Keywords: Hepatocellular carcinoma; Tumor markers; Survival; Recurrence; Curative treatment

1. Introduction

Hepatocellular carcinoma (HCC) is a common malignancy, especially in southern and eastern Asia. The incidence of HCC is also increasing in the United States [1,2]. The development of various scanning techniques and the identification of sensitive and specific tumor markers for HCC have contributed not only to the detection of HCC, but also to the evaluation of its progression and the determination of patient prognosis.

Three tumor markers specific for HCC are currently used in Japanese clinics: alpha-fetoprotein (AFP), *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), and des-gamma carboxy prothrombin (DCP), which is also referred to as protein induced by vitamin K absence-II (PIVKA-II). Previous reports have detailed the usefulness of each of these tumor markers in the detection and diagnosis of HCC, the evaluation of tumor progression, and the determination of patient prognosis [3–7]. The elevation of each tumor marker has been reported to indicate poor prognosis and decreased survival rates [8]. However, the prognostic value of these tumor markers in distinct patient subpopulations who underwent potential curative treatments for HCC has not been well studied.

In the present study, we attempted to evaluate the effect of the pretreatment elevation of these tumor markers for HCC (AFP, AFP-L3, and DCP) on the outcome of patients who underwent curative treatments: hepatectomy and locoregional thermal ablation that includes percutaneous microwave thermocoagulation (PMCT) and radiofrequency ablation (RFA).

2. Patients and methods

2.1. Patients

A total of 3725 patients were initially diagnosed with HCC at one of the five institutions that participated in the study between July 1994 and December 2004. Three tumor markers for HCC (AFP, AFP-L3, and DCP) were measured at the time of diagnosis, and drugs that would influence the serum DCP levels, such as warfarin or vitamin K, were not being taken by 2600 of the 3725 patients [8]. Of these 2600 patients, 736 received hepatectomy and 945 received locoregional ablative therapies as an initial treatment for HCC. Of the patients who underwent hepatectomy, 345 were enrolled in this study, while 391 were excluded as their maximum tumor size was greater than 3 cm or their number of tumors was greater than 3 (Fig. 1A). With regard to the patients who underwent locoregional ablative therapies, we first excluded 344 patients who received percutaneous ethanol injections because percutaneous ethanol injection is reported to be less effective as a therapy for HCC compared to other locoregional ablative therapies [9]. As a result, the patients in the study were primarily those who underwent PMCT ($n = 123$) or RFA ($n = 478$). We defined these patients as patients who underwent locoregional thermal ablation (LTA). We further excluded 139 patients in whom the maximum tumor size was greater than 3 cm or the number of tumors was greater than 3. Finally, we excluded 6 patients in whom the pretreatment remnant liver function was estimated as class C according to the Child-Pugh classification [10]. A total of 456 patients were therefore enrolled in this study (Fig. 1B). These patients were treated by hepatectomy and LAT solely, and no patients received an addition of other kinds of therapy for HCC as multimodality treatment until the recurrent HCC developed. The study protocol was approved by the Institutional Ethics Review Board at each of the participating institutions and was in compliance with the Declaration of Helsinki. Written informed consent for the use of information on the pretreatment tumor marker values for future study on patient outcome was obtained from each patient prior to treatment.

2.2. Diagnosis of HCC, treatment, and follow-up

Patients were diagnosed with HCC based on histologic examination of tumor tissue taken from resected specimens in 345 patients who underwent hepatectomy. In patients treated by LTA, the diagnosis of HCC had been made based on fine-needle biopsy of specimens from 198 of the 456 patients (43.4%). In the remaining 258 patients, the diagnosis was made based on 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 by B-mode ultrasonography; hypervascularity on angiographic images; and a high-density mass on arterial phase dynamic computed tomography (CT) images and a low-density mass on portal phase dynamic CT images obtained with a helical or multidetector row CT scanner. When findings typical of HCC were not obtained by means of dynamic CT or angiography, CT during hepatic arteriography and CT during arterial portography or T1- and T2-weighted imaging associated with superparamagnetic iron oxide-enhanced magnetic resonance imaging were performed.

All patients underwent the treatment for HCC within 2 weeks after the diagnosis of HCC. In patients who underwent LTA, dynamic CT was performed 1 to 3 days after the last session of ablation to evaluate the efficacy of treatment. Complete ablation was defined by CT findings as non-enhancement in the entire lesion with a safety margin in the surrounding liver parenchyma. Patients received additional sessions of ablation until complete ablation was confirmed in each nodule.

Patients were prospectively followed up from 0.8 months to 175.1 months (median follow-up period, 26.6 months). All 801 patients were followed up at one of the five participating institutions.

2.3. Measurement of tumor markers and cut-off levels

AFP, AFP-L3, and DCP were measured in serum samples obtained from each patient at the time of HCC diagnosis. The serum AFP levels were determined by enzyme-linked immunosorbent assay with a commercially available kit (ELISA-AFP, International Reagents, Kobe, Japan). Serum AFP-L3 levels were measured by lectin-affinity electrophoresis coupled with antibody-affinity blotting (AFP Differentiation Kit L, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and are expressed as a percentage (AFP-L3 level/total AFP level X 100) [13,14]. The serum DCP levels were determined by sensitive enzyme immunoassay (Eitest PIVKA-II kit, Eisai Laboratory, Tokyo, Japan) according to the manufacturer's instructions [15–17]. We designated

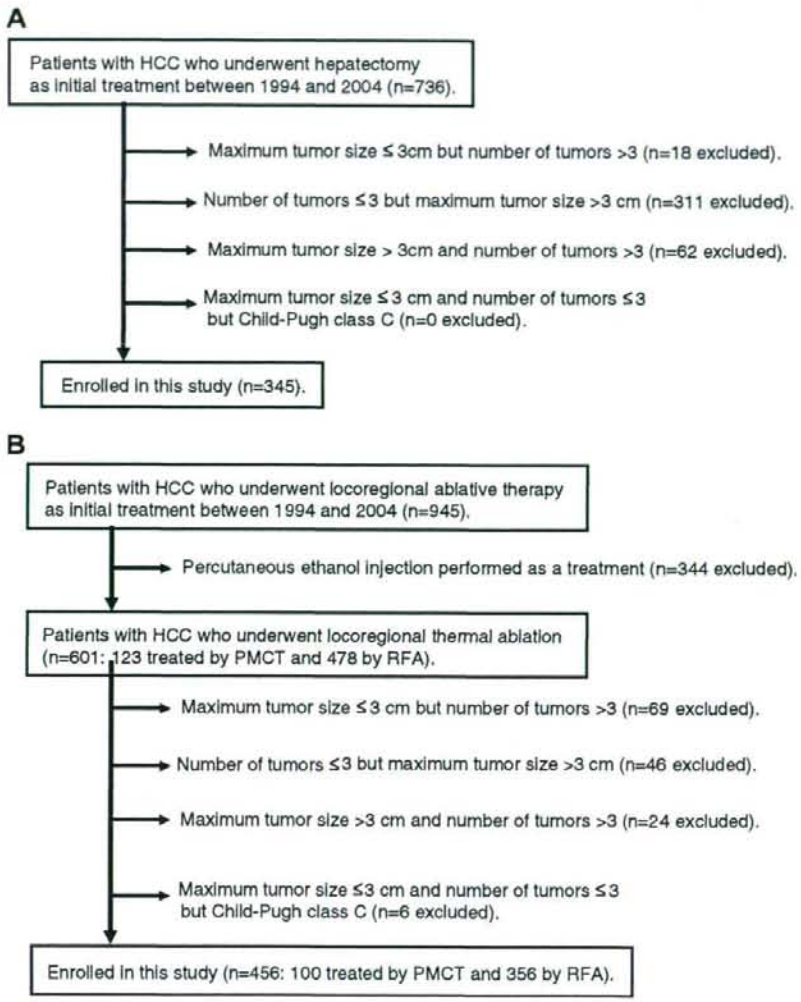

Fig. 1. Schematic representation of the enrolled patients treated by hepatectomy (A) and those treated by locoregional thermal ablation (LTA) (B). HCC, hepatocellular carcinoma; PMCT, percutaneous microwave thermocoagulation; RFA, radiofrequency ablation.

the cut-off level for each tumor marker as 400 ng/dL for AFP, 15% for AFP-L3, and 100 mAU/mL for DCP, respectively, based on our previous study [8]. Patients with pretreatment AFP of ⩾400 ng/dL, those with pretreatment AFP-L3 of ⩾15%, and those with pretreatment DCP of ⩾100 mAU/mL were considered as patients with pretreatment AFP elevation, those with pretreatment AFP-L3 elevation, and those with pretreatment DCP elevation, respectively. AFP-L3 of patients with AFP values not more than 10 ng/dL was decided as 0%, because AFP-L3 percent is normally quantifiable only in patients with AFP values above 10 ng/dL. Patients with AFP values not more than 10 ng/dL were, therefore, considered as those without pretreatment AFP-L3 elevation.

