TABLE I. Characteristics of Study Patients

Age (years), median (range)	60 (20–80)
Sex (male/female) (%)	150 (51.2)/143 (48.8)
BMI, median (range)	22.6 (15.8–33.3)
Prior treatment for HCV (no/yes) (%)	201 (68.6)/92 (31.4)
Initial dose of PEG-IFN (μg), median (range)	80.0 (40.0–150.0)
Initial dose of ribavirin (mg), median (range)	600 (200–1,000)
Pretreatment HCV RNA levels (log_{10} IU/ml), median (range)	6.1 (5.0–7.4)
Platelet count ($\times 10^3/\mu l$)	159 (43–373)
Hemoglobin (g/dl)	13.9 (8.6–18.1)
Neutrophil count (μl^{-1})	2,430 (4,670–7,480)
Alanine aminotransferase (IU/L)	49 (10–485)
Genetic polymorphisms of rs8099917 (TT/TG or GG) (%)	204 (69.6)/89 (30.4)
Amino acid at residue 70 of HCV core (arginine/glutamine or histidine) (%)	200 (68.3)/93 (31.7)
Amino acid sequence of ISDR (non-wild-type/wild-type) (%)	78 (26.6)/215 (73.4)

(N = 293).
BMI, body mass index; HCV, hepatitis C virus; PEG-IFN, peginterferon; ISDR, interferon sensitivity-determining region.

a high AUROC in all patients, in which sensitivity, specificity, PPV, NPV, and accuracy were more than 80%. The best cut-off for the prediction was 3.1-log_{10} reduction. When patients were stratified according to baseline predictive factors, AUROC remained above 0.85, indicating retention of high predictive ability. However, the best cut-off levels differ depending on baseline factors, and they were lower in patients with unfavorable baseline predictors (TG/GG genotype of rs8099917 near the *IL28B* gene, glutamine/histidine at residue 70 of the HCV core region, and wild-type of ISDR). Especially, when patients had the TG/GG rs8099917 genotype, the calculated best cut-off level was markedly lower than that of patients with the TT genotype. Sensitivity, specificity, PPV, NPV, and accuracy were more than 70% in all patient subgroups, except for patients with the TG/GG genotype in whom PPV was only 10%.

Association Between Week 12 Viral Reduction and Treatment Outcome Based on Baseline Predictive Factors

Table III shows the predictive value of a reduction in serum HCV RNA levels at week 12 of therapy in all patients and based on each baseline predictive variable. The predictive ability of week 12 viral reduction for sustained virologic response was decreased in comparison to that of week 4 with a low AUROC in all patients. The specificity, PPV, and accuracy of the prediction at week 12 were also lower than those at week 4. The best cut-off levels increased to 5.0-log_{10} reduction. When patients were stratified according to the genetic polymorphisms of rs8099917 near the *IL28B* gene and according to amino acid substitutions at residue 70 of the HCV core region, the differences of the best cut-off levels based on these baseline factors were less marked than those at week 4, although the best cut-off levels remained lower in patients with unfavorable baseline predictors. The difference of best cut-off levels between patients with TT genotype and with TG/GG genotype of rs8099917 also decreased, but PPV in patients with TG/GG genotype remained low (21%). In contrast, the difference in the best cut-off levels increased when patients were stratified according to amino acid sequences in ISDR. The best cut-off level of the reduction in HCV RNA levels at week 12 for predicting sustained virologic response was higher in patients with HCV of wild-type ISDR, an unfavorable baseline variable, than in patients with HCV of favorable non-wild-type ISDR, which was inverse to the evaluation with week 4 viral reduction in which the cut-off level was higher in patients with HCV of non-wild-type ISDR.

TABLE II. AUROC, Best Cut-Off Level, Sensitivity, Specificity, PPV, NPV, and Accuracy of the Reduction in Serum HCV RNA Levels 4 Weeks After Starting PEG-IFN and Ribavirin Combination Therapy From Pretreatment Levels for Predicting Sustained Virologic Response

	N	AUROC	Best cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Overall	293	0.92746	3.1 log_{10}	88	87	81	92	87
IL28B-TT	204	0.88353	3.2 log_{10}	87	78	82	84	83
IL28B-TG or GG	89	0.84302	1.1 log_{10}	100	69	10	100	70
Core 70-R	200	0.91023	3.2 log_{10}	86	83	82	87	85
Core 70-Q or H	93	0.94350	2.8 log_{10}	88	93	75	97	92
ISDR-non-wild type	78	0.93455	3.0 log_{10}	90	90	94	84	90
ISDR-wild type	215	0.92654	2.9 log_{10}	92	84	71	96	87

AUROC, area under the receiver-operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value; HCV, hepatitis C virus; PEG-IFN, peginterferon; R, arginine; Q, glutamine; H, histidine; ISDR, interferon sensitivity-determining region.

TABLE III. AUROC, Best Cut-Off Level, Sensitivity, Specificity, PPV, NPV, and Accuracy of the Reduction in Serum HCV RNA Levels 12 Weeks After Starting PEG-IFN and Ribavirin Combination Therapy From Pretreatment Levels for Predicting Sustained Virologic Response

	N	AUROC	Best cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Overall	293	0.86907	5.0 $\log_{10}$	88	73	67	91	79
IL28B-TT	204	0.79216	5.1l $\log_{10}$	81	61	70	73	71
IL28B-TG or GG	89	0.92829	4.6 $\log_{10}$	100	87	21	100	88
Core 70-R	200	0.81791	5.0 $\log_{10}$	88	63	69	86	75
Core 70-Q or H	93	0.94272	4.9 $\log_{10}$	100	84	59	100	87
ISDR-non-wild type	78	0.87298	5.0 $\log_{10}$	88	79	88	79	85
ISDR-wild type	215	0.89572	5.4 $\log_{10}$	84	79	63	92	81

AUROC, area under the receiver-operating characteristics curve; PPV, positive predictive value; NPV, negative predictive value; HCV, hepatitis C virus; PEG-IFN, peginterferon; R, arginine; Q, glutamine; H, histidine; ISDR, interferon sensitivity-determining region.

DISCUSSION

This study was conducted to confirm the predictive value of week 4 viral dynamics of HCV for predicting sustained virologic response to the combination therapy with PEG-IFN and ribavirin in patients infected with HCV genotype 1 and with pretreatment HCV RNA levels of $\geq$5.0 $\log_{10}$ IU/ml in a large multicenter study of Japan. The comparison of the predictability for sustained virologic response between week 4 and week 12 viral reductions revealed the higher predictive ability of week 4 viral response. In a recent study, Marcellin et al., [2012] suggested that a $\geq$3 $\log_{10}$ reduction in HCV RNA levels at week 4 of PEG-IFN and ribavirin combination therapy is a reliable factor for predicting sustained virologic response in patients with HCV genotype 1. Our current results are consistent with their analysis for patients with HCV genotype 1b and those with pretreatment HCV RNA levels $\geq$5.0 $\log_{10}$ IU/ml overall. The reduction in HCV RNA levels at week 4 appears to be a good and reliable predictor for a sustained virologic response. Although week 12 viral response (i.e., early virologic response) has been used as a pivotal decision criterion to extend treatment duration or to discontinue treatment, the predictive value is lower when the reduction in HCV RNA levels is compared to week 4 viral response.

When patients were stratified based on baseline predictive factors, however, the best cut-off levels for sustained virologic response were not constant. The cut-off levels decreased in patients with unfavorable baseline factors, that is, TG/GG genotype of rs8099917, glutamine/histidine at residue 70 of the HCV core region, and wild-type sequence of ISDR, indicating that the reduction in HCV RNA occurs slowly in patients with these unfavorable baseline variables. Conversely and paradoxically, the results may indicate that one can expect sustained virologic response in patients with a smaller reduction in HCV RNA levels at week 4 if they have unfavorable baseline variables.

When predictive value was evaluated using week 12 viral reduction, the best cut-off levels remained lower in patients with unfavorable TG/GG rs8099917 genotype and patients with HCV of unfavorable glutamine/histidine at residue 70 of the HCV core region. In contrast, the best cut-off level was higher in patients with HCV of unfavorable wild-type ISDR. Previous studies reported the association between the genetic polymorphisms near the *IL28B* gene (rs12979860 and rs8099917) and amino acid substitution at residue 70 of HCV core region [Abe et al., 2010; Kobayashi et al., 2010], whereas no associations were reported between these two variables and ISDR mutation. This might explain the difference in the relationship of early viral response during therapy between with two baseline predictive factors, *IL28B* genetic polymorphisms and amino acid substitution of HCV core region and with ISDR mutation.

The calculated PPV was markedly low in patients with the unfavorable TG/GG genotype of rs8099917 (CT/TT genotype of rs12979860) both by the evaluations at weeks 4 and 12 viral responses. Therefore, it appears to be difficult to identify patients in this subgroup who are likely to achieve a sustained virologic response by their week 4 viral response, although week 4 viral response can be a factor used to identify patients with a high likelihood of achieving sustained virologic response in other subgroups.

In conclusion, week 4 viral response can be a predictor of sustained virologic response in patients with HCV genotype 1. However, the cut-off levels should be modified based on baseline host and viral predictive variables. In addition, week 4 viral response is not predictive in patients with unfavorable genotype of genetic polymorphism near the *IL28B* gene.

