Journal of Medical Virology 86:131–138 (2014)

Correlation Between Hepatitis B Virus Surface Antigen Level and Alpha-Fetoprotein in Patients Free of Hepatocellular Carcinoma or Severe Hepatitis

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Alfa-fetoprotein (AFP) is used as a marker of early hepatocarcinogenesis. However, the impact of hepatitis B virus surface antigen (HBsAg) on this relationship in patients with HBV infection is not clear. The present study evaluated the relation between HBsAg and AFP levels at the initial visit in 1,610 untreated HBV patients, free of hepatocellular carcinoma (HCC) or severe hepatitis. The cumulative rate of HCC was significantly lower in patients with a low AFP level (≤10 μg/L; below the upper limit of normal) than in those with a high AFP level (≥11 μg/L) at the initial visit. In patients with HBsAg levels more than 500 IU/ml, HBsAg levels correlated significantly and negatively with AFP levels, and significantly with platelet count. Multivariate analysis of data of patients with HBsAg more than 500 IU/ml identified HBsAg (<7,000 IU/ml), albumin (<3.9 g/dl), platelet count ($<20.0 \times 10^4/mm^3$), gamma-glutamyl transpeptidase (≥50 IU/L), aspartate aminotransferase (≥34 IU/L), HBeAg (positive), and HBV core-related antigen (≥3.0 log U/ml) as determinants of a high AFP. Especially, in patients with HBsAg more than 500 IU/ml and low transaminase levels (below the upper limit of normal), HBsAg was identified as significant determinant of a high AFP. On the other hand, in patients with HBsAg less than 500 IU/ml, multivariate analysis identified albumin, gamma-glutamyl transpeptidase, and HBV core-related antigen as determinants of a high AFP. The results indicated that HBsAg level seems to affect, at least in part, the AFP levels, and that it can be used as a surrogate marker of early hepatocarcinogenesis. ***J. Med. Virol. 86:131–138, 2014.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: HBV; AFP; HBsAg; HBcrAg; genotype; hepatocellular carcinoma

INTRODUCTION

Hepatitis B virus (HBV) is a small, enveloped DNA virus known to cause chronic hepatitis and often leads to liver cirrhosis and hepatocellular carcinoma (HCC) [Viola et al., 1981; Kobayashi et al., 2002; Yao, 2003]. Evidence suggests that the use of elevated alpha-fetoprotein (AFP) for the prediction of early hepatocarcinogenesis in non-HCC patients could be clinically useful. AFP is a fetal glycoprotein produced by the yolk sac and fetal liver [Bergstrand and Czar, 1956] and has been widely used as a serum marker for the diagnosis of HCC [Sato et al., 1993; Johnson, 2001]. Furthermore, high serum AFP levels are also associated with various chronic liver diseases and hepatic regeneration [Kew et al., 1973; Silver et al., 1974; Elftherious et al., 1977; Alpert and Feller, 1978]. Many patients with chronic hepatitis B who are free of HCC have high AFP levels [Chen and Sung, 1979; Di Bisceglie and Hoofnagle, 1989], and some patients with cirrhosis and concomitant high

Grant sponsor: Ministry of Health, Labor and Welfare, Japan

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Accepted 21 August 2013

DOI 10.1002/jmv.23790
Published online 12 October 2013 in Wiley Online Library (wileyonlinelibrary.com).

inflammatory activity have very high AFP levels [Yao, 2003; Cheema et al., 2004]. On the other hand, some patients with small HCC lesions have only moderately elevated levels of AFP [Shinagawa et al., 1984; Ebara et al., 1986; Bruix and Sherman, 2005]. At present, however, there are no cutoff levels for serum AFP used to predict HCC in patients with HBV infection.

There is growing interest in the use of hepatitis B surface antigen (HBsAg) level as a prognostic marker in chronic hepatitis B patients [Chan et al., 2010]. The HBsAg levels are useful for identifying the stage of disease [Jaroszewicz et al., 2010; Nguyen et al., 2010], to distinguish true inactive carriers from patients with HBe antigen-negative disease [Brunetto et al., 2010; Martinot-Peignoux et al., 2010; Chan et al., 2011; Liaw, 2011], and to predict the response to interferon therapy [Brunetto et al., 2009; Moucari et al., 2009]. Recent studies has also demonstrated that the HBsAg levels are associated with the risk of progression to HCC, especially in patients with low HBV DNA levels [Chan, 2012; Tseng et al., 2012], and that there is a potential correlation between the HBsAg levels and the stage of liver fibrosis [Seto et al., 2012; Martinot-Peignoux et al., 2013]. However, the impact of viral factors, such as the HBsAg level, on serum AFP level as a predictor of early HCC is not clear at present.

The present study included 1,610 untreated patients with HBV infection, free of HCC or severe hepatitis. The present study was designed to provide answers to the following questions: (1) what is the relation between a high serum AFP level at the initial outpatient visit and subsequent development of hepatocarcinogenesis in antiviral-therapy-naive patients with hepatitis B viral infection? (2) What is the impact of viral factors, such as the HBsAg level, on serum AFP level in such patients, and (3) What is a good surrogate marker for a high serum AFP at the initial visit.

PATIENTS AND METHODS

Patients

Among 6,466 consecutive patients who were diagnosed with HBV infection between March 1972 and December 2012 at Toranomon Hospital, 1,610 were selected in the present study based on the following criteria: (1) They were positive for HBsAg (radioimmunoassay, Dainabot, Tokyo, Japan) and negative for anti-HCV (third-generation enzyme immunoassay, Chiron, CA). (2) They were free of HCC at the initial visit. (3) HBV hepatitis was assessed as less than severe at the initial visit, in order to minimize the potential effects of high inflammatory activity. Severe hepatitis was defined as serum transaminase level of $\geq$300 IU/L, and/or total bilirubin level of $\geq$3.0 mg/dl. (4) They had not received antiviral therapy in the past (e.g., interferon and/or nucleot(s)ide analogs) at the initial visit. (5) They underwent examination of the AFP level (upper limit of normal, 10 μg/L) at the initial visit. Furthermore, the HBsAg level, HBV core-related antigen (HBcrAg) level, and HBV DNA were also assayed using stored frozen sera obtained at the initial visit. (6) They were free of coinfection with human immunodeficiency virus. (7) They were free of other types of chronic liver disease, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, autoimmune liver disease, inherited liver disease including alpha-1 antitrypsin deficiency, and hepatic venous outflow block. (8) They consented to the study.

Table I summarizes the profile and laboratory data at the initial visit of the 1,610 patients included in the present study. They included 1,047 males and 563 females, with a median age of 40 years (range: 18–83 years). The median AFP level was 4 μg/L (range, 1–1,770 μg/L) and the median follow-up time (from the initial visit until the last visit) was 6.0 years (range, 0.0–34.6 years). The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Laboratory Tests

HBsAg, HBcrAg, and HBV DNA levels were assayed using stored frozen sera obtained at the initial visit. Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. Serum HBsAg level was measured using Architect HBsAg QT assay kit (Abbott Laboratories, Tokyo, Japan), which has a lower limit of detection of

TABLE I. Profiles and Laboratory Data at the Initial Visit of 1,610 Patients Infected With HBV

Demographic data	
Number of patients	1,610
Sex (male/female)	1,047/563
Age (years)*	40 (18–83)
Family history of liver disease[a]	836 (51.9%)
Lifetime cumulative alcohol intake (≥500 kg)	112 (7.0%)
Laboratory data*	
Total bilirubin (mg/dl)	0.6 (0.1–2.9)
Aspartate aminotransferase (IU/L)	37 (5–220)
Alanine aminotransferase (IU/L)	48 (5–297)
Albumin (g/dl)	4.2 (1.0–5.6)
Gamma-glutamyl transpeptidase (IU/L)	37 (2–2,370)
Hemoglobin (g/dl)	14.5 (6.9–18.2)
Platelet count (×104/mm^3)	19.1 (2.7–44.7)
Alpha-fetoprotein (μg/L)	4 (1–1,770)
Virological data	
HBeAg (No. of positive)	690 (42.9%)
HBsAg (IU/ml)*	2,845 (0.09 to >125,000)
HBcrAg (log U/ml)*	4.9 (<3.0 to >6.8)
HBV DNA (log copies/ml)*	5.7 (<2.1 to >9.1)
HBV genotype (A/B/C/others/ND)	65/218/1,119/6/202

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.
[a]Family history of positivity for hepatitis B surface antigen including third-degree relatives.

0.05 IU/ml and upper limit of detection of 250 IU/ml. To expand the upper range from 250 to 125,000 IU/ml, serum samples with the HBsAg levels above the upper range were diluted in a stepwise fashion to 1:20 and 1:500 with Architect diluents using the information supplied by the manufacturer. HBeAg was determined by enzyme-linked immunosorbent assay kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). Serum HBcrAg level was measured using a Cleia HBcrAg assay kit (Fujirebio, Tokyo, Japan) using a fully automated analyzer system (Lumipulse System; Fujirebio). The cut-off value of HBcrAg was 3.0 log U/ml. HBV DNA was quantified using the Cobas TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan), which has a dynamic range of 2.1–9.0 log copies/ml.

A commercial kit (HBV Genotype EIA; Institute of Immunology) was used to determine serologically the HBV genotypes using the combination of epitopes expressed on the pre-S2 region product, which is specific to each of the major genotypes.

Follow-Up and Diagnosis of Future Hepatocellular Carcinoma

After the initial visit, patients were followed-up once or three times a month. Imaging studies (ultrasonography, computed tomography, or magnetic resonance imaging) were conducted once or more per year.

