FIGURE LEGENDS

Fig.1 Comparison of the relative ratios of the three classifications of lipids based on the reference values for Japanese clinical examination, between the HCV-negative and -positive patients. The respective lipids were divided into under, within, and over normal ranges. ‡ $P<0.001$ shows a significant difference of the relative ratio between the HCV-negative and -positive subjects by Pearson's χ^2-analysis. Abbreviations: Total-C; total cholesterol, HDL-C; HDL cholesterol, LDL-C; LDL cholesterol, TG; Triglyceride.

Fig.2 The composition of serum HDL-C, LDL-C, and TG for each gender between the HCV-positive and -negative subjects. Data are shown as mean ± SD of the respective percentage in TG, LDL, and HDL for sum of them. See the legend for Fig.1 for abbreviations.

Fig.3 Serum lipid levels classified by the healthy limits of serum aminotransferases in the HCV-negative and -positive subjects. The NORMAL and ABNORMAL populations were classified by cut off points; ALT≤30 and AST≤30, and ALT>30 and/or AST>30, respectively. Data are expressed the mean ± SEM. ANOVA *P*-value was less than 0.0001 for all lipid parameters in both genders. ***P*<0.01, †*P*<0.001 by Bonferroni's post-hoc test. The symbols inside the columns of ABNORMAL without the bar indicate the comparison against the

respective NORMAL. See the legend for Fig.1 for abbreviations.

Fig.4 The serum lipid levels stratified by age-ranges in the HCV-negative and -positive subjects. Data are expressed as the mean ± SEM, and the age-ranges were divided into 5-year increments. Significant difference was analyzed by Mann-Whitney *U*-test between the HCV-negative and -positive subjects in each age-range; **P*<0.05, ***P*<0.01, †*P*<0.001, ‡*P*<0.0001. See the legend for Fig.1 for abbreviations.

Fig.5 Serum lipid levels classified by the WHO-defined BMI class in the HCV-negative and positive subjects. Data are expressed as the mean ± SEM. Abbreviations: Under Wt; under weight (BMI<18.5), Normal Wt; normal range of weight (18.5≤BMI<25), Over Wt; over weight (25≤BMI<30), Obese Cls.; obese classes 1~3 (BMI≥30), and see the legend for Fig.1 for other abbreviations. Significant difference between the HCV-negative and -positive was analyzed by Mann-Whitney *U*-test; **P*<0.05, ***P*<0.01, †*P*<0.001, ‡*P*<0.0001.

Table 1 Profile of serum lipids between the HCV-negative and -positive subjects by gender

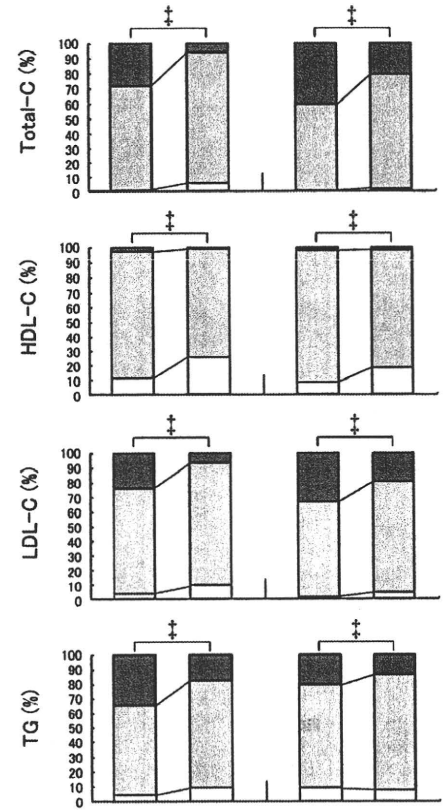
		Total-C (mg/dL)		HDL-C (mg/dL)		LDL-C (mg/dL)		TG (mg/dL)	
All									
HCV-positive	(*n*=1,317)	179.2 ± 1.0		52.9 ± 0.4		105.2 ± 0.3		107.6 ± 1.9	
HCV-negative	(*n*=145,540)	209.3 ± 0.1	**	60.3 ± 0.04	**	124.3 ± 0.1	**	124.5 ± 0.2	**
titer≥1	*(n=1,326)*	*204.2 ± 1.0*	**	*57.3 ± 0.4*	**	*121.6 ± 0.9*	**	*127.5 ± 2.2*	**
titer<1	*(n=144,214)*	*209.4 ± 0.1*	**	*60.3 ± 0.04*	**	*124.3 ± 0.1*	**	*124.5 ± 0.2*	**
Male									
HCV-positive	(*n*=679)	168.1 ± 1.2		48.3 ± 0.5		98.0 ± 1.1		112.0 ± 3.1	
HCV-negative	(*n*=49,720)	202.1 ± 0.2	**	54.9 ± 0.1	**	118.3 ± 0.1	**	155.7 ± 0.5	**
titer ≥ 1	*(n=638)*	*195.4 ± 1.3*	**	*53.3 ± 0.6*	**	*114.7 ± 1.3*	**	*139.3 ± 3.5*	**
titer < 1	*(n=49,082)*	*202.3 ± 0.2*	**	*54.9 ± 0.1*	**	*118.4 ± 0.1*	**	*155.9 ± 0.5*	**
Female			(vs. male)		(vs. male)		(vs. male)		(vs. male)
HCV-positive	(*n*=638)	191.0 ± 1.4	(**)	57.8 ± 0.6	(**)	112.8 ± 1.2	(**)	102.9 ± 2.3	(*)
HCV-negative	(*n*=95,820)	213.0 ± 0.1	** (**)	63.1 ± 0.1	** (**)	127.3 ± 0.1	** (**)	113.3 ± 0.2	** (**)
titer ≥ 1	*(n=688)*	*212.4 ± 1.3*	** (**)	*61.0 ± 0.5*	** (**)	*128.1 ± 1.2*	** (**)	*116.5 ± 2.5*	** (*)
titer < 1	*(n=95,132)*	*213.0 ± 0.1*	** (**)	*63.1 ± 0.1*	** (**)	*127.3 ± 0.1*	** (**)	*113.2 ± 0.2*	** (**)

Footnote: The titer ≥ 1 and < 1 show the HCV-negative subjects with HCV antibody titer over than 1 and more, and less than 1, respectively. Data are shown the mean ± SEM. Significant differences between the HCV-positive and HCV-negative subjects and between genders were analyzed by Mann-Whiteney *U*-test. **P*<0.05, ***P*<0.0001. Symbols in the parenthesis in female show the significant difference compared to that in male. LDL-C value was calculated using the Friedewald formula (LDL-C = Total-C - HDL-C - TG/5). Abbreviation: HCV; hepatitis virus C, Total-C; total cholesterol, HDL-C; HDL cholesterol, LDL-C; LDL cholesterol, TG; triglyceride.

Table 2 Multivariate logistic regression analysis of factors associated with hypolipidemia

	Total-C			HDL-C			LDL-C			TG		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Male												
HCV-(+)	10.75	7.00-16.50	<0.0001	3.09	2.55-3.74	<0.0001	2.10	1.62-2.72	<0.0001	3.17	2.37-4.26	<0.0001
age	1.01	0.99-1.03	0.1180	1.02	1.01-1.02	<0.0001	0.98	0.99-0.99	<0.0001	0.99	0.98-0.99	<0.0001
ALT	1.01	1.01-1.02	<0.0001	0.98	0.98-0.99	<0.0001	1.04	1.04-1.05	<0.0001	1.02	1.02-1.02	<0.0001
AST	1.00	0.99-1.00	0.1870	1.02	1.01-1.02	<0.0001	0.98	0.98-0.99	<0.0001	0.96	0.96-0.97	<0.0001
BMI	0.89	0.85-0.94	<0.0001	1.15	1.14-1.16	<0.0001	0.95	0.94-0.97	<0.0001	0.80	0.78-0.81	<0.0001
Female												
HCV-(+)	14.93	6.90-32.27	<0.0001	2.24	1.81-2.78	<0.0001	3.67	2.40-5.63	<0.0001	1.38	1.00-1.99	0.0479
age	0.94	0.92-0.96	<0.0001	1.03	1.03-1.03	<0.0001	0.93	0.93-0.94	<0.0001	0.94	0.94-0.94	<0.0001
ALT	1.02	1.00-1.04	0.1034	0.98	0.98-0.99	<0.0001	1.01	1.00-1.02	0.0566	1.03	1.03-1.04	<0.0001
AST	0.99	0.97-1.01	0.4201	1.02	1.01-1.02	<0.0001	1.00	1.00-1.01	0.5252	0.96	0.96-0.97	<0.0001
BMI	0.88	0.82-0.94	0.0002	1.13	1.12-1.14	<0.0001	0.96	0.94-0.97	<0.0001	0.84	0.84-0.85	<0.0001

Footnote: The lower level of each serum lipid was defined as below the normal range of the respective reference value for Japanese, and see Fig.1 for the values. Abbreviation: HCV-(+); positive for hepatitis C virus, OR; odds ratio, CI; confidence interval, *P*; *p*-value, and see footnote of Table 1 for other abbreviations.