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Predictive Value of Early Viral Dynamics During Peginterferon and Ribavirin Combination Therapy Based on Genetic Polymorphisms Near the *IL28B* Gene in Patients Infected With HCV Genotype 1b

Hidenori Toyoda,[1]* Takashi Kumada,[1] Toshifumi Tada,[1] Kazuhiko Hayashi,[2] Takashi Honda,[2] Yoshiaki Katano,[2] Hidemi Goto,[2] Takahisa Kawaguchi,[3] Yoshiki Murakami,[3] and Fumihiko Matsuda[3]

[1]*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan*
[2]*Department of Gastroenterology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[3]*Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan*

A study was carried out to determine whether early viral dynamics retain prediction of the outcome of peginterferon (PEG-IFN) and ribavirin combination therapy based on different genetic polymorphisms near the *IL28B* gene, the strongest baseline predictor of response to this therapy. A total of 272 patients infected with hepatitis C virus (HCV) genotype 1b were grouped according to genetic polymorphisms near the *IL28B* gene (rs8099917). The ability of reduced HCV RNA levels at 4 and 12 weeks after starting therapy to predict a sustained virologic response was evaluated based on these genotypes. Among patients with the TT genotype for rs8099917 (associated with a favorable response), the rates of sustained virologic response were higher in patients with a ≥ 3 $\log_{10}$ reduction in serum HCV RNA levels at 4 weeks after starting therapy ($P < 0.0001$). In contrast, among patients with the TG/GG genotype (associated with an unfavorable response), there were no differences in this rate based on the reduction in HCV RNA levels at 4 weeks. Early viral dynamics at 4 weeks after starting therapy retains its predictive value for sustained virologic response in patients with the TT genotype for rs8099917, but not in patients with the TG/GG genotype. Patients who are likely to achieve sustained virologic response despite unfavorable TG/GG genotype cannot be identified based on early viral dynamics during therapy. In contrast, lack of early virologic response at 12 weeks retains a strong predictive value for the failure of sustained virologic response regardless of *IL28B* polymorphisms, which remains useful as a factor to stop therapy. ***J. Med. Virol. 84:61–70, 2012.*** © 2011 Wiley Periodicals, Inc.

KEY WORDS: chronic hepatitis C; early viral dynamics; genetic polymorphisms near the *IL28B* gene; peginterferon; response-guided therapy; ribavirin

INTRODUCTION

The current standard antiviral therapy for patients with chronic hepatitis C is combination therapy with peginterferon (PEG-IFN) and ribavirin [Ghany et al., 2009]. Although this treatment regimen has increased markedly the number of patients with a sustained virologic response, i.e., the eradication of hepatitis C virus (HCV), only 50% of patients infected with HCV genotype 1 achieved a sustained virologic response approximately.

Many investigators have examined factors that predict the treatment outcome of PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1. In addition to the baseline factors, the response of HCV during combination therapy, i.e., the changes in serum HCV RNA levels after starting therapy, has been shown to be an important predictor of the treatment outcome [Zeuzem et al., 2001; Buti

Conflict of interest: None.

*Correspondence to: Hidenori Toyoda, MD, PhD, Department of Gastroenterology, Ogaki Municipal Hospital 4-86, Minaminokawa, Ogaki, Gifu, 503-8502, Japan.
E-mail: hmtoyoda@spice.ocn.ne.jp

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et al., 2002; Berg et al., 2003], with the emphasis on "response-guided therapy" [Lee and Ferenci, 2008; Marcellin and Rizzetto, 2008]. Recent reports have emphasized the importance of evaluating the viral dynamics at 4 weeks after starting therapy to predict a sustained virologic response. A rapid virologic response, in which serum HCV RNA is undetectable at 4 weeks after starting therapy, has been the strongest predictive factor of a sustained virologic response reportedly [Martinez-Bauer et al., 2006; Poordad et al., 2008; de Segadas-Soares et al., 2009; Martinot-Peignoux et al., 2009]. In addition, the predictive value of reduced serum HCV RNA levels at 4 weeks after starting therapy has been clarified further, and a ≥ 3 $\log_{10}$ reduction in HCV RNA levels at 4 weeks after starting therapy has high predictive value that a patient will achieve a sustained virologic response as a final outcome, even in the absence of a rapid virologic response [Toyoda et al., 2011].

In contrast, the lack of an early virologic response, defined as either undetectable serum HCV RNA or HCV RNA levels decreased by >2.0 $\log_{10}$ from the pretreatment level at 12 weeks after starting therapy, has been the most important predictor for the failure of a sustained virologic response in patients infected with HCV genotype 1 reportedly [Fried et al., 2002; Davis et al., 2003]. Therefore, treatment may be discontinued in patients without an early virologic response at 12 weeks of treatment, according to the recommendation in the AASLD guidelines [Ghany et al., 2009].

More recently, several studies reported that genetic polymorphisms near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 affect the virologic response to PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; McCarthy et al., 2010; Rauch et al., 2010]. Furthermore, genetic polymorphisms near the *IL28B* gene are the strongest baseline predictive factor of the final outcome of combination therapy. An additional report showed the effects of genetic polymorphisms near the *IL28B* gene on HCV viral dynamics during PEG-IFN and ribavirin combination therapy [Thompson et al., 2010].

Although early HCV viral dynamics during therapy was shown originally to have a high predictive value for a sustained virologic response in HCV genotype 1-infected patients before genetic polymorphisms near the *IL28B* gene were linked to a therapeutic response, it is not clear whether early viral dynamics retain their predictive value in light of this additional information. The purpose of the present study was to investigate whether response-guided therapy based on viral dynamics at 4 or 12 weeks after initiating therapy retains its ability to predict the final outcome of PEG-IFN and ribavirin combination therapy after accounting for genetic polymorphisms near the *IL28B* gene.

MATERIALS AND METHODS

Patients and Treatment

Between January 2007 and June 2008, a total of 402 patients with chronic hepatitis C received antiviral combination therapy with PEG-IFN and ribavirin for HCV infection at the Ogaki Municipal Hospital or the Nagoya University Hospital. Among these patients, 272 were infected with HCV genotype 1b and had pretreatment HCV RNA levels >5.0 $\log_{10}$ IU/ml based on a quantitative real-time PCR-based method for HCV (HCV COBAS AmpliPrep/COBAS TaqMan System; Roche Molecular Systems, Pleasanton, CA; Lower limit of quantification, 1.7 $\log_{10}$ IU/ml: Lower limit of detection, 1.0 $\log_{10}$ IU/ml) [Colucci et al., 2007; Pittaluga et al., 2008]. This study did not include any patients infected with HCV genotype 1a because this genotype is not found in the general Japanese population.

All patients were given PEG-IFN alpha-2b (Pegintron, Schering-Plough, Tokyo, Japan) weekly and ribavirin (Rebetol, Schering-Plough, Kenilworth, NJ) daily. The PEG-IFN and ribavirin doses were adjusted based on the patient's body weight. Patients weighing $\leq$45 kg were given 60 μg of PEG-IFN alpha-2b once a week, those weighing >45 and $\leq$60 kg were given 80 μg, those weighing >60 and $\leq$75 kg were given 100 μg, those weighing >75 and $\leq$90 kg were given 120 μg, and those weighing >90 kg were given 150 μg. Patients weighing $\leq$60 kg were administered 600 mg of ribavirin per day, those weighing >60 and $\leq$80 kg were given 800 mg per day, and those weighing >80 kg were administered 1000 mg per day. The PEG-IFN and ribavirin doses were modified based on the manufacturer's recommendations. All patients were scheduled to undergo 48 weeks of treatment. The treatment duration was extended up to 72 weeks in some patients. In addition, treatment was discontinued before 48 weeks in some patients who had a low likelihood of achieving an eradication of HCV due to the presence of serum HCV RNA at 24 weeks after starting therapy.

A sustained virologic response was defined as undetectable serum HCV RNA at 24 weeks after ending the therapy. A patient was considered to have relapsed when serum HCV RNA was detectable between the end of treatment and 24 weeks after completing treatment, although serum HCV RNA was undetectable during and at the end of therapy. Patients were considered to have non-response if serum HCV RNA was detectable at 24 weeks after initiating therapy (i.e., null response or partial response according to the American guidelines [Ghany et al., 2009]). Patients were considered to have a rapid virologic response if they had undetectable serum HCV RNA at 4 weeks after starting therapy. An early virologic response was defined as the disappearance or decrease in serum HCV RNA levels by at least 2 $\log_{10}$ at 12 weeks after starting therapy. Patients were considered to have a complete early virologic response if serum HCV RNA was undetectable at 12 weeks after starting therapy and a partial early virologic response if the serum

HCV RNA levels had decreased by at least 2 $\log_{10}$ at 12 weeks after initiating therapy. Patients were considered not to have an early virologic response if their HCV RNA levels did not decrease by more than 2 $\log_{10}$ at 12 weeks compared to the pretreatment levels. Patients were considered to have a slow virologic response if the serum HCV RNA became undetectable between 12 and 24 weeks.

The study protocol was in compliance with the Helsinki Declaration and was approved by the ethics committee of the Ogaki Municipal Hospital and the Nagoya University School of Medicine. Prior to initiating the study, each patient provided written informed consent to use the laboratory data, analyze genetic polymorphisms near the *IL28B* gene, and test stored serum samples.

