Table 3 Independent risk factors influencing the development of hepatocellular carcinoma

Factors	Category	Hazard ratio (95% CI†)	*P*-value
AST (IU/l)	1: < 70	1	0.016
	2: ≥ 70	6.21 (1.40–27.5)	
YMDD mutants	1: YVDD or YV/IDD	1	0.012
	2: YIDD	3.97 (1.36–11.6)	
Age (years)	1: < 50	1	0.023
	2: ≥ 50	3.24 (1.17–8.95)	
Cirrhosis	1: Absent	1	0.030
	2: Present	1.42 (1.04–1.96)	

†Confidence interval.

adefovir was 59 (0–896) days for the patients who developed HCC and 54 (0–3240) days for those who did not ($P = 0.330$). Hence, exacerbation of hepatitis was not a risk factor for the development of HCC.

Age-specific risk factors for the development of HCC were evaluated by the multivariate analysis. In the patients < 50 years, platelet counts < $13 \times 10^3/mm^3$ was the only significant risk factor for HCC (hazard ratio 6.88 [95% confidence interval; 1.26–37.6]), while AST levels ≥ 70 IU/L was that in those ≥ 50 years (hazard ratio: 9.50 [95% confidence interval 1.20–74.9]).

Factors increasing the cumulative incidence of hepatocellular carcinoma

AST levels ≥ 70 IU/L at the start of adefovir increased the development of HCC during follow-ups ranging to 5 years (Fig. 1). HCC developed more frequently in the patients with YIDD mutants than in those with YVDD or the mixture of YVDD and YIDD mutants (Fig. 2). The cumulative incidence of HCC in the patients with YIDD mutants alone was: 4% at 1 year, 10% at 3 years and 43% at 5 years. In contrast, HCC never developed in the patients with the mixture of YIDD and YVDD mutants through 5 years of follow-up. HCC developed more frequently in the patients with cirrhosis and those aged ≥ 50 years (Figs 3,4, respectively).

DISCUSSION

HCC DEVELOPED IN 18 of the 247 (7.3%) patients who had received adefovir add-on lamivudine during a long-term ranging to 5 years. There were some differences in the characteristics at the start of adefovir dipivoxil between the patients who did and who did not develop HCC. The patients who developed HCC were older, more frequently had signs of early cirrhosis with less platelet counts, as well as higher levels of AST, ALT and AFP, than those who did not develop HCC. By multivariate analysis, AST ≥ 70 IU/L, YIDD mutants in

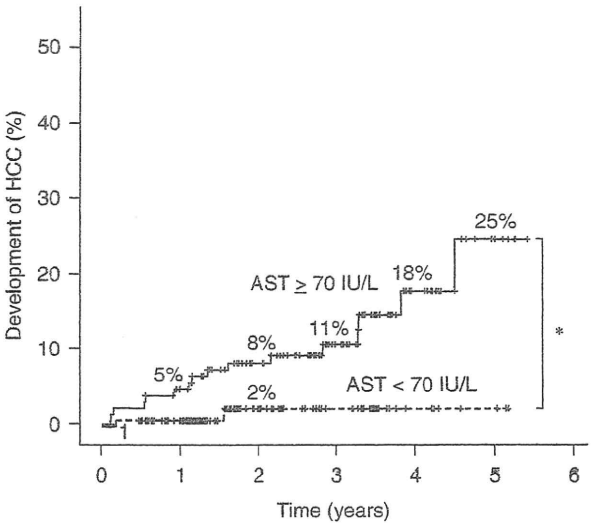

Figure 1 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with different baseline aspartate aminotransferase (AST) levels. *$P = 0.009$.

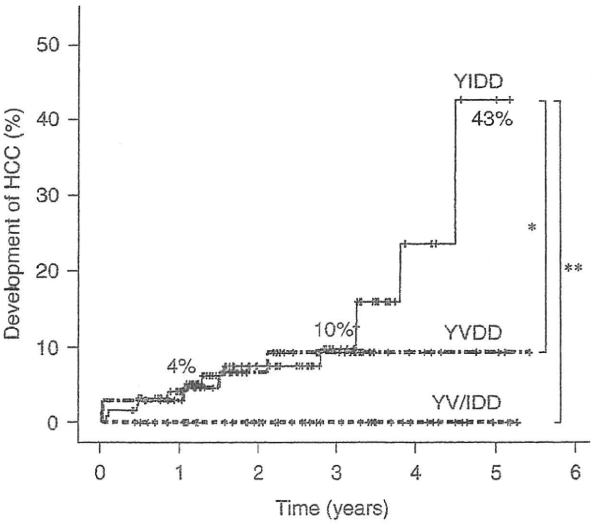

Figure 2 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adeforvir add-on lamivudine in the patients with distinct YMDD mutants. *$P = 0.035$; **$P = 0.003$.

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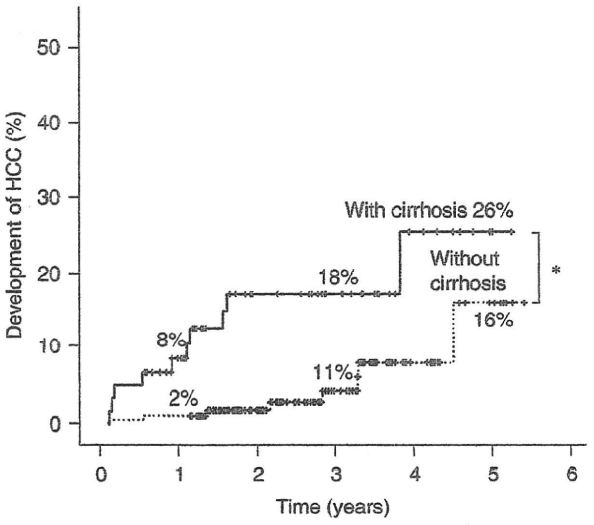

Figure 3 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with and without cirrhosis at the baseline. $^*P = 0.002$.

comparison with YVDD or the mixture of YVDD and YIDD mutants, age ≥ 50 years and cirrhosis were independent risk factors for the development of HCC. By the Kaplan-Meier life-table analysis, the cumulative incidence of HCC during 5 years in the patients receiving

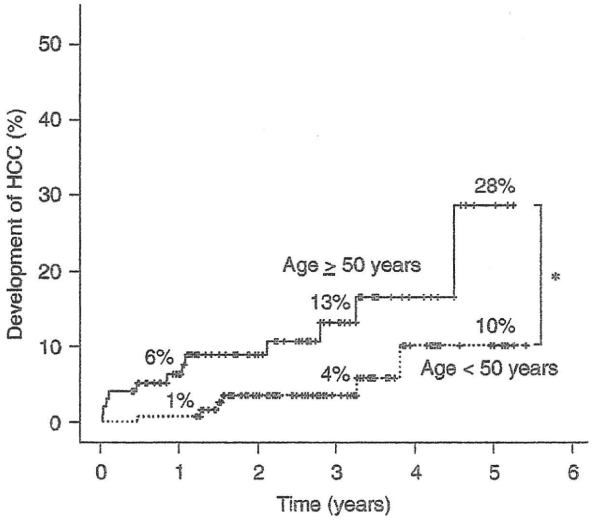

Figure 4 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients aged ≥ 50 years and < 50 yeas at the baseline. $^*P = 0.014$.

adefovir add-on lamivudine was significantly higher in those with AST ≥ 70 IU/L, YIDD mutants, cirrhosis and aged ≥ 50 years at the start of adefovir.

