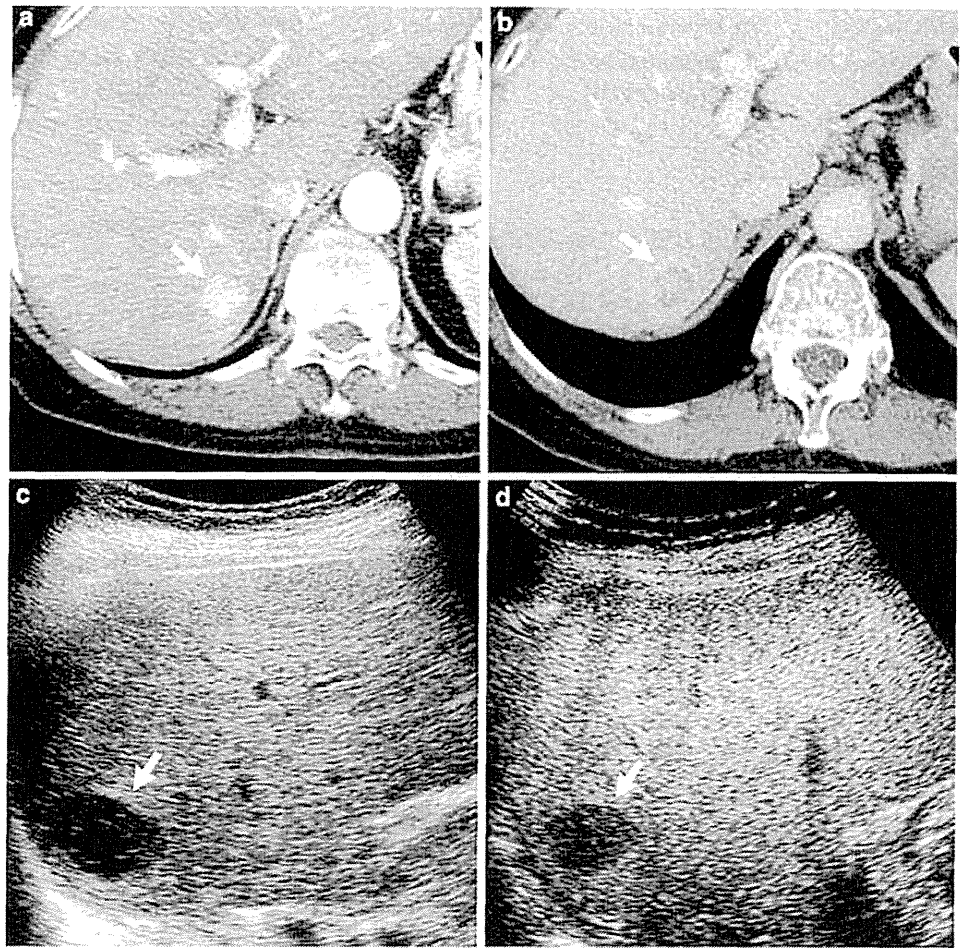

Fig. 1 Typical appearance of HCC (*arrow*) on dynamic CT and unenhanced and enhanced US. Appearance of HCC on dynamic CT [early enhancement (**a**) with late washout (**b**)]. The nodule was detected as a hypoechoic lesion by unenhanced US (**c**) and visualized as an enhancement defect in Kupffer imaging Sonazoid CEUS (**d**)

sterile water for injection, and 0.0075 mL/kg body weight of the solution was injected as a bolus via the antecubital vein and immediately flushed with 10 mL normal saline. Kupffer imaging was defined as the period starting 15 min after injection of Sonazoid (Fig. 1d).

Imaging technique and storage

US operators (EG, JI), each of whom had more than 8 years' experience in liver ultrasonography, were blinded with regard to subjects' clinical information including CT findings. All ultrasonographic images were recorded on movie files according to the following conditions. First, unenhanced ultrasonography was performed, and the images were stored in four separate movie files for each subject, one each for the left lateral, left medial, right anterior, and right posterior segments. Each segment was scanned from two directions in a standardized fashion, taking approximately 1 min. Sonazoid was then injected as described above, and after 15 min, Kupffer imaging scanning was performed and stored as for movie files. Vascular imaging images were not recorded. All movie files were formatted on uncompressed audio/video interleaving files with a resolution of 800 × 600 pixels and frame rate of 15 frames per second. Personal information was completely deleted from the movie files using the Toshiba Clip Washer ver. 3.5 software package (Toshiba Medical, Tokyo, Japan).

Movie file review

A total of 800 movie files, four each for unenhanced and Sonazoid-enhanced US per subject, were examined in a randomized order. Two readers (RT, RM), each of whom had more than 11 years' experience in liver ultrasonography, reviewed each movie file independently. The readers were blinded to all clinical and demographic information. After reviewing each movie file, the reviewers recorded the number of HCC nodules in the file, if any, and the confidence of diagnosis was graded on a four-point scale: 1, definitely absent; 2, probably absent; 3, probably present; 4, definitely present. The location of detected nodules and frame timestamp were also recorded.

After all movie files had been evaluated, the file order was restored. The evaluation by each reader was compared

with the findings of contrast-enhanced dynamic CT, using the latter as the reference standard. In some cases, nodules on a segment were also visualized in another movie file (e.g., a nodule in the medial segment was also visualized in a movie file for the anterior segment). In such cases, the third reader (EG) reassigned the score by referring to the readers' comments (e.g., "definitely present; however, the nodule seems to be located in the medial segment rather than the anterior segment"). In cases where two or more nodules were located in the same segment, the third reader assessed whether the first and second readers accurately pointed out the nodule in the same segment for nodule-based subgroup analysis according to the readers' comments.

To assess the characteristics of false-positive and -negative nodules, the third reader reviewed the corresponding movie files. Management of data regarding subject characteristics, scoring, and linking scores to randomized movie files was processed using Microsoft Access 2007 (Microsoft Corporation; Redmond, WA, USA).

Statistical analysis

Variables were expressed as mean ± standard deviations unless otherwise specified. Categorical variables were compared using Fisher's exact probability test. Sensitivity and specificity of unenhanced and enhanced US in the detection of HCC nodules were calculated using the presence or absence of HCC in the corresponding liver segment on dynamic CT as the reference standard. Confidence intervals were calculated based on the F distribution. We also calculated positive and negative likelihood ratios defined as sensitivity/(1 − specificity) and (1 − sensitivity)/specificity, respectively. Differences in proportion were assessed using Fisher's exact probability test. The concordance between the two readers was evaluated by weighted kappa statistics. The optimal cutoff in transforming the four-point scale to a dichotomous variable was validated by calculating the Youden index [15]. In addition to these segment-based analyses, tumor nodule-based analysis was also performed to evaluate tumor-associated factors that affected sensitivity for HCC detection. Statistical analyses were performed with S-plus ver. 7 (TIBCO Software, Inc., Palo Alto, CA, USA).

Results

Subject characteristics and CT findings

The study population consisted of 60 men and 40 women, with a mean age of 67.5 years. Seventy-seven subjects were hepatitis C virus antibody (HCVAb)-positive, and nine were hepatitis B surface antigen (HBsAg)-positive. During the study period, no treatment-naïve HCC patients were excluded, and only one patient refused to participate in the study. The total number of HCC nodules detected was 138, with a mean diameter of 23.1 ± 7.7 mm determined by dynamic CT. Subject characteristics are summarized in Table 1 [16]. A total of 10 segments had two and three segments had three HCC nodules. Thus, 123 of 400 segments (13 lateral, 11 medial, 63 anterior, and 36 posterior segments) were HCC-positive, whereas the other 277 were negative.

Evaluation of movie files

Weighted kappa statistics related to the concordance of evaluations between the two readers were 0.732 for B-mode US and 0.718 for CEUS, indicating fair reproducibility. In subsequent analyses, scores of one or two were considered as HCC absent, and three or four as HCC present according to Youden index analysis.

