of severe associated diseases in the elderly, or patients rejecting treatment, result in the selection of only TACE, even in candidates for local therapies. TACE is known to be effective for inoperable HCC [9, 10]; however, little information has been reported about TACE for early-stage HCC, and it is hard to decide whether to perform additional treatment following TACE in these difficult conditions.

The objective of this retrospective cohort study was to determine whether only TACE for HCC with 3 tumors or fewer of up to 3 cm could control HCC; to achieve this objective we examined the recurrence rate and the risk factors for local and intrahepatic distant recurrences in such cases.

Subjects and methods

Patients

Patients were enrolled from among 1,560 newly diagnosed HCC patients who were admitted to Okayama University Hospital between 2002 and 2010. Inclusion criteria were as follows: (1) no previous treatment of HCC; (2) 3 or fewer nodules of up to 3 cm (early-stage HCC); (3) at least one nodule treated only by TACE as an initial treatment; (4) no planned local treatment such as RFA, PEIT, or operation performed within 30 days of TACE; (5) no vascular invasion; and (6) no extrahepatic metastasis. Exclusion criteria were: (1) complete cure of all nodules by RFA, PEIT, or operation and (2) follow-up period less than 1 year. Finally, 43 patients were selected and enrolled in this study. Of these patients, 6 died in the follow-up period. Five of these patients died due to liver disease and 1 died of heart failure. Informed consent for the use of their clinical data was obtained from all patients in this study. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by the ethics committee of the institute (approval # 458).

Diagnosis

HCC was diagnosed in accordance with AASLD guidelines. The diagnostic criteria for HCC via imaging were based on hyperattenuation in the the arterial phase and hypoattenuation in the portal phase on dynamic computed tomography (CT) or magnetic resonance imaging (MRI), and tumor stain on angiography. Ultrasonography (US) with perfluorobutane (Sonazoid; Daiichi Sankyo, Tokyo, Japan) and/or gadolinium ethoxybenzyl MRI (Gd-EOB-MRI) was performed in 30 (69.8%) patients. When we could not diagnose HCC by imaging only, fine-needle biopsy using abdominal US was performed as histological proof (3 cases). Recurrence of HCC was diagnosed in the same way as at the initial diagnosis.

Treatment

TACE was performed using the Seldinger technique followed by arterial embolization. After introducing a 4-Fr catheter through the femoral artery, hepatic arteriography and superior mesenteric arterial portovenography were performed to evaluate portal flow and the location of the tumors. When portal flow was sufficient, a 1.8-Fr or a 2.0-Fr microcatheter was placed in the feeding arteries at the closest point to the HCC. An emulsion consisting of 30–60 mg of epirubicin (Kyowa-Hakko, Tokyo, Japan) and 2–6 mL of iodized oil (Lipiodol Ultrafluid; Terumo, Tokyo, Japan) was injected into the artery supplying blood to the tumor, followed by embolization with 1-mm gelatin sponge particles (Gelfoam; Nihonkayaku, Tokyo, Japan). After embolization, CT angiography was performed to determine the extent of vascular occlusion and to assess blood flow in other arterial vessels. Patients were observed carefully, and analgesia (pentazocine; Astellas, Tokyo, Japan) was administered if necessary.

Lipiodol uptake was categorized as either homogeneous or heterogeneous by plain CT after TACE. Homogeneous uptake was defined as complete uptake of Lipiodol in the tumor without any defect, and the CT value in these cases was more than 200 Hounsfield Units. Uptake that did not meet the definition of homogeneous was considered as heterogeneous. Two experienced investigators (K. N. and H. O.) reviewed the CT images and evaluated the Lipiodol uptake. If the two investigators had different diagnoses, they discussed the difference and reached agreement.

Follow up

Patients were assessed every 1–3 months by serum biochemistry, dynamic CT, dynamic MRI, or US. Local recurrence was defined as the appearance of a viable tumor in contact with or inside the treated area. Intrahepatic distant recurrence was defined as the occurrence of a new HCC in the liver that did not meet the definition of local recurrence. When recurrence was detected, TACE, RFA, PEIT, or surgical resection was performed depending on the condition of the recurrence and the background liver function. Patients were followed until loss to follow up, death, or 31 January 2011.

Statistical analysis

The following 12 parameters were used for analyzing the risk factors for recurrence: age, sex, viral markers (hepatitis B virus surface antigen and hepatitis C virus antibody), alcohol intake, liver function, size of tumors, number of tumors, location of tumors (within 10 mm of the surface of the liver or not), serum tumor markers [alpha-fetoprotein

(AFP) and des-gamma-carboxy prothrombin (DCP)], and the status of Lipiodol uptake.

Recurrence rates were estimated using the Kaplan–Meier method and differences between groups were compared using the log-rank test. The Cox proportional hazard model was used to analyze the predisposing factors for recurrence. All statistical analyses were performed using JMP version 9 (JMP Japan, Tokyo, Japan). All reported P values are 2-sided, with $P < 0.05$ considered statistically significant.

Results

Patient background

A total of 43 patients met the criteria of this study. The total number of HCC nodules was 54. There were 27 males (63%) and 16 females (37%) aged 50–85 years (mean: 71 years), and 34 patients (79%) were infected with hepatitis C virus and 6 (14%) with hepatitis B virus. Twenty-three patients (53%) were habitual drinkers. Thirty-four patients (79%) had a single tumor in the initial treatment. Thirty-six patients (84%) had recurrence (Table 1). Eleven (26%), 23 (53%), and 9 (21%) patients were treated with only TACE because of poor liver functional reserve, difficult location for RFA, and old age, respectively. Of the 11 patients who had poor liver function, 1 patient was Child C and 10 patients were Child B. Although the Japanese guidelines for treatment of HCC recommend RFA or operation for the treatment of small HCC in patients with Child A/B stage, these 10 Child B patients had poor conditions such as uncontrollable ascites or low albumin, so that it was quite difficult to perform RFA or operation. In the 23 patients who had a difficult location for RFA, the nodules were located beside the portal vein in 5 patients, beside the digestive tract in 4 patients, beside the gallbladder in 4 patients, beside the inferior vena cava in 3 patients, beside the collateral veins on the surface of liver in 3 patients, beside the bile duct in 3 patients, and beside the heart in 1 patient.

Table 1 Clinical background of 43 patients

Demographic variables	
Sex (male) (%)	27 (63)
Age (years)	71 (50–85)
Etiology (%)	
HCV	34 (79)
HBV	6 (14)
HCV + HBC	2 (4.7)
Alcohol	23 (53)
Unknown	1 (2)
Tumor size (mm)	18 (6–30)
Number of tumors (%)	
1	34 (79)
2	7 (16)
3	2 (5)
Location (distance from surface of the liver)	
≤10 mm	28 (65)
Recurrence (%)	
Local	22 (52)
Distant	7 (16)
Local + distant	7 (16)
No recurrence	7 (16)
Lipiodol uptake (%)	
Homogeneous	23 (53)
Heterogeneous	20 (47)
AFP (ng/mL)	124.1 (1.6–1,539)
DCP (mAU/mL)	157.7 (12–3,450)
Total bilirubin (mg/dL)	1.09 (0.37–3.13)
Albumin (g/dL)	3.47 (2.4–4.42)
ALT (IU/L)	43.5 (13–147)
AST (IU/L)	55.2 (23–119)
Child-Pugh score	
(A/B/C)	32/10/1

Numbers in the Tables are shown as medians (ranges) unless otherwise noted

HCV positive for hepatitis C virus antibody, *HBV* positive for hepatitis B virus antigen, *AFP* alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

Recurrence rate

Local recurrence and intrahepatic distant recurrence were observed in 29 patients and 14 patients, respectively, and 7 of these patients showed both local and intrahepatic distant recurrences at the same time.

The total recurrence rates at 3 months, 6 months, and 1 year were 20.9, 35.3, and 68.5%, respectively. The local recurrence rates and intrahepatic distant recurrence rates at 3 months, 6 months, and 1 year were 18.6, 33.4, and 61.8%, and 2.8, 2.8, and 10.2%, respectively (Fig. 1). Thirteen patients had local recurrences within 180 days. Eleven patients received RFA and and 11 patients received TACE for the therapy of local recurrence. Eight patients received RFA, 5 patients received TACE, and 1 patient received PEIT for the therapy of intrahepatic distant recurrence. No difference in the therapies was observed between the local recurrence group and the distant recurrence group.

Factors related to local recurrence

Of the 12 factors analyzed, heterogeneous Lipiodol uptake (risk ratio 3.19; 95% confidence interval 1.41–7.90; $P = 0.004$) and high serum DCP (2.37; 1.06–5.83; 0.034)

were correlated with local recurrence by univariate analysis. The factor of location was not associated with local recurrence on univariate analysis. Factors exhibiting significance in the univariate analysis and reported to be correlated with recurrence; namely, DCP, age, number of HCCs, liver function, size of HCC, extent of Lipiodol uptake, HCV, and location of HCC, were further analyzed using the Cox multivariate proportional hazard model [11–20]. On multivariate analysis, only heterogeneous Lipiodol uptake (risk ratio 3.38; 95% confidence interval 1.14–10.60; $P = 0.027$) and high serum DCP (2.58; 1.03–7.14; 0.042) were significantly correlated with local recurrence (Table 2).

