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Highly sensitive lens culinaris agglutinin-reactive α-fetoprotein is useful for early detection of hepatocellular carcinoma in patients with chronic liver disease

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Abstract. The fucosylated fraction of α-fetoprotein (AFP-L3) is a specific marker for hepatocellular carcinoma (HCC). However, conventional AFP-L3% (c-AFP-L3%) has not always been reliable in cases with low serum α-fetoprotein (AFP) levels. In this study, we evaluated the clinical utility of a newly developed assay, highly sensitive AFP-L3% (hs-AFP-L3%). Subjects included 74 patients with benign liver disease (BLD), including chronic hepatitis and cirrhosis, and 94 with HCC. Serum hs-AFP-L3% was significantly higher than c-AFP-L3% in patients with early-stage HCC (solitary or <20 mm in diameter). Additionally, hs-AFP-L3% was significantly increased in patients with well-differentiated HCC. In patients with serum AFP <20 ng/ml, the sensitivities of c-AFP-L3% and hs-AFP-L3% were 12.5 and 44.6%, respectively, at a cut-off value of 5%. In 59 BLD patients with serum AFP <20 ng/ml, the HCC-positive rate in patients with hs-AFP-L3% ≥5% was significantly higher compared to those with hs-AFP-L3% <5% during the follow-up period (median, 35 months; range, 5-48 months). Importantly, none of the BLD patients with both serum AFP <20 ng/ml and hs-AFP-L3% <5% developed HCC. These results indicated that hs-AFP-L3% is useful for early detection of HCC in BLD patients, even for those with serum AFP <20 ng/ml. Furthermore, since hs-AFP-L3% increases before HCC is detectable by various advanced imaging modalities, this assay may help identify BLD patients with a higher risk of HCC.

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Key words: α-fetoprotein, α-fetoprotein fucosylated fraction L3, hepatocellular carcinoma, hepatocarcinogenesis

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world, and the third most common cause of cancer-related death (1). Although it is more common in Asia and Africa, its incidence in the United States has increased over the past two decades, largely due to the spread of hepatitis C (HCV) infection, which is an underlying risk factor (2). Early detection of HCC increases the potential for curative treatment and improves prognosis. Several methods developed for the diagnosis of HCC, including evaluation of serum markers, ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI), have been tested clinically. α-fetoprotein (AFP) and des-γ carboxy prothrombin (DCP), serum proteins that are elevated in HCC, are the most widely used markers. Although routine screening offers the best chance for early tumor detection, the reported sensitivities and specificities of elevated serum AFP and DCP levels vary significantly (3-8). Furthermore, serum AFP levels increase in only 30-40% of patients with HCC, especially early in the disease process (5). Additionally, an increase in serum AFP is also seen in patients with non-cancerous conditions, including cirrhosis or exacerbation of chronic hepatitis (9). AFP-L3, the lectin lens culinaris agglutinin-bound fraction, is one of the three glycoforms of AFP, and is the major glycoform elevated in the serum of HCC patients. The reported sensitivities of AFP-L3 as a method of detecting HCC range from 75-97% with specificities of 90-92% (10,11). In cases of HCC, however, high percentage of AFP-L3 is closely associated with poor differentiation and biologically malignant characteristics, including portal vein invasion, of neoplastic cells (11,12). Therefore, it is not clear how useful this test is for the early detection of HCC. Additionally, measurement of AFP-L3 has not always been reliable for serum samples with low total AFP concentration, as determined by conventional lectin affinity system (LiBASys) (13).

Recently, a novel automated immunoassay for AFP-L3 has been developed. The new method uses on-chip electrokinetic reaction and separation by affinity electrophoresis (micro-total

analysis system; μ-TAS) (14). In patients with an AFP level of ≥20 μg/ml, μ-TAS AFP-L3% correlated well with LiBASys AFP-L3% (15). Furthermore, this system has enabled the accurate measurement of AFP-L3% at very low AFP concentrations. Therefore, in this retrospective study, we investigated the clinical utility of the new highly sensitive μ-TAS AFP-L3% assay for diagnosis of HCC in a population of patients with HCC or benign liver diseases (BLD), including chronic hepatitis or cirrhosis.

Patients and methods

Patients. Between December 2006 and September 2010, frozen serum samples were obtained from 94 patients with HCC, as well as from 74 patients with BLD, who had chronic hepatitis or liver cirrhosis, but not HCC (Table I). All patients met the eligibility criteria (availability of stored serum samples and written informed consent). Among the BLD patients, 20 were positive for hepatitis B surface antigen (HBsAg), 43 were positive for anti-hepatitis C virus (HCV) antibody, and 11 were negative for either HBsAg or anti-HCV antibody. The BLD patients were followed after serum sampling for 32.8±12.3 months (median, 35; range, 5-48); liver imaging was performed by US at 6- to 12-month intervals in most patients with chronic hepatitis, and CT, MRI, or US was performed at 3- to 6-month intervals in patients with liver cirrhosis.

HCC patients were diagnosed using imaging modalities such as US, MRI and CT during hepatic arteriography. Vascular invasion was evaluated by imaging modalities. In some cases that showed atypical features upon imaging, ultrasound-guided biopsies were performed. Based on imaging findings, tumor stage was ranked using the tumor-node-metastasis (TMN) staging system of the Liver Cancer Study Group of Japan (16,17): T1 (fulfilling the following three conditions: solitary, 2 cm, no vessel invasion), T2 (fulfilling two of the three conditions), T3 (fulfilling one of the three conditions), T4 (fulfilling none of the three conditions or showing presence of distant metastasis); N0 (no lymph node metastasis), N1 (metastasis to lymph nodes); M0 (no distant metastasis), M1 (distant metastasis); stage 1 (T1N0M0), stage II (T2N0M0), stage III (T3N0M0), and stage IV (T4N0M0 or any TN1M0, or any TN0-1M1).

Measurement of serum AFP and AFP-L3%. For the HCC group, AFP and AFP-L3% were measured in the same sample obtained at the time of HCC diagnosis, before any treatment. For the BLD without HCC group, measurements were made at the time of diagnosis of chronic liver disease. Highly sensitive AFP-L3% (hs-AFP-L3%) were measured by a microchip capillary electrophoresis and liquid-phase binding assay on a μ-TASWako i30 auto analyzer (Wako Pure Chemical Industries, Ltd., Osaka, Japan) (15). Conventional AFP-L3% (c-AFP-L3%) was examined using a column chromatography and liquid-phase binding assay on a LiBASys auto analyzer (Wako Pure Chemical Industries, Ltd.) (13). The analytical sensitivity of the μ-TASWako i30 auto analyzer is 0.3 μg/ml AFP; the AFP-L3% can be measured when AFP-L3 is over 0.3 μg/ml. Although the analytical sensitivity of the LiBASys is 0.8 μg/ml AFP, AFP-L3% cannot be measured at AFP <10 ng/ml. Therefore, the correlation between μ-TAS-L3% and LiBA-L3% was poor at AFP <20 ng/ml.

Statistical analysis. We used the Mann-Whitney U test, Z test and Chi-square test for evaluation of the statistical significance of each finding. SPSS version 17.0J (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis; $p<0.05$ was considered to indicate statistical significance.

Results

Clinical feature of patients. The demographics, etiology of liver disease, hepatic functional reserve ranked by Child-Pugh classification, tumor stage, tumor size and tumor number of the study patients are summarized in Table I. The HCC group included 94 patients: 35 patients with stage I, 35 with stage II, 14 with stage III, and 10 with stage IV; thus, ~75% of HCC cases were stage I or II. The incidence of cirrhosis in HCC patients (55.3%) was significantly higher than in BLD (25.7%), whereas the hepatic reserve expressed by Child-Pugh classification of HCC patients was significantly preserved compared with BLD patients.

Serum AFP levels in patients with HCC were significantly higher than those with BLD (Table I and Fig. 1A). hs-AFP-L3% was measurable in 47.3 and 78.7% of patients with BLD and HCC, respectively, whereas c-AFP-L3% was detected in 31.1 and 63.8% of patients. Thus, hs-AFP-L3% was significantly higher than c-AFP-L3% in both BLD and HCC patients (Table I and Fig. 1B). Since a cut-off value of 5% has been reported to be useful for diagnosis of HCC using hs-AFP-L3% (18), the cut-off value for AFP-L3% was set at 5% in the present study. The sensitivity and specificity of hs-AFP-L3% were 57.0 and 63.5%, respectively, whereas those of c-AFP-L3% were 40.4 and 81.1%.

hs-AFP-L3% significantly increases in HCC patients at early stage. Next, we analyzed serum AFP levels, c-AFP-L3% and hs-AFP-L3%, and compared early and advanced stages of HCC (Fig. 2). When compared with HCC patients with stage I or II cancer, serum AFP levels were significantly increased in patients with stage III and IV disease (Fig. 2A). Both c-AFP-L3% and hs-AFP-L3% in HCC patients with advanced stages were also significantly higher than in patients with early stages (Fig. 2B). Although 86% of HCC patients with stage I (n=35) exhibited serum AFP <20 ng/ml, c-AFP-L3% and hs-AFP-L3% were measurable in 46 and 69% of these patients, respectively; hs-AFP-L3% was significantly higher than c-AFP-L3%. Consequently, in HCC patients at stage I, the sensitivity of c-AFP-L3% or hs-AFP-L3% at a cut-off level of 5% were 17.1 or 48.6%, respectively.

Next, we evaluated the relationship between AFP-L3% and tumor number or size (Fig. 3). hs-AFP-L3% was significantly higher than c-AFP-L3%, even in patients with single or small HCC (<20 mm in diameter) (Fig. 3). Conversely, when compared to HCC patients with solitary or small HCC, both c-AFP-L3% and hs-AFP-L3% were increased in cases with multiple or ≥20 mm HCC, and there was no statistical difference between c-AFP-L3% and hs-AFP-L3%. These results indicate that hs-AFP-L3% is a useful biomarker for detecting early-stage HCC.