		UNDER the normal range	WITHIN the normal range	OVER the normal range
Total-C		≤119	120–219	≥220
HDL-C	male	≤39	40–86	≥87
	female	≤44	45–96	≥97
LDL-C		≤64	65–139	≥140
TG		≤49	50–149	≥150

Comparison of the relative ratios of the three classifications of lipids based on the reference values for Japanese clinical examination, between the HCV-negative and -positive patients. The respective lipids were divided into under, within, and over normal ranges. ‡ $P<0.001$ shows a significant difference of the relative ratio between the HCV-negative and -positive subjects by Pearson's χ^2-analysis. Abbreviations: Total-C; total cholesterol, HDL-C; HDL cholesterol, LDL-C; LDL cholesterol, TG; Triglyceride.
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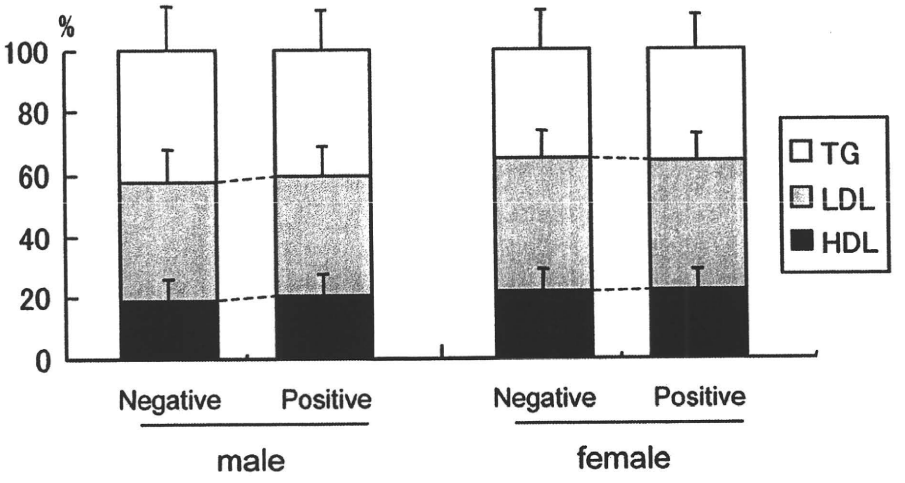

The composition of serum HDL-C, LDL-C, and TG for each gender between the HCV-positive and -negative subjects. Data are shown as mean±SD of the respective percentage in TG, LDL, and HDL for sum of them. See the legend for Fig.1 for abbreviations.
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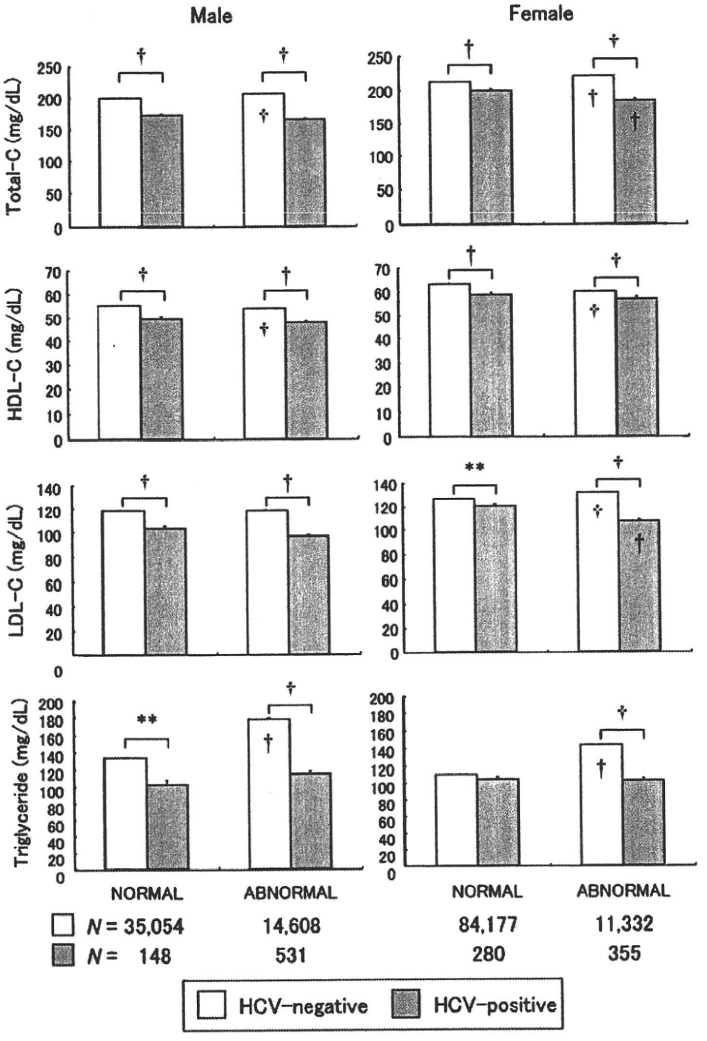

Serum lipid levels divided by the healthy limits of serum aminotransferases in the HCV-negative and -positive subjects. The NORMAL and ABNORMAL populations were classified by cut off points; ALT≤30 and AST≤30, and ALT>30 and/or AST>30, respectively. Data are expressed the mean ± SEM. ANOVA P-value was less than 0.0001 for all lipid parameters in both genders. $^{**}P<0.01$, $^{\dagger}P<0.001$ by Bonferroni's post-hoc test. The symbols inside the columns of ABNORMAL without the bar indicate the comparison against the respective NORMAL. See the legend for Fig.1 for abbreviations.
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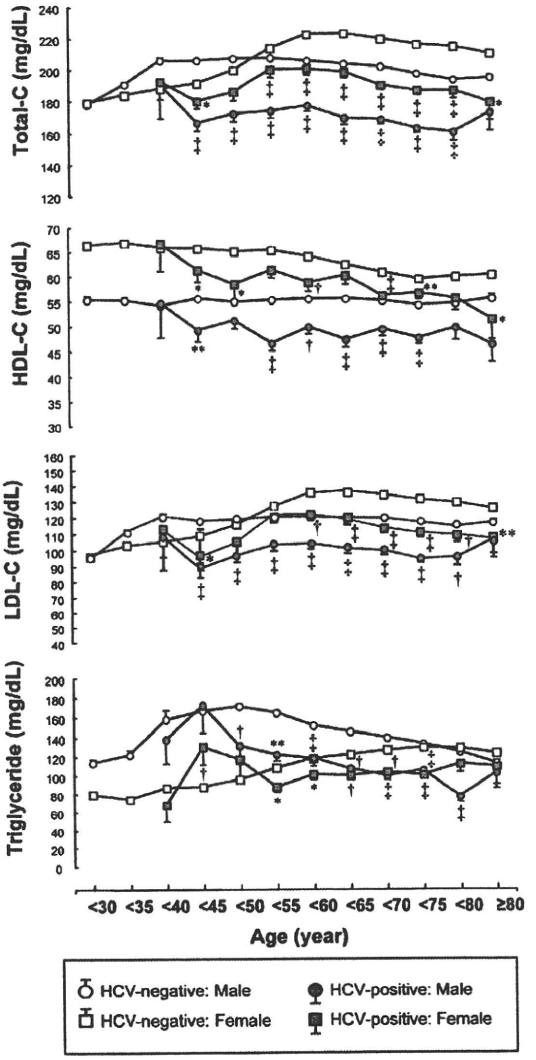