Statistical Analysis

Non-parametric tests (Mann–Whitney *U*-test, chi-squared test and Fisher's exact probability test) were used to compare differences between two groups. Correlation analysis was evaluated by the Spearman rank correlation test. The cumulative rate of hepatocarcinogenesis was calculated using the Kaplan–Meier technique; differences between cumulative carcinogenesis curves between groups were tested using the log-rank test. Statistical analyses of the rate of hepatocarcinogenesis according to groups were calculated using the period from the initial visit. Univariate and multivariate logistic regression analyses were used to determine the independent surrogate markers of elevated AFP at the initial visit. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. A two-tailed *P*-value less than 0.05 was considered significant. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors for elevated AFP. Potential surrogate markers of elevated AFP at the initial visit included the following pretreatment variables: age, sex, family history of liver disease, lifetime cumulative alcohol intake, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (GGT), hemoglobin, platelet count, HBV genotype, HBeAg, HBsAg levels, HBcrAg levels, and HBV DNA levels. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL).

RESULTS

Cumulative Rate of Hepatocarcinogenesis According to the AFP Level at the Initial Visit

A total of 1,061 patients naïve to antiviral therapy from the initial visit until the last visit were evaluated for the rate of development of HCC based on the AFP levels at the initial visit. During the follow-up period, HCC was diagnosed in 31 of 905 patients (3.4%) with a low AFP level (≤10 μg/L; below the upper limit of normal) and 37 of 156 patients (23.7%) with a high AFP level (≥11 μg/L) at the initial visit. The cumulative hepatocarcinogenesis rates for patients with low and high AFP levels at the initial visit were 4.7% and 30.2% at the end of 10-year follow-up; 9.1% and 36.5% at the end of 20-year follow-up; and 13.2% and 42.9% at the end of 30-year follow-up, respectively. These results indicate that the rate of hepatocarcinogenesis is significantly higher in patients with HBV infection and high AFP levels than their counterparts with low AFP levels ($P < 0.001$; Log-rank test) (Fig. 1).

HBsAg and AFP Levels at the Initial Visit

Blood samples from all patients were analyzed to determine the relationship between the HBsAg and the AFP levels at the initial visit. The proportions of patients with high AFP levels among those with the HBsAg levels below 500 IU/ml, from 500 to 1,999 IU/ml, from 2,000 to 6,999 IU/ml, from 7,000 to 24,999 IU/ml, and above 25,000 IU/ml were 12.6% (42 of 333 patients), 26.7% (89 of 333), 22.6% (94 of 416), 10.4% (29 of 278), and 6.4% (16 of 250), respectively (Fig. 2A). The relationship between the HBsAg and

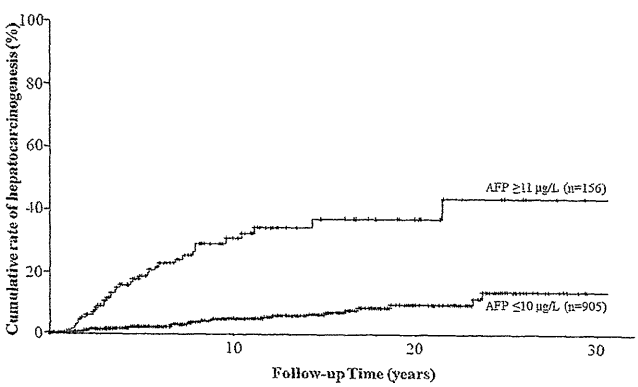

Fig. 1. Cumulative rate of hepatocarcinogenesis according to the AFP level at the initial visit in patients naïve to antiviral therapy from the initial visit until the last visit. The rate of hepatocarcinogenesis was significantly higher in patients with high AFP levels (≥11 μg/L) than in those with low levels (≤10 μg/L) at the initial visit ($P < 0.001$; Log-rank test).

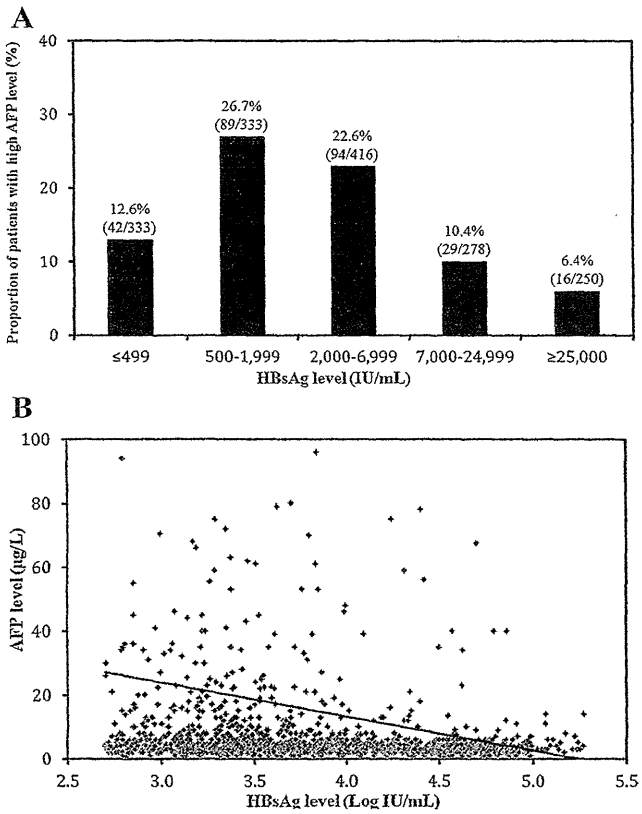

Fig. 2. **A**: Proportions of patients with the high AFP levels (≥11 μg/L) at the initial visit, stratified according to the HBsAg levels. Patients with the HBsAg levels above 500 IU/ml included a significantly lower proportions of patients with the high AFP levels and the HBsAg levels above 7,000 IU/ml (8.5%) than those with the HBsAg levels below 7,000 IU/ml (24.4%) ($P < 0.001$). **B**: Analysis of data of patients with the HBsAg levels above 500 IU/ml at the initial visit, showed a significant negative correlation between logarithmically transformed HBsAg and AFP levels ($r = -0.225$, $P < 0.001$).

the AFP levels at the initial visit suggested the presence of two distinct populations within the study group. Especially, in 1,277 patients with the HBsAg levels above 500 IU/ml, a significantly smaller proportion of patients with high AFP levels were noted among those with HBsAg of more than 7,000 IU/ml (8.5%) than those with the HBsAg levels less than 7,000 IU/ml (24.4%) ($P < 0.001$). Furthermore, the HBsAg levels correlated negatively but significantly with the AFP levels ($r = -0.225$, $P < 0.001$) (Fig. 2B).

The HBsAg Levels and the Platelet Count at the Initial Visit

Blood samples from all patients were analyzed to determine the relationship between the HBsAg levels and the platelet count at the initial visit. The median platelet counts among patients with the HBsAg levels below 500 IU/ml, from 500 to 1,999 IU/ml, from 2,000 to 6,999 IU/ml, from 7,000 to 24,999 IU/ml, and above 25,000 IU/ml were $19.1 \times 10^4/mm^3$, $17.2 \times 10^4/mm^3$, $18.0 \times 10^4/mm^3$, $20.9 \times 10^4/mm^3$, and $21.2 \times 10^4/mm^3$, respectively (Fig. 3A). The relationship between the HBsAg levels and the platelet count at the initial visit also suggested the presence of two distinct populations within the study group. Especially, in 1,277 patients with the HBsAg levels of more than 500 IU/ml, significantly higher platelet counts were noted among those with the HBsAg levels of more than 7,000 IU/ml (the median platelet count; $21.0 \times 10^4/mm^3$) than those with the HBsAg levels less than 7,000 IU/ml (the median platelet count; $17.6 \times 10^4/mm^3$) ($P < 0.001$). Furthermore, the HBsAg

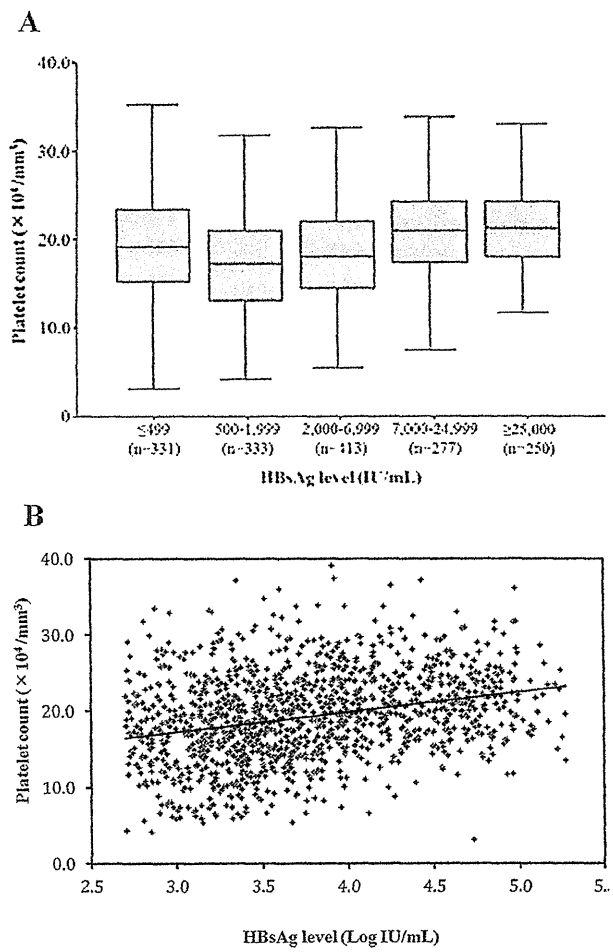

Fig. 3. **A**: The platelet count at the initial visit, stratified according to the HBsAg levels. Bars within the boxes indicate the median platelet count. The boxes denote the 25th to 75th percentiles, the lower and upper bars the 10th and 90th percentiles, respectively. Among patients with the HBsAg levels above 500 IU/ml at the initial visit, those with the HBsAg levels above 7,000 IU/ml had significantly higher platelet count (the median platelet count; $21.0 \times 10^4/mm^3$) compared to those with the HBsAg levels below 7,000 IU/ml (the median platelet count; $17.6 \times 10^4/mm^3$) ($P < 0.001$). **B**: Among patients with the HBsAg levels above 500 IU/ml at the initial visit, logarithmically transformed the HBsAg levels correlated significantly with the platelet count ($r = 0.293$, $P < 0.001$).

levels correlated significantly and positively with the platelet count ($r = 0.293$, $P < 0.001$) (Fig. 3B).