An increase in hs-AFP-L3% is observed in both BLD and HCC patients with AFP <20 ng/ml. We analyzed c-AFP-L3%

Table I. Clinical features of patients with BLD and HCC.

	BLD (n=74)	HCC (n=94)	p-value
Age	56.23±13.88	65.76±12.98[a]	<0.001
Gender (male/female)	30/44	56/38[a]	0.015
CH/LC	55/19	42/52[a]	<0.001
HBV/HCV/NBNC	20/43/11	5/61/28[a]	<0.001
Child-Pugh class (A/B/C/unknown)	39/5/4/26	75/19/0/0[a]	<0.001
TNM stage (I/II/III/IV)		35/35/14/10	
Tumor size (mean ± SD)		22.35±16.42	
<20 mm/≥20 mm		58/36	
Tumor number (single/multiple)		50/44	
AFP (ng/ml)	46.17±163.6	2871.5±9882.7[a]	<0.001
c-AFP-L3%	2.96±6.45	18.19±26.95[a]	<0.001
hs-AFP-L3%	3.84±5.59	21.12±29.01[a]	<0.001
Platelet count ($x10^4/\mu l$)	14.98±6.82	11.39±4.73[a]	0.001
AST (IU/l)	70.55±95.87	55.78±22.92	0.099
ALT (IU/l)	85.38±144.71	48.28±24.13	0.783

BLD, benign liver disease; HCC, hepatocellular carcinoma; CH, chronic hepatitis; LC, liver cirrhosis; HBV, hepatitis B virus; HCV, hepatitis C virus; hs-AFP-L3%, hypersensitive-AFP-L3%; c-AFP-L3%, conventional-AFP-L3%.

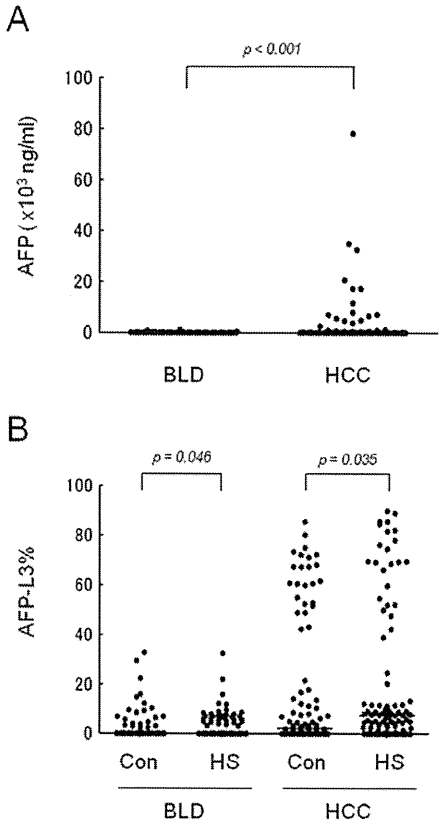

Figure 1. Serum levels of AFP, c-AFP-L3% and hs-AFP-L3% in patients with BLD or HCC. (A) Serum AFP concentrations in HCC patients (n=94) were significantly higher than those in BLD (n=74). (B) hs-AFP-L3% (HS) significantly increased in comparison with c-AFP-L3% (Con) in both BLD and HCC patients.

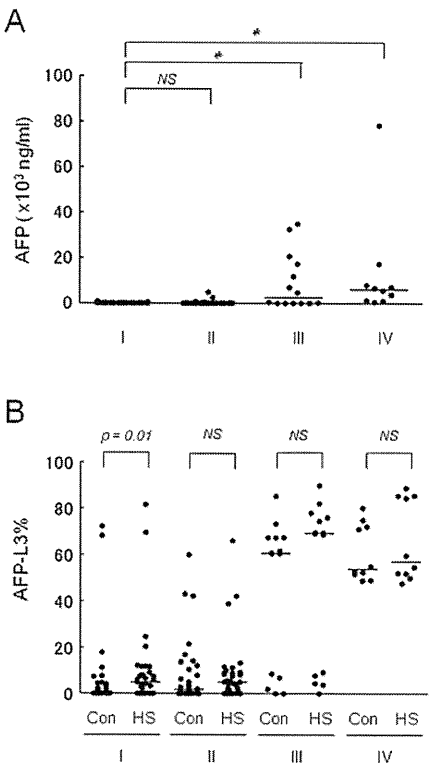

Figure 2. Serum levels of AFP, c-AFP-L3% and hs-AFP-L3% in patients with early or advanced HCC. (A) Serum AFP levels in HCC patients at stage III (n=14) or IV (n=10) were significantly higher than those at stage I (n=35) or II (n=35). *p<0.05. (B) hs-AFP-L3% (HS) was significantly higher than c-AFP-L3% (Con) in patients with HCC at stage I, whereas there was no significant difference between c- and hs-AFP-L3% in HCC patients at stages II, III and IV.

Table II. Clinical features of BLD and HCC patients with AFP <20 ng/ml.

	BLD (n=59)	HCC (n=56)	p-value
Age	56.78±13.51	68.88±12.05[a]	<0.001
Gender (male/female)	23/36	26/30	0.422
CH/LC	45/14	25/31[a]	0.001
HBV/HCV/NBNC	14/35/10	5/32/19[a]	0.008
Child-Pugh class (A/B/C/unknown)	31/4/1/23	50/6/0/0[a]	<0.001
TNM stage (I/II/III/IV)		30/21/5/0	
Tumor size (mean ± SD)		16.16±11.59	
<20 mm/≥ 20 mm		47/9	
Tumor number (single/multiple)		35/21	
AFP (ng/ml)	4.68±3.6	8.92±5.23[a]	<0.001
c-AFP-L3%	0.83±3.92	1.86±3.16[a]	0.002
hs-AFP-L3%	2.7±5.15	4.86±5.19[a]	0.003
Platelet count (x$10^4/\mu$l)	15.93±6.67	11.93±4.49[a]	0.001
AST (IU/l)	43.91±25.72	54.32±21.61[a]	0.003
ALT (IU/l)	49.21±51.7	48.66±24.41	0.184

BLD, benign liver disease; HCC, hepatocellular carcinoma; CH, chronic hepatitis; LC, liver cirrhosis; HBV, hepatitis B virus; HCV, hepatitis C virus; hs-AFP-L3%, hypersensitive-AFP-L3%; c-AFP-L3%, conventional-AFP-L3%.

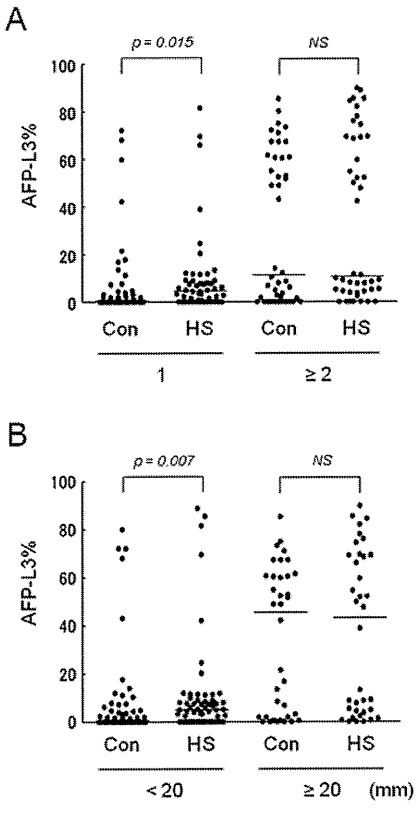

Figure 3. hs-AFP-L3% significantly increased in patients with solitary or small HCC, but not multiple or HCC ≥20 mm in diameter. (A) hs-AFP-L3% (HS) was significantly higher than c-AFP-L3% (Con) in patients with solitary HCC (n=50), but not in patients with multiple HCC (n=44). (B) hs-AFP-L3% significantly increased in comparison with c-AFP-L3% in patients with small HCC (<20 mm in diameter) (n=58), but not in patients with large HCC (≥20 mm) (n=36).

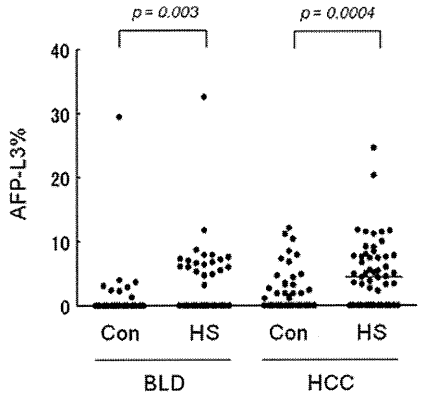

Figure 4. Higher levels of hs-AFP-L3% were observed in both BLD and HCC patients with serum AFP <20 ng/ml. c-AFP-L3% (Con) and hs-AFP-L3% (HS) in BLD and HCC patients with AFP <20 ng/ml (n=59 and 56, respectively) were analyzed. c-AFP-L3% was detectable in 13.6 and 39.3% of BLD and HCC patients, respectively, whereas hs-AFP-L3% was measurable in 33.9 and 64.3% of BLD and HCC patients, respectively; hs-AFP-L3% was significantly higher than c-AFP-L3%.

and hs-AFP-L3% in BLD and HCC patients with AFP <20 ng/ml (Table II). Forty-seven of 56 (83.4%) HCC patients exhibited small HCCs (<20 mm in diameter); 35 patients (62.5%) exhibited solitary tumors. c-AFP-L3% was detectable in 13.6 and 39.3% of BLD and HCC patients, respectively. Conversely, hs-AFP-L3% was measurable in 33.9 and 64.3% of BLD and HCC patients, respectively, and the levels of hs-AFP-L3% were significantly higher than those of c-AFP-L3% [BLD: mean ± SD (range) 0.83±3.92 (1.3-29.5) vs. 2.70±5.15%, p=0.003, and HCC: 1.86±3.16 (1.1-12.1) vs. 4.86±5.19% (2.3-24.6), p=0.004] (Fig. 4). The sensitivity and specificity of hs-AFP-L3%

Table III. Characterization of seven BLD patients, who developed HCC.