A marked difference in the development of HCC between the present study (7.3% [18/247]) and two studies reported from Europe and the US (0/70 and 0/65, respectively)[16,17] would be accounted for, at least in part, by the age of patients who developed HCC in this study that was older than in those in previous reports (the median of 52 years vs. means of 36 and 47 years, respectively). This view would be supported by the age of patients with long-term adefovir add-on lamivudine that was higher in those with than without the development of HCC (52 vs. 45 years [median], $P = 0.008$). HBV infection in Asia is acquired by the perinatal infection, while that in Western countries is gained after the adolescence ~20 years after birth. Hence, the duration of HBV infection would have been > 20 years longer in Japanese than Western patients. In addition, genotypes of HBV may give an additional account on the difference in development of HCC between them. All the 18 patients who developed HCC in this study were infected with genotype C; it is associated with HCC more closely than the other genotypes.[20–23] By contrast, by far the most patients from Western countries would have been infected with genotypes A and D.[24,25]

HCC developed more frequently in patients with than without cirrhosis at the start of adefovir (10/61 [16.4%] vs. 8/186 [4.3%], $P = 0.002$). Hence, cirrhosis increased the risk of HCC in patients receiving adefovir add-on lamivudine. This view is supported by the development of HCC in 11 of the 94 (11.7%) patients with cirrhosis who received adefovir add-on lamivudine from Italy.[10] Although HCC did not develop in any of the 39 Italian patients with chronic hepatitis, it did in eight of the 186 (4.3%) Japanese patients in the present study. There were, however, marked differences in the median baseline ALT levels between Italian and Japanese patients (58 vs. 108 IU/L); the grade of liver inflammation would have been higher in the Japanese patients. In actuality, all the eight patients with chronic hepatitis who developed HCC had high AST and ALT levels at the start of adefovir (Table 2).

In the natural history of persistent HBV infection, HCC develops more frequently in the patients with persistently high ALT levels than in those with normal levels. Hence, necroinflammation in the liver would contribute to carcinogenesis.[26,27] Although adefovir add-on lamivudine may prevent virological breakthroughs, it would not be able to suppress the pre-

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neoplastic state induced by exacerbation of hepatitis. It would be necessary therefore to identify the patients with chronic hepatitis at an increased risk for HCC during adefovir add-on lamivudine, such as those with cirrhosis or aged ≥ 50 years, and take special care of them toward early detection of HCC and immediate therapeutic intervention. They need to be monitored frequently for any increase in HBV DNA and aminotransferase levels that herald breakthrough hepatitis during lamivudine therapy.

In the present study, HCC developed more frequently in the patients with YIDD mutants than in those with YVDD or the mixture of YVDD and YIDD; there have been no studies correlating YMDD mutants and the development of HCC. No patients with the mixture of YVDD and YIDD mutants developed HCC, despite the predominance of YIDD mutants in the patients with HCC. This might have been due to the assay used for YMDD mutants by the commercial kit; it can miss YVDD mutants in samples in which YIDD mutants account for the great majority. By the assay method specific for either mutant, YIDD was detected either alone or accompanied by small amount of YVDD in the patients who have received adefovir add-on lamivudine treatment.[28] Sensitive and specific quantification of YIDD and YVDD mutants are necessary for further evaluating a role for YIDD mutants in hepatocarcinogenesis, as well as for identifying factors promoting the generation of both YIDD mutants and HCC.

Some points of clinical importance have emerged in the present study. First, patients who receive a long-term adefovir add-on lamivudine and have developed YMDD mutants need to be screened for HCC on the regular basis. This is required especially for the patients who have signs of cirrhosis and/or high AST levels, or aged ≥ 50 years. In these high-risk patients, adefovir has to be started promptly when HBV DNA levels increase, even before transaminase levels elevate in them. Secondly, it would be a matter of concern if adefovir is involved in the development of HCC. Should it be the case, tenofovir or newer potent antivirals, either as a monotherapy or add-on lamivudine, would deserve considerations. Thirdly, it needs to be evaluated if YIDD mutants have any significance in the development of HCC. Although nucleot(s)ide analogues may suppress hepatic inflammation and are expected to improve the prognosis of patients with chronic hepatitis B, they need to be monitored closely for HCC. The development of HCC has to be identified, as early as possible, for timely treatment toward longevity with minimal morbidity and improvement of the quality of life.

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REFERENCES

1 Lee WM. Hepatitis b virus infection. *N Engl J Med* 1997; 337: 1733–45.
2 Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118–29.
3 Dienstag JL. Hepatitis B virus infection. *N Engl J Med* 2008; 359: 1486–500.
4 Jarvis B, Faulds D. Lamivudine. A review of its therapeutic potential in chronic hepatitis B. *Drugs* 1999; **58**: 101–41.
5 Dando T, Plosker G. Adefovir dipivoxil: a review of its use in chronic hepatitis B. *Drugs* 2003; **63**: 2215–34.
6 Akuta N, Suzuki F, Kobayashi M *et al.* Virological and biochemical relapse according to YMDD motif mutant type during long-term lamivudine monotherapy. *J Med Virol* 2003; **71**: 504–10.
7 Suzuki F, Suzuki Y, Tsubota A *et al.* Mutations of polymerase, precore and core promoter gene in hepatitis B virus during 5-year lamivudine therapy. *J Hepatol* 2002; **37**: 824–30.
8 Keeffe EB, Dieterich DT, Han SH *et al.* A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. *Clin Gastroenterol Hepatol* 2006; **4**: 936–62.
9 Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507–39.
10 Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E, Colombo M. Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. *Gastroenterology* 2007; **133**: 1445–51.
11 Yatsuji H, Suzuki F, Sezaki H *et al.* Low risk of adefovir resistance in lamivudine-resistant chronic hepatitis B patients treated with adefovir plus lamivudine combination therapy: two-year follow-up. *J Hepatol* 2008; **48**: 923–31.
12 Kumada H. Continued lamivudine therapy in patients with chronic hepatitis B. *Intervirology* 2003; **46**: 377–87.
13 Hosaka T, Suzuki F, Suzuki Y *et al.* Factors associated with the virological response of lamivudine-resistant hepatitis B virus during combination therapy with adefovir dipivoxil plus lamivudine. *J Gastroenterol* 2007; **42**: 368–74.
14 Hosaka T, Suzuki F, Suzuki Y *et al.* Adefovir dipivoxil for treatment of breakthrough hepatitis caused by lamivudine-resistant mutants of hepatitis B virus. *Intervirology* 2004; **47**: 362–9.
15 Delaney WE IV. Progress in the treatment of chronic hepatitis B: long-term experience with adefovir dipivoxil. *J Antimicrob Chemother* 2007; **59**: 827–32.

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16 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. *Gastroenterology* 2006; **131**: 1743–51.
17 Marcellin P, Chang TT, Lim SG *et al.* Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2008; **48**: 750–8.
18 Usuda S, Okamoto H, Iwanari H *et al.* Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. *J Virol Methods* 1999; **80**: 97–112.
19 Usuda S, Okamoto H, Tanaka T *et al.* Differentiation of hepatitis B virus genotypes D and E by ELISA using monoclonal antibodies to epitopes on the preS2-region product. *J Virol Methods* 2000; **87**: 81–9.
20 Livingston SE, Simonetti JP, Bulkow LR *et al.* Clearance of hepatitis B e antigen in patients with chronic hepatitis B and genotypes A, B, C, D, and F. *Gastroenterology* 2007; **133**: 1452–7.
21 Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* 2000; **118**: 554–9.
22 Orito E, Ichida T, Sakugawa H *et al.* Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* 2001; **34**: 590–4.
23 Tsubota A, Arase Y, Ren F, Tanaka H, Ikeda K, Kumada H. Genotype may correlate with liver carcinogenesis and tumor characteristics in cirrhotic patients infected with hepatitis B virus subtype adw. *J Med Virol* 2001; **65**: 257–65.
24 Chu CJ, Keeffe EB, Han SH *et al.* Hepatitis B virus genotypes in the United States: results of a nationwide study. *Gastroenterology* 2003; **125**: 444–51.
25 Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes. *Intervirology* 2003; **46**: 329–38.
26 Chen CJ, Yang HI, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**: 65–73.
27 Wu CF, Yu MW, Lin CL *et al.* Long-term tracking of hepatitis B viral load and the relationship with risk for hepatocellular carcinoma in men. *Carcinogenesis* 2008; **29**: 106–12.
28 Suzuki F, Kumada H, Nakamura H. Changes in viral loads of lamivudine-resistant mutants and evolution of HBV sequences during adefovir dipivoxil therapy. *J Med Virol* 2006; **78**: 1025–34.

