 patients.

Informed consent was obtained from all patients before enrollment in this study. The study was approved by the institutional ethics committees of the hospitals involved and conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization of guidelines for good clinical practice.

Treatment regimen

All patients were treated with a weight-based, 1.5 μg/kg weekly dose of subcutaneous peg-IFN alpha-2b (PegIntron A; Schering-Plough, Osaka, Japan). In combination with peg-IFN alpha-2b, RBV (Rebetol; Schering-Plough) was given orally at a daily dose of 600–1000 mg based on bodyweight (600 mg for patients weighing < 60 kg, 800 mg for those weighing 60–80 kg, and 1000 mg for those weighing ≥ 80 kg). The lengths of treatment were 48 weeks and 24 weeks for HCV genotypes 1 and 2 patients, respectively. The above durations and dosages are those approved by the Japanese Ministry of Health, Labor and Welfare. Patients were considered to have RBV-induced anemia if the hemoglobin level decreased to < 100 g/L. In such cases, a reduction in the dose of RBV was required. Some patients also had peg-IFN alpha-2b-induced psychological adverse effects or a decrease of white blood cell and platelet count. In such cases, a reduction in the dosage of peg-IFN alpha-2b was required. Both peg-IFN alpha-2b and RBV were discontinued if the hemoglobin level, white blood cell count, or platelet count fell below 85 g/L, 1×10^9/L, and 2.5×10^9/L, respectively. The treatment was discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia, or severe hemolytic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient desired discontinuation of treatment.

Clinical and laboratory assessment

Body mass index (BMI) was calculated as weight in kilograms/height in square meters. Blood samples were taken on enrollment, in the morning after 12 h overnight fasting. Serum levels of alanine aminotransferase (ALT), γ-GTP, white blood cell count, hemoglobin, and platelet count were measured by standard laboratory techniques at a commercial laboratory.

Determination of baseline HCV-RNA level and HCV genotype

The pretreatment, baseline, serum HCV-RNA level was measured by a quantitative HCV-RNA polymerase chain reaction (PCR) assay (COBAS Amplicor HCV Monitor Test v2.0 using the 10-fold dilution method; Roche Diagnostics, Tokyo, Japan), which has a lower limit of quantitation of 5000 IU (1350 copies)/mL (5 kIU/mL) and an outer limit of quantitation of 5 100 000 IU/mL (5100 kIU/mL). The HCV genotype was determined by a type-specific primer from the core region of the HCV genome. The protocol for genotyping was carried out as previously described.[23]

Efficacy of treatment

Sustained virological response was defined as serum HCV-RNA undetectable at 24 weeks after the end of treatment. Patients who had undetectable HCV-RNA within the initial 12 weeks of treatment were considered to have had an early virological response (EVR). These efficacy variables, SVR and EVR, were defined as non-detectable HCV-RNA as measured by the COBAS Amplicor HCV Monitor Test v2.0, and the results were labeled as positive or negative. The lower limit of detection was 50 IU/mL (0.5 kIU/mL). The analysis of SVR and EVR was done on an intention-to-treat basis.

Statistical analysis

Continuous data were expressed as mean values, the values ± standard deviation (SD), or the values ± standard error (SE) of the mean. The following statistics were done using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA, USA) for the IBM 3090 system computer. The chi-squared or Fisher's exact test was used to examine the association between baseline characteristics and SVR. The Mann–Whitney *U*-test was also used to compare responders and non-responders with regard to various characteristics, when appropriate. The Cochran-Mantel-Haenszel test was used to test for statistical significance among the subgroups. A *P*-value of less than 0.05 was considered significant.

Results

Discontinuation of peg-IFN alpha-2b plus RBV treatment and adverse effects

Of the 715 patients, 152 (21.2%) did not complete peg-IFN alpha-2b plus RBV treatment due to adverse effects or for other reasons (Table 2). Although anemia, as a cause of discontinuation, was followed by general fatigue and depression, most patients discontinued the treatment because of general fatigue and depression together with anemia (hemoglobin 85–100 g/L).

The discontinuation rate was higher for patients with genotype 1 (138 of 586, 23.5%) than those with genotype 2 (14 of 129, 10.8%). The genotype 1 patients included 55 who stopped receiving treatment without virological effect (positive for serum HCV-RNA or no more than 2-$\log_{10}$ reduction from the pretreatment viral level) at 24 or more weeks after the start ($n = 22$), economic problems related to the high cost of treatment ($n = 6$), and other reasons (drop out, moving, nursing ill family members, and being arrested for a crime) ($n = 27$). Thus, the discontinuation rates for patients with adverse effects were only 14.1% (83 of 586) and 7.7% (10 of 129) for genotypes 1 and 2, respectively, with no significant difference. The majority were patients aged 65 years or

Table 2 Reasons for discontinuation of pegylated IFN plus ribavirin treatment, classified by HCV genotype

	Genotype 1	Genotype 2	Total
Adverse effects			
General fatigue	29	0	29
Depression	10	1	11
Encephalopathy	2	0	2
Anemia	11	0	11
Thrombocytopenia	1	1	2
Hyperthyroidism	5	1	6
Rash	6	3	9
Retinopathy	2	0	2
Interstitial pneumonia	1	1	2
Articular rheumatism	1	0	1
Brain infarction	0	1	1
Proteinuria	1	0	1
Hepatocellular carcinoma	11	2	13
Malignancy (extra-liver)†	2	0	2
Pulmonary tuberculosis	1	0	1
Other reasons			
No effect of treatment	22	2	24
Economic problems	6	0	6
Others‡	27	2	29
Total	138	14	152

†Includes one patient with gastric cancer and one patient with lung cancer.

‡Includes drop out ($n = 16$), patients who moved ($n = 6$), who nursed ill family members ($n = 3$), or who were arrested for criminal activity ($n = 2$).

over: 68 (73.1%) of the 93 discontinued due to adverse effects. The discontinuation rate due to adverse effects was significantly higher for patients aged 65 years or over (68 of 198, 34.3%) than for those aged under 65 years (25 of 517, 4.8%) ($P < 0.0001$). The mean times to discontinuation (± SD) were 23.0 ± 13.1 weeks and 20.2 ± 15.4 weeks for patients with genotypes 1 and 2, respectively.

SVR by intention-to-treat analysis

Of the 715 patients, 345 (48.2%) achieved SVR in the intention-to-treat analysis. SVR was significantly higher in genotype 2 (96 of 129, 74.4%) than in genotype 1 (249 of 586, 42.4%) ($P < 0.0001$). No significant differences in SVR were found between patients with and without prior non-peg-IFN monotherapy or non-peg-IFN plus RBV treatment between the genotype-classified patients.

An analysis of the association between SVR and the length of treatment showed that patients who completed the combination treatment had a significantly higher rate of SVR than did those with a shortened period of treatment (Fig. 1). Completing the 48-week combination treatment resulted in a significantly higher rate of SVR than either 1–11-week and 12–23-week treatments (both $P < 0.0001$), but there was no significant difference between 24 and 47 weeks and the complete 48 weeks of treatment ($P = 0.1260$). The SVR of patients with genotype 1 was significantly associated with a ≥ 24-week treatment period when compared with treatment < 24 weeks (244 of 481, 50.7% *vs* 5 of 105, 4.7%, $P < 0.0001$). In genotype 2 patients, SVR significantly increased with the treatment period: 28.6%, 57.1%, and 78.3% by 1–11-week, 12–23-week, and 24-week periods, respectively ($P = 0.0018$ by the Cochran-Mantel-Haenszel test).

The combination treatment was done for 443 (75.5%) and 110 (85.2%) of genotype 1 and 2 patients, respectively (Fig. 2). The rates of SVR for genotype 1 and 2 patients were significantly higher in those who continued the combination treatment than in those who discontinued RBV treatment: 230 of 443 (51.9%) versus 19 of 143 (13.2%) genotype 1 ($P < 0.0001$) and 89 of 110 (80.9%) versus seven of 19 (36.8%) genotype 2 ($P = 0.0002$). In genotype 1, 286 patients who required a reduced dosage during treatment (Groups B, C, and D) were able to complete the full 48 weeks of combination treatment. There were no significant differences in SVR among Groups A to D patients with genotypes 1 and 2. Of the patients who discontinued RBV treatment (143 with genotype 1 and 19 with genotype 2), most patients (138 (96.5%) with genotype 1 and 14 (73.7%) with genotype 2) did not complete combination treatment because there was no viral effect, because of adverse effects, or because they dropped out. The remaining patients discontinued the RBV treatment but completed the combination treatment without a reduction of the peg-IFN alpha-2b target dosage (three with genotype 1 and five with genotype 2), or discontinued the RBV treatment and completed their peg-IFN alpha-2b treatment with a reduction of the target dosage (two with genotype 1 and none with genotype 2).