Clinical Profiles and Laboratory Data According to the HBsAg Level at the Initial Visit

Table II summarizes the clinical profiles and laboratory data according to the HBsAg level at the initial visit of 1,610 patients infected with HBV. Patients with the HBsAg levels below 500 IU/ml were significantly older and exhibited lower inflammatory activity (lower levels of AST and ALT), and had lower viral levels (they were HBeAg negative and had lower levels of HBcrAg/HBV DNA), compared to those with the HBsAg levels above 500 IU/ml ($P < 0.001$).

Factors Associated With High AFP Levels at the Initial Visit, Stratified According to the HBsAg Levels

Blood samples from all 1,610 patients were analyzed to determine the factors that affect the AFP level at the initial visit. Among 1,277 patients with the HBsAg levels more than 500 IU/ml at the initial visit, high AFP levels were detected in 228 (17.9%) patients. Univariate analysis identified 12 parameters that correlated significantly with a high AFP level at the initial visit. These included age (≥30 years; $P < 0.001$), AST (≥34 IU/L; $P < 0.001$), ALT (≥43 IU/L; $P < 0.001$), albumin (<3.9 g/dl; $P < 0.001$), GGT (≥50 IU/L; $P < 0.001$), total bilirubin (≥1.0 mg/dl; $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; $P < 0.001$), HBV genotype (C; $P < 0.001$), HBsAg levels (<7,000 IU/ml; $P < 0.001$), HBeAg (positive; $P < 0.001$), HBV DNA (≥5.0 log copies/ml; $P < 0.001$), and HBcrAg (≥3.0 log U/ml; $P < 0.001$). Multivariate analysis that included the above variables identified seven factors that influenced independently the elevated AFP level at the initial visit. These included HBsAg level (<7,000 IU/ml; OR 3.69, $P < 0.001$), albumin (<3.9 g/dl; OR 3.09, $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; OR 2.50, $P = 0.001$), GGT (≥50 IU/L; OR 2.28, $P = 0.001$), AST (≥34 IU/L; OR 2.77, $P = 0.003$), HBeAg (positive; OR 2.07, $P = 0.005$), and HBcrAg (≥3.0 log U/ml; OR 5.10, $P = 0.031$) (Table III).

Among 333 patients with the HBsAg levels less than 500 IU/ml, a high AFP at the initial visit was detected in 42 (12.6%) patients. Univariate analysis identified nine parameters that correlated significantly with a high AFP level at the initial visit. These included AST (≥34 IU/L; $P < 0.001$), ALT (≥43 IU/L; $P = 0.001$), albumin (<3.9 g/dl; $P < 0.001$), GGT (≥50 IU/L; $P < 0.001$), platelet count ($<20.0 \times 10^4/mm^3$; $P = 0.001$), HBV genotype (C; $P < 0.001$), HBeAg (positive; $P < 0.001$), HBV DNA (≥5.0 log copies/ml; $P = 0.001$), and HBcrAg (≥3.0 log U/ml; $P < 0.001$). Multivariate analysis that included the above variables identified three factors that influenced independently the elevated AFP level at the initial visit. These included albumin (<3.9 g/dl; OR 12.8, $P < 0.001$), GGT (≥50 IU/L; OR 6.95, $P = 0.002$), and HBcrAg (≥3.0 log U/ml; OR 5.62, $P = 0.010$) (Table III).

Factors Associated With High AFP Levels at the Initial Visit According to the HBsAg Levels in Patients With Low Transaminase Levels

To minimize the effect of inflammatory activity, we examined the data of 618 (among 1,610 patients) who

TABLE II. Profiles and Laboratory Data of Patients Infected With HBV According to the HBsAg Level at the Initial Visit

	HBsAg <500 IU/L	HBsAg ≥500 IU/L	*P*
Demographic data			
Number of patients	333	1,277	
Sex (male/female)	227/106	820/457	NS
Age (years)*	49 (18–75)	38 (18–83)	<0.001
Family history of liver disease[a]	130 (39.0%)	706 (55.3%)	<0.001
Lifetime cumulative alcohol intake (≥500 kg)	32 (9.6%)	80 (6.3%)	0.037
Laboratory data*			
Total bilirubin (mg/dl)	0.7 (0.2–2.9)	0.6 (0.1–2.9)	0.033
Aspartate aminotransferase (IU/L)	29 (12–175)	40 (5–220)	<0.001
Alanine aminotransferase (IU/L)	32 (7–289)	56 (5–297)	<0.001
Albumin (g/dl)	4.2 (1.1–5.6)	4.2 (1.0–5.5)	NS
Gamma-glutamyl transpeptidase (IU/L)	36 (2–2,370)	38 (4–1,638)	NS
Hemoglobin (g/dl)	14.4 (8.4–17.4)	14.6 (6.9–18.2)	NS
Platelet count ($\times 10^4/mm^3$)	19.1 (2.7–39.6)	19.2 (3.1–44.7)	NS
Alpha-fetoprotein (μg/L)	4 (1–968)	4 (1–1,770)	0.005
Virological data			
HBeAg (No. of positive)	37 (11.1%)	653 (51.1%)	<0.001
HBsAg (IU/ml)*	123 (0.09–498)	4,680 (503 to >125,000)	<0.001
HBcrAg (log U/ml)*	<3.0 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	<0.001
HBV DNA (log copies/ml)*	3.7 (<2.1 to >9.1)	6.6 (<2.1 to >9.1)	<0.001
HBV genotype (A/B/C/others/ND)	7/104/141/0/81	58/114/978/6/121	<0.001

NS; not significant.
Data are number/percentages of patients, except those denoted by *, which represent the median (range) values.
[a]Family history of positivity for hepatitis B surface antigen including third-degree relatives.

TABLE III. Results of Multivariate Logistic Analysis for Factors Associated With the High AFP Levels at the Initial Visit

Factor	Category	Risk ratio (95%CI)	P
Patients with the HBsAg levels above 500 IU/ml (n = 1,277)			
HBsAg (IU/ml)	1: ≥7,000	1	
	2: <7,000	3.69 (2.12–6.41)	<0.001
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	3.09 (1.88–5.05)	<0.001
Platelet count ($\times 10^4$/mm^3)	1: ≥20.0	1	
	2: <20.0	2.50 (1.47–4.24)	0.001
Gamma-glutamyl transpeptidase (IU/L)	1: <50	1	
	2: ≥50	2.28 (1.40–3.72)	0.001
Aspartate aminotransferase (IU/L)	1: <34	1	
	2: ≥34	2.77 (1.42–5.39)	0.003
HBeAg	1: Negative	1	
	2: Positive	2.07 (1.24–3.45)	0.005
HBcrAg (log U/ml)	1: <3.0	1	
	2: ≥3.0	5.10 (1.16–22.4)	0.031
Patients with the HBsAg levels below 500 IU/ml (n = 333)			
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	12.8 (4.02–41.7)	<0.001
Gamma-glutamyl transpeptidase (IU/L)	1: <50	1	
	2: ≥50	6.95 (2.06–23.5)	0.002
HBcrAg (log U/ml)	1: <3.0	1	
	2: ≥3.0	5.62 (1.51–21.0)	0.010

Low transaminase levels were defined as transaminase levels below the upper limit of normal.

had low transaminase levels (AST ≤33 IU/L and ALT ≤42 IU/L, i.e., below the upper limits of normal) to further determine those factors that determine the high level of AFP at the initial visit. High AFP was detected in 26 (6.1%) patients among 426 with the HBsAg levels above 500 IU/ml and low transaminase levels. Using the data of these patients, univariate analysis identified three parameters that correlated significantly with a high AFP level at the initial visit. These included albumin (<3.9 g/dl; $P = 0.004$), platelet count (<20.0 × 10^4/mm^3; $P = 0.012$), and HBsAg levels (<7,000 IU/ml; $P = 0.004$). Multivariate analysis that included the above variables identified albumin (<3.9 g/dl; OR 3.92, $P = 0.001$) and HBsAg levels (<7,000 IU/ml; OR 4.33, $P = 0.004$) as independent determinants of a high AFP level at the initial visit (Table IV).

Among 192 patients with the HBsAg levels below 500 IU/ml and low transaminase levels, high AFP levels were detected at the initial visit in 12 (6.3%). Univariate analysis identified three parameters that influenced significantly the elevated AFP level at the initial visit. These included albumin (<3.9 g/dl; $P = 0.010$), GGT (≥50 IU/L; $P = 0.011$), and platelet count (<20.0 × 10^4/mm^3; $P = 0.020$). Multivariate analysis that included these variables identified albumin (<3.9 g/dl; OR 7.19, $P = 0.004$) as the only independent determinant of a high AFP level at the initial visit (Table IV).

TABLE IV. Results of Multivariate Analysis for Factors Associated With the High AFP Levels at the Initial Visit

Factor	Category	Risk ratio (95%CI)	P
Patients with HBsAg >500 IU/ml and low transaminase levels (n = 426)			
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	3.92 (1.71–9.01)	0.001
HBsAg (IU/ml)	1: ≥7,000	1	
	2: <7,000	4.33 (1.58–11.9)	0.004
Patients with HBsAg <500 IU/ml and low transaminase levels (n = 192)			
Albumin (g/dl)	1: ≥3.9	1	
	2: <3.9	7.19 (1.87–27.8)	0.004

Low transaminase levels were defined as transaminase levels below the upper limit of normal.

