

total AFP (AFP-L3%) has been reported to be useful for HCC diagnosis in many studies,<sup>17-20</sup> but is not sufficiently sensitive because it has been conventionally determined by lectin affinity electrophoresis and antibody affinity blotting method,<sup>21</sup> or liquid-phase binding assay on an auto-analyzer (LiBASys),<sup>22</sup> with a clinical sensitivity of about 20% among patients with curable small HCC.<sup>17-19</sup> Recently, a micro-total analysis system ( $\mu$ TAS) based on lectin-affinity electrophoresis using microfluidics technology has been put into clinical use to quickly determine the AFP-L3% with high sensitivity.<sup>23</sup> The  $\mu$ TAS is a system enabling simultaneous determination of AFP, AFP-L3%, and DCP, and is expected to be useful in assistance of detecting HCC.<sup>24,25</sup>

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**

**B**ETWEEN 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  $-80^{\circ}\text{C}$ .

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.<sup>9</sup> 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)	35-84 (64)	35-80 (62)	38-87 (67)	0.004
Gender	179(72)/71(28)	72(77)/21(23)	107(68)/50(32)	NS
Infection of hepatitis virus	169(68)/52(21)/29(11)	46(49)/32(34)/15(16)	123(78)/20(13)/14(9)	<0.001
Tumor size (mm)	8-83 (20)	10-83 (25)	8-40 (17)	<0.001
Tumor number	193(77)/57(23)	71(76)/22(24)	122(78)/35(22)	NS
Albumin (g/dL)	2.4-4.7 (3.6)	2.4-4.7 (3.7)	2.6-4.4 (3.6)	0.006
Bilirubin (mg/dL)	0.3-4.1 (0.9)	0.3-3.1 (0.8)	0.3-4.1 (1.0)	0.001
AST (IU/L)	15-446 (48)	15-446 (40)	16-258 (54)	0.001
PLT ( $\times 10^4/\text{mm}^3$ )	2.7-31.6 (12.0)	3.8-31.6 (14.5)	2.7-24.6 (10.7)	<0.001
PT (%)	39-125 (91)	67-124 (94)	39-125 (89)	0.026
Preoperative AFP (ng/mL)	1.1-20 893 (12)	1.3-20 893 (11.8)	1.1-2388 (12.0)	NS
Preoperative DCP (mAU/mL)	1-1774 (18)	7-1774 (23)	1-1253 (16)	<0.001

AFP,  $\alpha$ -fetoprotein; AST, aspartate aminotransferase; 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.<sup>26</sup>

### Measurements of AFP, AFP-L3%, and DCP

$\alpha$ -fetoprotein, AFP-L3%, and DCP were assayed using a microchip capillary electrophoresis and liquid-phase binding assay on the  $\mu$ TASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The minimal detection limit of the  $\mu$ TAS 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  $\chi^2$  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  $\geq$  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)	Stage 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).

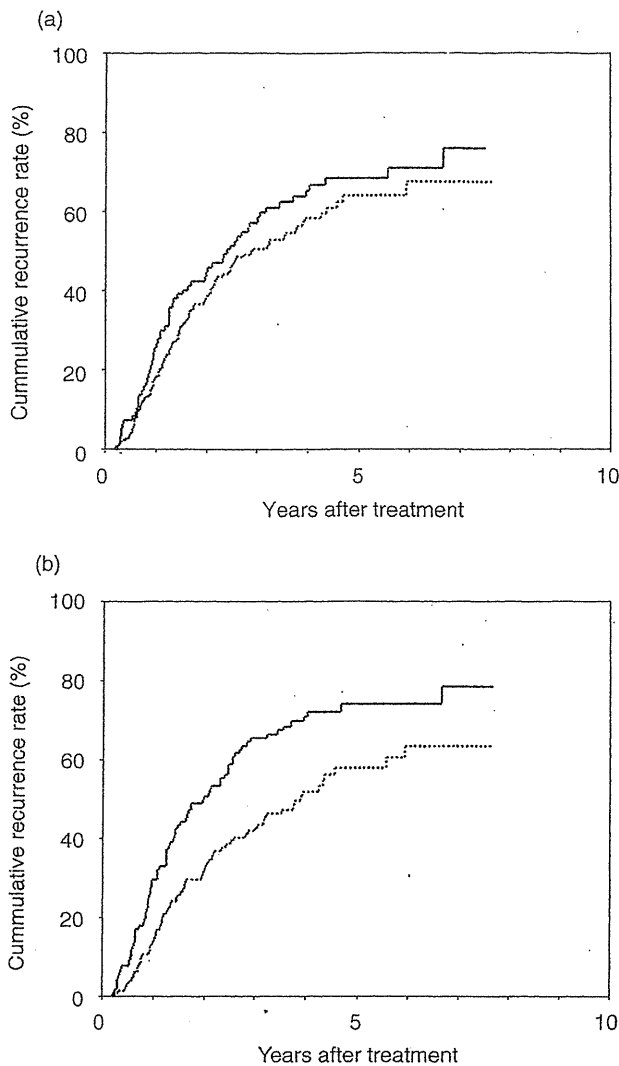
There was no significant difference in recurrence rate between the patient groups with AFP greater than and less than 20 ng/mL (Fig. 1a). On the other hand, the 1- and 3-year recurrence rates were 29.4% and 65.5% in patients with AFP-L3% greater than 5% and 14.5% and 42.7% in patients with AFP-L3% less 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).