An analysis of the association between SVR and the total dosage of peg-IFN alpha-2b and RBV during the treatment showed that patients with a higher total dosage of the combination treatment had a significantly higher rate of SVR than those with a lower dosage only for genotype 1 patients, although no significant difference was found in genotype 2 (Fig. 3). In genotype 1, reducing the total dosage of peg-IFN alpha-2b during the treatment significantly reduced the rate of SVR: 52.9% (187 of 353) for patients with ≥ 80% of the peg-IFN alpha-2b dosage, 36.5% (30 of 82) for those ≥ 60% but < 80% of the peg-IFN alpha dosage, and 21.1% (32 of 151) for those < 60% of the peg-IFN alpha dosage (both $P < 0.0001$). In genotype 1, the SVR rate of patients < 60% of the RBV dosage (91 of 307, 29.6%) was significantly lower than that of patients ≥ 80% of the RBV dosage (112 of 189, 59.2%) and those ≥ 60% but < 80% of the RBV dosage (46 of 90, 51.1%) (both $P < 0.0001$), although no significant difference was found between those ≥ 80% of the RBV dosage and those ≥ 60% but < 80% of the dosage. In genotype 2, neither a dosage reduction of peg-IFN nor RBV significantly influenced SVR.

An analysis of the association between SVR and the total combined dosage of peg-IFN alpha-2b plus RBV showed that patients with a higher total combined dosage of the combination treatment had a significantly higher rate of SVR than those with a lower dosage for genotype 1 patients only, although no significant difference was found in genotype 2 (Fig. 4). In genotype 1, the SVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 80% of RBV was significantly higher (78 of 122, 63.9%) than those without these combined dosages (171 of 464, 36.8%) ($P < 0.0001$). Moreover, the SVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 60% of RBV was significantly higher (116 of 187, 62.0%) than those without these dosages (133 of 399, 33.3%) ($P < 0.0001$). However, in genotype 2, neither a dosage reduction of peg-IFN nor RBV significantly influenced SVR.

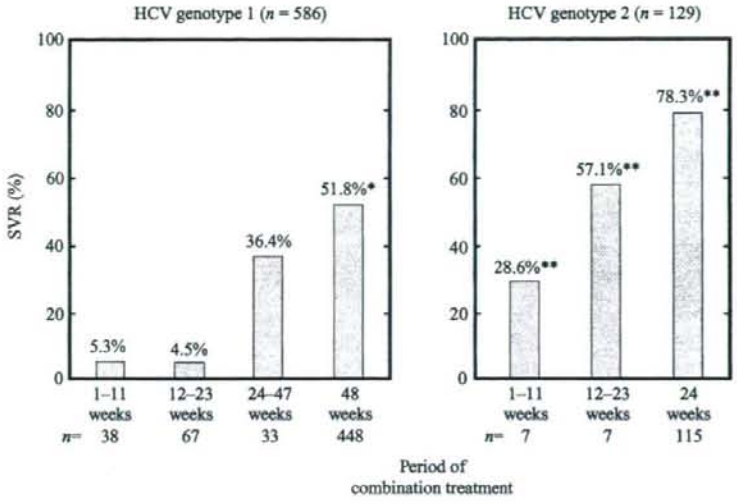

Figure 1 Sustained virological response (SVR) rates classified by length of pegylated interferon-alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) combination treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis.

Analysis of EVR and the first 12-week adherence

An EVR was significantly higher in patients with genotype 2 (119 of 129, 92.2%) than in those with genotype 1 (307 of 586, 52.3%) ($P < 0.0001$). An analysis of the association between SVR and EVR showed that patients with EVR had a significantly higher rate of SVR than did patients without EVR for both genotypes 1 and 2: 220 of 309 (71.1%) versus 29 of 277 (10.4%) in genotype 1, and 96 of 119 (80.6%) versus none of 10 (0%) in genotype 2 (all $P < 0.0001$).

An analysis of the association between EVR and the first 12-week combined dosage of peg-IFN alpha-2b plus RBV showed that patients with a higher total combined dosage of the combination treatment had a significantly higher rate of SVR than those with a lower dosage for genotype 1 patients only, although no significant difference was found in genotype 2 (Fig. 5). In genotype 1, the EVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 80% of RBV was significantly higher (217 of 357, 60.7%) than those without these dosages (92 of 229, 40.1%) ($P < 0.0001$). Moreover, the SVR rate of patients ≥ 80% of peg-IFN alpha-2b and ≥ 60% of RBV was significantly higher (262 of 445, 58.8%) than those without these dosages (47 of 141, 33.3%) ($P < 0.0001$). However, in genotype 2, neither a dosage reduction of peg-IFN nor RBV influenced EVR.

Discussion

To the best of our knowledge, no reports have been written on the efficacy and safety of peg-IFN alpha-2b plus RBV treatment for a large number of Japanese HCV patients. The present study by intention-to-treat analysis included over 700 Japanese patients with chronic hepatitis C, a sufficient number to provide a meaningful statistical analysis and to be of interest to clinical physicians. Our findings show that in peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C it is important to complete the target treatment duration and to use the full dosage to achieve virological efficacy.

A recent study showed Asian patients with chronic hepatitis C were more likely to achieve an SVR by treatment with peg-IFN alpha-2a and RBV than were Caucasian patients, suggesting a genetic influence on the antiviral response.[16] A significant difference between Asian and Caucasian patients with genotype 1 infections (65% and 36%) was also reported. However, the study included only 52 Asian patients and had no analysis concerning dosage of peg-IFN and RBV. Because our study included a large number of Japanese patients and an analysis of the complete combination treatment and the dosage of peg-IFN alpha and RBV, the present study provides for meaningful statistical analysis.

Our analysis showed that the discontinuation of RBV was significantly associated with a marked decline in SVR. We also showed that a < 60% reduction of the total dosage was associated with a poor outcome. Several adverse reactions are strongly associated with RBV. One of the most significant problems is hemolytic, especially anemia.[14] Most patients with anemia have general fatigue. Careful administration is necessary for patients > 60 years old, female patients, and patients receiving an RBV dosage by bodyweight of ≥ 12 mg/kg.[24] In fact, most of our patients who required a reduction in the total dosage or who discontinued RBV had anemia or fatigue. Also, discontinuation in this study was frequently found in patients aged ≥ 65 years. In Japan, many older patients with chronic hepatitis C are candidates for antiviral treatment, different from other countries. It is important to reduce the dosage of RBV at an early a stage as possible to allow the safe continuation of the combination treatment, as shown by data that a reduction of up to 60% of the total dosage of RBV does not appear to adversely influence SVR in Japanese patients.

The duration and dose of antiviral treatment are the most important factors influencing treatment outcome, especially in

