beta and ribavirin has the possibility to show the strong effect for hepatitis C virus (HCV) and mild side effects originating from the treatment (13-15). We have reported that the combination of IFN-beta plus ribavirin therapy is effective and safety for HCV patients with high virus load and depressive state (14). However, the previous study was retrospective and a prospective study is necessary to evaluate the efficacy and safety of combination therapy of IFN-beta and ribavirin for HCV patients with high virus load and depressive state.

Thus, in the present study, we performed a prospective study to examine the efficacy and safety of combination therapy of IFN-beta and ribavirin in HCV genotype 1b patients who had stopped the IFN therapy due to depression induced by IFN-alpha. At the same time, depression states, reflected by Beck depression inventories (BDI) and Hamilton depression rating scale (Ham-D), were assessed during combination therapy (16, 17).

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of $\geq$100 KIU/mL before treatment; 3) stopping of IFN-alpha therapy due to depression appearance during the prior IFN-alpha treatment; 4) Ham-D of <18; 5) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 6) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 7) white blood cell (WBC) >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/mL; follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy of IFN-beta and ribavirin as well as the potential adverse reactions to each patient and informed consent was obtained from each patient. This study was approved by the Human Ethics Review Committee of Toranomon Hospital.

From December 2007 to May 2008, 14 HCV patients were enrolled in this prospective cohort study at the study hospital. A sustained virological response (SVR) was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (18).

Laboratory investigation

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80℃ until analysis. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (19). Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (18). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (20). The core protein of HCV-1b was determined by the previous report (21). Next, the genetic variations near the IL28B gene (rs8099917), reported as the pretreatment predictors of treatment efficacy and clinical outcome, were investigated (22-26). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Clinical evaluation and biochemical and hematological tests were performed at 1, 2, and 4 weeks in the first month after the initiation of combination therapy. After that, these evaluations were done at monthly intervals. The patients were followed by both physicians of hepatology and psychiatry.

Combination therapy of IFN-beta and ribavirin

Treatment was provided for 48 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) by six times a week for 4 weeks, followed by three times a week for 44 weeks. The total dose was 936MU. Ribavirin (Rebetol, MSD KK., Tokyo, Japan) was given at the dose prescribed based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for $\leq$60 kg, 800 mg for >60 kg and $\leq$80 kg, and 1,000 mg for >80 kg).

Evaluation of the psychic state

The psychiatrist in charge evaluated the scores of BDI and Ham-D prospectively. BDI shows the subjective symptom of the depressive patients and Ham-D shows the objective evaluation by the psychiatrist. Scores on the BDI were divided the following; severe, 29-63; moderate, 20-28; mild, 14-19; and minimal, 0-13. Scores on the Ham-D were divided the following; very severe, >23; severe, 19-22; moderate, 14-18; mild, 8-13; and normal $\leq$7 (27).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher's exact test. The following variables were evaluated as prognostic factors: sex, age, BDI score, Ham-D score, a HCV RNA level, IL28B (genetic variation in rs8099917), variation of HCV-core, biochemical factors (AST, ALT, gamma glutamyltransferase, total cholesterol), white blood cell (WBC), hemoglobin, platelet count, HCV RNA 4, 12, 24 week after the initiation of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Table 1. **The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR ***

	Total	SVR (n=5)	Non-SVR (n=9)	p value†
Age (years old)	62.1 ± 4.3	62.4 ± 4.2	61.9 ± 4.6	0.797
Sex (male/female)	6/8	2/3	4/5	0.898
Previous IFN therapy (combination/monotherapy)	8/6	3/2	5/4	0.898
Duration of previous IFN therapy (week)	11.9 ± 7.8	11.6 ± 10.2	12.0 ± 7.1	0.699
HCV-RNA (KIU/mL)	2588± 1455	2228± 1807	2788± 1296	0.759
Core aa70 (Wild/Mutant)	6/8	3/2	3/6	0.438
BDI score	11.9 ± 10.3	12.2 ± 14.2	11.7 ± 8.4	0.518
Ham-D score	3.5 ± 4.1	3.6 ± 5.5	3.4 ± 3.5	0.606
IL28B (genetic variation in rs8099917, genotype TT/TGorGG)	7/7	5/0	2/7	0.042
AST (IU/L)	50 ± 24	46 ± 37	52 ± 17	0.112
ALT (IU/L)	68 ± 33	60 ± 35	72 ± 32	0.518
GGT (IU/L)	55 ± 59	25 ± 5	72 ± 69	0.813
Total cholesterol (mg/dL)	175 ± 30	166 ± 35	179 ± 28	0.298
White blood cell(10^3/mm^3)	4.39 ± 1.24	4.16 ± 1.02	4.52 ± 1.39	0.898
Hemoglobin (g/dL)	14.1 ± 1.1	14.2 ± 1.5	14.0 ± 0.9	0.898
Platelet(10^4/mm^3)	15.8 ± 4.8	19.9 ± 2.4	13.5 ± 4.1	0.019
HCV RNA (+/-) 4W	11/3	2/3	9/0	0.083
HCV RNA (+/-) 12W	10/4	1/4	9/0	0.012
HCV RNA (+/-) 24W	8/6	0/5	8/1	0.004

Data are number of patients (percentage) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDI, Beck depression inventories; GGT, gamma-glutamyltransferase; Ham-D, Hamilton depression rating; HCV, hepatitis C virus;
*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 44 weeks.
†Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher' exact test.

Result

Clinical characteristics of the patients

A total of 14 patients treated with IFN-beta +ribavirin were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age =62.1 years, male/female =6/8, and HCV-RNA =2,588±1,455 KIU/mL. Patients were classified into two groups according to the difference of response: SVR (n=5), Non-SVR (n=9).

Efficacy of treatment

Five of 14 patients (37.5%) had SVR by the intention to treat analysis. Table 1 shows the differences in the clinical background between patients with SVR and those without SVR. The negativity rate of HCV RNA 12 weeks after the initiation of combination therapy was 80% (4/5) in SVR group and 0%(0/9) in Non-SVR group (p=0.012). The negativity rate of HCV RNA 24 weeks after the initiation of combination therapy was 100% (5/5) in SVR group and 11.1% (1/9) in Non-SVR group (p=0.004). Next, the platelet count in SVR group was significantly higher than that in Non-SVR group.

On the IL28B (genetic variation in rs8099917), all seven patients with TG or GG at IL28B showed non-SVR. On the other hand, five of the seven patients with TT at IL28B showed SVR. The TT at IL28B that is associated with SVR was statistically significant in the present study (p=0.042).

Safety and tolerance of combination therapy

Of the 14 patients treated with IFN-beta + ribavirin included in this study, four patients necessitated a reduced dose of ribavirin due to the appearance of hemoglobin level <10 g/dL and two patients needed a reduced dose of IFN-beta due to WBC count of <2,000/mm^3. Three patients had dipstick proteinuria of +1 at 4 week after the initiation of combination therapy. This proteinuria continued during combination therapy. However, no patient discontinued combination therapy because of treatment related adverse events related to exacerbation of depression. Fig. 1 shows the changes of BDI scores in 14 patients treated with IFN-beta + ribavirin. BDI scores during combination therapy were lower than that at the initiation time of treatment. Fig. 2 shows the changes of Ham-D scores in 14 patients. There was no statistically significant difference in changes of Ham-D scores during combination therapy compared to that at the initiation time of treatment.

Regarding the prescription of antidepressant and anti-anxiety drugs, antidepressants, such as sulpiride, and amitriptyline hydrochloride, were given to three patients at the

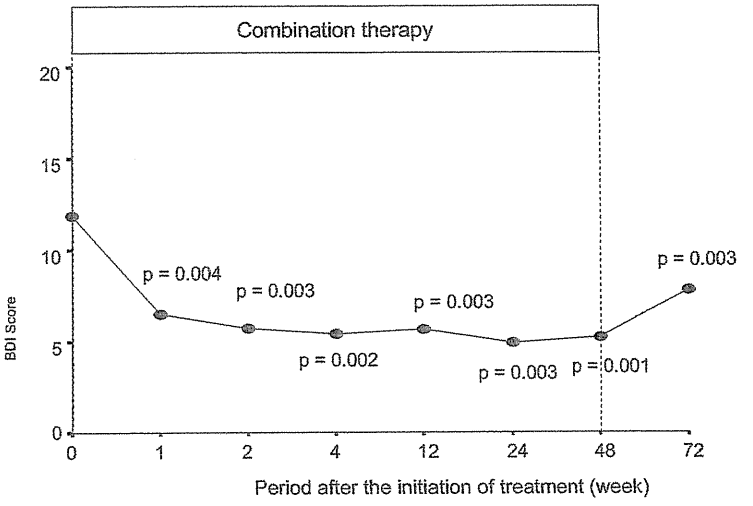

Figure 1. The change of BDI score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the BDI-2 score at the initiation time of combination therapy by the use of Mann-Whitney U test.