Case no.	1	2	3	4	5	6	7
Age	58	70	63	70	53	60	59
Gender	M	F	F	F	M	M	F
CH/LC	LC	CH	LC	LC	LC	LC	CH
HCV/NBNC	HCV	HCV	HCV	NBNC	HCV	HCV	HCV
AFP (ng/ml)	5.3	8.3	10.7	10.9	27.8	28.5	32.0
c-AFP-L3%	ND	ND	29.5	4.9	15.9	12.2	3.4
hs-AFP-L3%	6.0	7.0	32.6	8.4	12.2	9.6	3.7
ALT (IU/l)	31	48	23	39	41	65	116
Months until HCC detection	13	31	5	13	18	8	31

F, female; M, male; ND, not detectable.

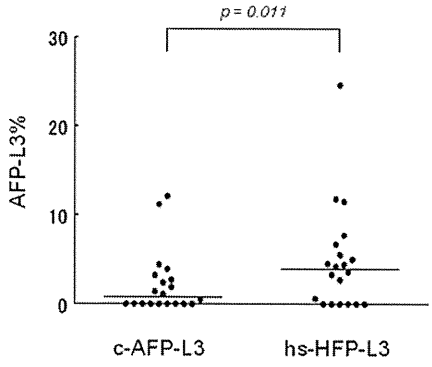

Figure 5. Patients with well-differentiated HCC showed an increase in hs-AFP-L3%. hs-AFP-L3% (HS) was significantly higher than c-AFP-L3% in patients with well-differentiated HCC; this was confirmed by histological examination.

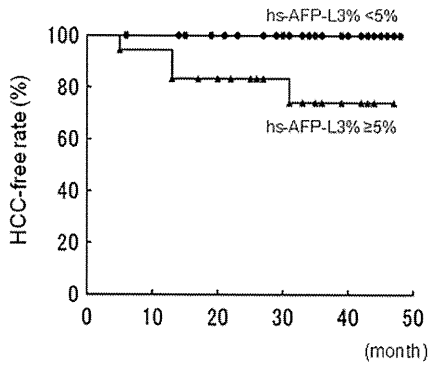

Figure 6. No patients with both serum AFP <20 ng/ml and hs-AFP-L3% <5% developed HCC. Patients with BLD (n=74) were periodically followed by US, CT, or MRI during the follow-up period (median, 35 months; range, 5-48 months). In cases of BLD with AFP <20 ng/ml (n=59), HCC was newly detected in 4 patients with hs-AFP-L3% ≥5%. The HCC-free rate in patients with hs-AFP-L3% ≥5% (▲) was significantly higher than in patients with hs-AFP-L3% <5% (●) (log-rank test and Wilcoxon test; p=0.0012 and p=0.0017, respectively). Importantly, no patients with hs-AFP-L3% <5% developed HCC.

at a cut-off level of 5% were 44.6 and 71.2%, whereas those of c-AFP-L3% were 12.5 and 98.3%, respectively. These results suggest that hs-AFP-L3% is useful for early detection of HCC, even when serum AFP is <20 ng/ml.

Serum hs-AFP-L3% increases in patients with well-differentiated HCC. Most HCC, initially present as well-differentiated HCC, develops in patients with chronic liver disease. Therefore, we evaluated c-AFP-L3% and hs-AFP-L3% in 20 patients with well-differentiated HCC, which was confirmed by histological examination. Fifteen patients (75.0%) exhibited small HCCs (<20 mm), and 9 (45.0%) suffered from liver cirrhosis. Serum AFP was 14.2±12.4 ng/ml (1.4-54.1), and 18 patients (90%) exhibited serum AFP levels <20 ng/ml. hs-AFP-L3% was measurable in 14 patients (70%), while 11 patients (55%) exhibited detectable levels of c-AFP-L3% (Fig. 5). Consequently, hs-AFP-L3% was significantly higher than c-AFP-L3% [4.81±5.91 (0.6-24.6) vs. 2.24±3.53% (0.5-12.1), p=0.011]. These results support the possible utility of hs-AFP-L3% for detection of early-stage HCC.

hs-AFP-L3% increases prior to detection of HCC in patients with BLD. Seven of 74 patients with BLD developed HCC during the follow-up period (median, 35 months; range, 5-48) (Table III). Five patients suffered from liver cirrhosis, and 6 exhibited hepatitis C virus infection. Two of the patients with chronic hepatitis required a longer period (31 months) for appearance of HCC than did the 5 patients with cirrhosis (5-18 months). Five patients exhibited measurable c-AFP-L3%, and an increase in c-AFP-L3% (≥5%) was observed in 3 patients. In contrast, hs-AFP-L3% was measurable in all 7 patients prior to detection of HCC, and 6 patients (85.7%) exhibited hs-AFP-L3% ≥5%. In 59 BLD patients with serum AFP <20 ng/ml, 4 patients developed HCC (Table III). An increase in c-AFP-L3% (≥5%) was observed only in 1 patient, who developed HCC during the follow-up period, whereas the other three patients exhibited undetectable levels or <5% of c-AFP-L3%. Conversely, all 4 patients with serum AFP <20 ng/ml exhibited an increase in hs-AFP-L3% (≥5%) prior to detection of HCC.

Next, we analyzed the HCC-free rate in BLD patients with serum AFP <20 ng/ml during the follow-up period (Fig. 6). The HCC-free rate in patients with hs-AFP-L3% ≥5% was

significantly higher than those with hs-AFP-L3% <5%. Of importance, HCC was not detected in BLD patients with both serum AFP <20 ng/ml and hs-AFP-L3% <5%, whereas 3 out of 58 patients with both serum AFP <20 ng/ml and <5% of c-AFP-L3% developed HCC. These results suggest that an increased hs-AFP-L3% allows prediction of HCC development; measurement of hs-AFP-L3% is useful for selecting BLD patients with higher risk of HCC.

Discussion

Most HCC occurs in patients with chronic liver diseases, especially cirrhosis. Therefore, periodical measurement of tumor markers for HCC, such as AFP and DCP, is recommended in patients who are at high risk for HCC. However, recent advances in diagnostic imaging techniques, including US, CT and MRI, facilitate the detection of small and early-stage HCC (19-21), resulting in an increase in the number of HCC patients diagnosed without an observed increase in serum AFP. Indeed, the 18th survey and follow-up study of primary liver cancer in Japan has reported that most patients with HCC exhibited low levels of serum AFP, <15 ng/ml. Additionally, although AFP-L3% status is known to be a specific marker for HCC, measurement of c-AFP-L3% has not always been reliable in patients with AFP <20 ng/ml.

In this study, we investigated the clinical utility of hs-AFP-L3%, which was measured by a newly developed and highly sensitive method, μ-TAS, in patients with BLD and HCC. Here, we showed that although most HCC patients with stage I cancer did not exhibit an increase in serum AFP levels (≥20 ng/ml), hs-AFP-L3% was measurable in ~70% of the patients, and was significantly increased in comparison with c-AFP-L3% (Fig. 2). Since hs-AFP-L3% is reliable even when serum AFP is <20 ng/ml, it is possible to set the cut-off value for hs-AFP-L3% at 5-7% (18,22,23). We show here that at a cut-off level of 5%, the sensitivity and specificity of hs-AFP-L3% were 44.6 and 71.2%, respectively, in HCC patients with serum AFP <20 ng/ml (Fig. 4). Recent investigations have shown that diagnostic sensitivity of hs-AFP-L3% at a cut-off level of 5 or 7% was 41.5 or 41.1%, respectively, in HCC patients with serum AFP <20 ng/ml (18,22). Therefore, our findings in this study support the specificity of hs-AFP-L3% in patients with serum AFP <20 ng/ml, as previously reported.

The sensitivity of c-AFP-L3% is relatively low (22.2-38.6%) in early-stage HCCs <20 mm in diameter (24,25). In this study, although the sensitivity of c-AFP-L3% was <20% in patients with HCC at stage I, hs-AFP-L3% was significantly higher than c-AFP-L3% in patients with solitary or small (<20 mm) HCC or with stage I HCC (Figs. 2 and 3); consequently, ~50% of HCC patients at stage I exhibited hs-AFP-L3% ≥5%. Additionally, in patients with well-differentiated HCC, hs-AFP-L3% was also significantly higher than c-AFP-L3%. Conversely, patients with stage III or IV HCC (multiple or larger (≥20 mm) tumors) exhibited an increase in both hs- and c-AFP-L3%, with no statistical difference. HCC initially develops as well-differentiated HCC, and then progresses to moderately- to poorly-differentiated HCC via a process of dedifferentiation. Thus, an increase in hs-AFP-L3% in patients with well-differentiated HCC and early-stage HCC supports the conclusion that measurement of hs-AFP-L3% is useful for early detection of HCC.

HCC often develops in patients with chronic infection of hepatitis B or C virus; especially in patients with chronic HCV infection, the annual incidence of HCC increases as a function of the stage of liver fibrosis, from 0.5% at stages F0 to F1 to 7.9% at stage F4 (cirrhosis) (26). Recently, Tateyama *et al* demonstrated that elevated AFP levels are a risk factor for the development of HCC in patients with HCV infection; the 10-year cumulative incidence rates of HCC in the patients with AFP levels of <6, 6-20 and ≥20 ng/ml at entry were 6.0, 24.6 and 47.3%, respectively, and that AFP levels may be used as a non-invasive and predictive marker in place of stage of fibrosis (27). In this study, all 7 BLD patients who developed HCC during the follow-up period exhibited measurable hs-AFP-L3% prior to detection of HCC, and 6 patients exhibited hs-AFP-L3% ≥5%. Of particular note, even when serum AFP levels increased to up to 20 ng/ml, HCC was not detected in patients with hs-AFP-L3% <5% (Fig. 6).