HCC detection sensitivity

HCC detection sensitivity was evaluated in the 123 segments that contained HCC nodules (Table 2). Sensitivities of B-mode US were 0.837 [95% confidence interval (CI) 0.761–0.893] by reader A and 0.846 (95% CI 0.770–0.899) by reader B. Sensitivities of Sonazoid CEUS were 0.732 (95% CI 0.646–0.803) and 0.831 (95% CI 0.681–0.831), respectively. Reader A correctly detected HCC on unenhanced ultrasonography, but failed to detect the corresponding nodule on Sonazoid CEUS in 23 segments, and reader B did so in 18 segments.

Table 1 Baseline characteristics (n = 100)

Variables	Value
Age (years)[a]	67.5 ± 10.6 (33–86)
Gender	
Male/female, n (%)/n (%)	60 (60.0)/40 (40.0)
Etiology	
HBsAg positive, n (%)	9 (9.0)
HCVAb positive, n (%)	77 (77.0)
Liver function	
Child-Pugh A/B, n (%)/n (%)	88 (88.0)/12 (12.0)
BMI (kg/m^2)[a]	23.3 ± 3.1 (17.7–34.4)
Number of tumor nodules (n)[a]	1.38 ± 0.79 (1–4)
Maximum diameter of tumor (mm)[a]	23.1 ± 7.7 (8–41)
Serum albumin concentration (g/dL)[a]	3.79 ± 0.6 (2.4–4.7)
Total bilirubin concentration (mg/dL)[a]	0.97 ± 0.44 (0.4–2.4)
AFP level >100 ng/mL, n (%)	15 (15.0)

BMI body mass index

[a] Data are expressed as mean ± SD (min–max)

Table 2 Sensitivity of HCC detection

	Conventional US			Sonazoid CEUS			*P* value
	Sensitivity		95% CI	Sensitivity		95% CI	
Reader A							
Total	0.837	(103/123)	0.761–0.893	0.732	(90/123)	0.646–0.803	0.062
Lateral	1.000	(13/13)	0.768–1.000	0.769	(10/13)	0.495–0.919	0.22
Medial	0.909	(10/11)	0.619–0.984	0.727	(8/11)	0.452–0.904	0.586
Arterial	0.841	(53/63)	0.731–0.912	0.714	(45/63)	0.592–0.812	0.133
Posterior	0.750	(27/36)	0.588–0.863	0.750	(27/36)	0.588–0.863	1.000
Reader B							
Total	0.846	(104/123)	0.770–0.899	0.756	(93/123)	0.672–0.824	0.110
Lateral	1.000	(13/13)	0.768–1.000	1.000	(13/13)	0.768–1.000	1.000
Medial	0.909	(10/11)	0.619–0.984	0.636	(7/11)	0.351–0.850	0.311
Arterial	0.857	(54/63)	0.749–0.923	0.746	(47/63)	0.625–0.838	0.179
Posterior	0.750	(27/36)	0.588–0.863	0.722	(26/36)	0.558–0.842	1.000

Numbers in parentheses indicate the number of segments considered as positive by a reader/number of segments with HCC by dynamic CT

Table 3 Factors affecting sensitivity

	Conventional US			Sonazoid CEUS		
	Sensitivity	95% CI	*P* value	Sensitivity	95% CI	*P* value
Size of tumor (*n*)						
<19 mm (69)	0.768	0.651–0.861	0.001	0.710	0.588–0.813	0.215
≥19 mm (69)	0.957	0.878–0.991		0.870	0.767–0.939	
Depth of tumor (*n*)						
<53 mm (69)	0.857	0.753–0.929	0.858	0.771	0.656–0.863	0.590
≥53 mm (69)	0.868	0.764–0.938		0.809	0.695–0.894	
BMI (*n*)						
<23 kg/m^2 (69)	0.855	0.750–0.928	0.805	0.800	0.683–0.884	0.834
≥23 kg/m^2 (69)	0.870	0.767–0.939		0.780	0.667–0.873	

Factors affecting sensitivity were assessed based on HCC nodules (total of 138 nodules). Continuous variables were divided by the median value. When the two readers disagreed, a nodule was considered detected when the averaged four-point scale score exceeded two. As shown in Table , sensitivity was significantly lower for small nodules (<19 mm) with B-mode US, and a similar trend was observed with Sonazoid CEUS. Neither nodule depth from body surface nor subject BMI had a significant effect on the sensitivity of either B-mode US or Sonazoid CEUS. We then assessed the B-mode US echo pattern of a total of 101 nodules correctly detected by both readers on B-mode US; eight nodules were hyperechoic; the remaining 93 were hypoechoic. The readers failed to detect 15 of these on Sonazoid CEUS. Of these, four were hyperechoic and 11 were hypoechoic on Sonazoid CEUS, suggesting that detection of a defect in Kupffer imaging by Sonazoid CEUS was relatively difficult when the nodule was hyperechoic on B-mode US ($P = 0.016$ by Fisher's exact test; Fig.).

HCC detection specificity

A total of 277 segments did not contain HCC nodules, as determined by dynamic CT. The readers' evaluation of movie files is summarized in Table . The specificity of B-mode US as evaluated by readers A and B was 0.902 (95% CI 0.861–0.932) and 0.949 (95% CI 0.917–0.970) and that of Sonazoid CEUS was 0.986 (95% CI 0.963–0.994) and 0.978 (95% CI 0.953–0.990), respectively. Sonazoid CEUS showed a higher specificity than B-mode US, irrespective of reader.

Both readers made a false-positive evaluation with Sonazoid CEUS in three segments, and one reader did so in five other segments. Of these eight segments, five had a cystic lesion (as shown by dynamic CT), which may have

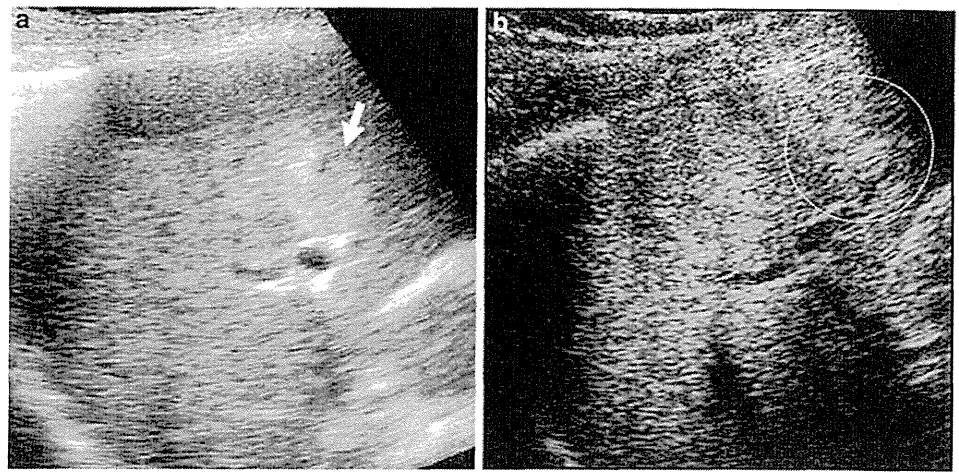

Fig. 2 A false-negative detected by Sonazoid CEUS. A hypervascular nodule was detected in segment 8. The nodule was visualized as a hyperechoic lesion (*arrow*) by unenhanced US (**a**), but disappeared (*circle*) after Kupffer imaging by Sonazoid CEUS (**b**)

Table 4 Specificity of HCC detection

	Conventional US			CEUS			*P*
	Specificity		95% CI	Specificity		95% CI	
Reader A							
Total	0.892	(247/277)	0.861–0.932	0.982	(272/277)	0.963–0.994	<0.001
Lateral	0.885	(77/87)	0.800–0.937	0.977	(85/87)	0.919–0.994	0.032
Medial	0.944	(84/89)	0.874–0.976	1.000	(89/89)	0.958–1.000	0.059
Arterial	0.865	(32/37)	0.718–0.941	0.973	(36/37)	0.860–0.995	0.199
Posterior	0.844	(54/64)	0.734–0.913	0.969	(62/64)	0.892–0.991	0.03
Reader B							
Total	0.949	(263/277)	0.917–0.970	0.978	(271/277)	0.953–0.990	0.109
Lateral	0.920	(80/87)	0.842–0.961	0.966	(84/87)	0.903–0.988	0.329
Medial	0.989	(88/89)	0.938–0.998	1.000	(89/89)	0.958–1.000	1.000
Arterial	0.919	(34/37)	0.785–0.972	0.946	(35/37)	0.821–0.985	1.000
Posterior	0.953	(61/64)	0.870–0.984	0.984	(63/64)	0.915–0.997	0.619

Numbers in parentheses correspond to the number of segments without HCC nodules by a reader/number of segments without HCC nodules by dynamic CT

been judged to be an enhancement defect in Kupffer imaging on Sonazoid CEUS (Fig. 3). Based on these sensitivities and specificities, the likelihood ratios for HCC presence when findings were positive were 11.4 and 43.4 with unenhanced and Sonazoid CEUS, respectively, and those for HCC presence when findings were negative were 0.171 and 0.222, respectively.