Assessments of Serum HCV RNA Levels and Genetic Polymorphisms Near the *IL28B* Gene

After a patient provided informed consent, serum samples were obtained at the patient's regular hospital visits, just prior to initiating treatment, every 4 weeks during the treatment period, and during the 24-week follow-up period after treatment. Serum samples were stored at −80°C until further use. The HCV RNA levels were measured using a quantitative real-time PCR-based method for HCV (HCV COBAS AmpliPrep/COBAS TaqMan System).

Genotyping of rs 8099917 polymorphisms near the *IL28B* gene was performed using the TaqMan SNP assay (Applied Biosystems, Foster City, California) according to the manufacturer's guidelines. A predesigned and functionally tested probe was used for rs8099917 (C__11710096_10, Applied Biosystems).

Statistical analyses. Quantitative values are reported as the mean ± SD. In between-group differences were analyzed by the chi-square test. Univariate and multivariate analyses using a logistic regression model were performed to identify factors that predict a sustained virologic response, including age, sex, body weight, serum alanine aminotransferase activity, serum aspartate aminotransferase activity, serum gamma-glutamyl transpeptidase levels, serum alkaline phosphatase values, serum albumin levels, total serum bilirubin values, white blood cell counts, hemoglobin, platelet counts, hepatitis activity grade (A0 and A1 vs. A2 and A3), liver fibrosis grade (F0 and F1 vs. F2 and F3), pretreatment HCV RNA levels ($\geq$6.5 $\log_{10}$ vs. <6.5 $\log_{10}$), reduction in peginterferon dose and ribavirin dose, reduction in HCV RNA levels at 4 weeks after starting therapy ($\geq$3 $\log_{10}$ vs. <3 $\log_{10}$), and the type of an early virologic response. All *P*-values are two-tailed, and $P < 0.05$ was considered significant statistically.

RESULTS

The characteristics of the patients examined in this study are shown in Table I. Liver histology was evaluated according to the METAVIR score [The French METAVIR Cooperative Study Group, 1994]. Although some patients had a reduction in their PEG-IFN and ribavirin doses during therapy, respectively, all patients except for those who discontinued the therapy had more than 80% adhesion to both the PEG-IFN and ribavirin regimens. No patients discontinued the therapy because of adverse effects. The treatment duration was extended up to 72 weeks in 51 of 71 patients (71.8%) who exhibited a slow virologic response. As a final outcome, 118 patients (43.4%) achieved a sustained virologic response, 84 patients (30.9%) relapsed, and the remaining 70 patients (25.7%) had no response.

TABLE I. Characteristics of all Study Patients (n = 272)

Age (years)	56.0 ± 10.9
Sex (female/male)	139 (51.1)/133 (48.9)
Body weight (kg)	57.8 ± 10.5
Alanine aminotransferase (IU/L)	64.6 ± 56.4
Aspartate aminotransferase (IU/L)	53.9 ± 42.7
Gamma-glutamyl transpeptidase (IU)	48.5 ± 43.9
Alkaline phosphatase (IU/L)	267.9 ± 101.3
Albumin (g/dl)	4.04 ± 0.37
Total bilirubin (mg/dl)	0.79 ± 0.30
White blood cell count (/μl)	4892 ± 1333
Hemoglobin (g/dl)	14.0 ± 1.3
Platelet count ($\times 10^3$/μl)	163 ± 51
Liver histology-activity (A0/A1/A2/A3)*	3 (1.2)/136 (55.3)/ 92 (37.4)/15 (6.1)
Liver histology-fibrosis (F0/F1/F2/F3)*	27 (11.0)/114 (46.3)/ 70 (28.5)/35 (14.2)
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	6.35 ± 0.79
Reduction in the peginterferon dose	81 (29.8)
Reduction in the ribavirin dose	130 (47.8)
Final outcomes (sustained virologic response /relapse/ no response)	118 (43.4)/ 84 (30.9)/70 (25.7)

HCV, hepatitis C virus.
Percentages are shown in parentheses.
*Liver biopsy was not performed in 26 patients.

Reduction in Serum HCV RNA Levels at 4 Weeks after Starting Therapy and Treatment Outcome According to Genetic Polymorphisms Near the *IL28B* Gene

An analysis of genetic polymorphisms at rs8099917 near the *IL28B* gene indicated that 207 patients (76.1%) had a TT genotype, 3 patients had a GG genotype (1.1%), and the remaining 62 patients were TG heterozygote (22.8%). Table II shows the comparison of the background characteristics between patients with the favorable TT genotype and those with the unfavorable TG/GG genotype. As reported previously [Abe et al., 2010], gamma-glutamyl transpeptidase level was higher significantly in patients with the TG/GG genotype. As a final outcome, the rate of a sustained virologic response was higher significantly in patients with the TT genotype. Among 207 patients with the TT genotype, serum HCV RNA became undetectable in 19 patients (9.2%) at 4 weeks after starting therapy (a rapid virologic response). In the remaining 188 patients, the decrease in serum HCV RNA levels at 4 weeks after starting therapy ranged from 0.12

TABLE II. Characteristics of Study Patients According to the Genetic Polymorphisms Near the *IL28B* Gene

	Patients with TT genotype of rs8099917 (n = 207)	Patients with TG/GG genotype of rs8099917 (n = 65)	*P*-value
Age (years)	56.5 ± 10.4	54.4 ± 12.4	0.4112
Sex (female/male)	107 (51.7)/100 (48.3)	32 (49.2)/33 (50.8)	0.8384
Body weight (kg)	57.8 ± 10.9	57.8 ± 9.4	0.8361
Alanine aminotransferase (IU/L)	65.1 ± 53.3	62.8 ± 65.6	0.2548
Aspartate aminotransferase (IU/L)	53.6 ± 34.8	54.7 ± 62.0	0.3339
Gamma-glutamyl transpeptidase (IU)	44.2 ± 37.1	62.3 ± 59.0	0.0003
Alkaline phosphatase (IU/L)	263.1 ± 90.3	282.8 ± 129.9	0.3875
Albumin (g/dl)	4.04 ± 0.36	4.05 ± 0.43	0.8020
Total bilirubin (mg/dl)	0.79 ± 0.30	0.76 ± 0.32	0.3010
White blood cell count (/μl)	4826 ± 1333	5100 ± 1320	0.1608
Hemoglobin (g/dl)	13.9 ± 1.3	14.1 ± 1.4	0.3339
Platelet count ($\times 10^3/\mu l$)	161 ± 49	169 ± 57	0.3871
Liver histology-activity (A0/A1/A2/A3)*	2 (1.1)/98 (52.4)/ 74 (39.6)/13 (6.9)	1 (1.7)/38 (64.4)/ 18 (30.5)/2 (3.4)	0.3241
Liver histology-fibrosis (F0/F1/F2/F3)*	21 (11.2)/83 (44.4)/ 57 (30.5)/26 (13.9)	6 (10.2)/31 (52.5)/ 13 (22.0)/9 (15.3)	0.6401
Pretreatment HCV RNA concentration (log_{10} IU/ml)	6.37 ± 0.85	6.29 ± 0.55	0.0582
Reduction in the peginterferon dose	61 (29.5)	20 (30.8)	0.9644
Reduction in the ribavirin dose	101 (48.8)	29 (44.6)	0.5565
Final outcomes (sustained virologic response /relapse/ no response)	106 (51.2)/ 69 (33.3)/32 (15.5)	12 (18.4)/15 (23.1)/ 38 (58.5)	<0.0001

HCV, hepatitis C virus.
Percentages are shown in parentheses.
*Liver biopsy was not performed in 26 patients.

log_{10} to 5.71 log_{10} (mean, 3.12 log_{10}). The reduction in serum HCV RNA levels was ≥3 log_{10} in 98 patients (47.3%), <3 log_{10} and ≥2 log_{10} in 52 patients (25.1%), <2 log_{10} and ≥1 log_{10} in 23 patients (11.1%), and <1 log_{10} in 15 patients (7.3%). Figure 1A shows the rate of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy in patients with the TT genotype. The rates were higher significantly in patients who achieved a rapid virologic response or had a ≥3 log_{10} decrease in