DISCUSSION

There is little information on the cutoff value of AFP that can be used to predict the future probability of HCC in patients with HBV infection. The present study followed-up patients naïve to antiviral therapy from the initial visit and showed that the rate of hepatocarcinogenesis was significantly higher in those with high AFP levels at the baseline than those with low levels. To our knowledge, the present study is the first to report the hepatocarcinogenesis rate stratified according to the AFP level in patients infected with HBV but free of HCC at the initial visit, based on a large-scale long-term follow-up cohort. The results indicated that patients with high AFP levels at the initial visit are at high risk of HCC, and emphasize the need to determine the factors that could affect the AFP level as surrogate markers of early hepatocarcinogenesis. Previous studies in patients with HCV infection indicated that suppression of the AFP level by treatment with interferon reduced the HCC risk even in those without complete eradication of HCV [Arase et al., 2007; Asahina et al., 2013]. However, there is little

evidence that suppression of the AFP level by antiviral therapy reduces the HCC risk in patients with HBV infection. Further prospective studies are needed to investigate this issue in detail.

In the present study, the relationship between the HBsAg levels and the AFP levels detected at the initial visit suggested the presence of two distinct groups within the study patients. Interestingly, in patients with the HBsAg levels above 500 IU/ml, a significant negative correlation was observed between the HBsAg and the AFP levels, and a significant positive correlation was observed between the HBsAg and the platelet count. Previous studies indicated that high serum AFP levels correlated with liver fibrosis Stage 3 and 4 [Bayati et al., 1998; Chu et al., 2001; Hu et al., 2002, 2004], and that lower thrombocytopenia was closely associated with advanced liver disease [Ikeda et al., 2009; Akuta et al., 2012]. Considered together, these results emphasize the importance of hyper-α-fetoproteinemia and thrombocytopenia in the prediction of severe liver fibrosis, respectively. Based on the present results and the recent reports suggesting the potential correlation between the HBsAg level and the stage of liver fibrosis [Seto et al., 2012; Martinot-Peignoux et al., 2013], it is possible that HBsAg levels could correlate with the stage of fibrosis in patients with the HBsAg levels above 500 IU/ml. Further studies are needed to determine the value of hyper-α-fetoproteinemia in patients with low and high HBsAgemia.

In addition to the HBsAg level, multivariate analysis also identified HBcrAg as another viral factor that influenced independently the AFP level at the baseline. HBcrAg comprises HBcAg, HBeAg and a 22-kDa precore protein coded with the precore/core gene [Kimura et al., 2002, 2005]. Previous studies reported a significant correlation between serum HBcrAg concentrations and intrahepatic levels of covalently closed circular DNA (cccDNA) [Wong et al., 2007; Suzuki et al., 2009]. Other studies indicated that HBcrAg is a useful predictor of HCC during antiviral therapy [Kumada et al., 2013], and post-treatment recurrence of HCC during antiviral therapy [Hosaka et al., 2010]. The present study, based on patients naïve to antiviral therapy showed that high serum HBcrAg concentrations also correlated with high AFP at the initial visit. This is the first report demonstrating the potential usefulness of HBcrAg as a surrogate marker for early hepatocarcinogenesis.

The impact of the HBsAg level on hepatocarcinogenesis is not clear at this stage. In this study, the effect of the HBsAg levels at the initial visit on HCC was assessed in 1,061 consecutive antiviral therapy-naive patients infected with HBV. Analysis of data of 794 patients with the HBsAg levels above 500 IU/ml at the initial visit (after exclusion of patients on antiviral therapy) showed a significantly lower cumulative HCC rate in patients with the HBsAg levels above 7,000 IU/ml than those with levels below 7,000 IU/ml ($P < 0.001$, Log-rank test, Fig. 4). This

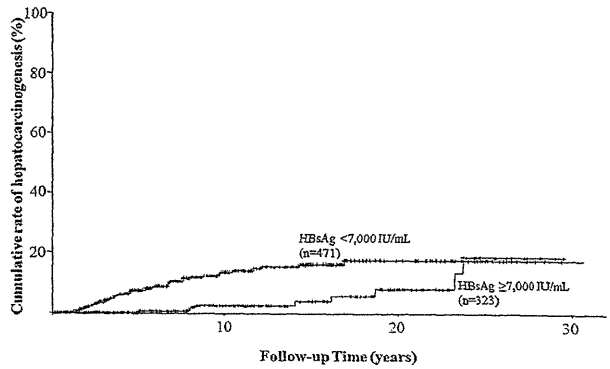

Fig. 4. Cumulative rate of hepatocarcinogenesis stratified according to the HBsAg levels at the initial visit in patients naïve to antiviral therapy from the initial visit until last visit. In a preliminary study based on 794 patients with the HBsAg levels above 500 IU/ml at the initial visit, the cumulative hepatocarcinogenesis rate for patients with the HBsAg levels more than 7,000 IU/ml was significantly lower than for those with levels below 7,000 IU/ml ($P < 0.001$; Log-rank test).

result suggests that HBsAg levels at the baseline do not only influence AFP, but also play a role in hepatocarcinogenesis. Further studies need to be performed to determine the pathomechanisms of HBsAg in hepatocarcinogenesis.

The present study has certain limitations. First, the study did not examine the effects of other genotypes, apart from HBV genotype B or C. Second, the study population was limited to Japanese and did not include other races, and thus generalization of the results to other races cannot be made based on the results. Third, the study did not investigate the effects of antiviral therapy (interferon and/or nucleot(s)ide analogs) on the outcome since such therapy suppressed the AFP levels and thus reduce the risk of HCC in patients with HBV infection.

In conclusion, the present studies demonstrated that the HBsAg level seem to influence the AFP levels and can be used as a surrogate marker for early hepatocarcinogenesis in patients with hepatitis B viral infection.

REFERENCES

Akuta N, Suzuki F, Seko Y, Kawamura Y, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Hara T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. 2012. Complicated relationships of amino acid substitution in hepatitis C virus core region and IL28B genotype influencing hepatocarcinogenesis. Hepatology 56:2134–2141.

Alpert E, Feller ER. 1978. α-fetoprotein (AF) in benign liver disease. Gastroenterology 74:856–858.

Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kobayashi M, Kumada H. 2007. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol 79:1095–1102.

Asahina Y, Tsuchiya K, Nishimura T, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Hosokawa T, Ueda K, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Nakagawa M, Kakinuma S, Watanabe M, Izumi N. 2013. α-Fetoprotein levels after interferon therapy and risk of hepatocarcinogenesis in chronic hepatitis C. Hepatology 58:1253–1262.

Bayati N, Silverman Al, Gordon SC. 1998. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. Am J Gastroenterol 93:2452–2456.

Bergstrand CG, Czar B. 1956. Demonstration of a new protein fraction in serum from the human fetus. Scand J Clin Lab Invest 8:174.

Bruix J, Sherman M. 2005. Practice guidelines committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 42:1208–1236.

Brunetto MR, Moriconi F, Bonino F, Lau GK, Farci P, Yurdaydin C, Piratvisuth T, Luo K, Wang Y, Hadziyannis S, Wolf E, McCloud P, Batrla R, Marcellin P. 2009. Hepatitis B virus surface antigen levels: A guide to sustained response to peginterferon alfa-2a in HBeAg-negative chronic hepatitis B. Hepatology 49:1141–1150.

Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, Romagnoli V, Cherubini B, Moscato G, Maina AM, Cavallone D, Bonino F. 2010. Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. Gastroenterology 139:483–490.

Chan HL. 2012. Identifying hepatitis B carriers at low risk for hepatocellular carcinoma. Gastroenterology 142:1057–1060.

Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, Sung JJ. 2010. A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. Hepatology 52:1232–1241.

Chan HL, Thompson A, Martinot-Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, Tillmann HL, Kao JH, Jia JD, Wedemeyer H, Locarnini S, Janssen HL, Marcellin P. 2011. Hepatitis B surface antigen quantification: Why and how to use it in 2011—A core group report. J Hepatol 55:1121–1131.

Cheema AW, Hirschtritt T, Van Thiel DH. 2004. Markedly elevated alpha-fetoprotein levels without hepatocellular carcinoma. Hepatogastroenterology 51:1676–1678.

Chen DS, Sung JL. 1979. Relationship of hepatitis B surface antigen to serum alpha-fetoprotein in nonmalignant diseases of the liver. Cancer 44:984–992.

Chu CW, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, Wu JC, Chang FY, Lee SD. 2001. Clinical, virological, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. J Clin Gastroenterol 32:240–244.

Di Bisceglie AM, Hoofnagle JH. 1989. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. Cancer 64:2117–2120.

Ebara M, Ohto M, Shinagawa T, Sugiura N, Kimura K, Matsutani S, Morita M, Saisho H, Tsuchiya Y, Okuda K. 1986. Natural history of minute hepatocellular carcinoma smaller than three centimeters complicating cirrhosis. A study in 22 patients. Gastroenterology 90:289–298.

Elftherious N, Heathcote J, Thomas HC, Sherlock S. 1977. Serum alpha-fetoprotein levels in patients with acute and chronic liver disease. J Clin Pathol 30:704–708.

Hosaka T, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. 2010. HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy. Liver Int 30:1461–1470.

Hu KQ, Esrailian E, Thompson K, Chase R, Kyulo N, Hassen M, Abdelhalim F, Hillebrand DJ, Runyon BA. 2002. Hepatic steatosis is associated with disease progression of chronic hepatitis C: A large cohort study in the United States. Hepatology 36:349A.

Hu KQ, Kyulo N, Lim N, Elhazin B, Hillebrand DJ, Bock T. 2004. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol 99:860–865.

Ikeda K, Arase Y, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Saitoh S, Suzuki F, Suzuki Y, Kumada H. 2009. Necessities of interferon therapy in elderly patients with chronic hepatitis C. Am J Med 122:479–486.

Jaroszewicz J, Calle Serrano B, Wursthorn K, Deterding K, Schlue J, Raupach R, Flisiak R, Bock CT, Manns MP, Wedemeyer H, Cornberg M. 2010. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV)-infection: A European perspective. J Hepatol 52:514–522.

Johnson PJ. 2001. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liv Dis 5:145–159.

Kew MC, Purves LR, Bersohn I. 1973. Serum alpha-fetoprotein levels in acute viral hepatitis. Gut 14:939–942.