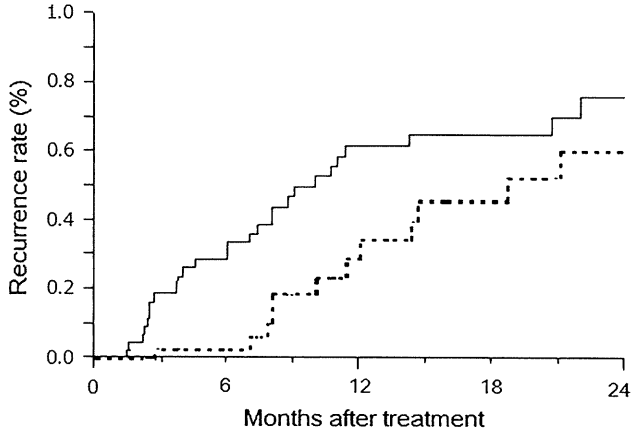

Fig. 1 Local and distant recurrences after transcatheter arterial chemoembolization. The local recurrence rates (*solid line*) and intrahepatic distant recurrence rates (*dotted line*) at 3 months, 6 months, and 1 year were 18.6, 33.4, and 61.8%, and 2.8, 2.8, and 10.2%, respectively

Factors related to intrahepatic distant recurrence

In the same way as for factors related to local recurrence, the 12 factors were analyzed, and the factor of multiple tumors (3.98; 1.02–13.50; 0.047) was correlated with intrahepatic distant recurrence by univariate analysis. On multivariate analysis with the factors serum DCP, age, tumor number, Child-Pugh score, tumor size, Lipiodol uptake, HCV, and tumor location, the presence of multiple tumors (10.64; 1.76–93.75; 0.010) was significantly correlated with recurrence (Table 3).

Discussion

While RFA is considered as the first choice for the treatment of early-stage HCC, TACE could be another option when RFA is unsuccessful or unfeasible. Livraghi [21] reported that RFA was not feasible in 6.0% of patients because of a high-risk tumor location or poor detection on ultrasonography (US). However, it is hard to decide whether to treat these patients with additional locoregional therapy because there have been few reported studies examining the outcomes of these patients in detail.

In the present study, we examined the clinical courses of 43 patients treated with palliative TACE for HCC with 3 tumors or fewer of up to 3 cm. More than 80% of the patients (36/43, 84%) had recurrence and most of them (29/36, 80%) exhibited local recurrence. The recurrence rate was higher than that for local ablation. The recurrence rates of the patients treated with RFA or operation at 3 months,

Table 2 Risk factors for local recurrence after TACE

Variables	Univariate		Multivariate	
	Risk ratio (95% CI)	*P* value	Risk ratio (95% CI)	*P* value
AFP (>10 ng/mL)	1.46 (0.69–3.23)	0.328		
DCP (>28 mAU/mL)	2.37 (1.06–5.83)	0.034*	2.58 (1.03–7.14)	0.042*
Age (≥75 years)	1.04 (0.50–2.24)	0.900	1.16 (0.42–3.11)	0.769
Tumor number (≥2)	1.72 (0.66–4.04)	0.249	1.18 (0.37–3.26)	0.759
Child-Pugh score (≥7)	1.25 (0.51–3.74)	0.650	1.65 (0.60–5.43)	0.347
Tumor size (≥20 mm)	1.58 (0.74–3.37)	0.230	1.66 (0.70–3.94)	0.245
Lipiodol uptake (heterogeneous)	3.19 (1.41–7.90)	0.004*	3.38 (1.14–10.60)	0.027*
Sex	1.01 (0.48–2.23)	0.976		
HCV	2.07 (0.74–4.99)	0.153	2.09 (0.55–7.65)	0.271
HBV	0.91 (0.32–3.85)	0.882		
Alcohol	0.99 (0.47–2.14)	0.975		
Location (within 10 mm from the liver surface)	0.66 (0.31–1.47)	0.302	0.80 (0.26–2.37)	0.687

TACE transcatheter arterial chemoembolization, *95% CI* 95% confidence interval. Other abbreviations are the same as those listed in Table 1 footnote

* Significant value

Table 3 Risk factors for distant recurrence after TACE

Variables	Univariate		Multivariate	
	Risk ratio (95% CI)	*P* value	Risk ratio (95% CI)	*P* value
AFP (>10 ng/mL)	1.22 (0.40–3.85)	0.721		
DCP (>28 mAU/mL)	1.19 (0.37–3.83)	0.765	1.73 (0.44–7.41)	0.425
Age (≥75 years)	2.07 (0.67–7.67)	0.211	4.30 (0.88–32.60)	0.073
Tumor number (≥2)	3.98 (1.02–13.50)	0.047*	10.64 (1.76–93.75)	0.010*
Child-Pugh score (≥7)	0.70 (0.21–3.21)	0.611	0.71 (0.16–4.21)	0.688
Tumor size (≥20 mm)	1.09 (0.33–3.34)	0.879	1.50 (0.33–7.08)	0.594
Lipiodol uptake (heterogeneous)	1.99 (0.61–6.53)	0.250	1.32 (0.08–15.76)	0.834
Sex	2.30 (0.76–7.66)	0.138		
HCV	0.45 (0.11–3.04)	0.360	0.64 (0.08–7.19)	0.688
HBV	0.49 (0.12–3.27)	0.405		
Alcohol	0.60 (0.19–1.83)	0.365		
Location (within 10 mm from the liver surface)	0.54 (0.17–1.84)	0.304	0.78 (0.04–14.50)	0.864

Abbreviations are the same as those listed in Table 2 footnote

* Significant value

6 months, and 1 year at our institution during the same period were 2.8, 9.6, and 24.5%, respectively.

Heterogeneous Lipiodol uptake and high serum DCP were significantly correlated with local recurrence, whereas the presence of multiple tumors was significantly correlated with intrahepatic distant recurrence.

The risk factors for recurrence after TACE in patients with HCC have been described in several reports. These include the extent of Lipiodol uptake, location of HCC, size of HCC, tumor markers, viral markers, number of HCCs, age, and liver function [11–20]. However, most of these studies were on TACE for advanced HCC. In the present study, we examined the risk factors for early-stage HCC and revealed that only the extent of Lipiodol uptake and the serum DCP level were correlated with local recurrence, which is the most common type of recurrence after TACE. In early-stage HCCs, most of the tumors might be highly differentiated and less invasive, so that the tumors can be controlled merely by complete obstruction of their blood supply. Thus, only high DCP would be an additional risk factor, because HCC with high DCP showed a poorer differentiation grade than HCC with low DCP [22]. Our results suggest that it is better to treat early-stage HCC showing high serum DCP as well as incomplete Lipiodol uptake not only with TACE but also with additional locoregional treatment if possible.

Of note, we found that intrahepatic distant recurrence was observed in patients with multiple tumors, indicating that some of these tumors were intrahepatic metastases and that undetectable small HCCs might have already existed before the TACE was performed.

A meta-analysis showed that chemoembolization could improve the survival of well-selected patients with unresectable HCC [23]; in addition, there is a report that chemoembolization had an effect on HCC even in patients with poor liver function [24]. However, the efficacy of TACE for treating HCC at an early stage has not been well elucidated. Although we could not show a survival benefit of TACE in early-stage HCC, to the best of our knowledge, this is the first report about the outcome of TACE for HCC with 3 tumors or fewer of up to 3 cm.

While new technologies such as artificial pleural effusion and ascites, or real-time virtual sonography (RVS), have increased the number of patients eligible for RFA, the age of HCC patients is gradually increasing, so that, when considering the treatment guideline algorithm for HCC, more patients would be excluded from such treatment owing to the presence of complications and poor performance status, among other factors. Eventually, we may not be able to avoid the selection of palliative TACE instead of RFA and operation. This study helped us to decide whether additional treatment should be considered in patients with difficult conditions according to the treatment algorithms .

In conclusion, palliative TACE could be effective for HCC with 3 tumors or fewer of up to 3 cm. Lipiodol uptake, serum DCP, and the number of tumors (≥2) are the most important risk factors for recurrence in these HCCs treated with palliative TACE. Patients showing heterogeneous Lipiodol uptake after TACE should not be left untreated if at all possible.

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Conflict of interest None.

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HEPATOLOGY

Predicting the treatment effect of sorafenib using serum angiogenesis markers in patients with hepatocellular carcinoma

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Abstract

Background and Aim: Sorafenib, the first agent demonstrated to have efficacy to improve the survival of patients with advanced hepatocellular carcinoma (HCC), is an active multikinase inhibitor affecting angiogenesis and tumor proliferation. We analyzed cytokines related to angiogenesis or cell proliferation, and tried to determine their utility as biomarkers of sorafenib treatment effect for HCC.

Methods: Nine serum cytokines (angiopoietin-2 [Ang-2], follistatin, granulocyte colony-stimulating factor [G-CSF], hepatocyte growth factor [HGF], interleukin-8 [IL-8], leptin, platelet-derived growth factor-BB, platelet endothelial cell adhesion molecule-1, and vascular endothelial growth factor) were measured in 30 HCC patients treated with sorafenib, and the effects of treatment were compared using modified Response Evaluation Criteria in Solid Tumors.

Results: All but IL-8 were significantly higher at baseline in patients with progressive disease. Progression-free survival was significantly shorter in patients with high levels of Ang-2, G-CSF, HGF, and leptin, and the hazard ratios were 2.51, 6.89, 2.55, and 4.14, respectively. As the number of cytokines at a high level increased, the treatment response deteriorated. Disease progression was seen in three of 12 (25.0%) patients with zero to two high biomarkers, two of six (33.3%) patients with 3–5 high biomarkers, and 10 of 12 (83.3%) patients with six to eight high biomarkers ($P = 0.008$). The prognosis of all patients with eight high biomarkers was progressive disease.

