Hepatology Research

Hepatology Research 2011; 41: 1036–1045 doi: 10.1111/j.1872-034X.2011.00858.x

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
Supported in part by a Research Grant from the Japanese Ministry of Health, Labor and Welfare, and Okinaka Memorial Foundation of Toranomon Hospital.
Received 29 March 2011; revision 10 June 2011; accepted 13 June 2011.

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 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.[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.

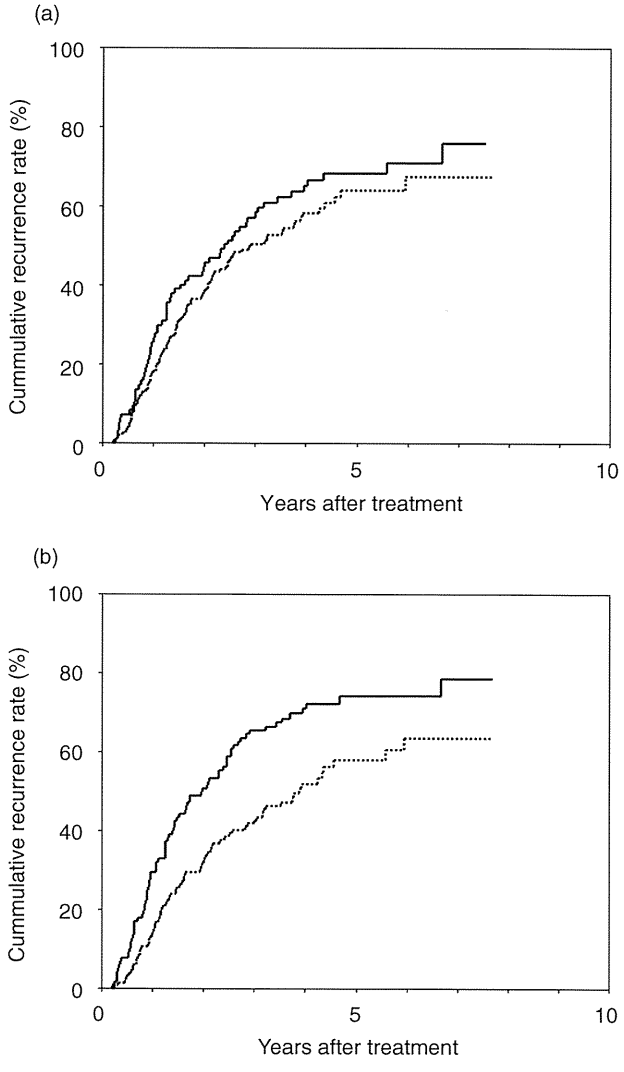

Figure 1 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for α-fetoprotein (AFP) and AFP-L3% in all patients. (a) Recurrence rate for AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL. (b) Recurrence rate for AFP-L3%: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

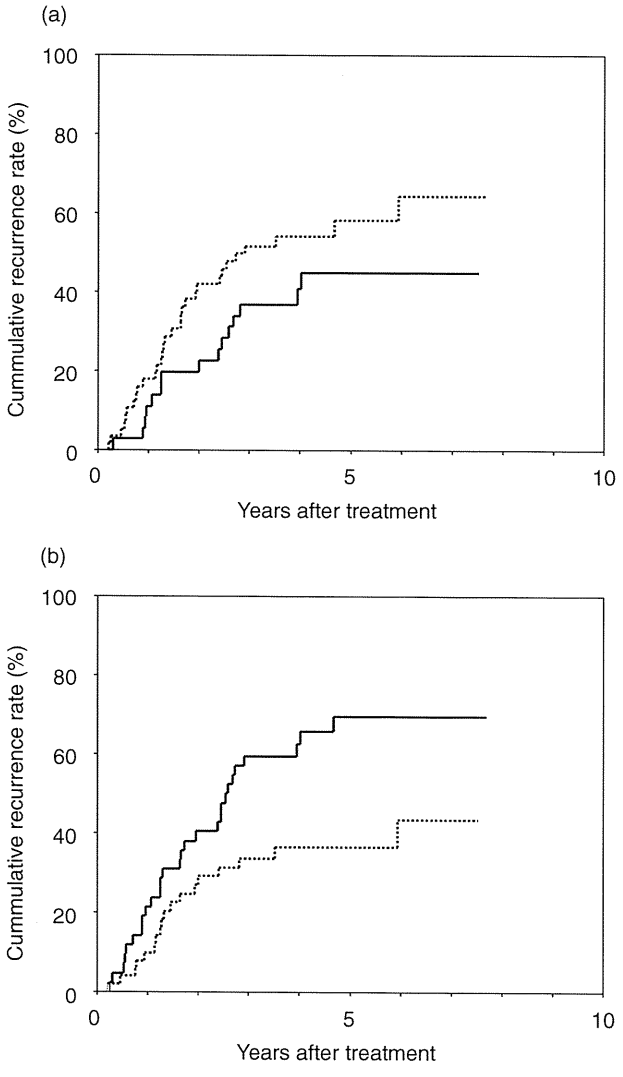

Figure 2 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for preoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for preoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20 ng/mL.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