The serum lipid levels stratified by age-ranges in the HCV-negative and -positive subjects. Data are expressed as the mean ± SEM, and the age-ranges were divided into 5-year increments. Significant difference was analyzed by Mann-Whitney *U*-test between the HCV-negative and -positive subjects in each age-range; **P*<0.05, ***P*<0.01, †*P*<0.001, ‡*P*<0.0001. See the legend for Fig.1 for abbreviations.
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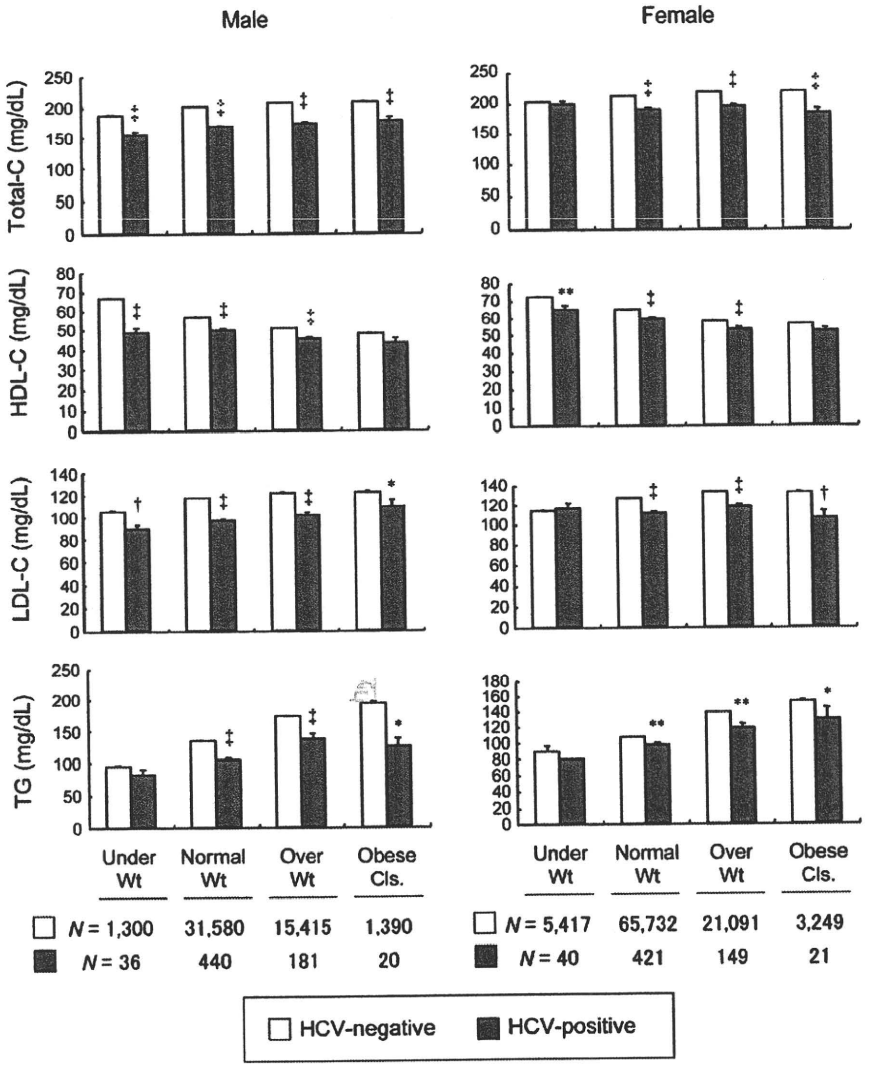

Serum lipid levels classified by the WHO-defined BMI class in the HCV-negative and positive subjects. Data are expressed as the mean ± SEM. Abbreviations: Under Wt; under weight (BMI<18.5), Normal Wt; normal range of weight (18.5≤BMI<25), Over Wt; over weight (25≤BMI<30), Obese Cls.; obese classes 1~3 (BMI>30), and see the legend for Fig.1 for other abbreviations. Significant difference between the HCV-negative and -positive was analyzed by Mann-Whitney *U*-test; **P*<0.05, ***P*<0.01, †*P*<0.001, ‡*P*<0.0001.
176x214mm (300 x 300 DPI)

Journal of Medical Virology 82:1537–1544 (2010)

An Early Viral Response to Standard Interferon-Alpha Identifies Resistance to Combination Therapy With Peginterferon and Ribavirin in Patients Infected by HCV Genotype 1

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As combination therapy with peginterferon (PEG-IFN) and ribavirin has a high morbidity, identifying individuals with hepatitis C virus (HCV) who will not respond to the treatment would be beneficial. The early responses of serum HCV RNA levels to standard interferon (IFN) and PEG-IFN were examined to determine if it was possible to identify resistance to combination therapy. One hundred thirty-one patients infected with HCV genotype 1b were enrolled. Patients were given 6 MU of standard IFN alpha-2b at least 2 weeks before initiating combination therapy. Serum HCV RNA levels were measured before, 24 hr after the administration of standard IFN, and 24 hr after the administration of PEG-IFN (at the start of the combination therapy). The association between reductions in HCV RNA levels at 24 hr after the administration of standard IFN and PEG-IFN and the outcome of combination therapy were analyzed. Reductions in HCV RNA levels were poorer in patients who did not respond than in those with a sustained virologic responses or relapses ($P < 0.0001$), both 24 hr after the administration of standard IFN and 24 hr after the administration of PEG-IFN. Reductions in HCV RNA levels 24 hr after the administration of standard IFN were an independent factor associated with non-response by multivariate analysis. An early reduction in viral load to a single administration of standard IFN is a useful predictor of non-response in patients with HCV genotype 1, allowing for pretreatment identification of patients who will not benefit from combination therapy. ***J. Med. Virol. 82:1537–1544, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis C; standard interferon; peginterferon and ribavirin; non-response; resistance to interferon

INTRODUCTION

Current standard antiviral therapy for patients with chronic hepatitis C is combination therapy with peginterferon (PEG-IFN) and ribavirin [Ghany et al., 2009]. The rate of sustained virologic response, indicating the eradication of hepatitis C virus (HCV), has increased markedly with this regimen. Patients with HCV genotype 1, however, have a rate of sustained virologic response of only 50%, making this subset of patients more resistant to therapy than patients with genotype 2 or 3.

In patients failing to achieve sustained virologic responses, two patterns of response are seen. Some patients relapse, characterized by an initial response to treatment, during which serum HCV RNA becomes undetectable, but it becomes detectable subsequently after cessation of treatment. The remaining patients exhibit a non-response, wherein HCV RNA is not cleared from serum even after 24 weeks of therapy. Non-response is thought to result from the presence of HCV resistant to PEG-IFN and ribavirin therapy, with a low likelihood that these patients will achieve sustained virologic response with current treatment modalities. There are many adverse effects associated with antiviral therapy with PEG-IFN and ribavirin, and the treatment course is costly. For these reasons, it would be beneficial to identify patients who would not respond to combination therapy with PEG-IFN and ribavirin to prevent unnecessary treatment.

Jessner et al. [2001] reported previously the utility of measuring serum HCV RNA level 24 hr after the

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Accepted 20 April 2010
DOI 10.1002/jmv.21858
Published online in Wiley InterScience (www.interscience.wiley.com)

administration of standard IFN to identify non-responders to monotherapy with standard IFN. Boulestin et al. [2006] demonstrated the utility of this measurement to identify patients with non-response to combination therapy with standard IFN and ribavirin. In contrast, Jessner et al. [2003] reported that non-response to IFN predicted by reductions in HCV RNA levels 24 hr after the administration of standard IFN could be overcome by the use of PEG-IFN with ribavirin. However, the current study only examined a small number of patients, making the clinical utility of this information for combination therapy with PEG-IFN and ribavirin unclear.