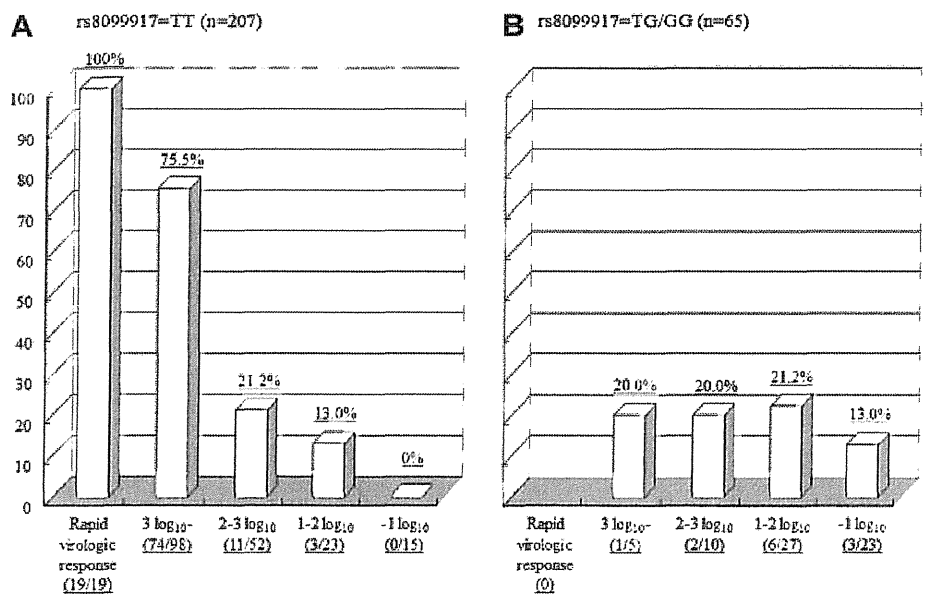

Fig. 1. The rate of sustained virologic responses (%) based on the reduction in serum HCV RNA levels at 4 weeks after starting therapy. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

serum HCV RNA levels at 4 weeks compared to those with a <3 $\log_{10}$ decrease in serum HCV RNA levels ($P < 0.0001$). When a 3 $\log_{10}$ decrease in serum HCV RNA levels was defined as the cut-off point, 56.5% of patients were considered to have a ≥3 $\log_{10}$ decrease in serum HCV RNA levels. The sensitivity, specificity, positive predictive value, and negative predictive value for a sustained virologic response were 86.8, 75.2, 78.6, and 84.4%, respectively.

Among the 65 patients who had the TG/GG genotype, no patient achieved a rapid virologic response at 4 weeks after initiating therapy. The decrease in serum HCV RNA levels at 4 weeks after starting therapy ranged from 0.11 $\log_{10}$ to 4.75 $\log_{10}$ (mean, 1.66 $\log_{10}$). The reduction in serum HCV RNA levels at 4 weeks after starting the therapy were smaller in patients with the TG/GG genotype than those with the TT genotype (1.66 ± 1.02 $\log_{10}$ in patients with the TG/GG genotype vs. 3.12 ± 1.37 $\log_{10}$ in patients with TT genotype excluding RVR, $P < 0.0001$). The reduction in serum HCV RNA levels was ≥3 $\log_{10}$ in five patients (7.7%), <3 $\log_{10}$ and ≥2 $\log_{10}$ in 10 patients (15.4%), <2 $\log_{10}$ and ≥1 $\log_{10}$ in 27 patients (41.5%), and <1 $\log_{10}$ in 23 patients (35.4%). Figure 1B shows the rates of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy in patients with the TG/GG genotype. There were no differences in the rate of a sustained virologic response based on the reduction in HCV RNA levels at 4 weeks after starting therapy; the rate of a sustained virologic response remained at 20% approximately regardless of the reduction in HCV RNA levels in 42 patients with a ≥1 $\log_{10}$ reduction in serum HCV RNA levels.

Association Between an Early Virologic Response at 12 Weeks and Treatment Outcome Based on Genetic Polymorphisms Near the *IL28B* Gene

Figure 2 shows the rate of patients with the TT genotype or TG/GG genotype for rs8099917 who achieved a complete early virologic response, a partial early virologic response, and those who did not achieve early virologic response at 12 weeks after starting therapy based on the reduction in serum HCV RNA level at 4 weeks after initiating therapy. Nearly 75% of patients with the TT genotype whose HCV RNA levels were reduced by ≥3 $\log_{10}$ at 4 weeks after starting the therapy achieved a complete early virologic response. In contrast, 80% of patients with the TG/GG genotype whose HCV RNA levels were reduced by ≥3 $\log_{10}$ at 4 weeks after starting the therapy showed a partial early virologic response. The majority of patients with the TT or TG/GG genotypes achieved a partial early virologic response when their reduction in HCV RNA levels was <3 $\log_{10}$ and ≥2 $\log_{10}$ or <2 $\log_{10}$ and ≥1 $\log_{10}$.

Figure 3 shows the rates of a sustained virologic response according to the type of early virologic response in patients with the TT genotype (Fig. 3A) and TG/GG genotype (Fig. 3B). Among patients with the TT genotype, the rate of sustained virologic response was significantly higher in patients with a complete early virologic response than in those with a partial early virologic response ($P < 0.0001$). In contrast, there was no difference in the rate of a sustained virologic response between patients with a complete early virologic response and those with a partial early virologic response ($P = 0.8917$) among patients with

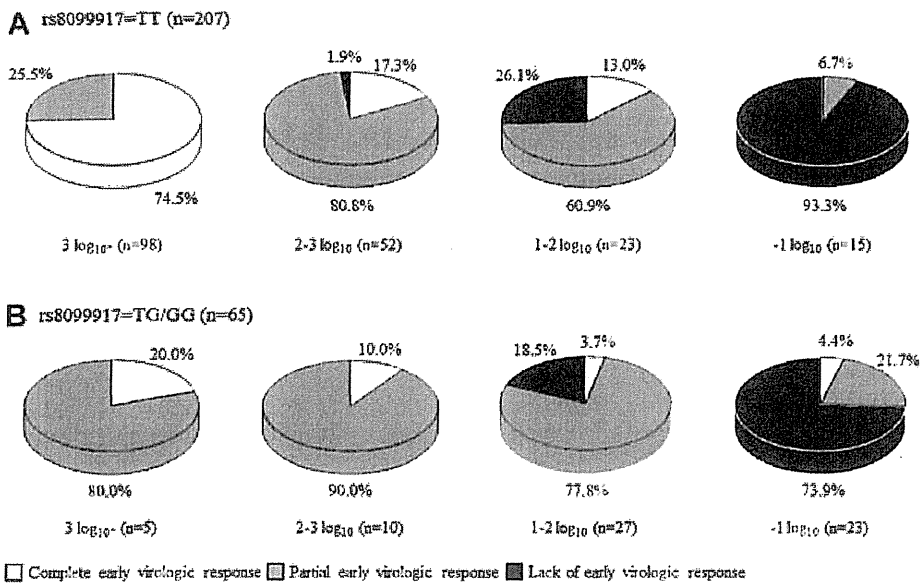

Fig. 2. The association between the virologic responses at 12 weeks after starting therapy and the reduction in serum HCV RNA levels at 4 weeks after starting therapy. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

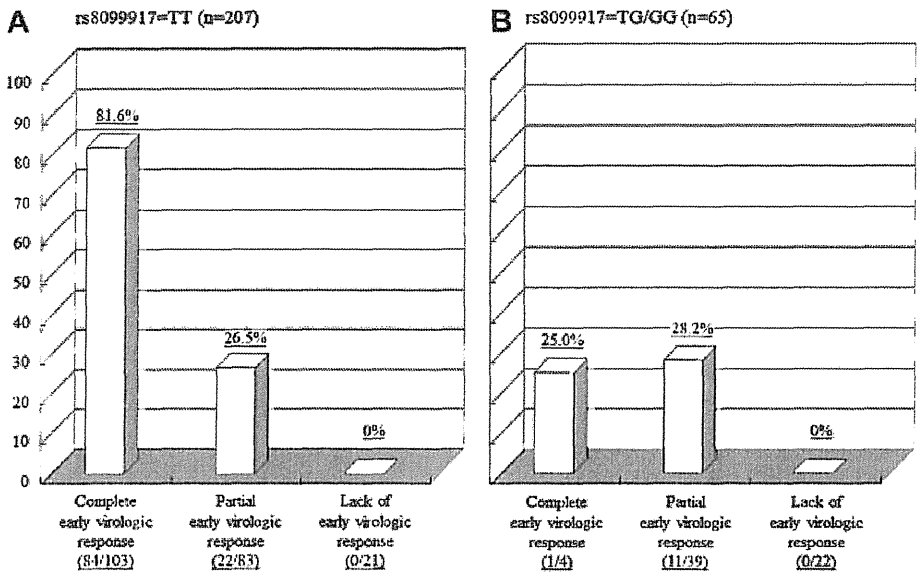

Fig. 3. The rate of sustained virologic responses based on the type of early virologic response. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

the TG/GG genotype. None of the patients with the TT genotype or TG/GG genotype who yielded a lack of an early virologic response reached a sustained virologic response.

Univariate and Multivariate Analyses for Factors Associated With a Sustained Virologic Response to Peginterferon and Ribavirin Combination Therapy in Patients With the TT and the TG/GG Genotype for the rs8099917

Univariate and multivariate analyses were conducted for factors associated with a sustained virologic response based on different genetic polymorphisms near the *IL28B* gene. In patients with the TT genotype, the factors that were associated with a sustained virologic response included serum alkaline phosphatase levels, serum albumin, platelet counts, hepatitis activity grade, liver fibrosis grade, reduction in HCV RNA levels at 4 weeks after starting therapy, and a complete early virologic response based on a univariate analysis (Table IIIA). In a multivariate analysis, the serum albumin levels, reduction in HCV RNA levels 4 weeks after starting therapy, and a complete early virologic response were independent factors that were significantly associated with a sustained virologic response (Table IIIB). A reduction in HCV RNA levels 4 weeks after starting therapy was the strongest factor that affected a sustained virologic response. In patients with the TG/GG genotype, the factors that were associated with a sustained virologic response included patient age, platelet counts, and pretreatment HCV RNA levels based on a univariate analysis (Table IIIA). A reduction in the HCV RNA levels at 4 weeks after starting therapy was not associated with a sustained virologic response. In a multivariate analysis, patient age and pretreatment HCV RNA levels were independent factors that were significantly associated with a sustained virologic response (Table IIIC).