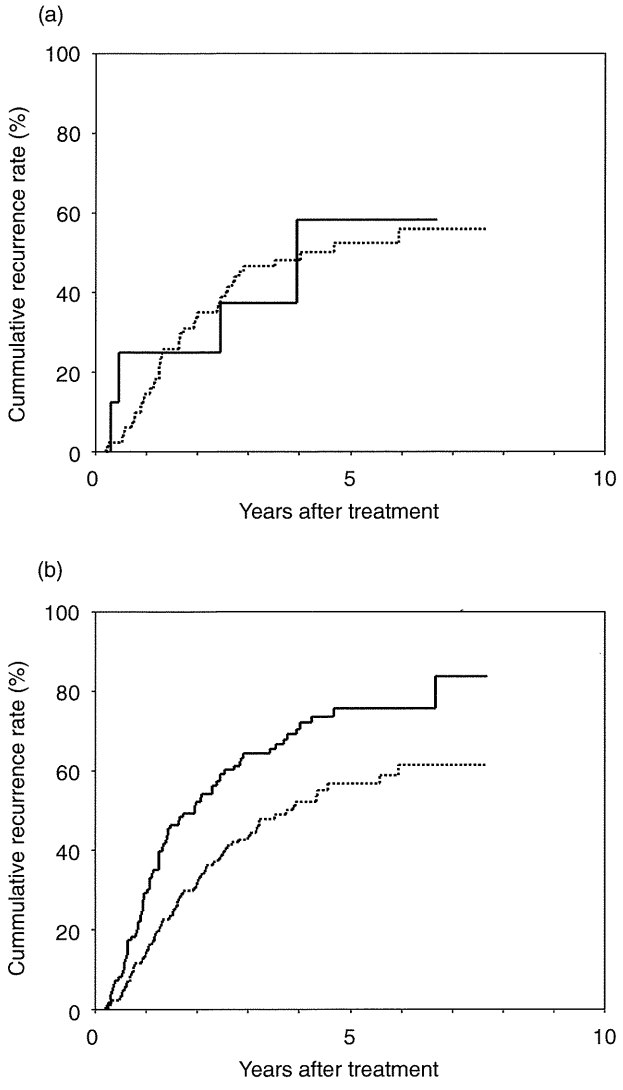

Figure 3 Cumulative recurrence rate of hepatocellular carcinoma (HCC) for postoperative α-fetoprotein (AFP) and AFP-L3% in resected patients. (a) Recurrence rate for postoperative AFP: solid line, recurrence rate in patients with AFP ≥ 20 ng/mL; broken line, recurrence rate in patients with AFP < 20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

REFERENCES

1 Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71–96.
2 Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of alpha-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994; **19**: 61–6.
3 Daniele B, Bencivenga A, Megna AS, Tinessa V. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S108–12.
4 Marrero JA, Feng Z, Wang Y *et al.* Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 110–18.
5 Saitoh S, Ikeda K, Koida I *et al.* Diagnosis of hepatocellular carcinoma by concanavalin A affinity electrophoresis of serum alpha-fetoprotein. *Cancer* 1995; **76**: 1139–44.
6 Bruix J, Sherman M, Llovet JM *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. *J Hepatol* 2001; **35**: 421–30.
7 Omata M, Lesmana LA, Tateishi R *et al.* Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010; **4**: 439–74.
8 Makuuchi M, Kokudo N. Surveillance algorithm and diagnostic algorithm for hepatocellular carcinoma. *Hepatology Research* 2010; **40**: 6–7.
9 Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020–2.
10 Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC. High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus infection, age, p53 and beta-catenin mutations. *Int J Cancer* 2004; **112**: 44–50.
11 Imamura H, Matsuyama Y, Miyagawa Y *et al.* Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. *Br J Surg* 1999; **86**: 1032–8.
12 Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. *Cancer* 1989; **64**: 2117–20.
13 Liaw YF, Tai DI, Chen TJ, Chu CM, Huang MJ. Alpha-fetoprotein changes in the course of chronic hepatitis: relation to bridging hepatic necrosis and hepatocellular carcinoma. *Liver* 1986; **6**: 133–7.
14 Chu CW, Hwang SJ, Luo JC *et al.* Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. *J Clin Gastroenterol* 2001; **32**: 240–4.
15 Aoyagi Y, Isemura M, Suzuki Y *et al.* Fucosylated alpha-fetoprotein as marker of early hepatocellular carcinoma. *Lancet* 1985; **2**: 1353–4.
16 Taketa K, Sekiya C, Namiki M *et al.* Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. *Gastroenterology* 1990; **99**: 508–18.
17 Toyoda H, Kumada T, Kaneoka Y *et al.* Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. *J Hepatol* 2008; **49**: 223–32.
18 Tamura Y, Igarashi M, Suda T *et al.* Fucosylated fraction of alpha-fetoprotein as a predictor of prognosis in patients with hepatocellular carcinoma after curative treatment. *Dig Dis Sci* 2010; **55**: 2095–101.
19 Tateishi R, Shiina S, Yoshida H *et al.* Prediction of recurrence of hepatocellular carcinoma after curative ablation using three tumor markers. *Hepatology* 2006; **44**: 1518–27.
20 Sterling RK, Jeffers L, Gordon F *et al.* Clinical utility of AFP-L3% measurement in North American patients with HCV-related cirrhosis. *Am J Gastroenterol* 2007; **102**: 2196–205.
21 Shimizu K, Taniichi T, Satomura S, Matsuura S, Taga H, Taketa K. Establishment of assay kits for the determination of microheterogeneities of alpha-fetoprotein using lectin-affinity electrophoresis. *Clin Chim Acta* 1993; **214**: 3–12.
22 Yamagata Y, Katoh H, Nakamura K, Tanaka T, Satomura S, Matsuura S. Determination of alpha-fetoprotein concentration based on liquid-phase binding assay using anion exchange chromatography and sulfated peptide introduced antibody. *J Immunol Methods* 1998; **212**: 161–8.
23 Kagebayashi C, Yamaguchi I, Akinaga A *et al.* Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. *Anal Biochem* 2009; **388**: 306–11.
24 Toyoda H, Kumada T, Tada T *et al.* Clinical utility of high sensitive lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein less than 20 ng/mL. *Cancer Sci* 2011; **102**: 1025–31. [Epub ahead of print].
25 Tamura Y, Igarashi M, Kawai H, Suda T, Satomura S, Aoyagi Y. Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. *Dig Dis Sci* 2010; **55**: 3576–83.
26 Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer. English Edn.* Tokyo: Kanehara, 2003.
27 Kobayashi M, Ikeda K, Kawamura Y *et al.* High serum des-gamma-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. *Cancer* 2009; **115**: 571–80.