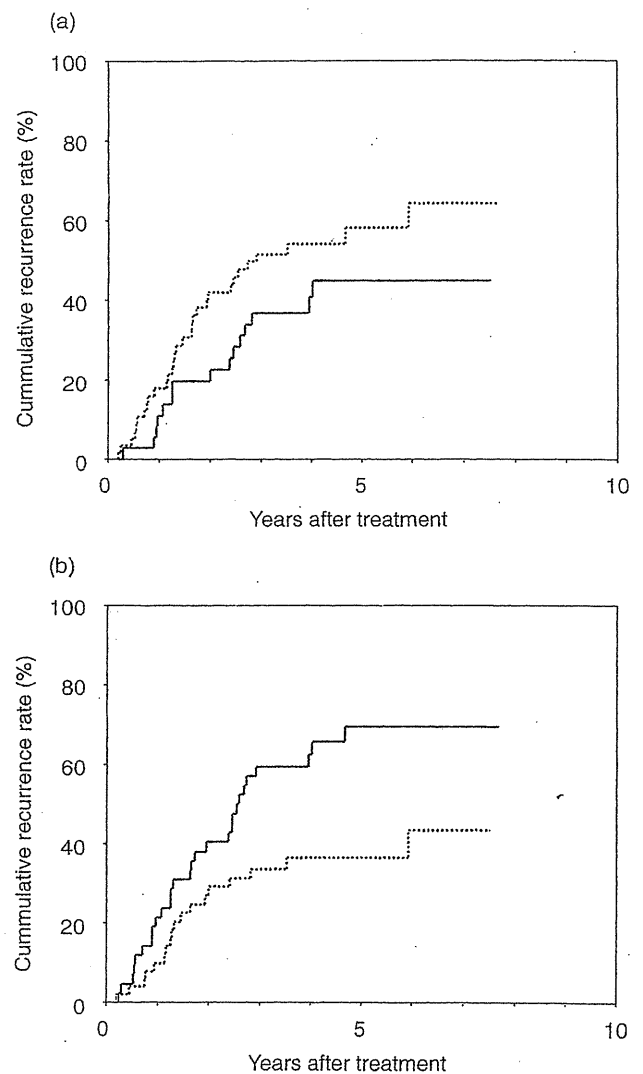
Similar analyses were performed using the serum samples obtained from 91 of 93 patients after resection. Preoperative level of AFP greater than 20 ng/mL decreased to the level of less than 20 ng/mL in 29 of 37 patients (78.4%). On the other hand, preoperative AFP levels below 20 ng/mL turned positive 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.