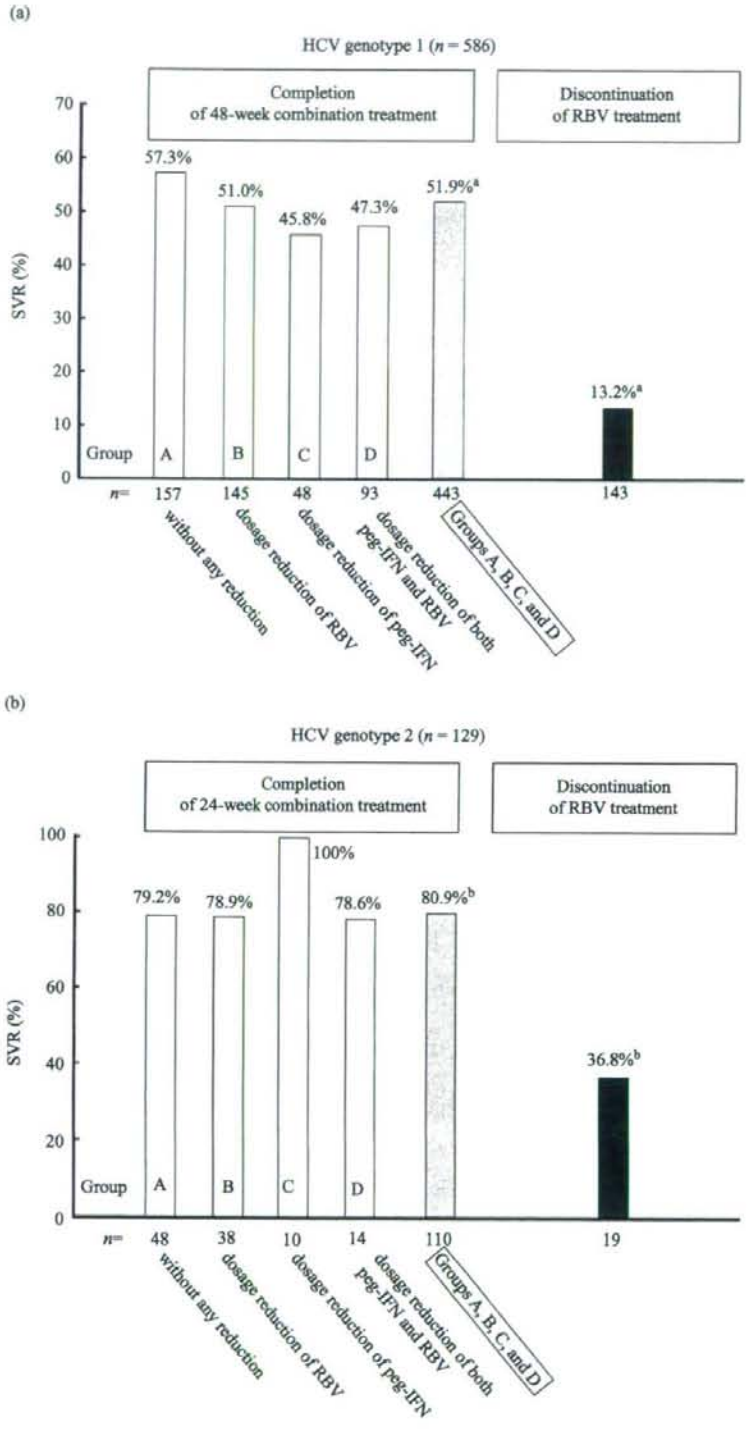

Figure 2 Sustained virological response (SVR) rates classified by continuation, reduction of the dosage, discontinuation of pegylated interferon-alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment, and hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 2a for genotype 1 and Fig. 2b for genotype 2). The following groups A, B, C, and D consisted of patients who completed their scheduled combination treatment (48 weeks for genotype 1 patients [$n = 443$] and 24 weeks for genotype 2 patients [$n = 110$]) and patients who discontinued RBV treatment (genotype 1 patients [$n = 143$] and genotype 2 patients [$n = 19$]). Group A patients well tolerated the combination treatment with peg-IFN alpha-2b and RBV without any reduction in the target dosage of either drug; Group B patients completed the combination treatment and had no reduction of peg-IFN alpha-2b dose, but needed a reduction of the RBV target dosage; Group C patients completed the combination treatment and had no reduction of RBV dosage, but needed a reduction of the target dosage of peg-IFN alpha-2b; Group D patients completed the combination treatment, but needed a reduction of the target dosage of both peg-IFN alpha-2b and RBV. 'a' and 'b' indicate significant differences between completion of the full combination treatment and discontinuation of RBV treatment ($P < 0.0001$ and $P = 0.0002$, respectively).

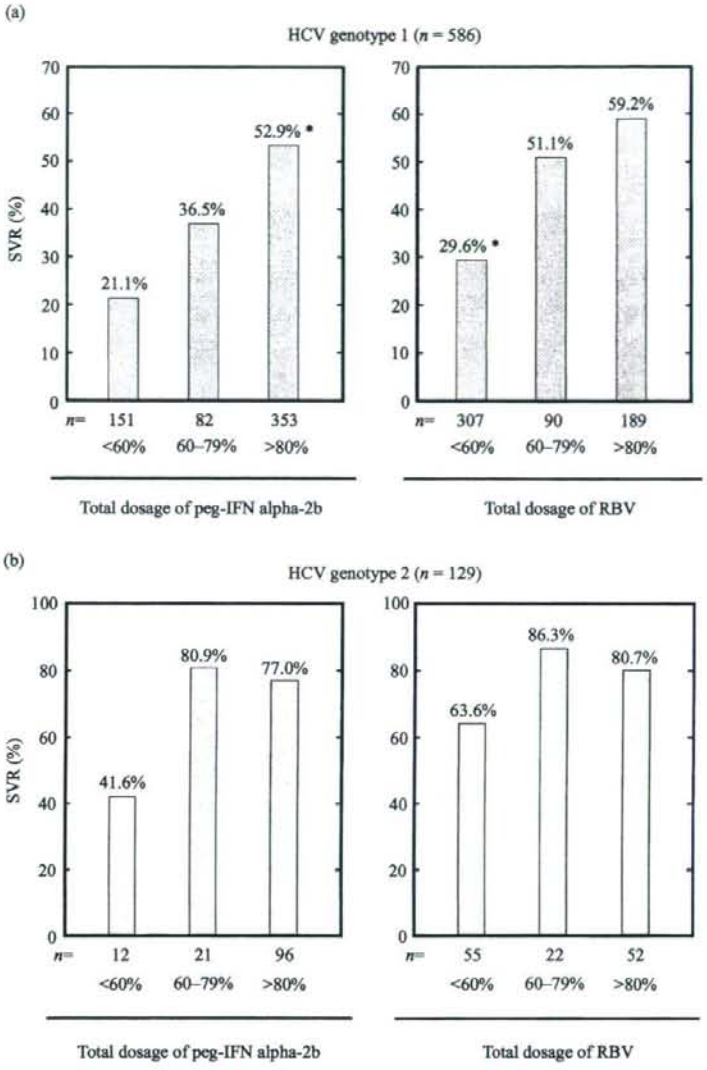

Figure 3 Sustained virological response (SVR) rates classified by percentage of total dosage of pegylated interferon-alpha2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 3a for genotype 1 and Fig. 3b for genotype 2). *indicates a significant difference between the groups.

HCV genotype 1-infected patients.[25,26] Shiffman and colleagues reported that reducing the total dose of peg-IFN alpha-2a to < 80% within the first 20 weeks of therapy significantly reduced SVR, but reducing the dose of RBV appeared to have little impact on SVR.[25] For our patients with genotype 1, the treatment period and total dosage were important to gaining SVR with peg-IFN alpha-2b plus RBV treatment. The 48-week combination treatment is the minimum requirement for SVR by these patients. Moreover, it is necessary to give ≥ 80% of the target dosage of peg-IFN alpha-2b (suitable for the weekly ≥ 0.9–1.2 μg/kg) and ≥ 60% of the target RBV (suitable for the daily 6–8 mg/kg) throughout the treatment.

Our previous report showed that a 24-week non-peg-IFN alpha plus RBV treatment regimen produced a high rate of SVR in Japanese genotype 2-infected patients.[19] The 24-week peg-IFN alpha-2b plus RBV treatment regimen used in the present study also demonstrated a remarkable rate of SVR (74.4%) for genotype 2 patients, as expected. This can be explained by the fact that genotype 2 patients have an extremely high rate of EVR, over 80%, with this combination treatment. Another important finding was that the total dosages of peg-IFN alpha-2b and RBV during the treatment for genotype 2 patients did not significantly influence SVR, although a dosage < 60% of the target resulted in a lower rate of SVR than a dosage ≥ 60%, without

Journal of Gastroenterology and Hepatology **23** (2008) 1094–1104

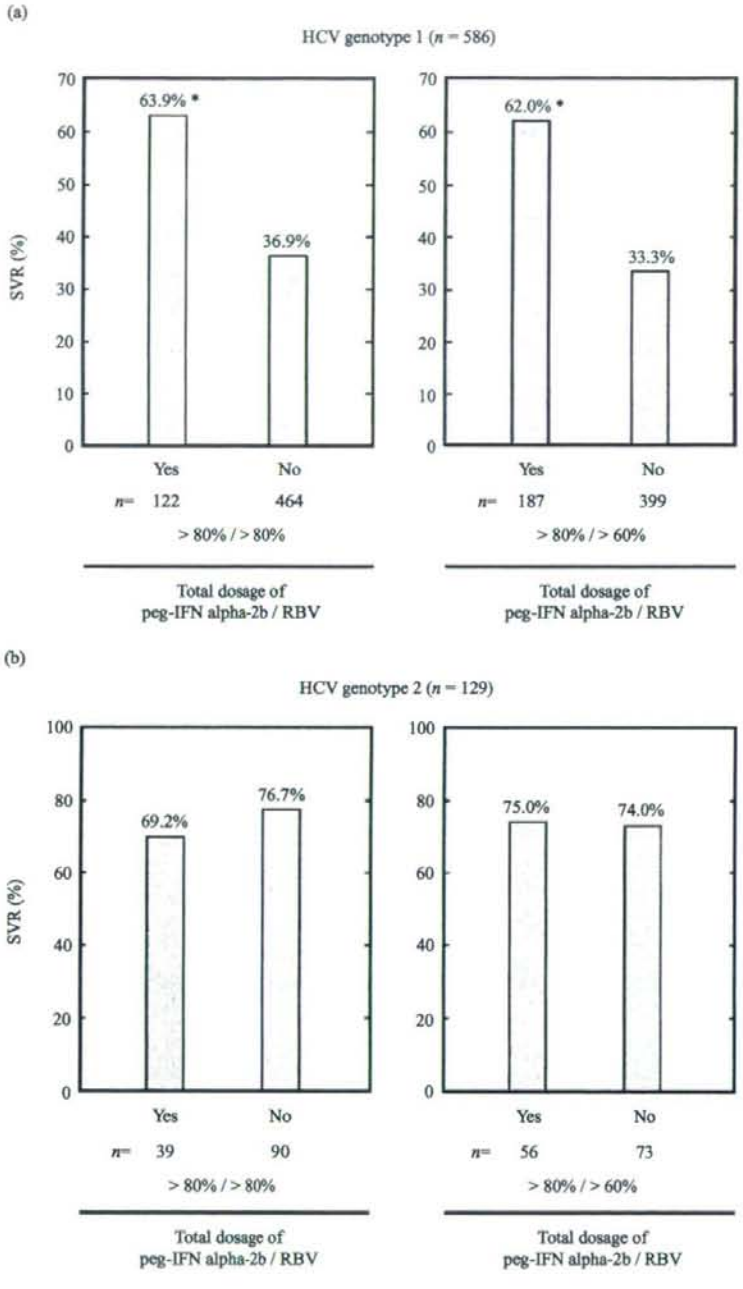

Figure 4 Sustained virological response (SVR) rates classified by percentage of total combined dosage of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 4a for genotype 1 and Fig. 4b for genotype 2). *indicates a significant difference between the groups.