In the present study, a single dose of standard IFN was given to patients with HCV genotype 1b, measuring the changes in serum HCV RNA levels 24 hr later. All patients then underwent combination therapy and were followed-up to determine the outcome. An attempt was made to analyze whether non-response of HCV RNA levels 24 hr after administration of standard IFN could identify patients with non-response to combination therapy with PEG-IFN and ribavirin.

PATIENTS AND METHODS

Patients and Treatment

One hundred sixty-seven patients with chronic hepatitis C who were candidates for antiviral therapy with PEG-IFN and ribavirin underwent treatment between January 2005 and June 2007. Of these patients, 133 were infected with HCV genotype 1b and had pretreatment HCV RNA levels $>100 \times 10^3$ IU/ml by quantitative polymerase chain reaction (PCR) assay (Amplicor GT-HCV Monitor, Version 2.0; Roche Molecular Systems, Pleasanton, CA) and were considered for enrollment. Two patients refused enrollment leaving 131 patients in the study. Patients coinfected with hepatitis B virus or the human immunodeficiency virus and those abusing alcohol or using intravenous drugs were to be excluded, but none of the patients met these exclusion criteria.

All patients were given PEG-IFN alpha-2b (Pegintron, Schering-Plough, Tokyo, Japan) weekly and ribavirin (Rebetol, Schering-Plough) daily. The dose of PEG-IFN and ribavirin was adjusted by the body weight. Patients weighing ≤45 kg were given 60 μg of PEG-IFN alpha-2b once a week, those weighing >45 and ≤60 kg were given 80 μg, those weighing >60 and ≤75 kg were given 100 μg, those weighing >75 and ≤90 kg were given 120 μg, and those weighing >90 kg were given 150 μg. Patients weighing ≤60 kg were given 600 mg of ribavirin per day, those weighing >60 and ≤80 kg were given 800 mg of ribavirin per day, and those weighing >80 kg were given 1,000 mg of ribavirin per day. Dose modification or discontinuation of PEG-IFN or ribavirin was based on the manufacturer's recommendations. All patients were scheduled to undergo 48 weeks of treatment. No patient underwent prolonged treatment, as the effectiveness of a 72-week combination therapy regimen for patients with HCV genotype 1 with a slow virologic response [Berg et al., 2006; Pearlman et al., 2007] had not been established in Japan when this study was conducted. Sustained virologic response was defined as undetectable serum HCV RNA at 24 weeks after the end of therapy. Relapse was defined as positive serum HCV RNA between the end of treatment and 24 weeks thereafter, despite the disappearance of serum HCV RNA during therapy. Non-response was defined as positive serum HCV RNA 24 weeks after beginning of the therapy [i.e., null-response or partial non-response according to the American guidelines, Ghany et al., 2009]. Early virologic response was defined as disappearance or decrease in serum HCV RNA levels by two logs or more 12 weeks after the start of the therapy. HCV RNA in the serum was measured by the qualitative Amplicor Monitor HCV RNA assay (Amplicor Hepatitis C Virus (HCV) Test, version 2.0; Roche Molecular Systems) to confirm the non-detectability of serum HCV RNA, when it was unquantifiable (under the detection limit) by the quantitative Amplicor Monitor assay.

The study protocol was in compliance with the Helsinki Declaration and was approved by the hospital ethics committee. Written informed consents were obtained from all patients prior to the study for use of laboratory data.

Single Administration of Standard Interferon Before the Start of Combination Therapy and Measurement of Serum HCV RNA Level

All patients received a single dose of standard IFN-alpha 2b at least 2 weeks before initiating combination therapy with PEG-IFN and ribavirin (Fig. 1). Patients received an intramuscular administration of 6 MU of standard IFN-alpha 2b (Intron A; Schering-Plough). This dose was fixed, based on previous standards for monotherapy of patients with chronic hepatitis C in Japan [Toyoda et al., 1996]. HCV RNA levels were measured before and 24 hr after standard IFN administration, and the reduction in HCV RNA levels was calculated. HCV RNA levels were also measured before and 24 hr after PEG-IFN administration (24 hr after beginning combination therapy), and the reduction in HCV RNA levels was calculated.

Statistical Analyses

Quantitative values are displayed as the means ± SD. Between-group differences were analyzed by the chi-square test. Differences between two groups in quantitative values were analyzed by the Mann–Whitney *U*-test. Univariate and multivariate analyses using a logistic regression model were performed to identify factors predictive of non-response. Factors examined were age, sex, body weight, serum alanine aminotransferase activity, serum aspartate aminotransferase activity, serum gamma-glutamyl transpeptidase levels, serum alkaline phosphatase values, serum albumin levels, serum total bilirubin values, white blood cell counts, hemoglobin, platelet counts, hepatitis activity grade (A0 and A1 vs. A2 and A3), liver fibrosis

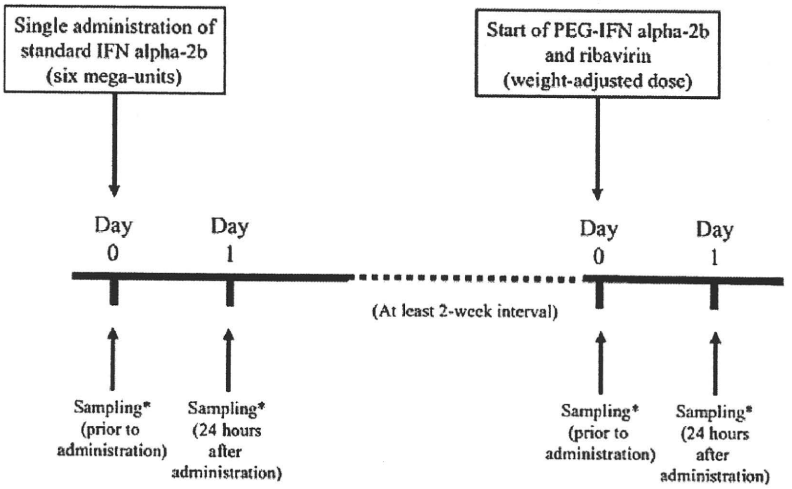

Fig. 1. Schematic representation of the single administration of standard interferon and combination therapy with peginterferon-alpha 2b and ribavirin, with the time points for measurement of serum HCV RNA levels. Serum HCV RNA levels were measured before and 24 hr after a single administration of standard interferon and before and 24 hr after the administration of peginterferon (start of combination therapy). Combination therapy with peginterferon and ribavirin was started at least 2 weeks after a single dose administration of standard interferon. IFN, interferon; PEG-IFN, peginterferon. Sampling*, sampling of serum samples for the measurement of HCV RNA levels.

grade (F0 and F1 vs. F2 and F3), pretreatment HCV RNA levels, reductions in serum HCV RNA levels 24 hr after the administration of standard IFN and PEG-IFN, reduction in PEG-IFN dose, and reduction in ribavirin dose. All *P*-values were two-tailed. $P < 0.05$ was accepted as statistically significant.

RESULTS

Outcome of Combination Therapy and Reduction in Serum HCV RNA Levels 24 hr After the Administration of Standard Interferon and Peginterferon

Pretreatment characteristics, reduction of PEG-IFN and ribavirin doses, and reductions in HCV RNA levels 24 hr after standard IFN and PEG-IFN administration of all patients and patients with sustained virologic response, relapse, or non-response are listed in Table I. One hundred twenty-eight patients (97.7%) completed the entire treatment regimen; three patients discontinued therapy. Although 37 patients (28.2%) required reduction of the dose of PEG-IFN and 69 patients (52.7%) required reduction of the dose of ribavirin, all patients received more than 80% of scheduled total dose for both drugs, with the exception of the three patients who discontinued therapy. Fifty-three patients (40.5%) had been treated previously with standard IFN monotherapy or by combination therapy with standard IFN and ribavirin; no patients had received PEG-IFN previously. Of the 125 patients who underwent pretreatment liver biopsy, the grade of liver fibrosis according to the METAVIR score [The French METAVIR Cooperative Study Group, 1994] was F0 in 5 (4.0%) patients, F1 in 77 (61.6%) patients, F2 in 30 (24.0%) patients, and F3 in 13 (10.4%) patients. Among 128 patients who completed the treatment regimen, 50 (39.1%) achieved a sustained virologic response, 45 (35.1%) relapsed, and 33 patients (25.8%) did not respond.