2.4. Statistical analyses

In both the patients who underwent hepatectomy and those who underwent LTA, we compared the survival and recurrence rates of patients stratified by the elevation of each tumor marker at the time of HCC diagnosis. The impact of each tumor marker on patient survival and HCC recurrence was assessed using univariate and multivariate analyses within each of the two groups. The date of HCC diagnosis was defined as time zero for calculations. Surviving patients and patients who died from causes other than liver disease, and patients without HCC recurrence were censored. Patients who died from HCC-related causes or HCC-related liver failure, and patients in whom recurrent HCC developed were not censored. Univariate survival and recurrence curves were calculated by the Kaplan-Meier method [18], and differences in survival and recurrence rates between groups were analyzed by a log-rank test [19]. To identify independent factors associated with the survival and recurrence rates, various likely predictors (p values ⩽0.1) associated with the survival or recurrence by univariate analysis were subjected to multivariate analysis. The Cox proportional hazards model [20] was used for multivariate analysis. The factors included for analyses were patient age, sex (female/male), etiology (hepatitis B virus [HBV]/hepatitis C virus [HCV]/non-HBV, non-HCV), Child-Pugh class (A/B), maximum tumor size (⩽2 cm/>2 cm), number of tumors (single/multiple), portal vein invasion (absent/present), pretreatment AFP level (<400 ng/dL/⩾400 ng/dL), pretreatment AFP-L3 level (<15%/⩾15%), and pretreat-

Table 1
Clinical characteristics of study patients who underwent hepatectomy ($n = 345$)

Age (median, years)	66 (range, 22–82)
Sex	
Male	195 (56.5%)
Female	150(43.5%)
Etiology of underlying liver disease	
HBV	55 (16.0%)
HCV	255 (73.9%)
HBV, HCV	6 (1.7%)
Non-HBV, non-HCV	29 (8.4%)
Child-Pugh class	
A	319 (92.5%)
B	26 (7.5%)
Albumin (mean ± S.D., g/dL)	3.81 ± 0.40
Total bilirubin (mean ± S.D., mg/dL)	0.87 ± 0.47
Tumor size	2.10 ± 0.33
≤2 cm	173 (50.1%)
>2 cm	172 (49.9%)
Tumor number	1.25 ± 0.28
Single	273 (79.1%)
Multiple	72 (20.9%)
Portal vein invasion[a]	
Absent	338 (98.0%)
Present	7 (2.0%)
AFP (median, ng/dL)	19.1 (range, 0.8–5810)
<400 ng/dL	300 (87.0%)
≥400 ng/dL	45 (13.0%)
AFP-L3 (median, %)	0.5 (range, 0–81.2)
<15%	282(81.7%)
≥15%	63 (18.3%)
DCP (median, mAU/mL)	42.0 (range, 3.0–118000)
<100mAU/mL	234 (67.8%)
≥100 mAU/mL	111 (32.2%)

Number of patients is shown unless otherwise indicated. HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.
[a] Evaluated on imaging findings.

Table 2
Clinical characteristics of study patients who underwent locoregional thermal ablation ($n = 456$)

Age (median, years)	68 (range, 34–89)
Sex	
Male	298 (65.4%)
Female	158 (34.6%)
Etiology of underlying liver disease	
HBV	34 (7.5%)
HCV	381 (83.5%)
HBV, HCV	7 (1.5%)
Non-HBV, non-HCV	34 (7.5%)
Child-Pugh class	
A	319 (70.0%)
B	137 (30.0%)
Albumin (mean ± S.D., g/dL)	3.58 ± 0.51
Total bilirubin (mean ± S.D., mg/dL)	1.07 ± 0.69
Tumor size	1.92 ± 0.26
≤2 cm	288 (63.2%)
>2 cm	168 (36.8%)
Tumor number	1.40 ± 0.31
Single	320 (70.2%)
Multiple	136 (29.8%)
Portal vein invasion*	
Absent	456 (100%)
Present	0
AFP (median, ng/dL)	19.4 (range, 0.8–3770)
<400 ng/dL	426 (93.4%)
≥400 ng/dL	30 (6.6%)
AFP-L3 (median, %)	0.5 (range, 0–83.8)
<15%	406 (89.0%)
≥15%	50 (11.0%)
DCP (median, mAU/mL)	30.0 (range, 5.0–27100)
<100 mAU/mL	348 (76.3%)
≥100 mAU/mL	108 (23.7%)

Number of patients is shown unless otherwise indicated. HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin. *Evaluated on imaging findings.

ment DCP level (<100 mAU/mL/≥100 mAU/mL). (Portal vein invasion was withdrawn from the factors in multivariate analysis of patients who underwent LTA.) All analyses were performed using SAS statistical software (version 8.2; SAS Institute, Cary, NC) or the SPSS Medical Pack for Windows (version 10.0; SPSS, Inc., Chicago, IL). All p values were derived from two-tailed tests, and p values <0.05 were considered statistically significant.

3. Results

3.1. Clinical backgrounds and survival rates of patients who underwent hepatectomy or locoregional thermal ablation

The clinical backgrounds of patients who underwent hepatectomy and LTA are shown in Tables 1 and 2, respectively. More than 90% of patients who underwent hepatectomy had Child-Pugh class A liver function, and the tumor size of half of the patients was greater than 2 cm. In patients who underwent LTA, 30% had Child-Pugh class B liver function, and the tumor size was not greater than 2 cm in more than 60% of patients. No patients who underwent LTA exhibited portal vein invasion of the tumor on imaging findings. AFP, AFP-L3, and DCP were equal to or above the cut-off levels in 13.0%, 18.3%, and 32.2% of hepatectomy patients, respectively, while they were equal to or above the cut-off levels in 6.4%, 11.0%, and 23.4% of LTA patients, respectively.

When focusing on patients with pretreatment AFP-L3 elevation, no significant difference was observed between patients who underwent hepatectomy and those who underwent LTA except for higher rate of Child-Pugh class A in patients treated by hepatectomy. When focusing on patients with pretreatment DCP elevation, in contrast, younger age, lower rate of HCV infection, greater tumor size, smaller tumor number were observed, in addition to higher rate of Child-Pugh class A, in patients treated by hepatectomy (data not shown).

During the follow-up period, 168 patients died, 64 patients lost for follow-up. Twenty patients died from causes other than liver disease, including pneumonia, brain hemorrhage, perforation of duodenal ulcer, acute myocardial infarction, and sepsis caused by cholangitis with common bile duct stone. The 3-year and 5-year survival rates were 80.0% and 66.3%, respectively, in patients who underwent hepatectomy, and they were 76.2% and 61.3%, respectively, in patients who underwent LTA. The recurrence of HCC was observed in 163 of 345 patients (47.2%) who underwent hepatectomy and 292 of 465 patients (62.8%) who underwent LTA. The 1-year and 3-year recurrence rates were 16.8% and 54.8%, respectively, in patients who underwent hepatectomy, and they were 27.9% and 72.6%, respectively, in patients who underwent LTA.

3.2. Impact of tumor markers on the survival of patients who underwent hepatectomy and of patients who underwent locoregional thermal ablation

In patients who underwent hepatectomy, 302 of 345 patients (87.5%) had a complete response and the other 43 patients did not. We found no correlation between pretreatment elevation of tumor markers and the response of hepatectomy. By univariate analysis, we found a significant difference in survival rates by pretreatment AFP-L3 elevation ($p = 0.0264$), as well as Child-Pugh class ($p = 0.0010$) and portal vein invasion of the tumor ($p = 0.0068$, left column of Table 3). In contrast, we found no difference by pretreatment AFP ($p = 0.8151$) or DCP ($p = 0.1919$) elevation (Fig. 2). According to multivariate analysis for the factors that could influence patient survival, Child-Pugh class B ($p = 0.0103$) was selected as a

Table 3
Univariate analysis for factors that influenced survival after treatment in patients who underwent hepatectomy ($n = 345$) and those who underwent locoregional thermal ablation ($n = 456$)

	Patients who underwent Hepatectomy ($n = 345$)			Patients who underwent locoregional thermal ablation ($n = 456$)		
	3-year[a]	5-year[a]	*p*-value	3-year[a]	5-year[a]	*p*-value
Age (years)						
<65	80.7	63.1	0.8459	74.7	58.2	0.5944
⩾65	79.7	70.1		77.1	63.4	
Sex						
Male	79.7	70.1	0.8459	77.1	63.4	0.5944
Female	80.7	63.1		74.4	58.2	
Etiology						
HBV	86.4	65.6	0.4027	86.7	74.3	0.1949
HCV	77.7	65.2		73.8	58.4	
Non-HBV, non-HCV	83.8	83.8		90.0	83.1	
Child-Pugh class						
A	81.9	69.8	0.0010	81.3	68.0	0.0011
B	60.9	35.5		64.7	44.9	
Tumor size						
⩽2 cm	81.9	66.1	0.5985	79.5	65.4	0.0160
>2 cm	78.1	66.5		70.0	54.4	
Tumor number						
Single	78.7	68.4	0.2557	80.7	67.7	0.0002
Multiple	84.3	59.0		64.9	45.1	
Portal vein invasion[b]						
Absent	80.4	66.6	0.0068			
Present	–	–				
AFP						
<400 ng/dL	80.5	66.2	0.8151	76.6	60.9	0.7702
⩾400 ng/dL	76.9	68.4		70.9	70.9	
AFP-L3						
<15%	81.4	66.9	0.0264	78.8	62.9	0.0005
⩾15%	73.8	58.4		50.0	50.0	
DCP						
<100 mAU/mL	81.8	68.6	0.1919	82.0	67.6	<0.0001
⩾100 mAU/mL	76.2	61.5		58.3	40.3	

HBV, hepatitis C virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.

[a] 3-year and 5-year survival rates.

[b] No patients with portal vein invasion who underwent hepatectomy were observed more than 3 years.