Characteristics of Patients who Achieved a Sustained Virologic Response to the Combination Therapy Despite the Unfavorable TG/GG Genotype Near the *IL28B* Gene

Table IV shows the characteristics of 12 patients who achieved a sustained virologic response despite having the unfavorable TG/GG genotype for rs8099917 near the *IL28B* gene. All but one patient was under 60 years old and had liver fibrosis not more than grade 2 (one patient did not undergo a liver biopsy). Except for one patient, the reduction in the serum HCV RNA levels at 4 weeks after starting therapy was less than 3 $\log_{10}$ and all but one patient showed a partial early virologic response at 12 weeks after starting the therapy. In all 11 patients with a partial early virologic response, the serum HCV RNA was undetectable up to 24 weeks after starting the therapy. All but one patient extended the treatment duration from 48 to 72 weeks (two patients discontinued therapy at 60 weeks during the extended treatment period). When the characteristics of patients who achieved a sustained virologic response were compared between those with the unfavorable TG/GG genotype and those with the favorable TT genotype, patients with the TG/GG genotype were younger (41.8 ± 14.4 years vs. 55.1 ± 10.4 years, $P = 0.0023$) and had lower pretreatment HCV RNA levels (5.91 ± 0.44 $\log_{10}$ IU/ml vs. 6.21 ± 1.05 $\log_{10}$ IU/ml, $P = 0.0199$).

TABLE III. Univariate and Multivariate Analyses for Factors Associated With a Sustained Virologic Response to Peginterferon and Ribavirin Combination Therapy in Patients With the TT and the TG/GG Genotype for the rs8099917

(A) Univariate analyses	*P*-value	
	Patients with TT genotype of rs8099917 (n = 207)	Patients with TG/GG genotype of rs8099917 (n = 65)
Age (years)	0.0505	0.0007
Sex (female/male)	0.1830	0.2296
Body weight (kg)	0.6891	0.2456
Alanine aminotransferase (IU/L)	0.7988	0.4032
Aspartate aminotransferase (IU/L)	0.5021	0.1705
Gamma-glutamyl transpeptidase (IU)	0.6340	0.6648
Alkaline phosphatase (IU/L)	0.0315	0.0599
Albumin (g/dl)	0.0002	0.6594
Total bilirubin (mg/dl)	0.2929	0.7130
White blood cell count (/μl)	0.2508	0.5549
Hemoglobin (g/dl)	0.0847	0.2289
Platelet count ($\times 10^3$/μl)	0.0454	0.0411
Liver histology-activity (A0–1/A2–3)	0.0445	0.1117
Liver histology-fibrosis (F0–1/F2–3)	0.0002	0.2283
Pretreatment HCV RNA concentration (≥ 6.5 $\log_{10}$ vs. <6.5 $\log_{10}$)	0.5279	0.0379
Reduction in the peginterferon dose	0.4316	0.5563
Reduction in the ribavirin dose	0.1823	0.4272
Reduction in HCV RNA levels at 4 weeks after starting the therapy (≥ 3 $\log_{10}$ vs. < 3 $\log_{10}$)	<0.0001	0.9265
Early virologic response (complete vs. partial)	<0.0001	0.9777
Early virologic response (partial vs. non)	0.8632	0.0686

(B) Multivariate analyses: Patients with TT genotype of rs8099917	*P*-value	Odds ratio (95% confidence interval)
Alkaline phosphatase (IU/L)	0.2617	
Albumin (g/dl)	0.0365	28.287 (1.4107–755.41)
Platelet count ($\times 10^3$/μl)	0.2599	
Liver histology-activity (A0–1/A2–3)	0.6678	
Liver histology-fibrosis (F0–1/F2–3)	0.2307	
Reduction in HCV RNA levels at 4 weeks after starting the therapy (≥ 3 $\log_{10}$ vs. <3 $\log_{10}$)	<0.0001	16.029 (6.8593–40.406)
Early virologic response (complete vs. partial)	0.0224	0.3685 (0.1557–0.8749)

(C) Multivariate analyses: Patients with TG/GG genotype of rs8099917	*P*-value	Odds ratio (95% confidence interval)
Age (years)	0.0022	0.0034 (0.0000–0.0840)
Platelet count ($\times 10^3$/μl)	0.3344	
Pretreatment HCV RNA concentration (≥ 6.5 $\log_{10}$ vs. <6.5 $\log_{10}$)	0.0304	0.0548 (0.0020–0.4950)

HCV, hepatitis C virus.

DISCUSSION

Several previous studies reported that patients who achieved a rapid virologic response, in which serum HCV RNA become undetectable at 4 weeks after starting therapy, had a high likelihood of achieving a sustained virologic response [Martinez-Bauer et al., 2006; Poordad et al., 2008; de Segadas-Soares et al., 2009; Martinot-Peignoux et al., 2009]. In addition, several recent studies reported the predictive value of the degree of reduction in serum HCV RNA levels at 4 weeks after starting therapy [Yu et al., 2007; Huang et al., 2010; Toyoda et al., 2011]. Therefore, the viral dynamics of HCV at 4 as well as 12 weeks after starting therapy is important for response-guided therapy.

Genetic polymorphisms near the *IL28B* gene have emerged as the strongest predictive factor of a sustained virologic response in patients infected with HCV genotype 1 [Hayes et al., 2011; Kurosaki et al., 2011]. In addition, Thompson et al. [2010 reported that genetic polymorphisms near the *IL28B* gene were associated strongly with early viral dynamics during PEG-IFN and ribavirin combination therapy. These findings raised an important issue of whether response-guided therapy, based on the reduction in serum HCV RNA levels at 4 or 12 weeks after starting

TABLE IV. Patients who Achieved a Sustained Virologic Response Despite the TG/GG Genotype for the rs8099917

	Age (years)	Sex	Liver histology	Pretreatment HCV RNA level (log_{10} IU/ml)	HCV RNA reduction at 4 weeks	Response at 12 weeks	HCV RNA became undetectable (weeks)	Treatment duration (weeks)
1.	31	Female	A1/F1	6.13	2.19	partial EVR	20	48
2.	55	Male	A1/F1	5.80	1.77	partial EVR	16	72
3.	57	Female	A1/F1	5.58	3.01	partial EVR	16	72
4.	57	Female	A1/F1	6.21	1.81	partial EVR	20	72
5.	62	Male	N.D.	6.23	1.13	partial EVR	24	72
6.	21	Male	A1/F2	6.04	1.83	partial EVR	24	72
7.	42	Male	A1/F1	6.27	0.57	partial EVR	24	72
8.	29	Female	A1/F2	5.83	1.83	partial EVR	20	60
9.	52	Male	A1/F0	5.91	2.12	complete EVR	12	48
10.	40	Male	A2/F1	5.84	1.34	partial EVR	20	72
11.	27	Male	N.D.	5.63	0.42	partial EVR	24	72
12.	28	Male	A1/F0	6.59	0.76	partial EVR	20	60

N.D., not done; HCV, hepatitis C virus; EVR, early virologic response.

therapy, retains a predictive value when considering genetic polymorphisms near the *IL28B* gene.

In the present study, the predictive value of the decrease in serum HCV RNA levels was evaluated at 4 and 12 weeks after starting therapy in Japanese patients infected with HCV genotype 1b based on genetic polymorphisms near the *IL28B* gene. Consistent with previous reports, patients with the TG/GG genotype for rs8099917 had a smaller reduction in serum HCV RNA levels at 4 weeks after starting treatment ($P < 0.0001$), which indicates an unfavorable response to the combination therapy. Patients with the TT genotype for rs8099917, which is associated with a favorable response to the combination therapy, exhibited a significant difference in the rate of a sustained virologic response based on the reduction in serum HCV RNA levels at 4 weeks after initiating the therapy. Patients with a rapid virologic response or with a ≥ 3 log_{10} reduction in HCV RNA levels had a higher likelihood of achieving a sustained virologic response.

In contrast, these factors did not have any predictive value in patients with the TG/GG genotype. Only 18.5% of patients achieved a sustained virologic response (12 of 65 patients), and it was difficult to identify these patients based on the reduction in HCV RNA levels at 4 weeks or the type of an early virologic response at 12 weeks after starting therapy. Patients who achieved a sustained virologic response, despite the TG/GG genotype for rs8099917, were identified among those with a <2 log_{10} and ≥ 1 log_{10} or even <1 log_{10} reduction in HCV RNA levels at 4 weeks after starting therapy. Interestingly and paradoxically, the possibility of a sustained virologic response can be expected in patients with a <1 log_{10} reduction in HCV RNA levels at 4 weeks after starting therapy only when they have the unfavorable TG/GG genotype.