Although prolonged observation will be required in order to clarify whether hs-AFP-L3% is useful for prediction of HCC, the findings presented here indicated that hs-AFP-L3% is useful for early detection of HCC in BLD patients even with serum AFP <20 ng/ml, and also that an increase in hs-AFP-L3% prior to detection of HCC by various advanced imaging modalities may contribute to more precisely identifying BLD patients with a higher risk of HCC.

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Sorafenib and hepatic arterial infusion chemotherapy for unresectable advanced hepatocellular carcinoma: A comparative study

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Abstract. Sorafenib is a kinase-targeted drug that has high efficacy for advanced hepatocellular carcinoma (HCC). The aim of the present study was to determine whether sorafenib is more effective than hepatic arterial infusion chemotherapy (HAIC) for HCC. Twenty patients treated with sorafenib (sorafenib group) initiated at 800 mg/day and 45 patients treated with HAIC (HAIC group) for unresectable Child-Pugh A advanced HCC were investigated retrospectively. The treatment effect was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST). As a result, the overall response rate was significantly lower in the sorafenib group than in the HAIC group (P=0.03), while the disease control and survival rates did not differ between the two groups. In the sorafenib group, treatment was discontinued in 19 patients, including 12 due to side effects. In subgroups of patients treated with sorafenib, the survival rate was significantly lower in patients (n=11) administered sorafenib for <60 days compared to those (n=9) treated for ≥60 days. A shorter treatment period (<60 days) was an independent risk factor for unfavorable survival [hazard ratio (HR), 3.34; 95% confidence interval (CI), 1.45-7.66 vs. HAIC], while survival in patients treated with sorafenib for ≥60 days did not differ from those treated with HAIC (HR, 0.79; 95% CI, 0.27-2.34). In conclusion, the disease control and survival rates of patients treated with sorafenib for advanced HCC were comparable to such rates in patients treated with HAIC. However, the prognosis was poor when long-term sorafenib treatment was not possible due to side effects, demonstrating the importance of patient selection for sorafenib treatment.

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Key words: hepatocellular carcinoma, sorafenib, hepatic arterial infusion chemotherapy, prognosis, side effect

Introduction

Hepatocellular carcinoma (HCC) is a highly prevalent cancer worldwide, and is frequently caused by infection with hepatitis B or C. Early-stage HCC can be cured by surgery or local ablation, and inhibition of recurrence has been achieved using antiviral agents. However, prevention of HCC recurrence after radical treatment remains insufficient. Many HCC cases are in an advanced stage or unresectable at the time of diagnosis. Moreover, although unresectable advanced HCC can be treated with hepatic arterial infusion chemotherapy (HAIC) and systemic chemotherapy, the therapeutic effects are limited (1-3) and the prognosis of advanced cases of HCC is poor.

Large-scale randomized placebo-controlled trials have shown that sorafenib, a multi-kinase inhibitor, prolongs overall and disease progression-free survival in patients with HCC (4,5). Based on these findings, sorafenib is recommended for treatment of advanced stage HCC (Child-Pugh A-B, grade 1-2 performance status cases with portal tumor thrombus, lymph node metastasis or distant metastasis) in the Barcelona Clinic Liver Cancer (BCLC) staging system-based therapeutic strategy for HCC (6). In Japan, the use of sorafenib for unresectable hepatocellular carcinoma was approved in May 2009, and the indication for sorafenib may be expanded in the future. However, to date, the effect of sorafenib has only been compared with untreated groups, and further evidence is required to position sorafenib in the treatment algorithm for HCC, for which various treatment methods are available (7,8).

The response rate of advanced HCC to HAIC is approximately 30-40% (9-16), and HAIC (as well as sorafenib) is recommended for treatment of advanced HCC, particularly in Japan (17,18). However, comparison of the effects of sorafenib with other treatment methods for HCC has not been

carried out. Therefore, in the present study, we retrospectively compared the efficacy of sorafenib for unresectable advanced HCC with that of HAIC.

Patients and methods

Patients. A total of 107 Child-Pugh class A patients with advanced HCC were treated at Kagoshima Kouseiren Hospital between July 1, 2004 and May 31, 2010; 72 patients were treated with HAIC and 35 with sorafenib. Diagnosis of HCC was established based on typical radiographic evidence and tumor markers such as α-fetoprotein (AFP) and des-γ-carboxy prothrombin [DCP, also known as protein induced by vitamin K absence or antagonist (PIVKA-II)].

Of the 107 patients, 65 were selected for further analysis based on the criteria below. These patients included 45 treated with HAIC and 20 treated with sorafenib. All 65 patients had advanced HCC unsuitable for surgical resection, liver transplantation, or nonsurgical interventions [such as radiofrequency ablation or transcatheter arterial chemoembolization (TACE)] because of multiple tumors involving both lobes of the liver or portal invasion in the first or main portal branch (19). Other eligibility criteria included Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1 for sorafenib or 0 to 2 for HAIC, no other serious medical condition, no history of systematic chemotherapy with sorafenib, no concurrent malignancy of another type, and previously described laboratory findings for sorafenib (20). In addition, we excluded patients who had more than two distant metastases or a distant metastasis of size >1 cm.

The average total and daily alcohol consumption was calculated assuming that 633 ml of beer or 120 ml of shochu (a traditional Japanese distilled spirit) contains 25 g of ethanol, which is the typical ethanol content of Japanese beer and shochu. Excess alcohol intake was defined as >75 g of ethanol per day, using data obtained by questionnaire. Body mass index (BMI) was calculated by dividing body weight (kg) by the square of the height (m^2). Informed consent was obtained from all patients before treatment. This study was performed retrospectively and was approved by the Ethics Committee of the Kagoshima Prefectural Federation of Agricultural Cooperatives for Health and Welfare.

Treatment and dose modification. Sorafenib for unresectable HCC was approved in Japan in May 2009. Before this date, all patients underwent HAIC, while after May 2009 patients were treated with sorafenib or HAIC. HAIC was administered in three regimens. Regimen A consisted of daily cisplatin (5 mg/m^2) followed by 5-fluorouracil (5-FU, 250 mg/body) on days 1-5, 8-12 and 15-19, with days 6, 7, 13, 14, 20 and 21 as rest days. Cisplatin and 5-FU were administered by a mechanical infusion pump through implanted reservoir over 1 and 23 h, respectively (21). Regimen B consisted of cisplatin (50 mg/body), mitomycin C (MMC, 10 mg/body) and epirubicin (EPI, 30 mg/body) as a bolus injection on day 1, and daily cisplatin (5 mg/m^2) followed by 5-FU (250 mg/body) on days 8-12 and 15-19. Cisplatin and 5-FU after day 8 were administered by a mechanical infusion pump through implanted reservoir over 1 and 23 h, respectively. Regimen C consisted of cisplatin (50 mg/body), MMC (10 mg/body) and EPI (30 mg/body) as a bolus injection through a catheter on day 1. All anticancer agents were administered through the common or proper hepatic artery. Regimens A, B and C were administered to 25, 12 and 8 patients, respectively, and the cycles were repeated when possible. Sorafenib was administered orally at 400 mg twice a day. Selection of the treatment was made by each physician, patient or family members after informed consent was obtained.

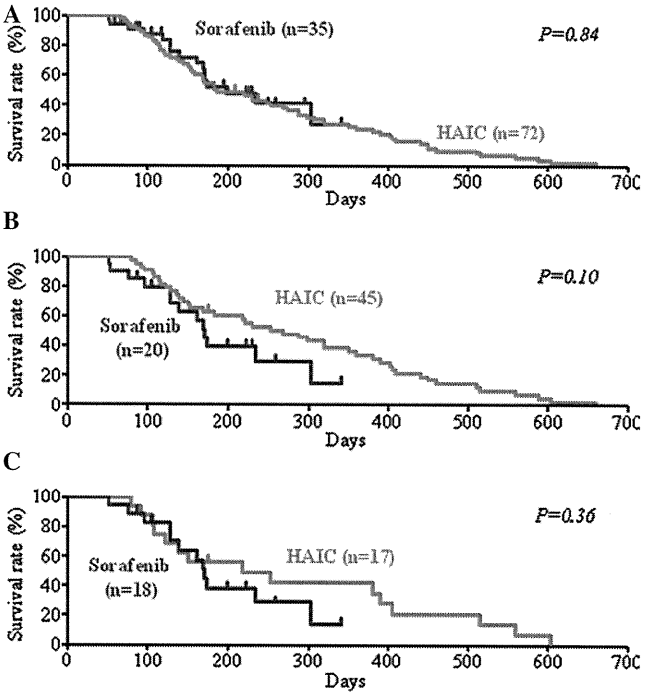

Figure 1. Accumulated survival rates of patients with advanced hepatocellular carcinoma treated with hepatic arterial infusion chemotherapy (HAIC) and sorafenib. (A) Seventy-two patients were treated with HAIC and 35 patients were treated with sorafenib at our hospital. There was no significant difference in the survival rate between the two groups (P=0.84). (B) Among the patients in A, 45 treated with HAIC and 20 treated with sorafenib were selected for further analysis using the criteria described in Materials and methods. The survival rate of these two groups did not differ significantly (P=0.10). (C) Among the patients in B, 17 treated with HAIC and 18 treated with sorafenib were previously treated by modalities such as transarterial chemoembolization. The survival rate of these two groups also did not differ significantly (P=0.36).