28 Miyaaki H, Nakashima O, Kurogi M, Eguchi K, Kojiro M. Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 962–8.

29 Tada T, Kumada T, Toyoda H *et al.* Relationship between Lens culinaris agglutinin-reactive alpha-fetoprotein and pathologic features of hepatocellular carcinoma. *Liver Int* 2005; **25**: 848–53.

Clinical Study

Oncology

Oncology 2011;80:188–194
DOI: 10.1159/000328749

Received: April 4, 2011
Accepted: April 4, 2011
Published online: June 27, 2011

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.

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

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/11/0804–0188$38.00/0

Accessible online at:
www.karger.com/ocl

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

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-subtraction 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

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, $\times 10^3/\mu l$	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.

TACE procedures was four (range 1–13), and the median interval between previous TACE and miriplatin administration was 4 months (range 1–41 months).

Treatment Protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and the catheter was inserted superselectively into the hepatic artery that supplied the target tumor for injection of the miriplatin-lipiodol suspension and 1-mm gelatin cubes (Gelpart; Nippon Kayaku, Tokyo). The miriplatin-lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

Assessment of Therapeutic Effects

The effect of chemotherapy was evaluated by dynamic CT 1 to 3 months after TACE with miriplatin, and was based on the change in the maximum diameter of the viable target lesions (i.e. showing enhancement in the arterial phase). Response categories, according to the criteria of Modified Response Evaluation Criteria in Solid Tumors (mRECIST) [22], are as follows: complete response (CR) = disappearance of any intratumoral arterial enhancement in all target lesions; partial response (PR) = at least a 30% decrease in the sum of diameters of viable target lesions; stable disease (SD) = any cases that do not qualify for either PR or progressive disease; and progressive disease (PD) = an increase of at least 20% in the sum of the diameters of viable target lesions.

Toxicity Evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria (version 4.0). Within 2 weeks before TACE with miriplatin, and at 3 to 7 days (three times during this period) and at 1 month afterward, the following toxicity evaluations were made: hematological assessments (i.e. leukocyte and thrombocyte counts) and clinical chemistry assessments (i.e. serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], albumin, total bilirubin, serum creatine, and prothrombin activity).

Statistical Analysis

The distribution of subject characteristics was assessed by the chi-square test or the Mann-Whitney's U test, as appropriate. Multivariate logistic regression analysis was used to evaluate significant factors for CR by TACE with miriplatin. All variables are expressed as mean (range). All tests were 2-sided, and p values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., IBM, Somers, N.Y., USA).

Results

Dosing of Study Drugs

Table 2 summarizes the profiles and study drug data of 122 HCC patients who were treated with miriplatin. The median dosage of miriplatin was 80 mg (range 20–120 mg), and the median dosage of lipiodol was 3 ml (range 1–6 ml). Twenty-two patients (18%) were injected with the miriplatin-lipiodol suspension from the peripheral to the segmental branch of the hepatic artery. Thirty patients (25%) were injected with the miriplatin-lipiodol suspension from the anterior or posterior segmental branch of the right hepatic artery. Sixty-six patients (54%) were injected with the miriplatin-lipiodol suspension from the right or left branch of the hepatic artery. And 4 patients (3%) were injected with the miriplatin-lipiodol suspension from the proper hepatic artery.

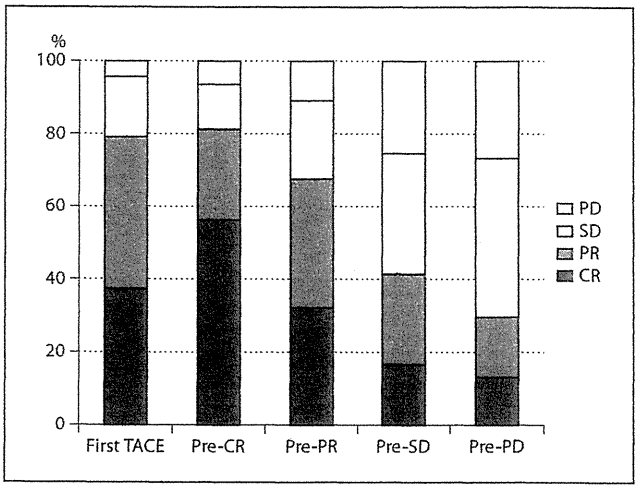

Fig. 1. The efficacy of TACE using miriplatin in patients with HCC according to response to previous TACE. Abbreviations used in the figure: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. pre-CR group (n = 16): patients who showed CR after previous TACE. pre-PR group (n = 28): patients who showed PR after previous TACE. pre-SD group (n = 24): patients who showed SD after previous TACE. pre-PD group (n = 30): patients who showed PD after previous TACE.

Treatment Effects

Thirty-five of the 122 treated patients (29%) showed CR, 35 patients (29%) showed PR, 33 patients (27%) showed SD, and 19 patients (15%) showed PD. Overall, 58% of patients showed an objective response (i.e. CR or PR).