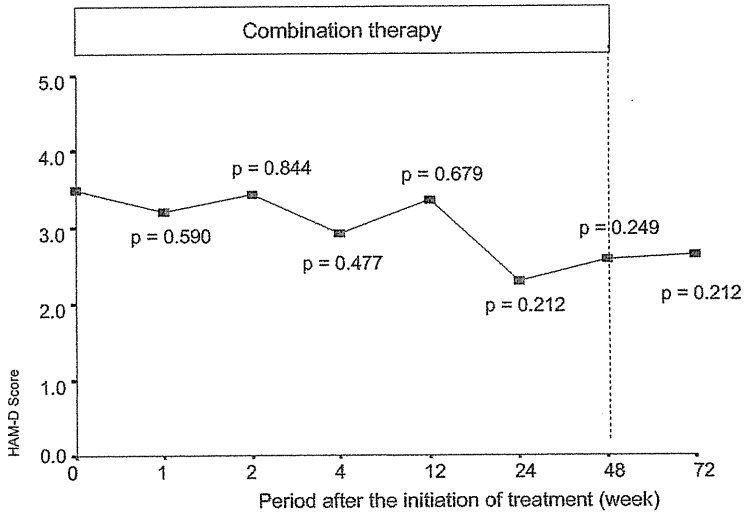

Figure 2. The change of Ham-D score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the HAM-D score at the initiation time of combination therapy by the use of Mann-Whitney U test.

start of IFN therapy and to four patients during IFN therapy. Anti-anxiety drugs, such as etizolam, alprazolam, were given to four patients at the start of IFN therapy and to five patients during IFN therapy.

The changes of WBC, hemoglobin, and platelet count after the initiation of combination therapy are shown in Fig. 3. WBC and hemoglobin levels were decreased during combination therapy. On the other hand, the platelet count decrease was statistically significant at 1, 2, and 4 weeks after the initiation of combination therapy compared to that at the initiation time of treatment. After that, the platelet count recovered to the base line at 12, 24, and 48 weeks after the initiation of combination therapy.

Discussion

In the present study, we have described the efficacy and safety of combination therapy of IFN-beta and ribavirin for patients for whom IFN therapy was discontinued due to depression induced by IFN-alpha. The patients with HCV genotype 1b and HCV-load of $\geq$100 KIU/mL were enrolled. We could evaluate the relationship between IL-28 or HCV core mutation and SVR in the combination therapy of IFN-beta and ribavirin for genotype 1b and high virus load. The present study was limited to exclude the subjects with Ham-D score of more than 18. Patients with Ham-D score of more than 18 were defined as severe depression state. It is possible that high score of Ham-D enhance the dropout

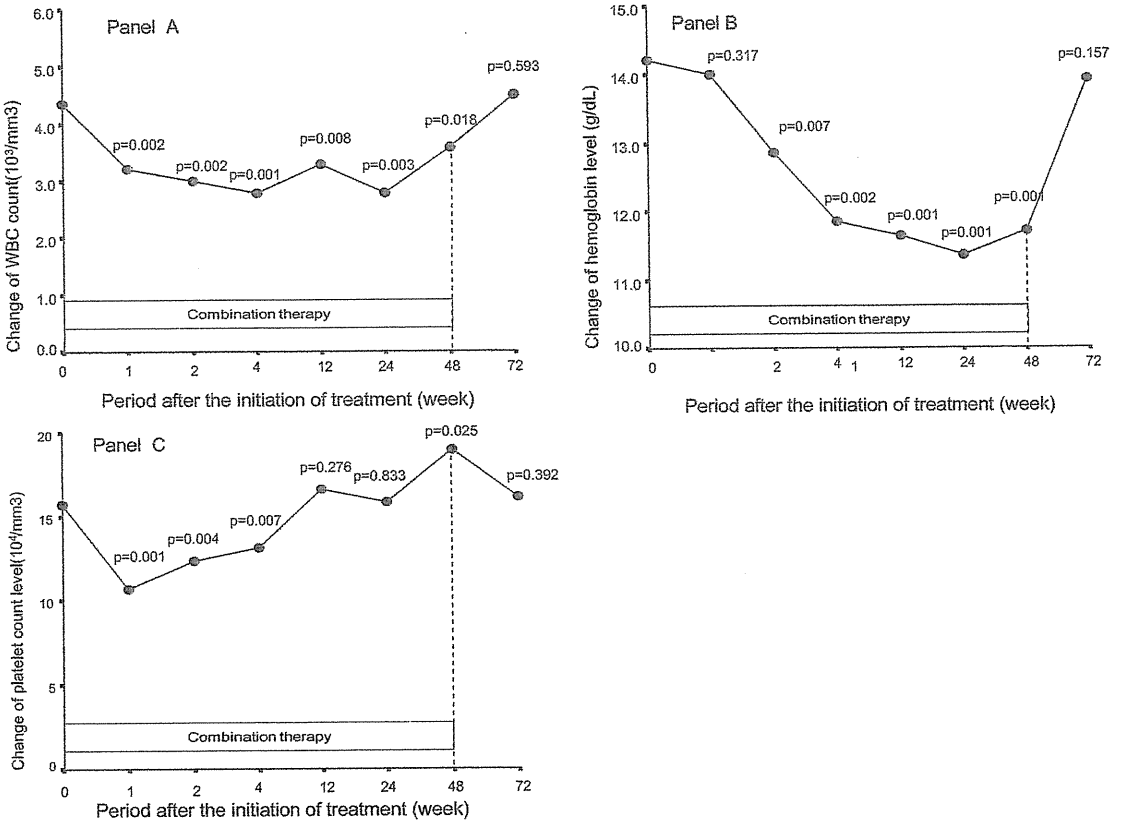

Figure 3. The change of complete blood cell count after the initiation of combination therapy. Panel A; The change of white blood cell count. Panel B; The change of hemoglobin level. Panel C; The change of platelet count.

due to combination therapy and aggravation of depressive state. Thus, we excluded the patients with Ham-D score of more than 18 in the present study. Moreover, the number of 14 patients enrolled was a small size. Another limitation is that the present study was not a randomized controlled study. Several findings from the present study have direct implications for combination therapy of IFN-beta and ribavirin for chronic hepatitis C in the future. First, the drop-out rate due to depressive state in combination therapy of IFN-beta and ribavirin was low. This result was similar to that in the previous study (14). The result by this prospective study confirmed that combination therapy of IFN-beta and ribavirin reduced the aggravation of depressive state compared with combination therapy of peginterferon-alpha and ribavirin.

Second, 5 out of 14 patients treated with combination therapy of IFN-beta and ribavirin had SVR. The SVR rate in the present study was almost the same to that in the previous study.

Third, SVR had a tendency to occur in patients with negativity of HCV RNA at 12 and/or 24 weeks after the initiation of combination therapy. All of the patients with positive HCV RNA at 24 weeks after the initiation of combination therapy showed non-SVR. This result agreed with our previous report (14). Thus, positive HCV RNA at 24 weeks after the initiation of combination therapy of IFN-beta and ribavirin suggests that the possibility of SVR is low. Next, patients with a high platelet count tended to show SVR. In general, a high platelet count suggests slight fibrosis of liver. Thus, the result raises the possibility that slight hepatic fibrosis enhance the efficacy of combination therapy.

Finally, SVR in combination therapy of IFN-beta + ribavirin was associated with IL-28B in the present study. None of the seven patients with genotype TG or GG at the genetic variation in rs8099917 near the IL28B gene had SVR. The results suggested that only patients with genotype TT might have the possibility of getting SVR. On substitution of core amino acid (aa) 70, two of eight patients with mutant type of core aa 70 showed SVR. The result shows that patients with mutant type of core aa 70 have the possibility of getting SVR. Several authors have reported that virus clearance in combination therapy of peginterferon-alpha and ribavirin is associated with HCV mutations in the core region and IL-28B (21-26). The present study confirmed that IL-28B was related with SVR for HCV patients with genotype 1b and high virus load.

IFN-beta is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to those of IFN-alpha. IFN-beta-induced mental disorders are mild compare to those induced by IFN-alpha. Out of 7,250 HCV patients treated with IFN in our hospital, 960 (13.2%) were

given IFN-beta. The mechanism of the better tolerability of IFN-beta and ribavirin is unclear. However, the following mechanism might be considered: 1) IFN-beta is not recombinant IFN but produced from human white blood cell. Thus, IFN-beta has a tendency not to produce some immune complex relating to IFN-related side effects. 2) IFN-beta might have different intracellular mechanisms compared to IFN-alpha. Although the receptor of IFN alpha and beta are common, intracellular mechanisms could differ. Our results described above suggest that combination therapy of IFN-beta and ribavirin is one possible method for patients who have HCV-genotype 1, high virus load and depressive state of Ham-D scale of <18. In conclusion, the combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

The authors state that they have no Conflict of Interest (COI).