In the analysis of early viral response, patients who did not respond had significantly poorer reductions in HCV RNA levels 24 hr after the administration of standard IFN and PEG-IFN in comparison to patients who had a sustained virologic response or a relapse (Fig. 2). There were no differences between patients with a sustained virologic response and those who relapsed. The results were similar when only the 78 treatment-naïve patients were evaluated (data not shown).

Univariate and Multivariate Analyses for Factors Affecting Non-Response to Combination Therapy With Peginterferon and Ribavirin

When univariate analysis was conducted to identify factors associated with non-response to combination therapy, only reductions in HCV RNA levels 24 hr after standard IFN and PEG-IFN administration (< 0.0001) were associated significantly with non-response. Pretreatment white blood cell counts ($P = 0.0695$) and reduction of the dose of ribavirin ($P = 0.0866$) tended to be associated with it but were not significant statistically. Multivariate analysis of these factors confirmed that only reduction in serum HCV RNA levels 24 hr after the administration of standard IFN was associated significantly with non-response ($P < 0.0001$, Table II).

When focusing on the 78 treatment-naïve patients, reduction of the dose of ribavirin ($P = 0.0280$)

TABLE I. Characteristics of All Patients, Sustained Virologic Responders, Relapsers, and Non-Responders to Combination Therapy With Peginterferon and Ribavirin

	All patients (n = 131)	Sustained virologic response (n = 50)	Relapse (n = 45)	Non-response (n = 33)
Age (years)	58.7 ± 8.6	57.0 ± 10.0	59.8 ± 7.2	59.5 ± 8.1
Sex (female/male)	66 (50.4)/65 (49.6)	22 (44.0)/28 (56.0)	24 (53.3)/21 (46.7)	18 (54.5)/15 (45.5)
History of interferon therapy (naive/retreatment)	78 (59.5)/53 (40.5)	30 (60.0)/20 (40.0)	25 (55.6)/20 (44.4)	20 (60.6)/13 (39.4)
Response to previous therapy[a]	24 (45.3)/29 (54.7)	10 (50.0)/10 (50.0)	9 (45.0)/11 (55.0)	5 (38.5)/8 (61.5)
History of transfusion (−/+)	100 (76.3)/31 (23.7)	34 (68.0)/16 (32.0)	37 (82.2)/8 (17.8)	26 (78.8)/7 (21.2)
Body weight (kg)	58.8 ± 9.9	59.1 ± 9.9	59.3 ± 10.1	57.8 ± 10.1
Alanine aminotransferase (IU/L)	60.2 ± 33.7	62.9 ± 36.0	58.5 ± 36.4	61.2 ± 26.2
Aspartate aminotransferase (IU/L)	50.6 ± 28.2	50.3 ± 27.5	50.1 ± 33.0	52.8 ± 23.1
Gamma-glutamyl transpeptidase (IU)	51.2 ± 38.1	45.4 ± 39.1	55.1 ± 40.8	56.8 ± 32.9
Alkaline phosphatase (IU/L)	257.3 ± 77.4	246.3 ± 65.1	261.8 ± 93.0	264.3 ± 74.1
Albumin (g/dl)	4.11 ± 0.34	4.19 ± 0.31	4.08 ± 0.33	4.02 ± 0.39
Total bilirubin (mg/dl)	0.66 ± 0.25	0.67 ± 0.25	0.66 ± 0.29	0.66 ± 0.21
White blood cell count (/μl)	5,082 ± 1,322	5,274 ± 1,464	5,115 ± 1,162	4,712 ± 1,225
Hemoglobin (g/dl)	14.1 ± 1.3	14.1 ± 1.3	14.0 ± 1.3	13.9 ± 1.4
Platelet count ($\times 10^3$/μl)	16.4 ± 5.0	17.8 ± 5.4	15.8 ± 3.6	15.4 ± 5.7
Liver histology activity (A0-1/A2-3)[b]	76 (60.8)/49 (39.2)	31 (66.0)/16 (34.0)	28 (62.2)/17 (37.8)	15 (50.0)/15 (50.0)
Liver histology fibrosis (F0-1/F2-3)[b]	82 (65.6)/43 (34.4)	35 (74.5)/12 (25.5)	28 (62.2)/17 (37.8)	17 (56.7)/13 (43.3)
Pretreatment HCV RNA concentration ($\times 10^3$ IU/ml)	1,855 ± 1,468	1,790 ± 253	1,815 ± 1,314	1,548 ± 999
Reduction of the peginterferon dose	37 (28.2)	15 (30.0)	13 (28.9)	9 (27.3)
Reduction of the ribavirin dose	69 (52.7)	26 (52.0)	25 (55.6)	18 (54.5)
Reduction of HCV RNA 24 hr after standard IFN ($\log_{10}$ IU/ml)	1.36 ± 0.58	1.61 ± 0.43	1.56 ± 0.51	0.85 ± 0.50
Reduction of HCV RNA 24 hr after PEG-IFN ($\log_{10}$ IU/ml)	1.27 ± 0.56	1.47 ± 0.42	1.36 ± 0.55	0.89 ± 0.53

HCV, hepatitis C virus; IFN, interferon; PEG-IFN, peginterferon.
Percentages are shown in parentheses.
[a]In patients with a history of interferon therapy.
[b]Liver biopsy was not performed in six patients.

and reduction in serum HCV RNA levels 24 hr after standard IFN and PEG-IFN administration (< 0.0001) were associated significantly with non-response by univariate analysis. By multivariate analysis, only reductions in serum HCV RNA levels 24 hr after the administration of standard IFN were associated with non-response ($P = 0.0061$, Table III).

Cut-Off for the Identification of Patients With Non-Response to Combination Therapy by Reduction of Serum HCV RNA Levels 24 hr After the Administration of Standard Interferon

Receiver-operating characteristic (ROC) analyses were performed to determine an appropriate cut-off for HCV RNA reductions that would identify patients with

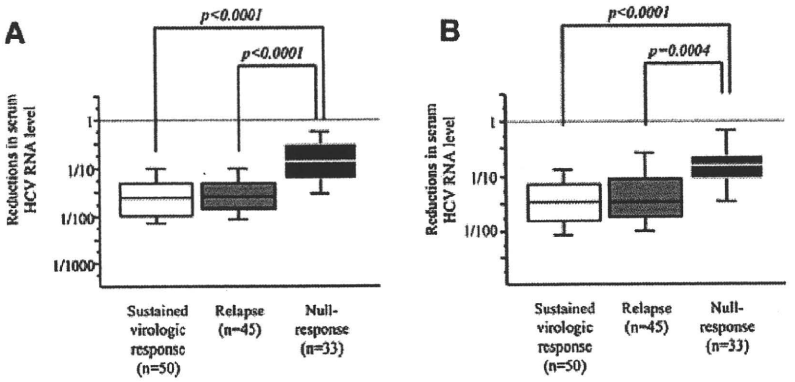

Fig. 2. Reductions in serum HCV RNA levels 24 hr after administration of standard interferon and peginterferon. Reductions 24 hr after the administration of (A) standard interferon and (B) peginterferon.

TABLE II. Univariate and Multivariate Analyses for Factors Associated With Non-Response to Combination Therapy With Peginterferon and Ribavirin

	Univariable analysis	Multivariate analysis	Odds ratio (95% confidence interval)
Age (years)	0.4988	—	
Sex (female/male)	0.5448	—	
Body weight (kg)	0.4673	—	
Alanine aminotransferase (IU/L)	0.9529	—	
Aspartate aminotransferase (IU/L)	0.6608	—	
Gamma-glutamyl transpeptidase (IU)	0.3763	—	
Alkaline phosphatase (IU/L)	0.4993	—	
Albumin (g/dl)	0.1069	—	
Total bilirubin (mg/dl)	0.8189	—	
While blood cell count (/μl)	0.0695	0.1338	
Hemoglobin (g/dl)	0.4578	—	
Platelet count ($\times 10^3$/μl)	0.1685	—	
Liver histology activity (A0-1/A2-3)	0.1716	—	
Liver histology fibrosis (F0-1/F2-3)	0.2394	—	
Pretreatment HCV RNA concentration ($\times 10^3$ IU/ml)	0.1703	—	
Reduction of the peginterferon dose	0.1841	—	
Reduction of the ribavirin dose	0.0866	0.2941	
Reduction of HCV RNA 24 hr after administration of standard IFN ($\log_{10}$ IU/ml)	<0.0001	<0.0001	5300.6 (136.3–436373.7)
Reduction of HCV RNA 24 hr after administration of PEG-IFN ($\log_{10}$ IU/ml)	<0.0001	0.1413	

HCV, hepatitis C virus; IFN, interferon; PEG-IFN, peginterferon.

non-response (Fig. 3). The area under the ROC curves for non-response were 0.8614 and 0.7980 for the reductions in serum HCV RNA levels 24 hr after the administration of standard IFN and PEG-IFN, respectively. Thus, a reduction in HCV RNA levels at 24 hr is an appropriate predictor for patients who have a subsequent non-response. A reduction in HCV RNA levels 24 hr after the administration of standard IFN was a better predictor with a higher area under the ROC curve than the reduction 24 hr after the administration of PEG-IFN. Based on this ROC analysis, the cut-offs for the reduction in HCV RNA for non-response were 1.24 $\log_{10}$ 24 hr after the administration of standard IFN. A 1.24 $\log_{10}$ reduction at 24 hr as the cut-off gave a sensitivity, specificity, positive predictive value, and negative predictive value of 0.81, 0.79, 0.59, and 0.91, respectively.

The cut-off values determined using ROC analysis provided reasonable test characteristics for identifying non-response. Using this cut-off to determine the

TABLE III. Univariate and Multivariate Analyses for Factors Associated With Non-Response to Combination Therapy With Peginterferon and Ribavirin in Treatment-Naive Patients

	Univariate analysis	Multivariate analysis	Odds ratio (95% confidence interval)
Age (years)	0.1804	—	
Sex (female/male)	0.2681	—	
Body weight (kg)	0.5122	—	
Alanine aminotransferase (IU/L)	0.9716	—	
Aspartate aminotransferase (IU/L)	0.8176	—	
Gamma-glutamyl transpeptidase (IU)	0.5234	—	
Alkaline phosphatase (IU/L)	0.4098	—	
Albumin (g/dl)	0.4077	—	
Total bilirubin (mg/dl)	0.5473	—	
White blood cell count (/μl)	0.5221	—	
Hemoglobin (g/dl)	0.9215	—	
Platelet count ($\times 10^3$/μl)	0.5957	—	
Liver histology activity (A0-1/A2-3)	0.5414	—	
Liver histology fibrosis (F0-1/F2-3)	0.8017	—	
Pretreatment HCV RNA concentration ($\times 10^3$ IU/ml)	0.1322	—	
Reduction of the peginterferon dose	0.2785	—	
Reduction of the ribavirin dose	0.0280	0.5304	
Reduction of HCV RNA 24 hr after administration of standard IFN ($\log_{10}$ IU/ml)	<0.0001	0.0061	1344.9 (12.1–434249.1)
Reduction of HCV RNA 24 hr after administration of PEG-IFN ($\log_{10}$ IU/ml)	<0.0001	0.0620	

HCV, hepatitis C virus; IFN, interferon; PEG-IFN, peginterferon.

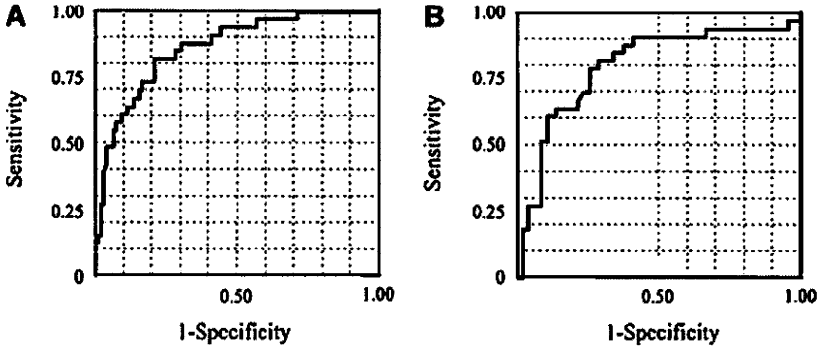

Fig. 3. Receiver-operating characteristic curves for prediction of non-response by the reduction 24 hr after the administration of (A) standard interferon and (B) peginterferon.

indications for combination therapy, however, would have denied treatment to 11 patients who would develop later a sustained virologic response. This cut-off would therefore lead to an unacceptable misidentification of patients with a response. The data were then re-examined to determine an appropriate cut-off that would incorporate all patients who achieved sustained virologic response. Such an approach would lead to the inappropriate treatment of more patients with non-response but would not miss any patients who could respond to therapy. The lowest reduction in serum HCV RNA levels seen in patients with sustained virologic response was 0.81 $\log_{10}$ 24 hr after the administration of single-dose standard IFN. The cut-off was therefore agreed as a reduction of 0.8 $\log_{10}$ 24 hr after the administration of standard IFN to determine eligibility for the initiation of combination therapy. Table IV shows the outcome of combination therapy using this value as a cut-off. Of the 109 patients with reductions in serum HCV RNA levels >0.8 $\log_{10}$, 50 patients (45.9%) achieved sustained virologic response, while 17 patients (15.6%) did not respond. In contrast, of the 19 patients with reduction in serum HCV RNA levels $\leq$0.8 $\log_{10}$, none achieved a sustained virologic response and 16 patients (84.2%) did not respond. When the 19 patients with reductions in serum HCV RNA levels 24 hr after the administration of standard IFN $\leq$0.8 $\log_{10}$ were excluded, the rate of sustained virologic response increased from 39.1% to 45.9%, while the rate of non-response decreased from 25.8% to 15.6%. Using this cut-off value would have prevented the initiation of combination therapy in 16 patients, which represented 48.5% of all patients with non-response.

DISCUSSION

It was reported previously that virologic responses after beginning therapy are important predictors of outcomes of combination therapy with PEG-IFN and ribavirin [Zeuzem et al., 2001; Buti et al., 2002; Berg et al., 2003; Lee and Ferenci, 2008]. The absence of an early virologic response is thought to be able to identify patients without sustained virologic response. Failure to observe decreases in serum HCV RNA levels by two logs or more 12 weeks after beginning therapy correlated strongly with a lack of therapeutic response in individuals with genotype 1 infection [Fried et al., 2002; Davis et al., 2003]. According to current American guidelines, treatment may be discontinued in patients who do not achieve an early virologic response. Discontinuation of therapy is recommended for patients with non-response in whom serum HCV RNA remains positive 24 weeks after beginning therapy [Ghany et al., 2009]. Although these responses would be useful to predict the outcome of therapy, these data can be observed only during treatment and cannot be obtained before treatment. All patients would have to undergo a treatment course of at least 12 weeks to predict a non-response, while this would lead to futile treatment for some patients. The early identification of patients with non-response would prevent unnecessary treatment with PEG-IFN and ribavirin in a subset of patients.

TABLE IV. Outcomes of Combination Antiviral Therapy With Peginterferon and Ribavirin by Reductions in Serum HCV RNA Levels 24 hr After the Administration of Standard IFN in Patients With Chronic Hepatitis C

		Reduction in serum HCV RNA level 24 hr after standard IFN administration	
Outcomes	All patients (n = 128)	>0.8 $\log_{10}$ IU/ml (n = 109)	<0.8 $\log_{10}$ IU/ml (n = 19)
Sustained virologic response	50 (39.1)	50 (45.9)	0
Non-response	33 (25.8)	17 (15.6)	16 (84.2)

HCV, hepatitis C virus; IFN, interferon.
Percentages are shown in parentheses.

In this study, the reduction in serum HCV RNA levels was measured 24 hr after the administration of standard IFN and PEG-IFN. Reductions in serum HCV RNA levels 24 hr after the administration of standard IFN and PEG-IFN were poorer significantly in patients with subsequent non-response. Reduction 24 hr after the administration of standard IFN, the only independent factor associated with non-response in multivariate analysis, had a higher area under the ROC curve than the reduction seen 24 hr after the administration of PEG-IFN. The reduction in HCV RNA levels 24 hr after the administration of standard IFN was a better predictor of non-response than the reduction seen 24 hr after the administration of PEG-IFN (24 hr after beginning combination therapy). The reduction in serum HCV RNA levels 24 hr after the administration of standard IFN remained an independent predictor when examining only the 78 treatment-naïve patients. Jessner et al. [2003] indicated previously that the value of reduction in serum HCV RNA levels 24 hr after the administration of standard IFN as a predictor of non-response, which they confirmed in case of IFN monotherapy [Jessner et al., 2001], could be overcome by combination therapy with PEG-IFN and ribavirin. In contrast to their report, the data of the present study indicate that viral response 24 hr after the administration of standard IFN is a strong predictor of non-response to combination therapy as well. The reduction in serum HCV RNA levels following single administration of standard IFN can be a baseline predictor of later non-response. Such a screening scheme can be performed easily in the outpatient setting, and the results will provide important information when considering the indications for combination therapy.

Reductions in HCV RNA levels that occur within 24 hr after the injection of IFN are considered to be the first phase, characterized by a rapid decrease in the serum HCV RNA concentrations of 0.5–2.0 $\log_{10}$. This is thought to be related to the ability of IFN to inhibit viral replication, facilitating the clearance of circulating virus [Layden-Almer et al., 2006]. Early failures to inhibit viral replication were associated with non-response as the outcome of PEG-IFN and ribavirin combination therapy as well as IFN monotherapy. The results of the present study suggest that the absence of a substantial first-phase viral decline was predictive of a non-response to combination therapy, even using of ribavirin. The resistance of HCV to combination therapy with PEG-IFN and ribavirin appeared to be related to the inability of IFN to clear circulating HCV partly.

There are several limitations to this study. While patients received a fixed dose of standard IFN, the dose of PEG-IFN used for combination therapy with ribavirin was weight-based. Although a significant effect of weight on non-response or the reduction in serum HCV RNA levels 24 hr after the administration of standard IFN were not found (data not shown), the results of HCV RNA reduction may be different if standard IFN had been administered on a weight-based basis. In addition, this study included only patients infected with HCV genotype 1b, because there are so few patients infected with HCV genotype 1a in Japan. While patients with genotype 1a are defined as difficult-to-treat cases, further studies will be needed to determine if reduction in serum HCV RNA applies as a predictor of non-response in patients bearing genotype 1a infections. Finally, only standard IFN-alpha 2b and PEG-IFN-alpha 2b were used in this study. Results may differ between IFN/PEG-IFN alpha-2b and alpha-2a, as the pharmacokinetics of PEG-IFN are different between PEG-IFN alpha-2b and PEG-IFN alpha-2a [Foster, 2004].

In conclusion, the failure to observe a reduction in HCV RNA levels 24 hr after the administration of standard IFN predicts a high likelihood of a non-response to PEG-IFN and ribavirin combination antiviral therapy. Pretreatment identification of patients likely to have non-response to combination antiviral therapy using a single administration of standard IFN will prevent some patients from undergoing unnecessary treatment.

REFERENCES

Berg T, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, Wiedenmann B, Hopf U, Zeuzem S. 2003. Prediction of treatment outcome in patients with chronic hepatitis C: Significance of baseline parameters and viral dynamics during therapy. Hepatology 37:600–609.

Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Pape GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S. 2006. Extended treatment duration for hepatitis C virus type 1: Comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. Gastroenterology 130:1086–1097.

Boulestin A, Kamar N, Sandres-Saune K, Legrand-Abravanel F, Alric L, Vinel JP, Rostaing L, Izopet J. 2006. Twenty-four hour kinetics of hepatitis C virus and antiviral effect of alpha-interferon. J Med Virol 78:365–371.

Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M, Esteban R. 2002. Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. Hepatology 35:930–936.

Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. 2003. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 38:645–652.

Foster GR. 2004. Pegylated interferons: Chemical and clinical differences. J Viral Hepat 20:825–830.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 345:975–982.

Ghany MG, Strader DB, Thomas DL, Seeff LB. 2009. Diagnosis, management, and treatment of hepatitis C: An update. Hepatology 49:1335–1374.

Jessner W, Gschwantler M, Steindl-Munda P, Hofer H, Watkins-Riedel T, Wrba F, Mueller C, Gangl A, Ferenci P. 2001. Primary interferon resistance and treatment response in chronic hepatitis C infection: A pilot study. Lancet 358:1241–1242.

Jessner W, Stauber R, Hackl F, Datz C, Watkins-Riedel T, Hofer H, Gangl A, Kessler H, Ferenci P. 2003. Early viral kinetics on treatment with pegylated interferon-α-2a in chronic hepatitis C virus genotype 1 infection. J Viral Hepat 10:37–42.

Layden-Almer JE, Cotler SJ, Layden TJ. 2006. Viral kinetics in the treatment of chronic hepatitis C. J Viral Hepat 13:499–504.

Lee SS, Ferenci P. 2008. Optimizing outcomes in patients with hepatitis C virus genotype 1 or 4. Antiviral Res 13:9–16.

Pearlman BL, Ehleben C, Saifee S. 2007. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis C genotype 1-infected slow responders. Hepatology 46:1688–1694.

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

Toyoda H, Nakano S, Kumada T, Takeda I, Sugiyama K, Osada T, Kiriyama S. 1996. Effect of daily administration of natural alpha interferon on response in patients with chronic hepatitis C. Am J Gastroenterol 91:743–747.

Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, Roth WK. 2001. Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alpha2a. Gastroenterology 120:1438–1447.

Hepatology Research

Hepatology Research 2010; 40: 667–685 doi: 10.1111/j.1872-034X.2010.00673.x

Special Report

Management of hepatocellular carcinoma: Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009)

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Hepatocellular carcinoma (HCC) is responsible for approximately 600 000–700 000 deaths worldwide. It is highly prevalent in the Asia–Pacific region and Africa, and is increasing in Western countries. The evidence-based guideline for HCC in Japan was published in 2005 and revised in 2009. Apart from this guideline, a consensus-based practice manual proposed by the HCC expert panel of the Japan Society of Hepatology (JSH), which reflects widely accepted daily practice in Japan, was published in 2007. At the occasion of the 45th Annual meeting of the JSH in Kobe 4–5 June 2009, a consensus meeting of HCC was held. Consensus statements were created based on 67% agreement of 200 expert members. This article describes the up-to-date consensus statements which largely reflect the real world HCC practice in Japan. We believe readers of this article will gain the newest knowledge and deep insight on the management of HCC proposed by consensus of the HCC expert members of JSH.

Key words: hepatocellular carcinoma, Japan Society of Hepatology, staging system, surveillance, treatment algorithm, consensus-based guideline

INTRODUCTION

THE LAST EVIDENCE-BASED guideline for hepatocellular carcinoma (HCC) for Japan was published in 2005,[1] and has prevailed nationwide. This document was developed by a committee composed of 14 experts (Chairman: Professor Masatoshi Makuuchi) and was based on a critical review of 7118 English reports published between 1966 and 2002. This guideline includes 58 research questions regarding important issues for the prevention, diagnosis, surveillance and treatment of HCC. The utility of this guideline is recognized by many Japanese clinicians and has provided a great contribution to clinical practice. However, there are several issues in which solid evidence is still lacking; thus, clear recommendations for clinical practice cannot be stated. In fact, 45% of the research questions are of grade C recommendation level, representing a lack of adequate evidence. These issues are left to the clinician's discretion within the clinical setting. Furthermore, because the guidelines did not include the most up-to-date articles, no recommendation or statements were made regarding newly established evidence. In addition, the clinical practices that follow these guidelines are considered to account for 70–80% of general practice institutions.

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Received 23 December 2009; revision 3 March 2010; accepted 15 March 2010.