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Review Article

Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan

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In the 2008 guidelines for the treatment of patients with cirrhosis, who are infected with hepatitis B virus (HBV), the main goal is to normalize levels of alanine and aspartate aminotransferases by eliminating HBV or reducing viral loads. In patients with compensated cirrhosis, the clearance of HBV from serum is aimed for by entecavir, as the main resort, for histological improvement toward the prevention of hepatocellular carcinoma (HCC). In patients with decompensated cirrhosis, by contrast, meticulous therapeutic strategies are adopted for the reversal to compensation, toward the eventual goal of decreasing the risk of HCC. For maintaining liver function and preventing HCC, branched chain amino acids and nutrient supplements are applied, in addition to conventional liver supportive therapies. For patients with chronic hepatitis B, separate guidelines are applied to those younger than 35 years and those aged 35 years or older. Even for patients with chronic hepatitis who are negative for hepatitis e antigen (HBeAg), but who harbor HBV DNA in titers of 7 log copies/mL or more, a "drug-free state" is aimed for by sequential treatment with interferon (IFN) plus entecavir as the first line. For patients with chronic hepatitis B aged 35 years or older, who are HBeAg-negative and carry HBV DNA in titers of less than 7 log copies/mL, long-term IFN for 24–48 weeks is adopted anew. To HBeAg-negative patients who have either or both platelet counts of less than $150 \times 10^3/mm^3$ and less than 7 log copies of HBV DNA, also, long-term IFN for 24–48 weeks is indicated.

Key words: chronic hepatitis, cirrhosis, hepatitis B virus, hepatocellular carcinoma, interferon, liver supportive therapies, nucleos(t)ide analogs

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INTRODUCTION

SINCE THE FISCAL year 2002, guidelines for the treatment of patients with viral hepatitis have been compiled annually by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, under the auspice of the Ministry of Health, Labor and Welfare of Japan, supported by enduring efforts of many specialists recruited from all over the nation. Guidelines have been improved every year with many supplementary issues, which had surfaced as our understanding of many facets of viral hepatitis deepened and treatment options widened increasingly with time. For the fiscal year 2008, guidelines have been worked out for a comprehensive standardization of the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in Japan. These guidelines have been observed by more than 70% of practicing hepatologists treating patients with viral liver disease in Japan. It is hoped that these guidelines will continue being widely accepted and implemented to help as many patients as possible who are suffering from sequelae of persistent hepatitis virus infections.

Here, we relate excerpts of the 2008 guidelines for the treatment of patients with liver disease due to HBV, covering a wide range from those with chronic hepatitis to those with decompensated cirrhosis. The 2008 guidelines for the treatment of liver disease due to HCV are reported in an accompanying paper.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS B

PATIENTS WITH CHRONIC hepatitis B can stabilize the activity of liver disease in their natural course, after they have seroconverted from hepatitis B e antigen (HBeAg) to the corresponding antibody (anti-HBe), accompanied by decrease in HBV DNA titers. For that reason, treatment guidelines were constructed separately for the patients younger than 35 years and those aged 35 years or older.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS B YOUNGER THAN 35 YEARS

PATIENTS WITH CHRONIC hepatitis B younger than 35 years are treated in accordance with the guidelines summarized in Table 1. Criteria for the treatment eligibility are: (i) serum levels of alanine aminotransferase (ALT) of 31 IU/L or more; and (ii) HBV DNA titers of 5 log copies of more in HBeAg-positive patients and 4 log copies or more in HBeAg-negative patients. In the 2008 guidelines, the indication of treatment is extended to the patients with cirrhosis due to HBV who carry HBV DNA in titers of 3 log copies/mL or more.

In Japan, most HBeAg-positive patients with 7 log copies or more of HBV DNA have been infected with HBV of genotype C by perinatal infection at birth;

Table 1 Guidelines for the treatment of patients with chronic hepatitis B younger than 35 years

Eligibility criteria	ALT	≥31 IU/L
	HBV DNA	HBeAg-positive patients: ≥5 log copies/mL HBeAg-negative patients: ≥4 log copies/mL Patients with cirrhosis: ≥3 log copies/mL
HBV DNA	≥7 log copies/mL	<7 log copies/mL
HBeAg-positive	(1) Long-term IFN for 24–48 weeks (2) Entecavir	(1) Long-term IFN for 24–48 weeks (2) Entecavir
HBeAg-negative	(1) Sequential treatment† (entecavir plus IFN) (2) Entecavir	(1) Regular follow up (2) Long-term IFN for 24 weeks
	Start with entecavir in HBeAg-negative patients who have platelet counts $<15 \times 10^3/mm^3$ and in those with advanced liver disease of stage F2 or higher.	

†Sequential treatment: patients who have lost hepatitis B virus (HBV) DNA after treatment with nucleos(t)ide analogs receive combined interferon (IFN) for 4 weeks, and then IFN monotherapy is continued for 20 weeks, and lifted thereafter. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen.

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Table 2 Guidelines for the treatment of patients with chronic hepatitis B aged 35 years or older

Eligibility criteria	ALT HBV DNA	≥31 IU/L HBeAg-positive patients: ≥5 log copies/mL HBeAg-negative patients: ≥4 log copies/mL Patients with cirrhosis: ≥3 log copies/mL
HBV DNA	≥7 log copies/mL	<7 log copies/mL
HBeAg-positive	(1) Entecavir (2) Sequential treatment† (entecavir plus IFN)	(1) Entecavir (2) Long-term IFN for 24–48 weeks
HBeAg-negative	Entecavir	(1) Entecavir (2) Long-term IFN for 24–48 weeks

†Sequential treatment: patients who have lost hepatitis B virus (HBV) DNA after treatment with nucleot(s)ide analog receive combined interferon (IFN) for 4 weeks, and then IFN monotherapy is continued for 20 weeks, and lifted thereafter. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen.

accordingly, they would be resistant to interferon (IFN) therapy. Should they receive nucleos(t)ide analogs, however, the duration would become inevitably longer, because they start the treatment when younger than 35 years old. Hence, IFN for 24–48 weeks is the first choice in their treatment. The standard treatment of 3 months is favored, which can be extended to the maximum of 6 months. Non-pegylated (standard) IFN-α is recommended to them, because self-injection at home is approved for preparations of IFN-α; it helps improve their quality of life (QOL). There are many patients who are refractory to IFN and in whom improvement of ALT levels and/or decrease in HBV DNA titers are hardly achievable. Therefore, as another option, monotherapy with entecavir can be applied for the purpose of clearing HBeAg from serum and lowering HBV DNA titers. For HBeAg-positive patients with lower HBV DNA titers (<7 log copies/mL), also, long-term IFN is endorsed as a rule.

There are HBeAg-negative patients in whom ALT levels increase to 31 IU/mL or more repeatedly. In the 2008 guidelines, sequential treatment with IFN and entecavir is introduced as a new arm of therapeutic options for such patients.[1]

For HBeAg-negative patients with less than 7 copies/mL of HBV DNA, in general, regular follow up without therapeutic intervention is deemed to suffice for the majority. For those of them in whom ALT levels flare to 31 IU/mL or more time after time, long-term IFN for 24 weeks is indicated. Because liver disease progresses in many HBeAg-negative patients, for those with platelet counts of less than $150 \times 10^3/mm^3$ or in fibrosis stage F2 or higher, treatment with entecavir is indicated.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS B AGED 35 YEARS OR OLDER

TABLE 2 SUMS up treatment modalities for patients with chronic hepatitis B who are aged 35 years or older. HBeAg-positive patients in this age range who carry HBV DNA in titers of 7 log copies/mL or more rarely, if ever, seroconvert to the loss of HBeAg by IFN-based therapies. Hence, entecavir is the first choice in their treatment.[2,3] Because HBV mutants resistant to entecavir can be elicited by it, sequential treatment with IFN plus entecavir is amended in the 2008 guidelines.[1] In view of low viral loads in patients who possess HBV DNA in titers of less than 7 log copies/mL, entecavir is selected as the first choice, followed by long-term IFN as the second choice of treatment in these patients. HBeAg-negative patients who have high viral loads (≥7 log copies/mL), on the other hand, can normalize ALT levels by monotherapy with entecavir. Therefore, entecavir becomes their first choice, and this is the case even in patients with HBV DNA titers less than 7 copies/mL.