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Original Article

Highly sensitive AFP-L3% assay is useful for predicting recurrence of hepatocellular carcinoma after curative treatment pre- and postoperatively

Masahiro Kobayashi, Tetsuya Hosaka, Kenji Ikeda, Yuya Seko, Yusuke Kawamura, Hitomi Sezaki, Norio Akuta, Fumitaka Suzuki, Yoshiyuki Suzuki, Satoshi Saitoh, Yasuji Arase and Hiromitsu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

Aim: The micro-total analysis system (μTAS), a fully automated immunoassay system using microchip capillary electrophoresis, is highly sensitive and able to quickly assay the AFP-L3%. The clinical usefulness of this system was studied.

Methods: We retrospectively enrolled 250 patients who underwent curative treatment for primary hepatocellular carcinoma (HCC) (93 patients underwent hepatic resection and 157, radiofrequency ablation [RFA]).

Results: The sensitivity for μTAS AFP-L3% was 40.3% at the cutoff value of 5% in a range of AFP less than 20 ng/mL where the conventional method was unable to determine AFP-L3%. The sensitivity for AFP-L3% remained high even at stage I and at tumor size less than 2 cm (42.5% and 46.0%, respectively). Recurrence rate of patients with AFP-L3% greater than 5% was significantly higher than that of patients with less than 5% ($P = 0.001$). Furthermore, in resected patients, the postoperative AFP-L3% remained elevated with value greater than 5% was related to HCC recurrence ($P = 0.001$). Multivariate analysis revealed that multiple tumors ($P = 0.004$), preoperative AFP-L3% greater than 5% ($P = 0.003$), albumin less than 3.5 g/dL ($P = 0.008$), and RFA ($P = 0.003$) were significant prognostic factors of recurrence.

Conclusions: The μTAS was found to be a highly sensitive assay for AFP-L3% in patients with curative treatment of HCC. A cutoff value of 5% was useful for predicting recurrence after the curative treatment and detecting small tumors and early stage HCC. Additionally, postoperative AFP-L3% was found to be a prognostic factor of HCC recurrence.

Key words: hepatocellular carcinoma, highly sensitive AFP-L3%, micro-total analysis system

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is the fifth most common malignancy and the third leading cause of cancer-related death in the world.[1] Assays of three tumor markers, α-fetoprotein (AFP), Lens culinaris agglutinin-reactive fraction of α-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin (DCP), are helpful for HCC surveillance and diagnosis in parallel with imaging.[2–5] Among such markers, AFP is the most frequently assayed in the world, and adopted in the guidelines of the European Association for the Study of the Liver (EASL)[6] and The Asian Pacific Association for the Study of the Liver (APASL)[7] and also in the surveillance guidelines in Japan,[8] while the markers are not yet recommended for HCC surveillance by the American Association for the Study of Liver Disease (AASLD).[9] AFP level has been reported to be related to both disease stage and histological progression of HCC.[10,11] However, AFP level is often elevated even in patients with benign liver disease, and the low specificity of AFP has thus been a cause of concern for use as a HCC marker.[12–14] Aoyagi *et al.*[15] and Taketa *et al.*,[16] who focused on HCC-specific glycoform, found that the carbohydrate chain of AFP derived from HCC is fucosylated, leading to the discovery of AFP-L3 fraction highly specific for HCC. The rate of AFP-L3 in

Correspondence: Dr Masahiro Kobayashi, Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: mshkobayashi@toranomon.gr.jp
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total AFP (AFP-L3%) has been reported to be useful for HCC diagnosis in many studies,[17–20] but is not sufficiently sensitive because it has been conventionally determined by lectin affinity electrophoresis and antibody affinity blotting method,[21] or liquid-phase binding assay on an auto-analyzer (LiBASys),[22] with a clinical sensitivity of about 20% among patients with curable small HCC.[17–19] Recently, a micro-total analysis system (μTAS) based on lectin-affinity electrophoresis using microfluidics technology has been put into clinical use to quickly determine the AFP-L3% with high sensitivity.[23] The μTAS is a system enabling simultaneous determination of AFP, AFP-L3%, and DCP, and is expected to be useful in assistance of detecting HCC.[24,25]

In the present study, AFP-L3% was assayed using this system in HCC patients who underwent curative resection or radiofrequency ablation (RFA) of HCC at our hospital, to investigate the clinical sensitivity and the relationship of the AFP-L3% with prognosis of HCC recurrence.

METHODS

Patients

BETWEEN 2003 AND 2007, a total of 724 patients were diagnosed with primary HCC at the Department of Hepatology, Toranomon Hospital. Of these, 250 patients who underwent curative resection ($n = 93$) or RFA ($n = 157$) for HCC were included in the present study. The demographic characteristics of patients are shown in Table 1. Serum samples were obtained immediately before treatment and 30 to 120 days (median 83 days) after surgical resection, and stored at −80°C.

The present study was retrospective in design and approved by the Toranomon Hospital Clinical Committee, with written consent obtained from patients or patients' legally acceptable representatives.

Diagnosis of HCC

Hepatocellular carcinoma was diagnosed by image modalities in most cases. If a hepatic nodular lesion was found on screening by ultrasonography (US), the patient underwent dynamic computed tomography (CT) and/or dynamic magnetic resonance imaging (MRI). Furthermore, when a liver nodule exhibited hyper-attenuation in the arterial phase of dynamic study and washout in the portal or delayed phase, or exhibited typical hyper vascular staining on digital subtraction angiography, the nodule was diagnosed as HCC according to the AASLD guidelines.[9] When the nodule did not

Table 1 Demographics of study population

Characteristics		All patients ($n = 250$)	Patients with resection ($n = 93$)	Patients with RFA ($n = 157$)	*P*-value
Age (years)	Range (Median)	35–84 (64)	35–80 (62)	38–87 (67)	0.004
Gender	Male/Female (%)	179(72)/71(28)	72(77)/21(23)	107(68)/50(32)	NS
Infection of hepatitis virus	HCV/HBV/Others (%)	169(68)/52(21)/29(11)	46(49)/32(34)/15(16)	123(78)/20(13)/14(9)	<0.001
Tumor size (mm)	Range (Median)	8–83 (20)	10–83 (25)	8–40 (17)	<0.001
Tumor number	Single/multiple (%)	193(77)/57(23)	71(76)/22(24)	122(78)/35(22)	NS
Albumin (g/dL)	Range (Median)	2.4–4.7 (3.6)	2.4–4.7 (3.7)	2.6–4.4 (3.6)	0.006
Bilirubin (mg/dL)	Range (Median)	0.3–4.1 (0.9)	0.3–3.1 (0.8)	0.3–4.1 (1.0)	0.001
AST (IU/L)	Range (Median)	15–446 (48)	15–446 (40)	16–258 (54)	0.001
PLT ($x10^4/mm^3$)	Range (Median)	2.7–31.6 (12.0)	3.8–31.6 (14.5)	2.7–24.6 (10.7)	<0.001
PT (%)	Range (Median)	39–125 (91)	67–124 (94)	39–125 (89)	0.026
Preoperative AFP (ng/mL)	Range (Median)	1.1–20 893 (12)	1.3–20 893 (11.8)	1.1–2388 (12.0)	NS
Preoperative DCP (mAU/mL)	Range (Median)	1–1774 (18)	7–1774 (23)	1–1253 (16)	<0.001

AFP, α-fetoprotein; AST, aspartate aminottransferase; DCP, des-gamma-carboxy prothrombin; NS, Not significance; PLT, platelet count; PT, prothrombin time; RFA, radiofrequency ablation.

appear with the above-noted typical imaging features, a fine needle aspiration biopsy was carried out, followed by histological examination and diagnosis. Tumor stage on imaging findings was assessed on the basis of the Tumor Node Metastasis (TNM) classification of the Liver Cancer Study Group of Japan.[26]

Measurements of AFP, AFP-L3%, and DCP

α-fetoprotein, AFP-L3%, and DCP were assayed using a microchip capillary electrophoresis and liquid-phase binding assay on the μTASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The minimal detection limit of the μTAS was 0.3 ng/mL for AFP, and AFP-L3% was measurable when its concentration was above 0.3 ng/mL.

Follow-up protocol

Physicians examined patients every 4 weeks after curative treatment, and liver function and tumor markers were also measured once every month. After completion of HCC eradication, recurrence was surveyed with contrast-enhanced three-phase CT every 3 months.

Statistical analysis

We determined sensitivity and recurrence rate of HCC at diagnosis with AFP at the cutoff value set to 20 ng/mL. AFP-L3% cutoff values was set to 3%, 5%, 7%, and 10%.