Treatment Effects according to Previous TACE Effect

The efficacy of TACE using miriplatin according to the treatment effect of previous TACE was as follows (and is illustrated in fig. 1). For the first TACE group (patients who received TACE for the first time), 9 of 24 patients (38%) showed CR; for the pre-CR group (patients who

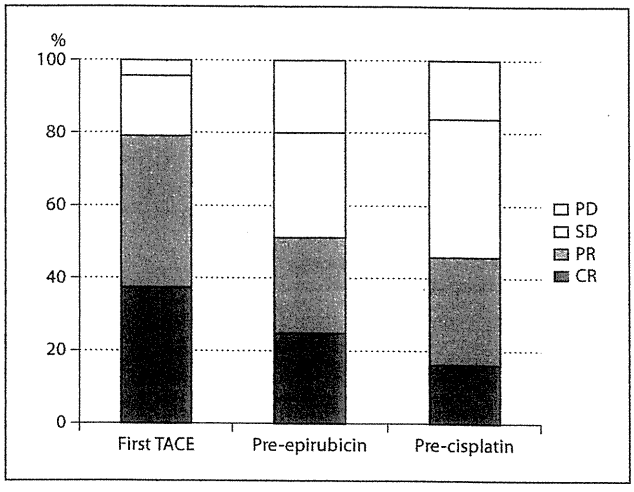

Fig. 2. The efficacy of TACE using miriplatin in patients with HCC according to previous TACE agent. Abbreviations used in the figure: CD = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. Pre-cisplatin group (n = 37): patients who had received TACE using cisplatin. Pre-epirubicin group (n = 80): patients who had received TACE using epirubicin.

showed CR response after previous TACE), 9 of 16 patients (56%) showed CR; for the pre-PR group (patients who showed PR response after previous TACE), 9 of 28 patients (32%) showed CR; for the pre-SD group (patients who showed SD response after previous TACE), 4 of 24 patients (17%) showed CR; and for the pre-PD group (patients who showed PD response after previous TACE), 4 of 30 patients (13%) showed CR.

Treatment Effects according to Previous TACE Agent

In patients who had received TACE using epirubicin, 20 of 80 patients (25%) showed CR and 21 of 80 patients (26%) showed PR. In patients who had received TACE using cisplatin, 6 of 37 patients (16%) showed CR and 11 of 37 patients (30%) showed PR. In each of the above groups, the objective response rate (sum of CR and PR) was significantly lower than that in patients who received their first TACE ($p = 0.015$ and $p = 0.010$, respectively), as illustrated in figure 2.

Univariate analysis identified the following six factors as influencing the rate of CR: response after previous TACE (pre-CR group vs. other groups, $p = 0.005$), tumor multiplicity (solitary vs. multiple, $p < 0.0001$), gamma-GTP concentration (≤40 vs. >40 IU/l, $p = 0.037$), AFP concentration (≤40 vs. >40 ng/ml, $p = 0.042$), DCP concentration (≤50 vs. >50 AU/l, $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, $p = 0.001$). These parameters were entered into multivariate logistic regression analysis, which revealed that response after previous TACE (pre-CR group vs. other groups, risk ratio: 4.76; 95% CI: 1.11–20.37; $p = 0.035$), tumor multiplicity (solitary vs. multiple, risk ratio: 9.69; 95% CI: 2.18–42.92; $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, risk ratio: 5.28; 95% CI: 1.07–25.95; $p = 0.040$) were significant independent predictors associated with CR after TACE using miriplatin (table 3).

Adverse Effects

Fever, anorexia, and elevation of serum transaminase levels were observed in most patients after miriplatin administration (table 4). The following Grade 4 events were observed: decreased neutrophil count in 1 patient (1%), increased AST in 4 patients (3%), and increased ALT in 1 patient (1%); all these cases resolved within 2 weeks. In this study group, no vascular complications of the hepatic artery were observed. No other serious complications or treatment-related deaths were observed after miriplatin administration.

Discussion

TACE is most widely performed in patients with HCC who are not eligible for curative therapy. The survival benefit of TACE has been confirmed by randomized controlled trials and meta-analyses. Various anti-cancer drugs, such as doxorubicin, epirubicin, mytomycin C, cisplatin, and neocarzinostatin, have been used as TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified [13–15].

Although TACE can be repeated in most patients, good therapeutic efficacy cannot be expected when the same anti-cancer drug is used more than once since various types of resistance to therapy can develop during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs [23, 24]. Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior anti-tumor effect in HCC with lower toxicity compared with cisplatin [16–

Table 3. Univariate and multivariate analysis of predictors of complete necrosis (logistic regression analysis)

Category	Univariate		Multivariate	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Tumor multiplicity, solitary vs. multiple	8.57 (3.08–23.8)	<0.0001	9.69 (2.19–42.9)	0.003
Response by pre-TACE, pre-CR vs. others	4.91 (1.59–15.1)	0.005	4.76 (1.11–20.3)	0.035
Injection artery, peripheral to segmental hepatic artery vs. others	2.50 (0.96–6.48)	0.001	5.28 (1.07–25.9)	0.040
DCP, ≤50 vs. >50 AU/l	4.04 (1.61–10.13)	0.003	3.55 (0.99–12.6)	0.051
gamma-GTP, ≤40 vs. >40 IU/l	2.39 (1.05–5.44)	0.037		
AFP, ≤40 vs. >40 ng/ml	2.50 (1.03–6.06)	0.042		