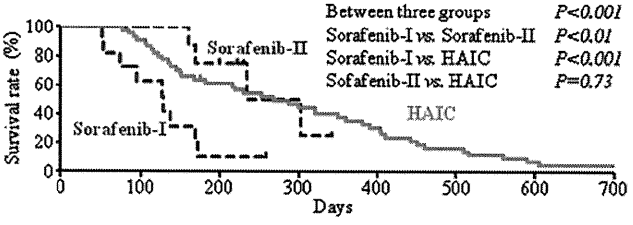

Figure 2. Accumulated survival rate of patients with advanced hepatocellular carcinoma treated with sorafenib for <60 days (n=11, sorafenib-I), sorafenib for ≥60 days (n=9, sorafenib-II), and hepatic arterial infusion chemotherapy (HAIC). The definition of the sorafenib-I and sorafenib-II subgroups is described in Materials and methods. The survival rate was lower in the sorafenib-I subgroup compared to the sorafenib-II subgroup and HAIC group.

Table I. Clinical characteristics and tumor-related background factors of the advanced hepatocellular carcinoma patients treated with HAIC and sorafenib[a].

Factor	HAIC (n=45)	Sorafenib (n=20)	P-value[b]
Age (range), in years	69.6 (47-84)	69.6 (44-83)	0.88
Gender (male/female)	0.71/0.29	0.85/0.15	0.35
Virus marker (HBV/HCV/NBNC)	0.24/0.40/0.36	0.25/0.50/0.25	0.74
Excess alcohol intake[c] (+/-)	0.31/0.69	0.35/0.65	0.77
Diabetes mellitus (+/-)	0.27/0.73	0.30/0.70	0.77
Body weight (kg)	54.6 (37.8-72.5)	55.9 (38.4-68.9)	0.87
Body mass index (kg/m^2)	20.6 (15.8-27.1)	21.9 (16.0-28.4)	0.69
Total bilirubin (mg/dl)	1.2 (0.3-2.7)	1.1 (0.5-1.9)	0.80
AST (IU/l)	74.7 (22-206)	80.6 (25-201)	0.39
ALT (IU/l)	53.2 (13-198)	53.1 (17-178)	0.74
Serum albumin (g/dl)	3.6 (2.4-4.5)	3.6 (2.8-4.4)	0.73
White blood cell (x10^3/μl)	3.8 (2.1-5.7)	4.2 (2.5-6.8)	0.88
Neutrophils (x10^3/μl)	2.3 (1.1-4.1)	2.6 (1.3-5.0)	0.82
Platelet count (x10^4/μl)	16.3 (5.3-47.7)	14.0 (6.1-26.2)	0.48
Prothrombin time (%)	81.1 (56-100)	87.4 (58-115)	<0.05
α-fetoprotein (x10^3 ng/ml)	8.8 (0-55.9)	7.3 (0-97.3)	0.16
DCP (x10^3 mAU/ml)	11.5 (0-176.0)	11.4 (0-86.8)	0.92
Tumor diameter (≥50 mm/<50 mm)	0.49/0.51	0.40/0.60	0.60
Tumor thrombus (Vp3 or 4; +/-)	0.64/0.36	0.50/0.50	0.29
Distant metastasis (+/-)	0.13/0.87	0.25/0.75	0.29
Previous treatment (+/-)	0.38/0.62	0.90/0.10	<0.001
Locoregional therapy[d]	0.9 (0-2)	1.8 (0-6)	0.23
TACE[e]	2.0 (0-4)	4.3 (0-8)	<0.001

[a]Data are presented as geometric means (range) or proportions. [b]P-values were obtained by the Mann-Whitney U test or Fisher's exact test as appropriate. [c]Defined as >75 g of ethanol per day based on data obtained by questionnaire. [d]Average frequency (number of times) of locoregional therapy including surgery or radiofrequency ablation was evaluated. [e]Average frequency (number of times) of transarterial chemoembolization (TACE) was evaluated. HAIC, hepatic arterial infusion chemotherapy; HBV, positive for hepatitis B virus antigen (HBsAg); HCV, positive for anti-hepatitis C virus antibody (HCV Ab); NBNC, negative for both HBsAg and HCV Ab; AST, asparate aminotransferase; ALT, alanine aminotransferase; DCP, des-γ-carboxy prothrombin.

Evaluation. The therapeutic effect was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) (22). HAIC was evaluated for every course (every 4 or 6 weeks), and sorafenib treatment was evaluated every month using computed tomography (CT) and tumor markers such as AFP and DCP. Side effects were evaluated following the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 (JCOG/JSCO edition) (23). The discontinuation criteria were as follows: difficulty with continuation of treatment due to disease progression or an adverse effect of grade 3 or higher, a Child-Pugh score ≥10 points or uncontrollable hepatic encephalopathy, intractable ascites, total bilirubin ≥4.0 mg/dl, or a performance status of grade 3 or 4 or worsening of the grade by ≥2 categories. Additional therapies were administered on the basis of performance status, hepatic reserve capacity, tumor responses to HAIC or sorafenib, and complications.

The primary endpoint was overall survival (OS), and the secondary efficacy endpoints were overall response rate [ORR = complete response (CR) + partial response (PR)] and disease control rate [DCR = CR + PR + stable disease (SD)]. OS was calculated from the time of the first treatment with HAIC or sorafenib until death or the last follow-up visit.

Statistical analysis. For comparison between two independent groups, the Mann-Whitney U test and Fisher's exact test were used as appropriate. For the cumulative survival and discontinuation rates, a log-rank test was performed using the Kaplan-Meier method. For multivariate analysis, logistic regression analysis and a Cox proportional hazards model were used. Cluster analysis was used to divide the sorafenib group into a limited number of maximally differing clusters based on the duration of sorafenib administration. This analysis was performed using the hierarchical agglomerative clustering method (24). A P-value <0.05 was considered to indicate a significant difference. The analyses were performed using XLSTAT version 2009 (Addinsoft Corp., New York, NY).

Table II. Comparison of the therapeutic effects and discontinuation of treatment between the HAIC- and sorafenib-treated groups[a].

Decision case (%)	HAIC (n=45)	Sorafenib (n=20)	P-value[b]
Effect: overall response			
Complete response (CR)	2 (4.4)	0 (0.0)	0.150
Partial response (PR)	8 (17.8)	0 (0.0)	
Stable disease (SD)	21 (46.7)	13 (65.0)	
Progressive disease (PD)	14 (31.1)	7 (35.0)	
ORR (CR+PR)	10 (22.2)	0 (0.0)	0.030
DCR (CR+PR+SD)	31 (68.8)	13 (65.0)	0.780
Discontinuation/continuation	41 (91.1)/4 (8.9)	19 (95.0)/1 (5.0)	1.000
Reason of discontinuation			
Disease progression	41 (91.1)	7 (35.0)	<0.001
Side effects	0 (0.0)	12 (60.0)	
Liver dysfunction	0	3	
Skin disorder	0	4	
Diarrhea	0	4	
Hepatic encephalopathy	0	1	

[a]Date are presented as number (proportions). [b]P-values were obtained by Fisher's exact test as appropriate. HAIC, hepatic arterial infusion chemotherapy; ORR, overall response rate; DCR, disease control rate.

Results

Comparison of background factors, tumor factors and survival rate between the HAIC and sorafenib groups. Of the 107 patients with advanced HCC treated at our hospital between July 1, 2004 and May 31, 2010, the survival rate did not differ between the 72 patients treated with HAIC and the 35 patients treated with sorafenib (Fig. 1A). Among these patients, 45 in the HAIC group and 20 in the sorafenib group were included in further analysis. A comparison of patient background factors between the HAIC and sorafenib groups showed significant differences in prothrombin time (PT) and pre-treatment, but not in age, gender, history of excess alcohol intake, serum albumin, tumor markers, size of the main tumor, and presence or absence of portal vein tumor thrombosis in the first branch or trunk (Vp3 or Vp4, respectively) (Table I). The mean follow-up period was 317 days (55-1438 days) in the HAIC group and 166 days (51-341 days) in the sorafenib group. There was no significant difference in survival rate between the two groups (Fig. 1B), or between subgroups of patients who received pre-treatment in the HAIC and sorafenib groups (Fig. 1C).

Comparison of the therapeutic effects and treatment discontinuation between the HAIC and sorafenib groups. Assessment of the therapeutic effect using RECIST criteria (22) indicated that CR, PR and SD were achieved in 2 (4.4%), 8 (17.8%) and 21 (46.7%) cases, respectively, in the HAIC group, and in 0 (0%), 0 (0%) and 13 (65.0%) cases, respectively, in the sorafenib group (Table II). Thus, the overall response rate (ORR) in the sorafenib group was significantly lower than that in the HAIC group (0 vs. 22.2%, P=0.03). However, there was no significant difference in the DCR between the 2 groups (65.0 vs. 68.8%, P=0.78).

The treatment course, including the discontinuation rate and reasons for discontinuation, were compared between the HAIC and sorafenib groups (Table II). Treatment was discontinued in 41 (91.1%) cases in the HAIC group and in 19 (95%) cases in the sorafenib group, with no significant difference between the groups. However, the reason for discontinuation was disease progression including depressed hepatic reserve function due to HCC progression in all cases in the HAIC group, while the reason for discontinuation was adverse effects of grade 3 or higher in 12 (60.0%) cases in the sorafenib group, showing a significantly higher occurrence of adverse effects in the sorafenib group (P<0.001).