Kimura T, Rokuhara A, Sakamoto Y, Yagi S, Tanaka E, Kiyosawa K, Maki N. 2002. Sensitive enzyme immunoassay for hepatitis B virus core-related antigens and their correlation to virus load. J Clin Microbiol 40:439–445.

Kimura T, Ohno N, Terada N, Rokuhara A, Matsumoto A, Yagi S, Tanaka E, Kiyosawa K, Ohno S, Maki N. 2005. Hepatitis B virus DNA-negative dane particles lack core protein but contain a 22-kDa precore protein without C-terminal arginine-rich domain. J Biol Chem 280:21713–21719.

Kobayashi M, Arase Y, Ikeda K, Tsubota A, Suzuki Y, Saitoh S, Kobayashi M, Suzuki F, Akuta N, Someya T, Matsuda M, Sato J, Kumada H. 2002. Clinical characteristics of patients infected with hepatitis B virus genotypes A, B, and C. J Gastroenterol 37:35–39.

Kumada T, Toyoda H, Tada T, Kiriyama S, Tanikawa M, Hisanaga Y, Kanamori A, Niinomi T, Yasuda S, Andou Y, Yamamoto K, Tanaka J. 2013. Effect of nucleos(t)ide analogue therapy on hepatocarcinogenesis in chronic hepatitis B patients: A propensity score analysis. J Hepatol 58:427–433.

Liaw YF. 2011. Clinical utility of hepatitis B surface antigen quantitation in patients with chronic hepatitis B: A review. Hepatology 54:E1–E9.

Martinot-Peignoux M, Lada O, Cardoso AC, Lapalus M, Boyer N, Ripault MP, Asselah T, Marcellin P. 2010. Quantitative HBsAg: A new specific marker for the diagnosis of HBsAg inactive carriage. Hepatology 52:992A.

Martinot-Peignoux M, Carvalho-Filho R, Lapalus M, Netto-Cardoso AC, Lada O, Batrla R, Krause F, Asselah T, Marcellin P. 2013. Hepatitis B surface antigen serum level is associated with fibrosis severity in treatment-naïve, E antigen-positive patients. J Hepatol 58:1089–1095.

Moucari R, Mackiewicz V, Lada O, Ripault MP, Castelnau C, Martinot-Peignoux M, Dauvergne A, Asselah T, Boyer N, Bedossa P, Valla D, Vidaud M, Nicolas-Chanoine MH, Marcellin P. 2009. Early serum HBsAg drop: A strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 49:1151–1157.

Nguyen T, Thompson AJ, Bowden S, Croagh C, Bell S, Desmond PV, Levy M, Locarnini SA. 2010. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: A perspective on Asia. J Hepatol 52:508–513.

Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y, Nagataki S. 1993. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 328:1802–1806.

Seto WK, Wong DK, Fung J, Ip PP, Yuen JC, Hung IF, Lai CL, Yuen MF. 2012. High hepatitis B surface antigen levels predict insignificant fibrosis in hepatitis B e antigen positive chronic hepatitis B. PLoS ONE 7:e43087.

Shinagawa T, Ohto M, Kimura K, Tsunetomi S, Morita M, Saisho H, Tsuchiya Y, Saotome N, Karasawa E, Miki M. 1984. Diagnosis and clinical features of small hepatocellular carcinoma with emphasis on the utility of real-time ultrasonography. A study in 51 patients. Gastroenterology 86:495–502.

Silver HK, Gold P, Shuster J, Javitt NB, Freedman SO, Finlayson ND. 1974. Alpha 1-fetoprotein in chronic liver disease. N Engl J Med 291:506–508.

Suzuki F, Miyakoshi H, Kobayashi M, Kumada H. 2009. Correlation between serum hepatitis B virus core-related antigen and intrahepatic covalently closed circular DNA in chronic hepatitis B patients. J Med Virol 81:27–33.

Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, Kuo SF, Liu CH, Chen PJ, Chen DS, Kao JH. 2012. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 142:1140–1149.

Viola LA, Barrison IG, Coleman JC, Paradinas FJ, Fluker JL, Evans BA, Murray-Lyon IM. 1981. Natural history of liver disease in chronic hepatitis B surface antigen carriers. Survey of 100 patients from Great Britain. Lancet 2:1156–1159.

Wong DK, Tanaka Y, Lai CL, Mizokami M, Fung J, Yuen MF. 2007. Hepatitis B virus core-related antigens as markers for monitoring chronic hepatitis B infection. J Clin Microbiol 45:3942–3947.

Yao FY. 2003. Dramatic reduction of the alpha-fetoprotein level after lamivudine treatment of patients with chronic hepatitis B virus infection and cirrhosis. J Clin Gastroenterol 36:440–442.

Reply

Title: Does long-term entecavir treatment really reduce hepatocellular carcinoma incidence in patients with hepatitis B virus infection?

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Word count: 500

Number of figures and tables: 1

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/hep.26774

We thank Dr. Lo for his comments on our paper "Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection" (1). We appreciate his concerns. In this study, we calculated the propensity score (PS) estimated for all patients treated with entecavir (ETV). Variables used in the model did not include alcohol consumption. We recalculated a PS including alcohol consumption and conducted PS matching using this revised PS. The revised PS matching process resulted in a matched sample size that consisted of 316 patients in each group. There was no difference in baseline characteristics between ETV and control groups, as was the case in our original cohort. We compared the incidence of hepatocellular carcinoma (HCC) with ETV vs. untreated control groups. The result was similar to our original report. Cumulative HCC incidence rates were significantly lower in ETV group than in the control group (Figure). Seven factors were associated with HCC development as determined by Cox proportional hazard regression at 5-year: age, alcohol consumption, cirrhosis, hepatitis B e antigen (HBeAg), platelet count, and ETV treatment. The multivariate adjusted hazard ratio of ETV treatment was 0.27 (95% confidence interval; 0.12-0.62). These results were re-analyzed using the update data until Jun. 2013. Median follow-up duration in the PS matched ETV group were extended to 4.5 years (original: 3.3 yrs).

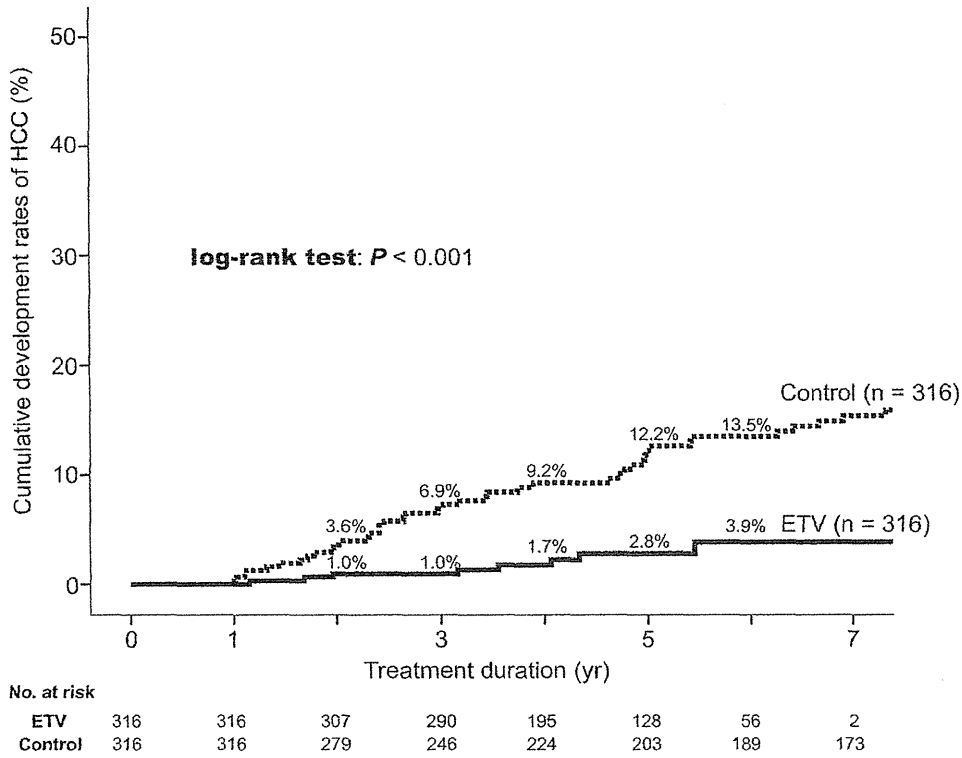
Because prothrombin time was lacking in our pooled data, we could not present cirrhotic severity in this study. This study end point is HCC incidence over 1 year after the start of observation. Patients who developed HCC within 1 year were included in those with follow-up duration < 1 year. Therefore, patients with HCC or suspicion of HCC on enrollment were excluded from this study. It is impossible to avoid any bias because this study is a retrospective cohort study. We think that the method of this study is the next best method of ensuring that the experimental and control groups are similar in the absence of randomization as Prof. Sherman described (2). Our findings are consistent with those recently published from Hong Kong (3). A recent meta-analysis, that included our results, demonstrated a reduction in HCC incidence with oral antiviral agents (4). The risk reduction of HCC by nucleos(t)ide analogues needs to be confirmed in other long-term studies of ETV or tenofovir with high antiviral potency.

References

1. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. Hepatology 2013;58:98-107.
2. Sherman M. Does hepatitis B treatment reduce the incidence of hepatocellular carcinoma? Hepatology 2013;58:18-20.
3. Wong GLH, Chan HLY, Mak CWH, Lee SKY, Ip ZMY, Lam ATH, et al. Entecavir treatment reduces hepatic events and deaths in chronic hepatitis B patients with liver cirrhosis. Hepatology 2013 Epub ahead of print.
4. Singal AK, Salameh H, Kuo YF, Fontana RJ. Meta-analysis: the impact of oral anti-viral agents on the incidence of hepatocellular carcinoma in chronic hepatitis B. Aliment Pharmacol Ther 2013;38:98-106.

Figure legend

Figure. Comparison of HCC cumulative incidence rates between the entecavir-treated group and the non-treated control group after propensity score matching (alcohol consumption included) using update data until Jun. 2013. The long-rank test revealed a statistically significant difference between the ETV and the control group in the incidence of HCC at 5 years time.