Conclusion: High levels of serum cytokines at baseline were correlated with poor effects of sorafenib treatment in patients with HCC.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, with more than half a million new cases each year, and it is the third highest cause of cancer-related death globally, behind only lung and stomach cancers.[1,2] Although potentially curative treatments are available for patients diagnosed at early stages, such as radiofrequency ablation, resection, and liver transplantation, disease that is diagnosed at an advanced stage or progresses after locoregional therapy has a dismal prognosis, owing to the underlying liver disease and lack of effective treatment options.[3–5] However, the recent development of molecular-targeted therapies is changing the landscape. The efficacy and safety of sorafenib in advanced HCC patients were assessed in the Sorafenib HCC Assessment Randomized Protocol study, which demonstrated that monotherapy with sorafenib prolonged overall survival (OS) and delayed the time to progression (TTP) in patients with advanced HCC[6]. The efficacy in prolonging OS and TTP in patients from the Asia–Pacific region was confirmed in a phase III randomized, double-blind, placebo-controlled study (Asia–Pacific study).[7]

Sorafenib is an orally-active multikinase inhibitor affecting angiogenesis and tumor proliferation. It inhibits the activity of targets, such as raf, the vascular endothelial growth factor (VEGF) receptor, and the platelet-derived growth factor (PDGF) receptor.[8] Some components of these proliferative and pro-angiogenic signaling pathways, or molecules related to other angiogenesis pathways, can be detected in serum or plasma, and might provide sensitive indicators of tumor activity, response to treatment with sorafenib, and drug-related adverse events.

In the present study, we simultaneously analyzed nine cytokines related to angiogenesis or cell proliferation in the serum of HCC patients before sorafenib treatment, and attempted to determine their utility as biomarkers of the treatment effect.

Methods

Patients and diagnosis of HCC

Between July 2009 and October 2010, 30 of 35 patients with advanced HCC who were treated with sorafenib in our institute and affiliated hospitals, and who could be evaluated for treatment response, were enrolled in this study (Table 1). All of the patients were treated by sorafenib as a first-line therapy. The patients enrolled in this study consisted of 24 males and six females, with a median age of 71.5 years. Hepatitis B surface antigen and anti-hepatitis C virus antibody were positive in eight and 18 patients, respectively. In accordance with the American Association for the Study of Liver Disease guidelines, we confirmed the diagnosis of HCC by at least two dynamic imaging modalities.[4] Written, informed consent was obtained from all patients. This study was approved by the ethical committee of the institutes (approval no. 850).

Treatment regimen, evaluation of response, and adverse events

All patients received continuous oral treatment with sorafenib. Twenty patients received 400 mg twice daily, and 10 patients received 200 mg twice daily. The reasons why some patients received a reduced dose of sorafenib were for due to their high age, high Child–Pugh score, high aminotransferases, or low white blood cell count. Patients were followed every month for routine surveillance, including serological and radiological examinations, such as dynamic computed tomography or magnetic resonance imaging. Patients had at least one untreated target lesion that could be measured in one dimension, and patients were evaluated for radiographic response in the primary tumor and metastatic lesions before and 33.6 ± 9.6 (mean ± standard deviation [SD]) days after starting therapy, in accordance with the conventional and modified Response Evaluation Criteria in solid Tumors (RECIST).[9,10] Adverse events were estimated with the use of version 4.0 of the National Cancer Institute's Common Terminology Criteria for Adverse Events.

Measurement of cytokines

Baseline serum of all patients was collected at the time of admission, just before starting the therapy. Serum was also collected at 7.0 ± 1.1 (mean ± SD) days after starting therapy in 21 patients. Serum samples were centrifuged for 10 min at 1000 × *g* prior to the analysis, and the supernatants were used for the assay.

Table 1 Clinicopathological characteristics of the patients with hepatocellular carcinoma

Variables	Total ($n = 30$)	PD ($n = 15$)	Non-PD ($n = 15$)	*P*-value
Age (years)	71.5 (36–84)	73 (36–84)	66 (63–84)	0.950†
Sex (%)				1.000
Male	24 (80.0)	12 (80.0)	12 (80.0)	
Female	6 (20.0)	3 (20.0)	3 (20.0)	
Viral infection (%)				
HBsAg (positive)	8 (26.7)	3 (20.0)	5 (33.3)	0.682
HCVAb (positive)	18 (60.0)	10 (66.7)	8 (53.3)	0.710
ECOG performance status (%)				1.000
0–1	29 (96.7)	14 (93.3)	15 (100)	
2	1 (3.3)	1 (6.7)	0 (0.0)	
Child–Pugh grade (%)				1.000
A	24 (80.0)	12 (80.0)	12 (80.0)	
B	6 (20.0)	3 (20.0)	3 (20.0)	
T category (%)				1.000
T1–T2	23 (76.7)	11 (73.3)	12 (80.0)	
T3–T4	7 (23.3)	4 (26.7)	3 (20.0)	
N category (%)				1.000
N0	19 (63.3)	10 (66.7)	9 (60.0)	
N1	11 (36.7)	5 (33.3)	6 (40.0)	
M category (%)				0.139
M0	13 (43.3)	4 (26.7)	9 (60.0)	
M1	17 (56.7)	11 (73.3)	6 (40.0)	
TNM stage (%)				0.651
II–III	6 (20.0)	2 (13.3)	4 (26.7)	
IV	24 (80.0)	13 (86.7)	11 (73.3)	
Tumor markers				
AFP (ng/mL)	50 (1.3–8 074)	45 (1.3–8 074)	57.5 (2.2–4 840)	0.852†
AFP-L3 (%)	21.6 (0–88.1)	26.7 (0–84.1)	20.4 (0–88.1)	0.867†
DCP (mAU/mL)	284.5 (15–226 930)	688 (15–11 577)	256 (15–226 930)	0.740†

Values are indicated as median (range) unless otherwise noted. †*P*-values from Wilcoxon rank sum test; all other *P*-values are from Fisher's exact test. AFP, α-fetoprotein; AFP-L3, Lens culinaris-reactive AFP; DCP, des-γ-carboxy prothrombin; ECOG, Eastern Cooperative Oncology Group; HBsAg, hepatitis B surface antigen; HCVAb, anti-hepatitis C virus antibody; PD, progressive disease.

Concentrations of nine molecules (angiopoietin-2 [Ang-2], follistatin [FST], granulocyte colony-stimulating factor [G-CSF], hepatocyte growth factor [HGF], interleukin-8 [IL-8], leptin, PDGF-BB, platelet endothelial cell adhesion molecule-1 [PECAM-1/CD31], and VEGF) were quantified using the BioPlex 200 System (Bio-Rad Laboratories, Hercules, CA, USA), following the manufacturer's protocol. Samples were tested in duplicate, and the mean value was used for the analysis. When out-of-range values were included in the analysis, the highest value across all samples was substituted for values above the upper limit of detection. For values below the lower limit of detection, half the lowest value was substituted.

Statistical analysis

Progression-free survival (PFS) and OS were calculated from the first day of therapy. The Wilcoxon rank sum test was used to compare the continuous data, and Fisher's exact test was used to compare categorical data. The survival of the patients was compared by the Kaplan–Meier method, and the differences were evaluated by the log–rank test. Cox's proportional hazards model was used to analyze hazard ratios (HR).

For these exploratory analyses, $P < 0.05$ was considered significant. All statistical analyses were carried out using JMP statistical software, version 8 (SAS Institute, Cary, NC, USA).

Results

Characteristics of patients

Treatment interruptions and dose reductions of sorafenib were allowed for drug-related toxicity in five and eight patients before radiographic evaluation, and in four and seven patients after the evaluation, respectively. There were no patients who needed treatment interruptions or dose reductions before serum collections at 1 week after starting sorafenib. Of the 30 patients, one had a complete response (CR), six had a partial response (PR), eight had a stable disease (SD), and 15 had a progressive disease (PD), an increase of at least 20% in the sum of the diameters of viable target lesions, according to modified RECIST. One patient with a CR and two patients with PR, by modified RECIST, were classified as having a SD by conventional RECIST, but there was no difference of PD classification between conventional and modified versions of RECIST. Patients who had a CR, PR, or SD in classical or modified RECIST were classified into the non-PD group. The distribution of the TNM stage was five, one, and 24 for TNM II, III, and IV respectively. There were no significant differences in demographics (age, sex), baseline clinical characteristics (viral infection, Eastern Cooperative Oncology Group [ECOG] performance status, Child–Pugh class, T category, N category, M category, TNM stage), and serum tumor markers (α-fetoprotein [AFP], Lens culinaris-reactive AFP, AFP-L3, and des-γ-carboxy prothrombin) between patients with PD and non-PD (Table 1).

Cytokine expression and treatment response

The expression of each cytokine at baseline was classified into two groups: high biomarker and low biomarker (the cut-off values were set at the median of each marker), and was compared with other clinical parameters. There were no significant differences in demographics (age, sex) or baseline clinical characteristics (viral infection, ECOG performance status, Child–Pugh class, T category, N category, M category, TNM stage) between patients with high and low biomarkers for all nine markers examined.

Baseline cytokine levels were compared between patients with PD ($n = 15$) and non-PD ($n = 15$) by Wilcoxon rank sum test, and eight of nine biomarkers were significantly higher in patients with PD (Fig. 1). These were Ang-2, FST, G-CSF, HGF, leptin, PDGF-BB, PECAM-1/CD31, and VEGF. PFS was significantly shorter in the group with high biomarkers of Ang-2, G-CSF, HGF, and leptin (Fig. 2). HR of the markers were 2.51 (95% confidence interval [CI]: 1.01–6.57; $P = 0.048$) for Ang-2, 6.89 (95% CI: 2.29–25.9, $P < 0.001$) for G-CSF, 2.55 (95% CI: 1.03–6.61, $P = 0.042$) for HGF, and 4.14 (95% CI: 1.52–13.3, $P = 0.005$) for leptin.