Differences in the patient characteristics and laboratory data between the resection and RFA groups were examined with the χ^2 test and Mann–Whitney's *U*-test. Differences in the positive rates of AFP and AFP-L3% were evaluated by the Cochran–Armitage trend test. Recurrence rates were analyzed using the Kaplan–Meier method, and differences in the curves were tested using the log-rank test. Independent risk factors associated with recurrence were studied using the Cox proportional hazards model. Probabilities of less than 0.05 were considered significant. The Cochran–Armitage trend test was performed using the JMP statistical software version 9 (SAS Institute, Cary, NC, USA). Other data analysis was performed using SPSS statistical software version 10 (SPSS Inc., Chicago, IL, USA).

RESULTS

Sensitivity for AFP and AFP-L3%

OVERALL, THE SENSITIVITY for AFP was 38.0% when the cutoff value was set to 20 ng/mL. The sensitivity for AFP-L3% was 66.4%, 47.2%, 31.6%, and 18.8% at a cutoff value of 3%, 5%, 7%, and 10%, respectively (Table 2A).

Table 2 Sensitivity (A) All patients ($n = 250$) (B) Patients with AFP < 20 ng/mL ($n = 154$), and (C) Patients with AFP ≥ 20 ng/mL ($n = 96$)

	Analyte	Cutoff value	Sensitivity (%)
	AFP	20 ng/mL	38.0
(A)	AFP-L3%	3%	66.4
		5%	47.2
		7%	31.6
		10%	18.8
(B)	AFP-L3%	3%	54.5
		5%	40.3
		7%	24.0
		10%	12.3
(C)	AFP-L3%	3%	85.4
		5%	58.3
		7%	43.8
		10%	29.2

We compared the sensitivities in the groups of 154 patients with AFP less than 20 ng/mL (Table 2B) and 96 patients greater than 20 ng/mL (Table 2C). The sensitivity for AFP-L3% was 54.5%, 40.3%, 24.0%, and 12.3% in the patient group with low AFP and 85.4%, 58.3%, 43.8%, and 29.2% in the patient group with high AFP, with the cutoff value at 3%, 5%, 7%, and 10%, respectively. The sensitivity for AFP-L3% was higher in the high AFP patient group at respective cutoff values, but relatively high even in the low AFP patient group.

Sensitivity for AFP-L3% by tumor stage and size

Table 3A shows the sensitivity for AFP and AFP-L3% by tumor stage and Table 3B shows the sensitivity by maximal tumor size. The sensitivity for AFP-L3% increased with tumor progression at the cutoff values of 7% and 10% ($P = 0.021$ and 0.011, respectively, by the Cochran–Armitage trend test); however, the sensitivities were 65.0% and 42.5% and remained at a high level even for patients with stage-I tumors when the cutoff values were 3% and 5%, respectively.

When analyzed by tumor size, no significant difference observed at all the cutoff values. The sensitivity was 68.0% and 46.0% in patients with tumor size less than 2 cm and remained high at AFP-L3% of cutoff 3% and 5% regardless of tumor size, respectively.

Relationship of AFP and AFP-L3% with HCC recurrence

Hepatocellular carcinoma recurred in 151 (60.4%) patients during a median follow-up period of 4.2 years

Table 3 Sensitivity by tumor stage and size (A) by tumor stage and (B) by tumor size

(A)

Analyte	Cutoff value	Stage I ($n = 120$)	Stage II ($n = 103$)	Stege III ($n = 27$)	*P*-value
AFP	20 ng/mL	38.3%	37.9%	40.7%	NS
AFP-L3%	3%	65.0%	67.0%	70.4%	NS
	5%	42.5%	50.5%	55.6%	NS
	7%	25.0%	35.9%	44.4%	0.021
	10%	12.5%	23.3%	29.6%	0.011

(B)

Analyte	Cutoff value	≤2 cm ($n = 150$)	2–3 cm ($n = 66$)	3–5 cm ($n = 25$)	>5 cm ($n = 9$)	*P*-value
AFP	20 ng/mL	42.7%	33.3%	36.0%	11.1%	0.057
AFP-L3%	3%	68.0%	71.2%	48.0%	55.6%	NS
	5%	46.0%	54.5%	36.0%	44.4%	NS
	7%	28.0%	42.4%	24.0%	33.3%	NS
	10%	15.3%	27.3%	16.0%	22.2%	NS

AFP, α-fetoprotein; NS, not significant.

(0.2 to 7.8 years) after curative treatment. The cumulative recurrence rate was 21.5% at year 1, 53.5% at year 3, and 65.6% at year 5 after treatment. In these patients, the recurrence rate was analyzed by preoperative AFP and AFP-L3% (Fig. 1).

There was no significant difference in recurrence rate between the patient groups with AFP greater than and less than 20 ng/mL (Fig. 1a). On the other hand, the 1- and 3-year recurrence rates were 29.4% and 65.5% in patients with AFP-L3% greater than 5% and 14.5% and 42.7% in patients with AFP-L3% less than 5%, respectively, and significantly different between the two patient groups ($P = 0.001$) (Fig. 1b). When the cutoff value for AFP-L3% was set to 7% and 10%, recurrence rate tended to be high in the patient group with AFP-L3% greater than the cutoff value, though not to a significant difference (data not shown).

Relationship of pre- and postoperative AFP and AFP-L3% with recurrence rate in patients undergoing resection

To exclude the improper matching of other potential risk factors for recurrence between the resected and the RFA patients, the relationships of pre- and postoperative AFP and AFP-L3% with the recurrence rate of HCC were analyzed for 93 resected patients. Figures 2 and 3 show the recurrence rates with preoperative and postoperative, respectively.

On analysis by preoperative AFP, the 1- and 3-year recurrence rates were 17.9% and 51.7% in patients with AFP less than 20 ng/mL and 11.1% and 36.9% in patients with AFP greater than 20 ng/mL, respectively, showing that the recurrence was high in the patient group with lower AFP, but this is not statistically significant ($P = 0.121$) (Fig. 2a). In contrast, by preoperative AFP-L3% using a cutoff value of 5%, the 1- and 3-year recurrence rates were 10.0% and 33.6% in patients with AFP-L3% less than 5% and 21.4 and 59.5% in patients with AFP-L3% greater than 5%, with a significantly high recurrence rate in patients with AFP-L3% higher than 5% ($P = 0.013$) (Fig. 2b). In addition, using the cutoff values of 7% and 10%, there was no significant difference between groups (data not shown).

Similar analyses were performed using the serum samples obtained from 91 of 93 patients after resection. Preoperative level of AFP greater than 20 ng/mL decreased to the level of less than 20 ng/ml in 29 of 37 patients (78.4%). On the other hand, preoperative AFP levels below 20 ng/mL turned positive in only one of 54 (1.9%) patients after curative treatment. Similarly, preoperative level of AFP-L3% greater than 5% decreased to a level less than 5% only in 16 of 42 (38.1%) patients. Moreover, preoperative level of AFP-L3% less than 5% increased to a postoperative level of 5% or higher after treatment in seven of 49 patients (14.3%). Thereby AFP-L3% turning negative after treatment was rare.

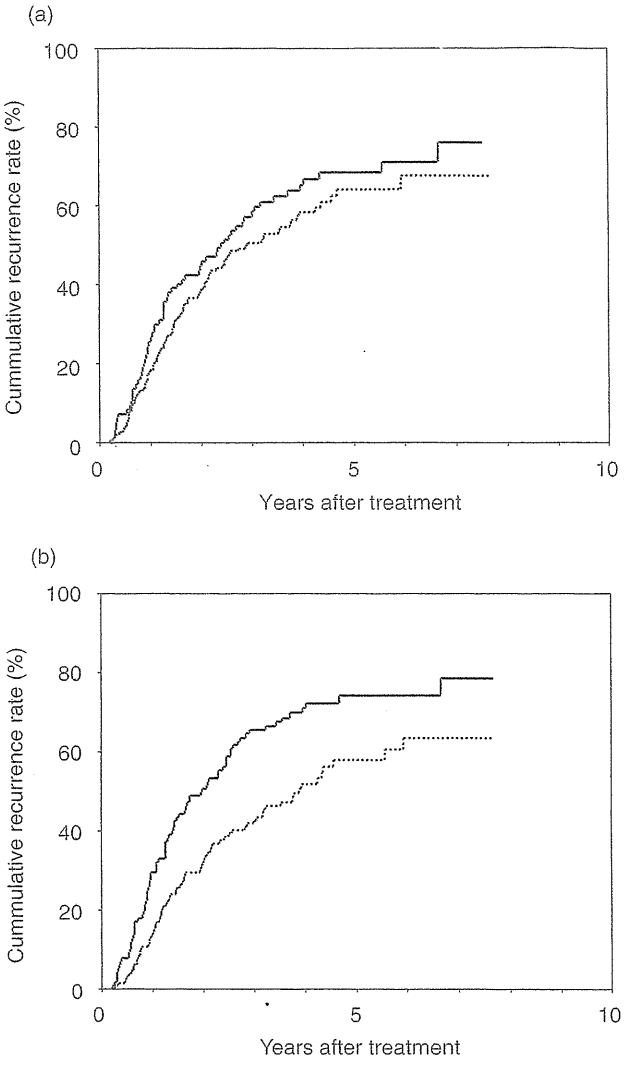

Figure 1 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for α-fetoprotein (AFP) and AFP-L3% in all patients. (a) Recurrence rate for AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for AFP-L3%: solid line, recurrence rate in patients with AFP-L3 ≥ 5%; broken line, recurrence rate in patients with AFP < 5%.