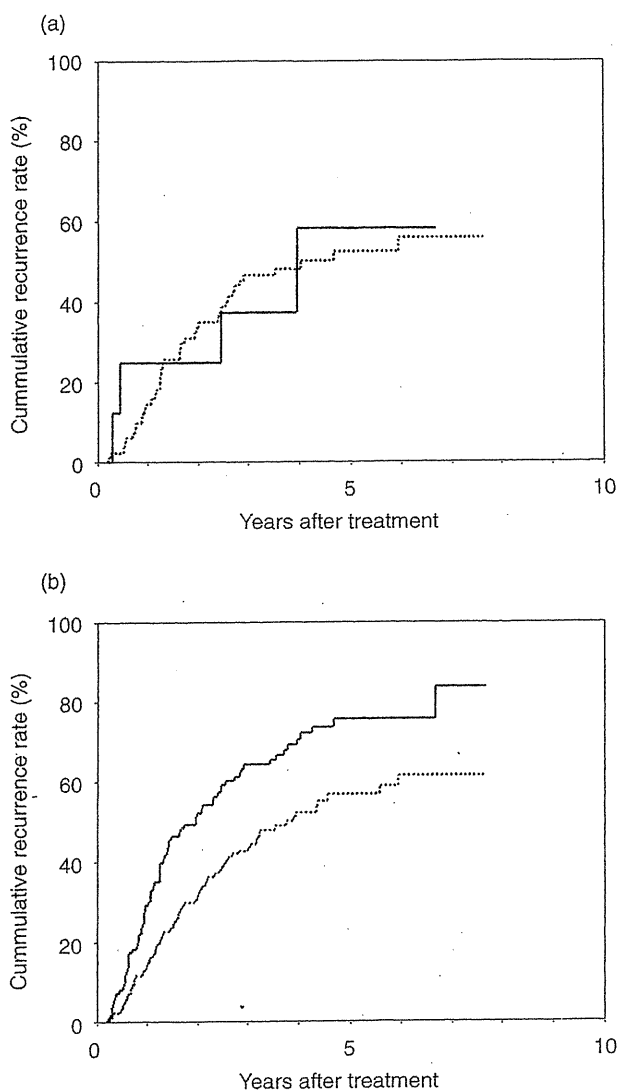
**Figure 1** Cumulative recurrence rate of hepatocellular carcinoma (HCC) for  $\alpha$ -fetoprotein (AFP) and AFP-L3% in all patients. (a) Recurrence rate for AFP: solid line, recurrence rate in patients with AFP  $\geq$  20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence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



**Figure 3** Cumulative recurrence rate of hepatocellular carcinoma (HCC) for postoperative  $\alpha$ -fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for postoperative AFP: solid line, recurrence rate in patients with AFP  $\geq 20$  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  $\geq 5\%$ ; 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  $\geq 50$  IU/L, platelets  $< 10 \times 10^4/\mu\text{L}$ , prothrombin time  $< 80\%$ , preoperative AFP-L3%  $\geq 5\%$ , and preoperative DCP  $\geq 40$  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%  $\geq 5\%$  ( $P = 0.003$ ), 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 ( $\leq 0.5$ –7.4) ( $P = 0.032$ ).

### DISCUSSION

**I**N THE PRESENT study, AFP-L3% assayed by the  $\mu\text{TAS}$  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	<i>n</i>	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 × 10 <sup>4</sup> /mm <sup>3</sup>	87	65.4	0.024
≥10 × 10 <sup>4</sup> /mm <sup>3</sup>	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.<sup>17–19</sup>

One of the advantages of the highly sensitive  $\mu$ TAS 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.<sup>27</sup> 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  $\mu$ TAS 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.*<sup>24</sup> assayed AFP-L3% in 270 patients with AFP less than 20 ng/mL and 396 patients with chronic liver diseases using the same  $\mu$ TAS 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.*,<sup>24</sup> although it was not possible to compare specificity, since in this study we included only HCC patients.

Similarly, Tamura *et al.*<sup>25</sup> 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 to be due to intra-hepatic multi-centric carcinogenesis or intra-hepatic micrometastasis. Miyaaki *et al.*,<sup>28</sup> 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.*<sup>29</sup> 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  $\mu$ TAS 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  $\geq 60$  mL/min per  $1.73$  m<sup>2</sup> after the termination of interferon therapy were enrolled. CKD was defined as an eGFR of  $< 60$  mL/min per  $1.73$  m<sup>2</sup>. End-stage-CKD was defined as an eGFR of  $< 15$  mL/min/ $1.73$  m<sup>2</sup>. 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<sup>2</sup> (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).<sup>1–4</sup> 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.<sup>5–8</sup>

Data supporting a link between hepatitis C infection and chronic kidney disease (CKD) have been reported.<sup>9–15</sup> 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<sup>2</sup> and/or the presence of proteinuria.<sup>16</sup> CKD is currently considered a serious worldwide public health problem.<sup>16,17</sup> Tsuji *et al.* have reported that HCV infection enhance the onset of end-stage renal disease.<sup>18,19</sup> 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.<sup>20</sup> We had reported that patients with severe fibrosis had high possibility of progressed kidney damage.<sup>11,12</sup> 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  $\geq 60$  (mL/min per 1.73 m<sup>2</sup>); (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  $\geq 40$  years; (v) period of  $\leq 1$  year on IFN therapy; (vi) negativity for hepatitis B surface antigen (HBsAg), anti-nuclear 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 erythematosus, 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.<sup>21</sup>