In the evaluation at 12 weeks after starting therapy, patients with the TT genotype who achieved a complete early virologic response had a higher rate of a sustained virologic response significantly than patients who achieved a partial early virologic response, whereas this difference was not found in patients with the TG/GG genotype. No patients who failed to achieve an early virologic response achieved a sustained virologic response regardless of the genetic polymorphisms near the *IL28B* gene. Thus, the lack of an early virologic response retained a strong predictive value for the failure of achieving a sustained virologic response. This result supports the recommendation in the AASLD guidelines, in which treatment may be discontinued in patients without an early virologic response at 12 weeks of treatment.

The characteristics of patients who achieved a sustained virologic response despite the unfavorable TG/GG genotype were younger in age and lower pretreatment HCV RNA levels. Most patients with the TG/GG genotype who achieved a sustained virologic response showed a partial early virologic response and extended the treatment duration. It was difficult to identify these patients according to viral dynamics at 4 or 12 weeks after starting therapy.

There are several limitations in this study. Some patients with a slow virologic response did not have their treatment period extended from 48 to 72 weeks. This is because the effectiveness of a 72-week combination therapy regimen in patients with HCV genotype 1 with a slow virologic response [Berg et al., 2006; Pearlman et al., 2007] had not been established in Japan in the earlier part of this study. This fact might have influenced the treatment outcome especially in patients with the unfavorable TG/GG genotype. Another limitation is a smaller sample size of patients with the TG/GG genotype in comparison to that of patients with the TT genotype. This sample size could have caused the lack of statistical significance in the rate of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy or according to the type of an early virologic response in patients with the TG/GG genotype. In addition, the data were based on Japanese patients infected with HCV genotype 1b. Therefore, these results should be confirmed in other ethnicities and patients infected with HCV genotype 1a.

In conclusion, among patients infected with HCV genotype 1b with the TT genotype for rs8099917, a rapid virologic response or a ≥ 3 $\log_{10}$ reduction in HCV RNA levels at 4 weeks after starting therapy, or a complete early virologic response indicate strongly that these patients will achieve a sustained virologic response as a final outcome for PEG-IFN and ribavirin combination therapy. Early viral dynamics retain the predictive value in this patient subpopulation. A reduction in HCV RNA levels at 4 weeks after starting therapy or the type of an early virologic response does not predict the likelihood that patients with the TG/GG genotype will achieve a sustained virologic response. In contrast, the lack of an early virologic response retains a strong predictive value for the failure to achieve a sustained virologic response regardless of *IL28B* polymorphisms, which remains useful as a factor to stop therapy.

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CLINICAL STUDY

Transarterial Chemoembolization for Hepatitis B Virus–associated Hepatocellular Carcinoma: Improved Survival after Concomitant Treatment with Nucleoside Analogues

Hidenori Toyoda, MD, Takashi Kumada, MD, Toshifumi Tada, MD, Yasuhiro Sone, MD, and Masashi Fujimori, MD

ABSTRACT

Purpose: To determine whether nucleoside analogue therapy is associated with improved survival in patients with hepatitis B virus (HBV)–associated hepatocellular carcinoma (HCC) who are treated solely with transarterial chemoembolization.

Materials and Methods: A retrospective chart review of patients diagnosed with HBV-associated HCC was performed to identify patients treated solely with chemoembolization. Relevant demographic and clinical data were extracted and recorded. The influence of therapy with nucleoside analogues (lamivudine, adefovir dipivoxil, or entecavir) was determined by estimating the survival function using the Kaplan-Meier product-limit method.

Results: The inclusion criteria for chemoembolization were met by 81 patients (67 men and 14 women, mean age 60.6 years ± 9.2); 21 (25.9%) of these patients had been treated with nucleoside analogues. The number of chemoembolization treatments was significantly greater in the patients who were treated with nucleoside analogues (3.43 ± 2.32) than in the patients who did not receive nucleoside analogues (1.82 ± 0.95; $P = .0022$). The 1-year, 3-year, and 5-year survival rates were 89.5%, 66.8%, and 40.5% in the patients treated with nucleoside analogues and 72.6%, 27.5%, and 14.3% in the patients not treated with nucleoside analogues. The survival rate was significantly higher in the patients who received nucleoside analogues ($P = .0051$). Nucleoside analogue intake was an independent factor that was associated with increased survival ($P = .0063$).

Conclusions: Administration of nucleoside analogues was associated with longer survival in patients with HBV-associated HCC who were treated with transarterial chemoembolization.

ABBREVIATIONS

AFP = alpha-fetoprotein, HBV = hepatitis B virus, HCC = hepatocellular carcinoma

Transarterial embolization was initially used to treat hepatocellular carcinoma (HCC) by Doyon et al (1) in 1974, and chemoembolization with gelatin sponge particles and anticancer agents was subsequently developed in Japan to treat inoperable HCC (2). Despite the increase in the number of patients who undergo complete curative treatments such as hepatectomy or radiofrequency ablation (3), transarterial chemoembolization continues to have an important role, both as an initial treatment and as a therapeutic alternative for recurrent disease (4) because of the advanced nature of HCC at diagnosis and the high rate of recurrent disease (5). The benefits resulting from chemoembolization have long been a subject of debate (6–10), but two randomized trials found that chemoembolization was associated with higher survival compared with symptomatic treatment (4,11,12).

Because of poor liver function, patients with HCC do not always receive chemoembolization. Repeated chemoembolization treatments for HCC may cause liver function to deteriorate despite the fact that the deterioration of liver function by each chemoembolization treatment would be mild (13). If repeated chemoembolization treatments are to be used in cases of HCC recurrence, it is important to

From the Departments of Gastroenterology (H.T., T.K., T.T.) and Radiology (Y.S., M.F.), Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu, 503-8502, Japan. Received May 31, 2011; final revision received November 12, 2011; accepted November 14, 2011. Address correspondence to H.T., E-mail: tkumada@he.mirai.ne.jp

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prevent the worsening of liver function in the intervals between the treatments for longer survival (14).

Nucleoside analogues against hepatitis B virus (HBV) have been used since the late 1990s to suppress the replication of HBV and to normalize transaminase levels. Therapy with nucleoside analogues against HBV is known to arrest the progression of hepatic dysfunction in patients with chronic hepatitis B. More recent studies have shown that these drugs prevent the development of liver failure, even in the patients with advanced liver fibrosis (15–19). However, it is unknown whether this beneficial effect of antiviral therapy translates into longer survival for patients with concomitant HCC who undergo chemoembolization. We conducted a retrospective review of our experiences using chemoembolization to treat HCC in patients with chronic HBV infection.

MATERIALS AND METHODS

Patients

The complete study protocol was approved by the institutional review board of our hospital and was performed in compliance with the Helsinki Declaration. Between July 1997 and December 2010, 1,359 patients were diagnosed with primary HCC at our institution. Chronic HBV infection was confirmed in 260 of these patients, and 95 of these 260 patients were treated with chemoembolization. Of these 95 patients, 14 underwent treatments other than chemoembolization for recurrent HCC (4 underwent hepatectomy and 10 underwent radiofrequency ablation), and the remaining 81 patients had been treated with chemoembolization alone for recurrent HCC tumors. Our study retrospectively examined these 81 patients.

HCC was diagnosed based on clinical criteria (20) in all 81 patients. Specifically, the patients had a pertinent clinical background (chronic HBV infection) and typical imaging results. The tumor usually was detected by B-mode ultrasonography with typical HCC imaging features, including a hypoechoic tumor or a tumor with a mosaic pattern with a halo. HCC was diagnosed when a high-density mass was detected on arterial phase dynamic computed tomography (CT) images combined with a low-density mass on portal phase dynamic CT images obtained with a single or multidetector helical CT scanner. All of the patients with possible HCC tumors underwent angiography using a unified CT-angiography system (Interventional-CT; Toshiba, Tokyo, Japan) (21,22). CT during arterial portography and CT during hepatic arteriography were also performed to evaluate the progression of HCC (23).

The patients included 67 men (82.7%) and 14 women (17.3%), with a mean age of 60.6 years ± 9.2. The liver function at diagnosis was Child-Pugh class A in 49 patients (60.5%). At the time of diagnosis, 52 patients (64.2%) had multiple initial HCC tumors. HCC was accompanied by branch portal vein invasion in 18 patients (22.2%), but no patients had HCC invasion of the main portal vein trunks or the left or right main portal vein (Table E1).

Chemoembolization for Hepatocellular Carcinoma and Follow-up after Treatment

The treatment decisions were based principally on the Japanese HCC treatment guidelines (24). The patients were initially assessed for their eligibility for hepatic resection and subsequent local ablative therapies, including percutaneous ethanol injection, percutaneous microwave thermocoagulation, and radiofrequency ablation. The patients who were not eligible for curative treatment with surgery, local ablative therapies, or a combination of both were offered chemoembolization. The patients with Child-Pugh class C (25) liver function and the patients with HCC invasion of the main portal vein trunks and left or right main portal vein were not offered chemoembolization. Chemoembolization was performed by injecting an emulsion of 50 mg of farmorubicin hydrochloride (Epirubicin; Adria Laboratories, Columbus, Ohio) or 100 mg of cisplatin (IA-Call; Nihon-Kayaku, Tokyo, Japan) dissolved in 5 mL of iopamidol (Iopamiron, 370 mg I/mL; Schering, Tokyo, Japan) and mixed with 5 mL of iodized oil (Lipiodol Ultra Fluid; Guerbet, Paris, France). This procedure was followed by an injection of gelatin sponge particles (Gelfoam; Upjohn, Kalamazoo, Michigan). The total dose of the injected emulsion was determined by the volume of the liver that would be embolized. An unenhanced CT scan was obtained to confirm complete deposition of the iodized oil in the lesion and to complete the treatment.