Comparing recurrence rates by postoperative AFP and AFP-L3%, the 1- and 3-year recurrence rates were 14.6% and 46.7% in patients with total AFP less than 20 ng/mL and 25.0% and 37.5% in patients with AFP greater than 20 ng/mL, with no significant difference between the two groups (Fig. 3a). In contrast, the 1- and 3-year recurrence rates were 14.7% and 43.5% in patients with AFP-L3% less than 5% and 29.3 and 64.4% in patients with AFP-L3% greater than 5%, with a significant difference between the two groups ($P = 0.001$) (Fig. 3b). With a cutoff value of 7% for AFP-L3%, no significant difference was observed between the two groups (data not shown). Only two patients had the postoperative AFP-L3% value greater than 10%. They developed HCC recurrence within 1 year and were suspected to have persistent HCC.

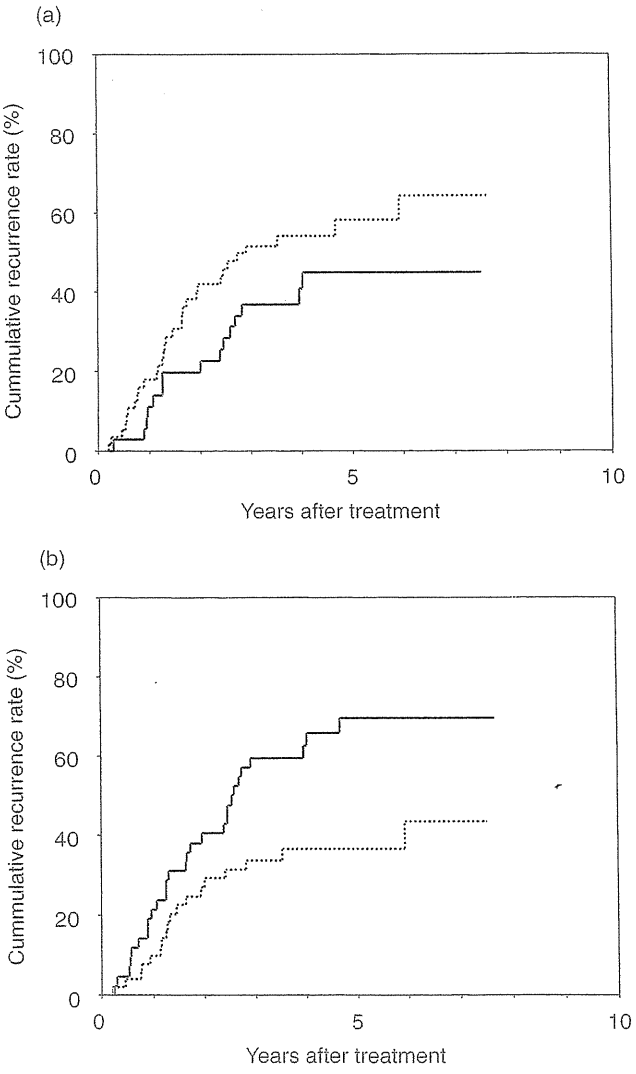

Figure 2 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for preoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for preoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for preoperative AFP-L3%: solid line, recurrence rate in patients with AFP-L3 ≥ 5%; broken line, recurrence rate in patients with AFP-L3 < 5%.

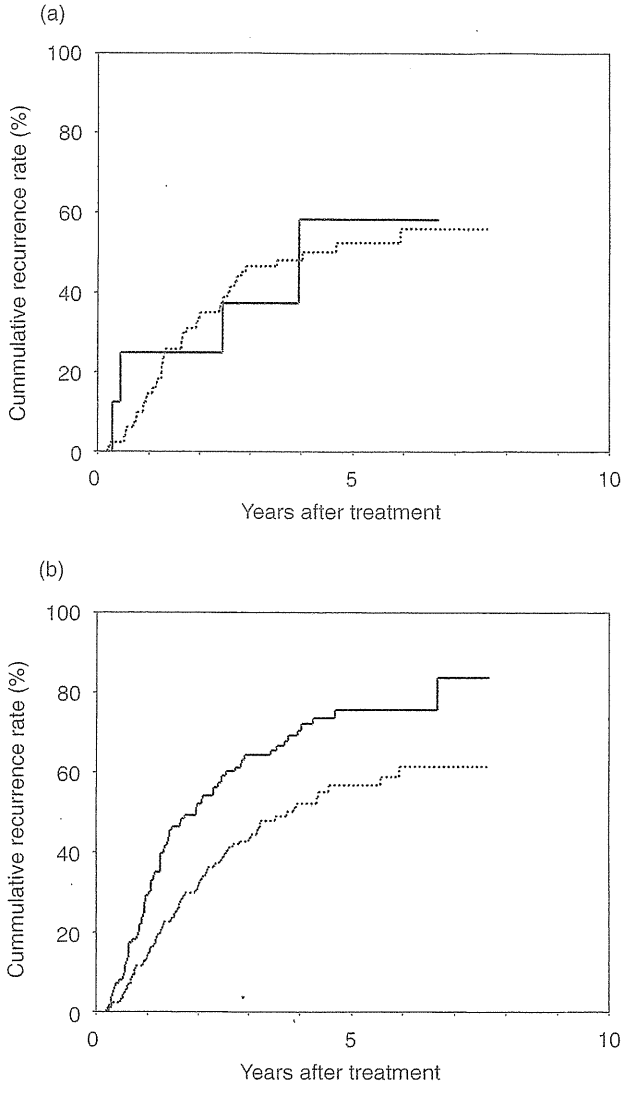

Figure 3 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for postoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for postoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for postoperative AFP-L3%: solid line, recurrence rate in patients with AFP-L3 ≥ 5%; broken line, recurrence rate in patients with AFP < 5%.

Prognostic factors for HCC recurrence

Factors related to HCC recurrence were analyzed by the Kaplan–Meier method and multivariate analysis (Table 4). Potential risk factors for recurrence included the following 15 variables: age, gender, etiology of background liver disease, amount of alcohol intake, albumin, bilirubin, aspartate aminotransferase (AST), platelet count (PLT), prothrombin time (PT), preoperative AFP, AFP-L3%, DCP, tumor size, tumor number, and treatment procedure (resection or ablation). In all of the patients ($n = 250$), factors that were significantly related to HCC recurrence were RFA therapy, multiple tumors, albumin <3.5 g/dL, AST ≥ 50 IU/L, platelets <10 × 104/μL, prothrombin time <80%, preoperative AFP-L3% ≥ 5%, and preoperative DCP ≥ 40 mAU/mL by the Kaplan–Meier method (Table 4A). On multivariate analysis, the following were significant prognostic factors: multiple tumors ($P = 0.004$), preoperative AFP-L3% ≥ 5% ($P = 0.003$), albumin <3.5 g/dL ($P = 0.008$), and RFA ($P = 0.003$) (Table 4B).

In the 93 resected patients, on multivariate analysis, factors contributing to HCC recurrence were tumor number and preoperative AFP-L3% ($P = 0.003$ and 0.019, respectively). In the 157 RFA patients, similarly the four factors of age, preoperative AFP, AFP-L3%, and albumin were identified ($P = 0.003$, 0.006, 0.009, and 0.011, respectively) (data not shown).

Histological features and serum AFP, AFP-L3%, and DCP levels

From the 93 patients who underwent resection, we were able to obtain 85 specimens and assess their histological features. Ten nodules were well-differentiated HCCs; 69, moderately differentiated HCCs; and the remaining six, poorly differentiated HCCs. The nodules were macroscopically classified: four nodules were of small nodular type with indistinct margin (SNIM); 50, of simple nodular type (SN); 24, of simple nodular type with extranodular growth (SNEG); and seven, of confluent multinodular type (CM). Microscopic vascular invasion was observed in 14 (16.5%) nodules, and microscopic intrahepatic metastasis was observed in four (4.7%) nodules.

The median (25–75 percentile) preoperative DCP level in moderately/poorly differentiated HCCs was 25 (15–113) AU/L, whereas that of the well-differentiated HCCs was 18 (14–20) AU/L, and this difference was statistically significant ($P = 0.041$). Similarly, a significant difference was observed in the preoperative AFP-L3% between groups: the median AFP-L3% in the SNEG/CM group was 6.4 (2.5–18.9), whereas in the SNIM/SN group, it was 2.5 (≤0.5–7.4) ($P = 0.032$).