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<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup>) =  $194 \times (\text{serum creatinine level [mg/dL]})^{-1.094} \times (\text{age [y]})^{-0.287}$ . 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<sup>2</sup> or dipstick proteinuria ( $\geq +1$ ) as follows: stage 1, eGFR  $\geq 90$  and proteinuria ( $\geq +1$ ); stage 2,  $90 > eGFR \geq 60$  and proteinuria ( $\geq +1$ ); stage 3,  $60 > eGFR \geq 30$ ; stage 4,  $30 > eGFR \geq 15$ ; 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.<sup>22,23</sup>

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  $-80^\circ\text{C}$  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<sup>2</sup>). The criteria for the diagnosis of diabetes include: (i) casual plasma glucose  $\geq 200$  mg/dL; (ii) fasting plasma glucose (FPG)  $\geq 126$  mg/dL; and (iii) 2 h post-glucose (oral glucose tolerance test)  $\geq 200$  mg/dL.<sup>24</sup>

Patients were regarded as hypertension by the confirmation of blood pressure  $\geq 140$  mmHg systolic and/or  $\geq 90$  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.<sup>25</sup>

### 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.<sup>26</sup> 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  $-80^{\circ}\text{C}$  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 per 1.73 m<sup>2</sup> (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*
<i>n</i>	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 (×10 <sup>4</sup> /mm <sup>3</sup> )	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 IFN $\alpha$  2a, 73 cases; recombinant IFN $\alpha$  2b, 52 cases; natural IFN $\alpha$ , 278 cases; natural IFN $\beta$ , 150 cases; total dose of IFN = 572 ± 165 megaunit.

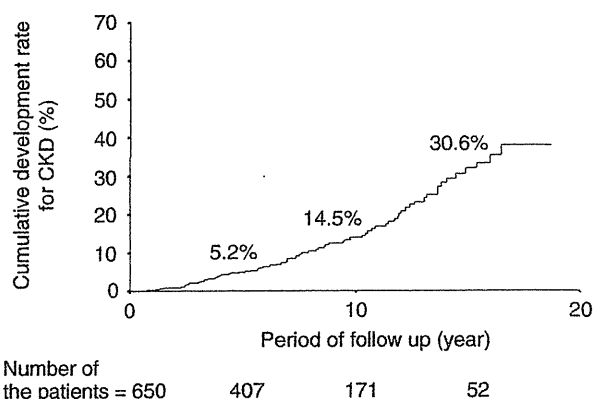
‡Outbreak of combination therapy: recombinant IFN $\alpha$  2b+ribavirin, 29 cases, total dose of IFN = 502 ± 182 megaunit, total dose of ribavirin = 160 ± 68 g; peg IFN $\alpha$  2b+ribavirin, 68 cases, total dose of peg IFN = 4.10 ± 1.08 mg, total dose of ribavirin = 202 ± 56 g. Data are number of 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 ( $\geq 25$ / $< 25$ )	1.35 (0.72–2.50)	0.347		
HCV load (KIU/mL, $\geq 1000$ / $< 1000$ )	1.39 (0.80–2.38)	0.173		
Genotype (1/2)	1.19 (0.78–1.89)	0.436		
AST (IU/L, $\geq 50$ / $< 50$ )	1.63 (0.92–2.94)	0.097		
ALT (IU/L, $\geq 50$ / $< 50$ )	2.01 (1.13–3.57)	0.016		
Platelet ( $\times 10^4$ /mm <sup>3</sup> , $\geq 15$ / $< 15$ )	0.70 (0.25–1.94)	0.487		
eGFR, per decrease of 10 mL/min/1.73 m <sup>2</sup>	2.00 (1.56–2.56)	<0.001	1.66 (1.27–2.16)	<0.001
Uric acid (mg/dL, $\geq 7.0$ / $< 7.0$ )	1.43 (0.81–2.47)	0.225		
Triglyceride (mg/dL, $\geq 150$ / $< 150$ )	1.61 (0.62–3.70)	0.336		
Cholesterol (mg/dL, $\geq 220$ / $< 220$ )	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 ( $\geq 1$ / $< 1$ )	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.