GUIDELINES FOR THE TREATMENT WITH NUCLEOS(T)IDE ANALOGS OF PATIENTS WITH CHRONIC HEPATITIS B WHO ARE RECEIVING LAMIVUDINE

TABLE 3 DETAILS guidelines for the treatment with nucleos(t)ide analogs of patients with chronic hepatitis B who are receiving lamivudine. Because a number of drug-resistant HBV mutants emerge increasingly with time in patients on long-term treatment with lamivudine, the fundamental rule is to switch them to ente-

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Table 3 Guidelines for the treatment with nucleos(t)ide analogs in patients with chronic hepatitis who are receiving lamivudine

Lamivudine	Less than 3 years	3 years or longer
HBV DNA		
<1.8 log copies/mL persistently	May be switched to entecavir 0.5 mg daily	Continued on lamivudine 100 mg daily
≥1.8 log copies/mL	VBT (−) May be switched to entecavir 0.5 mg daily	
	VBT (+) Adefovir 10 mg daily add-on lamivudine	Adefovir 10 mg daily add-on lamivudine

HBV, hepatitis B virus; VBT, virological breakthrough.

cavir. For this reason, patients are stratified by the duration of lamivudine treatment, less than 3 years and 3 years or more, as well as HBV DNA titers persistently below 1.8 log copies/mL and 1.8 log copies/mL or more, and separate treatment strategies have been worked out for the patients in each category. Because by far the majority of patients with a duration of lamivudine treatment of less than 3 years and HBV DNA titers of less than 1.8 copies/mL possess drug-resistant mutants in low frequencies, they are recommended to switch to entecavir 0.5 mg daily as soon as possible. Likewise, patients who have received lamivudine for 3 years or longer, but in whom drug-resistant mutants have never developed, are recommended to switch to entecavir 0.5 mg daily. By contrast, for patients in whom drug-resistant mutants have emerged already and who have undergone virological breakthroughs,[4] adefovir 10 mg daily add-on lamivudine is started for the purpose of stabilizing liver function.[5] In regard of the patients who have received lamivudine for 3 years or longer, those without drug-resistant mutants can stay on lamivudine 100 mg daily.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS B (PART I)

FOR THE FISCAL year 2008, the following three items have been added to previous guidelines for the treatment of chronic hepatitis B (Table 4).

1 In the treatment of patients with chronic hepatitis B, IFN is the first resort for those younger than 35 years, toward the eventual goal of gaining a "drug-free state". For the patients aged 35 years or older, persistently negative HBV DNA is the aim of nucleos(t)ide analogs, with the first choice being entecavir in their primary treatment. On the other hand, for patients with HBV mutants resistant to lamivudine and/or entecavir, combined treatment with adefovir and lamivudine is the principal rule (Table 3).[6–8]
2 Therapeutic responses to antiviral treatment are much different in patients with chronic hepatitis B who are infected with HBV of distinct genotypes. It is recommended therefore to determine HBV genotypes before making a decision on the treatment choice. In particular, the patients infected with HBV of genotype A or B respond to IFN in high rates, even if they are aged 35 years or older. For these reasons, IFN becomes the first choice in their antiviral treatment.
3 The duration of IFN treatment is 24 weeks basically. In the patients in whom the efficacy of IFN has been achieved with decrease in HBV DNA titers and normalization of ALT, the treatment duration is better extended to 48 weeks.

Table 4 Supplements to guidelines for the treatment of patients with chronic hepatitis B (part I)

1 Treatment of patients with chronic hepatitis B aims at a "drug-free state" by IFN-based therapies in those younger than 35 years, and at persistently negative HBV DNA in those aged 35 years or older, with entecavir as the first choice in the primary therapy. Lamivudine plus adefovir forms the basis for the treatment of HBV mutants resistant to lamivudine or entecavir.
2 In view of antiviral response much different in patients infected with HBV of distinct genotypes, it is desired to make treatment choices based on genotypes. In particular, because genotypes A and B respond to IFN with high efficacy, even in patients aged 35 years or older, IFN is recommended as the first treatment choice in these patients.
3 The duration of IFN is for 24 weeks basically, but extension to 48 weeks is recommended in patients who respond to IFN with decrease in HBV DNA titers and normalization of ALT levels.

ALT, alanine aminotransferase; HBV, hepatitis B virus; IFN, interferon.

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Table 5 Supplements to guidelines for the treatment of patients with chronic hepatitis B (part II)

- Self-injection of IFN at home is recommended to patients, who are eligible to do it, for improving their quality of life.
- Treatment with nucleos(t)ide analogs should be continued in patients in whom cirrhosis or HCC has been cured.
- Antiviral treatment is considered in patients with ALT levels of ≥31 IU/L. To patients aged 35 years or older in whom viral replication persists, even to those with normal ALT levels, antiviral treatments are indicated. It is possible, however, to follow for outcomes in patients who are elderly or HBeAg-negative and in whom antiviral treatments are difficult, while they receive liver supportive therapy (e.g. SNMC, UDCA).
- In patients co-infected with HBV and HIV, entecavir cannot be used due to the possibility for emergence of HIV variants resistant to antiretroviral therapies.
- Immunosuppressive and anticancer drugs should be used with utmost caution, even in patients with low HBV DNA titers and normal ALT levels, because they can induce severe liver damage along with elevation in HBV DNA titers.

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS B (PART II)

FURTHER, THE FOLLOWING five supplements have been added to the 2008 guidelines (Table 5).

To patients who are eligible, self-injection of IFN at home is recommended, taking into consideration their QOL. Because IFN-based therapies are not recommended for patients in whom HBV has been transmitted by perinatal infection, sequential treatment with IFN plus entecavir serves as another option in their antiviral treatment.

Treatment with nucleos(t)ide analogs should be extended to patients in whom cirrhosis or hepatocellular carcinoma (HCC) has been cured after successful therapies.

Antiviral treatment has to be considered in patients with ALT levels of 31 IU/L or more. Patients aged 35 years or older with normal ALT levels but in whom HBV replication persists, need to be considered for antiviral treatments. Elderly and HBeAg-negative patients, as well as those to whom the administration of antiviral drugs is difficult, can be followed regularly while they receive liver supportive therapy (e.g. stronger neo-minophagen C,[9] ursodeoxycholic acid [UDCA][10]).

Patients co-infected with HBV and HIV type 1 cannot receive entecavir due to the possibility of emergence of HIV mutants resistant to antiretroviral drugs.

Even in patients with low HBV DNA titers and normal ALT levels, HBV DNA loads can increase massively to induce severe liver damages in them, while they receive immunosuppressive or anticancer drugs. Hence, utmost caution should be exercised if they are to undergo antiviral treatments.

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CIRRHOSIS DUE TO HBV

TABLE 6 SUMMARIZES guidelines for the treatment of patients with type B cirrhosis. Patients with compensated or decompensated cirrhosis, who are infected with HBV, receive entecavir for persistent clearance of HBV DNA detectable by the real-time polymerase chain reaction and normalization of aspartate aminotransferase as well as ALT levels. Combined lamivudine plus adefovir therapy are indicated for patients in whom HBV mutants resistant to lamivudine or entecavir have developed. Guidelines for maintaining liver function, for preventing the development of HCC, include liver supportive therapy with glycyrrhizin and UDCA, either alone or in combination. For treatment toward sup-

Table 6 Guidelines for treatment of type B cirrhosis

Principles
- Compensated: termination of HBV infection by antiviral treatment with entecavir as the mainstay.
- Decompensated: reversal to compensation and prevention of HCC.

Methods
(1) Eradication of HBV and normalization of ALT/AST (compensated and decompensated cirrhosis).
  a) Entecavir.
  b) Combined lamivudine and adefovir (for patients with HBV mutants resistant to lamivudine or entecavir).
(2) Maintenance of liver function (improvement of ALT/AST and albumin) for preventing HCC.
  a) Liver supportive therapy such as SNMC or UDCA.
  b) Branched chain amino acids (Livact).
(3) Supplementation with nutrients (for stabilizing liver function in decompensated cirrhosis).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

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pressing the development of HCC, branched chain amino acids (BCAA)[11] are implemented. Also, nutrient supplements are utilized for stabilizing liver function.

DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HBV and HCV ranging from chronic hepatitis to cirrhosis of various severities annually, since the fiscal year 2002. The principal aim of these guidelines is to decrease the incidence of HCC due to hepatitis virus infections in Japan. In accordance with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic viral liver disease every fiscal year. This article summarizes guidelines for the treatment of liver disease due to HBV. Guidelines for the treatment of liver disease due to HCV for the fiscal year 2008 are reported in the accompanying paper. They are formulated on evidence-based data that have been accumulated by members and cooperators of the study group. It will be necessary to improve these guidelines in the next fiscal year and henceforth, in accordance with many pieces of new evidence that are expected to evolve through enduring efforts and keen insights of members and cooperators of the study group.

In the treatment of chronic hepatitis B, novel therapeutic strategies have continued to evolve in previous guidelines. In guidelines of the fiscal year 2008, diverse new treatment arms are introduced for gaining the eventual goal of the "drug-fee state".

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has been drafted and displayed on the web site (www.jsh.or.jp/medical/index.html [in Japanese]) as well, guidelines for the treatment of a spectrum of liver diseases due to HBV, ranging from chronic hepatitis to cirrhosis of various severities for the fiscal year 2008. In view of the eventual goal of decreasing the incidence of HCC due to HBV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been introduced to the treatment of cirrhosis due to HBV infection. As a general rule, antiviral treatments are the mainstay in guidelines for the treatment of chronic hepatitis B. In addition to them, it is necessary to always keep in mind the fundamental concepts of these guidelines. It is our sincere hope that, for the treatment of each patient, readers will conduct their clinical practice on the basis of these concepts, and then refer to appropriate individual guidelines, when they make decisions regarding treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in making treatment choices.

We can foretell that there is no end to the treatment of patients with chronic hepatitis and cirrhosis due to HBV, as it will keep evolving and improving in future guidelines. The enduring efforts of doctors and scientists, in pursuit of this goal, will fill in wide social and economic gaps in medical practices being served to the nation, and produce substantial and efficient interest in the medical economy on a national basis. In conducting treatment of patients with liver disease due to HBV infection, according to these guidelines, many new and unforeseen facets may surface that will require further improvements. Hence, it will be necessary to evaluate the therapeutic efficacy of these guidelines, and revise or add necessary supplements to them as required in the future.

REFERENCES

1 Serfaty L, Thabut D, Zoulim F *et al.* Sequential treatment with lamivudine and interferon monotherapies in patients with chronic hepatitis B not responding to interferon alone: results of a pilot study. *Hepatology* 2001; **34**: 573–7.
2 Shindo M, Chayama K, Mochida S. Antiviral activity,dose-responce relationship, and safety of entecavir following 24 week oral dosing in nucleoside-naive Japanese adult patients with chronic hepatitis B: a randomized, double-blind, phase II clinical trial. *Hepatol Int* 2009.
3 Kobayashi H, Fujioka S, Kawaguchi MK. Two cases of development of entecavir resistance during entecavir treatment for nucleoside-naive chronic hepatitis B. *Hepatol Int* 2009; **3**: 403–10.
4 Suzuki F, Toyoda J, Katano Y *et al.* Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. *J Gastroenterol Hepatol* 2008; **23**: 1320–6.
5 Shakado S, Watanabe H, Tanaka T. Combination therapy of lamivudine and adefovir in Japanese patients with chronic hepatitis B. *Hepatol Int* 2009; **2**: 361–9.
6 Akuta N, Suzuki F, Kawamura Y *et al.* Virological response and hepatocarcinogenesis in lamivudine-resistant hepatitis B virus genotype C patients treated with lamivudine plus adefovir dipivoxil. *Intervirology* 2008; **51**: 385–93.
7 Hosaka T, Suzuki F, Suzuki Y *et al.* Factors associated with the virological response of lamivudine-resistant hepatitis B virus during combination therapy with adefovir dipivoxil plus lamivudine. *J Gastroenterol* 2007; **42**: 368–74.

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8 Yatsuji H, Suzuki F, Sezaki H *et al.* Low risk of adefovir resistance in lamivudine-resistant chronic hepatitis B patients treated with adefovir plus lamivudine combination therapy: two-year follow-up. *J Hepatol* 2008; **48**: 923–31.
9 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; **79**: 1494–500.
10 Omata M, Yoshida H, Toyota J *et al.* A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007; **56**: 1747–53.
11 Muto Y, Sato S, Watanabe A *et al.* Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* 2006; **35**: 204–14.

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Review Article

Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan

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In the 2008 guidelines for the treatment of patients with chronic hepatitis C, pegylated interferon (Peg-IFN) combined with ribavirin for 48 weeks are indicated for treatment-naive patients infected with hepatitis C virus (HCV) of genotype 1. Treatment is continued for an additional 24 weeks (72 weeks total) in the patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA during 13–36 weeks on treatment. Re-treatment is aimed to either eradicate HCV or normalize transaminase levels for preventing the development of hepatocellular carcinoma (HCC). For patients with compensated cirrhosis, the clearance of HCV RNA is aimed toward improving histological damages and decreasing the development of HCC. The recommended therapeutic regimen is the initial daily dose of 6 million international units (MIU) IFN continued for 2–8 weeks that is extended to longer than 48 weeks, if possible. IFN dose is reduced to 3 MIU daily in patients who fail to clear HCV RNA by 12 weeks for preventing the development of HCC. Splenectomy or embolization of the splenic artery is recommended to patients with platelet counts of less than $50 \times 103/mm^3$ prior to the commencement of IFN treatment. When the prevention of HCC is at issue, not only IFN, but also liver supportive therapy such as stronger neo-minophagen C, ursodeoxycholic acid, phlebotomy, branched chain amino acids (BCAA), either alone or in combination, are given. In patients with decompensated cirrhosis, by contrast, reversal to compensation is attempted.

Key words: chronic hepatitis, cirrhosis, hepatocellular carcinoma, hepatitis C virus, interferon, liver supportive therapy, pegylated interferon, ribavirin

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INTRODUCTION

SINCE THE FISCAL year 2002, guidelines for the treatment of patients with viral hepatitis have been compiled annually by the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, under the auspice of the Ministry of Health Labor and Welfare of Japan, recruiting many specialists from all over the nation. They have been improved every year with many supplementary issues that have evolved, as our understanding of various aspects of viral hepatitis deepens and treatment options widen with time. For the fiscal year 2008, guidelines have been worked out for a comprehensive standardization of the treatment of chronic hepatitis and cirrhosis due to infection with hepatitis C virus (HCV) in Japan. It is hoped that these guidelines will be accepted widely and implemented for helping as many patients as possible who suffer from sequelae of persistent HCV infection.

Here, we relate excerpts of the 2008 guidelines for the treatment of patients with HCV-induced liver disease covering a wide range from those with normal aminotransferase levels to those with decompensated cirrhosis.

GUIDELINES FOR THE PRIMARY TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

TABLE 1 SUMMARIZES the antiviral therapy of treatment-naive patients with chronic hepatitis C. In comparison with previous guidelines, the duration of combined treatment with pegylated interferon (Peg-IFN) and ribavirin is extended to 48–72 weeks for patients infected with HCV of genotype 1 in high viral loads (HVL: ≥5 log IU/mL by the Japanese criteria).[1,2] For patients infected with HCV of genotype 2 in HVL, Peg-IFN-α2b and ribavirin for 24 weeks are indicated. To patients with HCV-1 in low viral loads (LVL: <5 log IU/mL), either the standard IFN (not conjugated with polyethylene glycol) for 24 weeks, or the weekly monotherapy with Peg-IFN-α2a for 24–48 weeks, is given.[3] Patients with HCV-2 in LVL receive either the standard IFN for 8–24 weeks, or the weekly monotherapy with Peg-IFN-α2a for 24–48 weeks.