Discussion

The absence of Kupffer cells is one of the distinctive characteristics of hepatic malignant nodules, including HCC [8, 17]. This feature was first put to practical application for discriminating benign and malignant liver nodules using superparamagnetic iron oxide (SPIO) in magnetic resonance imaging (MRI) [18, 19]. Signal intensity of nodules on SPIO-MRI was reported to reflect grades of differentiation [20, 21]. The first-generation contrast agent Levovist (a suspension of galactose microparticle stabilized with palmitic acid) is also taken up by Kupffer cells in the liver and enables Kupffer cell imaging, similar to SPIO-MRI [22]. However, successful visualization of Kupffer cell defects by CEUS using Levovist requires sufficient ultrasound acoustic pressure to disrupt microbubbles. Repeated observation becomes impossible after microbubbles have been disrupted. This is a critical disadvantage in using Levovist CEUS for whole-liver screening. In contrast, Kupffer imaging using Sonazoid CEUS can be performed for several hours, which is suitable for imaging the entire liver.

In an assessment of the diagnostic accuracy of a test, evaluation of both sensitivity and specificity is essential. As an inverse correlation exists between sensitivity and

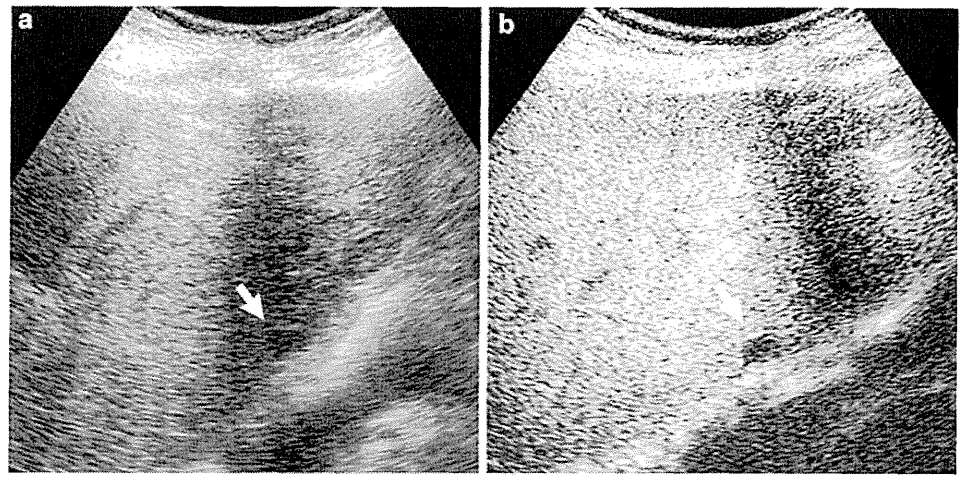

Fig. 3 A false-positive detected by Sonazoid CEUS. False-positive HCC detection by Sonazoid CEUS occurred mainly due to misrecognition of hepatic cysts (**a**, *arrow*) as HCC. Posterior enhancement was often obscured in Kupffer imaging by Sonazoid CEUS (**b**) and had an appearance (*arrow*) similar to that of an HCC nodule

specificity, reporting only sensitivity by recruiting those known to have the target disease is misleading and biased. Specificity of diagnostic imaging of liver nodules can be reported in at least three ways: (1) patient-, (2) segment-, or (3) nodule-based approaches. Calculation of specificity on a patient basis requires recruitment of individuals without HCC who undergo dynamic CT as a control. These controls should be patients with advanced liver fibrosis who are at a high risk for HCC. However, recruiting sufficient subjects to provide specificity with a narrow confidence interval is problematic. For a nodule-based study, controls should be benign nodules, such as hemangiomas, in patients with chronic liver disease. Previous reports on the diagnostic ability of Sonazoid CEUS on liver nodules were all based on this study design [11, 12, 25–26]. However, the diagnostic accuracy assessed by this type of study is appropriate for differential diagnosis, but not for detection. In the present study we adopted the second approach. Ultrasound movie files were obtained from patients with known HCC, but readers did not know whether each segment contained a cancer nodule, which made evaluation of specificity possible. This design had the additional advantage that matching ultrasonographic conditions such as parenchymal heterogeneous echogenicity or subjects' compliance was unnecessary, as "control" movie files were obtained from the same individuals. In addition, to reduce the likelihood of bias, ultrasound scanning of each segment was standardized, and B-mode US and Sonazoid CEUS movie files of the same segment were reviewed separately. These procedures were chosen for research purposes and obviously differ from clinical settings.

As a result, the sensitivity of Sonazoid CEUS for HCC detection was shown to be no greater than that of B-mode US. This may be due to several reasons. First, the lower acoustic power of Sonazoid CEUS compared with B-mode US (to avoid disrupting microbubbles) makes visualizing a deep nodule from the surface problematic. For enough sensitivity of CEUS, careful examination is demanded because CEUS is easily affected by the artifacts (such as bone and air) in low mechanical index (MI) mode. These artifacts were found especially in the right intercostal scan. Second, detection of nodules in obese patients is difficult due to attenuation of ultrasound by fatty tissue. However, subgroup analysis did not support this hypothesis. Hyperechoic nodules were unlikely to be detected by Kupffer imaging of Sonazoid CEUS, but this cannot entirely explain the low sensitivity. It should be noted that some nodules typical of HCC by dynamic CT are seen to possess Kupffer cells [27, 28]. However, Sonazoid CEUS can provide very stable post-vascular phase images for up to 60–120 min. Post-vascular phase obtained from later time (20–30 min) may increase the sensitivity of CEUS. The negative likelihood ratio of HCC presence by Sonazoid CEUS was insufficient, and thus caution should be taken when using this technique for diagnosis.

In contrast, the specificity of Sonazoid CEUS was shown to be about 98% irrespective of reader. The majority of false positives were due to misdiagnosis of cystic lesions as Kupffer imaging defects. Posterior enhancement, one of the major ultrasonographic characteristics of cystic lesions, may be obscured in Sonazoid CEUS. A positive likelihood ratio of at least 43 indicates that Sonazoid CEUS is suitable for confirmative diagnosis of HCC. Hyperechoic nodules were unlikely to be detected by post-vascular phase of Sonazoid US, but this cannot entirely explain the low sensitivity. Some new techniques can facilitate the diagnosis of tumor and should be combined with routine CEUS examination in clinical practice [29].