significant difference. These findings suggest that the target dosage can be reduced for genotype 2 patients to avoid the adverse effects such as general fatigue, depression, and anemia and that the 24-week combination treatment can still be successfully completed.

An EVR, a virological clearance by antiviral treatment in the initial 12 weeks, is significantly related with sustained response.[27] The present study also showed that the first 12-week combined dosage was significantly related with EVR in both genotype 1 and 2 patients, leading to the attainment of an SVR.

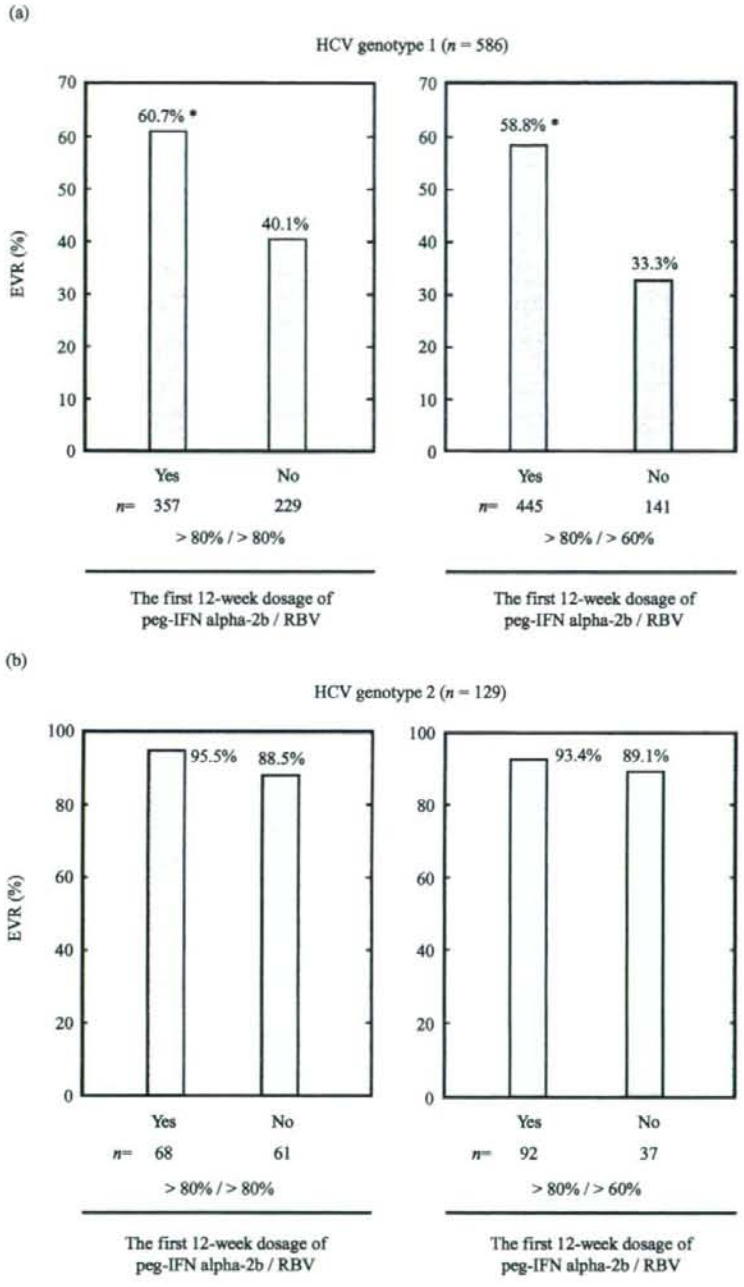

Figure 5 Early virological response (EVR) rates classified by percentage of the first 12-week combined dosage of pegylated interferon alpha-2b (peg-IFN alpha-2b) plus ribavirin (RBV) treatment and by hepatitis C virus (HCV) genotype: An intention-to-treat analysis (Fig. 5a for genotype 1 and Fig. 5b for genotype 2). *indicates a significant difference between the groups.

Because of the impact of medical adherence during the first 12-week dosage on EVR, it is important to continue the dosage from the early stage to the target period in peg-IFN alpha-2b plus RBV treatment.

Since the introduction of peg-IFN alpha plus RBV combination regimen, the treatment of chronic hepatitis C has dramatically improved over the past decade and can cure a significant proportion of the patients.[5,6] However, the combination treatment has its

limitations, especially for HCV genotype 1 patients. Although the limited efficacy and adverse effects necessitate the development of new therapeutics approaches, we must acknowledge the current situation in which many older Japanese patients with chronic hepatitis C are candidates for antiviral treatment. Therefore, a key to solving the problem is managing antiviral treatment for these older patients. Recent analysis suggests that using erythropoietic agents (epoetin and dartbepoetin) for the reduction of anemia may not be cost-effective for the majority of patients.[28] A new RBV analog, viramidine, is reported to be associated with a lower incidence of anemia than RBV (4% *vs* 27%),[29] and, if proven effective, may eventually be substituted for RBV in combination with peg-IFN alpha for patients with chronic hepatitis C.

In conclusion, in peg-IFN alpha-2b plus RBV treatment for chronic hepatitis C, it is important to complete the target duration and reach the target dosage to achieve virological efficacy, especially for genotype 1 patients.

Acknowledgments

We greatly thank Kazukuni Kawasaki, Tomohiro Kuwasaki, Naonori Ohe, and Toshihiro Ueda for their support of this study.