Comparison of sorafenib administration for less than and greater than 60 days. Since administration was discontinued due to side effects in more than half of the patients in the sorafenib group, the cumulative discontinuation rate and duration of administration were investigated using the Kaplan-Meier method. Treatment was discontinued in the early phase (within 60 days) in 11/20 (55%) of the patients. In addition, hierarchical agglomerative clustering identified two sorafenib subgroups, and on this basis the patients (n=20) were divided into those for whom administration was discontinued within a period of <60 days (n=11, sorafenib-I subgroup) and those who received sorafenib for ≥60 days (n=9, sorafenib-II subgroup; administration was discontinued after >60 days or continued). The mean durations (range) of sorafenib administration and follow-up were 31 (14-45) and 123 (51-259) days, respectively, in the sorafenib-I subgroup, and 106 (67-161) and 218 (104-341) days, respectively, in the sorafenib-II subgroup. There were no significant differences in background and tumor factors between the two subgroups (Table III). However, the survival rate differed significantly among the two sorafenib subgroups and the HAIC group (multi-group log-rank test,

Table III. Clinical characteristics of the advanced hepatocellular carcinoma patients treated with sorafenib[a]: Comparative evaluation of the sorafenib-I (administration <60 days) and sorafenib-II (administration ≥60 days) groups.

Factor	Sorafenib-I (n=11)	Sorafenib-II (n=9)	P-value[b]
Age (range), in years	69.9 (44-83)	69.2 (58-78)	0.57
Gender (male/female)	0.82/0.18	0.89/0.11	1.00
Virus marker (HBV/HCV/NBNC)	0.37/0.45/0.18	0.11/0.56/0.33	0.60
Excess alcohol intake[c] (+/-)	0.27/0.73	0.44/0.56	0.64
Diabetes mellitus (+/-)	0.18/0.82	0.44/0.56	0.34
Body weight (kg)	53.6 (38.4-68.4)	58.9 (48.0-68.9)	0.25
Body mass index (kg/m^2)	21.6 (16.0-28.4)	22.2 (18.4-24.8)	0.82
Initial dose of sorafenib (mg/kg/day)	15.5 (6.9-17.4)	13.6 (5.8-15.4)	0.24
Total bilirubin (mg/dl)	1.2 (0.5-1.8)	1.0 (0.6-1.9)	0.21
AST (IU/l)	93.5 (25-201)	64.9 (27-116)	0.32
ALT (IU/l)	56.4 (17-178)	51.2 (26-73)	0.47
Serum albumin (g/dl)	3.5 (2.8-4.4)	3.7 (3.0-4.2)	0.12
White blood cell (x10^3/μl)	4.2 (2.5-6.8)	4.2 (2.6-6.2)	0.88
Neutrophils (x10^3/μl)	2.6 (1.4-5.0)	2.5 (1.3-4.6)	0.88
Platelet count (x10^4/μl)	15.4 (7.0-26.2)	12.4 (6.1-19.1)	0.15
Prothrombin time (%)	85.1 (72-98)	90.2 (58-115)	0.29
α-fetoprotein (x10^3 ng/ml)	0.9 (0-5.2)	12.4 (0-97.3)	0.62
DCP (x10^3 mAU/ml)	17.8 (0-86.8)	4.2 (0-27.9)	0.40
Tumor diameter (≥50 mm/<50 mm)	0.36/0.64	0.44/0.56	1.00
Tumor thrombus (Vp3 or 4; +/-)	0.64/0.36	0.33/0.67	0.37
Distant metastasis (+/-)	0.27/0.73	0.22/0.78	1.00
Previous treatment (+/-)	0.91/0.09	0.89/0.11	1.00
Locoregional therapy[d]	1.9 (0-6)	1.6 (0-4)	0.72
TACE[e]	3.9 (0-7)	4.6 (0-8)	0.40

[a]Data are presented as geometric means (range) or proportions. [b]P-values were obtained by Mann-Whitney U test or Fisher's exact test as appropriate. [c]Defined as >75 g of ethanol per day based on data obtained by questionnaire. [d]Average frequency (number of times) of locoregional therapy including surgery or radiofrequency ablation was evaluated. [e]Average frequency (number of times) of transarterial chemoembolization (TACE) was evaluated. HBV, positive for hepatitis B virus antigen (HBsAg); HCV, positive for anti-hepatitis C virus antibody (HCV Ab); NBNC, negative for both HBsAg and HCV Ab; AST, asparate aminotransferase; ALT, alanin aminotransferase; DCP, des-γ-carboxy prothrombin.

P<0.001) (Fig. 2). A between-group comparison showed that survival was significantly shorter in the sorafenib-I subgroup compared to the sorafenib-II subgroup and the HAIC group. There was no significant difference in survival time between the sorafenib-II subgroup and the HAIC group.

The treatment discontinuation rate was 100% (11 cases) in the sorafenib-I subgroup and 89% (8 cases) in the sorafenib-II subgroup, with no significant difference between the subgroups. The reason for discontinuation was disease progression in 4 cases and side effects in 7 in the sorafenib-I subgroup, and disease progression in 3 cases and side effects in 5 in the sorafenib-II subgroup, with no significant difference between the subgroups (Table IV). However, the reasons for discontinuation of sorafenib differed from those for discontinuation of HAIC (Table II). After discontinuation of sorafenib, HAIC was performed in 2 (18.2%) cases in the sorafenib-I subgroup, and in 6 (66.7%) cases in the sorafenib-II subgroup (P=0.02). Of the 12 cases in which sorafenib was discontinued due to side effects, additional HAIC was performed in 1 of 7 cases in the sorafenib-I subgroup, but in all 5 cases in the sorafenib-II subgroup (14.3 vs. 100%, P=0.02).

Of the factors shown in Table III, body weight, dose of sorafenib/body weight, total bilirubin, serum albumin, platelet counts and prothrombin time (%) differed between the sorafenib-I and -II subgroups at a level of P<0.3. These factors were subjected to multivariate logistic regression analysis, but none was found to be an independent predictor of classification into either of the two subgroups.

Prognostic factors in advanced hepatocellular carcinoma. Prognostic factors were investigated in the 65 patients with advanced HCC. Univariate analysis (log-rank test) of the 18 factors shown in Table V revealed that the survival rate differed significantly between the different treatment methods and between high and low DCP levels. Multivariate analysis using a Cox proportional hazards model was performed using 7 factors with P<0.3 in the univariate analysis [age, gender,

Table IV. Comparison of treatment discontinuation and post-treatment in the sorafenib-I and sorafenib-II groups.

Decision case	Sorafenib-I (n=11)	Sorafenib-II (n=9)	P-value[a]
Discontinuation/continuation	11/0	8/1	0.45
Reason of discontinuation			
Disease progression	4	3	1.00
Side effects	7	5	
Liver dysfunction	2	1	
Skin disorder	2	2	
Diarrhea	2	2	
Hepatic encephalopathy	1	0	
Post-treatment with HAIC			
Yes	2 (1)[b]	6 (5)[b]	0.02
No	9 (6)[b]	2 (0)[b]	(0.02)[b]

[a]P-values were obtained by the Fisher's exact test. [b]The number of patients whose treatment was interrupted by side effects is indicated in parentheses. HAIC, hepatic arterial infusion chemotherapy.

alanine aminotransferase (ALT), DCP, tumor thrombus, tumor size, and treatment method]. From this analysis, a DCP level ≥1000 and discontinuation of sorafenib within 60 days (sorafenib-I subgroup) were independent risk factors contributing to a poor prognosis, and the hazard ratio in the sorafenib-I subgroup was 3.34 compared to HAIC (Table V). To eliminate the possible bias of the 4 cases in which treatment was discontinued due to marked disease progression in the sorafenib-I subgroup, survival rate and prognostic factors were analyzed for the 7 cases in which treatment was discontinued due to side effects and in which the disease condition was not markedly changed. These 7 patients were compared with the sorafenib-II subgroup and the HAIC group. In this analysis, administration of sorafenib for <60 days remained a poor prognostic factor by log-rank test (P=0.01 vs. sorafenib-II; P<0.01 vs. HAIC).

Discussion

Prolongation of survival by sorafenib compared to a placebo and the efficacy of HAIC for advanced HCC have been reported (1,2,4,5,10-12). However, comparison of the efficacy between sorafenib and HAIC has not been investigated. In this retrospective study, we demonstrated that the disease control rate (DCR=CR+PR+SD) and OS rate in patients with advanced unresectable HCC did not differ significantly between sorafenib treatment and HAIC, although the overall response rate (ORR=CR+PR) with sorafenib treatment was lower than that for HAIC.

The prognosis was also found to be very poor when sorafenib treatment was discontinued within 60 days due to serious side effects. Although it is possible that the Kaplan-Meier curves for subgroups established based on events during the follow-up period (i.e., treatment cessation) included serious bias, side effects led to the discontinuation of treatment in more than half of the the cases in the sorafenib group, and many of these cases received additional treatment that may have prolonged survival. Thus, the present study suggests that early discontinuation of sorafenib was the most important factor influencing survival of patients with advanced HCC of Child-Pugh A, even if the reason for discontinuation was not disease progression.

A prospective cohort study on the combination therapy of HAIC and sorafenib for advanced HCC is currently underway (25), but the therapeutic effects of regimens including combination therapy and monotherapy of HAIC or sorafenib remain unclear. In the present study, the survival rate was comparable between HAIC and sorafenib (Fig. 1), and there was no significant difference in the DCRs (Table II). However, the overall response rate for sorafenib was 0%, which was significantly lower than that for HAIC. In the SHARP study (4), the response rate of sorafenib was 2% and the DCR was 43%, suggesting that the therapeutic effect depended on control of disease progression, but not on tumor size reduction. Similar findings were observed in a phase II study of sorafenib conducted in the US and Europe (26), and in a phase I study conducted in Japan (20). Our study suggests that a similar survival rate may be achieved by different treatment methods independently of the response rate when the DCR is similar. Therefore, it is important to consider not only the response rate but also the DCR, including SD cases, in the treatment of advanced HCC.