In clinical practice, US is performed in real time, so ambiguous lesions may be scrutinized by changing patients' positions and requesting that they hold their breath. Final diagnoses are likely to be based on findings obtained by both contrasted and B-mode US. Thus, the sensitivity and specificity obtained in this study may be

inferior to those in actual practice. Indeed, when movie files were re-examined 3 months after first review and images from both B-mode US and Sonazoid CEUS were examined simultaneously, the sensitivity improved compared to that for Sonazoid CEUS alone (data not shown). Other factors that may have affected HCC detectability were (1) that we did not use information in arterial images obtained by Sonazoid CEUS, which may have facilitated the assessment of the vascularity of targeted nodules; and (2) by using dynamic CT as the gold standard, atypical HCC detected with US may have been judged as false positives. The present study only included classical HCC and dynamic CT has its limitation. Early HCC is hypovascular on dynamic imaging in most cases. Its accurate diagnosis has remained difficult even with CT during hepatic arteriography (CTHA) and CT during arterial portography (CTAP). Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced MRI should be considered regarding diagnosis of early HCC [30].

In conclusion, we have shown that Sonazoid CEUS detects HCC with high specificity, but its sensitivity is no higher than that of B-mode US. Sonazoid CEUS is more suitable for confirmative diagnosis of HCC and can be performed immediately after B-mode US for this purpose.

Conflict of interest The authors declare that they have no conflict of interest.

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CME

Open

Radiofrequency Ablation for Hepatocellular Carcinoma: 10-Year Outcome and Prognostic Factors

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OBJECTIVES: **Radiofrequency ablation (RFA) is widely performed for hepatocellular carcinoma (HCC). However, there has been no report on 10-year outcome of RFA. The objective of this study was to report a 10-year consecutive case series at a tertiary referral center.**

METHODS: **We performed 2,982 RFA treatments on 1,170 primary HCC patients and analyzed a collected database.**

RESULTS: **Final computed tomography images showed complete tumor ablation in 2,964 (99.4%) of 2,982 treatments performed for the 1,170 primary HCC patients. With a median follow-up of 38.2 months, 5- and 10-year survival rates were 60.2% (95% confidence interval (CI): 56.7–63.9%) and 27.3% (95% CI: 21.5–34.7%), respectively. Multivariate analysis demonstrated that age, antibody to hepatitis C virus (anti-HCV), Child-Pugh class, tumor size, tumor number, serum des-γ-carboxy-prothrombin (DCP) level, and serum lectin-reactive α-fetoprotein level (AFP-L3) were significantly related to survival. Five- and 10-year local tumor progression rates were both 3.2% (95% CI: 2.1–4.3%). Serum DCP level alone was significantly related to local tumor progression. Five- and 10-year distant recurrence rates were 74.8% (95% CI: 71.8–77.8%) and 80.8% (95% CI: 77.4–84.3%), respectively. Anti-HCV, Child-Pugh class, platelet count, tumor size, tumor number, serum AFP level, and serum DCP level were significantly related to distant recurrence. There were 67 complications (2.2%) and 1 death (0.03%).**

CONCLUSIONS: **RFA could be locally curative for HCC, resulting in survival for as long as 10 years, and was a safe procedure. RFA might be a first-line treatment for selected patients with early-stage HCC.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignant neoplasm in the world (1). Only 20% of HCC patients are candidates for resection (2). Furthermore, recurrence is frequent even after apparently curative resection. Liver transplantation is restricted by organ donor shortage. Thus, various nonsurgical therapies have been introduced (3–5). Among these, image-guided percutaneous ablation is considered best for early-stage HCC.

Ethanol injection was formerly the standard procedure among the various percutaneous ablation techniques. Randomized controlled trials, however, have demonstrated that radiofrequency ablation (RFA) has a more reliable local antitumor effect, leading to a lower local tumor progression risk and higher survival rates (6–9). RFA has largely replaced ethanol injection (10).

Several reports on 5-year outcome of RFA exist (11–17); however, no study has covered 10-year outcome. We report on a 10-year consecutive case series at a tertiary referral center. We analyzed antitumor effect, patient survival, local tumor progression, and distant recurrence rates, variables relevant to these outcomes, and complications. To our knowledge, this study documents the largest number of RFA treatments performed at a single institution.

METHODS

RFA indications

RFA was the treatment of choice in HCC patients satisfying the following criteria: (i) ineligible for surgical resection/liver transplantation or patient refusal for surgery; (ii) no extrahepatic metastasis/vascular invasion; and (iii) no other malignancies that

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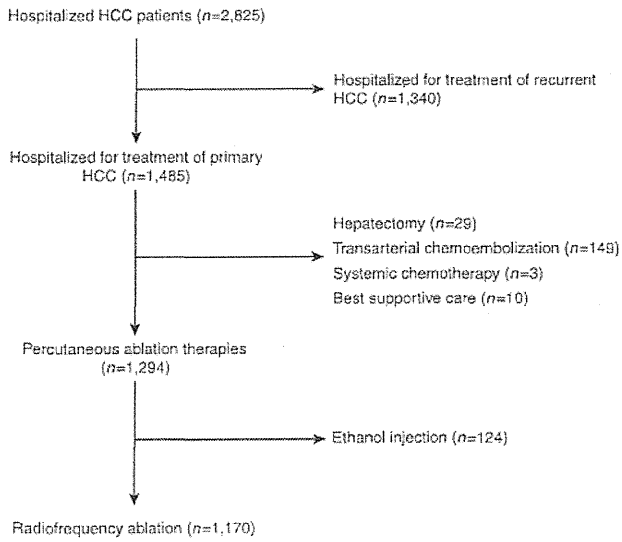

Figure 1. Flow of patients in this study. HCC, hepatocellular carcinoma.

may determine the patient's prognosis. Exclusion criteria were as follows: (i) tumor not visualized by ultrasonography/not accessible percutaneously; (ii) total bilirubin level ≥3.0 mg/dl; (iii) platelet count $< 50\times10^9$/l or prothrombin activity < 50%; (iv) refractory ascites; (v) enterobiliary reflux; and (vi) adhesion between the tumor and the gastrointestinal tract. In general, we performed RFA on Child-Pugh class A or B patients, a single tumor ≤5 cm in diameter, or three or fewer tumors ≤3 cm in diameter. In cases beyond these conditions, we performed RFA on patients who were likely to benefit from this procedure for possible cure or prolongation of life. No patients were excluded solely on account of tumor location (18). Informed consent was obtained from each patient. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and approved by the institutional review board (Registration ID: P98C05-11Y).

Patients

In this cohort study, we analyzed a prospectively collected computerized database. Between February 1999 and December 2009, 2,825 HCC patients were admitted once or more to the Department of Gastroenterology, the University of Tokyo (**Figure 1**). At initial hospitalization, 1,485 had primary HCC and the remaining 1,340 had recurrent HCC. In the recurrent HCC patients, primary HCC had previously been treated by therapies other than RFA.

Of the 1,485 primary HCC patients, 1,294 (87.1%) underwent percutaneous ablation as the initial treatment, including RFA. The remaining 191 patients underwent other therapies: hepatic resection, 29 patients with good liver function and who consented to an operation; transarterial chemoembolization, 149 with multinodular or large tumors that could not be treated by ablation therapies; systemic chemotherapy, three with extrahepatic metastasis; and only supportive care, 10 with decompensated cirrhosis or poor general condition.

Of the 1,294 patients treated by percutaneous ablation, 1,170 underwent RFA and the other 124 underwent ethanol injection. The choice of therapy was made as follows: between April 1999 and January 2001, 232 patients with three or fewer tumors, each ≤3 cm in diameter, and Child-Pugh class A or B liver function were entered into a randomized controlled trial to compare RFA with ethanol injection (6). Patients outside these inclusion criteria were mostly treated by RFA. After this trial, RFA was generally the treatment of choice, and ethanol injection was administered only to those considered unsuitable for RFA; ethanol injection was administered to those with either enterobiliary reflux or adhesion of the tumor to the gastrointestinal tract.

HCC was diagnosed based on typical imaging findings; that is, early-phase enhancement and late-phase contrast washout on dynamic computed tomography (CT) (19). HCC diagnosis was also confirmed by biopsy in 1,078 (92.1%) of the 1,170 patients with RFA-treated primary HCC. A total of 998 (85.3%) were diagnosed as having liver cirrhosis.