Table 4. Adverse effects after miriplatin administration

Grade:	1	2	3	4
White blood cell decreased	1 (1%)	27 (22%)	7 (6%)	0
Neutrophil count decreased	2 (2%)	21 (17%)	5 (4%)	1 (1%)
Anemia	40 (33%)	21 (17%)	3 (2%)	0
Platelet count decreased	72 (59%)	21 (17%)	11 (9%)	0
AST increased	55 (45%)	23 (19%)	30 (25%)	4 (3%)
ALT increased	54 (44%)	12 (10%)	19 (16%)	1 (1%)
Fever	67 (55%)	14 (11%)	0	0
Anorexia	56 (46%)	1 (1%)	0	0
Nausea	23 (19%)	0	0	0
Abdominal pain	22 (18%)	4 (3%)	0	0
Hepatic infection	0	0	1 (1%)	0

Values denote numbers of subjects. Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0.

19]. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miriplatin.

According to pharmacokinetic studies, the plasma concentration of total platinum is much lower in patients treated with miriplatin compared with that in patients treated with intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer for miriplatin than the corresponding values for intra-arterial cisplatin.

Miriplatin/lipiodol releases 1,2-diaminocyclohexane platinum (II) dichloride (DPC) as its active platinum compound, which binds to nuclear DNA and mediates miriplatin/lipiodol cytotoxicity. In a cisplatin-resistant rat hepatoma cell-line model, cross-resistance to DPC was not observed [25].

Prior to the current study, clinical trials have shown that miriplatin is effective for the treatment of HCC, but the efficacy of switching the TACE anti-cancer drug from epirubicin or cisplatin to miriplatin for a repeat TACE had not been evaluated.

In the present study, having a low number of tumors (solitary vs. multiple), receiving the treatment injection in the peripheral to segmental hepatic artery, and having shown complete tumor necrosis after prior TACE (pre-CR group) were highly correlated with complete tumor necrosis after TACE with miriplatin. A previous CR may be a surrogate marker for other factors, such as tumor sensitivity to anti-cancer agents and intra-hepatic metastasis. Among the 54 patients in this study who had shown no change or disease progression after previous TACE (pre-SD and pre-PD groups), 19 patients (35%) showed an

objective response by switching the TACE agent from epirubicin or cisplatin to miriplatin.

In repetition of TACE, vascular complications can cause development of parasitic feeding arteries for liver cancers leading to insufficient tumor embolization; rapid tumor growth may follow. In the present study, no vascular complications or other serious adverse events were observed. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Previous studies reported that complete tumor necrosis after TACE offered favorable long-term survival outcomes to HCC patients [7, 26]. In the current study, miriplatin administration was associated with a beneficial tumor response even in recurrent HCC after TACE. These results suggest that miriplatin administration may offer a favorable prognosis for recurrent HCC after TACE.

Conclusion

In repetition of TACE in HCC patients, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown CR after previous TACE. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Disclosure Statement

The following authors have received honoraria (lecture fee) from Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan: Hiromitsu Kumada, MD; Kenji Ikeda, MD; Yasuji Arase, MD; Yoshiyuki Suzuki, MD; Fumitaka Suzuki, MD; and Norio Akuta, MD.

References

1 Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2 Bosch X, Ribes J, Borras J: Epidemiology of primary liver cancer. Semin Liver Dis 1999; 19:271–285.
3 Okuda K, Fujimoto I, Hanai A, Urano Y: Changing incidence of hepatocellular carcinoma in Japan. Cancer Res 1987;47:4967–4972.
4 Kudo M: Early detection and curative treatment of early-stage hepatocellular carcinoma. Clin Gastroenterol Hepatol 2005; 3:S144–S148.
5 Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S: Hepatic artery embolization in 120 patients with unresectable hepatoma. Radiology 1983;148:397–401.
6 Lin DY, Liaw YF, Lee TY, Lai CM: Hepatic arterial embolization in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. Gastroenterology 1988;94:453–456.
7 Ikeda K, Kumada H, Saitoh S, Arase Y, Chayama K: Effect of repeated transcatheter arterial embolization on the survival time in patients with hepatocellular carcinoma. Cancer 1991;68:2150–2154.
8 Llovet JM, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J: Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002;359:1734–1739.
9 Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J: Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002;35:1164–1171.
10 Camma C, Schepis F, Orlando A, Albanese M, Shahied L, Trevisani F, Andreone P, Craxì A, Cottone M: Transarterial chemoembolization for unresectable hepatocellular carcinoma: Meta-analysis of randomized controlled trials. Radiology 2002;224:47–54.
11 Ikeda M, Maeda S, Shibata J, Muta R, Ashihara H, Tanaka M, Fujiyama S, Tomita K: Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. Oncology 2004; 66:24–31.
12 Takayasu K, Arii S, Ikai I, Omata M, Okita K, Ichida T, Matsuyama Y, Nakanuma Y, Kojiro M, Makuuchi M, Yamaoka Y; Liver Cancer Study Group of Japan. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. Gastroenterology 2006;131: 461–469.
13 Marelli L, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, Tibballs J, Meyer T, Patch DW, Burroughs AK: Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6–25.
14 Kamada K, Nakanishi T, Kitamoto M, Aikata H, Kawakami Y, Ito K, Asahara T, Kajiyama G: Long-term prognosis of patients undergoing transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: comparison of cisplatin lipiodol suspension and doxorubicin hydrochloride emulsion. J Vasc Interv Radiol 2001;12:847–854.
15 Ikeda M, Maeda S, Ashihara H, Nagahama H, Tanaka M, Sasaki Y: Transcatheter arterial infusion chemotherapy with cisplatin-lipiodol suspension in patients with hepatocellular carcinoma. J Gastroenterol 2010;45: 60–67.
16 Maeda M, Uchida NA, Sasaki T: Liposoluble platinum (II) complexes with antitumor activity. Jpn J cancer Res 1986;77:523–525.
17 Kishimoto S, Ohtani A, Fukuda H, Fukushima S, Takeuchi Y: Relation between intracellular accumulation and cytotoxic activity of cis[((1R,2R)-1,2-cyclohexanediamine-N,N′)bis(myristato)]platinum(II) suspended in lipiodol. Biol Pharm Bull 2003;26:683–686.
18 Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T: Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of human hepatoma cells orthotopically implanted in nude rats. Cancer Sci 2009;100:189–194.
19 Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T, Chiba N, Nishikaku F: Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of tumors implanted in rat livers by inducing platinum-DNA adducts to form and massive apoptosis. Cancer Chemother Pharmacol 2009;64:473–483.