After the first chemoembolization treatment, the patients were followed for 2.39–118.6 months (median follow-up period 19.3 months) at our institution with ultrasonography and CT or magnetic resonance imaging performed every 3–6 months. Serum tumor markers (alpha-fetoprotein [AFP], *Lens culinaris* agglutinin-reactive AFP, and des-gamma-carboxy prothrombin) were monitored every 3 months. When elevated tumor markers were detected, an additional imaging examination (usually CT or magnetic resonance imaging) was performed to check for recurrence or progression of HCC. If recurrence or progression was confirmed, retreatment was considered. Retreatment decisions were also based on the Japanese HCC treatment guidelines. Repeat chemoembolization was considered as a retreatment option in patients who had HCC recurrence or progression.

Statistical Analyses

The intergroup differences were analyzed using χ^2 and Mann-Whitney U tests for categorical and quantitative data. The date of the initial HCC treatment (chemoembolization) was defined as time zero when calculating the patient survival rates. Surviving patients and patients who died from causes other than liver disease were censored in the survival analysis. Patients whose death was caused by HCC

Table 1. Clinical Characteristics of Patients Who Did and Did Not Receive Nucleoside Analogues

	Nucleoside Analogues (+) (n = 21)	Nucleoside Analogues (−) (n = 60)	*P* Value
Age (mean ± SD, y) (range)	60.3 ± 8.9 (46–81)	60.6 ± 9.3 (37–78)	.7957
Sex ratio (female/male)	3 (14.3%)/18 (85.7%)	11 (23.3%)/49 (76.7%)	.9274
Child-Pugh class (A/B)	14 (66.7%)/7 (33.3%)	35 (58.3%)/25 (41.7%)	.6773
Albumin (mean ± SD, g/dL)	3.65 ± 0.45	3.33 ± 0.79	.0372
Total bilirubin (mean ± SD, mg/dL)	1.22 ± 0.72	0.98 ± 0.85	.0844
15-minute retention rate of ICG (%)*	24.8 ± 12.3	19.6 ± 13.8	.0691
Prothrombin (%)	81.1 ± 19.5	79.7 ± 20.4	.8209
Platelet count (× 1,000/mL)	112 ± 52	143 ± 82	.1867
Tumor size (mean ± SD, cm) (range)	4.30 ± 2.94 (1.2–11.5)	4.40 ± 3.24 (1.0–16.0)	.8083
Tumor size (≤ 2 cm/> 2 cm and ≤ 5 cm/> 5 cm)	4 (19.0%)/11 (52.4%)/6 (28.6%)	17 (28.3%)/25 (41.7%)/18 (30.0%)	.6282
Tumor number (single/multiple)	9 (42.9%)/12 (57.1%)	20 (33.3%)/40 (66.7%)	.4333
Portal vein invasion (absent/present)	18 (85.7%)/3 (14.3%)	45 (75.0%)/15 (25.0%)	.4744
AFP (median, ng/mL) (range)	56.7 (0.9–3,132)	61.4 (0.8–1,304,200)	.7836
AFP (≥ 20 ng/mL/< 20 ng/mL)	13 (61.9%)/8 (38.1%)	35 (58.3%)/25 (41.7%)	.9746
AFP-L3 (median, %) (range)	0.5 (0–64.0)	6.2 (0–60.7)	.3658
AFP-L3 (≥ 10%/< 10%)	7 (33.3%)/14 (66.7%)	24 (40.0%)/36 (60.0%)	.7769
DCP (median, mAU/mL) (range)	94.0 (16–8,000)	62.0 (10–75,000)	.7997
DCP (≥ 40 mAU/mL/< 40 mAU/mL)	13 (61.9%)/8 (38.0%)	41 (68.3%)/19 (31.7%)	.7854

AFP = alpha-fetoprotein; AFP-L3 = *Lens culinaris* agglutinin-reactive AFP; DCP = des-gamma-carboxy prothrombin; ICG = indocyanine green test.
* ICG test was not performed in 14 patients.

or liver failure were not censored. The survival function was estimated using the Kaplan-Meier product-limit method (26), and the log-rank test (27) was used to analyze the differences in survival.

The Cox proportional hazards model (28) was used to perform a multivariate analysis of the factors related to survival. The following variables were analyzed: patient age and sex, Child-Pugh class (A/B), tumor size (≤ 2 cm/> 2 cm and ≤ 5 cm/> 5 cm), number of tumors (single/multiple), portal vein invasion (absent/present), and treatment with nucleoside analogues against HBV. The data analyses were performed using JMP statistical software, version 6.0 (Macintosh version; SAS Institute, Cary, North Carolina). All *P* values were derived from two-tailed tests; $P < .05$ was considered statistically significant.

RESULTS

Comparison of Patient Characteristics According to Nucleoside Analogue Intake

The anti-HBV nucleoside analogues had been administered to 21 of the 81 patients (25.9%). Among the 21 patients who had received nucleoside analogues, 7 patients had already been taking nucleoside analogues at the initial HCC diagnosis, and the remaining 14 patients started nucleoside analogues after diagnosis of HCC. Seven patients were taking 100 mg of lamivudine (Zefix; GlaxoSmithKline, Tokyo, Japan), eight patients were taking 0.5 mg of entecavir (Baraclude; Bristol-Myers Squibb, Tokyo, Japan), and six patients were taking lamivudine and 10 mg of adefovir dipivoxil (Hepsera, GlaxoSmithKline) because of the emergence of lamivudine-resistant HBV. Table 1 compares the background characteristics of the patients who had and had not been treated with nucleoside analogues. There were no significant differences between these two groups in patient age and sex, liver function, and tumor progression, although the serum albumin levels were higher in the patients who received nucleoside analogues.

Influence of Nucleoside Analogue Treatment on Survival and Progression-free Survival

Table 2 shows the number of chemoembolization treatments that were performed for initial and recurrent HCC with respect to the nucleoside analogue intake. Chemoembolization could not be performed more than four times in the patients who had not received nucleoside analogues; however, it was performed more than four times in one-third of patients who did receive them. The number of chemoembolization treatments was significantly higher in the patients who had received nucleoside analogues than in the patients who were not treated with nucleoside analogues ($P = .0022$). In the patients who underwent chemoembolization treatments repeatedly, the interval between two chemoembolization treatment sessions did not differ significantly between the patients who were and were not treated with nucleoside analogues (6.27 months ± 2.66 in patients without nucleoside analogues vs 6.71 months ± 2.71 in

Table 2. Number of Transarterial Chemoembolization Procedures Performed as a Function of Treatment with Nucleoside Analogues

No. Transarterial Chemoembolization Procedures	1	2	3	4	5	6	7	8
Nucleoside analogues (−) (n = 60)	28 (46.7%)	20 (33.3%)	7 (11.7%)	5 (8.3%)	0	0	0	0
Nucleoside analogues (+) (n = 21)	5 (23.8%)	4 (19.0%)	5 (23.8%)	0	3 (14.3%)	1 (4.8%)	1 (4.8%)	2 (9.5%)

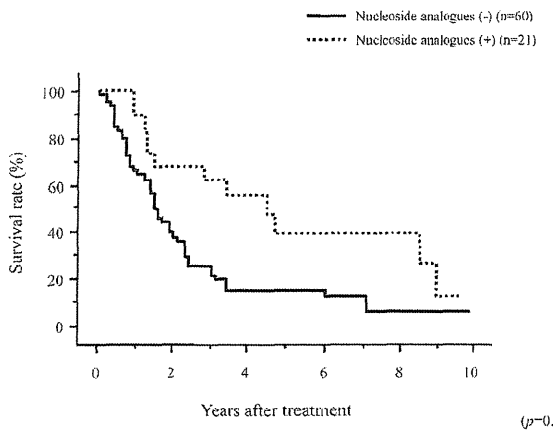

Figure 1. Plot of the Kaplan-Meier product-limit functions for survival after transarterial chemoembolization for initial HCC in the patients who did and did not receive nucleoside analogues.

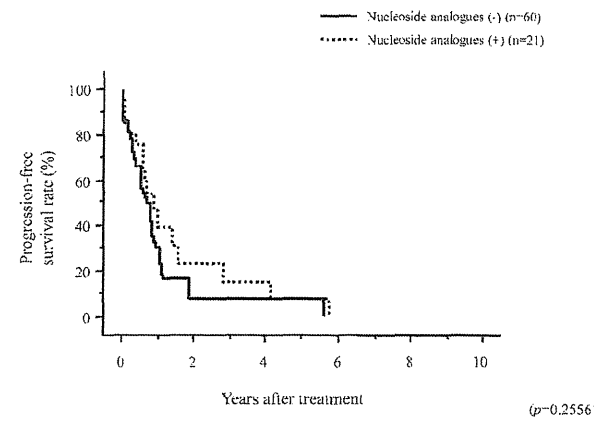

Figure 2. Plot of the Kaplan-Meier product-limit functions for progression-free survival after transarterial chemoembolization for initial HCC in the patients who did and did not receive nucleoside analogues.

patients with nucleoside analogues; $P = .3893$). The reasons for not offering further chemoembolization treatments to patients who did not receive nucleoside analogue therapy were emerging signs of liver failure (including ascites, jaundice, and hepatic coma) in 29 (48.3%) patients, progression to Child-Pugh C liver function in 18 (30.0%) patients, and progression of HCC (including extrahepatic metastases and invasion of the main portal vein trunks and left or right main portal vein) in 13 (21.7%) patients. The reasons for not offering further chemoembolization to the patients who did receive nucleoside analogue therapy were emerging signs of liver failure in 6 (28.6%) patients, progression to Child-Pugh C liver function in 4 (19.0%) patients, and HCC progression in 11 (52.4%) patients. Further chemoembolization was denied because of HCC progression more frequently in patients who were treated with nucleoside analogues ($P = .0174$).