In general, transarterial chemoembolization was combined with RFA in patients with either ≥4 tumors or those with even one tumor >3.0 cm in diameter, although indication criteria of this combination had changed over time. The combination of transarterial chemoembolization with RFA was performed in 324 primary HCC patients.

Treatment methods

RFA was performed on an inpatient basis. Preoperative planning including evaluation of all imaging studies, and careful ultrasound examination was performed to identify the tumors and determine the access routes.

The procedure was performed according to an institutional protocol and in the presence of three physicians. One physician inserted the electrode under ultrasound guidance while another assisted the procedure; at least one had 8-year or longer experience of percutaneous ablation therapies. The remaining physician was responsible for the ultrasound machine and data recording. Video recording was performed occasionally to improve and standardize the procedure.

The precise techniques of RFA are described elsewhere (6). Briefly, all RFA procedures were performed percutaneously under ultrasound guidance (Power Vision 8000, Aplio XV or Aplio XG; Toshiba, Tokyo, Japan). We used artificial pleural effusion (20) or artificial ascites (21) for tumors, which were in the hepatic dome or adjacent to the gastrointestinal tract. After administration of sedatives and local anesthesia, a 17-gauge cooled-tip electrode (Cool-Tip; RF Ablation System, Covidien, Boulder, Colombia, CO) was inserted. Radiofrequency energy was delivered for 6–12 min for each application. For large tumors, the electrode was repeatedly inserted into different sites, such that the entire tumor could be enveloped by assumed necrotic volumes. Following the procedure, the patient remained in bed until the next morning.

A CT scan with a 5-mm section thickness was performed 1–3 days after RFA to evaluate technique effectiveness (22). Complete ablation was defined as hypoattenuation of the entire tumor. We intended to ablate not only the tumor but also some of the liver

parenchyma surrounding it. When we suspected that unablated tumor portions remained, the procedure was repeated. We did not predefine the procedure number in a treatment: treatment was generally continued until CT imaging demonstrated necrosis of the entire tumor.

Follow-up

To detect recurrence at an early stage, serum α-fetoprotein (AFP), lectin-reactive AFP (AFP-L3), and des-γ-carboxy-prothrombin (DCP) levels were measured monthly, and CT and ultrasonography were performed every 4 months. Local tumor progression was defined as the appearance of viable cancer tissue touching the initially treated tumor (22) and distant recurrence as the emergence of one or several tumor(s) separate from the primary site. Chest CT or bone scintigraphy was performed if extrahepatic recurrence was suspected. RFA was used for recurrence if the patient still met the indication criteria. If multiple recurrences were not treatable with RFA, transarterial chemoembolization was generally performed.

Statistical analysis

This is a report of a consecutive case series: all RFA treatments performed on primary HCC patients at the Department of Gastroenterology, University of Tokyo between February 1999 and December 2009 were included and none was excluded. Data are presented as mean±s.d. for quantitative variables, and as absolute frequencies for qualitative variables.

A "procedure" was defined as a single intervention episode comprising one or more ablation performed on one or more tumors and a "treatment" as the completed effort to ablate one or more tumors. A treatment comprised one or more procedures (22).

"Technique effectiveness" rate was defined as the percentage of successfully eradicated macroscopic tumors, as evidenced by CT scan 1–3 days after the last procedure (22).

Overall survival was calculated in the 1,170 primary HCC patients. Survival curves were generated by the Kaplan–Meier method. In addition to overall survival, some subgroup analyses were performed with clinical characteristics including tumor size, tumor number, and liver function. Recurrence was evaluated in 1,138 of the 1,170 primary HCC patients; the remaining 32 patients were excluded from the recurrence analysis because some small tumors had been left untreated by RFA on account of detection failure by ultrasonography. Recurrence rates were calculated by the Gaynor's method (23). All time estimates were made from the date of the first RFA. The follow-up was finalized at either death or the last visit to the outpatient clinic before 31 December 2009. Transplanted patients were censored from this study at the date of transplantation.

The prognostic relevance of 19 baseline variables (**Table 1**), the combination of transcatheter arterial chemoembolization (TACE) with RFA, HCC recurrence, and the number of RFA sessions to survival was analyzed by univariate and multivariate Cox proportional hazards regression models. The prognostic relevance of 19 baseline variables (**Table 1**), the combination of TACE with RFA, and the number of RFA sessions to local tumor progression and distant recurrence was also analyzed by univariate and multivariate models. All variables with a P value <0.05 by univariate comparison were subjected to multivariate analysis. Some continuous variables in which log-linearity could not be assumed were transformed into categorical variables. In multivariate analysis, we evaluated two models that contained either Child-Pugh class or its components to avoid multicollinearity. A stepwise variable selection was performed with Akaike Information Criteria in multivariate analysis. The results of multivariate analyses were presented as a hazard ratio with corresponding 95% confidence interval (CI), with P values from the Wald test. All significance tests were two-tailed, and differences with a P value <0.05 were considered statistically significant.

Complications were defined according to the guidelines of the Society of Interventional Radiology (24).

RESULTS

Antitumor effect

We performed a total of 2,982 RFA treatments for the 1,170 primary HCC patients, comprising 4,514 procedures. Thus, procedure number per treatment was 1.52±0.78. Many patients undergoing RFA for treatment of primary HCC received iterative RFA treatments for recurrence. A total of 485 patients underwent RFA treatment once, 247 twice, 177 thrice, 94 four times, 70 five times, 35 six times, 23 seven times, 14 eight times, 7 nine times, 7 ten times, 6 eleven times, 2 twelve times, 2 thirteen times, and 1 fourteen times.

Technique effectiveness rate was 99.4% (2,964/2,982 treatments). It was similar between the initial RFA treatments and the other RFA treatments for recurrence ($P=0.98$). Complete ablation of the tumor was achieved in 1,163 (99.4%) of the 1,170 initial treatments and in 1,801 (99.4%) of the 1,812 other RFA treatments. However, technique effectiveness rate significantly differed with tumor size ($P=0.023$). No apparent viable portions remained in the treated tumor in 1,642 (99.6%) of 1,648 treatments for tumors ≤2.0 cm in diameter, in 923 (99.2%) of 930 treatments for tumors 2.1–3.0 cm, in 356 (98.9%) of 360 treatments for tumors 3.1–5.0 cm, and in 43 (97.7%) of 44 treatments for tumors >5.0 cm. Final CT imaging demonstrated residual cancer tissue in the remaining 18 treatments. We decided against performing additional procedures, because liver failure rather than HCC seemed to determine the prognosis in 10 treatments, and because additional RFA would have caused complications on account of poor visualization or inaccessibility in the other eight treatments.

Survival

The 19 baseline clinical characteristics of the 1,170 patients who underwent RFA for treatment of primary HCC are shown in **Table 1**. A total of 269 patients (23.0%) were >75 years old. In all, 422 patients had tumors ≤2.0 cm in diameter, 467 had tumors 2.1–3.0 cm, 246 had tumors 3.1–5.0 cm, and 35 had tumors >5.0 cm; 685 patients had 1 tumor, 395 had 2 or 3 tumors, and 90 had ≥4 tumors.