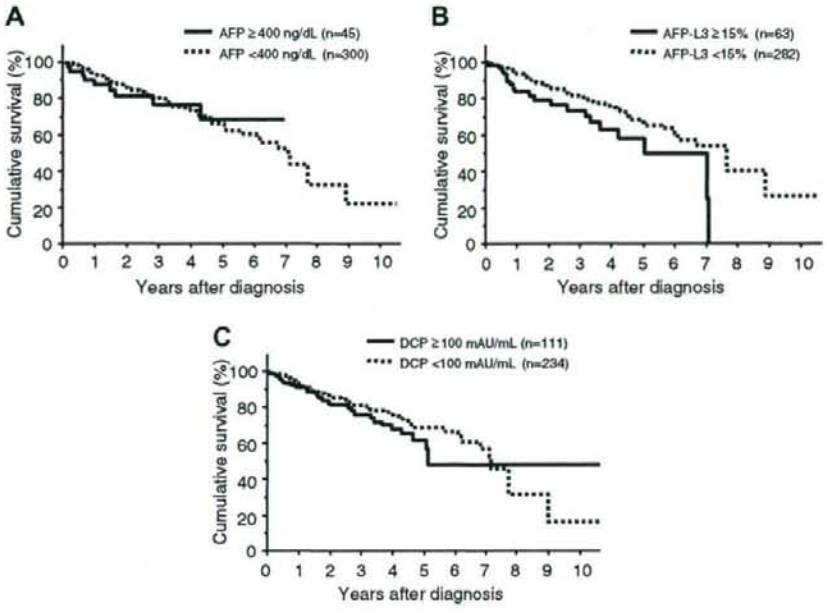

Fig. 2. Cumulative survival rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients treated by hepatectomy. No significant difference was found between patients with or without elevated pretreatment AFP or DCP (AFP, $p = 0.8151$; DCP, $p = 0.1919$). The survival rate of patients with elevated pretreatment AFP-L3 was significantly lower than that of patients without the elevation ($p = 0.0264$).

factor that significantly affected decreased survival rate. Portal vein invasion of the tumor ($p = 0.0732$) and AFP-L3 elevation ($p = 0.0657$) also tended to decrease survival rate (Table 4).

In patients who underwent LTA, all patients had complete response on the basis of the imaging evaluation, because patients received additional sessions of LTA until complete ablation was confirmed. According to univariate analysis, we found a significant difference in survival rates by pretreatment AFP-L3 ($p = 0.0005$) and DCP ($p < 0.0001$) elevation, as well as Child-Pugh class ($p = 0.0011$), tumor size ($p = 0.0160$), and tumor number ($p = 0.0002$, right column of Table 3). We found no difference by pretreatment AFP elevation ($p = 0.7702$, Fig. 3). According to multivariate analysis, Child-Pugh class B ($p = 0.0097$), multiple tumors ($p = 0.0049$), AFP-L3 elevation ($p = 0.0171$), and DCP elevation ($p = 0.0004$) were selected as factors that significantly affected decreased survival rate and tumor diameter >2 cm ($p = 0.0503$) tended to decrease survival rate (Table 5). The elevation of pretreatment DCP level had the strongest effect on decreased survival rate of patients who underwent LTA.

Table 4
Multivariate analysis for factors that influenced survival of patients who underwent hepatectomy ($n = 345$)

Factor	Parameter estimate	Standard error	X	Risk ratio (95% confidence interval)	*p* value
Child-Pugh class					
1: Class A				1	
2: Class B	0.410	0.147	6.589	1.5069 (1.1090–1.9827)	0.0103
Portal vein invasion					
1: Absent				1	
2: Present	0.817	0.368	3.211	2.2632 (0.9073–4.1450)	0.0732
AFP-L3					
1: <15%				1	
2: ⩾15%	0.252	0.131	3.386	1.2866 (0.9829–1.6495)	0.0657

AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein.

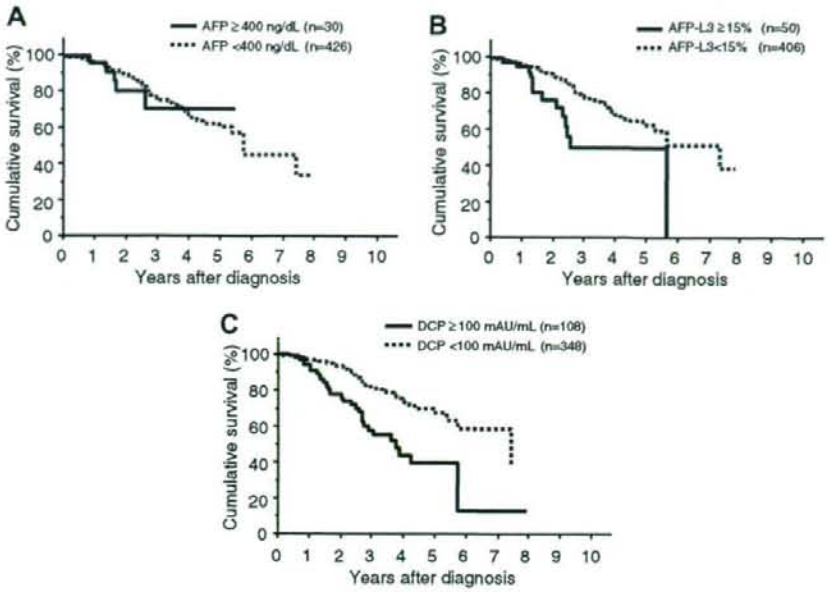

Fig. 3. Cumulative survival rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients who underwent LTA. No significant difference was found between patients with or without elevated pretreatment AFP (AFP, $p = 0.7702$). The survival rate of patients with elevated pretreatment AFP-L3 or DCP was significantly lower than that of patients without the elevation (AFP-L3, $p = 0.0005$; DCP, $p < 0.0001$).

3.3. Impact of tumor markers on the recurrence of HCC in patients who underwent hepatectomy and in patients who underwent locoregional thermal ablation

In patients who underwent hepatectomy, only multiple tumors affected increased recurrence rate in both univariate ($p = 0.0236$) and multivariate ($p = 0.0298$) analyses (data not shown). Pretreatment elevation of AFP, AFP-L3, and DCP did not affect recurrence rate (Fig. 4).

Pretreatment DCP elevation significantly affected increased recurrence rate ($p < 0.0001$, Fig. 5), as well as male sex ($p = 0.0131$) and multiple tumors ($p < 0.0001$), in univariate analysis of patients who underwent LTA. These 3 factors significantly affected increased recurrence rate also in multivariate analyses

Table 5
Multivariate analysis for factors that influenced survival of patients who underwent locoregional thermal ablation ($n = 456$)

Factor	Parameter estimate	Standard error	X	Risk ratio (95% confidence interval)	*p* value
Child-Pugh class					
1: Class A				1	
2: Class B	0.286	0.108	6.691	1.3310 (1.0731–1.6442)	0.0097
Tumor size					
1: ⩽2 cm				1	
2: > 2 cm	0.224	0.113	3.832	1.2508 (0.9997–1.5583)	0.0503
Tumor number					
1: Single				1	
2: Multiple	0.314	0.109	7.906	1.3687 (1.1016–1.6942)	0.0049
AFP-L3					
1: <15%				1	
2: ⩾ 15%	0.388	0.150	5.682	1.4743 (1.0768–1.9488)	0.0171
DCP					
1: < 100 mAU/mL				1	
2: ⩾ 100 mAU/mL	0.405	0.111	12.468	1.4992 (1.2022–1.8595)	0.0004

AFP-L3, *lens culinaris* agglutinin A-reactive fraction of alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin.

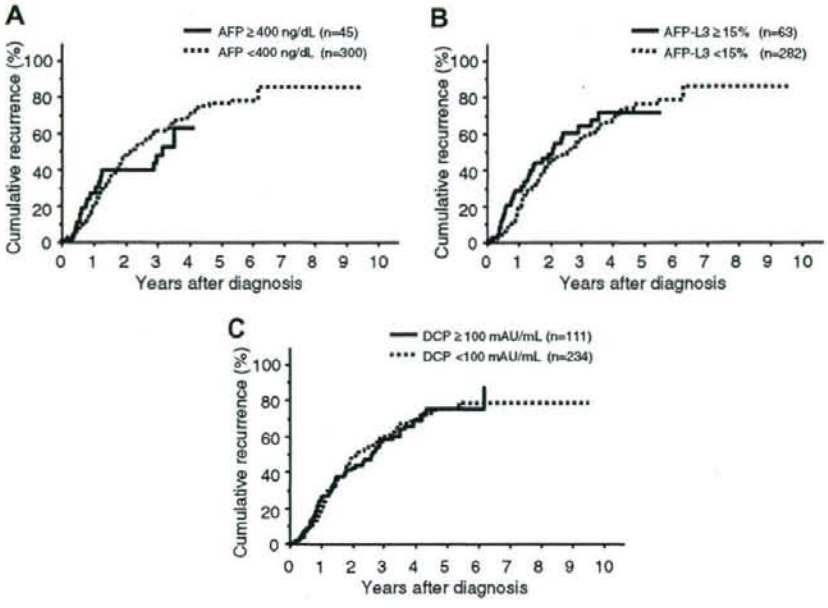

Fig. 4. Cumulative recurrence rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients treated by hepatectomy. No significant difference was found between patients with or without elevated pretreatment AFP, AFP-L3, or DCP (AFP, $p = 0.6924$; AFP-L3, $p = 0.2889$; DCP, $p = 0.8992$).