Subsequently, we examined whether simultaneous elevation of these molecules at baseline could be used as a predictive marker of disease progression after sorafenib treatment. For each patient, the number of biomarkers at high levels (above median) was counted and compared to the treatment response (Fig. 3). There was a tendency for the number to be higher with a worse treatment response. The prognosis of all patients with eight high biomarkers was PD. When the patients were classified into groups with zero to two, three to five, and six to eight high biomarkers, disease progression was seen in three of 12 (25.0%) patients with zero to two high biomarkers, two of six (33.3%) patients with three to five high biomarkers, and 10 of 12 (83.3%) patients with six to eight high biomarkers ($P = 0.008$). PFS was significantly different between these three groups ($P = 0.009$) (Fig. 4). HR of the group with six to eight high biomarkers compared to the other groups was 4.65 (95% CI: 1.59–15.5; $P = 0.005$).

We also measured the changes of nine biomarkers from baseline to 1 week after starting the therapy. Fold changes of cytokine levels were compared between patients with PD ($n = 9$) and non-PD ($n = 12$) by Wilcoxon rank sum test, and only the increase of PDGF-BB was significantly higher in patients with non-PD. The median fold change of PD and non-PD patients were 0.9 and 1.5, respectively ($P = 0.011$).

In addition, no correlation was observed between the cytokine levels and OS.

Baseline cytokine expression and adverse events

We also analyzed the correlations between cytokine levels and the known adverse events of sorafenib, such as hand–foot syndrome, hypertension, and diarrhea; however, no relationship was observed.

Discussion

To the best of our knowledge, this is the first report on the simultaneous measurement of cytokines in advanced HCC treated with sorafenib with a comparison of the response evaluated by conventional and modified RECIST. Modified RECIST is a new, proposed set of criteria measuring arterial enhanced lesions for the evaluation of "viable" tumors, such as HCC, especially when applied to molecular-targeted therapies or other therapeutic interventions, rather than to cytotoxic agents.[10] The decision about treatment response was different in three cases between conven-

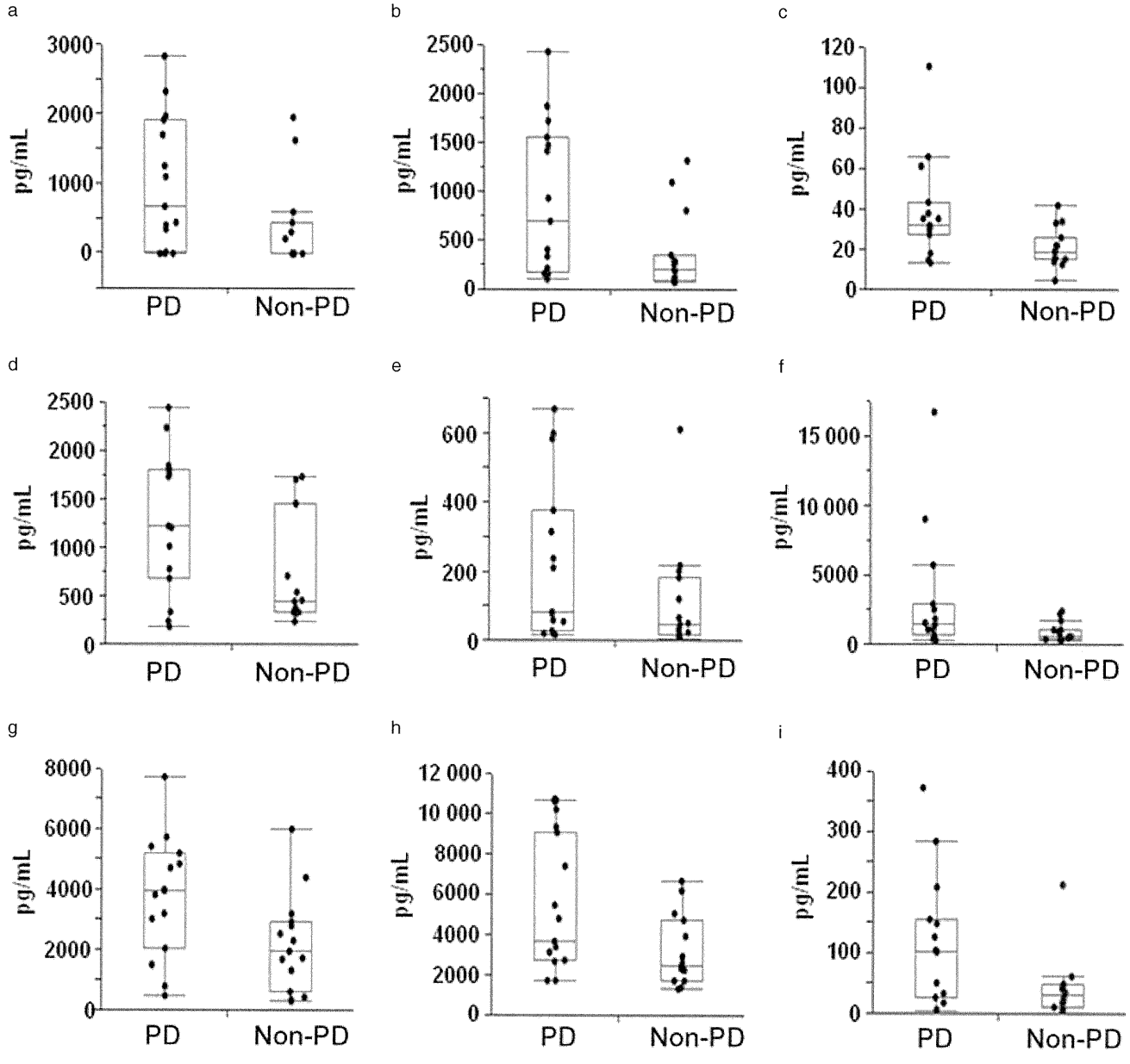

Figure 1 Box plot of baseline biomarker levels in patients with progressive disease (PD) and non-PD. All cytokines but interleukin-8 (IL-8) were higher in PD than in non-PD. (a) angiopoietin-2; (b) follistatin; (c) granulocyte colony-stimulating factor; (d) hepatocyte growth factor; (e) IL-8; (f) leptin; (g) platelet-derived growth factor-BB; (h) platelet endothelial cell adhesion molecule-1; (i) vascular endothelial growth factor. Horizontal bars in the boxes indicate median.

tional and modified RECIST. The differences were observed within the non-PD group and did not influence the disease control rate, which referred to the status of PD or non-PD.

We measured the expressions of Ang-2, FST, G-CSF, HGF, IL-8, leptin, PDGF-BB, PECAM-1/CD31, and VEGF, which are molecules related to angiogenesis. The reason why we chose these molecules is that sorafenib is a representative anti-angiogenic drug, and angiogenesis plays an important role in the aggressive biological behavior of HCC, which is one of the most hypervascular human cancers.[11] Our biomarker study identified eight serum markers at baseline, namely, Ang-2, FST, G-CSF, HGF, leptin, PDGF-BB, PECAM-1/CD31, and VEGF, which were correlated with the initial response, and also four markers, Ang-2, G-CSF, HGF, and leptin, which were correlated with PFS after sorafenib treatment. In addition, a large number of biomarkers at high levels were correlated with a worse response.

A few reports have been published demonstrating relationships between biomarkers and outcomes in anti-angiogenic therapy. Low HGF levels and high c-kit levels in serum at baseline were reported to be associated with longer survival in HCC patients