DISCUSSION

IN THE PRESENT study, AFP-L3% assayed by the μTAS method was detected with high clinical sensitivity

Table 4 Prognostic factors of hepatocellular carcinoma (HCC) recurrence. (A) Cumulative recurrence rate by variable and (B) Multivariate analysis

(A) Cumulative recurrence rate by variable

Variables	*n*	3-year Recurrence (%)	*P*-value
Treatment			
Resection	93	45.9	0.003
RFA	157	58.0	
Tumor number			
Single	193	50.8	0.003
Multiple	57	62.9	
Albumin			
<3.5 g/dL	105	64.9	0.001
≥3.5 g/dL	145	45.2	
AST			
<50 IU/L	131	48.3	0.009
≥50 IU/L	119	58.7	
PLT			
$<10 \times 10^4/mm^3$	87	65.4	0.024
$\geq 10 \times 10^4/mm^3$	163	47.4	
PT			
<80%	51	74.7	0.001
≥80%	199	48.1	
Preoperative AFP-L3%			
<5%	132	42.7	0.001
≥5%	118	65.5	
Preoperative DCP			
<40 mAU/mL	194	49.6	0.025
≥40 mAU/mL	56	67.0	

(B) Multivariate analysis

Variables		Hazard ratio (95% CI)	*P*-value
Tumor number	(multiple/single)	1.70 (1.19–2.43)	0.004
Preoperative AFP-L3%	(≥5%/<5%)	1.63 (1.18–2.26)	0.003
Albumin	(<3.5/≥3.5 g/dL)	1.55 (1.12–2.14)	0.008
Treatment	(RFA/resection)	1.09 (1.03–1.16)	0.003

AST, aspartate aminotransferase; CI, confidence interval; PLT, platelet count; PT, prothrombin time; RFA, radiofrequency ablation.

even in cases of HCC at a relatively early stage, which can be potentially cured by hepatic resection or RFA. It is worth noting that the sensitivity for HCC was as high as 47.2% when the cutoff value of AFP-L3% was set to 5%, compared to the sensitivity of 38.0% for total AFP. In addition, using a cutoff value of 10%, the sensitivity was 18.8%, which is comparable to that reported with the conventional method in patients whose HCC was curatively treated.[17–19]

One of the advantages of the highly sensitive μTAS method is measurement of AFP at low concentrations. Previously, the conventional method was unable to accurately determine AFP-L3% when total AFP concentration was less than 20 ng/mL, while in the present study detection of AFP-L3% was possible in 40.3%, 24.0%, and 12.3% of patients with AFP values less than 20 ng/mL when using the cutoff value for the AFP-L3% was set to 5%, 7%, and 10%, respectively. In our previous study of prognostic factors in patients that underwent hepatic resection or RFA with HCC of size less than 3 cm and not more than three tumors, it was reported that DCP was a significant prognostic factor in RFA

patients, while both AFP and DCP were not in resected patients.[27] During that study, we could not measure the highly sensitive AFP-L3%, and we measured the conventional AFP-L3% in only about half the patients. Therefore, we did not include the results of the AFP-L3% levels in that study. In the present study using the highly sensitive μTAS method to assay AFP-L3%, multivariate analysis revealed the AFP-L3% is a predictive factor for HCC recurrence with statistical significance both in the group of overall study population and surgically resected patients. These results showed that this highly sensitive assay method can increase clinical sensitivity and predict recurrence, suggesting that it is of additional clinical utility.

Toyoda *et al.*[24] assayed AFP-L3% in 270 patients with AFP less than 20 ng/mL and 396 patients with chronic liver diseases using the same μTAS method as in the present study, and reported that the AFP-L3% assayed by this method was useful for differential diagnosis of HCC and benign liver diseases with a sensitivity of 41.5% and specificity of 85.1% with the AFP-L3% cutoff value of 5%. He also found AFP-L3% to be related to survival rate. In the present study, the sensitivity was similar to that reported by Toyoda *et al.*,[24] although it was not possible to compare specificity, since in this study we included only HCC patients.

Similarly, Tamura *et al.*[25] reported a sensitivity of 60%, specificity of 90.3%, accuracy of 76.4%, positive predictive value (PPV) of 83.9%, and negative predictive value (NPV) of 72.8% at a cutoff value of 7% in 295 HCC patients and 350 patients with benign liver diseases. Comparison of cutoff values showed that the 7% was most clinically useful. Compared with the sensitivity of 60% reported by Tamura *et al.*, the sensitivity at 31.6% was relatively low in the present study with cutoff value at 7%. This appears to reflect differences in some fundamental patient characteristics between the two studies: for example, Stage III and IV HCC accounted for 50.2% of patients (148 of 295) in the report by Tamura *et al.* and 10.8% (27 of 250) in the present study.

The optimal cutoff value of a marker depends on the target disease under study and its intended use. We believed that the cutoff value for differential diagnosis between HCC and benign liver disease should achieve high specificity, preferably using receiver-operating characteristic (ROC) curve analysis. The purpose of the present study was to identify recurrence-predictive factors in a patient population with curatively treatable HCC at a relatively early stage; we determined that 5% AFP-L3% was most useful.

The relationships of postoperative AFP and AFP-L3% with HCC recurrence were also investigated in the present study. Notably, postoperative AFP-L3% remaining elevated greater than 5% was indicative of risk of HCC recurrence. Furthermore, it is noted that total AFP turned negative in 78.4% of patients after curative treatment, while AFP-L3% did in only 38.1% of patients (5% cutoff). Included in the present study of recurrence were all resected patients in whom radical cure was histologically confirmed. Therefore, all remnants of HCC should have been surgically removed. We speculate that lack of reduction in AFP-L3% after curative treatment appears was due to intra-hepatic multi-centric carcinogenesis or intra-hepatic micrometastasis. Miyaaki *et al.*,[28] who assayed AFP-L3% and protein induced by vitamin K absence-II (PIVKA-II), also known as DCP, by the conventional method in 110 resected patients, reported more cases of infiltrative growth-type HCC and poorly differentiated-type HCC in patients with postoperative AFP-L3% greater than 10%. Tada *et al.*[29] also reported a high rate of infiltrative growth, capsule infiltration, septum formation, portal vein invasion, and hepatic invasion in 111 patients with HCC with a high level of AFP-L3%. Regrettably, however, subsequent HCC recurrence was not followed. In our patients, the preoperative DCP level was related to the histological grade of the tumor, and a preoperative AFP-L3% greater than 5% was related to the macroscopic type of the nodule. In contrast, no relationship was observed between the postoperative markers and histological features in the current study. Unfortunately, we cannot clearly explain the discrepancies between the results of Tada *et al.* and this study; further examination with a larger number of patients is required to determine the relationship between highly sensitive AFP-L3% and the histological features of the tumors. In any case, patients with high level of AFP-L3% either before or after curative treatment should be followed closely.

The present study shows the high clinical sensitivity in diagnosis of HCC using μTAS AFP-L3% in patients with curative treatment of HCC. With a cutoff value of 5%, sensitivity was optimal in AFP less than 20 ng/mL where the conventional method was unable to determine the AFP-L3% value. Furthermore, both pre- and postoperative AFP-L3% were determined as prognostic factors of HCC recurrence. Since the high recurrence rate of HCC after even curative treatment is reported, it is of great importance to be able to predict such recurrence. Our study showed that the highly sensitive AFP-L3% is expected to be of clinical utility in predicting recurrence after curative treatments.

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Original Article

Development rate of chronic kidney disease in hepatitis C virus patients with advanced fibrosis after interferon therapy

Yasuji Arase, Fumitaka Suzuki, Yusuke Kawamura, Yoshiyuki Suzuki, Masahiro Kobayashi, Naoki Matsumoto, Norio Akuta, Hitomi Sezaki, Tetsuya Hosaka, Kyoko Ogawa, Norihiro Imai, Yuya Seko, Satoshi Saito, Kenji Ikeda, Mariko Kobayashi and Hiromitsu Kumada

Department of Hepatology and Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan

Aim: The aim of this retrospective cohort study is to assess the development incidence and predictive factors for chronic kidney disease (CKD) after the termination of interferon therapy in hepatitis C virus (HCV) positive Japanese patients with liver cirrhosis.

Methods: A total of 650 HCV positive, liver cirrhotic patients who were treated with interferon and showed an estimated glomerular filtration rate (eGFR) of ≥60 mL/min per 1.73 m^2 after the termination of interferon therapy were enrolled. CKD was defined as an eGFR of <60 mL/min per 1.73 m^2. End-stage-CKD was defined as an eGFR of <15 mL/min/1.73 m^2. The primary goal is the new development of CKD and end-stage-CKD.