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Hepatology Research

Hepatology Research 2014 doi: 10.1111/hepr.12240

Short Communication

Potential of a no-touch pincer ablation procedure for small hepatocellular carcinoma that uses a multipolar radiofrequency ablation system: An experimental animal study

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Aim: Treatment of hepatocellular carcinoma located on the liver surface is frequently difficult because direct puncture of the tumor must be avoided during needle insertion. The aim of this study was to investigate the utility of a no-touch pincer ablation procedure that uses a multipolar radiofrequency ablation (RFA) system for a tumor located on the liver surface.

Methods: The experimental animals were three pigs, and RFA was performed with two internally cooled bipolar electrodes. Three ablative procedures were compared: linear insertion at regular 13-mm intervals (pattern 1; virtual target tumor size, <10 mm); fan-shape insertion, maximum interval 20 mm (pattern 2; virtual target tumor size, <15 mm); and 25 mm (pattern 3; virtual target tumor size, <20 mm). All electrodes were inserted at a 30-mm depth. For patterns 1 and 2, ablation was performed on three other parts of the liver, and for pattern 3, ablation was performed on two other parts.

Results: For the median transverse and longitudinal diameter to the shaft, with the pattern 1 procedure, the ablative areas were 32 mm × 30 mm, and with the pattern 2 procedure, the ablative areas were 27 mm × 30 mm with carbonization of the liver surface. In contrast, with the pattern 3 procedure, the ablative areas were 45 mm × 26 mm; however, the ablative margin did not reach the surface, and carbonization was not apparent.

Conclusion: The no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small nodules that protrude from the liver surface.

Key words: bipolar, hepatocellular carcinoma, multipolar, no-touch ablation, radiofrequency ablation

INTRODUCTION

AMONG THE AVAILABLE treatment options for hepatocellular carcinoma (HCC), surgical resection is generally considered to be a local eradication method that can provide a satisfactory long-term outcome.[1–8] Recent advances in imaging procedures have led to increased detection of early-stage HCC and to improved survival due to the increased identification of patients in whom hepatic resection is possible.[9,10]

For patients who are not eligible for surgery for various reasons (e.g. lack of sufficient liver function for surgical resection), percutaneous local therapy is a viable therapeutic option. Several local ablation therapies are available, including percutaneous ethanol injection, percutaneous acetic acid injection, cryotherapy, percutaneous microwave coagulation therapy and radiofrequency ablation (RFA). In addition to surgical resection, local ablation therapies, particularly RFA, are considered to be local eradication methods for HCC that can provide good long-term outcomes.[11] Therefore, in recent years, RFA has become a widely used option for the primary treatment of small-size HCC. However, we often

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Funding: Okinaka Memorial Institute for Medical Research and Japanese Ministry of Health, Labor and Welfare.

Conflict of interest: The authors state that they have no conflicts of interest regarding the content of the article.

Author contribution: All authors had access to the data and played a role in writing this manuscript.

Received 29 May 2013; revision 26 August 2013; accepted 10 September 2013.

encounter cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver surface. In addition, a relationship between percutaneous local approaches to HCC (including tumor biopsy) and tumor seeding has been reported previously,[12,13] and with regard to the risk of treatment-related tumor seeding, the following risk factors have been reported: tumor size, tumor location (subcapsular portion), α-fetoprotein level, tumor stage and histopathological grade.[14,15] Therefore, a no-touch approach to local therapy may be considered an ideal treatment method for HCC.

Recently, a multipolar ablation system became available. Until now, in Japan, monopolar electrodes have typically been used, and the present cases are usually treated with some technical arrangement. For example, in the case of using a multi-tined expandable electrode, after obliquely inserting the electrode to avoid direct puncture of the target tumor, the multi needles are expanded toward the target tumor via non-tumor tissue, or in the case of using an internally cooled electrode, multiple insertions are made to avoid direct puncture of the target tumor, and RFA is performed after each insertion. However, these methods do not always provide enough of a treatment effect due to the influence of uncertain treatment procedures and natural, direct puncture to a tumor is indispensable. In contrast, a multipolar ablation system that uses an internally cooled bipolar electrode can combine the use of one to three electrodes at the same treatment session. When three electrodes are used, this system can treat large tumors; however, in the case of small tumors, it is not really necessary to use three electrodes to treat the target tumor. In addition, when we used this multipolar ablation system, usually electrodes were inserted into HCC, but in theory, this system can use no-touch ablation. However, to our knowledge, there are no technical reports that describe a non-direct punctual RFA method that uses a bipolar ablation system for HCC located on the liver surface. In this experimental animal study, we assumed that a small (<20 mm) HCC nodule protruded from the liver surface, and examined proper pincer ablation methods using two internally cooled bipolar electrodes.

METHODS

Summary of experimental procedures

WE USED A bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH [Telto, Germany]) and two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). RFA was applied in the livers of three normal female domestic pigs (each pig's weight was 60 kg) under general anesthesia maintained until killing. The abdomen was opened so that the needle could be inserted under an ultrasonography (US) guide directly into the upper region of the liver where the thickness was larger than 3.5 cm. As a pig liver consists of five thin lobes, RFA sessions were performed two to three times in each liver for evaluation of the "no-touch pincer ablation procedure". After the experiments were completed, the animal was killed, and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Toranomon Hospital.

Protocol of the no-touch pincer ablation procedure

We used a bipolar RFA device (CelonPOWER System; OLYMPUS Winter & Ibe GmbH), and all ablation procedures were performed with two internally cooled bipolar electrodes (30-mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH). Internal liquid circulation of the applicator enables the efficiency of coagulation to be increased. The delivery rate was set to 30 mL/min of saline solution at room temperature. The liquid flow was provided by a triple peristaltic pump, which is part of the system. The electrodes were operated by a power control unit working at 470 kHz and providing a maximum output power of 250 W (OLYMPUS Winter & Ibe GmbH). In this study, output power and total energy in each session were fixed at 60 W and 25 kJ, respectively, according to the dosimetry table for the bipolar RFA system (CelonPOWER System; OLYMPUS Winter & Ibe GmbH).

With regard to the ablation protocol, we performed the following three types of ablation procedure: linear insertion, at regular 13-mm intervals (pattern 1); fan-shape insertion, maximum interval of 20 mm (pattern 2); and 25 mm (pattern 3). All electrodes were inserted at a 30-mm depth from the liver surface under a US guide (Fig. 1). Each ablation procedure was performed for the following number of times: pattern 1, three sessions; pattern 2, three sessions; and pattern 3, two sessions. In this study, we assumed that the size of the virtual target tumor was less than 10 mm in pattern 1,

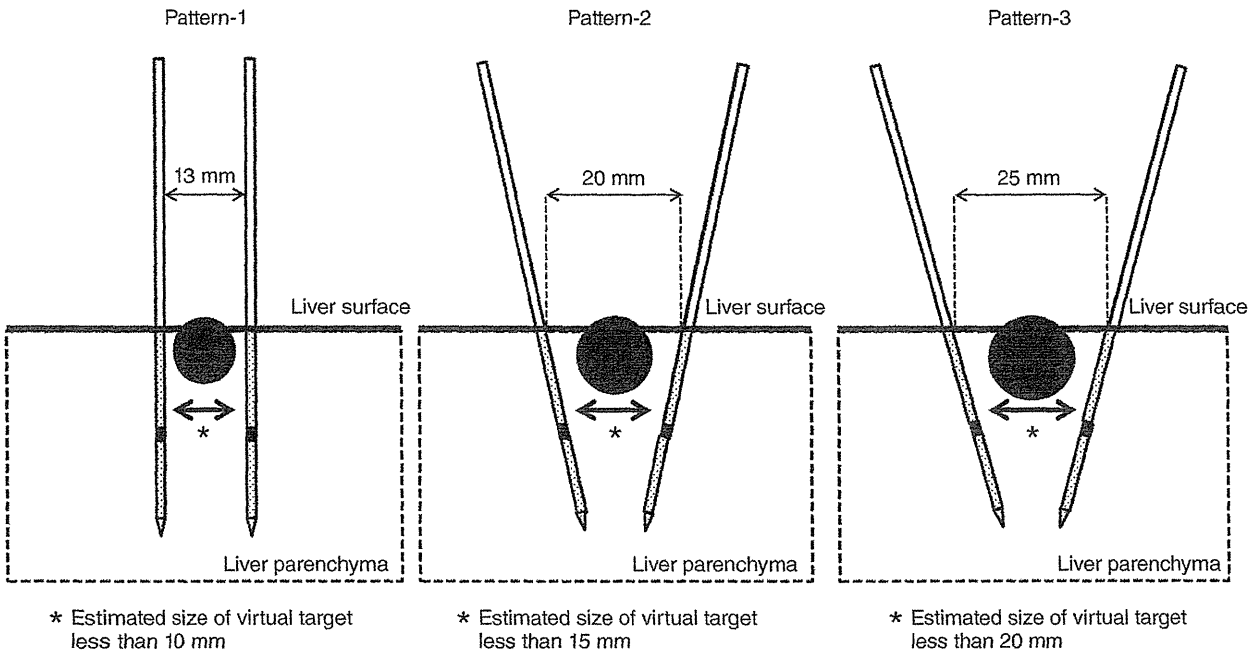


Figure 1 Protocol for a pincer ablation procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface.

less than 15 mm in pattern 2 and less than 20 mm in pattern 3.

Measurement procedure of the ablative margin

After completion of the experiments, the animal was killed and the ablated liver lobes were excised immediately. The specimen was cut in the plane of the needle tract and photographed to evaluate the shape and size of the ablated zone (white zone).

Statistical analysis

The size of the ablated zone and the duration of ablation were compared among the three groups with the Kruskal–Wallis test. All values are expressed as medians. A *P*-value of less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Features of the no-touch pincer ablation procedure

THE THREE TYPES of pincer ablation procedure applied to the pig liver were performed in the area shown in Figure 2(a).