References

1 Hayashi J, Kishihara Y, Yamaji K *et al.* Hepatitis C viral quasispecies and liver damage in patients with chronic hepatitis C virus infection. *Hepatology* 1997; **25**: 697–701.
2 Hayashi J, Furusyo N, Ariyama I, Sawayama Y, Etoh Y, Kashiwagi S. A relationship between the evolution of hepatitis C virus variants, liver damage, and hepatocellular carcinoma in patients with hepatitis C viremia. *J. Infect. Dis.* 2000; **181**: 1523–7.
3 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 1998; **28**: 1687–95.
4 Lindsay KL, Trepo C, Heintges T et al., Hepatitis Interventional Therapy Group. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; **34**: 395–403.
5 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958–65.
6 Wong JB, Davis GL, McHutchison JG, Manns MP, Albrecht JK, International Hepatitis Interventional Therapy Group. Economic and clinical effects of evaluating rapid viral response to peginterferon alfa-2b plus ribavirin for the initial treatment of chronic hepatitis C. *Am. J. Gastroenterol.* 2003; **98**: 2354–62.
7 Nomura H, Sou S, Tanimoto H *et al.* Short-term interferon-alfa therapy for acute hepatitis C: a randomized controlled trial. *Hepatology* 2004; **39**: 1213–19.
8 Hayashi J, Kishihara Y, Ueno K *et al.* Age-related response to interferon alfa treatment in women vs. men with chronic hepatitis C virus infection. *Arch. Intern. Med.* 1998; **158**: 177–81.
9 Furusyo N, Hayashi J, Ueno K *et al.* Human lymphoblastoid interferon treatment for patients with hepatitis C virus-related cirrhosis. *Clin. Ther.* 1997; **19**: 1352–67.
10 Furusyo N, Kubo N, Toyoda K *et al.* Helper T cell cytokine response to ribavirin priming before combined treatment with interferon alpha and ribavirin for patients with chronic hepatitis C. *Antiviral Res.* 2005; **67**: 46–54.
11 Furusyo N, Hayashi J, Ohmiya M *et al.* Differences between interferon-alpha and beta treatment for patients with chronic hepatitis C virus infection. *Dig. Dis. Sci.* 1999; **44**: 608–17.
12 Furusyo N, Hayashi J, Kashiwagi K *et al.* Hepatitis C virus (HCV) RNA level determined by second-generation branched-DNA probe assay as predictor of response to interferon treatment in patients with chronic HCV viremia *Dig. Dis. Sci.* 2002; **47**: 535–42.
13 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352**: 1426–32.
14 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N. Engl. J. Med.* 1998; **339**: 1485–92.
15 Muir AJ, Bornstein JD, Killenberg PG, Atlantic Coast Hepatitis Treatment Group. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. *N. Engl. J. Med.* 2004; **350**: 2265–71.
16 Missiha S, Heathcote J, Arenovich T *et al.* Impact of Asian race on response to combination therapy peginterferon alfa-2a and ribavirin in chronic hepatitis C. *Am. J. Gastroenterol.* 2007; **102**: 1–8.
17 Furusyo N, Takeoka H, Toyoda K *et al.* Long-term lamivudine treatment for chronic hepatitis B in Japanese patients: a project of Kyushu University Liver Disease Study. *World J. Gastroenterol.* 2006; **12**: 561–7.
18 Furusyo N, Katoh M, Tanabe Y *et al.* Interferon alpha plus ribavirin combination treatment of Japanese chronic hepatitis C patients with HCV genotype 2: a project of the Kyushu University Liver Disease Study Group. *World J. Gastroenterol.* 2006; **12**: 784–90.
19 Nakamuta M, Kotoh K, Tanabe Y *et al.* Body surface area is an independent factor contributing to the effects of lamivudine treatment. *Hepatol. Res.* 2005; **31**: 13–17.
20 Nakamuta M, Morizono S, Tanabe Y *et al.* Relationship between body surface area and ALT normalization after long-term lamivudine treatment. *World J. Gastroenterol.* 2005; **11**: 6948–53.
21 Nakamuta M, Kotoh K, Enjoji M *et al.* Effects of lamivudine on serum albumin levels correlate with pretreatment HBV-DNA levels in cirrhotic patients. *Comp. Hepatol.* 2007; **6**: 3.
22 Hayashi J, Kishihara Y, Yamaji K *et al.* Transmission of hepatitis C virus by health care workers in a rural area of Japan. *Am. J. Gastroenterol.* 1995; **90**: 794–9.
23 Simmonds P, Holmes EC, Cha TA *et al.* Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J. Gen. Virol.* 1993; **74**: 2391–9.
24 Nomura H, Tanimoto H, Kajiwara E *et al.* Factors contributing to ribavirin-induced anemia. *J. Gastroenterol. Hepatol.* 2004; **19**: 1312–17.
25 Shiffman ML, Ghany MG, Morgan TR *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; **132**: 103–12.
26 Hadziyannis SJ, Sette H Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann. Intern. Med.* 2004; **40**: 346–55.
27 Davis GL, Wong JB, McHutchison JG *et al.* Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 645–52.
28 McHutchison JG, Manns MP, Brown RS Jr, Reddy KR, Shiffman ML, Wong JB. *et al.* Strategies for managing anemia in

hepatitis C patients undergoing antiviral therapy. *Am. J. Gastroenterol.* 2007; **102**: 880–9.
29 Gish RG. Treating HCV with ribavirin analogues and ribavirin-like molecules. *J. Antimicrob. Chemother.* 2006; **57**: 8–13.

Appendix I

In addition to the authors, the following investigators of the KULDS Group were involved in the present study: M Tatsukawa and M Murata, Haradoi Hospital, Fukuoka; K Toyoda, Yokota Hospital, Hirokawa, Fukuoka; E Ogawa, Yagi Hospital, Fukuoka; T Koga, H Takeoka, T Kuga and A Mitsutake, Mitsutake Hospital, Iki, Nagasaki; R Sugimoto, Harasanshin Hospital, Fukuoka; H Amagase, S Tominaga, Mihagino Hospital, Kitakyushu; K Yanagita, Saiseikai Karatsu Hospital, Karatsu; K Ogiwara, Kyusyu Rosai Hospital, Kitakyushu; M Tokumatsu, Saiseikai Fukuoka Hospital, Fukuoka; S Tabata, Hayashi Hospital, Fukuoka; M Yokota, National Kyushu Cancer Center, Fukuoka; H Tanaka, Chihaya Hospital, Fukuoka; S Nagase, Fukuoka Teishin Hospital, Fukuoka; S Tsuruta, Nakabaru Hospital, Fukuoka; S Tada, Moji Rosai Hospital, Kitakyushu; M Nagano, Kyushu Koseinenkin Hospital, Kitakyushu; M Honda, Nishi-Fukuoka Hospital, Fukuoka; T Umeno, Sawara Hospital, Fukuoka; T Sugimura, National Hospital Organization Fukuoka Higashi Hospital, Fukuoka; S Ueno, Kitakyushu Municipal Wakamatsu Hospital, Kitakyushu; K Miki, Kitakyushu Municipal Moji Hospital, Kitakyushu; H Okubo, Shineikai Hospital, Kitakyushu; H Fujimoto, Mitsubishikagaku Hospital, Kitakyushu; N Higuchi, Shin-Nakama Hospital, Kitakyushu; S Shigematsu, Kouseikan Hospital, Saga; N Higashi, National Hospital Organization Beppu Hospital, Beppu, Japan.

Journal of Gastroenterology and Hepatology **23** (2008) 1094–1104

Hepatology Research 2008; **38**: 1063–1068 doi: 10.1111/j.1872-034X.2008.00386.x

Review Article

Transient elastography: Applications and limitations

Kentaro Yoshioka, Naoto Kawabe and Senju Hashimoto

Division of Liver, Biliary Tract and Pancreas Diseases, Department of Internal Medicine, Fujita Health University, Aichi, Japan

Transient elastgraphy with use of FibroScan is one of most accurate methods for assessment of liver fibrosis. FibroScan can be readily used with an operator with a short training. In many different studies, liver stiffness measured by transient elastgraphy correlates well with fibrosis stages, and cutoff values of liver stiffness for fibrosis staging are similar even among different diseases. However there is wide variation of stiffness values in the same fibrosis stage, and some overlap between the adjacent stages. In addition, inflammatory activity and size of nodule of cirrhosis affect the liver stiffness values. The reproducibility may be reduced by age, obesity, steatosis, narrow intercostal space and lower degrees of hepatic fibrosis in patients. Thus the estimation of fibrosis stages from liver stiffness should be cautiously done. To improve the accuracy of liver fibrosis staging, the combination of transient elastography with other noninvasive methods such as FibroTest should be required.

Key words: cirrhosis, FibroScan, fibrosis, FibroTest, inflammation, liver stiffness

The search for a noninvasive method to assess liver fibrosis has encouraged the development of a number of new approaches. The prognosis and treatment of chronic liver diseases depend on the stage of liver fibrosis. In chronic viral hepatitis, the presence of significant fibrosis ($F \geq 2$) requires the use of antiviral therapies, and the response to therapy is assessed by the alleviation of fibrosis. In liver cirrhosis, the risk of hepatocellular carcinoma (HCC) or bleeding from esophageal varices is high. Thus, in the patients with liver cirrhosis, the frequent screening for HCC by serum tumor markers and ultrasound sonography and endoscopic evaluation of varices is required. Liver biopsy, the gold standard of assessment of liver fibrosis, is an invasive and expensive procedure, the accuracy of which is sometimes questionable due to sampling errors, inadequate specimens, and subjective observer diagnosis. Recently transient elastography for the noninvasive measurement of liver stiffness was developed – employing the new apparatus, FibroScan.[1] This article reviews the applications and limitations of transient elastography.

Correspondence: Dr Kentaro Yoshioka, Division of Liver, Biliary Tract and Pancreas Diseases, Department of Internal Medicine, Fujita Health University, 1-98 Dengakugakubo, Kutsukake, Toyoake, Aichi 470-1192, Japan. Email: kyoshiok@fujita-hu.ac.jp
Received 3 March 2008; revision 10 April 2008; accepted 15 April 2008.

PRINCIPLE AND PROCEDURE OF TRANSIENT ELASTOGRAPHY

FIBROSCAN (ECHOSENS, FRANCE) is equipped with a probe including an ultrasonic transducer and a vibrator.[1] A vibration of mild amplitude and low frequency is transmitted from the vibrator placed on body surface toward the liver through the intercostal space. The vibration induces an elastic shear wave that propagates through the liver tissue. The pulse-echo ultrasound acquisitions follow the propagation of the shear wave and determine its velocity. The velocity is directly related to tissue stiffness; the harder the tissue, the faster the shear wave propagates. The liver stiffness is calculated from velocity and expressed in kilopascal (kPa).