Results: Eighty-five patients developed CKD, and six patients progressed to end-stage-CKD. The development rate of CKD was 5.2% at the 5th year, 14.5% at the 10th year and 30.6% at the 15th year. Multivariate Cox proportional hazards analysis showed that CKD occurred when patients had age increments of 10 years (hazard ratio: 2.32; 95% confidence interval [CI] 1.61–3.35; $P < 0.001$), eGFR decrements of 10 mL/min per 1.73 m^2 (hazard ratio: 1.66; 95% CI 1.27–2.16; $P < 0.001$), hypertension (hazard ratio: 2.00; 95% CI 1.13–3.53; $P = 0.017$), diabetes (hazard ratio: 1.79; 95% CI 1.02–3.14; $P = 0.042$), and non-clearance of HCV (hazard ratio: 2.67; 95% CI 1.34–5.32; $P = 0.005$). The development rate of end-stage-CKD was 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year.

Conclusions: The annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage-CKD is one order of magnitude lower than that of CKD.

Key words: chronic kidney disease, hepatitis C virus, liver cirrhosis

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; SVR, Sustained virological response.

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a major risk for hepatocellular carcinoma (HCC).[1–4] In addition, chronic HCV infection has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, lymphoproliferative disorders, autoimmune thyroiditis, sialadenitis, cardiomyopathy, and diabetes.[5–8]

Data supporting a link between hepatitis C infection and chronic kidney disease (CKD) have been reported.[9–15] CKD, a disease entity including mild to end-stage renal diseases due to any etiology, was recently defined as an estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m^2 and/or the presence of proteinuria.[16] CKD is currently considered a serious worldwide public health problem.[16,17] Tsuji *et al.* have reported that HCV infection enhance the onset of end-stage renal disease.[18,19] Dalrymple *et al.* have

Correspondence: Dr Yasuji Arase, Department of Hepatology, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: es9y-ars@asahi-net.or.jp
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showed that HCV-positive patients had a 40% higher likelihood for developing renal insufficiency compared with seronegative subjects.[20] We had reported that patients with severe fibrosis had high possibility of progressed kidney damage.[11,12] Although there is growing evidence to support the concept that HCV infection is a risk factor for CKD, there have been a few interventional studies confirming this issue. This issue needs to be confirmed with a long-term follow-up of patients.

With this background in mind, the retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of aggravation of renal function after prolonged follow-up in HCV-infected and cirrhotic patients treated with interferon (IFN) monotherapy or combination therapy of IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

METHODS

Patients

A TOTAL OF 982 HCV positive and cirrhotic patients with infection were treated with IFN monotherapy or combination therapy of IFN and ribavirin between September 1990 and December 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Out of 982 patients, 650 satisfied the following criteria: (i) an estimated glomerular filtration rate (eGFR) of ≥60 (mL/min per 1.73 m^2); (ii) features of cirrhosis diagnosed by laparoscopy and/or liver biopsy before the initiation of IFN therapy; (iii) positivity for serum HCV-RNA before the initiation of IFN therapy; (iv) age of ≥40 years; (v) period of ≤ 1year on IFN therapy; (vi) negativity for hepatitis B surface antigen (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay or indirect immunofluorescence assay; (vii) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; and (viii) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Next, we excluded from the study all the patients with a history of alcohol abuse or advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites.

Alcohol abuse is a pattern of drinking that involves one or more of the following problems within a one-year period: (i) failure to carry out major responsibilities at work, school, or home; (ii) drinking in physically dangerous situations, such as while driving; (iii) legal problems related to using alcohol; and (iv) continued drinking despite ongoing problems in relationships with other people that are related to alcohol use.[21]

The primary outcome was the new development of CKD and/or end-stage CKD. CKD was defined as the first time when eGFR of <60 mL/min per 1.73 m^2 persisted for up to 3 months. End-stage CKD was defined as the first time when eGFR of <15 mL/min per 1.73 m^2 persisted for up to 3 months. Serum creatinine level was also measured using an enzymatical method, and the eGFR was estimated from the Japanese Society of Nephrology CKD Practice Guide: eGFR (mL/min per 1.73 m^2) = 194 × (serum creatinine level [mg/dL])$^{-1.094}$ × (age [y])$^{-0.287}$. The product of this equation was multiplied by a correction factor of 0.739 for women. CKD's stages were defined from estimated eGFR of <60 mL/min per 1.73 m^2 or dipstick proteinuria (≥+1) as follows: stage 1, eGFR ≥90 and proteinuria (≥+1); stage 2, 90 > eGFR ≥60 and proteinuria (≥+1); stage 3, 60 > eGFR ≥ 30; stage 4, 30 > eGFR ≥ 15; and stage 5, eGFR of <15. In the present study, patients with stage 3–5 were regarded as having CKD regardless of the absence of other markers of kidney damage.[22,23]

The physicians in charge explained the methods and side effects of IFN therapy, the storage of serum samples, and the use of stored serum samples to each patient and/or patient's family before IFN therapy. Informed consent was obtained from 650 patients before the initiation of IFN therapy. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by the Institutional Review Board of our hospital.

Laboratory investigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL, USA). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). Diagnosis of HCV infection was based on detection of serum HCV antibody and positive HCV RNA. HCV genotype and HCV RNA level were determined by the serum samples stored at −80°C before the initiation of IFN therapy.

Height and weight were recorded at baseline and the body mass index was calculated as weight (in kg)/height (in m^2). The criteria for the diagnosis of diabetes include: (i) casual plasma glucose ≥200 mg/dL; (ii) fasting plasma glucose (FPG) ≥126 mg/dL; and (iii) 2 h post-glucose (oral glucose tolerance test) ≥200 mg/dL.[24]

Patients were regarded as hypertension by the confirmation of blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic on at least three visits. Blood pressure was measured by a physician with a mercury sphygmomanometer, with subjects sitting and relaxed for at least 10 min.

Evaluation of liver cirrhosis

Liver status of the 650 patients was determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.[25]

Follow-up

The starting time of follow up was 3 months after the termination of IFN therapy. After that, patients were followed-up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check-ups. Blood samples were taken for routine analyses. These included transaminase activities, total cholesterol, uric acid, glucose, complete blood cell count, serum HCV RNA, and creatinine level. Fifty-seven patients were lost to follow-up. Because the appearance of worsening renal function was not identified in the 57 patients, they were considered as censored data in statistical analysis.[26] Moreover, patients retreated with antiviral agents were regarded as withdrawals at the time of starting the retreatment of antiviral agents.

Statistical analysis

Clinical differences between sustained virological response (SVR) group and non-SVR group were evaluated by Wilcoxon rank sum test or Fisher's exact test. The cumulative development rate of CKD and end-stage CKD was calculated from 3 months after the termination of IFN treatment using the Kaplan–Meier method. Independent factors associated with the development rate of CKD and end-stage CKD were analyzed by the Cox proportional hazard model. The following 17 variables were analyzed for potential covariates for incidence of aggravation of renal function: age, sex, body mass index, eGFR, HCV RNA level, HCV genotype, alanine aminotransferase, aspartate aminotransferase, platelet count, type of IFN, combination of ribavirin, efficacy of IFN therapy, triglyceride, total cholesterol, uric acid, hypertension, diabetes, and frequencies of using contrast medium in computed tomography. HCV RNA level and HCV genotype were measured by the serum samples stored −80°C before the initiation of IFN therapy. Yearly frequencies of using contrast medium in computed tomography were determined by clinical records. The remaining 15 variables were determined at the starting time of follow up after IFN therapy. A *P*-value of less than 0.05 was considered significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Patients' characteristics

TABLE 1 SHOWS the characteristics of the 650 HCV-positive and cirrhotic patients treated with IFN monotherapy or combination therapy of IFN and ribavirin. There were several differences in clinical backgrounds between the SVR group and the non-SVR group. However, there was no significant difference in eGFR between SVR group and non-SVR group. The sustained virological response (SVR) rate was 30.6% (169/553) in IFN monotherapy and 42.2% (41/97) in combination therapy of IFN and ribavirin. Thus, the number of patients with SVR was 210. The mean follow-up period after the termination of anti-virus drugs was 6.5 years.

Incidence of CKD in cirrhotic patients with HCV

A total of 85 subjects (56 men and 29 women) developed CKD during the follow-up period. Of these, 14 were SVR and 67 were non-SVR. The cumulative development rate of CKD was determined to be 4.9% at the 5th year, 14.5% at the 10th year and 30.6% at the 15th year by the use of the Kaplan–Meier method (Fig. 1).