20 Fujiyama S, Shibata J, Maeda S, Noumaru S, Sato K, Tomita K: Phase I clinical study of a novel lipophilic platinum complex (SM-11355) in patients with hepatocellular carcinoma refractory to cisplatin/lipiodol. British Journal of Cancer 2003;89:1614–1619.
21 Okusaka T, Okada S, Nakanishi T, Fujiyama S, Kubo Y: Phase II trial of intra-arterial chemotherapy using a novel lipophilic platinum derivative (SM-11355) in patients with hepatocellular carcinoma. Investigastional New Drugs 2004;22:169–176.
22 Lencioni R, Llovet JM: Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52–60.
23 Kawamura Y, Ikeda K, Hirakawa M, Hosaka T, Kobayashi M, Saitoh S, Yatsuji H, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Arase Y, Kumada H: Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin. Hepatology Research 2009;39:346–354.
24 Maeda N, Osuga K, Higashihara H, Mikami K, Nakazawa T, Nakamura H, Tomiyama N: Transarterial chemoembolization with cisplatin as second-line treatment for hepatocellular carcinoma unresponsive to chemoembolization with epirubicin-lipiodol emulsion. Cardiovasc Intervent Radiol 2011, Epub ahead of print.
25 Kishimoto S, Miyazawa K, Terakawa Y, Ashikari H, Ohtani A, Fukushima S, Takeuchi Y: Cytotoxicity of cis-[(1R,2R)-1,2-cyclohexanediamine-N,N′)bis(myristato)]-platinum (II) suspended in Lipiodol in a newly established cisplatin-resistant rat hepatoma cell line. Jpn J Cancer Res 2000;91:1326–1332.
26 Shim JH, Kim KM, Lee YJ, Ko GY, Yoon HK, Sung KB, Park KM, Lee SG, Lim YS, Lee HC, Chung YH, Lee YS, Suh DJ: Complete necrosis after transarterial chemoembolization could predict prolonged survival in patients with recurrent intrahepatic hepatocellular carcinoma after curative resection. Ann Surg Oncol 2010;17:869–877.

Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

CLINICAL STUDIES

Stage progression of small hepatocellular carcinoma after radical therapy: comparisons of radiofrequency ablation and surgery using the Markov model

Kenji Ikeda[1,2], Masahiro Kobayashi[1,2], Yusuke Kawamura[1,2], Norihiro Imai[1,2], Yuya Seko[1,2], Miharu Hirakawa[1,2], Tetsuya Hosaka[1,2], Hitomi Sezaki[1,2], Norio Akuta[1,2], Satoshi Saitoh[1,2], Fumitaka Suzuki[1,2], Yoshiyuki Suzuki[1,2], Yasuji Arase[1,2] and Hiromitsu Kumada[1,2]

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

Keywords
hepatocellular carcinoma – Markov model – radiofrequency ablation – recurrence – surgery – survival

Correspondence
Kenji Ikeda, Department of Hepatology, Toranomon Hospital, Toranomon 2-2-2 Minato-ku, Tokyo, 105-8470, Japan
Tel: +81 3 3588 1111
Fax: +81 3 3582 7068
e-mail: ikedakenji@tora.email.ne.jp

Received 8 August 2010
Accepted 21 January 2011

DOI:10.1111/j.1478-3231.2011.02480.x

Abstract

Background: Stage progression of 374 small hepatocellular carcinomas (HCC) was retrospectively analysed. *Patients and methods:* During 8 years, 236 patients with the early stage of HCC received radiofrequency ablation (RFA), and 138 underwent surgery as an initial therapy. More patients of young age and with better liver function tended to undergo surgical treatment. Based on 1892 patient-year data, the Markov model analysed the stepwise progression of early stage (multiple up to three nodules, 3 cm or less each) to intermediate stage (four nodules or more, or larger than 3 cm), to advanced stage (portal invasion, extrahepatic metastasis or Child–Pugh C) and to death. *Results:* The recurrence rates after RFA and surgery were 53.3 and 40.6% in the third year. The annual progression rates from the early stage to the intermediate stage, advanced stage and death were 5.40, 1.63 and 1.73% in the RFA group and 3.90, 1.87 and 0.62% in the surgery group respectively. The progression rate from the early to the intermediate stage was significantly lower (2.34% annually) in the younger patient group (< 60 years) than that in the older group (≥60 years, 5.70%, $P = 0.0053$). In contrast, the progression rate from the intermediate to the advanced stage was significantly higher in the younger patient group (< 60 years, 37.50% annually) than that in the older groups (60–69 years, 30.30%, 70 years or older 22.09%, $P = 0.0011$). Multivariate hazard analysis showed that initial treatment did not significantly affect the stage progression rate (hazard ratio of RFA 1.09, $P = 0.70$) and the survival rate (hazard ratio of RFA 1.09, $P = 0.73$). *Conclusion:* Although the recurrence rate was slightly higher in the RFA group, additional ablation procedures could control the progression of HCC, with a rate comparable to the surgical group.