As of December 2009 (with a median follow-up of 38.2 months), 692 patients (59.1%) remained alive, 39 (3.3%) were lost to

Table 1. **Baseline characteristics of the 1,170 patients undergoing radiofrequency ablation for primary hepatocellular carcinoma**

Variable	
Age (years)	68.3±8.6
Males, *n* (%)	751 (64.1)
Viral infection	
HBs-Ag-positive, *n* (%)	127 (10.9)
Anti-HCV-positive, *n* (%)	870 (74.4)
Both positive, *n* (%)	13 (1.1)
Both negative, *n* (%)	159 (13.6)
Alcohol consumption >80 g/d	170 (14.5)
Ascites, *n* (%)	117 (10.0)
Encephalopathy, *n* (%)	24 (2.1)
Albumin (g/dl)	3.65±0.47
Total bilirubin (mg/dl)	0.95±0.49
Prothrombin time (%)	79.6±14.1
Platelet count ($\times 10^3$/mm^3)	11.9±5.6
AST (IU/l)	61.5±35.9
ALT (IU/l)	57.3±40.8
Child-Pugh class, n (%)	
A	868 (74.2)
B	291 (24.9)
C	11 (0.9)
Tumor size (cm)	2.54±1.04
Tumor number	1.8±1.2
Serum AFP (ng/dl), n (%)	
≤100	928 (793)
101–400	146 (12.5)
>400	96 (8.2)
Serum DCP (mAU/ml), n (%)[a]	
≤100	964 (83.1)
101–400	126 (10.9)
>400	70 (6.0)
Serum AFP-L3 (%), n (%)	
≤15	1,015 (86.8)
15.1–40	74 (6.3)
>40	81 (6.9)

AFP, α-fetoprotein; AFP-L3, lectin-reactive α-fetoprotein; ALT, alanine aminotransferase; AST, asparatate aminotransferase; DCP, des-γ-carboxy-prothrombin; HCV, hepatitis C virus.

Data are expressed as mean±s.d.

[a]Serum DCP level could not be measured in 10 patients because they were being administered warfarin.

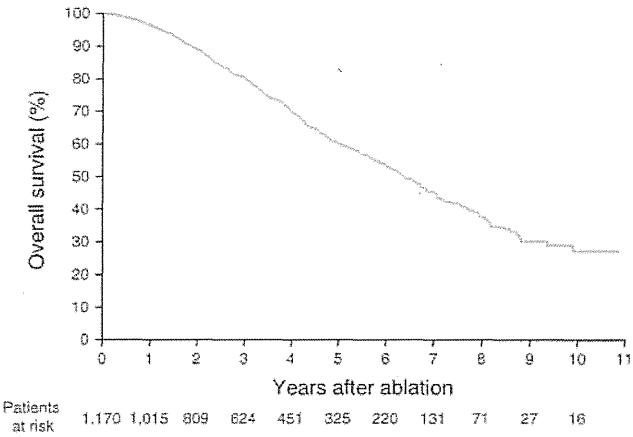

Figure 2. Overall survival in 1,170 primary hepatocellular carcinoma patients who underwent radiofrequency ablation.

follow-up, and 439 (37.5%) had died. Of the 1,170 patients, two were transplanted. The number of 5-, 7-, and 10-year survivors was 325, 131, and 16, respectively. The cause of death was HCC in 245 patients (55.8%), liver failure in 89 (20.3%), upper gastrointestinal bleeding in 11 (2.5%), complications related to the procedure in 3 (0.7%), liver-unrelated diseases in 81 (18.5%), and undetermined in 10 (2.3%).

The 1-, 3-, 5-, 7-, and 10-year survival rates of all 1,170 primary HCC patients were 96.6% (95% CI: 95.5–97.7%), 80.5% (95% CI: 78.0–83.1%), 60.2% (95% CI: 56.7–63.9%), 45.1% (95% CI: 40.9–49.6%), and 27.3% (95% CI: 21.5–34.7%), respectively (**Figure 2**; **Table 2**). Survival rates differed significantly with tumor size ($P<0.0001$), tumor number ($P=0.0003$), and Child-Pugh class ($P<0.0001$). In the Child-Pugh class A or B patients with a single tumor ≤5 cm in diameter, or three or fewer tumors ≤3 cm in diameter, the 5-year survival rate was 63.8% (95% CI: 59.9–67.9%), while in those outside these criteria, it was 46.4% (95% CI: 39.4–54.8%).

Univariate analysis showed 19 of the 22 variables relevant to survival. In multivariate analysis that contained Child-Pugh class but not its components, a model that contained age, antibody to hepatitis C virus (anti-HCV), Child-Pugh class, tumor size, tumor number, serum DCP level, and serum AFP-L3 level was selected (**Table 3**). The other model that contained the components of Child-Pugh class is shown in **Supplementary Table** online.

Recurrence

Recurrence developed in 741 patients. Local tumor progression alone was found in 25, local tumor progression with distant recurrence was found in 9, and distant recurrence alone was found in the other 707 patients. Of these 707 patients, 13 had the first recurrence in extrahepatic sites: 7 had lymph node metastasis, 3 had peritoneal seeding, 1 had lung metastasis, 1 had bone metastasis, and the remainder had both peritoneal seeding and lung metastasis. No recurrence developed in the remaining 397 patients.

Of the 741 patients, the first recurrence was treated by iterative RFA in 659 (88.9%), transarterial chemoembolization in 69 (9.3%), systemic chemotherapy in 4 (0.5%), surgical resection in 3 (0.4%), radiation therapy in 2 (0.3%), and supportive care in 4 (0.5%).

Table 2. Survival of patients undergoing radiofrequency ablation, based on tumor number, tumor size, and Child-Pugh class

Grading	*n*	Survival (%)					Median (years)	*P* value
		1-Year	3-Year	5-Year	7-Year	10-Year		
Overall survival	1,170	96.6	80.5	60.2	45.1	27.3	6.4	—
Tumor number								
Solitary	685	97.2	82.6	64.6	50.5	32.0	7.0	0.0003
2–3	395	95.7	77.9	54.4	39.4	19.9	5.6	
≥4	90	96.5	76.4	53.6	30.1	17.6	5.3	
Tumor size								
≤3 cm	889	97.2	83.8	65.1	47.3	30.7	6.7	<0.0001
>3 cm	281	94.8	71.0	46.5	38.0	18.6	4.6	
Child-Pugh class								
A	868	98.0	86.0	65.9	50.2	30.1	7.0	<0.0001
B	291	93.2	66.4	46.5	32.4	20.6	4.6	
C	11	81.8	58.4	23.4	23.4	—	3.1	
Combination of tumor number, tumor size, and Child-Pugh class								
Solitary, ≤3 cm	534	97.6	84.7	68.0	51.4	34.3	7.1	—
Solitary, ≤3 cm, Child-Pugh A	401	98.7	90.1	74.0	57.4	41.3	8.2	—
1–3 Tumors, ≤3 cm	822	97.1	83.7	65.2	48.8	32.5	6.9	—
Solitary, ≤5 cm, or 1–3 tumors, ≤3 cm	947	97.2	82.8	63.8	48.8	30.6	6.9	—
Child-Pugh A/B								
Satisfied the indication criteria of surgical resection proposed in the BCLC protocol[a]	237	98.6	90.5	75.9	61.1	38.1	8.7	—

BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.
[a]Child-Pugh class A with a normal level of bilirubin, no significant portal hypertension, and a single HCC.

The 1-, 3-, 5-, 7-, and 10-year rates of local tumor progression with or without distant recurrence were 1.4% (95% CI: 0.7–2.1%), 3.2% (95% CI: 2.1–4.3%), 3.2% (95% CI: 2.1–4.3%), 3.2% (95% CI: 2.1–4.3%), and 3.2% (95% CI: 2.1–4.3%), respectively (**Figure 3**). Univariate analysis demonstrated that prothrombin time and serum AFP, DCP, and AFP-L3 levels were correlated to local tumor progression, whereas multivariate analysis showed that serum DCP level alone was significantly correlated. Tumor size was not correlated to local tumor progression.

The 1-, 3-, 5-, 7-, and 10-year rates of distant recurrence without local tumor progression were 25.6% (95% CI: 23.0–28.2%), 63.3% (95% CI: 60.2–66.4%), 74.8% (95% CI: 71.8–77.8%), 78.1% (95% CI: 75.1–81.2%), and 80.8% (95% CI: 77.4–84.3%), respectively. Univariate analysis demonstrated 14 variables relevant to distant recurrence, whereas multivariate analysis showed that anti-HCV, Child-Pugh class, platelet count, tumor size, tumor number, serum AFP level, and serum DCP level were significantly related to distant recurrence (**Table 3**).