Table 1 summarizes the features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface.

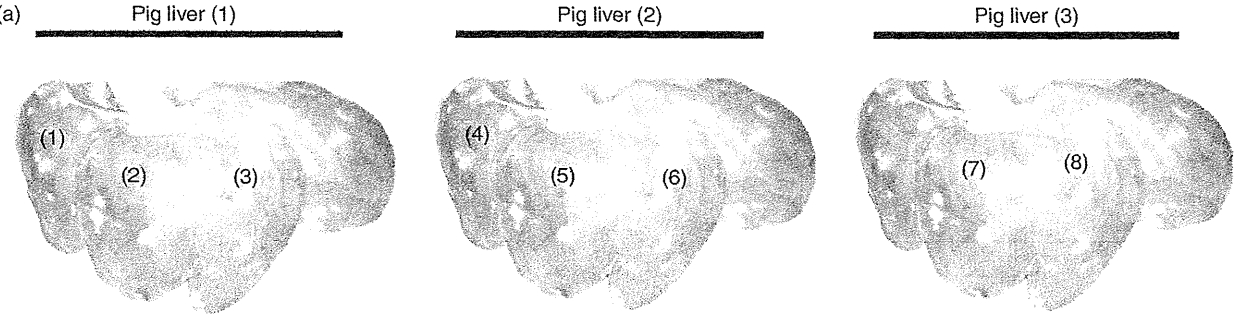
In the median (range) transverse and longitudinal diameter to the shaft, ablative areas were: pattern 1, 32 (27–35) mm × 30 (30–35) mm; pattern 2, 27 (25–35) mm × 30 (30–32) mm; and pattern 3, 45 (40–50) × 26 (25–27) mm. There were no significant differences in the size of each ablative area among the three ablation procedures. However, with the pattern 3 procedure, the transverse diameter to the shaft was larger than with the other procedures, and as a result, the ablative form was flatter. On the other hand, patterns 1 and 2 acquired sufficient ablative areas that covered the liver surface with carbonization of the surface; however, with pattern 3, the ablative areas did not reach the liver surface, and carbonization of the liver surface was not apparent (Fig. 2b–d).

In addition, there were no significant differences among ablation procedures in the duration of ablative time.

DISCUSSION

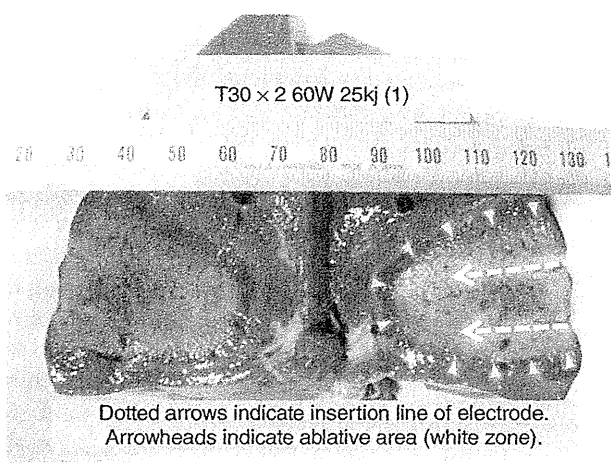
WE OFTEN ENCOUNTER cases of HCC that are difficult to treat with RFA as a result of tumor location, especially nodules that protrude from the liver

(a)



Ablative pattern	*Location*
Pattern-1: linear insertion, at regular 13-mm intervals	(1)(2)(5)
Pattern-2: fan-shape insertion, maximum interval, 20 mm	(3)(4)(7)
Pattern-3: fan-shape insertion, maximum interval, 25 mm	(6)(8)

(b) Representative ablative images: pattern-1



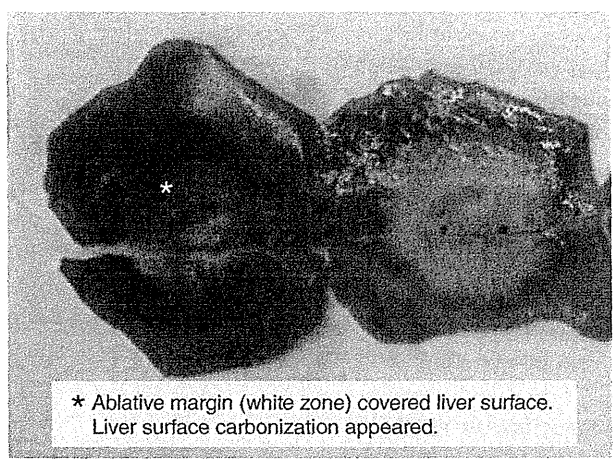
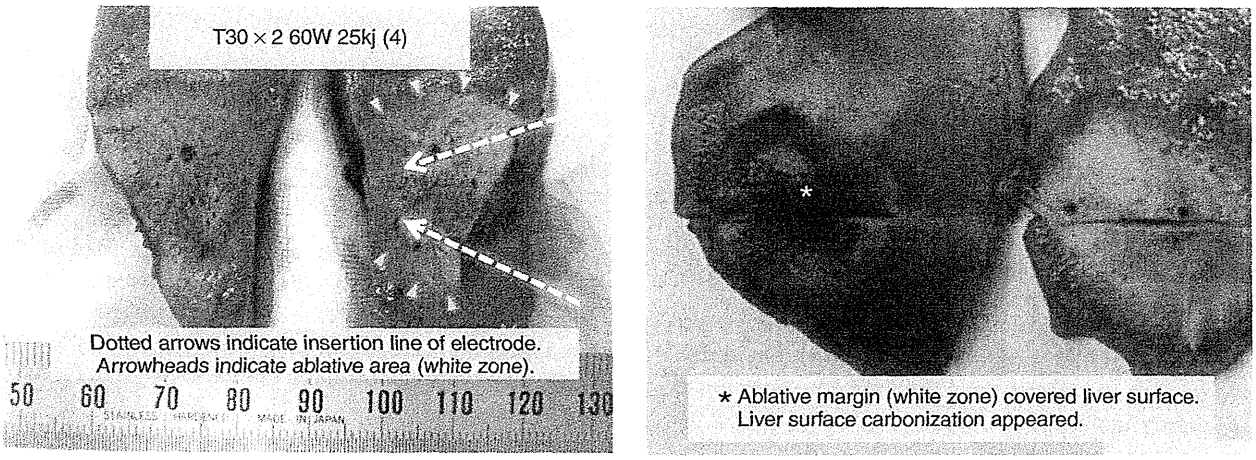


Figure 2 (a) Schema of the ablative areas of each pincer ablation procedure in the three pig livers. (b) One of the ablative shapes and the margin achieved with the pattern 1 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we inserted the electrodes linearly (maximum interval for each electrode was 13 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (c) One of the ablative shapes and the margin achieved with the pattern 2 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 20 mm). The ablative margin covered the liver surface with carbonization of the liver surface. (d) Ablative shape and margin achieved with the Pattern 3 procedure that uses two internally cooled bipolar electrodes for a virtual nodule that protrudes from the liver surface. With this pattern, we used a fan-shape insertion method (maximum interval for each electrode was 25 mm). The ablative area close to the liver surface was larger than with the other procedures. However, the ablative margin did not cover the liver surface, and carbonization of the liver surface was not apparent.