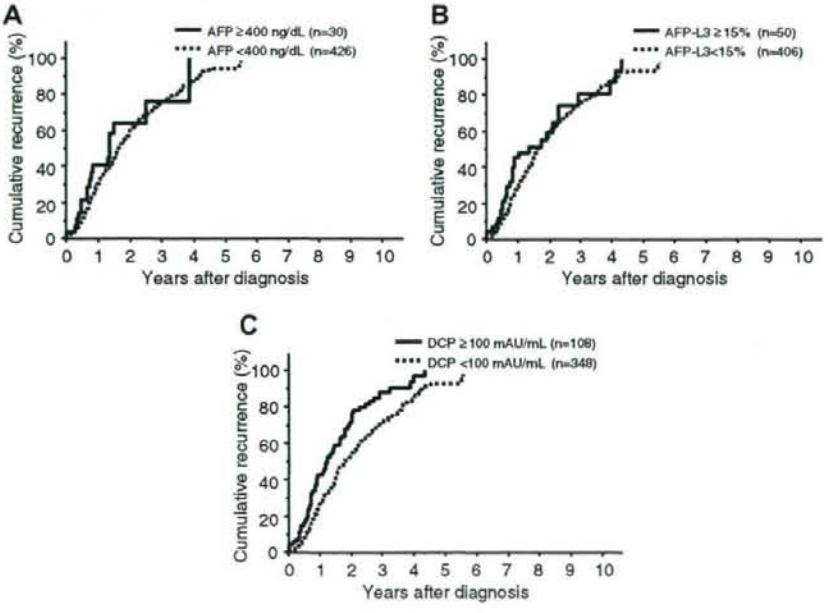

Fig. 5. Cumulative recurrence rates according to the elevation of pretreatment (A) alpha-fetoprotein (AFP), (B) *Lens culinaris* agglutinin A-reactive fraction of AFP (AFP-L3), or (C) des-gamma carboxy prothrombin (DCP) in patients who underwent LTA. No significant difference was found between patients with or without elevated pretreatment AFP or AFP-L3 (AFP, $p = 0.2323$; AFP-L3, $p = 0.2779$). The recurrence rate of patients with elevated pretreatment DCP was significantly higher than that of patients without the elevation ($p < 0.0001$).

($p = 0.0027$, $p = 0.0352$, and $p < 0.0001$, respectively, data not shown).

4. Discussion

In the present study, we evaluated the prognostic value of the pretreatment levels of three tumor markers for HCC (AFP, AFP-L3, and DCP) in patients who undergo treatments for HCC with curative intent, i.e., hepatectomy and LTA. We excluded patients treated by ethanol injections, which had been widely performed as a locoregional curative treatment for HCC before the emergence of thermal ablation, because a lower survival rate and a higher recurrence rate were reported in patients who underwent ethanol injection relative to those treated by LTA [9].

The survival rate was comparable between patients who underwent hepatectomy and those who underwent LTA. However, we cannot make any conclusions as to the differential benefits of these two treatment modalities. The backgrounds of patients were largely different between the two study populations. The remnant liver function was significantly better in patients who underwent hepatectomy than in those who underwent LTA, with a higher rate of Child-Pugh class A patients, higher serum albumin levels, and lower serum total bilirubin levels (all, $p < 0.0001$). In addition, the tumor size (of maximum tumor) was significantly larger in patients who underwent hepatectomy than in those who underwent LTA ($p < 0.0001$). The purpose of the present study is not to compare the impact of treatment modalities on patient survival, but rather to compare the prognostic value of the pretreatment elevation of tumor markers for HCC in different patient subgroups that underwent different treatments.

In univariate and multivariate analyses of patient survival, we found no difference in survival rates between patients with and without elevated serum AFP, in both patients treated by hepatectomy and those treated by LTA. AFP is currently the most widely used marker for monitoring the development of HCC [3,21,22]. However, AFP also increases in association with hepatocyte regeneration and is associated with serum alanine aminotransferase activity; AFP values above the normal limit (>20 ng) are observed in up to 20% of patients with chronic hepatitis and in 20% to 60% of patients with cirrhosis, even in the absence of HCC [6]. In addition, a recent study revealed the significance of AFP as a marker for liver fibrosis [23]. AFP, therefore, does not always directly reflect the development or progression of HCC. In our previous studies [7,8], the elevation of AFP was not associated with the progression of HCC, although its elevation predicted lower survival rates. These characteristics of AFP probably accounted for the lack of influence of AFP elevation on the survival of patients who underwent hepatectomy or LTA.

The elevation of pretreatment AFP-L3 levels significantly influenced decreased survival rate of patients who underwent LTA, and it also had a tendency to decrease survival rate of patients who underwent hepatectomy in multivariate analyses. In univariate analyses of hepatectomy patients, the survival rate of patients with elevated serum AFP-L3 was significantly lower than that in patients without AFP-L3 elevation. Our previous study revealed that pretreatment AFP-L3 elevation reflects greater number of tumors as well as larger HCC in size [7]. The pretreatment elevation of AFP-L3 might reflect the presence of small HCC tumors that cannot be detected in the liver by currently available imaging technologies, such as intrahepatic microscopic metastases of HCC, resulting in decreased survival rates in patients with elevated pretreatment AFP-L3 after the initial treatment of both hepatectomy and LTA. However, we found no difference in recurrence rate of HCC in both patient subgroups. Further study will be needed for the effect of pretreatment AFP-L3 elevation on patient survival and HCC recurrence in patients treated by curative therapies.

The elevation of pretreatment DCP level exhibited the most significant impact on decreased survival rate of patients who underwent LTA, whereas it did not affect the survival rate of hepatectomy patients. DCP elevation is reportedly an indicator of portal vein invasion of HCC [24]. Our previous study also revealed higher rates of HCCs with portal vein invasion as well as larger HCC in size in patients with elevated pretreatment DCP [7]. The presence of microscopic invasions of HCC tumors into the portal vein that was not detected by imaging modalities might be present more likely in patients with elevated pretreatment DCP. The presence of such invasions might affect decreased survival rate of patients who underwent LTA with increased recurrence rate, whereas hepatectomy might be able to overcome this microscopic invasion.

In summary, tumor markers for HCC had different impacts on the survival of HCC patients who underwent hepatectomy or LTA. Elevated pretreatment AFP-L3 levels affect decreased survival of patients who underwent hepatectomy and LTA but without increased recurrence. Elevated levels of pretreatment DCP had a significant impact on decreased survival only of patients who underwent LTA with increased recurrence rate. These data should be taken into consideration when selecting treatment options. In addition, further studies will be needed to evaluate the impact of other potential tumor markers for HCC, such as glypican-3, Golgi protein 73, hepatocyte growth factor, insulin growth factor 1, vascular endothelial growth factor, transforming growth factor-beta1, and alpha-L-fucosidase [25], on the survival of patients treated by different therapies.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2008.04.013.

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Correlation of serum ribavirin concentration with pretreatment renal function estimates in patients with chronic hepatitis C receiving combination antiviral therapy with peginterferon and ribavirin

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SUMMARY. Serum ribavirin concentration is an important factor in antiviral therapy in combination with peginterferon (PEG-IFN) and ribavirin for patients with chronic hepatitis C in terms of both beneficial and adverse effects. We evaluated whether the serum ribavirin concentration can be predicted on the basis of renal function estimates. Serum creatinine and cystatin C concentrations were measured at the start of treatment in a total of 148 patients with chronic hepatitis C who underwent combination PEG-IFN and ribavirin therapy. Creatinine clearance (CrCl) and total clearance of ribavirin (CL/F) were calculated on the basis of the serum creatinine level. The glomerular filtration rate was calculated with two different formulae on the basis of the serum cystatin C level. These values were compared with serum ribavirin concentrations 4 weeks after the start of therapy. The cystatin C level increased with the progression of liver fibrosis, whereas the creatinine level was constant regardless of the degree of liver fibrosis. Significant correlation was not observed between the serum ribavirin concentration and serum creatinine level, cystatin C level, or calculated renal function estimates. However, significant correlation was found between the serum ribavirin concentration and CrCl and CL/F in patients who were given ribavirin >800 mg/day. Overall, renal function estimates do not correlate with the serum ribavirin concentration in Japanese patients with chronic hepatitis C who undergo combination PEG-IFN and ribavirin therapy. Serum creatinine-based renal function estimates might be predictive for the serum ribavirin concentration only in patients with a daily ribavirin intake of 800 mg or more.

Keywords: chronic hepatitis C, creatinine, cystatin C, renal function, ribavirin.

INTRODUCTION

Combination interferon (IFN) and ribavirin therapy is the current standard antiviral therapy for chronic hepatitis C. The addition of ribavirin to IFN-α substantially increases the sustained virological response rate when compared with that of IFN-α alone among treatment-naïve [1–3] and nonresponding or relapsed IFN-α-experienced patients [4–6]. Recently, the combination of peginterferon (PEG-IFN)-α and ribavirin has been shown to be superior to IFN-α and ribavirin with a high sustained virological response rate [7,8].

Abbreviations: CrCl, creatinine clearance; CL/F, total clearance of ribavirin; HCV, hepatitis C virus; GFR, glomerular filtration rate.

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Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a synthetic guanosine nucleoside analogue, inhibits the replication of a wide range of RNA and DNA viruses, including those of the flavivirus family, of which hepatitis C virus (HCV) is a member [9]. Although ribavirin improves the antiviral effect of IFN against HCV infection, ribavirin accumulates in red blood cells, and haemolytic anaemia is one of the main adverse effects of ribavirin therapy. Therefore, maintaining an appropriate ribavirin concentration is important for providing a beneficial antiviral effect while avoiding or reducing the ribavirin-induced anaemia [10,11].

Ribavirin is normally eliminated by renal filtration [9]. Several studies have shown the importance of renal function for maintaining an appropriate serum ribavirin concentration during combination antiviral therapy with PEG-IFN and ribavirin for chronic hepatitis C [12,13]. Therefore, renal

function estimates can help to predict the serum ribavirin concentration in patients during treatment.

In the present study, we analyzed the correlation between pretreatment renal function estimates and the serum ribavirin concentration during treatment, and we evaluated renal function markers as pretreatment predictors of the serum ribavirin concentration.