Discontinuation of HAIC occurred in 41 cases and was due to disease progression in all cases, whereas discontinuation of sorafenib occurred due to side effects in 12 cases (60.0% of the patients treated with sorafenib) (Table II). Typical side effects of sorafenib include skin disorder (including hand-foot skin reactions), hypertension, liver dysfunction, hepatic encephalopathy, diarrhea, interstitial lung disease and hemorrhage; and the incidence of hand-foot skin reactions and diarrhea are high (25,27). In our study, discontinuation of treatment was sometimes avoided by prevention and countermeasures, but discontinuation due to liver dysfunction, diarrhea and erythema multiforme was required in many cases. The

Table V. Evaluation of the prognostic factors in the advanced hepatocellular carcinoma cases.

	Univariate[a]		Multivariate[b]		
Factor (categories)	n=65	P-value	HR	(95% CI)	P-value
Age (≥70/<70), in years	35/30	0.07	0.65	(0.35-1.19)	0.16
Gender (male/female)	49/16	0.10	1.37	(0.65-2.87)	0.41
Virus marker (HBV or HCV/NBNC)	44/21	0.77			
Excess alcohol intake[c] (+/-)	21/44	0.36			
Diabetes mellitus (+/-)	18/47	0.43			
TB (mg/dl) (≥1.2/<1.2)	31/34	0.35			
AST (IU/l) (≥50/<50)	41/24	0.56			
ALT (IU/l) (≥40/<40)	37/28	0.23	1.03	(0.57-1.87)	0.92
ALB (g/dl) (≥3.5/<3.5)	43/22	0.99			
PLT (x$10^4\mu$l) (≥15/<15)	31/34	0.70			
PT (%) (≥80/<80)	43/22	0.79			
AFP (ng/ml) (≥1000/<1000)	32/33	0.98			
DCP (mAU/ml) (≥1000/<1000)	36/29	0.02	1.87	(1.03-3.38)	0.04
Tumor thrombus (Vp3 or 4) (+/-)	39/26	0.19	1.25	(0.67-2.31)	0.48
Tumor diameter (mm) (≥50/<50)	29/36	0.10	1.37	(0.74-2.51)	0.31
Distant metastasis (+/-)	11/54	0.36			
Previous treatment (+/-)	36/29	0.37			
Treatment					
HAIC	45	<0.001	1		
Sorafenib-I[d]	11		3.34	(1.45-7.66)	<0.01
Sorafenib-II[d]	9		0.79	(0.27-2.34)	0.67

[a]Univariate analysis was conducted on the 18 factors by employing the log-rank test. [b]Multivariate analysis was conducted on 7 factors with P<0.3 in the univariate analysis by employing the Cox proportional hazards model. [c]Defined as >75 g of ethanol per day based on data obtained by questionnaire. [d]Duration of sorafenib administration was <60 days (sorafenib-I) or 60 days or longer (sorafenib-II). HR, hazard ratio; HBV or HCV, positive for hepatitis B antigen (HBsAg) or hepatitis C virus antibody (HCV Ab); NBNC, negative for both HBsAg and HCV Ab; TB, total bilirubin; AST, asparate aminotransferase; ALT, alanine aminotransferase; ALB, serum albumin, PLT, platelet count; PT, prothrombin time; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HAIC, hepatic arterial infusion chemotherapy.

incidence of adverse events of grade 3 or higher caused by sorafenib was 13% in the SHARP study and 9% in the Asia-Pacific study (4,5), and tolerability was favorable in these studies. However, complications of liver failure and hepatic encephalopathy have been reported, and a causal relationship with development of liver failure within 3 weeks of sorafenib administration and death has been suggested.

In our study, improvement of symptoms after discontinuation was slow in patients who developed severe side effects in the early phase (administration <60 days), and malaise, anorexia and fever developed. Many patients died without switching to other treatments due to concomitant malnutrition and disease progression. In contrast, patients who developed no or mild side effects in the early phase were able to tolerate long-term administration (≥60 days); even in cases in which drug administration was finally discontinued due to side effects, patients recovered from the side effects and a switch to another therapy was possible. These clinical differences may have influenced the differences in survival. Using the Kaplan-Meier method, the survival time was significantly shorter in the sorafenib-I subgroup compared to that in the sorafenib-II subgroup and the HAIC group (Fig. 2). In addition, short-term sorafenib administration (<60 days) was an independent factor predicting a poor prognosis in multivariate analysis using a Cox proportional hazards model (Table V). The DCP level has been reported to be a factor contributing to the prognosis of HCC (28). Since the condition of the patients in the sorafenib-I subgroup influenced the prognosis, in addition to DCP, it is likely that severe early side effects of sorafenib and the associated discontinuation of treatment markedly influence the survival of patients with advanced HCC.

Only a few treatment methods are available for advanced HCC accompanied by portal invasion in the first portal branch or main portal branch (29,30). In the treatment algorithm for HCC in Japan, sorafenib and HAIC are recommended for such cases of advanced HCC, but the first choice has not been specified. Based on our results, the survival rate did not differ significantly between sorafenib treatment and HAIC (Fig. 1), but the survival rate of patients was lower in patients who discontinued sorafenib treatment in the early phase of therapy

compared to the survival rate of patients who tolerated long-term sorafenib treatment (sorafenib-II) and those treated with HAIC (Fig. 2). In addition, HAIC was applicable after side effect-associated discontinuation in some cases in patients treated with long-term sorafenib, whereas sorafenib was not administered to any patients in the HAIC group because the discontinuation of HAIC was due to disease progression in all cases. Sorafenib in combination with other treatments, including HAIC, is likely to markedly prolong the overall survival of HCC patients, including those in an advanced stage (31). However, Abou-Alfa *et al* concluded that the combination of sorafenib and intravenous doxorubicin is not yet indicated for routine clinical use, based on the results of a double-blind phase II multinational study (32). Based on these findings, we speculate that patients with advanced HCC accompanied by portal invasion in the first portal branch or main portal branch should first be treated with sorafenib if they are likely to tolerate sorafenib for more than 60 days. HAIC should then be considered as an additional treatment for cases in which sorafenib cannot be continued.

The effect of sorafenib has been suggested to depend on the treatment period, which is influenced by the development of serious side effects. Vincenzi *et al* reported that the tumor control rate was higher in patients with skin toxicity of grade 1 or higher than in those without this toxicity (48.3 vs. 19.4%) (33). After dose reduction for skin toxicity, it has been suggested that the dose can be increased again in some cases after amelioration of the adverse effect (34). Thus, if long-term sorafenib administration can be achieved by controlling skin toxicity, the therapeutic effect may be further increased. Several background factors such as single nucleotide polymorphisms (SNPs) that contribute to the therapeutic effect of interferon on chronic hepatitis C have been reported (35,36). This suggests that tolerability of long-term sorafenib administration may also be predictable before treatment, which may allow the selection of patients for whom sorafenib is appropriate. In this study, we were unable to identify any factors that significantly discriminated between patients with and without tolerability of long-term sorafenib. Thus, further analysis, including examination of SNPs, is required for safe and effective sorafenib treatment for HCC in an increased number of cases (37).

There were several limitations to this study. First, this was a retrospective study, and the number of cases was small; therefore, a bias due to the unbalanced number of cases cannot be ruled out. However, no previous study has compared the effect of sorafenib on advanced HCC with those of other treatments, and makes the findings valuable in the absence of other information. Second, HAIC was performed after discontinuation of sorafenib treatment due to side effects in 8 cases; therefore, the effect of sorafenib alone could not be assessed in these cases. However, less than one cycle of HAIC was performed after discontinuation of sorafenib, suggesting that the additional effect of HAIC may have been limited.

In conclusion, treatment of advanced HCC with sorafenib may achieve a survival rate equivalent to that achieved by HAIC, through control of disease progression independent of tumor size reduction. However, early discontinuation of sorafenib due to adverse effects may be associated with a poor prognosis, and further investigation of the eligibility criteria for sorafenib administration is required.

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Impact of Radiation and Hepatitis Virus Infection on Risk of Hepatocellular Carcinoma

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In cohort studies of atomic bomb survivors and Mayak nuclear facility workers, radiation-associated increases in liver cancer risk were observed, but hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were not taken strictly into account. We identified 359 hepatocellular carcinoma (HCC) cases between 1970 and 2002 in the cohort of atomic bomb survivors and estimated cumulative incidence of HCC by radiation dose. To investigate contributions of radiation exposure and hepatitis virus infection to HCC risk, we conducted a nested case-control study using sera stored before HCC diagnosis in the longitudinal cohort of atomic bomb survivors. The study included 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, and time and method of serum storage, and countermatched on radiation dose. The cumulative incidence of HCC by follow-up time and age increased significantly with radiation dose. The relative risk (RR) of HCC for radiation at 1 Gy was 1.67 (95% confidence interval: 1.22-2.35) with adjustment for alcohol consumption, body mass index (BMI), and smoking habit, whereas the RRs for HBV or HCV infection alone were 63 (20-241) and 83 (36-231) with such adjustment, respectively. Those estimates changed little when radiation and hepatitis virus infection were fit simultaneously. The RR of non-B, non-C HCC at 1 Gy was 1.90 (1.02-3.92) without adjustment for alcohol consumption, BMI, or smoking habit and 2.74 (1.26-7.04) with such adjustment. *Conclusion:* These results indicate that radiation exposure and HBV and HCV infection are associated independently with increased HCC risk. In particular, radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, BMI, or smoking habit. (HEPATOLOGY 2011;53:1237-1245)

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) are recognized as critically important risk factors for HCC. Our previous study actually showed that about 63% of HCC in atomic bomb survivors is related to HCV infection, 14% to HBV infection, and 2% to both HBV and HCV infections.[1] However, an increase of non-B, non-C HCC without HBV and HCV infection has been noted recently in Japan.[2,3] The etiology of non-B, non-C HCC has been poorly understood, although alcoholic hepatitis, nonalcoholic fatty liver disease (NAFLD) including nonalcoholic steatohepatitis (NASH), and hemochromatosis[4,5] are known as risk factors. In Japan, NAFLD has increased along with Westernization of lifestyle, and most NASH cases have developed due to such lifestyle-related diseases such as obesity, diabetes mellitus, and hyperlipidemia.[6] Obesity and diabetes mellitus, as well as NAFLD, have also recently received increased attention as risk factors for HCC.[1,7-12]

Abbreviations: AHS, Adult Health Study; BMI, body mass index; CI, confidence interval; ERR: excess relative risk; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; RERF, Radiation Effects Research Foundation; RR, relative risk.