The patients are asked to lie in the dorsal decubitus position with the right arm in maximal abduction. The tip of the probe is placed on the body surface between the ribs. The operator, assisted by ultrasound time-motion and A-mode images, places the probe upon the liver that is at least 6 cm thick and free of large vascular structures. The operator presses the button, the vibration starts toward the liver, and an acquisition of the propagation of the shear wave made by vibration follows. The

measurement depth is between 25 and 45 mm. Ten successful acquisitions were performed on each patient. The median value is adopted as representative of the liver stiffness.

LIVER STIFFNESS AND THE NUMERICAL SYSTEM ASSESSMENT OF FIBROSIS IN LIVER BIOPSY SPECIMENS

IN LIVER BIOPSY specimens, fibrosis has been measured with numerical systems of Scheuer,[2] the Metavir group,[3] Ishak,[3] Knodell[4] or the new Inuyama criteria using stages that range from 0–4 for Scheuer, Metavir, Knodell and new Inuyama criteria or 0–6 for Ishak. The numbers, while intended to be semiquantitative, actually represent categories of increasing severity based on a combination of location and extent of fibrosis, and whether the fibrous tissue forms septa, bridges, or nodules. Direct measurement of the amount of fibrosis in the biopsy specimen by computer-assisted morphometric image analysis has also been reported, where the mean morphometric collagen content was 0.0552 in stage 4, 0.0856 in stage 5, and 0.1163 in stage 6 of Ishak scores.[5]

Many studies have reported the correlation of liver stiffness with the numerical system of assessment of fibrosis in liver biopsy specimens (Table 1).[1,6–20]

In patients infected with hepatitis C virus (HCV), optimal stiffness cutoff values for Metavir score F ≥ 2, F ≥ 3 and F = 4 were 7.0–8.8, 9.5–9.75 and 12.1–14.5 kPa, respectively.[1,6,8,9,15] In HCV patients with normal alanine aminotransferase (ALT), an optimal stiffness cutoff value for Metavir score F ≥ 2 was 8.74 kPa,[10] which was similar to that in the patients with abnormal ALT. However, in the patients in biochemical remission (either spontaneous or after antiviral therapy), liver stiffness was reported to be lower than in patients with an identical fibrosis stage with elevated ALT, which indicates that liver stiffness correlates not only with fibrosis but also with necroinflammatory activity.[21]

In HCV patients coinfected with human immunodeficiency virus (HIV), optimal stiffness cutoff values for fibrosis scores were similar to those in the patients with HCV single infection.[11,17]

In patients with various liver diseases, optimal stiffness cutoff values for Metavir score F ≥ 2, F ≥ 3 and F = 4 were 7.2–7.9, 8.85–12.5 and 11.9–17.6 kPa, respectively.[12,13,18,19] There was wide variation of stiffness values in the same fibrosis stage, and some overlap between the adjacent stages in the reports, which may be explained by the mixed study population with various liver diseases.

In patients with primary biliary cirrhosis (PBC) or primary sclerosing chorangitis (PSC), optimal stiffness cutoff values for Metavir score F ≥ 2, F ≥ 3 and F = 4 were 7.3, 9.8 and 17.3 kPa, respectively.[14] In patients with nonalcoholic fatty liver disease (NAFLD), optimal stiffness cutoff values for Brunt score F ≥ 1, F ≥ 2, F ≥ 3 and F = 4 were 5.6, 6.65, 8 and 17 kPa, respectively.[20] In both studies, optimal stiffness cutoff values for F = 4 was rather high compared with those in patients infected with HCV.

Barreiro *et al.* suggested the interpretation of liver stiffness according to Metavir score based on the study of Castera *et al.* as follows: F0-F1 if < 7 kPa, F2 if 7.1–9.4 kPa, F3 if 9.5–12.5 kPa, and F4 if > 12.5 kPa.[22] Cutoff values for fibrosis stages are similar among different studies on different diseases. However there is wide variation of stiffness values in the same fibrosis stage, and some overlap between the adjacent stages. Thus the estimation of fibrosis score from liver stiffness should be cautiously done.

OTHER NONINVASIVE METHODS FOR ASSESSMENT OF LIVER FIBROSIS AND LIVER STIFFNESS

SEVERAL ROUTINE LABORATORY tests have been used to assess the liver fibrosis, such as prothrombin time, albumin level, and platelet count. Direct fibrosis markers are also used for assessing fibrosis, such as hyaluronic acid, collagen IV, collagen VI, laminin, aminoterminal peptide of procollagen III, tissue inhibitor of metalloproteinase 1 (TIMP-1), and matrix metalloproteinase 2. An array of laboratory tests have been developed and have been demonstrated to be useful, including PGA (prothrombin index, gamma-glutamyl transpeptidase (GGT), and apolipoprotein),[23] PGAA (prothrombin index, GGT, apolipoprotein, and alpha-2-macroglobulin),[24] Bonacini index (Platelet count, ALT/aspartate aminotransferase (AST) ratio, and prothrombin time-international normalized ratio),[25] APRI index (AST/platelet ratio),[26] Forns fibrosis index (age, platelet count, GGT, and cholesterol),[27] FibroTest (alpha-2-macroglobulin, haptoglobin, gamma globulin, apolipoptotein, and bilirubin),[28] HepaScore (bilirubin, GGT, hyaluronic acid, alpha-2-macroglobulin, age, and sex)[29] and FibroSpect (hyaluronic acid, TIMP-1, and alpha-2-macroglobulin).[30]

Table 1 Summary of investigation of transient elastography for assessment of liver fibrosis

Author (year)	Disease	Number of patients (*n*)	System of fibrosis staging	Stage of fibrosis							
				F = 0	F ≥ 1		F ≥ 2		F ≥ 3		F = 4	
					Cutoff value (kPa)	AUROC	Cutoff value (kPa)	AUROC	Cutoff value (kPa)	AUROC	Cutoff value (kPa)	AUROC
Nitta (2005)[6]	HCV	165	New Inuyama		5.55	0.77	7	0.88	9.75	0.9	12.1	0.9
Sandrin (2003)[1]	HCV	106	Metavir			0.9	7.6	0.88		0.91		0.99
Saito (2004)[7]	HCV	75	New Inuyama		6.25 for F1 (median)		7.8 for F2 (median)		13.7 for F3 (median)		34.0 for F4 (median)	
Castera (2005)[8]	HCV	183	Metavir				7.1	0.83	9.5	0.9	12.5	0.95
Ziol (2005)[9]	HCV	327	Metavir				8.8	0.79	9.6	0.91	14.6	0.97
Colletta (2005)[10]	HCV with normal ALT	40	Metavir				8.74					
Ledinghen (2006)[11]	HIV/HCV	72	Metavir					0.72		0.91	11.8–14.5	0.97
Ganne-Carrie (2006)[12]	Various diseases	1007	Metavir								14.6	0.95
Foucher (2006)[13]	Various diseases	711	Metavir				7.2	0.8	12.5	0.9	17.6	0.96
Corpechot (2006)[14]	PBC and PSC	101	Metavir				7.3	0.92	9.8	0.95	17.3	0.96
Shaheen (2007)[15]	HCV	546 (meta-analyses)	Metavir				8	0.83				0.95
Ogawa (2007)[16]	HCV	161	Metavir	6.3 (median)	6.7 for F1 (median)		9.1 for F2 (median)		13.7 for F3 (median)		26.4 for F4 (median)	
Ogawa (2007)[16]	HBV	68	Metavir	3.5 (median)	6.4 for F1 (median)		9.5 for F2 (median)		11.4 for F3 (median)		15.4 for F4 (median)	
Vergara (2007)[17]	HIV/HCV	169	Scheuer				7.2	0.87			14.6–17.6	0.95
Kim (2007)[18]	Various diseases	47	Metavir				7.35		8.85		15.1	
Kim (2007)[18]	Potential liver donors	80	Metavir	Fibrosis and steatosis were not correlated with liver stiffness.								
Franquelli (2007)[19]	Various diseases	200	Metavir				7.9	0.86	10.3	0.87	11.9	0.9
Yoneda (2007)[20]	NAFLD	67	Brunt		5.6	0.881	6.65	0.876	8	0.914	17	0.997

ALT, alanine aminotransferase; AUROC, area under the receive operating characteristic curve; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficienty virus; kPa, kilopascal; NAFLD, nonalcoholic fatty liver disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing chorangitis.