GUIDELINES FOR THE RE-TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C

FOR PATIENTS WHO receive re-treatment, first, it is imperatively prerequisite to: (i) identify factors for non-response to previous treatments; and (ii) decide whether to aim for clearance of HCV or to prevent the progression of hepatitis that can accelerate the development of hepatocellular carcinoma (HCC), and this can be monitored by alanine aminotransferase (ALT) and α-fetoprotein (AFP) levels toward normalizing or stabilizing their levels (Table 2).[4] Second, IFN combined with ribavirin is the mainstay of re-treatment of patients with chronic hepatitis C. Third, long-term IFN monotherapy is recommended to patients who are not indicated to IFN/ribavirin or who have failed to respond to the combination therapy. However, some patients do not tolerate IFN due to side-effects or their complicating morbidities. In addition, IFN monotherapy does not always improve ALT levels. Such patients need to receive liver supportive therapy including stronger neominophagen C (SNMC)[5] and ursodeoxycholic acid (UDCA),[6] as well as phlebotomy, either alone or in combination. Therapeutic target ALT levels are: (i) within ×1.5 the upper limit of normal (ULN) for patients in fibrosis stage 1 (F1); and (ii) less than 30 IU/L in those in fibrosis stages 2 or 3 (F2/F3), as far as possible.

Table 1 Guidelines for the primary treatment of patients with chronic hepatitis C

Genotypes	Genotype 1	Genotype 2
Viral loads		
High viral load ≥5.0 log IU/mL ≥300 fmol/L ≥1 Meq/mL	• Peg-IFN-α2b (Peg-Intron) + ribavirin (Rebetol) for 48–72 weeks • Peg-IFN-α2a (Pegasys) + ribavirin (Copegus) for 48–72 weeks	• Peg-IFN-α2b (Peg-Intron) + ribavirin (Rebetol) for 24 weeks
Low viral load <5.0 log IU/mL <300 fmol/L <1 Meq/mL	• Standard IFN for 24 weeks • Peg-IFN-α2a (Pegasys) for 24–48 weeks	• Standard IFN for 8–24 weeks • Peg-IFN-α2a (Pegasys) for 24–48 weeks

Peg-IFN, pegylated interferon.

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Table 2 Guidelines for re-treatment of chronic hepatitis C

Principles
Selection has to be made between termination of HCV infection and normalization/stabilization of ALT as well as AFP levels (toward preventing aggravation of liver disease and development of HCC), after evaluating factors for non-response in the primary IFN treatment.

1 "IFN plus ribavirin" is the mainstay of re-treatment of patients who have failed to respond to the primary IFN therapy.
2 Long-term IFN is recommended to patients in whom ribavirin is not indicated or who have failed to respond to IFN/ribavirin; self-injection at home is approved for IFN-α (not for Peg-IFN).
3 Patients who are not indicated to IFN or have failed to improve ALT and AFP levels, in response to IFN, receive liver supportive therapy (SNMC, UDCA) and phlebotomy, either alone or in combination.
4 For preventing aggravation of liver disease (and development of HCC), ALT levels need to be controlled within 1.5 × ULN in patients in stage 1 fibrosis (F1), and as far as possible, 30 IU/L or lower in those in fibrosis stages 2–3 (F2/F3).
5 In treatment combined with ribavirin, dose and mode need to be selected, taking into consideration factors contributing to the response, such as age, sex, progression of liver disease, mutations in the HCV genome (amino acid substitutions in the core protein [aa70/aa91] and ISDR) and HCV RNA titers determined by the real-time PCR.

AFP, α-fetoprotein; ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ISDR, interferon sensitivity determining region; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CHRONIC HEPATITIS C

FOR THE FISCAL year 2008, the following items were supplemented to the treatment of chronic hepatitis C (Table 3).

1 The treatment of patients infected with HCV-1 in HVL with Peg-IFN/ribavirin for 72 weeks is modified by the early virological response (EVR) within 12 weeks after the start. Patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.[1,2]
2 Patients with HCV-1 in HVL who fail to clear HCV RNA detectable by real-time PCR but in whom ALT levels normalize are continued on Peg-IFN/ribavirin until 48 weeks, so that normalized ALT levels endure longer after the completion of therapy.[7]
3 Patients who are not indicated to Peg-IFN/ribavirin, or who have failed to respond to previous treatments, receive long-term IFN monotherapy. During the first 2 weeks, IFN in the conventional dose is given daily or three times a week. Patients who do not clear HCV RNA during the maximal treatment period of 8 weeks receive half the conventional dose of IFN indefinitely.[8]

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CHRONIC HEPATITIS C IN NORMAL ALT LEVELS

AS IN PREVIOUS guidelines, patients with chronic hepatitis C having normal ALT levels are stratified into four groups by ALT levels and platelet counts (Table 4). Patients with chronic hepatitis C who have normal ALT levels are reported to gain the sustained virological response (SVR) to antiviral treatments comparably frequently as those having elevated ALT levels. Taking this into consideration, patients with ALT levels of 30 IU/L or less and platelet counts of $150 \times 10^3/mm^3$ or more are followed for ALT every

Table 3 Supplements to guidelines for chronic hepatitis C

1 Criteria for extending the duration of Peg-IFN/ribavirin (to 72 weeks) in patients infected with HCV-1b in HVL: patients who have remained positive for HCV RNA detectable by the real-time polymerase chain reaction at 12 weeks after the start of treatment, but who turn negative for HCV RNA till 13–36 weeks on treatment.[1,2]
2 Patients with HCV-1b in HVL who fail to lose HCV RNA detectable by real-time PCR, but in whom ALT levels normalize by 36 weeks, Peg-IFN/ribavirin is given till 48 weeks for maintaining normalized ALT levels long after the completion of treatment.
3 Long-term IFN monotherapy in patients who are not indicated to Peg-IFN/ribavirin, or have failed to respond to it: the usual dose of IFN daily or three times in week is given for the first 2 weeks, and when HCV RNA does not disappear within the maximal duration of 8 weeks, long-term treatment with half the usual dose of IFN is continued indefinitely.

ALT, alanine aminotransferase; HCV, hepatitis C virus; HVL, high viral loads; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon.

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Table 4 Guidelines for the treatment of patients with normal ALT levels toward preventing the development of HCC

Platelets	$\geq 150 \times 10^3/mm^3$	$<150 \times 10^3/mm^3$
ALT		
≤30 IU/L	• Follow for ALT every 2–4 months. • If ALT levels elevate, start antiviral treatments taking into consideration the possibility of SVR and risk for HCC.	• Liver biopsy, if possible, and consider antiviral treatments for patients in A2/F2. • Follow for ALT every 2–4 months, and consider antiviral treatments when ALT levels elevate, for patients without biopsy.
31–40 IU/L	• Consider antiviral treatments for patients younger than 65 years.	• Start treatments for chronic hepatitis C. • Select treatments according to genotypes, viral load, age of patients, etc.

ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

2–4 months. If ALT levels increase in them, antiviral treatments are considered based on the possibility of resolving HCV infection and the risk for developing HCC. In view of significant fibrosis present in patients with platelet counts of less than $150 \times 10^3/mm^3$, they are recommended to receive liver biopsy, if this is possible. Patients in fibrosis stage F2 or higher are evaluated for the indication to antiviral treatments. Patients with ALT levels between 31 and 40 IU/L are classified by platelet counts. Antiviral treatments are considered in those aged younger than 65 years who have platelet counts of $150 \times 10^3/mm^3$ or more, while guidelines for patients with chronic hepatitis are applied to those with platelet counts of less than $150 \times 10^3/mm$.[9,10]

GUIDELINES FOR THE TREATMENT OF PATIENTS WITH CIRRHOSIS DUE TO HCV

PATIENTS WITH COMPENSATED cirrhosis who are not infected with HCV-1 in HVL receive either IFN-β or IFN-α (Table 5). Since the fiscal year 2008, IFN-α has been approved for the treatment of patients infected with HCV-1 in HVL, with the aim of resolving infection and normalizing ALT as well as AFP levels by long-term therapy. Treatment duration was set at 1 year or longer, and because the longer the treatment duration the higher the SVR rate, 36 weeks has been recommended as the optimal treatment duration. Because the normalization of ALT/AST is important, even in patients who fail to clear HCV infection by these therapeutic regimens, treatment is better conducted for maintaining normal ALT/AST levels. Guidelines for maintaining liver function for preventing the development of HCC include liver supportive therapy with glycyrrhizin[5] and UDCA,[6] either alone or in combination. For treatment toward suppressing the development of HCC, branched chain amino acids (BCAA)[11] or phlebotomy are adopted. Also, nutrient supplements are applied for stabilizing liver function.