PATIENTS AND METHODS

Patients

A total of 148 patients with chronic hepatitis C undergoing combination therapy with PEG-IFN-α and ribavirin were enrolled in the study. The clinical characteristics of the study patients are shown in Table 1. Patients included 85 women and 63 men with a mean age of 58.3 ± 10.2 years. Liver histology was evaluated by examining liver specimens obtained by fine-needle biopsy prior to antiviral therapy. METAVIR activity scores were A0 in 3 patients, A1 in 84, A2 in 42 and A3 in 11, and METAVIR fibrosis stages were F0 in 13 patients, F1 in 70, F2 in 38 and F3 in 19 [14]. Liver biopsy was not carried out in eight patients. One hundred and three patients were infected with HCV genotype 1b, 31 patients were infected with HCV genotype 2a and the other 14 patients were infected with HCV genotype 2b. The pretreatment HCV RNA concentration was shown to be $1747 \pm 1281 \times 10^3$ IU/mL by quantitative PCR assay (Amplicor GT-HCV Monitor, Version 2.0; Roche Molecular Systems, Pleasanton, CA, USA). Eighty-seven patients had no history of IFN therapy (naïve cases) and the other 61 patients had previous IFN therapy (retreatment cases).

The daily dose of ribavirin was adjusted by patient's body weight in accordance with the manufacturer's recommendations. 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 1000 mg of ribavirin per day. Patients weighing ≤45 kg were given 60 μg of PEG-IFN-α 2b (Schering-Plough, Osaka, Japan) 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. Eighty-four patients were given 600 mg of ribavirin per day, 58 patients were given 800 mg per day and 6 patients were given 1000 mg per day. No patient had the daily dose of ribavirin reduced during the first 4 weeks of therapy.

Measurement of serum renal function markers and evaluation of renal function

Serum creatinine and cystatin C were measured from the same serum samples. The serum creatinine level (mg/dL) was measured on the Dimension Clinical Chemistry System (Dade Behring, Marburg, Germany) with a commercially available assay. The serum cystatin C level (mg/L) was analyzed by a fully automated latex-enhanced immunonephelometric method (N Latex Cystatin C assay on the Nephelometer II System, Dade Behring).

Table 1 Clinical characteristics of the study patients (n = 148)

Age (years)	58.3 ± 10.2
Sex (female/male)	85 (57.4)/63 (42.6)
Body weight (kg)	58.7 ± 10.0
History of interferon therapy (naïve/retreatment)	87 (58.8)/61 (41.2)
History of transfusion (−/+)	119 (80.4)/29 (19.6)
Alanine aminotransferase (IU/L)	56.7 ± 48.1
Aspartate aminotransferase (IU/L)	50.2 ± 41.0
Gamma-glutamyl transpeptidase (IU)	50.2 ± 68.2
Alkaline phosphatase (IU/L)	265.4 ± 103.4
Albumin (g/dL)	4.21 ± 0.32
Total bilirubin (mg/dL)	0.68 ± 0.27
White blood cell counts (/μL)	5062 ± 1319
Haemoglobin (g/dL)	14.0 ± 1.3
Platelet counts (×103/μL)	17.6 ± 5.6
Liver histology-activity (A0/A1/A2/A3)	3 (2.1)/84 (60.0)/42 (30.0)/11 (7.9)*
Liver histology-fibrosis (F0/F1/F2/F3)	13 (9.3)/70 (50.0)/38 (27.1)/19 (13.6)*
HCV genotype (1b/2a/2b)	103 (69.6)/31 (20.9)/14 (9.5)
HCV RNA concentration (×103 IU/mL)	1747 ± 1281
Daily dose of ribavirin (600 mg/800 mg/1000 mg)	84 (56.8)/58 (39.2)/6 (4.0)

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

Renal function estimates were calculated on the basis of serum creatinine and cystatin C levels and patient's age, sex and body weight. Creatinine clearance (CrCl, mL/min) and total body clearance of ribavirin (CL/F, L/h) were calculated from the serum creatinine level by means of the Cockcroft–Gault formula [15]:

$$\mathrm{CrCl} = (140 - \mathrm{age}) \times \mathrm{body\ weight} \times \mathrm{sex}/72 \times \mathrm{serum\ creatinine}$$

where CrCl is in mL/min, body weight is in kg, sex = 1 for men and 0.85 for women and serum creatinine is in mg/dL; and the Kamar formula [13] is given as follows:

$$\mathrm{CL/F} = 32.3 \times \mathrm{body\ weight} \times [1 - (0.0094 \times \mathrm{age})] \times [1 - (0.42 \times \mathrm{sex})]/\mathrm{serum\ creatinine},$$

where CL/F is in L/h, body weight is in kg, sex = 0 for men and 1 for women, and serum creatinine is in μmol/L. Serum creatinine (mg/dL) was converted to serum creatinine (μmol/L) by the following formula:

$$\mathrm{Serum\ creatinine}(\mu\mathrm{mol/L}) = \mathrm{Serum\ creatinine}(\mathrm{mg/dL}) / 113.12 \times 10{,}000$$

The glomerular filtration rate (GFR, mL/min/1.73 m^2) was calculated from the serum cystatin C level by means of the Hoek formula [16]:

$$\mathrm{GFR} = -4.32 + 80.35 \times 1/\mathrm{serum\ cystatin\ C}$$

and the Larsson formula [17] is given as follows:

$$\mathrm{GFR} = 77.239 \times (\mathrm{serum\ cystatin\ C})^{-1.262}$$

where GFR is in mL/min/1.73 m^2 and serum cystatin C is in mg/L.

Measurement of the serum ribavirin concentration

Serum ribavirin concentrations were analyzed in serum samples obtained at 4 weeks after the start of ribavirin administration, because the serum ribavirin concentration reportedly reaches a plateau 4 weeks after the start of ribavirin intake [18,19]. Serum ribavirin concentrations (ng/mL) were determined by means of a high-performance liquid chromatography method which has been previously reported [20].

Statistical analyses

Data are shown as mean ± SD, unless otherwise indicated. Differences in quantitative values between two groups were analyzed by the Mann–Whitney *U*-test. Correlation between values was tested by the Spearman rank correlation coefficient. All *P*-values were two-tailed and $P < 0.05$ was accepted as statistically significant.

The study protocol was approved by the hospital ethics committee and was carried out in compliance with the Helsinki declaration.

RESULTS

Concentrations of serum creatinine and cystatin C and renal function

The median serum creatinine level was 0.62 mg/dL (range 0.30–1.30) and the median serum cystatin C level was 0.82 mg/L (range 0.60–1.43). These two values were well-correlated ($P < 0.0001$). The changes in serum creatinine and serum cystatin C levels are shown in Fig. 1 according to the grade of liver fibrosis evaluated by examining biopsy specimens. The serum creatinine level was constant regardless of the grade of liver fibrosis (0.65 ± 0.12 mg/dL in F0, 0.67 ± 0.15 mg/dL in F1, 0.65 ± 0.11 mg/dL in F2 and 0.61 ± 0.16 mg/dL in F3). In contrast, the serum cystatin C level increased with the degree of liver fibrosis (0.74 ± 0.11 mg/L in F0, 0.84 ± 0.15 mg/L in F1, 0.86 ± 0.14 mg/L in F2 and 0.94 ± 0.17 mg/L in F3; for F0 *vs* F1, $P = 0.0175$; for F0 *vs* F2, $P = 0.0006$; for F0 *vs*

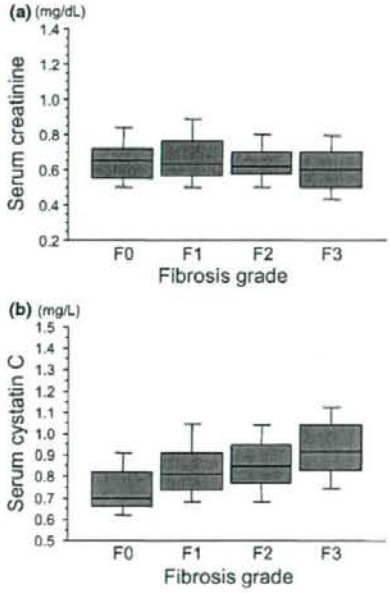

Fig. 1 Pretreatment serum creatinine and cystatin C levels in relation to histologic liver fibrosis grade ($n = 140$). Whereas the serum creatinine level is constant regardless of the grade of liver fibrosis, the serum cystatin C level increases with the degree of liver fibrosis (for F0 *vs* F1, $P = 0.0175$; for F0 *vs* F2, $P = 0.0006$; for F0 *vs* F3, $P = 0.0007$; for F1 *vs* F3, $P = 0.0128$; for F2 *vs* F3d, $P = 0.0482$).

F3, $P = 0.0007$; for F1 *vs* F3, $P = 0.0128$; for F2 *vs* F3, $P = 0.0482$).

The renal function estimates were calculated from serum creatinine and serum cystatin C levels by applying patient's age, sex and body weight to the appropriate formula. The median CrCl was 95.7 mL/min (range 49.7–209.7), calculated from the serum creatinine level according to the Cockcroft–Gault formula. The median CL/F was 11.7 L/h (range 2.5–56.9), calculated from the serum creatinine level according to the Kamar formula. The GFR calculated from the serum cystatin C level was 93.7 mL/min/1.73 m^2 (range 51.9–129.6) according to the Hoek formula and 99.2 mL/min/1.73 m^2 (range 49.2–147.2) according to the Larsson formula.

Correlation between the serum ribavirin concentration and renal function markers

The median serum ribavirin concentration was 2260 ng/mL (range 961–4394). Correlation between serum creatinine-based renal function estimates and the serum ribavirin concentration and between serum cystatin C-based renal function estimates and the serum ribavirin concentration are shown in Fig. 2. Significant correlation was not found between the serum ribavirin concentration and renal function estimates, with the exception of a mild correlation with creatinine-based CL/F ($P = 0.0240$).