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in the world today (1). Although routine imaging check-ups can often detect a small HCC at an early stage in high-risk patients with chronic hepatitis and cirrhosis, surgical resection is performed only in 20% or less of the cases because of the association of cirrhosis and tumour multiplicity (2–5). In the management of patients with HCC associated with cirrhosis, treatment repetition is common and inevitable for newly appearing multicentric tumours (6–8), and many practitioners hope each ablation procedure to be less invasive, less expensive and with a shorter hospitalization period.

Radiofrequency ablation (RFA) is currently considered the most effective percutaneous therapy for small HCCs, and certain centres now use it as a first-line treatment option (9), even in patients suitable for surgery. Indeed, RFA is sometimes considered as a less radical therapy compared with surgical resection because of the relatively high rate of local recurrence (10–12), but most of the local tumour progression can be completely treated through an additional RFA procedure. Surgical therapy, on the other hand, is an invasive mode of treatment with a higher cost (10), but achieves a lower recurrence rate. Only a few studies have evaluated the long-term outcome and prognostic factors of percutaneous RFA in comparison with surgical therapy (12–14).

When a recurrent tumour shows relatively advanced characteristics at an intermediate stage with a large tumour or multiples of four or more, transcatheter arterial chemoembolization (TACE) is preferred to surgical therapy or local ablation (15). We introduced the

Markov model to simulate the steps of stage progression of patients with small HCC under an intensive medical intervention. Here, we retrospectively evaluated the progression of HCC and the long-term prognosis of patients who had undergone RFA or surgical resection as the initial therapy for small HCCs, and assessed the prognostic factors of those patients.

The purposes of this study were, therefore, (i) to compare the recurrence rates, progression of tumour stage and survival rates between those patients who received percutaneous RFA and those who underwent surgery and (ii) to elucidate the significance of the selection of initial therapy for small HCCs from the viewpoints of stage progression and prognosis.

Patients and methods

Patients

A total of 468 patients were diagnosed as having a small HCC 3 cm or less in diameter, from March 1999 to April 2006, at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these 468 patients, 236 patients (50.4%) underwent percutaneous RFA therapy as a curative mode of treatment and the remaining 138 patients (29.5%) received surgical resection, 52 had TACE and the remaining 42 patients were treated with ethanol injection, microwave coagulation or other palliative methods of treatment.

A total of 374 consecutive patients with a small HCC, who underwent either RFA or surgery, were analysed in this study. None had been treated previously for HCC, and all had single or multinodular (up to three) HCCs 3 cm or less in diameter each, absence of portal venous thrombosis and known extrahepatic metastases, and Child–Pugh class A or B liver function.

The patients included 246 men and 128 women, and ranging in age from 29 to 87 years, with a median age of 65 years. The demography, laboratory data and features of cancer were compared between the two therapy groups (Table 1). Patients' age was lower in the surgery group by 4.5 years. The rate of HBV-positive disease was significantly higher in the surgery group, and liver function tests were also significantly better in the surgery group.

Hepatocellular carcinoma

Patients were required to have HCC with a definitive diagnosis by either typical hypervascular radiological features or histology through needle biopsy. Tumours had to be measurable by ultrasonography (US), computerized tomography (CT) and digital subtraction angiography. In order to elucidate the detailed characteristics of the HCC, CT during arterial portography and CT hepatic arteriography were performed in all the patients. Among 374 patients, HCC was confirmed by a resected specimen in 138 patients, by typical hypervascular characteristics on at least two modalities of imagings in 219 and by a fine-needle biopsy in 17.

Most patients (82.2%, 309 of 376) had a single tumour, and the median tumour diameter was 19 mm, ranging from 5 to 30 mm. The characteristics of the tumour in the subgroup of RFA and surgery are given in Table 1. The median size of the largest tumour was 18 mm in the RFA group and 20 mm in the surgery group ($P < 0.001$).

Table 1. Clinical features of the patients with small liver cancer

Initial therapy	Radiofrequency ablation ($n = 236$)	Hepatic resection ($n = 138$)	P
Demography			
Men:women	145:91 (38.6%)	101:37 (26.8%)	0.0021
Age (median, range)	67 (38–87)	62.5 (29–80)	< 0.001
Decompensated cirrhosis	16 (6.8%)	5 (3.6%)	0.20
HBsAg	24 (10.2%)	46 (33.3%)	< 0.001
Antibody to HCV	197 (83.5%)	84 (60.9%)	< 0.001
History of alcohol intake > 500 kg	21 (8.9%)	16 (11.6%)	0.40
Observation period (year)	3.7 (0.1–9.9)	4.5 (0.1–10.0)	0.041
Laboratory data (median, range)			
ICG R15 (%)*	28 (1–100)	21 (3–68)	< 0.001
Bilirubin (mg/dl)	1.0 (0.2–3.1)	1.0 (0.3–2.2)	0.003
Albumin (g/dl)	3.5 (2.2–4.2)	3.6 (2.8–4.4)	< 0.001
Aspartic transaminase (IU)	55 (17–311)	45 (17–386)	0.006
Platelet count ($\times 10^3/mm^3$)	97 (19–253)	127 (38–272)	< 0.001
Prothrombin time (%)	84 (31–125)	91 (59–115)	0.001
Liver cancer			
Median size (mm)	18 (8–30)	20 (5–30)	< 0.001
Single/multiple	195/41 (17.4%)	114/24 (17.4%) 1.00	
α-fetoprotein (ng/ml)	19 (1–2080)	17 (1–2610)	0.84
PIVKA-II (AU/L)†	17 (7–1470)	20 (9–1650)	0.008

*ICG R15, indocyanine green retention rate at 15 min.