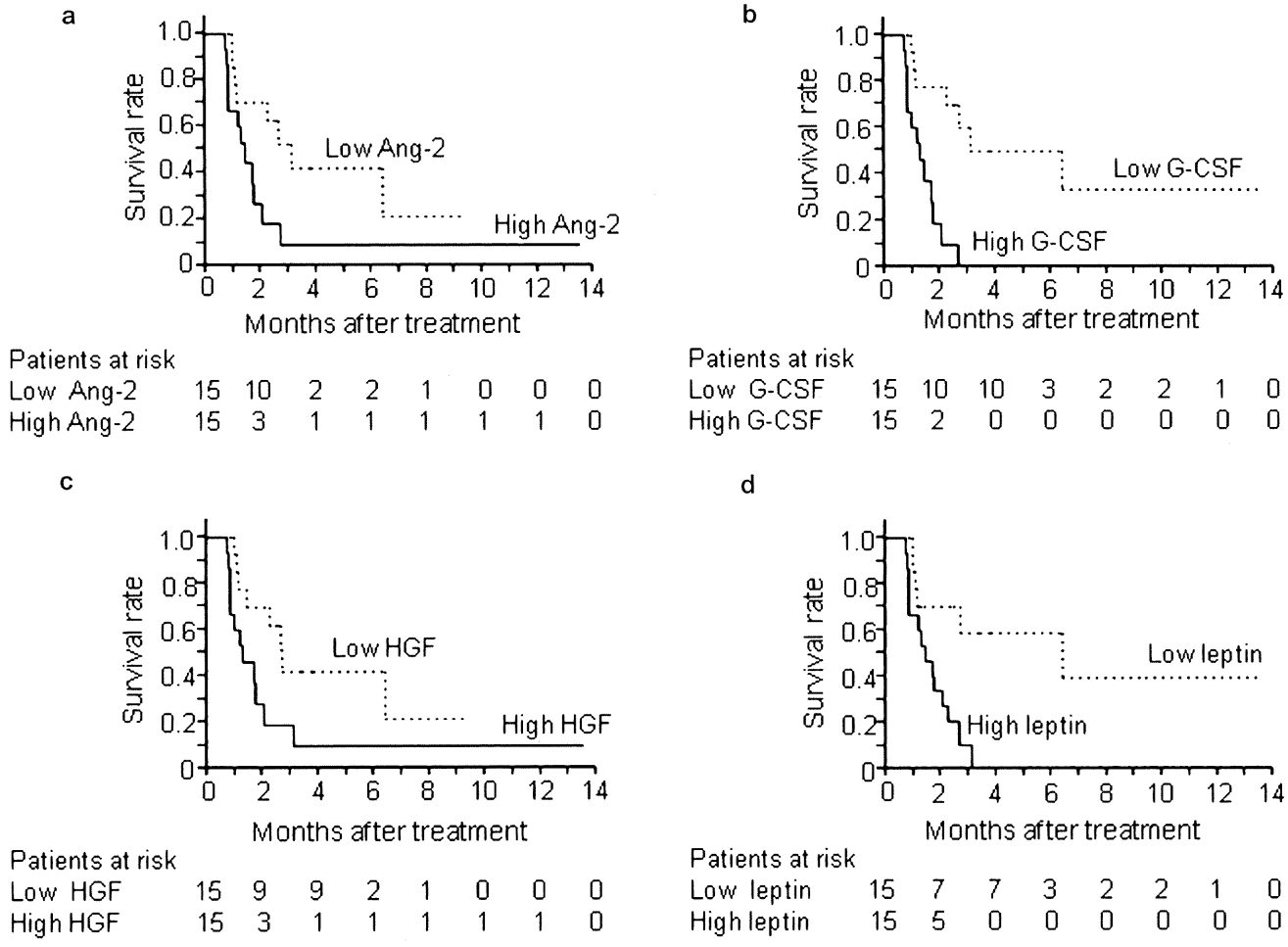

Figure 2 Progression-free survival of high and low biomarker groups. All high cytokine groups showed lower progression-free survival than low cytokine groups. (a) angiopoietin-2 (Ang-2); (b) granulocyte colony-stimulating factor (G-CSF); (c) hepatocyte growth factor (HGF); (d) leptin. (a) $p = 0.043$; (b) $p < 0.001$; (c) $p = 0.037$; (d) $p = 0.005$.

treated with sorafenib.[12] In addition, lower baseline VEGF levels were found to be correlated with increased PFS in HCC patients treated with sunitinib[13] and in metastatic breast cancer patients treated with bevacizumab combination therapy.[14] Several studies reported that no correlation was observed between VEGF levels and clinical outcome in metastatic colorectal cancer[15] and non-small cell lung cancer.[16] In fact, an inverse correlation was reported between high VEGF levels and PFS in advanced renal cell carcinoma.[17] One possible explanation for these differences is the pathological differences of tumors, or physiological differences associated with ethnicity, as we observed in the adverse events with sorafenib.[6,7]

Of the eight cytokines, serum VEGF and HGF were reported as molecules associated with tumor progression.[18–22] In addition, PDGF-B,[23,24] Ang-2,[25–28] leptin,[29] and G-CSF receptor[30–32] were overexpressed in at least some of the HCC tissues and were associated with tumor progression, angiogenesis, or dedifferentiation, among others. Therefore, a large number of cytokines at high levels might be representative of tumor aggressiveness as an additive effect of multiple cytokines. The elevation of the cytokines might also be correlated with the resistance to anti-angiogenic therapies in terms of the regulation of multiple pro-angiogenic mechanisms. For example, G-CSF was reported to induce tumor refractoriness to anti-VEGF therapy by recruiting myeloid cells to tumors and mediating tumor angiogenesis.[33] Molecules, such as vascular endothelial-cadherin and matrix metalloproteinases, play a role in another mechanism called vasculogenic mimicry,[34] although they were not measured in the present study. From this point of view, it is possible that monotherapies targeting limited numbers of molecules were not so effective when multiple pro-angiogenic molecules were activated, and it is important to investigate multiple molecules simultaneously as a reflection of the activation of multiple pro-angiogenic mechanisms.

We demonstrated that cytokines related to angiogenesis could be biomarkers predicting the effect of sorafenib treatment in patients with HCC, but there are several weak points in this study. We detected candidate markers related to tumor response and PFS; however, we could not strictly evaluate OS in this study. This study population was not adequate to analyze OS, because various treatments were performed after PD, such as transcatheter arterial

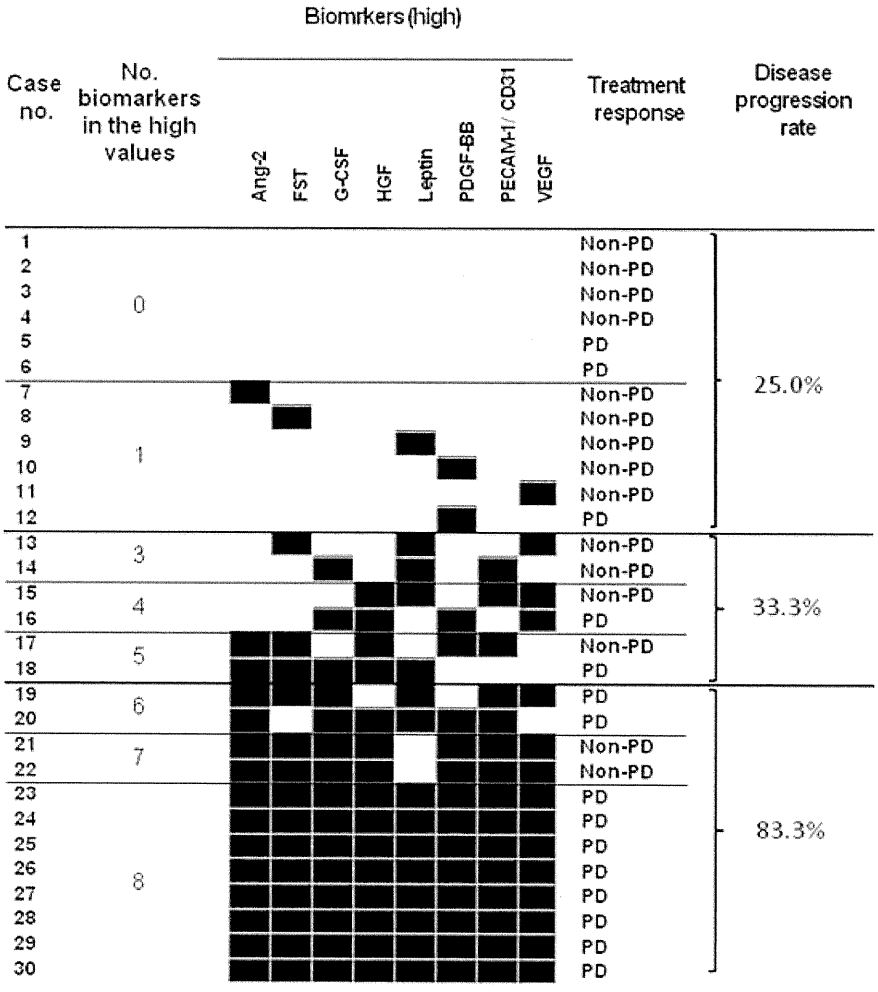

Figure 3 Combinations of biomarkers at high levels (cut-off values are the median). Closed square indicates that the expression was above the median. Progressive disease (PD) was seen in three of 12 (25%) patients with 0–2 high biomarkers, two of six (33.3%) patients with 3–5 high biomarkers, and 10 of 12 (83.3%) patients with 6–8 high biomarkers ($P = 0.008$). Ang-2, angiopoietin-2; FST, follistatin; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; PDGF-BB, platelet-derived growth factor-BB; PECAM-1/CD31, platelet endothelial cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

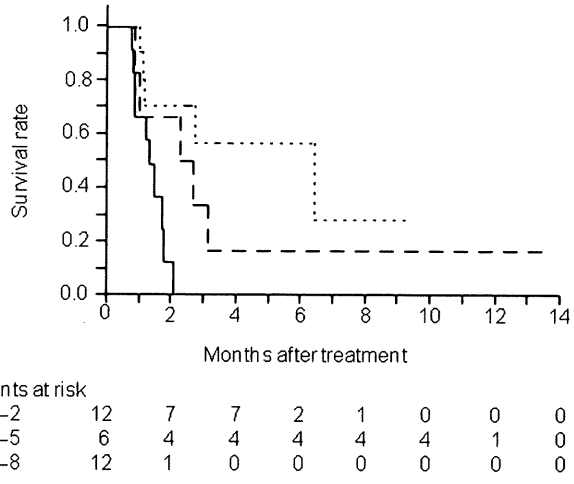

Figure 4 Progression-free survival of groups with 0–2, 3–5, and 6–8 high biomarkers. These refer to the number of biomarkers above the median at baseline. $P = 0.009$ (log-rank test). ·····, 0–2; ---, 3–5; —, 6–8.

embolization and chemoembolization. Another weak point is that this study is an exploratory analysis with a small sample size and a short observation period. There might be an effective combination of a few cytokines to predict the treatment effect; however, the number of patients in this study was too small to reach the conclusion. In addition, the difficulty in setting the standard values of these cytokines is another limitation. The values of serum cytokines in healthy controls differ between reports, and the difference extends from over 10-fold in VEGF to over 100-fold in PDGF-BB.[35–38] Therefore, it is necessary to measure them among a specific population with one method for obtaining a consistent result.

We demonstrated the expressions of angiogenesis-related factors in HCC patients who received sorafenib in this study, and we proposed a new concept of simultaneous measurement of serum markers of angiogenesis for the prediction of the treatment effect. Further examination is necessary to validate our findings by increasing the sample size and extending the observation period.

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HEPATOLOGY

Prognostic importance of fucosylated alpha-fetoprotein in hepatocellular carcinoma patients with low alpha-fetoprotein

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Key words
alpha-fetoprotein, fucosylation, hepatocellular carcinoma.