We evaluated correlations by dividing patients into two groups according to the daily dose of ribavirin: 84 patients who were given 600 mg of ribavirin daily (i.e., patients with body weight of ≤60 kg) and 64 patients who were given 800 mg or more of ribavirin daily (i.e., patients with body weight of >60 kg). In patients who were given 600 mg of ribavirin daily, we found no correlation between creatinin- or cystatin C-based renal function estimates and the serum ribavirin concentration. In contrast, we found significant correlations between creatinine-based CrCl and CL/F and the serum ribavirin concentration in patients who were given 800 mg or more of ribavirin daily (column A of Fig. 3; $P = 0.0059$ for CrCl and $P = 0.0082$ for CL/F). We found no correlation when cystatin C-based renal function estimates were compared with serum ribavirin concentration (column B of Fig. 3; $P = 0.2322$ for serum cystatin C, for GFR by the Hoek formula, and for GFR by the Larsson formula).

DISCUSSION

In the present study, we investigated correlation between serum ribavirin concentration during its plateau phase and renal function estimates. For renal function estimates, we measured the serum creatinine level and calculated CrCl and CL/F based on this value, and we measured the serum cystatin C level and the calculated GFR based on this value.

Cystatin C is a non-glycosylated cationic protein of 13.3 kDa belonging to the cystatin superfamily of cysteine protease inhibitors [21,22]. Recent studies have shown that measuring cystatin C allows detection of renal impairment, earlier than measuring serum creatinine does [23,24]. An increase in the serum cystatin C level preceding an increase in the serum creatinine level has been reported in patients with cirrhosis [25–29], in patients with diabetes [30] and in elderly persons without chronic kidney disease [31]. Use of the cystatin C level has been reported for prediction of the concentration and for dose adjustment of several drugs including digoxin, amikacin, gentamicin, tobramycin and vancomycin [32,33].

In the present study, serum cystatin C and cystatin C-based GFR did not correlate with the serum ribavirin concentration. Therefore, cystatin C-based renal function estimates cannot predict the serum ribavirin concentration in patients with chronic hepatitis C who undergo combination therapy with PEG-IFN and ribavirin. The increase in the cystatin C level with the progression of liver fibrosis, which was observed in our study patients and has previously been reported [34,35], might be one of the reasons for the lack of association between the serum cystatin C level or cystatin C-based GFR and serum ribavirin concentration, despite reports that cystatin C is a sensitive indicator of early renal impairment and that ribavirin is eliminated by renal filtration.

Bruchfeld *et al.* previously reported a significant link between renal function evaluated by CrCl and serum ribavirin concentration in Swedish patients with chronic hepatitis C [12]. In contrast, we failed to find a significant correlation between serum creatinine-based renal function and serum ribavirin concentration in our overall study patients. The body weight of patients between these two studies was largely different: 77 ± 16 kg in patients of the Swedish study and 58.7 ± 10.0 kg in patients of our study. In addition, a large number of patients (56.8%) from our study were given 600 mg of ribavirin daily, whereas all patients were given ≥800 mg of ribavirin daily in the Swedish study. These differences might account for the conflicting result between the study by Bruchfeld *et al.* and the present study. Indeed, we found significant correlation between serum creatinine-based CrCl and CL/F and serum ribavirin concentration in patients who were given ≥800 mg of ribavirin daily (patients with a body weight of >60 kg). Because of the lower body weight of Asian persons including the Japanese, in comparison to Western and African persons, larger percentages of Asian patients have a body weight of ≤60 kg, and 600 mg of ribavirin is given. However, in Western countries, most of the patients start combination therapy with a ribavirin dose of 800 mg or more. Serum creatinine-based CrCl and CL/F can, therefore, be more useful and predictive in Western countries. Further studies are needed to confirm creatinine-based CrCl and CL/F as predictors of serum ribavirin

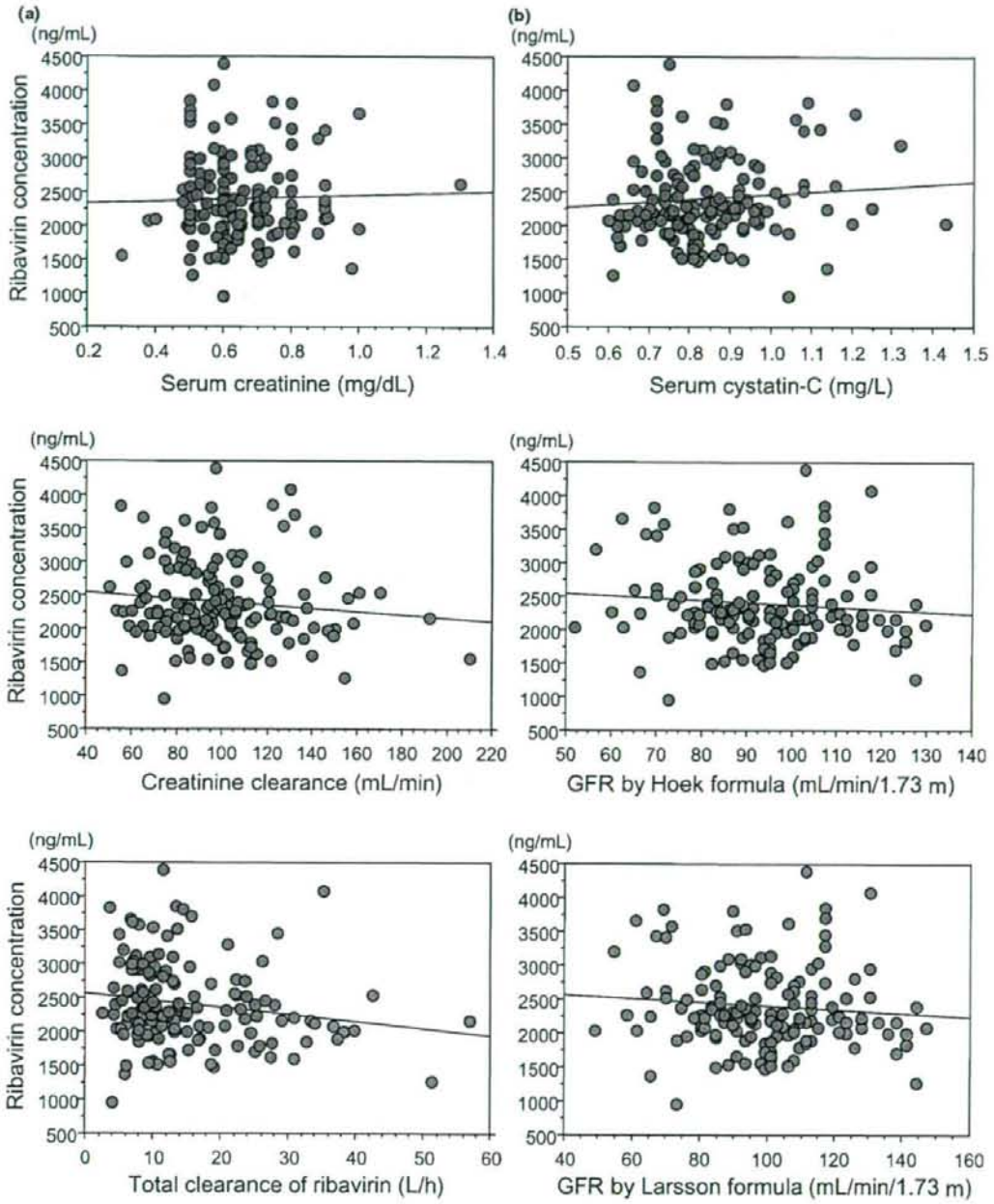

Fig. 2 Correlation between the serum ribavirin concentration 4 weeks after the start of combination therapy and pretreatment serum creatinine and creatinine-based renal function (CrCl and total clearance of ribavirin) (a), and pretreatment serum cystatin C and cystatin C-based renal function (GFR by the Hoek formula and by the Larsson formula) (b). No significant correlation was observed except for the correlation between ribavirin concentration and total clearance of ribavirin (for serum creatinine *vs* ribavirin concentration, $P = 0.9102$; for CrCl *vs* ribavirin concentration, $P = 0.1416$; for total clearance of ribavirin *vs* ribavirin concentration, $P = 0.0240$, for serum cystatin C *vs* ribavirin concentration, GFR by the Hoek formula *vs* ribavirin concentration and GFR by the Larsson formula *vs* ribavirin concentration, $P = 0.2122$).