SUPPLEMENTS TO GUIDELINES FOR THE TREATMENT OF CIRRHOSIS DUE TO HCV

THE FOLLOWING ITEMS have been appended to supplement guidelines for the treatment of type C cirrhosis (Table 6).

Table 5 Guidelines for treatment of type C cirrhosis

Principles
  Compensated: termination of HCV infection
  Decompensated: reversal to compensation and prevention of HCC
Methods
  (1) Eradication of HCV and normalization of ALT/AST (for patients with compensated cirrhosis).
    a) HCV-1b in HVL (≥5 log IU/mL)
      IFN-α (Sumiferon)
    b) Others
      IFN-α (Sumiferon)
      IFN-β (Feron)
  (2) Maintenance of liver function (improvement of ALT/AST and albumin) for preventing HCC.
    a) Liver supportive therapy
      Stronger neo-minophagen C (SNMC), ursodeoxycholic acid (UDCA), etc.
    b) Branched chain amino acids (BCAA [Livact])
    c) Phlebotomy
  (3) Supplementation with nutrients (for stabilizing liver function in decompensated cirrhosis).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HVL, high viral loads; IFN, interferon.

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Table 6 Supplements to guidelines for type C cirrhosis

1 To start with, IFN for compensated cirrhosis is desired at 6 MIU daily for 2–8 weeks, as far as possible, and to continue for 48 weeks or longer, as for chronic hepatitis C.
2 In patients with compensated cirrhosis who fail to clear HCV RNA within 12 weeks on IFN, long-term therapy at 3 MIU should be considered for preventing HCC.
3 In patients with platelet counts $<50 \times 10^3/mm^3$, splenectomy or embolization of splenic artery is recommended before re-treatment, and after thorough evaluation has been made on the response to IFN to be expected.
4 For the prevention of HCC, not only IFN, but also liver supportive therapy (SNMC, UDCA, etc.), phlebotomy and branched chain amino acids, either alone or in combination, are recommended for improving ALT/AST and AFP levels.

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; MIU, million international units; SNMC, stronger neo-minophagen C; UDCA, ursodeoxycholic acid.

1 For treatment of type C cirrhosis with IFN, the initial dose of 6 million international units (MIU) daily is continued as long as possible (2–8 weeks). Thereafter, long-term IFN for 48 weeks or longer is desired as in the treatment of chronic hepatitis C.
2 In the treatment of type C cirrhosis, patients who fail to achieve EVR with the clearance of HCV RNA from serum within 12 weeks should receive long-term IFN at a dose of 3 MIU.
3 For patients with type C cirrhosis who have platelet counts of less than $50 \times 10^3/mm^3$, splenectomy or embolization of the splenic artery is desirable before commencing IFN therapy, after the efficacy of IFN has been evaluated thoroughly.[12]
4 For preventing the development of HCC, improvement in ALT, AST and AFP levels are aimed. Toward this end, not only IFN, but also liver supportive therapy (SNMC and UDCA), phlebotomy and BCAA are used, either alone or in combination.

DISCUSSION AND CONCLUSION

THE STUDY GROUP for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, organized by the Ministry of Health, Labor and Welfare of Japan, has compiled a series of guidelines for the treatment of liver disease due to HCV ranging from chronic hepatitis to cirrhosis of various severities for the fiscal year 2008. The principal aim of these guidelines is to decrease the incidence of HCC due to HCV infection in Japan. In accord with this principle, supplements have been added to previous guidelines for the standardization of treatment of chronic hepatitis C. They are prepared on evidence-based data that have been accumulated by members and cooperators of the study group. It is necessary to improve these guidelines in the next fiscal year and thereafter, in accordance with many pieces of new evidence that are expected to emerge through enduring efforts of members and cooperators of the study group.

In the treatment of chronic hepatitis C, the duration of antiviral treatments is extended to 72 weeks, which has been approved as of the fiscal year 2008, and criteria for the eligibility of extended treatment duration are clearly defined. Long-term antiviral treatments, extended up to 72 weeks, are hoped to increase the SVR even further. In addition, comprehensive guidelines for the treatment of cirrhosis have been improved with substantial additions, and their criteria for the indication made explicit.

The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis has drafted, and also displayed online (www.jsh.or.jp/medical/index.html [in Japanese]), guidelines for a spectrum of liver diseases due to HCV, from chronic hepatitis to cirrhosis of various severities. In view of the eventual goal of decreasing the incidence of HCC due to HCV infection, supplementation and adjustment are appended to previous guidelines, and new guidelines have been constructed for the treatment of cirrhosis due to HCV infection. As a general rule, antiviral treatments constitute the main body of guidelines for the treatment of chronic hepatitis C. Furthermore, the fundamental concept of these guidelines would need to be kept in mind always. It is our sincere hope that, for the treatment of each patient, readers will base their clinical practice on these guidelines, and refer to appropriate individual guidelines, when they make a decision on the treatment strategy, on a case-by-case basis. With respect to guidelines for the treatment of patients with cirrhosis, above all, expected achievable outcomes have to be taken into account in treatment choice.

It is our sincere desire that treatment of patients with chronic hepatitis and cirrhosis due to HCV will proceed following these guidelines. Efforts along these lines will rectify a wide gap in medical treatment served to the nation and raise substantial and efficient interest in the medical economy on the national basis. In practicing treatment according to these guidelines, it will be nec-

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essary to evaluate their therapeutic efficacy, and revise or add necessary supplements to them as required in the future.

REFERENCES

1 Sanchez-Tapias JM, Diago M, Escartin P *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; **131**: 451–60.

2 Akuta N, Suzuki F, Hirakawa M *et al.* A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. *J Med Virol* 2009; **81**: 452–8.

3 Iwasaki Y, Shiratori Y, Hige S. A randomized trial of 24 versus 48 weeks of interferon alpha-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan. *Hepatol Int* 2009; **3**: 468–79.

4 Nomura H, Kashiwagi Y, Hirano R *et al.* Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: a pilot study. *Hepatol Res* 2007; **37**: 490–7.

5 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; **79**: 1494–500.

6 Omata M, Yoshida H, Toyota J *et al.* A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007; **56**: 1747–53.

7 Sezaki H, Suzuki F, Kawamura Y *et al.* Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatol Res* 2007; **37**: 787–92.

8 Akuta N, Suzuki F, Kawamura Y *et al.* Efficacy of low-dose intermittent interferon-alpha monotherapy in patients infected with hepatitis C virus genotype 1b who were predicted or failed to respond to pegylated interferon plus ribavirin combination therapy. *J Med Virol* 2008; **80**: 1363–9.

9 Okanoue T, Makiyama A, Nakayama M *et al.* A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. *J Hepatol* 2005; **43**: 599–605.

10 Okanoue T, Itoh Y, Minami M *et al.* Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts. *Hepatol Res* 2008; **38**: 27–36.

11 Muto Y, Sato S, Watanabe A *et al.* Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. *Hepatol Res* 2006; **35**: 204–14.

12 Morihara D, Kobayashi M, Ikeda K *et al.* Effectiveness of combination therapy of splenectomy and long-term interferon in patients with hepatitis C virus-related cirrhosis and thrombocytopenia. *Hepatol Res* 2009; **39**: 439–47.