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Abstract

Background and Aim: Fucosylated alpha-fetoprotein (AFP-L3) is known to be a marker of poor prognosis in patients with hepatocellular carcinoma (HCC). However, it has been difficult to measure AFP-L3 under low AFP (≤ 20 ng/mL). The aim of this study was to elucidate the role of AFP-L3 in HCC patients with low AFP conditions.

Methods: One hundred and ninety six consecutive newly developed HCC patients with low AFP (≤ 20 ng/mL) were examined for serum AFP-L3 expression by a newly-developed micro-total analysis system that could stably measure AFP-L3 in low AFP circumstances, and its clinical importance was analyzed.

Results: Positivity of AFP-L3 in HCC patients was 13.3% at a cut-off level of 10%. Five-year survivals of HCC patients with AFP-L3 (< 10%) and AFP-L3 (≥ 10%) were 69.4% and 41.1%, respectively ($P = 0.001$). Among 18 clinical parameters, low alanine aminotransferase, large tumor size, presence of portal vein tumor thrombus, high AFP and high des-gamma carboxy prothrombin were observed in the high AFP-L3 (≥ 10%) group. Multivariate analysis revealed that high aspartate aminotransferase (AST) (risk ratio [RR] = 3.24, 95% confidence interval [CI] = 1.27–8.26), the presence of ascites (RR = 3.44, 95% CI = 1.22–9.34), multiple tumor number (RR = 3.06, 95% CI = 1.33–7.17), and high AFP-L3 (RR = 8.36, 95% CI = 2.79–25.5) were risk factors for survival. High AFP-L3 was also a risk factor for survival in HCC patients who received radiofrequency ablation ($P = 0.048$).

Conclusions: AFP-L3 is a strong prognostic factor for survival even in HCC patients with low AFP (≤ 20 ng/mL).

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cause of cancer death in the world and represents a major health problem.[1] There are many reports about the risk factors for survival of the patients with HCC. They include tumor factors such as tumor size and tumor numbers, and background liver factors such as serum albumin and total bilirubin.[2–5] Alpha-fetoprotein (AFP), fucosylated AFP (AFP-L3), and des-gamma carboxy prothrombin (DCP) are three major tumor markers of HCC (tumor factors). All of these markers, especially AFP-L3, are closely correlated with the prognosis of patients with HCC.[6] Although the clinical importance of AFP-L3 has been reported, the level obtained by conventional measurement methods is unstable or even undetectable when the serum AFP level is less than 20 ng/mL.

Recently, a new method that can stably measure AFP-L3 in patients with low levels of AFP was developed.[7] The method uses the electrokinetic analyte transport assay technique that enables the on-tip integration of all assay steps, and can detect minute amounts of AFP-L3 by Laser-induced-fluorescence following lectin affinity electrophoresis.

In this report, we measured the AFP-L3 in HCC patients with low AFP levels (≤ 20 ng/mL) and analyzed the clinical importance of AFP-L3 in this patient population.

Methods

Patients

Among 776 consecutive newly diagnosed HCC patients who were admitted to Okayama University Hospital between 2002 and 2009,

196 patients with AFP less than or equal to 20 ng/mL were enrolled. The mean age of the patients was 70.3 years, 127 patients (64.8%) were male, and the median tumor diameter was 17 mm. The percentage of tumor < 2 cm, 2–5 cm and > 5 cm were 65%, 23%, and 12%, respectively. One hundred and sixty-six patients, 29, and one patient were Child–Pugh grade A, B, and grade C, respectively. Radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), surgical resection, chemotherapy, and percutaneous ethanol injection therapy were performed in 139, 29, 21, five and two patients, respectively. As a control, 87 patients with liver cirrhosis (LC) with low AFP (less than or equal to 20 ng/mL) who also visited our hospital during the same period were also examined for AFP-L3. All LC patients were confirmed not to have HCC with ultrasonography or contrast enhanced computed tomography (CT), and no patients developed HCC during a 6-month follow up period. Seven out of the 10 patients received liver transplantation and no clear evidence of HCC was found in the patients by the pathological examination of the explanted livers. Informed consent was obtained from all patients for the use of their clinical data. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by our institutional review board.

Diagnosis

Hepatocellular carcinoma was diagnosed by imaging modalities such as angiography, CT, and magnetic resonance imaging (MRI) ($n = 141$) or by tumor biopsy ($n = 55$). The criteria for HCC via imaging was based on previous reports of hyperattenuation at the arterial phase, hypoattenuation at the portal phase in dynamic CT or magnetic resonance imaging (MRI), and tumor staining on angiography. According to the American Association for the Study of Liver Disease guidelines, we confirmed the diagnosis at least by two dynamic imaging modalities.[8] The nodules without these findings were histologically confirmed as HCC via US-guided, fine-needle biopsy.

Treatment and follow up

The patients were treated basically according to the evidence-based clinical practice guidelines for HCC in Japan.[9] The rate of observance of the guidelines was 83.1%. After the initial treatments, patients were followed up with ultrasonography, dynamic CT, or MRI as well as by biochemical liver function tests at least every 3 months. Two patients received interferon therapy after the treatment. Recurrence was diagnosed with the same criteria used for the initial diagnosis, and re-treatment was performed based on the same decision criteria. The recurrence rates of RFA treated patients were 57.5% and 70.7% at 3 years and 5 years after treatment, respectively.

Measurement of AFP-L3

Serum AFP concentrations were determined using a commercially available EIA kit. The percentage of AFP-L3 was determined by a micro-total analysis system developed by Wako Pure Chemical Industries, Ltd. (Osaka, Japan).[7]

Statistical analysis

The Wilcoxon rank sum test was used to compare the continuous data and the χ^2 test was used to compare categorical data. The survival of the patients was compared by the Kaplan–Meier method and the differences were evaluated by the log-rank test. Cox proportional hazards regression analysis was used to examine prognostic factors including AFP-L3. Factors exhibiting significant values in univariate analysis were further analyzed by multivariate analysis. The reason for using 3 cm as a break point is that this tumor size is frequently used in many algorithms and studies including ours.[3] JMP (version 8.02) software packages (SAS Institute, Cary, NC, USA) were used for the analyses, and $P < 0.05$ was considered significant. Bonferroni correction was used for multiple-comparisons of three groups and $P < 0.05/3$ was considered significant.

Results

Positivity of AFP-L3 at different cut-off

Positivity of AFP-L3 in patients with HCC was 51.5%, 13.3%, and 8.7% at cut-off levels of 5%, 10%, and 15%, respectively (Table 1). The positivity in patients with LC was lower than that for those with HCC; however, no significant difference was observed between HCC and LC. The area under the receiver operating characteristic curve (AUROC) was 0.534.

Relationship between AFP and AFP-L3

A very weak correlation was observed between AFP and AFP-L3 ($P < 0.001$, $R^2 = 0.064$, Fig. 1). High AFP-L3 (≤ 10%) was not observed in patients with AFP below 3.2 ng/mL.

Survival of the patients with HCC

Patients were divided into two groups according to the percentage of AFP-L3 and the survival rates of each group were compared. The 3-year (5-year) survival rates of patients with AFP-L3 (< 10%) and AFP-L3 (≥ 10%) were 90.1% (69.4%), and 68.4% (41.1%), respectively ($P = 0.001$, Fig. 2). Because the cut-off value of AFP-L3 was 10% in our institute for AFP above 20 ng/mL, we adopted the cut-off value of 10% in subsequent studies.

Characteristics of HCC with high AFP-L3

Eighteen clinical parameters were analyzed in patients with different AFP-L3 (Table 2). In the high AFP-L3 (≥ 10%) group, alanine

Table 1 Positivity of fucosylated alpha-fetoprotein (AFP-L3) in patients with low AFP (≤ 20 ng/mL)

Cut-off	Positivity of AFP-L3		
	HCC	LC	*P*-value
5%	101/196 (51.5%)	40/87 (46.0%)	0.440
10%	26/196 (13.3%)	10/87 (11.5%)	0.846
15%	17/196 (8.7%)	3/87 (3.5%)	0.136

HCC, hepatocellular carcinoma; LC, liver cirrhosis.

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aminotransferase (ALT) was low ($P = 0.003$), tumor size was large ($P < 0.001$), the presence of portal vein tumor thrombus (PVTT) was high ($P = 0.026$) and AFP and DCP were high ($P = 0.049$ and 0.002, respectively). No differences were observed in total bilirubin, albumin, prothrombin time or presence of ascites, which represented liver function and factors in the Child–Pugh score.

Risk factors for survival

Among 18 parameters, Child–Pugh grade B/C, high T. Bil (≥ 1.5 mg/dL), low albumin (< 3.5 g/dL), high AST (≥ 80 IU/mL), low platelet count (< 10×10^4/mm^3), low prothrombin time (PT) (< 80%), the presence of ascites, multiple tumor number, and high AFP-L3 (≥ 10%) were risk factors for survival according to univariate analysis (Table 3). In multivariate analysis, high AST, the presence of ascites, multiple tumor number, and high AFP-L3 were risk factors for survival. The risk ratio of AFP-L3 (RR = 8.36, 95% confidence interval [CI] = 2.79–25.5) was the highest among the factors examined. Child–Pugh grade was not included in multivariate analysis to avoid multicollinearity.