(c) Representative ablative images: pattern-2



(d) Representative ablative images: pattern-3

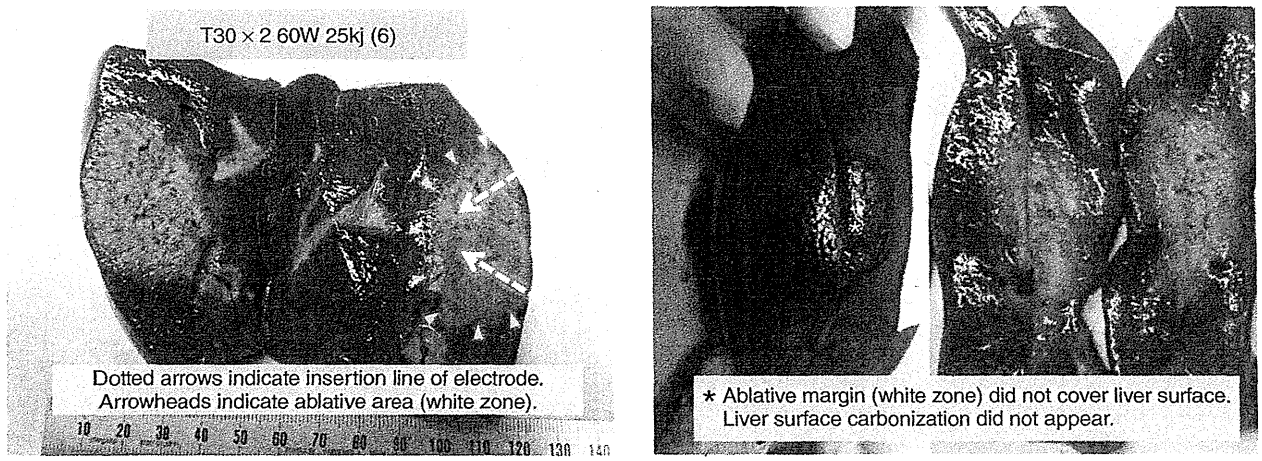


Figure 2 *Continued*

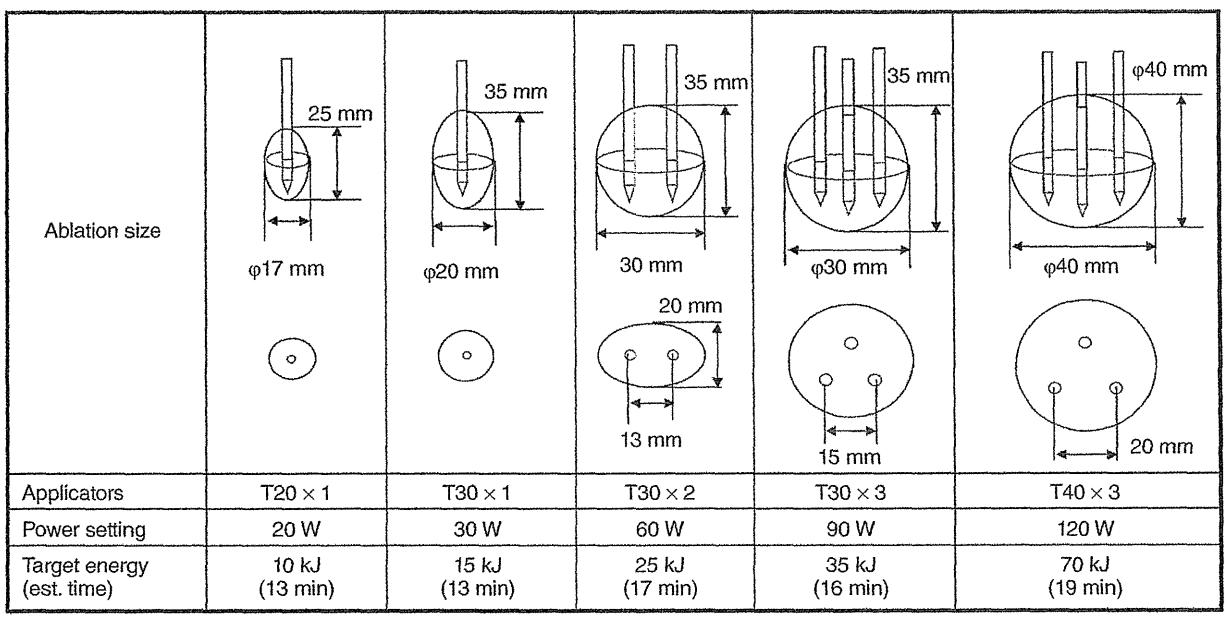
surface. In these situations, a multipolar ablation system that uses internally cooled bipolar electrodes may be suitable for treatment. With a multipolar ablation system, we can combine the use of one to three electrodes at the same treatment session, and when three electrodes are used, this system can treat a large tumor. However, in the case of small tumors (<20 mm), it is not really necessary to use three electrodes for treatment of the target tumor. However, in the dosimetry table of this bipolar system in Figure 3, which was made from previously reported early clinical data[16] and basic analysis, when two internally cooled bipolar electrodes are used (30 mm, 15-G, CelonProSurge; OLYMPUS Winter & Ibe GmbH), the recommended interval of each electrode in this system was 13 mm. With this regulation, we can treat only small tumors (<13 mm) when we perfume no-touch pincer ablation using two electrodes. Therefore, in this study we assumed a virtual target tumor with a tumor diameter less than 20 mm, and investigated the efficacy of a no-touch pincer ablation procedure and the maximum size of the tumor using two internally cooled bipolar electrodes for nodules that

Table 1 Features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface

	Pattern 1			Pattern 2			Pattern 3		*P*
	1	2	3	1	2	3	1	2	
Duration	13′46″	13′16″	12′58″	14′38″	13′50″	13′30″	13′05″	12′40″	$P = 0.151$
Ablated area									
Transverse diameter, mm	27	35	32	25	27	35	45	40	$P = 0.113$
Longitudinal length, mm	35	30	30	32	30	30	27	25	$P = 0.102$
Ablated area covered liver surface	Yes	Yes	Yes	Yes	Yes	Yes	No	No	
Liver surface carbonization appeared	Yes	Yes	Yes	Yes	Yes	Yes	No	No	

protrude from the liver surface. In addition, we investigated only the fan-shape insertion method at a maximum interval of 20–25 mm. The reason for this is that in an actual RFA procedure, it is occasionally difficult to insert two electrodes in the same intercostal space for slightly large nodules that protrude from the liver surface; therefore, in this study, we examined a fan-shape ablation method that assumed two different intercostal approaches. Our results showed that with the pattern 3 treatment procedure, we could not acquire a sufficient ablative margin to the side of the liver surface. From these results, tumors of 20 mm or more may not be suitable for a no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

In contrast, with the pattern 1 and 2 treatment procedures, we acquired a sufficient ablative margin to the side of the liver surface with carbonization of the liver



- The data are based on Frericks et al., Radiology (2005) 237: 1056–1062. The reported average efficacy was ~0.5 millitre ablation volume per kilojoule.
  From these data, the required energy for an ablation sphere or ellipsoid of given diameter was calculated.
- The application of blood flow interruption (e.g. Pringle's manoeuvre, embolization) allows for a significant reduction of the target energy.

Disclaimer: this dosimetry table does not replace the monitoring of actual ablation sizes. The ablation diameters are approximations based on statistical data; they are not guaranteed for individual clinical cases. Ablation size and shape as well as the procedure time may significantly vary due to tumor physiology and vascular structure. A deviation from the recommended applicator distances may also have an impact on the ablation dimensions.

Figure 3 Dosimetry table for the CelonPOWER system (in Japan).

surface. These results may indicate that tumors of less than 15 mm are candidates for the no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

Finally, this experimental animal study had some limitations. First, the number of animals was very small, and the target tumor was a virtual tumor. Second, an additional examination regarding a no-touch linear insertion procedure for maximum intervals of 20 mm and 25 mm for each electrode was not enforced. Third, we could not investigate the same fan-shape ablation procedure using monopolar RFA in this study, because we assumed it would be too difficult to carry out a two-step insertion method using a monopolar electrode under the influence of a first ablation for nodules that protrude from the liver surface. Fourth, we could not investigate the pathological changes in the ablative area in this study. Therefore, with only these study results, it may not be possible to draw conclusions regarding the utility of the fan-shape insertion method using a bipolar RFA device. To solve these problems, we must carry out an additional large-scale study that includes pathological examination in the near future.

Finally, to summarize the points to be noted at the time of performing the pincer ablation procedure, first, we should insert the needle carefully under US guidance, because in this procedure, measuring the distance of the needle tip from the liver surface and the two needle intervals on the liver surface correctly is the most important point.

Second, with this procedure, we should pay attention to the risk of thermal damage to the visceral peritoneum. Therefore, if possible, thermal protection using measures such as artificial ascites should be considered.

Third, in this study, we did not observe a portal or hepatic vein thrombus in the ablative area. However, this study was performed mainly in the vicinity of the liver surface, and usually this area does not include large vessels. Therefore, we need to use caution as with monopolar ablation when we ablate near large vessels.

In conclusion, the no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small HCC tumors that protrude from the liver surface.

REFERENCES

1 Poon RT, Fan ST, Lo CM *et al.* Hepatocellular carcinoma in the elderly: results of surgical and nonsurgical management. *Am J Gastroenterol* 1999; **94:** 2460–6.

2 Yamanaka N, Okamoto E, Toyosaka A *et al.* Prognostic factors after hepatectomy for hepatocellular carcinomas. A univariate and multivariate analysis. *Cancer* 1990; **65:** 1104–10.

3 Kawasaki S, Makuuchi M, Miyagawa S *et al.* Results of hepatic resection for hepatocellular carcinoma. *World J Surg* 1995; **19:** 31–4.

4 Shirabe K, Kanematsu T, Matsumata T, Adachi E, Akazawa K, Sugimachi K. Factors linked to early recurrence of small hepatocellular carcinoma after hepatectomy: univariate and multivariate analyses. *Hepatology* 1991; **14:** 802–5.

5 Jwo SC, Chiu JH, Chau GY, Loong CC, Lui WY. Risk factors linked to tumor recurrence of human hepatocellular carcinoma after hepatic resection. *Hepatology* 1992; **16:** 1367–71.

6 Nagasue N, Kohno H, Hayashi T *et al.* Lack of intratumoral heterogeneity in DNA ploidy pattern of hepatocellular carcinoma. *Gastroenterology* 1993; **105:** 1449–54.

7 Izumi R, Shimizu K, Ii T *et al.* Prognostic factors of hepatocellular carcinoma in patients undergoing hepatic resection. *Gastroenterology* 1994; **106:** 720–7.

8 Otto G, Heuschen U, Hofmann WJ, Krumm G, Hinz U, Herfarth C. Survival and recurrence after liver transplantation versus liver resection for hepatocellular carcinoma: a retrospective analysis. *Ann Surg* 1998; **227:** 424–32.

9 Takayama T, Makuuchi M, Hirohashi S *et al.* Early hepatocellular carcinoma as an entity with a high rate of surgical cure. *Hepatology* 1998; **28:** 1241–6.

10 Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004; **130:** 417–22.

11 Hong SN, Lee SY, Choi MS *et al.* Comparing the outcomes of radiofrequency ablation and surgery in patients with a single small hepatocellular carcinoma and well-preserved hepatic function. *J Clin Gastroenterol* 2005; **39:** 247–52.

12 Stigliano R, Marelli L, Yu D, Davies N, Patch D, Burroughs AK. Seeding following percutaneous diagnostic and therapeutic approaches for hepatocellular carcinoma. What is the risk and the outcome? Seeding risk for percutaneous approach of HCC. *Cancer Treat Rev* 2007; **33:** 437–47.

13 Kawamura Y, Ikeda K, Seko Y *et al.* Heterogeneous type 4 enhancement of hepatocellular carcinoma on dynamic CT is associated with tumor recurrence after radiofrequency ablation. *AJR Am J Roentgenol* 2011; **197:** W665–W673.

14 Llovet JM, Vilana R, Brú C *et al.*, Barcelona Clínic Liver Cancer (BCLC) Group. Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. *Hepatology* 2001; **33:** 1124–9.

15 Yu HC, Cheng JS, Lai KH *et al.* Factors for early tumor recurrence of single small hepatocellular carcinoma after percutaneous radiofrequency ablation therapy. *World J Gastroenterol* 2005; **11:** 1439–44.

16 Frericks BB, Ritz JP, Roggan A, Wolf KJ, Albrecht T. Multipolar radiofrequency ablation of hepatic tumors: initial experience. *Radiology* 2005; **237:** 1056–62.