Figure 1 shows the survival curves for the two patient groups. The 1-year, 3-year, and 5-year survival rates were 89.5%, 66.8%, and 40.5% in the patients treated with nucleoside analogues and 72.6%, 27.5%, and 14.3% in the patients who did not receive nucleoside analogues. The survival rate was significantly higher in the patients who were treated with nucleoside analogues ($P = .0051$). By contrast, there was no difference in the progression-free survival rates between the two groups ($P = .2556$) (**Fig 2**).

A multivariate analysis was performed to examine the factors that influenced survival after chemoembolization for the initial HCC (**Table 3**). Multiple tumors and portal vein invasion at the initial HCC diagnosis independently reduced the survival rate, and nucleoside analogue intake was an independent factor that increased the survival rate. When multivariate analysis included the number of chemoembolization treatments as an independent variable, the number of chemoembolization treatments was an independent factor associated with improved survival, and the statistical significance of nucleoside analogue intake disappeared (**Table E2**).

DISCUSSION

The results of the present study showed an association of nucleoside analogue therapy with longer survival in patients with HBV-associated HCC who were treated with chemoembolization for initial and recurrent disease. A multivariate analysis showed that nucleoside analogue intake was an independent factor that affected patient survival. However, the statistical significance of nucleoside analogue intake for improved survival disappeared when the multivariate analysis included the number of chemoembolization treatments as an independent variable, and the number of chemoembolization treatments was the factor that most affected survival. The patients who had received nucleoside analogues underwent a significantly greater number of chemoembolization treatments for HCC than the patients who were not treated with nucleoside analogues. Taken together, these results suggest that the association between nucleo-

Table 3. Multivariate Analyses of Factors Associated with Patient Survival

Factor		Parameter Estimate	Standard Error	Chi	Risk Ratio (95% Confidence Interval)	*P* Value
Age		−0.0188	0.0158	1.41	0.9814 (0.9512–1.0122)	.2342
Sex	Male				1	
	Female	0.0378	0.1804	0.04	1.0385 (0.7096–1.4504)	.8353
Child-Pugh class	A				1	
	B	0.1316	0.1428	0.84	1.1406 (0.8580–1.5057)	.3602
Tumor size	≤ 2 cm				1	
	> 2 cm and ≤ 5 cm	0.2868	0.1688	2.98	1.3322 (0.9625–1.8733)	.0842
	> 5 cm	0.0282	0.1939	0.02	1.0286 (0.7029–1.5113)	.8843
Tumor number	Single				1	
	Multiple	0.3492	0.1516	5.71	1.4179 (1.0631–1.9331)	.0169
Portal vein invasion	Absent				1	
	Present	0.3970	0.1852	4.31	1.4874 (1.0232-2.1235)	.0379
Nucleotide analogue	No				1	
	Yes	−0.4420	0.1727	7.46	0.6428 (0.4483–0.8871)	.0063

Note–Data on Child-Pugh class, tumor size, tumor number, and portal vein invasion refer to the status at initial diagnosis of hepatocellular carcinoma.

side analogue intake and improved patient survival was likely mediated by the increased number of chemoembolization treatments. The use of nucleoside analogues may have slowed the progressive decline in liver function that occurs even with repeated chemoembolization treatments, potentially allowing more sessions of chemoembolization treatment in patients who would otherwise have been excluded from chemoembolization treatment because of progressive liver dysfunction. Additional chemoembolization sessions may have explained the improved patient survival, although not the improved progression-free survival. Several groups have reported on the beneficial survival effects nucleoside analogues exert by preserving liver function in patients with HCC and HBV who undergo curative treatment (29,30). Our experience may suggest that this finding also applies to patients receiving chemoembolization as palliative therapy.

Although previous studies reported that nucleoside analogues can suppress the development of HCC (17,31), it has not been confirmed that nucleoside analogues can suppress HCC recurrence after treatment (30,32–34). Because the patients in the present study had been treated for both initial and recurrent HCC solely by chemoembolization, which is not a curative treatment, it is difficult to determine the extent to which nucleoside analogues prevent HCC progression or recurrence. Although there was no difference in the progression-free survival rate after the initial HCC treatment based on nucleoside analogue intake, further studies are needed to investigate whether the suppressive effects of nucleoside analogues on HCC recurrence or progression play a role in improving the survival of HBV-infected patients with HCC.

There are several limitations to this study. This was a retrospective study, and the patients were not randomly assigned to treatment arms. There may have been selection bias toward the patients who were administered nucleoside analogues. In addition, the data on liver function deterioration during the course of HCC recurrence and retreatment were insufficient, and the mechanisms behind the effect of nucleoside analogues on patients with HCC treated with chemoembolization were not elucidated. Additional studies are necessary to elucidate these mechanisms.

In conclusion, administering nucleoside analogues for chronic hepatitis B was associated with longer survival and more chemoembolization treatments in patients with HCC who were treated solely with chemoembolization. Additional studies are needed to examine these findings further and to clarify the mechanisms underlying this association.

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Table E1. Pretreatment Characteristics of Study Patients (n = 81)

Age (mean ± SD, y) (range)	60.6 ± 9.2 (37–81)
Sex ratio (female/male)	14 (17.3%)/67 (82.7%)
Child-Pugh class (A/B)	49 (60.5%)/32 (39.5%)
Albumin (mean ± SD, g/dL)	3.42 ± 0.73
Total bilirubin (mean ± SD, mg/dL)	1.04 ± 0.82
15-minute retention rate of ICG (%)*	20.0 ± 13.5
Prothrombin (%)	80.1 ± 20.0
Platelet count (× 1,000/mL)	135 ± 77
Tumor size (mean ± SD, cm) (range)	4.38 ± 3.15 (1.0–15.9)
Tumor size (≤ 2 cm/> 2 cm and ≤ 5 cm/> 5 cm)	21 (25.9%)/36 (44.5%)/24 (29.6%)
Tumor number (single/multiple)	29 (35.8%)/52 (64.2%)
Portal vein invasion (absent/present)	63 (77.8%)/18 (22.2%)
AFP (median, ng/mL) (range)	61.4 (0.8–1,304,200)
AFP (≥ 20 ng/mL/< 20 ng/mL)	48 (59.3%)/33 (40.7%)
AFP-L3 (median, %) (range)	6.1 (0–64.0)
AFP-L3 (≥ 10%/< 10%)	31 (38.3%)/50 (61.7%)
DCP (median, mAU/mL) (range)	62.0 (10–75,000)
DCP (≥ 40 mAU/mL/< 40 mAU/mL)	54 (66.7%)/27 (33.3%)

AFP = alpha-fetoprotein; AFP-L3 = *Lens culinaris* agglutinin-reactive AFP; DCP = des-gamma-carboxy prothrombin; ICG = indocyanine green test.
* ICG test was not performed in 14 patients.

Table E2. Multivariate Analyses of Factors Associated with Patient Survival (including Number of Chemoembolization Treatments)

Factor		Parameter Estimate	Standard Error	Chi	Risk ratio (95% Confidence Interval)	*P* Value
Age		−0.0250	0.0150	2.79	0.9753 (0.9469–1.0047)	.0949
Sex	Male				1	
	Female	−0.0013	0.1794	0.00	0.9987 (0.6836–1.3912)	.9943
Child-Pugh class	A				1	
	B	−0.0173	0.1476	0.01	0.9828 (0.7329–1.3106)	.9064
Tumor size	≤ 2 cm				1	
	> 2 cm and ≤ 5 cm	0.2361	0.1668	2.06	1.2662 (0.9183–1.7740)	.1512
	> 5 cm	0.0940	0.1920	0.24	1.0986 (0.7529–1.6069)	.6242
Tumor number	Single				1	
	Multiple	0.4285	0.1562	8.23	1.5350 (1.1415–2.1140)	.0041
Portal vein invasion	Absent				1	
	Present	0.3841	0.1843	4.05	1.4683 (1.0107–2.0898)	.0440
Nucleotide analogue	No				1	
	Yes	−0.1040	0.1903	0.31	0.9013 (0.6067–1.2859)	.5793
No. chemoembolization procedures		−0.3658	0.1194	10.00	0.6936 (0.5450–0.8720)	.0016

Note–Data on Child-Pugh class, tumor size, tumor number, and portal vein invasion refer to the status at initial diagnosis of hepatocellular carcinoma.