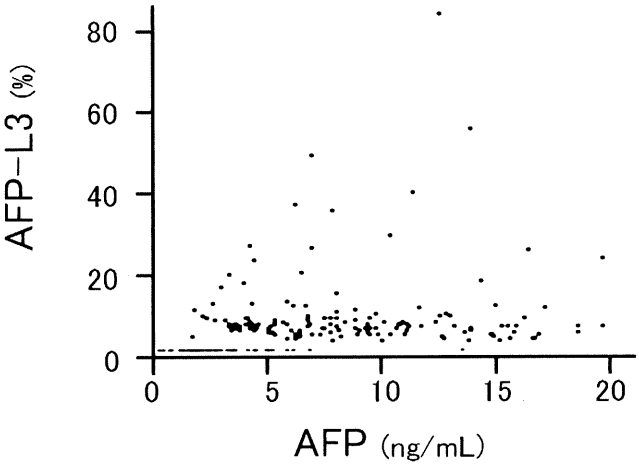

Figure 1 Relationship between fucosylated alpha-fetoprotein (AFP-L3) and alpha-fetoprotein (AFP) under 20 ng/mL in patients with hepatocellular carcinoma (HCC). A weak correlation was observed between AFP and AFP-L3 ($P < 0.001$, $R^2 = 0.064$). High AFP-L3 (10% ≤) was not observed in patients with AFP below 3.2 ng/mL. AFP-L3 under the lowest detection limit was considered as zero.

Survival and recurrence of RFA-treated patients

In this study, 139 patients were treated by RFA. Curative ablation could be performed in 129 of them, and these 129 were enrolled in the subsequent examination. The same 18 variables were analyzed. In the high AFP-L3 (≥ 10%) group, tumor size was larger (18 mm *vs* 15 mm, median), DCP was higher (68 mAU/mL *vs* 24 mAU/mL), AST was lower (33 IU/L *vs* 40 IU/L), and PT was lower (91% *vs* 98%) than in the low AFP-L3 (< 10%) group. Multivariate analysis revealed that high AST (≥ 80 IU/L, RR = 11.6, 95% CI = 3.14–47.6), low prothrombin time (< 80%, 3.50, 1.14–11.1), presence of ascites (RR = 5.37, 95% CI = 1.14–20.3), and high AFP-L3 (≥ 10%, 13.5, 2.99–69.1) were risk factors for survival.

The 3-year (5-year) recurrence free survival rates of patients with AFP-L3 (< 10%) and AFP-L3 (≥ 10%) were 42.7% (30.7%), and 43.8% (21.9%), respectively. The 3-year (5-year) survival rates of patients with AFP-L3 (< 10%) and AFP-L3 (≥ 10%) were 88.4% (66.3%), and 72.9% (43.8%), respectively ($P = 0.048$, Fig. 3).

The recurrence free survival curve tended to be higher in the low AFP-L3 group in the first 3 years ($P = 0.031$, Wilcoxon); however, no significant difference was observed between the two groups ($P = 0.123$, Log-rank test, Fig. 4). RFA and TACE were, respectively, performed in 62.1% and 12.1% for the treatment of recurrent HCC; however, no significant difference of the treatment was observed between high AFP-L3 (≥ 10%) group and low AFP-L3 (< 10%) group.

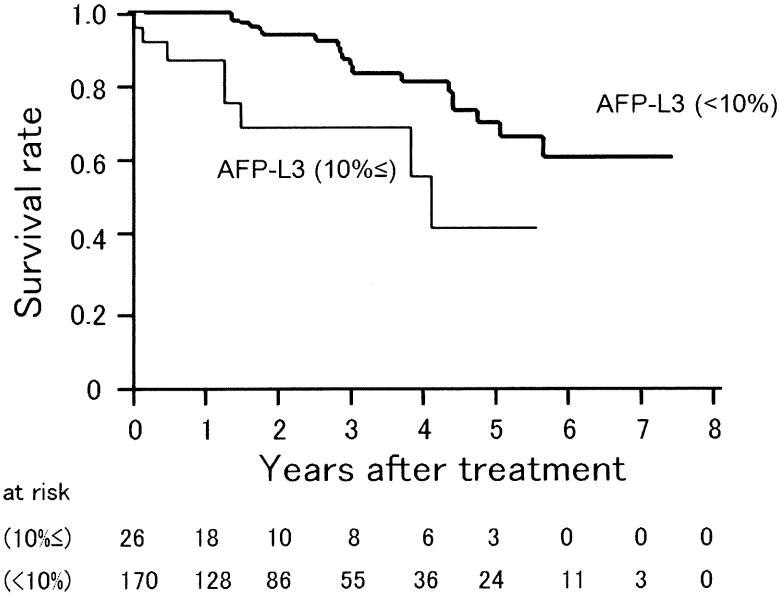

Figure 2 Survival of hepatocellular carcinoma (HCC) patients with different fucosylated alpha-fetoprotein (AFP-L3) level. The 3-year (5-year) survivals of patients with AFP-L3 (< 10%, thick solid line), and AFP-L3 (≥ 10%, thin solid line) were 90.1% (69.4%), and 68.4% (41.1%), respectively ($P = 0.001$).

Table 2 Characteristics of patients with hepatocellular carcinoma (HCC) at different fucosylated alpha-fetoprotein (AFP-L3) levels

Variables	AFP-L3		*P*-value
	< 10%	10% ≤	
Patient number	170 (86.7%)	26 (13.3%)	
Age (years)	70.2 (38.1–85.4)	71.4 (46.3–87.7)	0.988
Sex (male)	109 (64.1%)	18 (69.2%)	0.611
HCVAb (positive)	124 (72.9%)	17 (65.4%)	0.665
HBsAg (positive)	16 (9.4%)	4 (15.4%)	0.312
Child–Pugh A	145 (85.3%)	19 (76.0%)	0.116
Total bilirubin (mg/dL)	0.78 (0.36–2.88)	0.78 (0.33–4.44)	0.611
Albumin (g/dL)	3.8 (2.5–4.9)	3.6 (2.5–4.4)	0.278
AST (IU/L)	48 (19–198)	48 (16–243)	0.406
ALT (IU/L)	44 (13–235)	32 (14–115)	0.003
Platelet ($\times 10^4/mm^3$)	12.2 (3.4–74.0)	12.2 (3.5–29.8)	0.630
Prothrombin time (%)	98 (10–146)	92 (62–126)	0.166
Ascites (present)	22 (12.9%)	6 (23.1%)	0.169
Alcohol (≥ 90 g/day)	16 (9.6%)	4 (15.4%)	0.372
Tumor size (mm)	16 (7–87)	26 (9–170)	< 0.001
Tumor number (multiple)	61 (35.9%)	14 (53.8%)	0.079
PVTT (present)	11 (6.5%)	5 (19.2%)	0.026
Alpha-fetoprotein (ng/mL)	6.4 (0.9–20)	7.5 (3.2–20)	0.049
Des-gamma carboxy prothrombin (mAU/mL)	26 (10–363 350)	86 (11–75 000)	0.002

All numbers are medians (inter-quartile range) unless otherwise noted.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBsAg, hepatitis B virus surface antigen; HCVAb, hepatitis C virus antibody; PVTT, portal vein tumor thrombus.

Table 3 Risk factors for survival in patients with hepatocellular carcinoma (HCC) with low alpha-fetoprotein (AFP)

	Univariate			Multivariate		
	RR	95%CI	*P*-value	RR	95%CI	*P*-value
Age (≥ 65 years)	1.39	0.60–3.79	0.455			
Sex (male)	1.80	0.77–4.89	0.179			
HCVAb (positive)	1.55	0.60–5.33	0.385			
HBsAg (positive)	0.47	0.02–2.25	0.415			
Child–Pugh B/C	2.74	1.18–5.90	0.020			
Total bilirubin (≥ 1.5 mg/dL)	3.94	1.68–8.54	0.002	1.24	0.43–3.36	0.804
Albumin (< 3.5 g/dL)	2.17	1.02–4.62	0.042	0.87	0.31–2.35	0.796
AST (≥ 80 IU/L)	3.22	1.48–6.80	0.003	3.24	1.27–8.26	0.014
ALT (≥ 80 IU/L)	2.05	0.85–4.51	0.104			
Platelet (< $10 \times 10^4/mm^3$)	2.15	1.02–4.67	0.044	2.07	0.70–6.08	0.180
Prothrombin time (< 80%)	2.35	1.06–5.02	0.035	1.51	0.54–4.00	0.417
Ascites (present)	4.39	1.87–9.53	0.001	3.44	1.22–9.34	0.019
Alcohol (≥ 90 g/day)	0.55	0.08–1.85	0.377			
Tumor size (≥ 3 cm)	1.38	0.46–3.39	0.524			
Tumor (multiple)	2.20	1.03–4.68	0.040	3.06	1.33–7.17	0.008
PVTT (present)	3.40	0.99–8.92	0.051			
AFP L3 (≥ 10%)	3.50	1.44–7.75	0.007	8.36	2.79–25.5	< 0.001
Des-gamma carboxy prothrombin (≥ 40 mAU/mL)	1.18	0.52–2.51	0.674			

95% CI, 95% confidence interval; RR, risk ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBsAg, hepatitis B virus surface antigen; HCVAb, hepatitis C virus antibody; PVTT, portal vein tumor thrombus.