Complications

A total of 67 complications were encountered (**Table 4**). The incidence rates of complications per treatment and per procedure were 2.2% (67/2,982) and 1.5% (67/4,514), respectively. One patient died of hepatic failure on account of massive hepatic infarction 7 days after the last RFA procedure. He had undergone 12 RFA treatments in 8 years. The treatment mortality rate was 0.03%.

DISCUSSION

This study describes our 10-year clinical experience with RFA at a high-volume center. We performed the 2,982 RFA treatments on a total of the 1,170 primary HCC patients, showing that RFA has a high antitumor effect. Tumors were judged to have been completely ablated by final CT imaging in 99.4% of the treatments. Complete response was achieved not only in the first RFA but also in iterative RFA for recurrence. Although complete response rate differed with tumor size, there was not a sharp drop-off in effectiveness. The complete response rate may be higher in this study than others probably because we generally repeated the procedure until CT imaging demonstrated complete tumor necrosis, whereas many other studies limited the procedure number of RFA to 2–3 (11,13,15). Complete ablation of tumors has been reported to be related to improved survival (25). There were the 18 treatments in which we did not perform additional RFA for residual cancer tissue. In those treatments, usefulness of RFA had been unclear at the initial session because of liver dysfunction or tumor burden.

Table 3. **Multivariate analysis of variables relevant to survival, local tumor progression, and distant recurrence**

Variable	Multivariate analysis Hazard ratio (95% CI)	*P* value
Survival		
Age (per year)	1.03 (1.02–1.04)	<0.0001
Anti-HCV-positive	1.34 (1.03–1.76)	0.03
Child-Pugh class		
A	1	
B or C	2.08 (1.69–2.56)	<0.0001
Tumor size (cm)		
≤2.0	1	
2.1–3.0	1.40 (1.10–1.80)	0.007
3.1–5.0	1.80 (1.37–2.38)	<0.0001
>5.0	1.50 (0.90–2.49)	0.12
Tumor number		
Solitary	1	
2–3	1.28 (1.04–1.59)	0.02
≥4	1.58 (1.13–2.21)	0.008
Serum DCP (mAU/ml)		
≤100	1	
101–400	1.22 (0.88–1.69)	0.24
>400	1.66 (1.14–2.42)	0.008
Serum AFP-L3 (%)		
≤15	1	
>15	1.45 (1.11–1.91)	0.008
Local tumor progression		
Serum DCP (mAU/ml)		
≤100	1	
101–400	2.51 (1.02–6.20)	0.05
>400	6.52 (2.63–16.1)	<0.0001
Distant recurrence		
Anti-HCV-positive	1.44 (1.19–1.75)	0.0002
Child-Pugh class		
A	1	
B or C	1.23 (1.03–1.45)	0.02
Platelet count (/l)		
$>10^{11}$	1	
$\leq 10^{11}$	1.36 (1.12–1.64)	0.002
Tumor size (cm)		
≤2.0	1	
2.1–3.0	1.30 (1.10–1.55)	0.003
3.1–5.0	1.29 (1.05–1.60)	0.02
>5.0	1.25 (0.75–2.08)	0.4

Table 3. **Continued**

Variable	Multivariate analysis Hazard ratio (95% CI)	*P* value
Tumor number		
Solitary	1	
2–3	1.36 (1.16–1.59)	0.0002
≥4	2.02 (1.53–2.66)	<0.0001
Serum AFP (ng/dl)		
≤100	1	
101–400	1.15 (0.92–1.44)	0.22
>400	1.36 (1.03–1.81)	0.03
Serum DCP (mAU/ml)		
≤100	1	
101–400	1.19 (0.92–1.54)	0.19
>400	1.72 (1.22–2.42)	0.002

AFP, α-feloprotein; CI, confidence interval; DCP, des-γ-carboxy-prothrombin; HCV, hepatitis C virus.

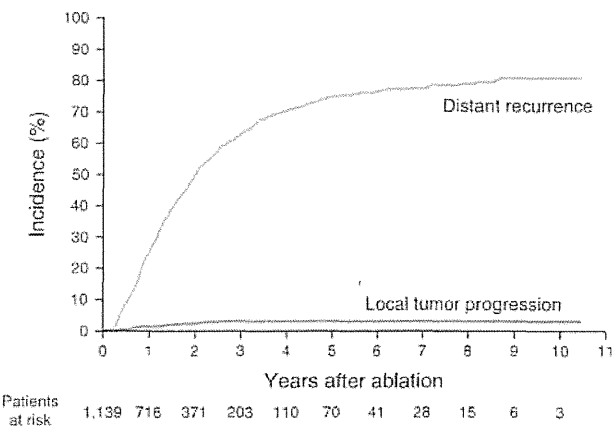

Figure 3. Local tumor progression and distant recurrence in patients who underwent radiofrequency ablation.

This study shows that RFA could achieve long-term survival for as long as 10 years. Sixteen patients treated by RFA survived for >10 years. The variables relevant to survival were similar to those found in previous studies on ethanol injection (26,27), RFA, hepatic resection (28), and transarterial chemoembolization (29). Both liver function and tumor-related factors were associated with survival. In addition, age and anti-HCV were relevant to survival in this study. Age was among the prognostic factors, probably because 23.0% of the patients were >75 years old, which resulted in a higher percentage (18.5%) of liver-unrelated deaths in this study compared with others. Anti-HCV was among the prognostic factors, probably because anti-HCV-positive patients developed distant recurrence more frequently.

HCC frequently recurred after RFA; most recurrences were, however, not local tumor progression but distant recurrence. Frequent recurrence is not specific to RFA. After hepatic resection, the

Table 4. Complications in 2,982 treatments of radiofrequency ablation for hepatocellular carcinoma

Complication	No. of complications
Neoplastic seeding	24
Liver abscess	6
Hemoperitoneum	12
Hemothorax	5
Symptomatic pleural effusion	1
Massive hepatic infarction	6
Gastrointestinal perforation or penetration	5
Hemobilia	2
Skin burn	1
Pneumothorax	3
Gallbladder injury	1
Cerebral infarction	1

tumor recurrence rate exceeds 70% at 5 years (30,31). In this study, periodic follow-up detected most recurrences at limited stage. RFA was performed again for first recurrence in almost 90% of cases, although multimodal treatments were used in a long-term follow-up. On the other hand, repeat resection rate for first recurrence has been reported to range from 10.4 to 30.6% (31,32). Because RFA is less invasive than hepatic resection, iterative RFA can be performed for recurrence more easily.

Local tumor progression was found less frequently in this study than in other studies, having been reported to be around 10% at 3 years following RFA (13,14). Furthermore, different from the findings in previous reports (33,34), tumor size was not related to local tumor progression in this study. These differences are probably because we repeated RFA until we considered we had ablated not only the tumor itself but also some of the liver tissue surrounding it. Furthermore, to avoid local tumor progression, we were more cautious in the treatment of larger tumors when deciding whether sufficient ablation had been performed. Only serum DCP level was significantly related to local tumor progression in this study. Elevated serum DCP level may be related to the malignant potential of HCC such as the development of portal venous invasion (35).

The frequency of distant recurrence in this study was similar to that reported in other studies (13). Among the variables significantly related to distant recurrence, tumor size, tumor number, serum AFP level, and serum DCP level were probably related to micrometastasis, which had not been detected by imaging modalities before the treatment, while anti-HCV, Child-Pugh class, and platelet count were related to metachronous multicentric carcinogenesis, which developed based on underlying chronic liver disease.

From the viewpoint of survival and distant recurrence, patients with 2.1–5.0 cm tumors had significantly worse outcomes than those with ≤2.0 cm tumors while those with tumors >5.0 cm did not have worse rates than those with tumors ≤2 cm. This is probably because the number of patients with tumors >5.0 cm ($n=35$) were not large enough for the difference to be statistically significant. Another possibility is selection bias. It is possible that patient with tumors >5.0 cm who underwent RFA had more favorable conditions for survival and distant recurrence except tumor size than those with 2.1–5.0 cm tumors.