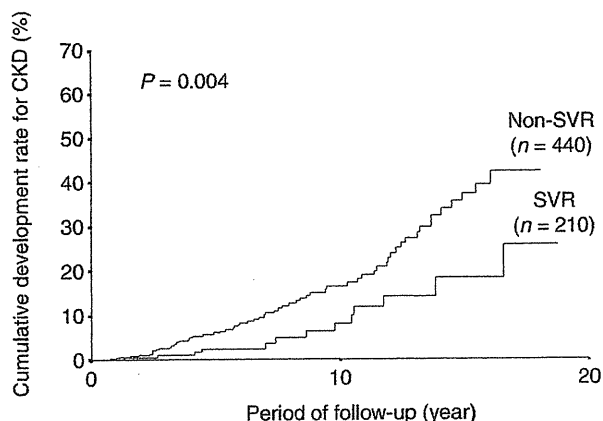
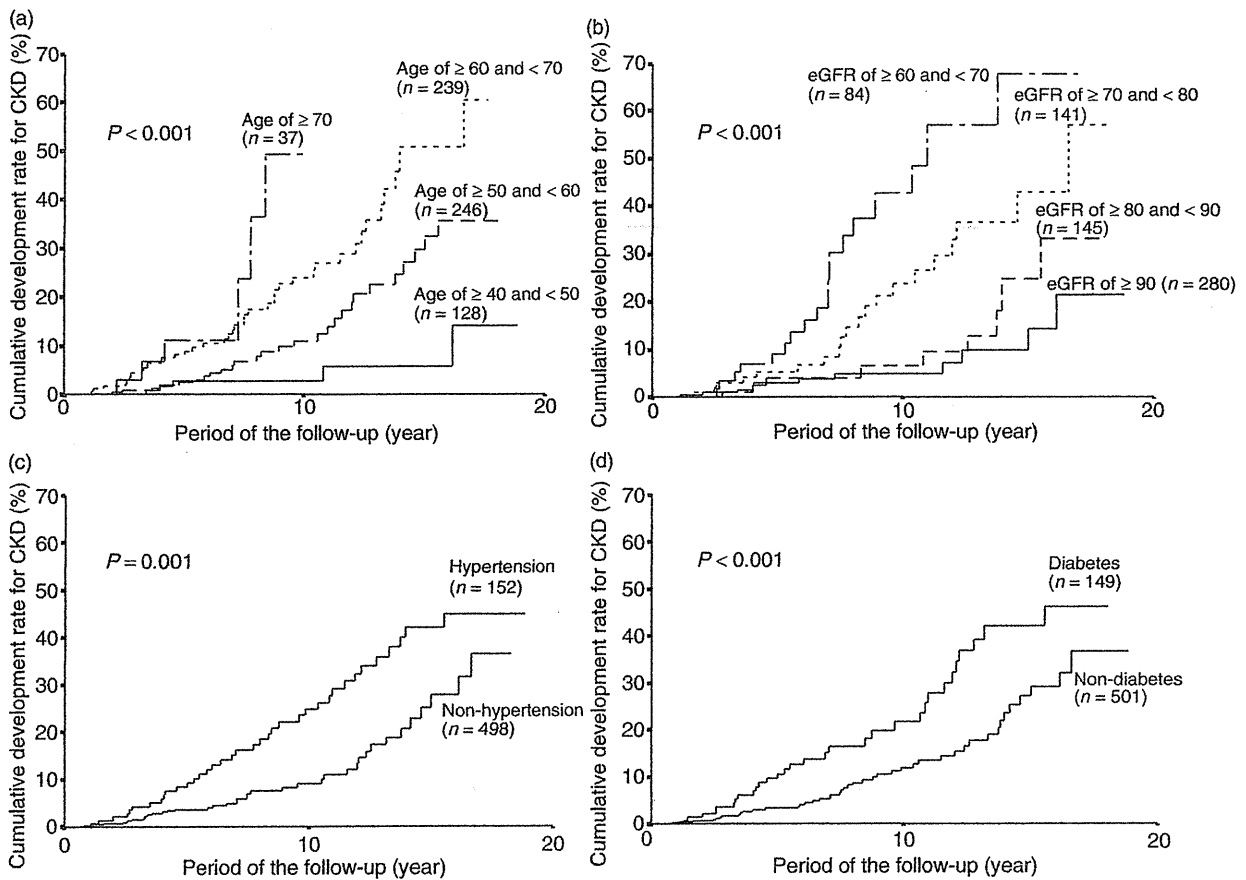


Figure 2 Cumulative development rate for chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

## 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.<sup>27</sup> To exclude kidney damage originated from IFN-related side effects, patients with eGFR of  $\geq 60$  (mL/min per 1.73 m<sup>2</sup>) 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<sup>2</sup> 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.