The comparison of liver stiffness with some of these serum fibrosis markers has been reported. In a study of HCV patients by Castera *et al.*, the area under the receiver operating characteristic curve (AUROC) of FibroScan, FibroTest, and APRI values were of the same order for the prediction of either F ≥ 2, F ≥ 3, or F = 4.[8] Castera *et al.* demonstrated that the combined use of FibroTest and FibroScan evaluates fibrosis more efficiently. Where FibroScan and FibroTest results agreed, liver biopsy confirmed the findings in 84% of cases for F ≥ 2, in 95% for F ≥ 3, and in 94% for F = 4. In the meta-analysis of HCV patients by Shaheen *et al.*, the sensitivity and specificity for the prediction of F2-F4 fibrosis of the FibroTest at threshold of 0.58–0.60 were 47% and 90%, while those of FibroScan at threshold of 7.1–8.8 kPa were 64% and 87%, respectively.[15] Thus the accuracy of the two methods was equally less dependent, while the identification of cirrhosis of the two was excellent. Colletta *et al.* demonstrated that, among HCV carrier with normal ALT, FiroScan is superior to the FibroTest in the identification of fibrosis.[10]

Some other methods such as FibroTest showed the same order of accuracy of for assessment of liver fibrosis as FibroScan. The combined use of these methods could improve the efficiency of fibrosis staging.

FAILURE OF MEASUREMENT OF LIVER STIFFNESS BY TRANSIENT ELASTOGRAPHY

KETTANEH *ET AL.* showed that success rate of shots decreased with age and obesity of the patients, and increased with an operator with more than 50 prior exams.[31] Fraquelli *et al.* analyzed the intraoperator and interoperator agreement using the intraclass correlation coefficient (ICC) and correlated with different patient-related and liver disease-related covariates.[19] The overall rate of indeterminate results was 2.4%, which was due to high body mass index (BMI; >28 kg/m^2) in four patients and narrow intercostal space in one patient. The interoperator agreement ICC was 0.98, which indicated that FibroScan is highly reproducible and operator-friendly apparatus. The reproducibility of FibroScan was significantly reduced in patients with steatosis, increased BMI and lower degrees of hepatic fibrosis.

FibroScan can be readily used with an operator with a short training. However, the reproducibility may be reduced by age, obesity, steatosis, narrow intercostal space and lower degrees of hepatic fibrosis in patients.

FACTORS AFFECTING LIVER STIFFNESS OTHER THAN FIBROSIS

RECENTLY SEVERAL REPORTS questioned the generally accepted supposition that liver stiffness is determined entirely by the stage of hepatic fibrosis. As mentioned in the former section, Franquelli *et al.* cautioned that the clinical use of FbroScan as a surrogate for liver biopsy because of the significant reduction of reproducibility of transient elastography in patients with steatosis, increased BMI and lower degrees of hepatic fibrosis.

The association between liver stiffness and necroinflammatory activity has been reported. Coco *et al.* reported that, in the patients with biochemical remission either spontaneous or after antiviral therapy, liver stiffness was lower than in patients with identical fibrosis stage, but elevated ALT.[21] In the study of Sagir *et al.*, 15 of 20 patients with acute liver damage of different etiologies showed high values of liver stiffness suggestive of liver cirrhosis, while none of them had any other signs of liver cirrhosis, and six of them who were followed up showed the decrease of liver stiffness values below the cutoff value of liver cirrhosis.[32] Arena *et al.* also reported that 18 patients with acute liver damage showed high liver stiffness values over the cutoff value of liver cirrhosis and then the progressive decrease of liver stiffness values in the follow-up period.[33] They also described the significant correlation between aminotransferases and liver stiffness at the onset of acute viral hepatitis. Thus liver stiffness measurement is not always a reliable means by which to detect liver cirrhosis when patients are suffering from acute hepatitis.

Ganne-Carrie *et al.* showed that FibroScan is reliable method for the diagnosis of cirrhosis in patients with chronic liver diseases, better at excluding than at predicting cirrhosis using threshold of 14.6 kPa and that most of false-negative diagnoses of cirrhosis by FibroScan are attributable to inactive or macronudular cirrhosis.[12]

So far, no well-controlled studies of transient elastography in patients with NAFLD have been done. Kim *et al.* reported that hepatic steatosis does not affect liver stiffness.[18] However it is likely that different cutoff values will be required for patients with nonalcoholic steatohepatitis.

Inflammatory activity and size of nodule of cirrhosis affect the liver stiffness values. Thus caution is needed in the clinical use of FibroScan as a surrogate for liver biopsy.

OTHER METHODS FOR MEASUREMENT OF LIVER STIFFNESS

REAL-TIME ELASTOGRAPHY is also a new noninvasive method for the assessment of liver fibrosis. Real-time elastography is done with conventional ultrasound probes; the Hitachi EUB-8500 and EUB-900 machines (Hitachi, Japan).[34] The examined tissue is divided in up to 30 000 finite elements before compression. During compression, the displacement of each element is measured. In hard tissue, the amount of displacement is low, whereas in soft tissue, the amount of displacement is high. The calculation of tissue elasticity distribution is performed in real time, and the examination results are presented as color-coded images with conventional B-mode image in the background. In the study of patients with HCV or hepatitis B virus and healthy volunteers, the cutoff elasticity scores and AUROC for Metavir score $F \geq 2$, $F \geq 3$ and $F = 4$ were 100.1, 102.5 and 111.75, and 0.75, 0.73, and 0.69, respectively. Since the AUROC of APRI for Metavir score $F \geq 2$, $F \geq 3$ and $F = 4$ were 0.87, 0.88 and 0.88 in the same study, the diagnostic efficiency of APRI was superior to that of real-time elastography. The new method of elastography using heart beats instead of manual compression for displacement also has been developed.

Novel techniques including magnetic resonance (MR) spectroscopy, diffusion weighted MR, and MR elastography have also developed for detecting liver fibrosis.[35]

CONCLUSIONS

A NUMBER OF noninvasive methods for assessment of liver fibrosis are now available, and many which are reasonably dependable. Transient elastgraphy is one of most accurate methods available. However, there are many limitations in transient elastography. The reproducibility of transient elastography is significantly reduced in patients with steatosis, increased BMI and lower degrees of hepatic fibrosis. Inflammatory activity affects liver stiffness values. To improve the accuracy of liver fibrosis staging, the combination of transient elastography with other noninvasive methods should be employed.

REFERENCES

1 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705–13.

2 Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991; **13**: 372–4.

3 Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. *Hepatology* 1994; **20**: 15–20.

4 Knodell RG, Ishak KG, Black WC *et al.* Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431–5.

5 Goodman ZD, Becker RL Jr, Pockros, PJ, Afdhal NH. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. *Hepatology* 2007; **45**: 886–94.

6 Nitta Y, Yoshioka K, Sakamoto K *et al.* Clinical usefulness of Fibroscan. *Acta Hepatologica Japonica* 2005; **46**: A438.

7 Saito H, Tada S, Nakamoto N *et al.* Efficacy of non-invasive elastometry on staging of hepatic fibrosis. *Hepatol Res* 2004; **29**: 97–103.

8 Castera L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343–50.

9 Ziol M, Handra-Luca A, Kettaneh A *et al.* Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48–54.

10 Colletta C, Smirne C, Fabris C *et al.* Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. *Hepatology* 2005; **42**: 838–45.

11 de Ledinghen V, Douvin C, Kettaneh A *et al.* Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**: 175–9.

12 Ganne-Carrie N, Ziol M, de Ledinghen V *et al.* Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006; **44**: 1511–17.

13 Foucher J, Chanteloup E, Vergniol J *et al.* Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403–8.

14 Corpechot C, El Naggar A, Poujol-Robert A *et al.* Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; **43**: 1118–24.

15 Shaheen AA, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. *Am J Gastroenterol* 2007; **102**: 2589–600.

16 Ogawa E, Furusyo N, Toyoda K *et al.* Transient elastography for patients with chronic hepatitis B and C virus infection: non-invasive, quantitative assessment of liver fibrosis. *Hepatol Res* 2007.

17 Vergara S, Macias J, Rivero A *et al.* The use of transient elastometry for assessing liver fibrosis in patients with HIV

and hepatitis C virus coinfection. *Clin Infect Dis* 2007; **45**: 969–74.
18 Kim KM, Choi WB, Park SH *et al.* Diagnosis of hepatic steatosis and fibrosis by transient elastography in asymptomatic healthy individuals: a prospective study of living related potential liver donors. *J Gastroenterol* 2007; **42**: 382–8.
19 Fraquelli M, Rigamonti C, Casazza G *et al.* Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968–73.
20 Yoneda M, Yoneda M, Fujita K *et al.* Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). *Gut* 2007; **56**: 1330–1.
21 Coco B, Oliveri F, Maina AM *et al.* Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; **14**: 360–9.
22 Barreiro P, Rodriguez-Novoa S, Labarga P *et al.* Influence of liver fibrosis stage on plasma levels of antiretroviral drugs in HIV-infected patients with chronic hepatitis C. *J Infect Dis* 2007; **195**: 973–9.
23 Poynard T, Aubert A, Bedossa P *et al.* A simple biological index for detection of alcoholic liver disease in drinkers. *Gastroenterology* 1991; **100**: 1397–402.
24 Naveau S, Poynard T, Benattar C, Bedossa P, Chaput JC. Alpha-2-macroglobulin and hepatic fibrosis. Diagnostic interest. *Dig Dis Sci* 1994; **39**: 2426–32.
25 Bonacini M, Hadi G, Govindarajan S, Lindsay KL. Utility of a discriminant score for diagnosing advanced fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1997; **92**: 1302–4.
26 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518–26.
27 Forns X, Ampurdanes S, Llovet JM *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986–92.
28 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069–75.
29 Adams LA, Bulsara M, Rossi E *et al.* Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; **51**: 1867–73.
30 Patel K, Gordon SC, Jacobson I *et al.* Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; **41**: 935–42.
31 Kettaneh A, Marcellin P, Douvin C *et al.* Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *J Hepatol* 2007; **46**: 628–34.
32 Sagir A, Erhardt A, Schmitt M, Haussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology* 2008; **47**: 592–5.
33 Arena U, Vizzutti F, Corti G *et al.* Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; **47**: 380–4.
34 Friedrich-Rust M, Ong MF, Herrmann E *et al.* Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. *AJR Am J Roentgenol* 2007; **188**: 758–64.
35 Talwalkar JA, Yin M, Fidler JL, Sanderson SO, Kamath PS, Ehman RL. Magnetic resonance imaging of hepatic fibrosis: emerging clinical applications. *Hepatology* 2008; **47**: 332–42.