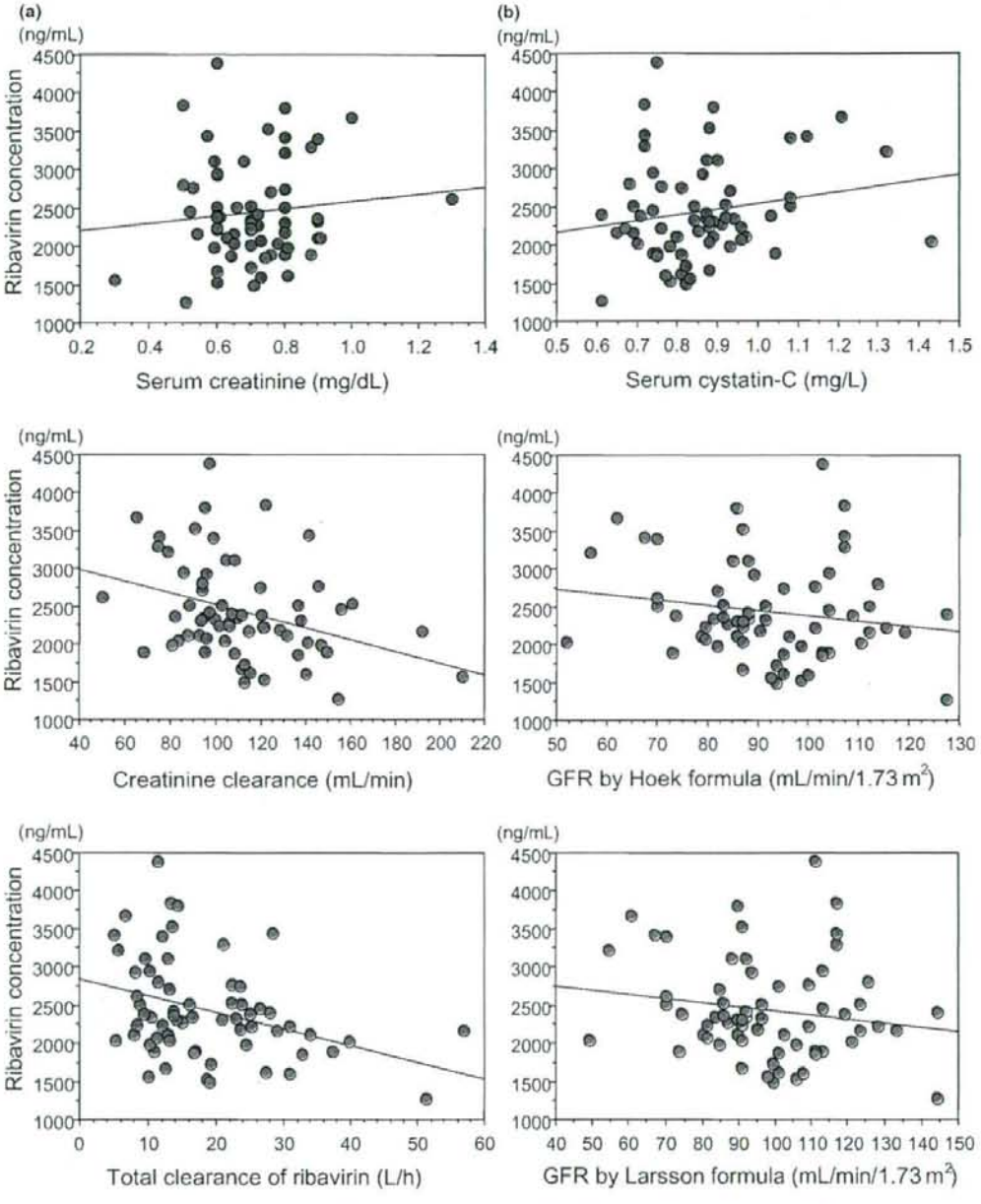

Fig. 3 Correlation between the serum ribavirin concentration 4 weeks after the start of combination therapy and pretreatment serum creatinine and creatinine-based renal function (CrCl and total clearance of ribavirin) (a), and pretreatment serum cystatin C and cystatin C-based renal function (GFR by the Hoek formula and by the Larsson formula) (b) in patients with a daily ribavirin intake of ≥800 mg. Significant correlation was observed between the ribavirin concentration and CrCl and between the ribavirin concentration and total clearance of ribavirin (for serum creatinine *vs* ribavirin concentration, $P = 0.9186$; for CrCl *vs* ribavirin concentration, $P = 0.0059$; for total clearance of ribavirin *vs* ribavirin concentration, $P = 0.0082$). In contrast, no significant correlation was observed between the ribavirin concentration and any of serum cystatin C-based renal function markers (for serum cystatin C *vs* ribavirin concentration, GFR by the Hoek formula *vs* ribavirin concentration and GFR by the Larsson formula *vs* ribavirin concentration, $P = 0.2322$).

concentrations in Asian patients who were given ≥800 mg of ribavirin daily.

In summary, renal function estimates did not correlate with the serum ribavirin concentration in patients with chronic hepatitis C undergoing combination therapy with PEG-IFN and ribavirin. However, serum creatinine-based renal function estimates might be predictive of the serum ribavirin concentration for patients taking 800 mg or more of ribavirin daily. Recent studies have shown that dose adjustment of ribavirin according to renal function improves the antiviral effect and reduces adverse effects [36,37]. Further studies are needed to find better predictors of the ribavirin concentration during combination antiviral therapy for chronic hepatitis C to allow for individualized adjustment of the ribavirin dose aimed at a greater antiviral effect and fewer adverse effects.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Figure S1. Correlation between pretreatment serum creatinine and serum cystatin C levels in 148 patients with chronic hepatitis C undergoing combination antiviral therapy with peginterferon and ribavirin ($P < 0.0001$).

Figure S2. Correlation between serum ribavirin concentration 4 weeks after the start of combination therapy and creatinine- and cystatin C-based renal function in patients who were given 600 mg of ribavirin.

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CLINICAL STUDIES

Eight-week regimen of antiviral combination therapy with peginterferon and ribavirin for patients with chronic hepatitis C with hepatitis C virus genotype 2 and a rapid virological response

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Abstract

Background: It remains unclear how we can shorten the treatment duration of antiviral combination therapy with peginterferon and ribavirin for patients with chronic hepatitis C virus (HCV) genotype 2 infection who achieved a rapid virological response (RVR). *Aim:* We compared the efficacy of antiviral combination therapy with peginterferon and ribavirin for 8 vs. 24 weeks for the treatment of patients with HCV genotype 2 infection and with RVR. *Methods:* Sixty-one patients were enrolled. Serum HCV RNA was not detected at 4 weeks after the start of treatment in 32 patients with an RVR. These 32 patients were randomly assigned to 8-week ($n = 15$) or 24-week ($n = 17$) treatment regimens. Patients in the 8-week group who relapsed underwent a 24-week retreatment. *Results:* No significant difference in patient characteristics was observed between the 8- and the 24-week treatment groups. A sustained virological response (SVR) was seen in five of 15 patients (33.3%) in the 8-week treatment group and 14 of 17 (82.4%) in the 24-week treatment group; the rate was significantly higher in the 24-week treatment group ($P = 0.0140$). Nine of 10 relapsed patients in the 8-week treatment group underwent a 24-week retreatment, and seven achieved an SVR. *Conclusion:* An 8-week regimen of combination antiviral therapy with peginterferon and ribavirin yielded an increase in the relapse rate, indicating the limitation of a reduction of treatment below 12 weeks in patients with genotype 2, after RVR.

The currently recommended treatment regimen for patients with chronic hepatitis C who are infected with hepatitis C virus (HCV) genotype 2 is combination therapy comprising weekly administration of peginterferon and daily administration of ribavirin for 24 weeks (1). Approximately 80% of patients have a sustained virological response (SVR) with this regimen (2, 3). Increasing the duration of treatment from 24 to 48 weeks does not appear to increase the rate of SVR (2–4). Several studies have attempted to shorten the treatment period for this patient population. SVR rates of 80–85% were observed with just 12–16 weeks of treatment in patients in whom serum HCV RNA was undetectable within 4 weeks after the start of therapy (5–8). These results suggest that patients with HCV genotype 2 infection and a rapid virological response (RVR, undetectable serum HCV RNA by 4 weeks after the start of therapy) may be treated for < 24 weeks. However, the results of a recent large clinical trial reported a lower overall SVR rate with a 16-week treatment regimen than with the standard 24-week treatment regimen in patients with HCV genotype 2 or 3, although the results suggested that the difference in SVR rate was smaller in patients with an RVR (9).

Antiviral therapy with peginterferon and ribavirin can cause various adverse effects, some of which can be severe. Adverse effects reportedly increase with the length of treatment (10). Investigation into further shortening of the treatment period is therefore important in terms of adverse effects and medical economy. In the present study, we evaluated the efficacy of an 8-week regimen of antiviral therapy with peginterferon and ribavirin for patients with HCV genotype 2 and an RVR.

Patients and methods

Patients

A total of 61 patients with chronic HCV genotype 2 infection who underwent antiviral combination therapy with peginterferon and ribavirin were enrolled in this study during the period January – June 2006. The patient characteristics are listed in Table 1. Patients comprised 24 men and 37 women with a mean age of 56.7 ± 11.2 years. Forty patients had no history of interferon therapy (naïve cases), and 21 patients had previously undergone interferon therapy (retreatment cases). HCV genotype was 2a in 46 patients and 2b in 15 patients, and the pretreatment HCV RNA concentration was $1919 \pm 1618 \times 10^3$ IU/ml by a quantitative polymerase chain reaction assay (Amplicor GT-HCV Monitor, version 2.0; Roche Molecular Systems, Pleasanton, CA, USA). All patients had a pretreatment serum HCV RNA concentration of $> 100 \times 10^3$ IU/ml because the addition of ribavirin to peginterferon is not allowed by Japanese National Medical Insurance for patients with a pretreatment HCV RNA concentration $\leq 100 \times 10^3$ IU/ml. Liver histology was evaluated before antiviral therapy in specimens obtained by a fine-needle biopsy. According to METAVIR scoring (11), the activity grade was A0 in one patient, A1 in 39, A2 in 13

and A3 in four, and the fibrosis grade was F0 in three patients, F1 in 39, F2 in 11 and F3 in four. Liver biopsy was not performed for four patients.

Study design

All patients were given peginterferon α-2b (Schering-Plough, Osaka, Japan) weekly and ribavirin (Schering-Plough) daily. For peginterferon, patients weighing ≤ 45 kg were given 60 μg, those > 45 kg and ≤60 kg were given 80 μg, those >60 kg and ≤75 kg were given 100 μg, those >75 kg and ≤90 kg were given 120 μg and those >90 kg were given 150 μg according to the manufacturer's recommendations. The ribavirin dosage was also adjusted according to body weight. Patients ≤60 kg were given 600 mg, those >60 kg and ≤80 kg were given 800 mg and those >80 kg were given 1000 mg according to the manufacturer's recommendations.