†PIVKA-II, protein induced by vitamin K antagonist-II.

HCV, hepatitis C virus.

Treatment for initial hepatocellular carcinoma

Physicians and surgeons usually held a conference about the choice of therapy in individual patients. RFA or surgical therapy were selected considering the site, size and number of tumours, liver function and the patient's general status. Both RFA and the surgical procedure were explained fully to all the patients, and informed consent was obtained. Despite the feasibility and availability of surgery, some patients voluntarily preferred RFA under informed consent.

Radiofrequency ablation therapy was performed percutaneously under US or CT guidance, under conscious sedation with fentanyl citrate (0.1–0.2 mg, Fentanyl; Daiichi-Sankyo, Tokyo, Japan) or pethidine hydrochloride (35–70 mg, Opystan; Tanabe-Mitsubishi, Osaka, Japan) administered intravenously. RFA was performed using three kinds of apparatus: a radiofrequency interstitial tumour ablation system (RITA, RITA Medical Systems Inc., Mountain View, CA, USA), a cool-tip system (Tyco Healthcare Group LP, Burlington, VT, USA) and a radiofrequency tumour coagulation system (RTC system, Boston-Scientific Japan Co., Tokyo, Japan).

Hepatic resection was performed under intra-operative US monitoring and guidance. In the cases of small and superficial HCC, arterial and portal vein clumping at the hepatic hilum was not usually performed for maintenance of liver perfusion.

Evaluation of the therapeutic effect

To evaluate the efficacy of local ablation, a dynamic CT was performed at 2–7 days after treatment with RFA, and 8–21 days after surgery. CT findings were confirmed by consensus among at least two hepatologists and radiologists. On dynamic CT images, the non-enhancing area was measured as the ablated area. When the diameter of the non-enhancing area was greater than that of the ablated nodule, RFA was considered to have had a complete effect, and the treatment was terminated. When patients had a smaller ablated area or a positively enhanced area in the original tumour based on CT results after RFA therapy, they usually underwent an additional RFA within several days.

Follow-up of patients

Physicians observed the patients every 4–8 weeks after the first treatment. Liver function test, haematology and tumour markers were measured every 1–2 months. After the completion of eradication of HCC, recurrence was surveyed with CT or magnetic resonance imagings (MRI) every 3–4 months. Serum α-fetoprotein (AFP) and des-γ-carboxy prothrombin were also measured every 1–2 months to detect recurrence as early as possible.

During a median observation period of 4.2 years, four patients (1.1%) were lost to follow-up.

Statistical analysis and the Markov model

Standard statistical measures and procedures were used. The χ^2-test, Fisher's exact test and Mann–Whitney's *U*-test were used to analyse the differences in the demography, laboratory findings and tumour characteristics between the RFA group and the surgery group. The recurrence rate, progression rates and survival rate were analysed using the Kaplan–Meier technique (16) with the log-rank test. Cox's proportional hazard analysis was performed to evaluate independent predictors of the outcomes.

The Markov model (17) was adopted to analyse the transition rates from the early stage to the intermediate stage of HCC, intermediate to advanced stage and advanced stage to death. A homologous Markov chain consisted of four states (Fig. 1). These were the early stage of HCC (solitary or multiple up to three nodules, 3 cm or less each), the intermediate stage (four nodules or more, or larger than 3 cm), the advanced stage (portal vein

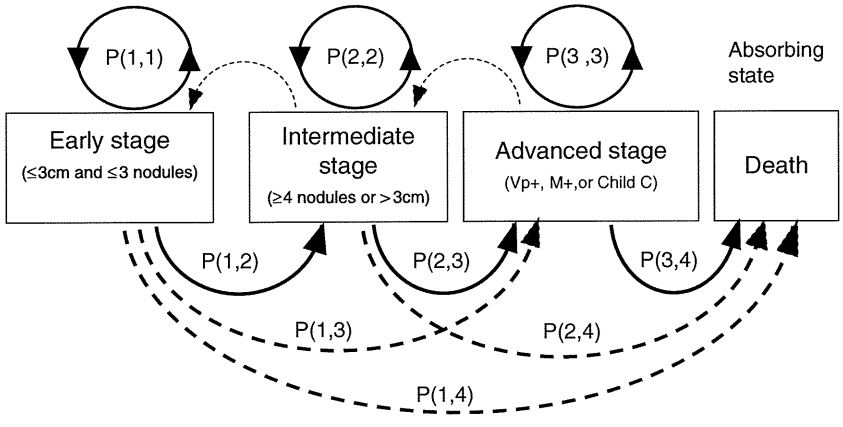

Fig. 1. The Markov state transition diagram of hepatocellular carcinoma. Four states were defined: early stage (solitary or multiple up to three nodules, 3 cm or less in diameter each), intermediate stage (multiple nodules of four or more, or 3.1 cm or more), advanced stage (main portal vein invasion, extrahepatic metastasis or Child–Pugh C) and death. Of these, death was the absorbing state from which no transitions to the other states occurred. The transition in one cycle (1 year) is shown. Arrows connecting two different states indicate the transitions observed.

Liver International (2011)