Discussion

AFP-L3 is known to be a predictor of poor prognosis; however, it has been difficult to evaluate its value in cases with low AFP under 20 ng/mL, because conventional measuring methods yield unstable or even undetectable AFP-L3 levels under these circumstances. Methodological development enabled us to overcome this difficulty and revealed that AFP-L3 was closely related to poor prognosis even in HCC patients with low AFP. The risk ratio for survival was 3.50 and was comparable with the ratio (2.43) in

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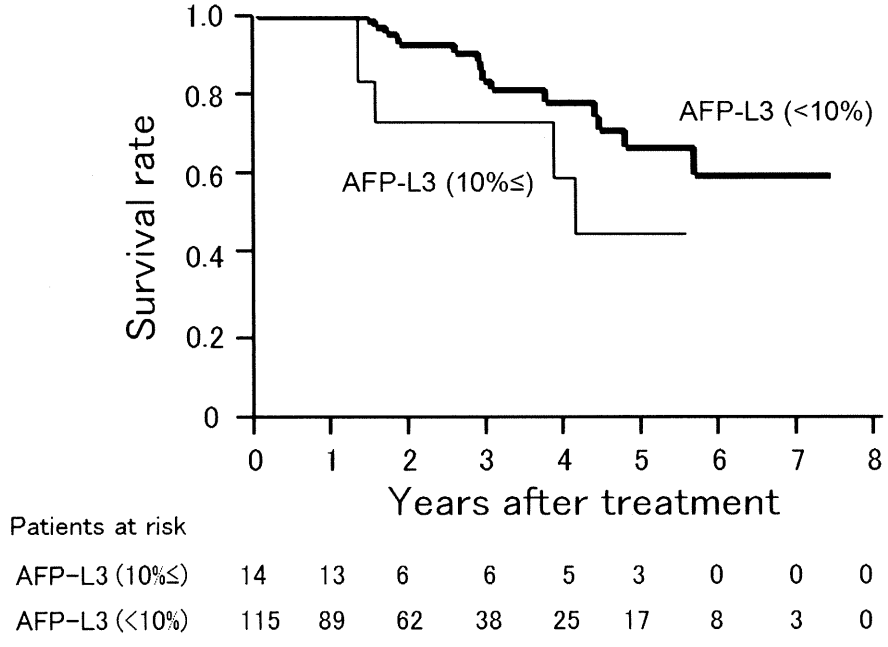
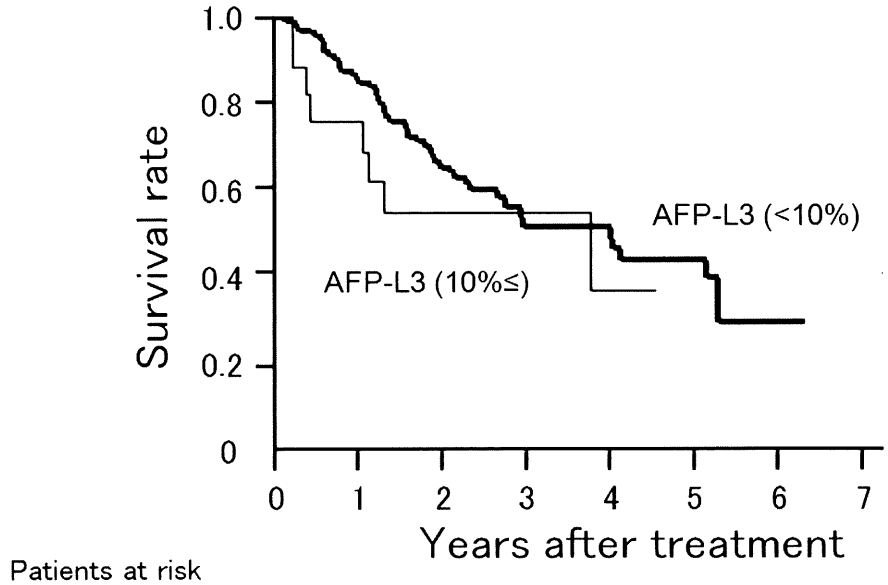


Patients at risk

AFP-L3 (10%≤)	14	13	6	6	5	3	0	0	0
AFP-L3 (<10%)	115	89	62	38	25	17	8	3	0

Figure 3 Survival of radiofrequency ablation (RFA)-treated patients. The 3-year (5-year) survivals of RFA-treated patients with fucosylated alpha-fetoprotein (AFP-L3) (< 10%, thick solid line), and AFP-L3 (≥ 10%, thin solid line) were 88.4% (66.3%), and 72.9% (43.8%), respectively ($P = 0.048$).



Patients at risk

AFP-L3 (10%≤)	14	9	4	3	2	0	0	0
AFP-L3 (<10%)	113	75	39	16	14	8	4	0

Figure 4 Recurrence free survival of radiofrequency ablation (RFA)-treated patients. The 3-year (5-year) recurrence free survivals of RFA-treated patients with AFP-L3 (< 10%, thick solid line), and AFP-L3 (≥ 10%, thin solid line) were 42.7% (30.7%), and 43.8% (21.9%), respectively.

HCC patients with high AFP in our institute (Nouso, unpubl. data, 2010). There were other differences in patients' backgrounds at different AFP-L3 levels. Total AFP level and DCP were high and tumor size was large in the high AFP-L3 group. The factors were known to correlate with the prognosis of patients with HCC.

Recently, many fucosylated glycoproteins other than AFP-L3 have been reported to be useful for the diagnosis of HCC. They include fucosylated hemopexin and fucosylated kininogen;[10,11] however, the mechanism of the increase of fucosylated glycoproteins is not fully understood. Fut 8 is known to be a key enzyme of fucosylation.[12] This enzyme might act to produce AFP-L3 but an activation of the enzyme is not the only reason for increased AFP-L3 in HCC, because Fut8 was also reported to exist in the non-cancerous cirrhotic liver.[13]

The sensitivity of AFP-L3 was quite low (13.3%) in this study, meaning that the diagnostic role of AFP-L3 is limited when AFP is below 20 ng/mL. This low specificity of AFP-L3 was completely different from the results of previous reports that demonstrated a high specificity of AFP-L3.[14–19] The reports analyzed AFP-L3 in patients with HCC with high AFP. We analyzed only patients with

low AFP (≤ 20 ng/mL), meaning that a small amount of AFP-L3 production could have easily raised the percentage of AFP-L3. Small constitutive expression of AFP-L3 by FUT8 activation in cirrhotic liver as well as the possibility of occult HCC in some patients might have led to the low specificity in cases with low AFP.

Despite the low sensitivity and specificity of AFP-L3, its increase was closely related to the prognosis of HCC patients; the risk ratio was 8.36 and was the highest among the factors examined in this study. The sensitivities of AFP at 20 ng/mL cut-off were reported to be approximately 25–55% and 53–70% when the tumor size was less than or equal to 2 cm and 5 cm, respectively.[14,15,20–23] The sensitivity of AFP in our department is 62%,[14] meaning that approximately 40% of our patients have previously undergone unreliable AFP-L3 measurements. Measuring AFP-L3 with the new method provides a good prediction of prognosis in large patient populations with normal AFP (≤ 20 ng/mL).

In the present study, we demonstrated the prognostic value of AFP-L3 in patients with low AFP (≤ 20 ng/mL). The outcome of LC patients with high AFP-L3 is a future issue that must be solved.

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Original Article

Long-term outcome and hepatocellular carcinoma development in chronic hepatitis B or cirrhosis patients after nucleoside analog treatment with entecavir or lamivudine

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Aim: We conducted this prospective study to elucidate the long-term outcome and incidence of hepatocellular carcinoma (HCC) development after nucleos(t)ide analog (NA) treatment in patients with chronic hepatitis B (CHB) or cirrhosis.

Methods: CHB or cirrhosis patients without past NA treatment or HCC were started on entecavir (ETV) or lamivudine (LVD), and prospectively followed up with monthly blood tests, and with abdominal imaging every 6 months in CHB and every 3 months in cirrhosis patients.

Results: A total of 256 subjects with CHB ($n = 194$) or cirrhosis ($n = 62$) received ETV ($n = 129$) or LVD ($n = 127$) for 4.25 years (range: 0.41–10.0). After NA treatment, serum HBV DNA, alanine aminotransferase and α-fetoprotein (AFP) dropped significantly, along with significant increases in serum albumin and prothrombin time. Drug-resistance developed in 60 cases in the LVD group and in only one case in the ETV group. HCC developed in 35 patients, and the incidence at years 1, 3, 5, 7 and 10 was significantly higher in patients with cirrhosis (8.1%, 17.5%, 43.2%, 46.7% and 53.4%, respectively) than chronic hepatitis (1.6%, 3.5%, 3.5%, 7.1% and 29.6%, respectively), with no difference between ETV and LVD. After NA treatment, the sensitivity/specificity for HCC of AFP and des-γ-carboxy prothrombin (DCP) was 45.7%/97.3% and 33.3%/96.2%, respectively, with the specificity of AFP being higher than at baseline (64.4%), at the cut-off of 10 ng/mL.

Conclusion: NA exerted a long-term efficacy and improved hepatic reservation in CHB and cirrhosis. After NA treatment, AFP dropped to lower than 10 ng/mL with marked elevation of specificity, leading to an earlier detection of HCC.

Key words: α-fetoprotein, chronic hepatitis B, entecavir, hepatitis B virus, hepatocellular carcinoma, lamivudine

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a serious health problem because of its potential to induce a variety of liver diseases, namely, acute hepatitis, chronic hepatitis (CH), liver cirrhosis, hepatocellular carcinoma (HCC) and fulminant hepatic failure.

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Conflicts of interest: None.

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The prevalence of HBV carriage is reported to be 350–400 million people worldwide, and the prevalence rate of HBV infection in Japan is estimated to be 0.8%.[1,2] It has been reported that 15–20% of chronic hepatitis B (CHB) patients progress to cirrhosis within 5 years and that the annual incidence of HCC is 2.8%.[3] Nucleos(t)ide analogs (NA) suppress HBV DNA replication by inhibiting HBV DNA polymerase activity in the reverse transcription process from pregenomic RNA derived from a HBV closely covalent circular (ccc)DNA template.[4] Three NA anti-HBV agents, namely lamivudine (LVD), adefovir-dipivoxil (ADV) and entecavir (ETV), have been approved for coverage by the health