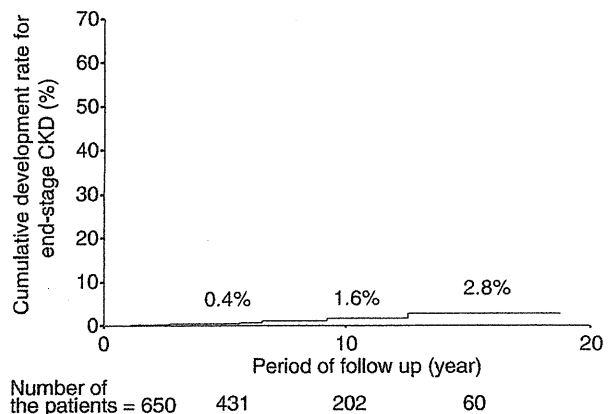
Imai *et al.* have reported that about 20% of the Japanese adult population have stage 3 to 5 CKD by the use of database for 527 594 (male, 211 034; female, 316 560) participants obtained from the general adult population aged over 20 years who received annual



**Figure 3** Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon: (a) Cumulative development rate for CKD based on difference of age; (b) Cumulative development rate for CKD based on the difference of estimated glomerular filtration rate (eGFR); (c) Cumulative development rate for CKD based on the difference of blood pressure; (d) Cumulative development rate for CKD based on the difference of glucose level.

health check programs in 2000–2004, from seven different prefectures in Japan. Next, the prevalence of CKD stage 3 in the study population, stratified by age groups of 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80–89 years, were 1.4%, 3.6%, 10.8%, 15.9%, 31.8%, 44.0%, and 59.1%, respectively. Moreover, they provided that the prevalence of stage 4+5 was <0.2%. Our results agreed with Imai’s report in the fact that end-stage CKD patients were few.

The present study was limited by a retrospective cohort trial. This cohort is over 10 years; hence, many patients had complications, such as diabetes and hypertension. However, the development of CKD was mainly evaluated based on the clinical characteristics at the initiation of follow-up. Second limitation of the study was that we defined CKD according to eGFR alone. Gener-



**Figure 4** Cumulative development rate for end-stage chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

**Table 3** Predictive factors for end-stage chronic kidney disease (CKD) development

Variables	Univariate analysis	
	HR (95%CI)	P
Age, per 10 years	2.13 (0.86–5.30)	0.104
Sex (female/male)	0.24 (0.03–1.92)	0.182
Body mass index ( $\geq 25$ / $< 25$ )	0.80 (0.16–4.10)	0.782
HCV load (KIU/mL, $\geq 1000$ / $< 1000$ )	1.58 (0.37–6.67)	0.535
Genotype (1/2)	2.74 (0.66–11.50)	0.167
AST (IU/L, $\geq 50$ / $< 50$ )	1.45 (0.18–11.76)	0.730
ALT (IU/L, $\geq 50$ / $< 50$ )	1.89 (0.45–7.93)	0.382
Platelet ( $\times 10^4$ /mm <sup>3</sup> , $\geq 15$ / $< 15$ )	0.67 (0.16–2.86)	0.586
eGFR, per decrease of 10 mL/min/1.73 m <sup>2</sup>	1.70 (0.89–3.23)	0.105
Uric acid (mg/dL, $\geq 7.0$ / $< 7.0$ )	1.27 (0.23–6.96)	0.784
Triglyceride (mg/dL, $\geq 150$ / $< 150$ )	1.33 (0.15–11.87)	0.802
Cholesterol (mg/dL, $\geq 220$ / $< 220$ )	1.03 (0.12–8.67)	0.980
Diabetes (+/-)	1.89 (0.45–7.93)	0.382
Hypertension (+/-)	2.83 (0.70–11.41)	0.143
Combination of ribavirin (+/-)	0.88 (0.10–7.66)	0.908
Kind of IFN (beta/alpha)	2.08 (0.52–8.37)	0.300
Efficacy (non-SVR/SVR)	3.25 (0.40–26.4)	0.269
Frequencies of contrast imaging per year ( $\geq 1$ / $< 1$ )	3.72 (0.70–19.72)	0.123

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.

ally, a recent definition of CKD also includes proteinuria.<sup>28,29</sup> Although the use of both eGFR and proteinuria might lead to a more accurate classification of CKD, we could not assess proteinuria in this study. Third, prescribed agents during the follow-up were not considered in the present study. However, therapy intervention is very important for protecting new development for CKD. In future, the intervention therapy for protecting the development of CKD should be evaluated. Finally, in the present study, patients were treated with different types of antiviral therapy (IFN monotherapy or combination therapy of IFN and ribavirin) for different durations (4 weeks to 52 weeks). This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