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Amino Acid Substitutions in the Hepatitis C Virus Core Region of Genotype 1b Affect Very Early Viral Dynamics During Treatment With Telaprevir, Peginterferon, and Ribavirin

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Substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG-IFN/ribavirin is not clear. The aims of this study were to investigate the rate of HCV RNA loss following 12-week triple therapy, and determine the effect of aa substitutions on very early (within 48 hr) viral dynamics. Sixty-seven patients infected with HCV genotype 1b (HCV-1b) and high viral load who received 12-week triple therapy were studied. RNA loss could be achieved in 2%, 34%, 80%, 92%, 95%, 94%, and 90% of the patients after 1, 2, 4, 6, 8, 10, and 12 weeks of triple therapy, respectively. After 24-hr treatment, the proportion of patients with Arg70 and Leu91 substitutions with ≥3.0 log fall in HCV RNA was significantly higher than those with <3.0 log fall ($P=0.008$). However, the aa substitution patterns in the core region did not influence the fall in HCV RNA after 48-hr treatment. Multivariate analysis identified substitutions of aa 70 and 91 ($P=0.014$) and level of viremia at baseline (≥7.0 log IU/ml; $P=0.085$) as independent parameters that determined the ≥3.0 log fall in HCV RNA level after 24-hr triple therapy. It is concluded that 12-week triple therapy achieved high rates of loss of HCV RNA in Japanese patients infected with HCV-1b and high viral load, and that the aa substitution pattern in the core region seems to influence very early viral dynamics. ***J. Med. Virol. 82:575–582, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: HCV; core region; NS5A-ISDR; telaprevir; peginterferon; ribavirin; very early viral dynamics

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INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [Dusheiko, 1998; Ikeda et al., 1998; Niederau et al., 1998; Kenny-Walsh, 1999]. At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) with high viral loads (>100 KIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Iino et al., 2005; Tsubota et al., 2005]. Such background calls for efficient treatment of patients with chronic HCV infection.

Even with pegylated interferon (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b with high viral loads [Manns et al., 2001; Fried et al., 2002]. Recently, a new strategy was introduced for the treatment of chronic HCV infection by inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these drugs, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [Lin et al., 2006]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, results in a robust antiviral activity [Modi and Hoofnagle, 2007; Zeuzem, 2008]. Specifically, HCV RNA disappears in

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almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin [Lawitz et al., 2008; Suzuki et al., 2009]. However, patients resistant to treatment who do not achieve sustained virological response by the triple therapy, have been reported [Lawitz et al., 2008; Hézode et al., 2009; McHutchison et al., 2009]. The underlying mechanism of the response to the treatment is still not clear.

It is useful to evaluate treatment efficacy based on viral dynamics as an early predictor of PEG-IFN plus ribavirin combination therapy. Previous reports showed that decreases in HCV RNA levels were significantly greater in patients with than without sustained virological response from 24 hr to 12 weeks after the start of PEG-IFN plus ribavirin combination therapy in patients infected with HCV-1b and high viral load. Very early dynamics within 48 hr of such treatment is particularly important for early prediction of response to therapy [Tsubota et al., 2005; Makiyama et al., 2006; Akuta et al., 2007b]. Accordingly, the pretreatment predictors of very early dynamics during triple therapy of telaprevir with PEG-IFN and ribavirin were investigated in the present study.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with genotype 1b and high viral load are pretreatment predictors of poor virological response to 48- and 72-week PEG-IFN plus ribavirin combination therapy [Akuta et al., 2005, 2007a,b, 2009a; Donlin et al., 2007; Okanoue et al., 2009], and also affect the clinical outcome, including insulin resistance and hepatocarcinogenesis [Akuta et al., 2007c, 2009b; Fishman et al., 2009; Nakamoto et al., 2009]. However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict the very early dynamics and response to triple therapy of telaprevir with PEG-IFN and ribavirin.

The present study included 67 patients with HCV-1b and high viral load, who received triple therapy of telaprevir with PEG-IFN plus ribavirin and followed-up for 12 weeks or more after the start of treatment. The aims of the study were to determine the rate of loss of HCV RNA during treatment, and to identify the pretreatment factors that could predict very early viral dynamics (within 48 hr) after the start of treatment, including aa substitutions in the HCV core, the NS3, and the NS5A regions.

PATIENTS AND METHODS

Study Patients

Between May 2008 and May 2009, 67 patients infected with HCV were recruited to the study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the Institutional Review Board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (30%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN, and ribavirin], and 47 patients (70%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks. All patients were followed-up for at least 12 weeks after the start of triple therapy.

All patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C; (2) HCV-1b confirmed by sequence analysis; (3) HCV RNA levels of $\geq$5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (4) Japanese (Mongoloid) ethnicity; (5) age at study entry of 20–65 years; (6) body weight $\geq$35 and $\leq$120 kg at the time of registration; (7) lack of decompensated cirrhosis; (8) absence of hepatitis B surface antigen (HBsAg) in serum; (9) no history of HCC; (10) no previous treatment for malignancy; (11) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C; (12) no history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of $\leq$50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of $\geq$110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication, or anaphylaxis at baseline; and (13) hemoglobin level of $\geq$12 g/dl, neutrophil count $\geq$1,500/mm^3, and platelet count of $\geq$100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study.

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at a dose of 750 or 500 mg three times a day at an 8-hr (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, NJ) was injected subcutaneously with a median dose 1.5 μg/kg (range: 1.3–2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1,000 mg). All participating patients received these three drugs in the initial 12 weeks of the study.

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1,500/mm^3, neutrophil count below 750/mm^3, or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1,000/mm^3, 500/mm^3, or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800–600, and 1,000–600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir

(MP-424) remained the same throughout the 12-week protocol, though the drug was discontinued altogether following the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at nine time points: 24 hr, 48 hr, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, and 12 weeks after the commencement of treatment. HCV RNA levels during treatment was evaluated in 66 (99%), 66 (99%), 65 (97%), 67 (100%), 64 (96%), 60 (90%), 58 (87%), 50 (75%), and 58 (87%) of the 67 patients, at the above time intervals, respectively. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative. Reduction in HCV RNA levels at 24 and 48 hr relative to the baseline were investigated as very early dynamics.

Detection of Amino Acid Substitutions in Core, NS3, and NS5A Regions

In the present study, aa substitutions of the core, NS3, and NS5A-ISDR regions were analyzed by direct sequencing. AA sequences in the upstream site (1027–1318 aa) of the NS3 region, including aa positions reported as resistance for telaprevir [Lin et al., 2005; Forestier et al., 2007; Zhou et al., 2007], were determined. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids were amplified by PCR using the following primers. (a) Nucleotide sequences of the core region: the first-round PCR was performed with CE1 (sense: 5′-GTC TGC GGA ACC GGT GAG TA-3′; nucleotides: 134–153) and CE2 (antisense: 5′-GAC GTG GCG TCG TAT TGT CG-3′; nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense: 5′-ACT GCT AGC CGA GTA GTG TT-3′; nucleotides: 234–253) and CE6 (antisense: 5′-GGA GCA GTC GTT CGT GAC AT-3′; nucleotides: 934–953) primers. (b) Nucleotide sequences of NS3 region: the first-round PCR was performed with NS33F (sense: 5′-ACT TCT AGG ACC GGC CGA TA-3′; nucleotides: 3359–3378) and NS34R (antisense: 5′-GCT CGT CAC ACT TCT TCT TG-3′; nucleotides: 4517–4536) primers, and the second-round PCR with NS33F (sense) and NS36R (antisense: 5′-GTC TGT GAA GAC CGG AGA CC-3′; nucleotides: 3946–3965) primers. (c) Nucleotide sequences of NS5A-ISDR: the first-round PCR was performed with ISDR1 (sense: 5′-ATG CCC ATG CCA GGT TCC AG-3′; nucleotides: 6662–6681) and ISDR2 (antisense: 5′-AGC TCC GCC AAG GCA GAA GA-3′; nucleotides: 7350–7369) primers, and the second-round PCR with ISDR3 (sense: 5′-ACC GGA TGT GGC AGT GCT CA-3′; nucleotides: 6824–6843) and ISDR4 (antisense: 5′-GTA ATC CGG GCG TGC CCA TA-3′; nucleotides: 7189–7208) primers ([a,c]; nested PCR. [b]; hemi-nested PCR). All samples were denatured initially at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of genotype 1b was determined and then compared with the consensus sequence constructed on 67 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2209–2248 aa in the NS5A of genotype 1b (IFN-sensitivity determining region, ISDR) reported by Enomoto et al. [1995, 1996] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (≤ 1) or mutant-type (≥ 2).

Statistical Analysis

Nonparametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to very early viral dynamics. The odds ratios and 95% confidence intervals (95% CI) were also calculated. All P-value <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. The potential pretreatment factors associated with very early dynamics included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alfa-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, and aa substitution in the core, NS3, and the NS5A-ISDR regions. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL).