Table 2. Characteristics of rapid virological responders and non-rapid virological responders*

	Rapid virological responders ($n = 32$)	Non-rapid virological responders ($n = 28$)	*P* value
Gender (years)	55.6 ± 12.7	57.8 ± 9.6	0.7553
Sex (female/male)	20 (62.5)/12 (37.5)	16 (57.1)/12 (42.9)	0.8741
History of interferon therapy (naïve/retreatment)	19 (59.4)/13 (40.6)	21 (75.0)/7 (25.0)	0.3142
History of transfusion (no/yes)	2 (6.3)/30 (93.7)	16 (57.1)/12 (42.9)	0.0004
Alanine aminotransferase (IU/L)	49.3 ± 57.7	54.4 ± 56.8	0.9409
Aspartate aminotransferase (IU/L)	46.3 ± 54.8	45.6 ± 43.9	0.2354
γ-glutamyl transpeptidase (IU)	64.3 ± 149.4	53.7 ± 90.7	0.6037
Alkaline phosphatase (IU/L)	244.7 ± 99.6	302.0 ± 187.8	0.1848
Albumin (g/dl)	4.35 ± 0.26	4.25 ± 0.41	0.2368
Total bilirubin (mg/dl)	0.67 ± 0.25	0.63 ± 0.23	0.7411
White blood cell count (/μl)	4961 ± 1269	5052 ± 1963	0.9882
Haemoglobin (g/dl)	13.9 ± 1.3	14.1 ± 1.7	0.6944
Platelet count (× 10^3/μl)	19.0 ± 7.1	20.0 ± 6.0	0.3660
Body weight (kg)	56.6 ± 9.2	62.2 ± 12.1	0.1044
Liver histology – activity (A0/A1/A2/A3)†	1 (3.3)/22 (73.4)/6 (20.0)/1 (3.3)	0/17 (65.4)/6 (23.1)/3 (11.5)	0.4997
Liver histology – fibrosis (F0/F1/F2/F3)†	2 (6.6)/22 (73.3)/5 (16.7)/1 (3.3)	1 (3.9)/16 (61.5)/6 (23.1)/3 (11.5)	0.5526
HCV genotype (2a/2b)	27 (84.4)/5 (15.6)	18 (64.3)/10 (35.7)	0.1352
HCV RNA concentration (× 10^3 IU/ml)	1197 ± 979	2713 ± 1848	0.0002
Treatment duration (8 weeks/24 weeks)	15/17	0/28	

Percentages are shown in parentheses.

*Rapid virological responders, serum HCV RNA was negative at 4 weeks after the start of therapy; non-rapid virological responders, serum HCV RNA remained positive at 4 weeks after the start of therapy.

†Liver biopsy was not performed in four patients.

HCV, hepatitis C virus.

8-week group compared with reported rates with treatment periods of 12–16 weeks (5, 6, 8); the relapse rate in RVR patients was 0% in those on a 16-week regimen (8), 9.5% in those on a 14-week regimen (5), 9.2% in those on a 12-week regimen (6) and 66.7% in those on the 8-week regimen in the present study. (The study of the 14-week regimen involved patients with undetectable HCV RNA at 8 weeks after the start of therapy in addition to RVR patients.) This marked increase in the relapse rate in the 8-week group strongly indicates the limitations of shortening the treatment period in patients with HCV genotype 2, even when an RVR is achieved. Although the appropriate duration of combination therapy for patients with HCV genotype 2 remains controversial (5–9), shortening the treatment period to 8 weeks is definitely insufficient as an antiviral therapy.

The very low SVR rate in the 8-week group could be accounted for, in part, by the higher pretreatment HCV RNA concentration. Our study did not include patients with a pretreatment HCV RNA concentration $\leq 100 \times 10^3$ IU/ml because only peginterferon monotherapy is allowed by Japanese National Medical Insurance for this patient population. SVR rates reportedly decrease in inverse proportion to pretreatment HCV RNA concentrations (2, 3, 12), and patients with a low pretreatment HCV RNA concentration, as well as those with RVR, reportedly have the highest likelihood of an SVR in response to short-duration therapy (9). Evaluation of the 8-week regimen in patients with HCV genotype 2 and with a low pretreatment HCV RNA concentration ($\leq 100 \times 10^3$ IU/ml) is necessary. Another reason for the very low SVR rate in the 8-week group could be the slightly lower dose of ribavirin used, in comparison with that in previous reports. The dosage of ribavirin was decided according to the manufacturer's recommendations for Japanese patients, which was slightly lower than that for patients in Western countries. The increase in the ribavirin dose, therefore, might have increased the SVR rate in this patient population.

In summary, an 8-week regimen of antiviral combination therapy with peginterferon and ribavirin yielded a very high relapse rate, indicating the limitation of shortened treatment in patients with HCV genotype 2 and who achieved an RVR. However, as well as the report by Mangia *et al.* (6), retreatment of relapsers with the standard 24-week regimen yielded a high SVR rate comparable to that in patients treated initially with the standard 24-week regimen. Reduced durations of therapy may, therefore, be reasonable in patients who experience adverse events and are unlikely to tolerate 24 weeks of therapy as initial treatment. Currently, in patients with HCV genotype 2 and 3 infection and with RVR, a 12-week treatment regimen remains the shortest duration proven to maintain an acceptable rate of relapse as a short course of antiviral combination therapy with peginterferon and ribavirin (6). Further studies will be necessary to investigate the appropriate treatment duration with a sufficient SVR rate and with less adverse effect and less medical expense.

Table 3. Characteristics and outcomes of patients receiving the 8-week regimen

Patient	Age (years)	Gender	Liver histology	HCV genotype	HCV RNA* concentration	HCV RNA at 2 weeks	Result	Time to relapse after the end of treatment (weeks)	Retreatment	Time to retreatment after the end of treatment (months)	HCV RNA† concentration	Result
1	65	M	A1/F1	2a	4400	Positive	Relapse	4	Yes	2	1900	SVR
2	64	F	Not done	2b	2500	Positive	Relapse	4	Yes	1	2100	Replace
3	29	F	A1/F1	2b	660	Negative	SVR					
4	65	M	A2/F1	2b	120	Positive	SVR					
5	28	M	A1/F1	2a	1800	Positive	SVR					
6	63	F	A2/F2	2a	1300	Positive	Relapse	4	Yes	2	590	SVR
7	59	F	A2/F2	2a	160	Positive	Relapse	4	No (refused)			
8	53	M	A2/F2	2a	1100	Positive	SVR					
9	46	F	A1/F1	2a	1000	Positive	Relapse	4	Yes	2	1300	SVR
10	58	F	A1/F1	2a	2100	Positive	Relapse	4	Yes	1.5	1400	SVR
11	66	F	A1/F1	2a	110	Negative	SVR					
12	68	F	A1/F1	2a	1400	Positive	Relapse	4	Yes	1.5	1700	Replace
13	21	F	A1/F1	2a	110	Negative	Relapse	8	Yes	6	70	SVR
14	68	M	A1/F1	2a	1000	Positive	Relapse	4	Yes	1	1200	SVR
15	51	F	A1/F1	2b	550	Negative	Relapse	4	Yes	3	2200	SVR

*Before initial treatment (× 10^3 IU/ml).

†Before retreatment (× 10^3 IU/ml).

No patient required reduction of peginterferon or ribavirin during the initial treatment.

Patient 6 required reduction of the peginterferon dose, and patients 4 and 15 required reduction of the ribavirin during retreatment.

No patient required discontinuation of peginterferon or ribavirin during initial treatment or retreatment.

HCV, hepatitis C virus; SVR, sustained virological response.

References

1. Strader DB, Wright T, Thomas DL, Seeff LB. American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147–71.
2. Hadziyannis SJ, Sette H Jr, Morgan TR, *et al.* Peginterferon-α2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346–55.
3. Manns MP, McHutchison JG, Gordon SC, *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958–65.
4. Zeuzem S, Hultcrantz R, Bourliere M, *et al.* Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients with HCV genotypes 2 or 3. *J Hepatol* 2004; **40**: 993–9.
5. Dalgard O, Bjoro K, Hellum KB, *et al.* Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* 2004; **40**: 1260–5.
6. Mangia A, Santoro R, Minerva N, *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; **352**: 2609–17.
7. von Wagner M, Huber M, Berg T, *et al.* Peginterferon-α-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; **129**: 522–7.
8. Yu M-L, Dai C-Y, Huang J-F, *et al.* A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; **56**: 553–9.
9. Shiffman ML, Suter F, Bacon BR, *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; **357**: 124–34.
10. McHutchison JG, Manns M, Patel K, *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061–9.
11. The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. *Hepatology* 1994; **20**: 15–20.
12. Fried MW, Shiffman ML, Reddy KR, *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.

Journal of Medical Virology 81:446–451 (2009)

Long-Term Follow-Up of Patients With Hepatitis C With a Normal Alanine Aminotransferase

Takashi Kumada,* Hidenori Toyoda, Seiki Kiriyama, Yasuhiro Sone, Makoto Tanikawa, Yasuhiro Hisanaga, Akira Kanamori, Hiroyuki Atsumi, Makiko Takagi, Satoshi Nakano, Takahiro Arakawa, and Masashi Fujimori

Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Gifu, Japan

An attempt was made to identify factors influencing the cumulative probability of an increased alanine aminotransferase (ALT) level and hepatocarcinogenesis in hepatitis C patients with a normal ALT level initially. A total of 398 consecutive patients with a normal ALT level initially for 6 months or more and follow-up period longer than 3 years during the period January 1995 to December 2004 were included. Patients were classified by ALT level into three groups: Group A (3–20 IU/L), Group B (21–30 IU/L), and Group C (31–35 IU/L). Factors associated with the cumulative probability of increased ALT level and hepotocarcinogenesis were evaluated. Women in groups B and C and men in Group C showed high cumulative probabilities of increased ALT levels. Factors associated with increased ALT were a high ALT level (Group B, relative risk; 1.758 [95% confidence interval: 1.290–2.392], $P < 0.001$, Group C, 3.328 [2.256–4.909], $P < 0.001$), high lactate dehydrogenase level (2.352 [1.445–3.829], $P = 0.001$), or low total cholesterol level (1.957 [1.330–2.882], $P = 0.001$). Factors associated with incidence of hepatocellular carcinoma were increased age (3.088 [1.025–9.308], $P = 0.045$), high ALT level (Group C, 5.803 [1.530–22.066], $P = 0.010$), and high total bilirubin level (8.309 [2.235–30.888], $P = 0.002$). In patients with hepatitis C with a normal ALT level initially, an ALT level of 21–35 IU/L is a risk factor for an increased ALT level and hepatocarcinogenesis. ***J. Med. Virol. 81:446–451, 2009.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis C; hepatitis C virus; normal alanine aminotransferase; hepatocarcinogenesis; long-term follow-up

INTRODUCTION

Hepatitis C virus (HCV) results in numerous complications including chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Disease progression and clinical manifestations are heterogeneous, and the underlying mechanisms are not understood fully [Alberti et al., 1999; Marcellin, 1999; Alberti, 2005]. Approximately 30% of patients with chronic HCV infection show persistently normal alanine aminotransferase (ALT) levels, but the majority of these patients have some degree of histologic liver damage, which is usually mild [Marcellin et al., 1997; Tassopoulos, 1999; Bacon, 2002].

ALT activity is the laboratory marker that is used most extensively for the evaluation of liver disease [Craxi and Almasio, 1996; Pratt and Kaplan, 2000]. However, particularly in the case of chronic hepatitis C, ALT measurement often fails to identify patients with minimal to mild necroinflammatory activity [Zanella et al., 1995; Prati et al., 1996; Puoti et al., 1997]. Thus, determination of the true normal range of ALT activity is important for screening of large populations and for the recognition of occult liver abnormalities.

Recently, combination therapy with pegylated interferon and ribavirin has produced sustained virologic response rates of 53–63% in patients with chronic hepatitis C infection and an increased ALT level [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. Zeuzem et al. [2004] reported that pegylated interferon plus ribavirin combination therapy was safe and effective in patients with persistently normal ALT levels.

Many studies have provided evidence that, although the majority of HCV carriers with a normal ALT level have minimal hepatic changes, a subgroup may have active and progressive liver disease that is difficult to predict based on clinical or biochemical parameters [Puoti, 2003; Alberti et al., 2004]. The association

*Correspondence to: Takashi Kumada, 4-86, Minaminokawa-cho, Ogaki, Gifu 503-8052, Japan.
E-mail: tkumada@he.mirai.ne.jp

Accepted 20 August 2008
DOI 10.1002/jmv.21342
Published online in Wiley InterScience (www.interscience.wiley.com)

between ALT level and prognosis is not established clearly in HCV carriers with normal ALT levels. The aim of this retrospective study was to identify factors influencing the cumulative probability of an increased ALT level and hepatocarcinogenesis in patients with a normal ALT level initially.

MATERIALS AND METHODS

Patients

Five thousand three hundred consecutive patients positive for anti-HCV antibody visited the Department of Gastroenterology of Ogaki Municipal Hospital during the period from January 1995 to December 2004. The long-term prognosis of patients with a normal ALT level initially was evaluated in these cases. Normal ALT was defined at our institution as 3–35 IU/L. All patients included in this study fulfilled the following criteria: (1) positive for anti-HCV by a second- or third- generation enzyme-linked immunosorbent assay, (2) no evidence of hepatitis B virus infection, (3) exclusion of other causes of chronic liver disease (alcohol, hepatotoxic drugs, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, or Wilson's disease), (4) detectable HCV-RNA and normal ALT level for longer than 6 months, (5) follow-up period longer than 3 years, (6) no evidence of hepatocellular carcinoma for at least 3 years from the start of the follow-up period, (7) no interferon treatment, and (8) measurement of ALT more than twice in 1 year. A total of 398 consecutive patients fulfilled these criteria.

All patients were followed-up at least every 6 months. During each follow-up examination, platelets, ALT, aspartate aminotransferase (AST), gamma glutamyl transpeptidase (gamma-GTP), prothrombin time (PT), total bilirubin, cholinesterase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total protein, albumin, and total cholesterol were measured. The patients were classified into the following three groups according to initial ALT values: Group A (3–20 IU/L), Group B (21–30 IU/L), and Group C (31–35 IU/L). In some cases, the HCV genotype was determined and quantitation of HCV-RNA (Amplicor 2, Roche Diagnostics K.K., Tokyo, Japan) was undertaken. To detect early-stage hepatocellular carcinoma, ultrasonography (US), computed tomography (CT), and measurement of tumor markers (alpha-fetoprotein [AFP], *Lens culinaris* agglutinin-reactive AFP, des-γ-carboxyprothrombin) were carried out in all patients at least every 6 months. The median follow-up period was 8.8 years (range: 3.0–14.7 years). The total number of blood examinations was 21,259, and the median number was 23 (range: 6–241).

Statistical Analysis

Statistical analysis was performed with the Statistical Program for Social Science (SPSS 11.5 for Windows, SPSS Japan, Inc., Tokyo, Japan). Continuous variables are expressed as median (range). The Kruskal–Wallis test was used to assess continuous variables with a skewed distribution, and the chi-square test was used to assess categorical variables. Actuarial analyses of cumulative increased ALT levels and hepatocarcinogenesis were performed by the Kaplan–Meier method, and differences were tested with the log-rank test. Bonferroni correction was performed for multiple comparisons. The Cox proportional hazard model and forward selection method were used for univariate and multivariate analyses. Statistical significance was defined as $P < 0.05$.

RESULTS

Baseline patient characteristics are listed in Table I for the three groups. Viral concentration, AST, total

TABLE I. Patient Characteristics[a]

	Group A (n=180)	Group B (n=165)	Group C (n=53)	p
Age (years)	61 (15–89)	60 (11–93)	58 (23–79)	NS
Sex (women/men)	109/71	93/72	21/32	0.0265
Genotype (type 1/type 2)	30/28	57/32	19/12	NS
Viral concentration (KIU/ml)	440 (0.5–3,500)	570 (0.5–7,700)	960 (9.1–4,800)	0.0082
Measurement frequency of ALT	17 (6–118)	25 (6–241)	27 (6–101)	<0.0001
AST (IU/L)	20 (10–60)	26 (15–58)	32 (15–121)	<0.0001
Platelets (10^4/mm^3)	19.9 (5.8–58.8)	18.5 (4.5–31.8)	16.9 (4.9–32.5)	0.0018
PT (%)	100 (26–140)	99 (22–145)	102 (58–125)	NS
Gamma-GTP (IU/L)	18 (6–175)	22 (6–174)	29 (12–475)	NS
Total bilirubin (mg/dl)	0.4 (0.2–2.1)	0.5 (0.2–2.7)	0.5 (0.3–2.0)	0.0027
Cholinesterase (IU/L)	276 (34–622)	280 (65–710)	279 (34–710)	NS
ALP (IU/L)	230 (84–3458)	246 (110–768)	220 (84–659)	0.0091
LDH (IU/L)	174 (92–567)	182 (106–763)	183 (107–360)	0.0300
Total protein (g/dl)	7.1 (4.5–8.0)	7.2 (5.6–9.0)	7.4 (5.3–8.4)	0.0037
Albumin (g/dl)	4.1 (2.0–4.8)	4.2 (2.6–4.9)	4.2 (2.8–4.7)	NS
Total cholesterol (mg/dl)	177 (66–289)	174 (72–301)	164 (66–301)	NS

ALT, alanine aminotransferase, Group A (ALT, 3–20 IU/L), Group B (ALT, 21–30 IU/L), Group C (ALT, 31–35 IU/L); AST, aspartate aminotransferase; PT, prothrombin time; Gamma-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.
[a]Continuous variables are quoted as median (range).

bilirubin, LDH, and total protein were significantly higher in Groups B and C compared to Group A. Platelets were significantly lower in Groups B and C compared to Group A. ALP was significantly higher in Group B compared to Groups A and C.

Incidence of Increased ALT Level

Factors associated with an increased ALT level are listed in Table II. Male sex (risk ratio: 1.389 [95% confidence interval: 1.062–1.817], $P = 0.016$), high ALT level (Group B, 1.732 [1.297–2.346], $P < 0.001$; Group C, 3.400 [2.318–4.896], $P < 0.001$), high AST level (1.675 [1.020–2.750], $P = 0.042$), high total bilirubin level (2.257 [1.287–3.961], $P = 0.005$), high LDH level (2.151 [1.340–3.452], $P = 0.002$), low albumin level (1.712 [1.621–2.717], $P = 0.022$), and low total cholesterol level (2.217 [1.513–3.247], $P < 0.001$) were significantly associated with increased ALT by univariate analysis. The 3-, 5-, and 10-year cumulative incidences of increased ALT were 22.9%, 33.2%, and 48.1%, respectively, in Group A, 37.6%, 50.4%, and 70.3% in Group B, and 69.4%, 73.6%, and 82.9% in Group C. Increased ALT occurred at higher rates in Groups B and C than in Group A and occurred at a higher rate in Group C than in Group B ($P < 0.001$, Fig. 1). Among women, there was a significant difference between Group A and Groups B and C in the cumulative incidence of increased ALT ($P = 0.021$, $P = 0.036$, respectively, Fig. 2), but there was no significant difference between Group B and Group C. In contrast, among men, there were significant differences in the cumulative incidence of increased ALT between Groups A and B and Group C ($P < 0.001$, Fig. 3), but there was no significant difference between Group A and Group B.

TABLE II. Factors Associated With an Increased ALT Level (Univariate Analyses)

	Relative risk (95% CI)	P
Age		
≤60 years	1	0.879
>60 years	0.979 (0.748–1.282)	
Sex		
Women	1	0.016
Men	1.389 (1.062–1.817)	
Genotype		
Type 1	1	0.783
Type 2	1.054 (0.726–1.529)	
Viral concentration		
≤100 KIU/ml	1	0.783
>100 KIU/ml	1.030 (0.678–1.523)	
ALT		
<20 IU/L	1	<0.001
21–30 IU/L	1.732 (1.297–2.346)	<0.001
31–35 IU/L	3.400 (2.318–4.986)	<0.001
AST		
≤40 IU/L	1	0.042
>40 IU/L	1.675 (1.020–2.750)	
Platelets		
$\geq 12.0 \times 10^4/mm^3$	1	0.113
$< 12.0 \times 10^4/mm^3$	1.030 (0.678–1.523)	
PT		
<70%	1	0.958
≥70%	0.984 (0.546–1.774)	
Gamma-GTP		
≤56 IU/L	1	0.667
>56 IU/L	0.894 (0.537–1.489)	
Total bilirubin		
≤1.2 mg/dl	1	0.005
>1.2 mg/dl	2.257 (1.287–3.961)	
Cholinesterase		
≤431 IU/L	1	0.099
>431 IU/L	0.723 (0.491–1.063)	
LDH		
≤250 IU/L	1	0.002
>250 IU/L	2.151 (1.340–3.452)	
Total protein		
≥6.5 g/dl	1	0.644
<6.5 g/dl	1.136 (0.661–1.955)	
Albumin		
≥3.5 g/dl	1	0.022
<3.5 g/dl	1.712 (1.621–2.717)	
Total cholesterol		
≥130 mg/dl	1	
<130 mg/dl	2.217 (1.513–3.247)	0.001

CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PT, prothrombin time; Gamma-GTP, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

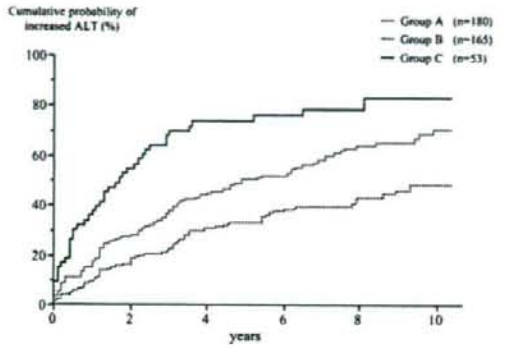

Fig. 1. Incidence of increased ALT for different initial ALT levels. Patients were classified into three groups according to ALT level: Group A (3–20 IU/L), Group B (21–30 IU/L), and Group C (31–35 IU/L). ALT increases at greater rates in Groups B and C compared to the increase in Group A and occurred at a greater rate in Group C compared to that in Group B ($P < 0.001$).

Factors associated with increased ALT analyzed by the Cox proportional hazard model and the forward selection method are listed in Table III. High ALT level (Group B, 1.758 [1.290–2.392], $P < 0.001$; Group C, 3.328 [2.256–4.909], $P < 0.001$), high LDH level (2.352 [1.445–3.829], $P = 0.001$), and low total cholesterol level (1.957 [1.330–2.882], $P = 0.001$) were factors associated significantly with an increased ALT level.

Incidence of Hepatocellular Carcinoma

Hepatocellular carcinoma occurred in 16 of 398 patients (4%) in this follow-up study. The 3-, 5-, and 10-year cumulative incidences of hepatocellular carcinoma were none, none, and 4%, respectively, in Group A, none, 1.3%, and 4.3% in Group B, and none, 7.8%, and

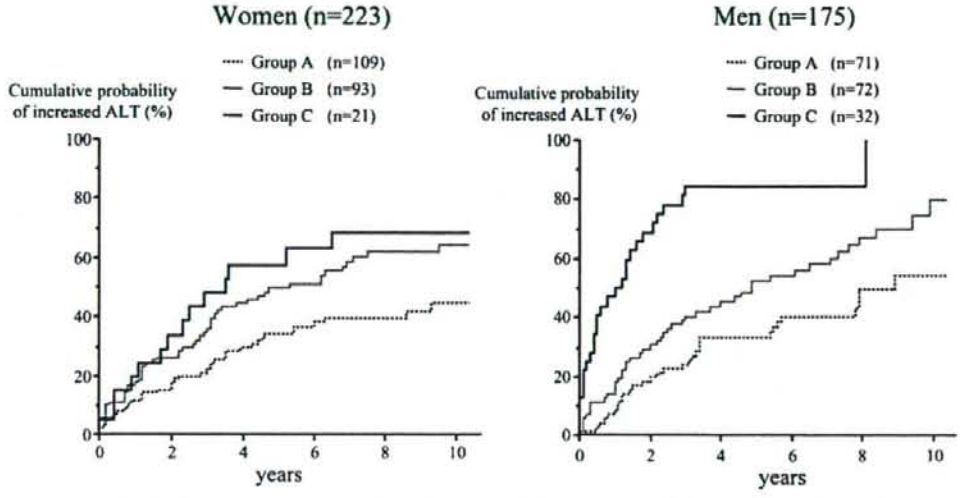

Fig. 2. Relation between sex and incidence of increased ALT. In women (left), the cumulative incidence of increased ALT was significantly different between Group A and Groups B and C ($P = 0.021$, $P = 0.036$) but not between Group B and Group C. In men (right), the cumulative incidence of increased ALT differed significantly between Groups A and B and Group C ($P < 0.001$) but not between Group A and Group B.

14.4% in Group C. The cumulative incidence of hepatocellular carcinoma differed significantly between Group A and Group C ($P = 0.017$, Fig. 3) but not between Group A and Group B, or between Group B and Group C. Factors associated with the incidence of hepatocellular carcinoma analyzed by Cox proportional hazards modeling and the forward selection method are listed Table IV. Increased age (3.088 [1.025–9.308], $P = 0.045$), a high ALT level (Group C, 5.803 [1.530–22.066], $P = 0.010$), and high total bilirubin level (8.309 [2.235–30.888], $P = 0.002$) were factors associated significantly with the incidence of hepatocellular carcinoma.

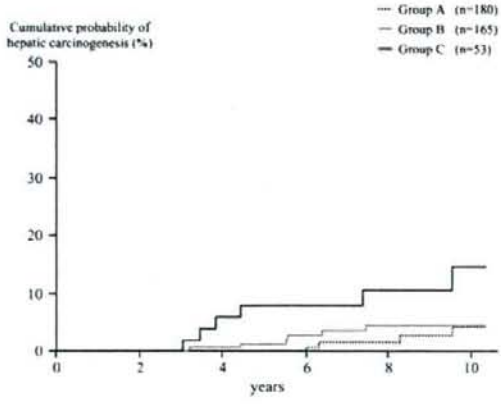

Fig. 3. Incidence of hepatic carcinogenesis in HCV carriers with a normal ALT level initially. The cumulative incidence of hepatic carcinogenesis differed significantly between Group A and Group C ($P = 0.017$) but not between Group A and Group B or between Group B and Group C.

DISCUSSION

Criteria for the subgroup of HCV patients with a persistently normal ALT level differ with respect to the most appropriate cut-off for defining ALT normality in patients infected chronically with HCV and the most appropriate time frame and algorithm for defining a persistently normal ALT level. The current upper limit for normal ALT in patients with HCV is approximately 40 IU/L (range: 30–50 IU/L) [Alberti et al., 1992; Zanella et al., 1995; Prati et al., 1996; Pratt and Kaplan, 2000]. Studies have suggested that the normal values currently used clinically may be low [Piton et al., 1998; Hayashi et al., 2000; Prati et al., 2002]. Prati et al. [2002] reported that the upper limit for normal ALT was 19 IU/L in women and 30 IU/L in men after excluding patients with undiagnosed HCV infection and those with behavioral risk for blood-borne disease. In this study,

TABLE III. Factors Associated With an Increased ALT Level (Multivariate Analysis)

	Relative risk (95% CI)	P
ALT		
<20 IU/L	1	<0.001
21–30 IU/L	1.758 (1.292–2.392)	<0.001
31–35 IU/L	3.328 (2.256–4.909)	<0.001
LDH		
≤250 IU/L	1	0.001
≥250IU/L	2.352 (1.445–3.829)	
Total cholesterol		
≥130 mg/dl	1	0.001
<130 mg/dl	1.957 (1.330–2.882)	

CI, confidence interval; ALT, alanine aminotransferase; LDH, lactate dehydrogenase.

TABLE IV. Factors Associated With Hepatic Carcinogenesis (Multivariate Analysis)

	Relative risk (95% CI)	P
Age (years)		
≤60	1	0.045
>60	3.088 (1.025–9.308)	
ALT		
<20 IU/L	1	0.017
21–30 IU/L	1.515 (0.415–5.530)	0.529
31–35 IU/L	5.803 (1.530–22.066)	0.010
Total bilirubin		
≤1.2 mg/dl	1	0.002
>1.2 mg/dl	8.309 (2.235–30.888)	

CI, confidence interval; ALT, alanine aminotransferase.

there was a significant difference in the cumulative incidence of increased ALT between Group A (ALT 3–20 IU/L) and Groups B (21–30 IU/L) and C (31–35 IU/L) among women. In contrast, among men, there were significant differences between Groups A and B and Group C. This is an interesting and important finding, suggesting that the upper limit for a normal ALT level should be revised to 30 IU/L for male patients and 20 IU/L for female patients with HCV infection. These data are consistent with the healthy limits in women and men proposed by Prati et al. [2002].

A persistently normal ALT level in patients with chronic hepatitis C has been defined generally as consecutive measurements within the normal range during a 6-month period [Marcellin et al., 1997; EASL International Consensus Conference on Hepatitis C, 1999]. In this study, patients with a normal ALT level for at least 6 months were selected according to this definition.

The natural course of HCV infection in patients with a normal ALT level is not understood fully. HCV patients with an initially and persistently normal ALT level may show disease progression on long-term follow-up as a consequence of transient ALT flares or of persistent biochemical reactivation of their liver disease. The incidence of ALT flares and/or of durable reactivation reported in HCV patients with a normal ALT level initially is variable and ranges from 15% to 27.5%, mainly as a consequence of different inclusion criteria and follow-up periods [Ohmiya et al., 2000; Tsuji et al., 2001; Puoti et al., 2002; Okanoue et al., 2005]. In the present study, the 3-, 5-, and 10-year cumulative incidences of increased ALT were 35.3%, 45.8%, and 62.5%, respectively. Thus, the longer the follow-up period, the more the incidence increased. Factors associated with increased incidence of increased ALT were gender, ALT, AST, total bilirubin, LDH, albumin, and total cholesterol level. Among these, the ALT level was associated most strongly with increased ALT. However, Puoti et al. [2002] reported that baseline ALT levels (<20 IU/L vs. >21 IU/L) did not correlate with the incidence of ALT flares. Further research is necessary to reconcile these data.

Hepatocellular carcinoma occurred in 16 of 398 patients (4%) in the present study. Factors associated with hepatic carcinogenesis were increased age, gender, platelets, ALT, ALP, total cholesterol, and albumin in patients with abnormal ALT level [Kumada et al., 2007]. Thus, it is important to maintain a normal or low ALT level to prevent hepatic carcinogenesis.

Recently, the results of the first large multinational trial of a pegylated interferon plus ribavirin combination regimen in patients with a persistently normal ALT level became available [Zeuzem et al., 2004]. A total of 491 patients were assigned at random to three groups: either 24 or 48 weeks of treatment with pegylated interferon alfa-2a plus 800 mg/day ribavirin, or no treatment for 72 weeks. The overall sustained response rates were 30% and 52% in patients treated for 24 and 48 weeks, respectively, and the treatment outcome was equal in patients with an increased ALT level initially [Manns et al., 2001; Fried et al., 2002]. Median ALT levels were decreased consistently from the baseline level in treatment responders, confirming that, in many HCV patients with a normal ALT level according to current cut-off limits, ALT levels were in fact abnormal, reflecting possible underlying liver disease, and were normalized following successful antiviral therapy to what might be considered a true healthy level.

In conclusion, Groups B and C of women with ALT levels of 21–35 IU/L (corresponding to Groups B and C), Group C of men with ALT levels of 31–35 IU/L (corresponding to Group C) should be treated, taking into consideration patient age, motivation, and the possibility of complications. Maintenance of a low ALT level may prevent hepatic carcinogenesis, even in patients with ALT levels in the normal range.

REFERENCES

Alberti A. 2005. Towards more individualized management of hepatitis C virus patients with initially or persistently normal alanine aminotransferase levels. J Hepatol 42:266–274.

Alberti A, Morsica G, Chemello L, Cavalletto D, Noventa F, Pontisso P, Ruol A. 1992. Hepatitis C viraemia and liver disease in symptom-free individuals with anti-HCV. Lancet 340:697–698.

Alberti A, Chemello L, Benvegnu L. 1999. Natural history of hepatitis C. J Hepatol 31:S17–S24.

Alberti A, Benvegnu L, Boccato S, Ferrari A, Sebastiani G. 2004. Natural history of initially mild chronic hepatitis C. Dig Liver Dis 36:646–654.

Bacon BR. 2002. Treatment of patients with hepatitis C and normal serum aminotransferase levels. Hepatology 36:S179–S184.

Craxi A, Almasio P. 1996. Diagnostic approach to liver enzyme elevation. J Hepatol 25:S47–S51.

EASL International Consensus Conference on Hepatitis C. 1999. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. J Hepatol 30:956–961.

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 347:975–982.

Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Jr., Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM, PEGASYS International Study Group. 2004. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: A randomized study of treatment duration and ribavirin dose. Ann Intern Med 140:346–355.

Hayashi J, Furusyo N, Ariyama I, Sawayama Y, Etoh Y, Kashiwagi S. 2000. A relationship between the evolution of hepatitis C virus variants, liver damage, and hepatocellular carcinoma in patients with hepatitis C viremia. J Infect Dis 181:1523–1527.

Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kanamori A, Kondo J, Yamauchi T, Nakano S. 2007. Relation between incidence of hepatic carcinogenesis and integration value of alanine aminotransferase in patients with hepatitis C virus infection. Gut 56:738–739.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomized trial. Lancet 358:958–965.

Marcellin P. 1999. Hepatitis C: The clinical spectrum of the disease. J Hepatol 31:S9–S16.

Marcellin P, Levy S, Erlinger S. 1997. Therapy of hepatitis C: Patients with normal aminotransferase levels. Hepatology 26:S133–S136.

Ohmiya M, Hayashi J, Ueno K, Furusyo N, Sawayama Y, Kawakami Y, Kinukawa N, Kashiwagi S. 2000. Effectiveness of interferon treatment for patients with chronic hepatitis C virus infection and normal aminotransferase levels. Dig Dis Sci 45:1953–1958.

Okanoue T, Makiyama A, Nakayama M, Sumida Y, Mitsuyoshi H, Nakajima T, Yasui K, Minami M, Itoh Y. 2005. A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. J Hepatol 43:599–605.

Piton A, Poynard T, Imbert-Bismut F, Khalil L, Delattre J, Pelissier E, Sansonetti N, Opolon P. 1998. Factors associated with serum alanine transaminase activity in healthy subjects: Consequences for the definition of normal values, for selection of blood donors, and for patients with chronic hepatitis C. MULTIVIRC Group. Hepatology 27:1213–1219.

Prati D, Capelli C, Zanella A, Mozzi F, Bosoni P, Pappalettera M, Zanuso F, Vianello L, Locatelli E, de Fazio C, Ronchi G, del Ninno E, Colombo M, Sirchia G. 1996. Influence of different hepatitis C virus genotypes on the course of asymptomatic hepatitis C virus infection. Gastroenterology 110:178–183.

Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. 2002. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med 137:1–10.

Pratt DS, Kaplan MM. 2000. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 342:1266–1271.

Puoti C. 2003. HCV carriers with persistently normal aminotransferase levels: Normal does not always mean healthy. J Hepatol 38:529–532.

Puoti C, Magrini A, Stati T, Rigato P, Montagnese F, Rossi P, Aldegheri L, Resta S. 1997. Clinical, histological, and virological features of hepatitis C virus carriers with persistently normal or abnormal alanine transaminase levels. Hepatology 26:1393–1398.

Puoti C, Castellacci R, Montagnese F, Zaltron S, Stornaiuolo G, Bergami N, Bellis L, Precone DF, Corvisieri P, Puoti M, Minola E, Gaeta GB. 2002. Histological and virological features and follow-up of hepatitis C virus carriers with normal aminotransferase levels: The Italian prospective study of the asymptomatic C carriers (ISACC). J Hepatol 37:117–123.

Tassopoulos NC. 1999. Treatment of patients with chronic hepatitis C and normal ALT levels. J Hepatol 31:S193–S196.

Tsuji K, Yamasaki K, Yamanishi M, Kawakami M, Shirahama S. 2001. Risk of alanine aminotransferase flare-up among asymptomatic hepatitis C virus RNA carriers: A 10-year follow-up study. J Gastroenterol Hepatol 16:536–540.

Zanella A, Conte D, Prati D, Mozzi F, Capelli C, Zanuso F, Fraquelli M, Bosoni P, Vianello L, Pappalettera M, Velio P, Montagnolo G, Bianchi PA, Sirchia G. 1995. Hepatitis C virus RNA and liver histology in blood donors reactive to a single antigen by second-generation recombinant immunoblot assay. Hepatology 21:913–917.

Zeuzem S, Diago M, Gane E, Reddy KR, Pockros P, Prati D, Shiffman M, Farci P, Gitlin N, O'Brien CB, Lamour F, Lardelli P. PEGASYS Study NR16071 Investigator Group. 2004. Peginterferon alfa-2a (40 kiloDaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. Gastroenterology 127:1724–1732.

Journal of Medical Virology 81:459–466 (2009)

Mutations in the Interferon Sensitivity-Determining Region of Hepatitis C Virus Genotype 2a Correlate With Response to Pegylated-Interferon-Alpha 2a Monotherapy

Kazuhiko Hayashi,[1] Yoshiaki Katano,[1]* Takashi Honda,[1] Masatoshi Ishigami,[1] Akihiro Itoh,[1] Yoshiki Hirooka,[1] Isao Nakano,[1] Fumihiro Urano,[2] Kentaro Yoshioka,[3] Hidenori Toyoda,[4] Takashi Kumada,[4] and Hidemi Goto[1]

[1]*Department of Gastroenterology, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya, Japan*
[2]*Department of Gastroenterology, Toyohashi Municipal Hospital, Toyohashi, Japan*
[3]*Division of Liver and Biliary Diseases, Department of Internal Medicine, Fujita Health University, Kutsukake-cho, Toyoake, Japan*
[4]*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki, Japan*

The interferon sensitivity-determining region (ISDR) is thought to be inhibited by the double-stranded RNA-dependent protein kinase (PKR). Several studies have reported a relationship between the ISDR and interferon (IFN) responsiveness. However, this relationship is controversial. The aim of this study was to investigate whether genomic heterogeneity of the ISDR among patients with hepatitis C virus (HCV) genotype 2a affects the response to pegylated-IFN-alpha 2a monotherapy. Eighty patients (47 men, 33 women; mean age: 54.2 ± 12.9 years) infected with HCV genotype 2a were evaluated. HCV viral loads were determined by real-time PCR. The ISDR (amino acids 2193–2228) was examined by direct sequencing. Thirty-one patients received subcutaneous injections of pegylated-IFN-alpha 2a (180 μg) once weekly for 24 weeks, and 35 patients received injections for 48 weeks. Fourteen patients withdrew from treatment. Of the remaining 66 patients, 51 (77.3%) showed a sustained virologic response. Factors related to sustained virologic response on multivariate analysis were rapid virologic response (negative HCV at 4 weeks; odds ratio: 0.033; 95% confidence interval (95% CI) 0.003–0.363; $P = 0.0052$) and the number of mutations in the ISDR (odds ratio: 0.025; 95% CI 0.001–0.476; $P = 0.0141$). There were no significant differences in other factors, including sex, age, aspartate aminotransferase, alanine aminotransferase, platelet count, duration of treatment, and HCV viral load. Rapid virologic response and the ISDR sequence variations are significantly associated with response to pegylated-IFN-alpha 2a monotherapy in Japanese patients with HCV genotype 2a. ***J. Med. Virol. 81:459–466, 2009.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: sustained virologic response; rapid virologic response; chronic hepatitis C

INTRODUCTION

Hepatitis C virus (HCV) is a member of the *Flaviviridae* family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma (HCC) [Seeff, 2002]. HCV infection is a significant global health problem, affecting 170 million individuals worldwide. HCV consists of three structural proteins (core, envelope 1, and envelope 2) and six non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). HCV NS5A protein was reported to have a domain associated with interferon (IFN) response. This domain, located in the NS5A region of HCV, is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR) [Enomoto et al., 1996; Murakami et al., 1999; Nakano et al., 1999; Pascu et al., 2004]. There are several modes of IFN action

Grant sponsor: Hori Information Science Promotion Foundation.

*Correspondence to: Yoshiaki Katano, MD, PhD, Department of Gastroenterology, Nagoya University Graduate School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466-8550, Japan. E-mail: ykatano@med.nagoya-u.ac.jp

Accepted 21 October 2008
DOI 10.1002/jmv.21407
Published online in Wiley InterScience (www.interscience.wiley.com)

against HCV infection, and the final mode is still under debate. However, one mechanism of IFN action involves inhibition of viral replication by inducing the double-stranded RNA-dependent protein kinase (PKR). The ISDR is located in the 5′ end of the PKR-binding domain and is inhibited by PKR in vitro [Gale et al., 1998]. Therefore, ISDR heterogeneity is an important factor that may affect response to IFN. The utility of ISDR sequences for predicting IFN responsiveness has been investigated for HCV genotype 1b, as well as for genotypes 2 and 3, because HCV genotypes, which vary in prevalence around the world, influence IFN responsiveness [Manns et al., 2001; Fried et al., 2002; Simmonds et al., 2005]. HCV genotype 2a is relatively common in Japan [Enomoto et al., 1990; Hayashi et al., 2003]. However, there are few reports regarding the ISDR and IFN responsiveness in HCV genotype 2a [Murakami et al., 1999; Kobayashi et al., 2002; Akuta et al., 2005], and the association of mutations in the ISDR and response to IFN therapy among patients with HCV genotype 2a is not well understood. The aim of the present study was to determine whether genomic heterogeneity of the ISDR among patients with HCV genotype 2a affects the response to pegylated-IFN-alpha 2a monotherapy.

MATERIALS AND METHODS

This prospective analysis involved 80 patients with chronic hepatitis C who received pegylated-IFN-alpha 2a monotherapy between January 2004 and December 2005. Patients who were previously treated with IFN were excluded. All patients were positive for serum anti-HCV antibody on a commercial enzyme-linked immunosorbent assay (Dinabot, Tokyo, Japan) and for HCV-RNA on a commercial polymerase chain reaction (PCR) test (Roche Diagnostic Systems, Tokyo, Japan). No patients had hepatitis B surface antigen, coinfection with human immunodeficiency virus, autoimmune disease, or chronic alcohol abuse.

Schedule of IFN Therapy

Patients received pegylated-IFN-alpha 2a (Pegasys Roche, Tokyo, Japan) at a dose of 180 μg injected subcutaneously once weekly for 24 or 48 weeks. The patients were allocated, at the discretion of the physician in charge, to a protocol lasting either 24 or 48 weeks. Laboratory tests and evaluation of adverse events were performed once weekly during treatment. The pegylated-IFN-alpha 2a dose was dropped to 90 μg when clinically significant adverse events or laboratory abnormalities such as neutropenia (<750 cells/mm^3) or thrombocytopenia (<50,000 cells/mm^3) occurred. Pegylated-IFN-alpha 2a was discontinued when neutropenia (<250 cells/mm^3) or a platelet count below 25,000 cells/mm^3 was observed. Patients who did not receive 80% of the ideal total dose of IFN were defined as the reduced-dose group. Serum HCV-RNA levels were examined at 4, 12 weeks, at the end of IFN therapy, and 6 months after the end of treatment. Serum was stored at −80 °C for virologic examination. Patients who were persistently negative for serum HCV-RNA and who had a normal serum alanine aminotransferase (ALT) level 24 weeks after withdrawal of IFN treatment were considered to have a sustained virologic response. Patients who were HCV-negative at the end of the treatment but returned to HCV-positive status after withdrawal of IFN were defined as virologic relapsers. Patients who did not become HCV-negative with IFN therapy were defined as virologic non-responders. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virologic Tests

The HCV-RNA quantitative viremia load was determined using real-time PCR [Takeuchi et al., 1999]. HCV was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions, as described previously [Otagiri et al., 2002; Hayashi et al., 2003]. The genotypes were classified according to the nomenclature proposed by a previous report [Simmonds et al., 2005]. Direct sequencing of the ISDR region was performed using serum samples taken within 2 days before the first administration of pegylated-IFN-alpha 2a. In brief, RNA was extracted from 140 μl of sera with a commercial kit (QIAamp Viral RNA Kit; Qiagen, Valencia, CA) and dissolved in 50 μl of diethylpyrocarbonate-treated water. Ten nanograms of the RNA was used for reverse transcription using the oligo and random hexamer primers of a commercial kit (iScript cDNA Synthesis Kit; Bio-Rad, Hercules, CA). The ISDR was amplified by hemi-nested PCR. In brief, each 50-μl PCR reaction contained 100 nM of each primer, 1 ng template cDNA, 5 μl of GeneAmp 10× PCR buffer, 2 μl of dNTPs, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primer sequences were sense, 5′-ACGTCCATGCTAACAGACCC-3′ and antisense, 5′-GGGAATCTCTTCTTGGGGAG-3′. Amplification conditions consisted of 10 min at 94 °C followed by 40 cycles of 94 °C for 10 sec, 55 °C for 30 sec, and 72 °C for 30 sec in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The second PCR was done in the same reaction buffer with the first-round PCR product as the template, the sense primer from the first-round PCR, and a new antisense primer, 5′-CGAGAGAGTC-CAGAACGACC-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers using a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems). The neighbor-joining method was used for phylogenetic analysis of the ISDR (amino acids 2193–2228) in the NS5A region [Saitou and Nei, 1987], and bootstrap analysis (1,000 replicates) was performed [Felsenstein, 1985].

Statistical Analysis

The data are expressed as mean ± standard deviation (SD). The paired *t*-test was used to analyze differences in the variables. A *P*-value of <0.05 was considered statistically significant. Multiple logistic regression models were used to identify factors predictive of sustained virologic response. Statview 5.0 software (SAS Institute Inc., Cary, NC) was used for all analyses.

RESULTS

The patients' clinical characteristics are summarized in Table I. All patients were infected with HCV genotype 2a, and 27 of 80 (33.8%) patients had a serum HCV-RNA level higher than 1 million copies/ml. Eighty patients were initially entered, but 14 patients withdrew from IFN therapy, and 4 of these 14 patients could not be followed-up. The remaining 66 patients were followed-up for 6 months after the end of treatment. The completion rate was 82.5% (66/80). Thirty-one patients were treated with pegylated-IFN-alpha 2a for 24 weeks, and 35 patients were treated for 48 weeks. Virologic response is shown in Table II. The rapid virologic response rate, which was defined as negativity for HCV after 4 weeks of treatment, was 74.2% (49/66). The early virologic response rate, which was characterized by undetectable HCV at 12 weeks, was 92.4% (61/66). The virologic response rate at the end of the treatment was 97.0% (64/66). Finally, 51 of 66 (77.3%) patients achieved sustained virologic response. There were no significant differences in clinical characteristics and virologic response between patients treated for 24 weeks and those treated for 48 weeks. ISDR sequences were obtained in 62 patients, and the sequence alignments of the ISDR according to virologic response are shown in Figure 1. The mean number of ISDR mutations in patients with non-sustained virologic response was 1.2 ± 0.6, and that in patients with sustained virologic response was 2.8 ± 2.1. Patients with sustained virologic response had a significantly higher number of mutations in the ISDR than did patients with non-sustained virologic response ($P = 0.0090$). Codon 2205 was frequently changed. The association of this single mutation with sustained virologic response was examined; however, there was no significant relationship between a single mutation at codon 2205 and sustained virologic response. Sequences of the HCJ6 strain and the HCJ6 strain with all nucleotide substitutions in codon 2205 were defined as the wild type, and ISDR sequences that deviated from these strains were defined as mutant type. A rapid virologic response was achieved in 7 of 33 patients with wild-type ISDR and 5 of 41 patients with mutant-type ISDR. There were no correlations between rapid virologic response and ISDR sequence. Mutant-type ISDR was detected more frequently in sustained virologic response patients (66.7%) than in non-SVR patients (28.6%) (odds ratio: 0.200; 95% confidence interval (95% CI) 0.054–0.738; $P = 0.015$). Phylogenetic analyses of the ISDR (amino acids 2193–2228) of the 62 patients were performed, and the results are shown in Figure 2. There were differences in distinctive clustering between the wild type and the mutant type defined by counting the number of substitutions in the ISDR, but no distinctive clustering was observed in wild types with A2205 and with T2205 and with V2205. The phylogenetic analyses did not show a significant relationship between the ISDR sequences and sustained virologic response. The clinical characteristics of the patients who achieved sustained virologic response are compared to those without sustained virologic response in Table III. There were significant differences in four factors (age, HCV-RNA level, the number of mutations in the ISDR, and rapid virologic response) between the sustained virologic response group and the non-sustained virologic response group on univariate analysis. The results of the multivariate analyses of factors predictive of sustained virologic response are shown in Table IV. The variables were recorded categorically as ordinal data. The background factors were: age (<60 years vs. ≥60 years); sex (male vs. female); platelet count ($<15 \times 10^4/mm^3$ vs. $\geq 15 \times 10^4/mm^3$); HCV-RNA level ($<10^6$ copies/ml vs. $\geq 10^6$ copies/ml); ALT levels (<70 IU/L vs. ≥70 IU/L); AST levels (<60 IU/L vs. ≥60 IU/L); length of IFN therapy (24 weeks vs. 48 weeks); reduction of IFN dose (yes or no); ISDR (wild type vs. mutant type); and rapid virologic response (yes or no). Rapid virologic response at 4 weeks was the most influential factor ($P = 0.0052$), followed by mutations in the ISDR ($P = 0.0141$). No other factors achieved statistical significance. Analysis of rapid virologic response in combination with the ISDR revealed that 28 of 29 patients with mutant-type ISDR and rapid virologic response achieved sustained virologic response. The positive predictive value for sustained virologic response was 96.6% (28/29). IFN therapy was withdrawn from 14 patients. The reasons for discontinuing therapy, length of IFN therapy, ISDR sequences, rapid virologic response, and outcomes are shown in Figure 3. Ten patients discontinued therapy within 16 weeks, but 4 of the 10 patients achieved sustained virologic response. All sustained virologic response patients who withdrew from therapy within 16 weeks had at least three ISDR mutations.

TABLE I. Clinical Characteristics

	N = 80
Age (y.o.)	54.2 ± 12.9
Sex: male/female	47/33
AST (IU/L)	57.9 ± 37.5
ALT (IU/L)	81.1 ± 65.3
Platelet count ($10^4/\mu l$)	20.7 ± 22.2
HCV-RNA level (copies/ml)	360,000 (540–63,000,000)
Body weight (kg)	60.8 ± 9.8

Data are expressed as mean ± standard deviation. HCV-RNA level was shown by median (range). AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

TABLE II. Virologic Response Rates

	All (n = 66)	24 weeks (n = 31)	48 weeks (n = 35)
Rapid virologic response	74.2% (n = 49)	77.4% (n = 24)	71.4% (n = 25)
Early virologic response	92.4% (n = 61)	96.8% (n = 30)	88.6% (n = 31)
End of treatment response	97.0% (n = 64)	96.8% (n = 30)	97.1% (n = 34)
Sustained virologic response	77.3% (n = 51)	77.4% (n = 24)	77.1% (n = 27)

Rapid virologic response as HCV-negative at 4 weeks. Early virologic response as HCV-negative at 12 weeks. End of treatment response as HCV-negative at the end of the treatment. Sustained virologic response as HCV-negative at 24 weeks after withdrawn of treatment.

DISCUSSION

HCV genotype is one of the most important factors that predict response to IFN therapy. Genotypes 1 and 4 respond poorly to IFN therapy, whereas genotypes 2 and 3 show a sustained virologic response to IFN therapy. However, patients infected with HCV genotype 2 respond differently to IFN therapy, suggesting that an additional viral factor associated with resistance to IFN exists. The ISDR sequence in the HCV NS5A region may influence the IFN response of patients with HCV genotype 1b [Enomoto et al., 1996; Nakano et al., 1999; Pascu et al., 2004]. The influence of the ISDR sequence in response to IFN has been investigated in patients with HCV genotypes 2a and 2b [Murakami et al., 1999; Kobayashi et al., 2002; Akuta et al., 2005]. In the present study, it was hypothesized that the amino acid variations in ISDR would explain differences in IFN resistance in patients infected with HCV genotype 2a. Multivariate analyses showed that mutation of the ISDR is one of the most influential factors for sustained virologic response (odds ratio: 0.025; 95% CI 0.001–0.476; $P = 0.0141$). The sustained virologic response rate of patients with more than three mutations in the ISDR was 100% (23/23) in the present study. The results confirmed that the number of mutations in the ISDR is an important determinant of the effectiveness of pegylated-IFN-alpha 2a monotherapy in patients with

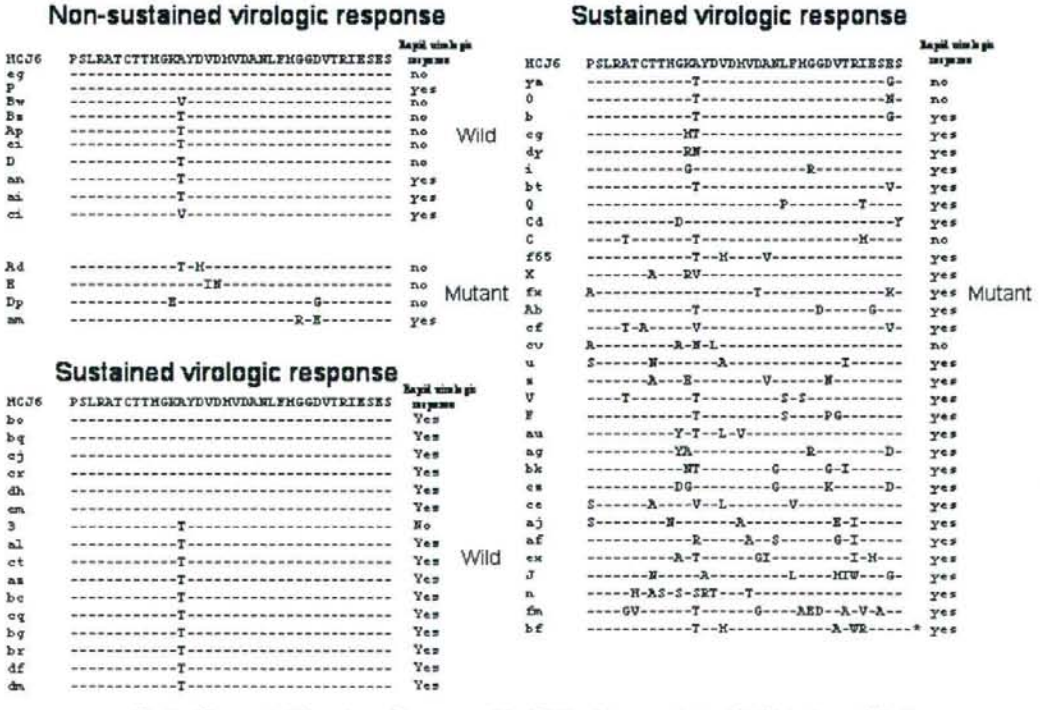

Fig. 1. Alignment of the amino acid sequence of the ISDR and response to pegylated-interferon-alpha 2a therapy. In the sequence alignment, dashes indicate amino acids identical to consensus sequence HCJ6. Sequences of the HCJ6 strain and the HCJ6 strain with all nucleotide substitutions in codon 2205 were defined as wild-type ISDR, and the other strains were defined as mutant-type ISDR. The strain marked with an asterisk had an insertion mutation. ISDR, interferon sensitivity-determining region.

Fig. 2. Results of phylogenetic analysis of 62 sequences from the interferon sensitivity-determining region (amino acids 2193–2228) and relationship with the response to pegylated-interferon-alpha 2a therapy. Phylogenetic analysis was performed by the neighbor-joining method. HCVJ, which is the prototype of genotype 1b, was used as the outer group. The scale bar indicates genetic distance. Each strain from the present study is shown with original code followed by the virologic response. All strains without description of virologic response were rapid virologic response and sustained virologic response. Definition of wild type was counting the number of substitution in the ISDR.

TABLE III. Clinical Characteristics of Patients With or Without Sustained Virologic Response

Factors	Sustained virologic response (n=51)	Non-sustained virologic response (n=15)	*P*-value
Age (y.o.)	52.7 ± 13.1	60.3 ± 6.8	0.0356
Gender: male/female	33/18	6/9	0.1346
ALT (IU/L)	75.6 ± 57.7	66.8 ± 14.1	0.6002
AST (IU/L)	51.8 ± 29.4	56.5 ± 40.1	0.6218
PLT ($\times 10^4$/mm^3)	18.5 ± 6.0	15.5 ± 5.4	0.0866
HCV-RNA level (copies/ml)	340,000 (2,600–63,000,000)	1,400,000 (50,000–22,000,000)	0.0067
Reduction: yes/no	8/43	6/9	0.0691
Duration: 24 weeks/48 weeks	24/27	7/8	0.9999
Mutations in the ISDR	2.8 ± 2.1	1.2 ± 0.6	0.0090
Rapid virologic response: yes/no	44/7	5/10	0.0001

Data are expressed as mean ± standard deviation. HCV-RNA level was shown by median (range). AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

HCV genotype 2a. In addition, 10 patients discontinued IFN therapy within 16 weeks in the present study; 4 of these 10 patients achieved sustained virologic response. All sustained virologic response patients who discontinued IFN therapy were infected with mutant-type ISDR. Thus, the mutant-type ISDR appears to be associated with good response to IFN. The ISDR sequence variation of HCV genotype 2a may also play an important role as a predictor of IFN responsiveness. However, most Western reports have not confirmed the clinical usefulness of ISDR analysis for predicting response to IFN therapy [Zeuzem et al., 1997; Chung et al., 1999; Squadrito et al., 2002]. Bias relating to the IFN therapy regimens, racial differences, and HCV strains may have produced this conflicting result. To investigate the role of the ISDR while avoiding bias, all of the patients in the present study were infected with genotype 2a and received pegylated-IFN-alpha 2a monotherapy. Most studies that did not find ISDR analysis useful had a lower dose of IFN than those that reported that ISDR analysis was useful (3 million units vs. 6–10 million units). A low IFN dose was associated with a low sustained virological response rate. The present study and the studies that confirmed the usefulness of ISDR analysis had a higher sustained virological response rate (mean 50.5%) than those that did not confirm the usefulness of ISDR analysis (mean 9.6%) [Enomoto et al., 1996; Zeuzem et al., 1997; Chung et al., 1999; Murakami et al., 1999; Nakano et al., 1999; Squadrito et al., 2002]. The low sustained virological response rate, as well as the low IFN dose, would not favor the use of ISDR analysis for predicting IFN responsiveness. The number of substitutions in the ISDR in reports with negative results was significantly smaller than in studies that confirmed the correlation between ISDR mutations and IFN responsiveness [Herion and Hoofnagle, 1997]. The present study and other studies that confirmed the association between ISDR mutations and IFN sensitivity frequently found that the patients had ISDR mutant type [Saiz et al., 1988; Murakami et al., 1999; Nakano et al., 1999]. The prevalence of patients infected with ISDR mutant type would affect the association between ISDR sequence and IFN responsiveness. Thus, a study including a large number of patients with two or more amino acid substitutions in the ISDR would be suitable for using the ISDR system to predict sustained virologic response. The original classification for the ISDR sequence of genotype 1b included three categories (wild, intermediate, and mutant) according to the number of amino acid substitutions compared to the HCVJ strain. In the present study, sequences of the HCJ6 strain and the HCJ6 strain with all amino acid substitutions in codon 2205 were defined as the wild type, and the other strains were mutant type. The classification for the ISDR sequence was minimally modified for ease of analysis

TABLE IV. Multivariate Analysis: Factors Predictive of Sustained Virologic Response

Factors	*P*-value	Risk ratio	95% CI	
Age: <60 years	0.0554	8.306	0.952	72.486
Sex: male	0.8270	1.228	0.194	7.778
ALT: <70 IU/L	0.5065	0.227	0.003	17.976
AST: <60 IU/L	0.9923	1.020	0.018	58.089
PLT: <15 $\times 10^4$/mm^3	0.1528	0.154	0.012	2.001
HCV-RNA level: <10^6 copies/ml	0.4830	0.437	0.043	4.425
Reduction: yes	0.2242	0.187	0.013	2.790
Duration: 48 weeks	0.1016	8.100	0.662	99.135
ISDR: wild	0.0141	0.025	0.001	0.476
Rapid virologic response: no	0.0052	0.033	0.003	0.363

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region.

Characteristics of the 14 patients withdrawing the treatment

length	reasons	HCJ6	PSLRATCTTHGKAYDVDMVDANLFMGGDVTRIESES	Rapid virologic response	effect
3wks	economic	none	//////////////////////////////////////	no	relapse
4wks	fatigue	none	//////////////////////////////////////	yes	Sustained virologic response
5wks	economic	ah	----------T----------	no	No virologic response
9wks	rash	bu	----------D----------R----W-----	yes	Sustained virologic response
10wks	ALT elevate	G	S------A----T----LT----------I------	yes	Sustained virologic response
10wks	moving	ca	----------	yes	dropout
11wks	ALT elevate	ec	----------R----------	no	relapse
12wks	unknown	fv	----------	yes	dropout
13wks	fatigue	aq	----------M----S--S-------G-	yes	Sustained virologic response
15wks	ineffective	Y	----T-------T----------	no	No virologic response
20wks	unknown	cs	----T--A--------A----------I------	yes	dropout
27wks	depression	L	A----------V----------G-V------	yes	Sustained virologic response
28wks	moving	h	----------YC----------	yes	dropout
40wks	pneumonia	r	--------YG---H-M----------	yes	Sustained virologic response

Fig. 3. Clinical characteristics of the 14 patients who withdrew from pegylated-interferon-alpha 2a therapy. Reasons for discontinuing therapy, length of therapy, alignment of the amino acid sequence of the ISDR, rapid virologic response, and response to IFN therapy are shown. ISDR, interferon sensitivity-determining region.

and adjusted for genotype and IFN protocol. Adjustment for racial differences, diversity between the HCV strains with respect to genotype and ISDR sequence, and IFN regimen would be needed to use the ISDR as a simple diagnostic tool to predict sustained virologic response. Nevertheless, the present study had a few limitations. Only the correlation between mutations within the ISDR and sustained virologic response was analyzed, although other parts of NS5A have been reported to be associated with IFN response [Nousbaum et al., 2000; Murphy et al., 2002]. The approach of counting the number of mutations to the chosen consensus sequence in the ISDR, originally reported by Enomoto, was used for the present analysis; however, this method may not be the best way to measure sequence variation. Phylogenetic analyses of the ISDR were used to evaluate the diversity of the ISDR sequence, but distinctive clustering was not found in the wild types with A2205 and with T2205 and with V2205. The ISDR interacts with PKR and inactivates replication of HCV in vitro [Gale et al., 1998]. However, some reports have not confirmed the interaction between PKR and NS5A [Podevin et al., 2001; Tan and Katze, 2001]. PKR-independent effects of NS5A have been reported [Polyak et al., 2001; Evans et al., 2004]. Although the effect of amino acid substitutions of the ISDR was unclear, the ISDR system could be used clinically as a simple diagnostic tool to predict sustained virologic response in patients infected with genotype 2a who received pegylated-IFN-alpha 2a monotherapy.

The current recommended therapy for patients with HCV genotype 2 is a combination of pegylated-IFN and ribavirin for 24 weeks [Strader et al., 2004]. However, pegylated-IFN-alpha 2a monotherapy in patients with HCV genotype 2a resulted in a high sustained virologic response rate (77.3%). Most reports dealing with pegylated-IFN-alpha and ribavirin combination therapy did not differentiate between HCV genotypes 2 and 3 or did not classify subgenotypes 2a and 2b [Zeuzem et al., 2004; Mangia et al., 2005; von Wagner et al., 2005; Shiffman et al., 2007]. There is also limited information regarding sustained virologic response in patients with HCV genotype 2a treated with pegylated-IFN-alpha and ribavirin combination therapy. Thus, it is difficult to compare the present results to those obtained with pegylated-IFN-alpha and ribavirin combination therapy. Large, randomized, prospective studies of pegylated-IFN-alpha with or without ribavirin for patients with genotype 2a, especially ISDR mutant, are needed to clarify these issues. The present study combined two predictive factors: rapid virologic response and the amino acid variations in ISDR compared to the reference sequence. Rapid virologic response is considered to be a strong indicator of progression to sustained virologic response for patients with HCV genotype 2a. Knowledge of both the ISDR sequence and rapid virologic response would be useful for individualization of IFN regimens for chronic hepatitis C patients, but rapid virologic response cannot be assessed before treatment. In the present study, there were no predictive factors associated with rapid virologic response on multivariate analyses (data not shown). Thus, it is impossible to predict which patients will be rapid virologic responders before IFN therapy. With respect to assessment before starting treatment, the number of mutations in the ISDR is a better predictor than rapid virologic response.

In conclusion, the present results indicate that pegylated-IFN-alpha 2a monotherapy is effective for achieving sustained virologic response in Japanese patients with HCV genotype 2a, particularly in those with rapid virologic response and mutant-type ISDR.

The ISDR sequence variation of HCV genotype 2a is useful for predicting IFN responsiveness.

ACKNOWLEDGMENTS

The authors thank Ms. Miho Kawaguchi, Ms. Mika Torii, and Ms. Yukiyo Hasegawa for their assistance.

REFERENCES

Akuta N, Suzuki F, Suzuki Y, Sezaki H, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. 2005. Hepatocyte steatosis is an important predictor of response to interferon (IFN) monotherapy in Japanese patients infected with HCV genotype 2a: Virological features of IFN-resistant cases with hepatocyte steatosis. J Med Virol 75:550–558.

Chung RT, Monto A, Dienstag JL, Kaplan LM. Mutations in the NS5A region do not predict interferon-responsiveness in American patients infected with genotype 1b hepatitis C virus.1999. J Med Virol 58:353–358.

Enomoto N, Takada A, Nakao T, Date T. 1990. There are two major types of hepatitis C virus in Japan. Biochem Biophys Res Commun 170:1021–1025.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Evans MJ, Rice CM, Goff SP. 2004. Phosphorylation of hepatitis C virus nonstructural protein 5A modulates its protein interactions and viral RNA replication. Proc Natl Acad Sci USA 101:13038–13043.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

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 347:975–982.

Gale M, Jr., Blakely CM, Kwieciszewski B, Tan SL, Dossett M, Tang NM, Korth MJ, Polyak SJ, Gretch DR, Katze MG. 1998. Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: Molecular mechanisms of kinase regulation. Mol Cell Biol 18:5208–5218.

Hayashi K, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayakawa T, Morita K, Nishimura D, Kato K, Urano F, Takamatsu J. 2003. Prevalence and characterization of hepatitis C virus genotype 4 in Japanese hepatitis C carriers. Hepatol Res 25:409–414.

Herion D, Hoofnagle JH. 1997. The interferon sensitivity determining region: All hepatitis C virus isolates are not the same. Hepatolgy 25:769–770.

Kobayashi M, Watanabe K, Ishigami M, Murase K, Ito H, Ukai K, Yano M, Takagi K, Hattori M, Kakumu S, Yoshioka K. 2002. Amino acid substitutions in the nonstructural region 5A of hepatitis C virus genotypes 2a and 2b and its relation to viral load and response to interferon. Am J Gastroenterol 97:988–998.

Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A. 2005. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 352:2609–2617.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

Murakami T, Enomoto N, Kurosaki M, Izumi N, Marumo F, Sato C. 1999. Mutations in nonstructural protein 5A gene and response to interferon in hepatitis C virus genotype 2 infection. Hepatology 30:1045–1053.

Murphy MD, Rosen HR, Marousek GI, Chou S. 2002. Analysis of sequence configurations of the ISDR, PKR-binding domain, and V3 region as predictors of response to induction interferon-alpha and ribavirin therapy in chronic hepatitis C infection. Dig Dis Sci 47:1195–1205.

Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T. 1999. Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? J Hepatol 30:1014–1022.

Nousbaum J, Polyak SJ, Ray SC, Sullivan DG, Larson AM, Carithers RL, Jr., Gretch DR. 2000. Prospective characterization of full-length hepatitis C virus NS5A quasispecies during induction and combination antiviral therapy. J Virol 74:9028–9038.

Otagiri H, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayashi K, Hayakawa T, Fukuda Y, Kinoshita M, Takamatsu J. 2002. Evaluation of a new assay for hepatitis C virus genotyping and viral load determination in patients with chronic hepatitis C. J Virol Methods 103:137–143.

Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, Berg T. 2004. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: A meta-analysis focused on geographical differences. Gut 53:1345–1351.

Podevin P, Sabile A, Gajardo R, Delhem N, Abadie A, Lozach PY, Beretta L, Bréchot C. 2001. Expression of hepatitis C virus NS5A natural mutants in a hepatocytic cell line inhibits the antiviral effect of interferon in a PKR-independent manner. Hepatology 33:1503–1511.

Polyak SJ, Khabar KS, Paschal DM, Ezelle HJ, Duverlie G, Barber GN, Levy DE, Mukaida N, Gretch DR. 2001. Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. J Virol 75:6095–6106.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Saiz JC, Lopez LF, Ampurdanes S, Dopazo J, Forns X, Sanchez TJ, Rodes J. 1988. The prognostic relevance of the nonstructural 5A gene interferon sensitivity determining region is different in infections with genotype 1b and 3a isolates of hepatitis C virus. J Infect Dis 177:839–847.

Seeff LB. 2002. Natural history of chronic hepatitis C. Hepatology 36:S35–S46.

Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Sola R, Shafran SD, Barange K, Lin A, Soman A, Zeuzem S, ACCELERATE Investigators. 2007. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med 357:124–134.

Simmonds P, Bukh J, Combet C, Deleage G, Enomoto N, Feinstone S, Halfon P, Inchauspe G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. 2005. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 42:962–973.

Squadrito G, Raffa G, Restuccia T, Pollicino T, Brancatelli S, Raimondo G. 2002. Is investigation of hepatitis C virus NS5A gene heterogeneity a tool for predicting long-lasting response to interferon therapy in patients with HCV-1b chronic hepatitis? J Viral Hepat 9:360–369.

Strader DB, Wright T, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. 2004. Diagnosis, management, and treatment of hepatitis C. Hepatology 39:1147–1171.

Takeuchi T, Katsume A, Tanaka T, Abe A, Inoue K, Tsukiyama-Kohara K, Kawaguchi R, Tanaka S, Kohara M. 1999. Real-time detection system for quantification of hepatitis C virus genome. Gastroenterology 116:636–642.

Tan SL, Katze MG. 2001. How hepatitis C virus counteracts the interferon response: The jury is still out on NS5A. Virology 284:1–12.

von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, Bergk A, Bernsmeier C, Haussinger D, Herrmann E, Zeuzem S. 2005. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 129:522–527.

Zeuzem S, Lee JH, Roth WK. 1997. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alfa. Hepatology 25:740–744.

Zeuzem S, Hulterantz R, Bourliere M, Goeser T, Marcellin P, Sanchez-Tapias J, Sarrazin C, Harvey J, Brass C, Albrecht J. 2004. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. J Hepatol 40:993–999.

Impact of a Unified CT Angiography System on Outcome of Patients with Hepatocellular Carcinoma

Hidenori Toyoda[1]
Takashi Kumada
Yasuhiro Sone

OBJECTIVE. The purpose of our study was to evaluate the impact of a unified CT angiography (CTA) system for the management of patients with hepatocellular carcinoma (HCC).

SUBJECTS AND METHODS. A total of 1,312 patients with HCC who had been diagnosed and treated between 1990 and 2003 were studied. The clinical characteristics and survival rate were compared between patients who underwent pretreatment evaluation of tumor progression by a unified CTA system and those who underwent evaluation by a conventional angiography system. In addition, the survival rates for 438 patients who underwent transcatheter arterial chemoembolization (TACE) as initial treatment were compared between patients who were treated using a unified CTA system and those who were treated without the system.

RESULTS. Overall, the survival rate was higher in patients who underwent pretreatment examination using a unified CTA system than in those who underwent examination without it ($p < 0.0001$). The survival rate was higher when focusing on patients with HCC of stage I ($p = 0.0093$). In patients who underwent TACE as an initial treatment, the survival rate was higher in patients treated by TACE using a unified CTA system than in those without it ($p = 0.0023$).

CONCLUSION. The more accurate and detailed pretreatment evaluation of HCC progression using a unified CTA system contributed to the improvement of survival of patients with HCC. In addition, it contributed to the improved efficacy of TACE with an increased survival rate.

Hepatocellular carcinoma (HCC) is one of the most common malignancies, especially in southern and eastern Asia. The incidence of HCC is also increasing in the United States [1, 2]. The prognosis of patients with HCC has improved because of improvements in the management of such patients, including increased early detection of HCC, development of novel treatment options and enhanced treatment techniques, and the accurate evaluation of tumor progression [3]. Detailed and accurate evaluation of the progression of HCC, including the determination of the size, number, and location of tumors at diagnosis and the accurate evaluation of treatment response, is an important factor in the management of patients with HCC and contributes to the overall improved outcome.

Examination of liver tumor with CT during angiography, that is, CT during arterial portography (CTAP) [4] and CT during hepatic arteriography (CTHA) [5], reportedly provides precise and detailed information, including the size, number, and location of HCC in the entire liver and vascular invasion by HCC [6–8]. In addition, in patients who underwent transcatheter arterial chemoembolization (TACE) as a treatment for HCC, the confirmation of the feeding arteries toward the tumor using CT in addition to digital subtraction arteriography (DSA) will improve the accuracy of the selection of arteries that should be embolized. Also, combination of TACE with simultaneous evaluation of its results using CT will improve the treatment efficacy.

A unified CT angiography (CTA) system was developed in 1996 [9, 10] and has been used in several liver centers in Japan. This system integrates CT and angiography and allows immediate and repeated CT examination during angiography examinations or the TACE procedure. We have applied this system for all angiography examinations or the TACE procedure for HCC in our institution since July 1997. In the present study, we evaluated the usefulness of a unified CTA system for the management of patients with HCC.

Keywords: hepatocellular carcinoma, survival, transcatheter arterial chemoembolization, tumor progression, unified CT angiography system

DOI:10.2214/AJR.08.1368

Received June 8, 2008; accepted after revision July 29, 2008.

[1]All authors: Department of Gastroenterology, Ogaki Municipal Hospital, 4-86 Minaminokawa, Ogaki, Gifu 503-8502, Japan. Address correspondence to T. Kumada (tkumada@he.mirai.ne.jp).

AJR 2009; 192:766–774

0361–803X/09/1923–766

Subjects and Methods

Patients and Angiography Examination

A total of 1,312 patients were diagnosed with an initial HCC (not a recurrence) and underwent treatment for HCC at our institution between 1990 and 2003. The background characteristics of the study patients are shown in Table 1 (348 women and 964 men; mean age, 65.5 ± 9.3 years). Patients were diagnosed with HCC on the basis of histologic examination of tumor tissue taken from resected or biopsy specimens in 401 cases (30.6%). In the remaining 911 patients, diagnosis was made on the basis of clinical criteria [11, 12]: a pertinent clinical background (association with liver cirrhosis or viral hepatitis) and typical imaging findings. Typical imaging features of HCC include a mosaic pattern with a halo on B-mode sonographic images, hypervascularity on angiographic images, and a high-density mass on arterial phase dynamic CT images with a low-density mass on portal phase dynamic CT images obtained with a helical or MDCT scanner. CTAP and CTHA were performed when typical findings for HCC were not obtained by means of dynamic CT or angiography before July 1997, at which time a unified CTA system was introduced in our institution. CTAP and CTHA were performed in all patients after July 1997, and T1- and T2-weighted imaging associated with superparamagnetic iron oxide–enhanced MRI was performed when typical findings for HCC were not obtained by means of CTAP and CTHA during this period. In cases without typical features on imaging studies, a biopsy was performed to confirm the diagnosis of HCC. Of the 1,312 patients, 294 (22.4%) underwent hepatectomy, 271 (20.7%) were treated by locoregional ablative therapy including ethanol injection, microwave thermocoagulation, or radiofrequency ablation; and 438 (33.4%) were treated using TACE.

All patients underwent angiography for the diagnosis of HCC before treatment, regardless of the treatment options applied and including patients who did not undergo treatment as a consequence. The size, number, and location of tumors and portal vein invasion by the tumor were evaluated at angiography through hepatic arteriography and arterial portography. Angiography was performed without a unified CTA system in 603 patients before July 1997. CTAP was performed only when necessary for the diagnosis of HCC, with transportation of the patient from angiography to CT during this period. After July 1997, angiography was performed using the unified CTA system in all 709 patients. CTAP and CTHA were performed for all patients during this period. Patients were followed up from 0.4 months to 100.8 months (median, 16.9 months) at our institution.

The study protocol was approved by the hospital ethics committee and was in compliance with the Helsinki Declaration. Written informed consent was obtained from all patients before the study for use of the clinical, laboratory, and outcome data.

A Unified CTA System

A unified CTA system was introduced in our institution in July 1997. The unified system of helical CT and angiography (Interventional-CT, Toshiba Medical Systems) [9, 10] consists of helical CT (X-Vision Real, Toshiba Medical Systems) and angiography units in which DSA equipment with a C-arm (Angiorex, BLA-800A, Toshiba Medical Systems) is arranged in a linear configuration to form a common couch, facilitating quick transportation of the patient from one unit to the other for CT, including CTAP and CTHA, without risking dislodgment of the catheter.

TACE

Individual decisions regarding treatment of HCC were determined by the treating physicians and according to each patient's wishes. Patients were assessed initially for eligibility for hepatectomy. Principally, patients with three or fewer HCC tumors were recommended to undergo hepatectomy when they had Child-Pugh class A liver function. Those who declined or were deemed not eligible for hepatectomy were considered for locoregional ablative therapy with percutaneous ethanol injection, percutaneous microwave thermocoagulation, or radiofrequency ablation. Locoregional ablative therapy was selected as a treatment in patients with three or fewer HCC tumors that were < 3 cm in maximum size. Patients who were not eligible for either hepatectomy or locoregional ablative therapy and had no extrahepatic spread or portal vein thrombosis were offered TACE. This treatment policy was constant throughout the study period. No patient underwent liver transplantation as a treatment for HCC.

As a result, a total of 438 patients underwent TACE as an initial primary treatment for HCC. TACE was performed immediately after angiography for the diagnosis of HCC or at the latest within 2 weeks after diagnosis. TACE was performed solely with an angiography apparatus in 219 patients before July 1997 and with a unified CTA system in the other 219 patients after July 1997.

Before TACE, a baseline angiography with arterial portography and hepatic arteriography was performed in all patients. Hepatic arteriography in the right anterior oblique position was performed if deemed necessary. On the basis of the baseline study findings, the feeding artery was determined. Subsequently, a 2.5- or 2.8-French, 135-cm-long microcatheter (Tracker-18 or Renegade, Boston Scientific) with a double angle-shaped 0.016-inch, 180-cm-long guidewire covered by hydrophilic polymer (Radifocus, Terumo) was advanced through a 5-French catheter into the peripheral portion of the feeding artery as close to the lesion as possible. If the predicted noncancerous hepatic portion to be embolized was large enough

TABLE 1: Background Characteristics of Study Patients (n = 1,312)

Parameter	Value
Age (y)	65.5 ± 9.3
Sex (%)	
M	964 (73.5)
F	348 (26.5)
Total bilirubin (mg/dl)	1.3 ± 1.9
Albumin (g/dl)	3.3 ± 0.6
Prothrombin time (%)	81.9 ± 18.6
Child-Pugh classification	
A	794 (60.5)
B	402 (30.6)
C	116 (8.9)
Maximum tumor size (cm)	
< 2	442 (33.7)
2–5	469 (35.7)
> 5	401 (30.6)
Number of tumors	
Single	643 (49.0)
Multiple	669 (51.0)
Portal vein invasion	
Absent	1,025 (78.1)
Present	287 (21.9)
Tumor stage[a]	
I	288 (21.9)
II	421 (32.2)
III	315 (24.0)
IV	288 (21.9)
Initial treatment	
Surgery	294 (22.4)
Locoregional ablative therapies[b]	271 (20.6)
Transcatheter arterial chemoembolization	438 (33.4)
Other	97 (7.4)
None	212 (16.2)

Note—Data in parentheses are percentages.
[a]TNM tumor stage according to the Liver Cancer Study Group of Japan.
[b]Locoregional ablative therapies include percutaneous ethanol injection, percutaneous microwave thermocoagulation, and radiofrequency ablation.

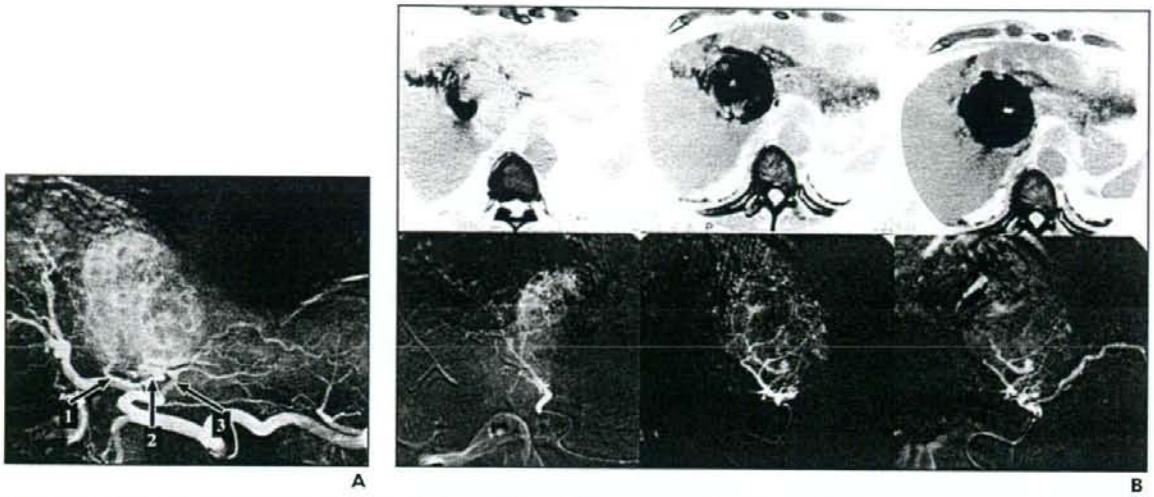

Fig. 1—72-year-old man who underwent transcatheter arterial chemoembolization (TACE) for hepatocellular carcinoma (HCC).
A, Angiography image shows three arteries (*arrows* 1, 2, and 3) feeding to HCC.
B, TACE images show each artery was embolized with mixture of anticancer agent and lipiodol followed by gelatin sponge particles (lower row, numbers correspond to numbered arteries in **A**) that was confirmed by retention of lipiodol on CT images (upper row) immediately after TACE. TACE procedure was completed after confirmation of retention of lipiodol in entire HCC tumor.

to deteriorate the hepatic functional reserve, the catheter tip was advanced to a more peripheral part of the feeding artery. TACE was performed with an injection into the feeding artery of an emulsion of 50 mg of farmorubicin hydrochloride (Epirubicin, Adria) dissolved in 5 mL of iopamidol (Iopamiron, 370 mg I/mL, Schering Tokyo) mixed with 5 mL of iodized oil (Lipiodol Ultra-Fluid, Guerbet), followed by an injection of gelatin sponge particles (Gelfoam, Upjohn). The end point of TACE was cessation of arterial blood flow. If deposition of iodized oil in the lesion was inadequate, another feeding artery was sought and TACE was performed again.

In 219 patients who underwent TACE with a unified CTA system after July 1997, both CTAP and CTHA examinations were performed for all patients as the baseline study. When the catheter tip was advanced into the peripheral portion of the feeding artery, follow-up CT arteriography was performed before TACE to confirm the possible feeding artery supplying the targeted lesion and the expected extent of embolization of the noncancerous portion. The deposition of the iodized oil in the lesion was examined using unenhanced CT immediately after TACE. If deposition of iodized oil in the lesion was inadequate on CT, another feeding artery was sought and CT arteriography was performed again to confirm the feeder supplying the lesion with an undeposited portion before performing TACE again (Fig. 1). When the feeding artery was not found by arteriography through the hepatic artery, other arteries that were not the branch of the hepatic artery, including the infraphrenic artery, renal artery, or adrenal artery, were examined as the possible feeding artery (Fig. 2). The complete deposition of iodized oil in the lesion was confirmed using unenhanced CT to complete treatment.

Statistical Analyses

Numeric data are expressed as mean ± SD values unless otherwise specified. Differences in proportions of the number of patients between groups were analyzed by a chi-square test. Differences in quantitative values were analyzed by the Student's *t* test if the data were normally distributed; otherwise, differences were analyzed by the Mann-Whitney *U* test.

In the analysis of overall study patients, the date of HCC diagnosis was defined as time zero for calculations of patient survival. In the analysis of patients who underwent TACE, the date of TACE was defined as time zero for calculations of patient survival. Surviving patients and patients who died from causes other than liver disease were censored. Patients who died from HCC-related causes or liver failure were not censored. The Kaplan-Meier method was used to calculate survival rates, and the log-rank test was used to analyze differences in survival. The Cox proportional hazards model was used for multivariate analysis for factors that influenced patient survival. The variables analyzed were age, sex, Child-Pugh class, TNM tumor stage according to the Liver Cancer Study Group of Japan (see Appendix 1) [13], and the use of the unified CTA system for TACE. The JMP statistical software package, version 4.0 (SAS Institute), was used for all statistical analyses. All *p* values were derived from two-tailed tests, and $p < 0.05$ was accepted as statistically significant.

Results

Characteristics and Survival Rates of Patients Examined by Angiography With or Without a Unified CTA System

The background characteristics of patients who underwent angiographic evaluation for HCC with a unified CTA system and those who underwent angiographic evaluation for HCC without it are compared in Table 2. With regard to liver function at the diagnosis of HCC, patients examined with a unified CTA system had lower serum albumin levels ($p = 0.0079$) than those examined without it. As for the progression of HCC, the prevalence of patients with multiple tumors at diagnosis tended to be higher in patients examined with a unified CTA system than those examined without it ($p = 0.0864$). However, the prevalence of findings at the earliest stage of HCC (HCC stage I according to the Liver Cancer Study Group of Japan [13]) was higher in patients examined with a unified CTA system than those without it ($p = 0.0337$). In contrast, the prevalence of findings at the most advanced HCC (HCC stage IV) was higher in patients examined without a uni-