J Gastroenterol 2008; 43:558–564
DOI 10.1007/s00535-008-2197-2

Journal of Gastroenterology

Association of hepatitis B virus subgenotypes and basal core promoter/precore region variants with the clinical features of patients with acute hepatitis

Kazuhiko Hayashi[1], Yoshiaki Katano[1], Yasushi Takeda[1], Takashi Honda[1], Masatoshi Ishigami[1], Akihiro Itoh[1], Yoshiki Hirooka[1], Isao Nakano[1], Kentaro Yoshioka[2], Hidenori Toyoda[3], Takashi Kumada[3], and Hidemi Goto[1]

[1]Department of Gastroenterology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan
[2]Division of Liver and Biliary Diseases, Department of Internal Medicine, Fujita Health University, Toyoake, Japan
[3]Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan

Background. In endemic areas, including Japan, basal core promoter (BCP) and precore (PC) variants of hepatitis B virus (HBV) have been reported to be associated with the clinical outcome of acute hepatitis B patients. However, the associations of BCP/PC variants with clinical outcomes have not been observed in non-endemic areas. HBV subgenotypes, which show geographic variations in prevalence, may underlie this discrepancy in clinical outcomes. Little is known about the differences in the clinical and virological features of HBV subgenotypes and BCP/PC variants. The aim of this study was to investigate the distributions of subgenotypes and BCP/PC variants to identify clinical differences in acute hepatitis B patients. ***Methods.*** One hundred thirty-nine patients with acute hepatitis were enrolled. Nested polymerase chain reaction was used to amplify the pre-S region of HBV for genotyping and the BCP/PC regions for variant screening. ***Results.*** HBV subgenotypes A1 ($n = 3$), A2 ($n = 28$), B1 ($n = 3$), B2 ($n = 9$), C1 ($n = 5$), C2 ($n = 84$), C variant ($n = 1$), D2 ($n = 3$), and H ($n = 3$) were detected. BCP/PC variants were not associated with progression to chronic hepatitis. Patients infected with subgenotype C2 who progressed to fulminant hepatic failure frequently carried variants at nucleotides non-T1753 and non-T1754 and T1762, A1764, and A1896. ***Conclusions.*** BCP/PC variants would be associated with progression to fulminant hepatitis in subgenotype C2. Knowledge of HBV subgenotypes and BCP/PC variants is useful for developing strategies to treat acute hepatitis B patients.

Key words: hepatitis B virus, fulminant hepatic failure, subgenotypes, basal core promoter/precore region variants

Received: October 22, 2007 / Accepted: March 21, 2008
Reprint requests to: Y. Katano

Introduction

Approximately 350 million people worldwide are infected with hepatitis B virus (HBV).[1] HBV infection has a variety of clinical courses, including self-limited acute hepatitis, fulminant hepatic failure, chronic hepatitis, and progression to cirrhosis and hepatocellular carcinoma.[2] Therefore, HBV infection is a significant global health problem. HBV has been classified into eight major genotypes on the basis of divergence of 8% of the full-length nucleotide sequence, and the prevalence of each genotype differs by region.[3,4] Each genotype shows different responses to antiviral treatments and different virological characteristics;[5–7] therefore, HBV genotype information may be useful for developing strategies to treat HBV-related liver disease. Moreover, HBV genotypes have been subdivided into subgenotypes that differ in their geographic distribution.[4] Therefore, HBV subgenotypes can be used to study geographic distributions in greater detail than can simple genotypes. Recently, the prevalence and geographic distribution of HBV subgenotypes in Japanese HBV carriers, including patients with acute hepatitis, were reported.[8,9] However, the effects of HBV subgenotypes on the clinical course of acute hepatitis have not been well documented. Several studies have reported that variants of the basal core promoter (BCP) and precore (PC) regions may be associated with progression to fulminant hepatic failure.[10–12] However, the roles of BCP and PC variants in acute hepatitis are controversial.[13,14] The prevalences of BCP and PC region variants depend on genotype, and some researchers have proposed that genotype differences influence clinical outcome.[15–17] Therefore, one reason that may explain the discrepant results for the roles of BCP and PC variants in acute hepatitis may be HBV genotype and subgenotype differences. Therefore, the relationships of BCP and PC region variants with clinical features need to be considered with respect to the HBV subgeno-

テノホビルのHBV感染症に対する効果
（海外での最近の知見）

Efficacy of Tenofovir Disoproxil Fumarate for Hepatitis B Virus

四柳　宏

東京大学医学部　生体防御感染症学

2008年40巻13号（通巻532号）p.37～45

細　胞

ニューサイエンス社

テノホビルのHBV感染症に対する効果
(海外での最近の知見)

Efficacy of Tenofovir Disoproxil Fumarate for Hepatitis B Virus

東京大学医学部　生体防御感染症学
四柳　宏

Key words
HBV感染症,
テノホビル,
TDF

要　約

フマル酸テノホビル ジソプロキシル（TDF）のB型肝炎ウイルス（HBV）単独感染患者に対する2つの第III相試験の72週の結果が海外にて報告された。HBe抗原陰性およびHBe抗原陽性のHBV単独感染患者において，TDFは，アデホビル（ADV）に比較して優れた抗HBV作用を示した。また，HBe抗原陰性およびHBe抗原陽性のHBV単独感染患者にADVを投与し，48週目以降，ADVからTDFに切り替えることによって，さらに強いHBV DNA抑制作用が認められた。TDFはADVと同様に忍容性や安全性が良好であった。

海外においては，これまで，HBVに対する核酸アナログは4剤が使用可能であったが，これらの結果を受けて，今年，TDFがヨーロッパおよび米国において承認に至っている。

はじめに

現在，選択可能なB型慢性肝炎の治療戦略としては，①HBV DNAの複製を直接，阻害する「核酸アナログ」および②ウイルス複製の抑制に加え，免疫増強作用を有する「インターフェロンα（標準およびペグ化）」が主なものであるが，「long-term treatment」としては経口薬で副作用も少ない核酸アナログが有望である。これまで，海外においては，ラミブジン（LAM），アデホビル（ADV），エンテカビル（ETV）およびテルビブジン（L-dT）の4つの核酸アナログが承認されていたが，今年，新たに，フマル酸テノホビル ジソプロキシル（TDF）が承認された（2008年4月：ヨーロッパ，2008年8月：米国）。これらの薬剤の短期的なベネフィットは認められているが，長期的な有効性を検証するデータはまだ十分ではない。TDFについては，第III相試験が進行中であり，5年間（240週間）の観察が計画されているが，2008年4月に72週までの結果が報告された。今回，これらの内容を概説する。

TDFは開環（acyclic）した糖鎖をもつアデニン誘導体のヌクレオチド系逆転写酵素阻害剤であり，本邦では2004年3月にHIV-1感染症を適応として承認され，鳥居薬品株式会社より「ビリアード®錠」として販売されている。

1．HBe抗原陰性のHBV単独感染患者におけるTDFの抗HBV作用（Study GS-US-174-0102；102試験）[1, 2]

本試験は，未治療のHBV単独感染患者（HBe抗原陰性：pre-core変異を有する患者）375例を対象に，TDF 300mgの1日1回投与（以下，TDF群。N=250）とADV 10mgの1日1回投与（以下，ADV群。N=125）を比較した多施設二重盲検の無作為化コントロール試験である。主要評価項目としては，肝線維化の悪

Hiroshi Yotsuyanagi
：Division of Microbiology Graduate School of Medicine and Faculty of Medicine University of Tokyo
〒113-8655　東京都文京区本郷7-3-1