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An increased risk of liver cancer with radiation dose among atomic bomb survivors has been reported based on tumor registries, mortality studies, and pathology review,[13-16] but hepatitis virus infection status was not taken into account. In three previous HBV studies at the Radiation Effects Research Foundation (RERF), the HBV surface antigen (HBsAg)-positive proportion increased with radiation dose.[17-19] Previous research at RERF demonstrated no increase in the prevalence of anti-HCV antibody (anti-HCV Ab) with radiation dose,[20] but reported supermultiplicative effects between radiation exposure and chronic HCV infection in the etiology of HCC without cirrhosis.[21]

On the other hand, the cohort study in workers at the Mayak nuclear facility demonstrated that the risk of liver cancer mortality was significantly associated with plutonium exposure,[22] and that the incidence of HCC was marginally significantly associated with plutonium exposure.[23] In the latest analysis, a significant plutonium dose-response relationship was observed for liver cancer mortality, with risk reasonably described by a linear function.[24] However, liver cancer in those analyses included hepatoblastoma and intrahepatic cholangiocarcinoma as well as HCC. In addition, hepatitis virus infection status was not taken into account in a strict and in-depth manner, although HCC accounted for most of the liver cancer.

A lifespan study using B6C3F1 mice exposed to continuous low-dose-rate γ rays demonstrated that the incidence of HCC was significantly increased in male mice exposed to total doses equivalent to 8,000, 400, and 20 mGy and in females exposed to 8,000 mGy. However, the incidence of other liver tumors did not significantly increase except for that of hepatoblastoma in males exposed to 400 mGy.[25]

With the aim of determining whether radiation exposure is an independent risk factor for HCC, even after adjusting for hepatitis virus infection, alcohol consumption, body mass index (BMI), and smoking habit, we conducted a nested case-control study among atomic bomb survivors using stored sera. We also evaluated whether radiation, alcohol consumption, increase of BMI, and smoking habit contribute to increased risk for non-B, non-C HCC.

Patients and Methods

Cohorts. The Atomic Bomb Casualty Commission (ABCC) and its successor, the RERF, established the Adult Health Study (AHS) longitudinal cohort in 1958, in which more than 20,000 gender-, age-, and city-matched proximal and distal atomic bomb survivors and persons not present in the cities at the time of bombings are examined biennially in outpatient clinics in Hiroshima and Nagasaki.

Cases and Controls. Incident cancer cases were identified through the Hiroshima Tumor and Tissue Registry and Nagasaki Cancer Registry, supplemented by additional cases detected by way of pathological review of related diseases.[26] As described in our previous study,[1] 359 primary HCC cases were diagnosed among 18,660 AHS participants between 1970 and 2002 who visited our outpatient clinics before their diagnosis. Of these, 229 cases had serum samples obtained within 6 years before HCC diagnosis. After excluding five cases with inadequate stored serum, 224 cases remained for our study. There were no important differences in characteristics such as gender, age at HCC diagnosis, city, alcohol consumption, BMI, or radiation dose to the liver (among exposed persons) between HCC cases excluded due to nonavailability of stored serum and those included in the present study.

Three control sera per case were selected from the at-risk cohort members matched on gender, age, city, and time and method of serum storage, and countermatched on radiation dose in nested case-control fashion.[27] Countermatching (to increase statistical efficiency for studying joint effects of radiation and other factors) was performed using four strata based on whole-body (skin) dose: zero dose (<0.0005 Gy), <0.05 Gy, <0.75 Gy, and ≥ 0.75 Gy (nonzero categories correspond roughly to tertiles of skin dose among all eligible exposed cases). At the time of each case diagnosis, one control serum was selected for each of the three dose strata not occupied by the case. Although the total number of potential matched control serum samples is 672, due to occasional lack of subjects with stored sera who met the matching and countermatching criteria, the total number of control serum samples actually selected was 644, which comprised 488 sera from unique noncase subjects and 156 sera from subjects sampled on repeated occasions.

Laboratory Tests. Virological assays were performed on 211 case and 640 control sera, because 13 case samples and four control samples had insufficient stored sera for these assays. HBsAg and antibody to hepatitis B core antigen (anti-HBc Ab) were measured by enzyme immunoassay (EIA), and anti-HCV Ab was measured by second-generation EIA as described.[28,29] Qualitative detection of HCV RNA among anti-HCV-positive samples was performed using a thermocycler (Whatman Biometra, Goettingen, Germany) based on the nested polymerase chain reaction (PCR) method, as described.[29] HBV infection (HBV+) status was

defined as positive for HBsAg or having a high titer of anti-HBc Ab. HCV infection (HCV+) status was defined as positive for HCV RNA. Non-B, non-C status was defined as negative for HBsAg and not having a high titer of anti-HBc Ab (HBV−) as well as negative for HCV RNA (HCV−).

Radiation Dose. Radiation dose to the liver was estimated for each subject according to Dosimetry System DS02.[30] A weighted sum of the gamma dose in gray plus 10 times the neutron dose in gray was used. Because of the countermatched selection of cases, direct comparison of doses between cases and controls in the study requires that control doses be weighted by the inverses of their selection probabilities.

Information on Alcohol Consumption, BMI, and Smoking Habit. Information on alcohol consumption was obtained from the 1965 AHS questionnaire when available, with missing data complemented using the 1978 mail survey. Alcohol consumption was quantified as volume of each type of alcoholic beverage; mean ethanol amounts were calculated as grams per day as described.[31] BMI (kg/m^2) was calculated from height and weight measured at the AHS examination. Subjects were classified based on BMI quintiles with cutpoints of 19.5, 21.2, 22.9, and 25.0. Following the recommendations for Asian people by the World Health Organization (WHO), the International Association for the Study of Obesity, and the International obesity Task Force,[32] 21.3 to 22.9 kg/m^2 was considered normal, 23.0 to 25.0 kg/m^2 as overweight, and >25.0 kg/m^2 as obese. We used information on BMI obtained 10 years before the time of HCC diagnosis or control matching because this condition is subject to change due to disease progression in the later stages before development of HCC. Information on smoking habit was obtained from the 1965 questionnaire; subjects were categorized as never, current (at time of survey), or former smoker.

Ethical Considerations. This study (RERF Research Protocol 1-04) was reviewed and approved by the Research Protocol Review Committee and the Human Investigation Committee of RERF.

Statistical Analyses. The nested case-control design was analyzed using a partial likelihood method analogous to that used for cohort follow-up studies,[33] which is in practice the same as the conditional binary data likelihood for matched case-control studies[34] except that the subjects (cases and "controls") in the study are not completely independent due to repeated selection. Cumulative incidence of HCC by follow-up time (year) and age was derived according to the method of Nelson and Aalen, using Cox regression to adjust for age at start of follow-up. Cumulative incidence by radiation dose groups (0-0.0009, 0.001-0.999, and 1.0+ Gray) was compared using the Gehan/Breslow generalized Wilcoxon test. All factors other than radiation were analyzed using relative risks (RRs) estimated by a log-linear model. Although radiation exposure could have been adjusted by matching on radiation dose as an additional matching factor in the control selection,[35] in addition to assessing effects of lifestyle factors and viral hepatitis, another purpose of the present study was to examine the effects of radiation exposure after adjustment for possible confounding and interaction by these factors, so matching on radiation—which precludes analysis of radiation risk—was not desirable; rather, we countermatched on radiation.[27,33,36] Radiation risk was analyzed using an excess relative risk (ERR) model (ERR = RR-1) as done previously.[37] The cumulative hazard estimator and comparisons by radiation dose groups were computed using Stata (StataCorp, College Station, TX; v. 11.1); all other analyses were conducted using Epicure (HiroSoft International, Seattle, WA; v. 1.81).

Results

Characteristics of Cases and Controls. Characteristics of the 224 HCC cases and 644 matched controls are shown in Table 1. HCC cases and controls were comparable with respect to gender, age, city, and time and method of serum storage by design. Prevalence of HBV and/or HCV infection status in HCC cases is higher than those in controls. Higher proportions of HCC cases had a history of alcohol consumption of more than 40 g of ethanol per day, were obese (BMI >25.0 kg/m^2), and were current smokers, compared with the controls. HCC cases also received on average higher radiation doses to the liver, compared with the controls.

Cumulative Incidence of HCC by Radiation Dose. Figure 1A,B shows the cumulative incidence of HCC by radiation dose using either follow-up time (adjusted for age at start of follow-up) or age. Of 359 HCC cases diagnosed among 18,660 AHS subjects between 1970 and 2002, the analysis was performed using 322 HCC cases, based on 16,766 subjects with known radiation dose. A significant increase with radiation dose was seen with cumulative incidence both by follow-up time (P = 0.028) (Fig. 1A) and by age (P = 0.0003) (Fig. 1B). The effect of radiation was especially evident at age 60 years or later.

Risk of HCC for Radiation and Hepatitis Virus Infection. Table 2 shows risk of HCC with and without adjustment for categorical alcohol consumption,