As mentioned above, Congress President, Professor Masatoshi Kudo, at the 45th Annual Meeting of the Japan Society of Hepatology organized the Consensus Meeting of Hepatocellular Carcinoma. The program was chaired by Professors M. Sata and S. Arii and covered the updated problems and clarified some controversial issues. Eight experts were selected to contribute to the meeting and they were assigned the following topics based on their specialties. Professor M. Sakamoto presented recommendations regarding diagnostic problems for small-sized HCC from the clinicopathological point of view. Professor M. Shimada discussed the utility of clinical staging and prognosis. Dr T. Kumada reviewed the current status of diagnostic imaging and tumor markers. Dr S. Shiina discussed important issues on ablative treatment. Dr Yamashita reviewed transarterial chemoembolization and chemotherapy. Professor N. Kokudo discussed surgical treatment, including liver transplantation. Dr M. Tanaka presented a treatment algorithm from the point-of-view of hepatologists. Finally, Professor T. Takayama comprehensively discussed the appropriateness of the present treatment algorithm.

In each presentation, the speakers raised clinical questions regarding the remaining problems that needed to be clarified in the present guidelines, and the HCC specialists (a total of 200 physicians: hepatologists, 70%; surgeons, 24%; radiologists, 2%; and pathologists, 4%) answered these questions using a question and answer analyzer system. Recommendations were approved when at least 67% of the HCC experts reached agreement. For instances where agreement was between 50% and 67%, the statements were considered informative, and are cited here as "informative statements".

In this consensus paper, each presenter has provided a summary of the recommendations and consensus. It is highly expected that this Consensus Statement established by the Japan Society of Hepatology (JSH)will provide valuable insight, and will greatly contribute to the future improvement of the guidelines and appropriate clinical practices for patients with HCC worldwide.

PATHOLOGICAL ASSESSMENT

PATHOLOGICAL ASSESSMENT OF HCC is described in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer.[2] It focuses on macroscopic typing and tumor grading based on tumor differentiation and reflects the aggressiveness of the tumors; differential diagnosis between multicentric development and intrahepatic metastasis of multiple tumors; and diagnosis of early HCC and precancerous lesions. Historically, careful and detailed histological evaluation of surgical specimens enabled us to understand the clinicopathological features of HCC development and extension, and to establish the above-mentioned diagnostic criteria. However, the recent increase in non-surgical treatments for HCC, such as radiofrequency ablation (RFA), is rapidly changing the role and position of pathological diagnosis. Thus, we discussed the indications for liver tumor biopsy for the diagnosis and treatment of HCC.

When we consider the indications for liver biopsy, the risk and benefit of this procedure must be considered.[3–8] The risk includes complications caused by the procedure itself, such as hemorrhage by needle insertion, and by tumor seeding. The incidence of tumor seeding has been reported in approximately 1–5% of cases. Certainly, we have to note that the incidence depends on the characteristics of the tumor such as tumor size and tumor differentiation. Liver biopsy is important in terms of tumor diagnosis, assessment of prognosis and decision making for treatment. For example, for a typical HCC larger than 2 cm in size with a typical vascular pattern on imaging, and elevated tumor markers such as α-fetoprotein (AFP) and/or des-γ-carboxy prothrombin (DCP), the benefit of performing tumor biopsy to confirm the diagnosis of HCC seems minimal. In contrast, only liver biopsy can be used to confirm the diagnosis of cancer in cases with suspected HCC or borderline lesions on clinical and imaging diagnosis. However, controversy remains because of the inconsistent treatment strategy for suspected lesions, particularly in cases with poor liver function.

Previous follow-up data of suspected HCC and borderline lesions showed that the tumors grow slowly during the precancerous or early HCC stages, but grow rapidly in some early HCC cases or in progressed HCC.[9] The transition from slow growing to rapidly growing tumors was supposed to take place once the tumor reaches approximately 1.5 cm in size. Therefore, the proposed recommendations for liver biopsy are as follows.

Recommendation 1. Liver biopsy should be discouraged in cases with a typical HCC over 1.5 cm in size, which shows typical pattern on imaging.

Recommendation 2. Liver biopsy should be considered in cases with a suspected HCC or borderline lesions/early HCC of 1.5 cm in size or less, which does not show typical pattern on imaging.

In addition to these recommendations, the requirement of liver biopsy should increase if the detection and diagnostic ability of imaging techniques increases for

smaller lesions. The emergence of new contrast agents such as gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) are expected to reveal suspected HCC nodules, including early HCC at approximately 1 cm in size. Tumor biopsy should then be performed to confirm the diagnosis of early cancer before it can progress to overt HCC. It is also expected that the increase in therapeutic options will increase the need for more detailed information of the tumor characteristics, such as tumor differentiation and immunophenotype reflecting tumor aggressiveness, which can only be determined by tumor biopsy.

PROGNOSTIC STAGING SYSTEM

IN TERMS OF estimating the prognosis of HCC, there are currently insufficient evidence-based data; therefore, no definite recommendations can be made, unlike other fields of HCC management. It is well known that the prognosis of HCC is defined by the behavior of the HCC itself, and by host factors such as hepatic functional reserve. The major questions that still need to be answered in terms of estimating the prognosis of HCC are: (i) whether an integrated staging system is necessary for the management of HCC; (ii) what is the best integrated staging system; and (iii) should the integrated staging system be included in the algorithm for HCC treatment?

Tumor staging (TNM staging)

There are two major classifications used for tumor staging of HCC. One is the tumor–node–metastasis (TNM) stage, developed by the American Joint Committee on Cancer (AJCC). This classification can also be applied to liver transplant recipients. However, the cut-off value for tumor diameter of 5 cm is too large to define small HCC, which are frequently found in Japan.

The other is the TNM stage proposed by the Liver Cancer Study Group of Japan (LCSGJ). The cut-off of 2 cm is very appropriate for patients in countries such as Japan, where small HCC are often found in an established nationwide screening system. However, in this system, the weighting of the strongest prognostic factor, vascular invasion, is equal to that of other factors used to estimate prognosis, which might not be adequate.

Staging for hepatic functional reserve

There are two major classifications for estimating liver functional reserve. One is the Child–Pugh classification, which is widely used worldwide, but is difficult to apply for decision making for hepatectomy. The other is the Liver Damage Classification scheme proposed by the LCSGJ, which is useful for hepatectomy. However, this scheme is not widely accepted because of the need to perform the indocyanine green retention at 15 min test ($ICGR_{15}$).

Integrated staging system for HCC

The combined classification of TNM stage and liver function stage, namely, an integrated staging system, is extremely important to estimate patient prognosis and guide decision making for patient management. The integrated staging system contributes to: (i) estimate patient prognosis; (ii) select the best treatment option for each patient; (iii) compare different treatment modalities; and (iv) compare treatment outcomes among different institutions.

Since the Okuda classification in 1985,[10] several integrated staging systems have been reported, including the Cancer of the Liver Italian Program (CLIP) score,[11] the Barcelona Clinic Liver Cancer (BCLC) stage[12] and the Japan Integrated Staging (JIS) score.[13] The Okuda classification scheme is simple and has been found to be suitable in the past, but does not seem to be suitable at the present time, now that relatively small HCC can be detected. The CLIP score is popular in Western countries, but its discriminating power is weak for small HCC, particularly at higher scores of 4–6, and over 50% of Japanese HCC patients are classified as score 0. The BCLC staging is thought to be useful as an integrated staging system and for guiding treatment. Therefore, it is recommended as an integrated treatment algorithm by the European Association for the Study of the Liver and the American Association for the Study of Liver Disease (AASLD). However, it is not suitable for the estimation of patient prognosis, and a large number of variables are used. In contrast, the JIS score essentially consists of the Child–Pugh score and the LCSGJ TNM stage, and is widely accepted in Japan. The discriminating power for relatively small HCC is excellent, and is particularly suitable for countries such as Japan, where many small HCC are detected.

In terms of a comparison of these integrated staging systems, Cillo *et al.*[14] reported that the BCLC was the best system among the Okuda, CLIP, BCLC and French classifications. Meanwhile, Tateishi *et al.*[15] reported that the Tokyo score was superior to BCLC staging and comparable to the CLIP score in predicting prognosis after hepatectomy and ablation. Kudo *et al.*[16] reported that the JIS score was better than the CLIP score, particularly in terms of discriminating power for each subgroup. Similarly, Chung *et al.*[17] reported that the JIS score was