The present study shows several findings with regard to development incidence for CKD or end-stage CKD

after the termination of IFN therapy for HCV positive and cirrhotic patients. First, SVR is effective for protecting the development incidence for CKD in HCV patients with liver cirrhosis. Though the role of HCV in the pathogenesis of aggravation of renal function remains speculative, the following possible mechanism have been reported: (i) systemic immune response to HCV infection mediated by cryoglobulins, HCV-antibody immune complexes, or amyloid deposition,<sup>8,30,31</sup> (ii) toll-like receptors increased expression in glomeruli induce immune response;<sup>32</sup> and (iii) insulin resistance and hyperinsulinemia cause excess intrarenal production of insulin-like growth factor-1 and transforming growth factor  $\beta$ , thus induce oxidative stress.<sup>33</sup> In addition, patients with liver cirrhosis might have the possibility of kidney damage such as hypovolemia due to fluid loss or hemorrhage, hepatorenal syndrome, and drug-induced renal failure. Second, in addition to non-SVR, the present study suggests that aging, low eGFR, hypertension, and diabetes enhanced the development of worsening renal function in cirrhotic patients with HCV infection after the termination of IFN. The repeated use of contrast imaging of computed tomography might worsen renal function. However, in the present study, SVR, aging, low eGFR, hypertension, and diabetes were the main predictive factors for the development of CKD compared to the repeated use of contrast imaging of computed tomography. The result that aging, hypertension and diabetes were associated with the development of worsening renal function agreed with several studies.<sup>16–19</sup>

In the present study, the predictive factors for end-stage CKD (stage 5) were not similar to those for CKD 3–5. The possible reason for this discrepancy is as follows. First, the number of patients who had progressed to end-stage CKD was six. Because of so few patients, we could not show the statistical significance in the predictive factors for end-stage CKD. Second, development of end-stage CKD might be robust to the several factors at the initiation of the follow-up. Development of end-stage CKD might be associated with the accidents during follow-up, such as the repeated use of contrast medium and hypovolemia due to bleeding. In fact, four of six patients who progressed to end-stage CKD had been given the repeated use of contrast medium. Next, whether HCV eradication in patients whose renal function progressed to stage of CKD 3–5 improves the mortality due to cardiovascular disease and stroke is a very important issue. However, this problem was not evaluated in the present study. This should be clarified by further examination.



In conclusion, our study suggests that 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.

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## Previous Chemoembolization Response after Transcatheter Arterial Chemoembolization (TACE) Can Predict the Anti-Tumor Effect of Subsequent TACE with Miriplatin in Patients with Recurrent Hepatocellular Carcinoma

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

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

### Key Words

Hepatocellular carcinoma · Miriplatin · Transcatheter arterial chemoembolization

### Abstract

**Aim:** The purpose of this retrospective study was to evaluate the efficacy and safety of transcatheter arterial chemoembolization (TACE) with miriplatin in patients with unresectable hepatocellular carcinoma (HCC). **Methods:** From 2007 to 2010, 122 consecutive patients with unresectable HCC were treated by TACE with miriplatin-lipiodol suspension in our institute. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. Ninety-eight patients (80%) had a history of TACE. **Results:** Thirty-five of the 122 treated patients (29%) showed complete response (CR). And no serious complications were observed. Patients who had shown CR after previous TACE (pre-CR) were significantly more likely to show CR in the current study compared with patients who had shown less successful responses after previous TACE (56 vs. 20%,  $p = 0.003$ ). Multivariate analysis revealed that response after previous TACE

(pre-CR, risk ratio: 4.76;  $p = 0.035$ ), tumor multiplicity (solitary, risk ratio: 9.69;  $p = 0.003$ ), and injection artery (peripheral to segmental hepatic artery, risk ratio: 5.28;  $p = 0.040$ ) were significant independent predictors associated with CR after TACE using miriplatin. **Conclusion:** In repetition of TACE treatment, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown a CR after previous TACE.

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### Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide [1]. Since it is well known that more than 80% of HCC cases are associated with liver cirrhosis, a routine check-up including ultrasound for cirrhotic patients could potentially lead to the detection of early HCC [2–4]. Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation) are applicable in only 30–40% of

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Fax +41 61 306 12 34  
E-Mail [karger@karger.ch](mailto:karger@karger.ch)  
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Norihiro Imai  
Department of Hepatology  
Toranomon Hospital  
2-2-2 Toranomon, Minato-ku, Tokyo 105-8470 (Japan)  
Tel. +81 3 3588 1111, E-Mail [norihiro.imai@gmail.com](mailto:norihiro.imai@gmail.com)

HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC [5–12].