The factors associated with the development of CKD in all 650 patients treated with IFN are shown in Table 2. Multivariate Cox proportional hazards analysis showed that CKD development after the termination of IFN therapy occurred when patients had age increments of 10 years (hazard ratio: 2.32; 95% confidence interval [CI] 1.61–3.35; $P < 0.001$), eGFR decrements of 10 mL/min pe 1.73 m^2 (hazard ratio: 1.66; 95% CI 1.27–2.16; $P < 0.001$), hypertension (hazard ratio: 2.00; 95% CI 1.13–3.53; $P = 0.017$), diabetes (hazard ratio: 1.79; 95% CI 1.02–3.14; $P = 0.042$), and non-SVR (hazard ratio:

Table 1 Patients characteristics

Characteristic	Total	SVR	Non-SVR	*P**
n	650	210	440	
Sex (male/female)	405/245	134/76	271/169	0.604
Age (years)	57.4 ± 11.7	57.0 ± 11.9	57.6 ± 12.8	0.185
Height (cm)	162.8 ± 9.1	163.3 ± 9.2	162.1 ± 9.1	0.270
Body weight (kg)	63.1 ± 13.7	63.6 ± 13.9	62.1 ± 13.7	0.387
Body mass index	23.6 ± 3.1	23.7 ± 3.2	23.6 ± 3.2	0.654
Blood pressure (systolic, mmHg)	132 ± 17	130 ± 17	133 ± 18	0.334
Blood pressure (diastolic, mmHg)	78 ± 12	78 ± 11	79 ± 11	0.929
Hypertension (+/−)	152/498	48/162	104/336	0.844
HCV-genotype (1b/2a/2b/others)	389/159/56/46	92/84/19/15	297/75/37/31	<0.001
HCV RNA level (KIU/mL)	659 ± 508	435 ± 476	728 ± 532	<0.001
eGFR	85.2 ± 15.5	86.2 ± 15.9	84.7 ± 15.7	0.141
Fasting plasma glucose (mg/dL)	100 ± 31	99 ± 25	102 ± 34	0.888
Diabetes	149/501	42/168	107/333	0.232
Total cholesterol (g/dL)	156 ± 30	158 ± 38	154 ± 30	0.486
Triglyceride (mg/dL)	104 ± 46	108 ± 56	102 ± 45	0.764
Uric Acid (mg/dL)	5.6 ± 2.1	5.5 ± 2.1	5.7 ± 2.2	0.433
AST (IU/L)	62 ± 50	39 ± 19	73 ± 55	<0.001
ALT (IU/L)	68 ± 72	36 ± 20	80 ± 80	<0.001
Platelet ($\times 10^4/mm^3$)	11.6 ± 4.7	12.2 ± 5.0	11.3 ± 4.5	0.040
Frequencies of contrast imaging per year (≥1/<1)	252/398	28/182	224/216	<0.001
IFN monotherapy†/combination therapy‡	553/97	169/41	384/56	0.026

*Clinical differences between SVR group and Non-SVR group were evaluated by Wilcoxon rank sum test or Fisher's exact test.
†Outbreak of IFN monotherapy: recombinant IFNα 2a, 73 cases; recombinant IFNα 2b, 52 cases; natural IFNα, 278 cases; natural IFNβ, 150 cases; total dose of IFN = 572 ± 165 megaunit.
‡Outbreak of combination therapy: recombinant IFNα 2b+ribavirin, 29 cases, total dose of IFN = 502 ± 182 megaunit, total dose of ribavirin = 160 ± 68 g; peg IFNα 2b+ribavirin, 68 cases, total dose of peg IFN = 4.10 ± 1.08 mg, total dose of ribavirin = 202 ± 56 g.
Data are number of patients, median (range) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response.

2.67; 95% CI 1.34–5.32; $P. = 0.005$). The cumulative development rate for CKD based on difference of efficacy of the IFN therapy is shown in Figure 2. In addition to non-SVR, the four factors of aging, low eGFR, hypertension, and diabetes are high risk of developing the CKD. The development rates for CKD based on difference of age, eGFR, blood pressure, and blood glucose level at the starting time of follow-up are shown in Figure 3.

Incidence of end-stage CKD in cirrhotic patients with HCV

A total of six subjects (five male and one female) developed end-stage CKD during the follow-up period. The cumulative development rate of end-stage CKD was determined to be 0.4% at the 5th year, 1.6% at the 10th year and 2.8% at the 15th year by the use of the Kaplan–

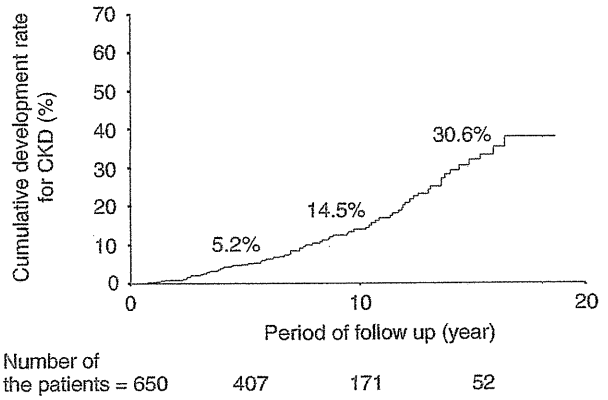

Figure 1 Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

Table 2 Predictive factors for chronic kidney disease (CKD) development

Variables	Univariate analysis		Cox-regression	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, per 10 years	2.30 (1.72–3.12)	<0.001	2.32 (1.61–3.35)	<0.001
Sex (female/male)	0.90 (0.57–1.40)	0.628		
Body mass index (≥25/<25)	1.35 (0.72–2.50)	0.347		
HCV load(KIU/mL, ≥1000/<1000)	1.39 (0.80–2.38)	0.173		
Genotype (1/2)	1.19 (0.78–1.89)	0.436		
AST (IU/L, ≥50/<50)	1.63 (0.92–2.94)	0.097		
ALT (IU/L, ≥50/<50)	2.01 (1.13–3.57)	0.016		
Platelet ($\times 10^4/mm^3$, ≥15/<15)	0.70 (0.25–1.94)	0.487		
eGFR, per decrease of 10 mL/min/1.73 m^2	2.00 (1.56–2.56)	<0.001	1.66 (1.27–2.16)	<0.001
Uric acid (mg/dL, ≥7.0/<7.0)	1.43 (0.81–2.47)	0.225		
Triglyceride (mg/dL, ≥150/<150)	1.61 (0.62–3.70)	0.336		
Cholesterol (mg/dL, ≥220/<220)	1.22 (0.48–3.12)	0.678		
Diabetes (+/−)	2.76 (1.79–4.22)	0.001	1.79 (1.02–3.14)	0.042
Hypertension (+/−)	2.82 (1.80–4.39)	<0.001	2.00 (1.13–3.53)	0.017
Combination of ribavirin (+/−)	0.75 (0.36–1.58)	0.453		
Kind of IFN (beta/alpha)	0.91 (0.53–1.57)	0.729		
Efficacy (non-SVR/SVR)	2.10 (1.21–3.58)	0.008	2.67 (1.34–5.32)	0.005
Frequencies of contrast imaging per year (≥1/<1)	1.83 (1.17–2.87)	0.009		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

Meier method (Fig. 4). The factors associated with the incidence of end-stage CKD in all 650 patients are shown in Table 3. There were no significant factors associated with the incidence of end-stage CKD as shown in Table 3.

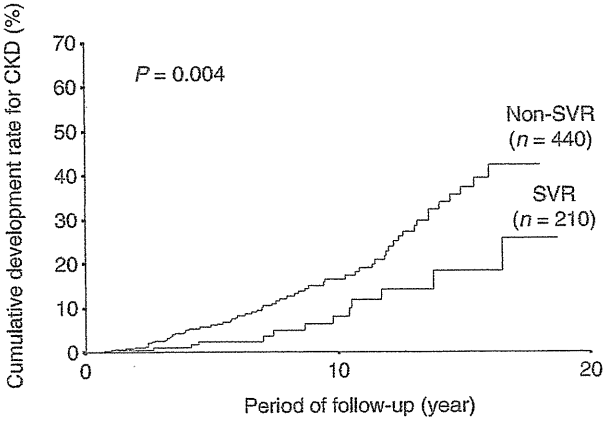

Figure 2 Cumulative development rate for chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

DISCUSSION

WE HAVE DESCRIBED the development incidence for CKD and end-stage CKD after the termination of IFN therapy in HCV positive and liver cirrhotic patients treated with IFN. In the present study, the liver cirrhotic patients were enrolled to evaluate the new onset of CKD or end-stage CKD. Moreover, kidney damage has been reported in patients treated with IFN.[27] To exclude kidney damage originated from IFN-related side effects, patients with eGFR of ≥60 (mL/min per 1.73 m^2) for 3 months after the termination of IFN were enrolled in the present study. Our results indicate that the annual incidence for CKD as defined by a GFR of less than 60 mL/min per 1.73 m^2 for a prolonged follow-up after the termination of IFN therapy in HCV positive and cirrhotic patients is about 1.0–1.5% based on the development incidence for CKD at the 5th year and the 10th year. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of a total of CKD.

Imai *et al.* have reported that about 20% of the Japanese adult population have stage 3 to 5 CKD by the use of database for 527 594 (male, 211 034; female, 316 560) participants obtained from the general adult population aged over 20 years who received annual