Serum HCV RNA was measured 4 weeks after the start of therapy. Patients with detectable serum HCV RNA underwent the standard 24-week treatment. Patients with no detectable serum HCV RNA were randomly assigned to one of two groups; one group underwent the 8-week treatment, and the other underwent the standard 24-week treatment. When relapse occurred after completion of treatment in the 8-week group, it was recommended that the patients undergo retreatment with the same dose of peginterferon α-2b and ribavirin for an additional standard 24 weeks.

All patients were monitored during the treatment period and for an additional 6 months after the end of treatment. They underwent weekly outpatient evaluations during the first 2 months and monthly evaluations during the rest of the treatment period and during the 6-month follow-up period. Patients underwent a physical examination, complete blood count, laboratory tests and serum HCV RNA measurement. Serum HCV RNA was measured using a COBAS TaqMan HCV test (Roche Molecular Systems); the detection limit was 15 copies/ml. SVR was defined as a continuous absence of serum HCV RNA up to 6 months after the completion of therapy.

Table 1. Clinical characteristics of the study patients ($n = 61$)

Age (years)	56.7 ± 11.2
Gender (female/male)	37 (60.7)/24 (39.3)
History of interferon therapy (naïve/retreatment)	40 (65.6)/21 (34.4)
History of transfusion (no/yes)	44 (72.1)/17 (27.9)
Alanine aminotransferase (IU/L)	51.4 ± 56.4
Aspartate aminotransferase (IU/L)	45.7 ± 49.2
γ-glutamyl transpeptidase (IU)	58.7 ± 123.7
Alkaline phosphatase (IU/L)	270.5 ± 147.9
Albumin (g/dl)	4.30 ± 0.33
Total bilirubin (mg/dl)	0.65 ± 0.24
White blood cell count (/μl)	5001 ± 1602
Haemoglobin (g/dl)	14.0 ± 1.5
Platelet count (× 10^3/μl)	19.5 ± 6.5
Body weight (kg)	59.1 ± 10.9
Liver histology – activity (A0/A1/A2/A3)*	1 (1.8)/39 (68.4)/ 13 (22.8)/4 (7.0)
Liver histology – fibrosis (F0/F1/F2/F3)*	3 (5.3)/39 (68.4)/ 11 (19.3)/4 (7.0)
HCV genotype (2a/2b)	46 (75.4)/15 (24.6)
HCV RNA concentration (× 10^3 IU/ml)	1919 ± 1618

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

Statistical analysis

Quantitative values are shown as mean ± standard deviation. Between-group differences were analysed using the χ^2 test. Differences in quantitative values between two groups were analysed using the Mann–Whitney *U*-test. All *P* values were two tailed, and $P < 0.05$ was accepted as being statistically significant.

The study protocol was approved by the hospital ethics committee and was carried out in compliance with the Helsinki declaration. Written informed consent was obtained from each patient before enrolment in this study.

Results

One patient dropped out of this study within 4 weeks after the start of treatment because of severe general malaise (Fig. 1). Therefore, serum HCV RNA at 4 weeks after the start of therapy was measured in 60 patients. Serum HCV RNA was detected in 28 (46.7%) patients and was not detected in 32 (53.3%) patients (RVR). The characteristics of RVR and non-RVR patients are listed in Table 2. A history of transfusion was most prevalent and the HCV RNA concentration was the lowest in RVR patients. The 32 RVR patients were randomized at 4 weeks after the start of therapy: 8-week group ($n = 15$) and 24-week group ($n = 17$, Fig. 1). Patients in the 8-week group underwent an additional 4 weeks of treatment. Patients in the 24-week group underwent an additional 20 weeks of treatment. No difference was found in the background characteristics between these two groups. The single dose of peginterferon and ribavirin was reduced in one and five of 17 patients, respectively, in the 24-week group, whereas no patient required reduction of the dose of peginterferon or ribavirin in the 8-week group. In non-RVR patients, three of 28 patients required reduction of the peginterferon dose, and six of 28 patients required reduction of the ribavirin dose during the treatment period. No patient required discontinuation of peginterferon or ribavirin during the treatment regimen and no patient dropped out of this study during the treatment or follow-up periods.

In patients who achieved an RVR, the SVR rate was 82.4% (14 of 17 patients) in the 24-week group and 33.3% (five of 15 patients) in the 8-week group. The SVR rate was significantly lower in the 8-week group than that in the 24-week group ($P = 0.0140$). Nine of 10 patients with relapse after treatment in the 8-week group subsequently underwent retreatment with the standard 24-week regimen (one patient declined retreatment). The SVR rate in this retreatment group was 77.8% (seven of nine patients). The SVR rate of the entire 8-week group, when including seven SVR patients by retreatment, was 80.0% (12 of 15 patients). The SVR rate between the 8- and the 24-week groups was similar when patients with an SVR by retreatment were included in the 8-week group ($P = 0.8650$). The SVR rate of patients without an RVR (non-RVR) was 53.6% (15 of 28 patients), which was between that of the 24-week group with an RVR and that of the 8-week group.

The characteristics and outcomes of patients in the 8-week group are listed in Table 3. There was no difference in patient

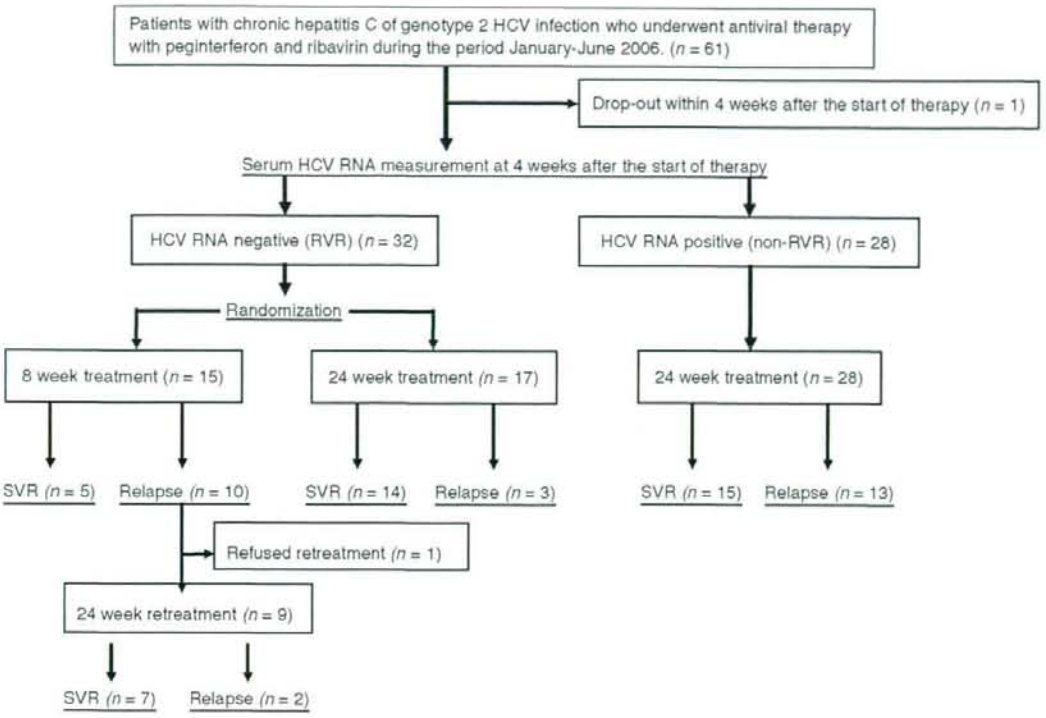

Fig. 1. Schematic of the trial profile. Serum hepatitis C virus (HCV) RNA was negative in 32 patients (rapid virological responders), and they were assigned to the 8-week regimen (15 patients) or the standard 24-week regimen (17 patients). Nine of 10 relapsers in the 8-week group underwent retreatment with the standard 24-week regimen. SVR, sustained virological response; RVR, rapid virological response.

age, gender, liver histology, HCV genotype, pretreatment HCV RNA concentration or detection of serum HCV RNA at 2 weeks after the start of therapy between patients with an SVR and those who relapsed. Serum HCV RNA was detected at 4 weeks after the end of therapy in all patients with relapse, with the exception of one patient with serum HCV RNA detected at 8 weeks after the end of therapy. Nine of 10 relapsers underwent retreatment within 1–6 months after the end of the initial treatment. The HCV RNA concentration just before retreatment was higher than that before the initial treatment in four patients, and was lower than that before the initial treatment in five patients. Two patients relapsed after retreatment, and the other seven patients achieved an SVR. One patient required reduction of the peginterferon dose, and two patients required reduction of the ribavirin dose during retreatment. No patient required discontinuation of peginterferon or ribavirin during either the initial treatment or the retreatment period.

Discussion

Shortening the period of antiviral therapy with peginterferon and ribavirin is important in terms of adverse effects and medical costs. A shortened regimen may be less expensive and better tolerated by the majority of patients. Studies on shorten treatment performed so far are different in terms of the design, the characteristics of the population and the virological methods used; these differences may account for the different relapse rates reported so far after an abbreviated course (7–9). Moreover, the possibility to further reduce to an 8-week treatment duration had not been investigated so far.

In the present study, we evaluated an 8-week regimen for patients with HCV genotype 2 and RVR. It has been reported recently that antiviral therapy is more beneficial for patients with HCV genotype 2 than for those with HCV genotype 3 (4, 6). In addition, achievement of an RVR is a reported indicator of the highest likelihood of SVR in response to short-duration therapy. Patients who were assigned to the 8-week regimen were, therefore, the population with the highest likelihood of achieving an SVR.

The results of the present study clearly showed that reduction of the treatment duration to 8 weeks is not sufficient as an antiviral therapy for patients with chronic infection of HCV genotype 2 (2a or 2b), at least in patients with pretreatment HCV RNA concentrations $> 100 \times 10^3$ IU/ml, even though an RVR is achieved. A marked increase in the rate of relapse after the completion of treatment was observed in patients in the