Although many chemotherapeutic agents (e.g. doxorubicin, epirubicin, mitomycin C, and cisplatin) are used with the ethyl ester of iodized fatty acids from poppy-seed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) in TACE, the best choices for first- and second-line drugs remain uncertain [13–15]. Miriplatin (cis-[[[(1R,2R)-1,2-cyclohexanediamine-N,N']bis(myristato)]-platinum(II)monohydrate; Dainippon Sumitomo Pharma Co., Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent [16–19]. When lipiodol is injected into an artery feeding HCC nodules, it selectively accumulates in the tumor. Accordingly, a miriplatin-lipiodol emulsion is deposited within the HCC nodules and gradually releases active platinum compounds into tumor tissues. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC, but the efficacy of TACE using miriplatin for patients with recurrent HCC after TACE has not been evaluated [20, 21]. The purpose of this retrospective study was to evaluate the efficacy and safety of TACE using miriplatin for patients with HCC.

## Patients and Methods

### Study Population

From December 2007 to December 2010, 122 consecutive patients with unresectable HCC were treated by TACE with a miriplatin-lipiodol suspension at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. The study group consisted of 79 men and 43 women ranging in age from 48 to 87 years (median, 72 years). They included 11 patients (9%) positive for HBs-Ag, 103 patients (84%) positive for HCV antibody, and 8 patients (7%) negative for both. At the time of the miriplatin administration, median values were as follows: total bilirubin level = 1.1 mg/dl; serum albumin concentration = 3.3 g/dl; indocyanin-green retention rate at 15 min = 29%; prothrombin activity = 82.5%; alpha-fetoprotein (AFP) concentration = 31.2 ng/ml; and des-gamma-carboxyprothrombin (DCP) concentration = 53 AU/l. As for Child-Pugh classification, 92 patients (75%) were Class A and 30 patients (25%) were Class B. The clinical characteristics of the study group are summarized in table 1. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

### Hepatocellular Carcinoma

Before treatment with miriplatin, all patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver-imaging studies (dynamic computerized tomography [dynamic CT], ultrasonography [US], digital-sub-

**Table 1.** Demographic characteristics and pretreatment assessments of 122 patients who underwent TACE using a miriplatin/lipiodol suspension for unresectable HCC

Number of cases	122
Age, years	72 (48–87)
Gender, male	65%
Etiology, HCV/HBV/others	103/11/8
Child-Pugh Class, A/B/C	92/30/0
ICG-R15, %	29 (4–78)
Albumin, g/dl	3.3 (2.0–4.2)
Total bilirubin, mg/dl	1.1 (0.4–4.9)
Prothrombin activity, %	82.5 (45.7–123.1)
Platelet, $\times 10^3/\mu\text{l}$	93 (29–282)
AFP, ng/ml	31.2 (1.8–152,800)
DCP, AU/l	53 (6–65,290)

HCV = Hepatitis C virus; HBV = hepatitis B virus; ICG-R15 = indocyanine-green retention rate at 15 min.

Variables are expressed as medians with ranges in parentheses.

**Table 2.** Tumor profiles, treatment history, and study drug dosages of 122 patients who underwent TACE using miriplatin for unresectable HCC

Tumor size, mm	20 (10–100)
Intrahepatic multiplicity, solitary	22 (18%)
Number of tumors	4 (1–100)
Presence of portal vein invasion	3 (2%)
History of TACE	98 (80%)
History of TACE with epirubicin	80 (66%)
History of TACE with cisplatin	37 (30%)
Median interval between previous TACE and miriplatin administration, months	4 (1–41)
Dosage of miriplatin, mg	80 (20–120)
Dosage of lipiodol, ml	3 (1–6)
Injection from peripheral to segmental branch of the hepatic artery	22 (18%)

Variables are expressed as medians with ranges in parentheses or number of cases.

traction angiography [DSA]), complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of dynamic CT, US and DSA. Patients who had extrahepatic metastasis of HCC or other malignancies were excluded.

Tumor profiles and TACE treatment history for the study group are summarized in table 2. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. The median diameter of the largest tumor was 20 mm (range 10–100 mm). Ninety-eight patients (80%) had a history of TACE. Thirty-seven patients had received cisplatin, and 80 patients had received epirubicin. Among these patients, the median number of