In this study, 324 of the 1,170 patients were treated with combination of TACE and RFA at the initial treatment. Thus, we evaluated the combination as a possible variable that influences survival or recurrence. Univariate analysis demonstrated that the combined therapy was significantly correlated to overall survival, whereas multivariate analysis did not show the relationship. TACE was generally combined with RFA in patients with either ≥4 tumors or those with even one tumor >3.0 cm in diameter. This is why the correlation was significant in univariate analysis, while it was not in multivariable model in which the effect of other risk factors, such as tumor number and tumor size were adjusted. The combination of TACE and RFA was not significantly related to either local tumor progression or distant recurrence.

RFA was a safe procedure. Although many patients treated by RFA in this study were at high risk for surgical treatment because of advanced cirrhosis or other comorbidities, complications occurred in only 2.2% of the treatments. Other investigators have also reported low complication rates of 0–6.1% (11,13–16). For hepatic resection, morbidity rates of 38–47% have been reported even in recent studies (36–38).

To date, percutaneous ethanol injection has been considered the standard in ablation (5). However, randomized controlled trials have demonstrated the superiority of RFA (6–9), with RFA now largely replacing ethanol injection. We have also shifted from ethanol injection to RFA (10). At our department, RFA is currently the first option and ethanol injection is performed only on patients on whom RFA cannot be performed safely because of either enterobiliary reflux, adhesion between the tumor and the gastrointestinal tract, or other reasons.

Surgical resection has been considered the treatment of choice for HCC. Our first option for resectable HCC was also surgery. However, most patients who came to our department visited us because they did not want surgical resection. Thus, many patients in this study underwent RFA not because of unresectable tumor but because of refusal of surgery. Those who preferred surgery would have directly gone to the surgical department that has extensive experience in hepatic resection (38).

It is not easy to compare outcomes between RFA and surgical resection; the indications are different between the two treatments. Furthermore, indications for each treatment are different from institution to institution. Thus, a case adjudged to be treatable by RFA or surgical resection at an institution may not be given the same treatment at another. The best known indication criteria for surgical resection may be those proposed in the Barcelona Clinic Liver Cancer (BCLC) protocol (5), which states that surgical resection should be restricted to patients with performance status 0, Child-Pugh class A, single HCC, normal portal pressure, and normal serum bilirubin level. In patients satisfying those criteria, the 5-year survival rate is expected to be >70% (30). In this study, 237

(20.3%) of 1,170 patients satisfied those criteria and were thus considered good candidates for surgical resection; their 5-year survival rate was 75.9%, which appears satisfactory when compared with outcomes following surgical resection. Furthermore, in all 1,170 primary HCC patients treated by RFA, 5- and 10-year survival rates were 60.2% and 27.3%, respectively. In patients treated by surgical resection, 5- and 10-year survival rates were 34.4–70.0% and 10.5–52.0%, respectively (32,39–45). Although this is an observational study with no control, survivals following RFA appear comparable to those reported following surgical resection.

Two recent randomized controlled trials showed no significant difference in survival between RFA and surgical resection (46,47). Several nonrandomized controlled trials reported that RFA had similar overall survival rates to resection (48–50), while others found resection to be associated with higher survival rates (51–53). Further studies are necessary to resolve comparison of RFA with resection.

We have made strenuous efforts to standardize the RFA procedure. Although many physicians have participated in RFA at our institution, the procedure was invariably performed according to the institutional protocol and in the presence of experienced physicians. Video recording was also used to monitor the procedure. Additionally, preoperative planning and postoperative evaluation of technique effectiveness were also carried out by at least three physicians. We also believe that not only proficient practice of RFA but also detailed preoperative planning, cautious postoperative evaluation of therapeutic effect, and careful follow-up are vital to achieve satisfactory outcomes.

Source population in this study may represent selection bias, as we performed RFA on most patients who were hospitalized at our department; however, many patients with unfavorable tumor conditions for RFA might not have been referred to us. Therefore, caution is required when extrapolating our findings to the general population of HCC patients.

A second limitation is that study population cannot be clearly defined. This study was based on daily clinical practice over a 10-year period. Indication criteria of RFA have changed over time, mainly because another percutaneous ablation, that is, ethanol injection has also been performed. Furthermore, various treatments besides percutaneous ablation were available for HCC, such as surgical resection and transarterial chemoembolization, with frequently overlapping indications.

One further limitation is the fact that this was a single-center study; these results might not be reproducible consistently in other settings. To extrapolate the findings in this study to patients at other institutions, careful consideration should be given to differences in the indications, methods, expertise, performance of available ultrasound and CT equipment, and others. Treatment outcome may be influenced by the physicians' expertise and the institution's volume of care. We started ethanol injection in 1985 and microwave ablation in 1995, that is, before the introduction of RFA. Recently, we have performed over 900 RFA treatments per year, which may represent a far greater number of treatments than those in most other institutions. We would not recommend any change in daily clinical practice solely on the strength of our study findings.

In conclusion, our 10-year clinical experience shows that RFA could be locally curative, resulting in survival for as long as 10 years, and was a safe procedure. RFA might be a first-line treatment for selected patients with early-stage HCC.

CONFLICT OF INTEREST

Guarantor of the article: Shuichiro Shiina, MD, PhD.

Specific author contributions: Study concept and design, analysis and interpretation of data, and drafting of the manuscript: Shuichiro Shiina; analysis and interpretation of data and statistical analysis: Ryosuke Tateishi; study execution and data acquisition: Toru Arano, Koji Uchino, Kenichiro Enooku, Hayato Nakagawa, Yoshinari Asaoka, Takahisa Sato, Ryota Masuzaki, Yuji Kondo, and Tadashi Goto; revised the article critically for important intellectual content: Haruhiko Yoshida; Masao Omata, and Kazuhiko Koike. All authors have read and approved the submitted manuscript.

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Potential competing interests: None.

WHAT IS CURRENT KNOWLEDGE

- ✓ Radiofrequency ablation (RFA) has been widely performed for hepatocellular carcinoma (HCC).
- ✓ RFA has a more reliable local antitumor effect and higher survival than ethanol injection.
- ✓ There has been no report on 10-year outcome of RFA.

WHAT IS NEW HERE

- ✓ Five- and 10-year survival rates in 1,170 patients with primary hepatocellular carcinoma (HCC) were 60.2 and 27.3%, respectively.
- ✓ Age, antibody to hepatitis C virus, Child-Pugh class, tumor size, tumor number, serum des-γ-carboxy-prothrombin level, and serum lectin-reactive α-fetoprotein level were significantly related to survival.
- ✓ Five- and 10-year local tumor progression rates were both 3.2%. Five- and 10-year distant recurrence rates were 74.8 and 80.8%, respectively.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Evaluation of molecular targeted cancer drug by changes in tumor marker doubling times

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Abstract
Background We evaluated the usefulness of tumor marker doubling time (DT) as an efficacy indicator of a molecular targeted anticancer agent.
Methods Twenty-five patients with advanced hepatocellular carcinoma (HCC) received TSU-68, a multiple tyrosine kinase inhibitor. Exponential increase in HCC-specific tumor marker levels (alpha-fetoprotein or des-gamma-carboxyprothrombin) was seen in 15 of them prior to TSU-68 administration. The relationship between tumor marker DT and tumor volume DT was evaluated. Next, tumor marker DT in the first 8 weeks of TSU-68 administration was compared with tumor marker DT before treatment. Efficacy evaluation based on changes in tumor marker DT was compared with Response Evaluation Criteria In Solid Tumors (RECIST).
Results Tumor marker DT and tumor volume DT were almost identical ($r^2 = 0.94$, $P < 0.001$) in each patient before TSU-68 administration. Efficacy evaluation based on changes in tumor marker DT on TSU-68 administration was in accordance with RECIST in 12/15 cases. Discordance was observed in three cases, for which RECIST indicated disease progression in spite of elongated tumor marker DT. Those cases showed substantial tumor necrosis without volume shrinkage or appearance of new lesions in spite of apparent effects on target lesions.
Conclusions Serum tumor marker DT can be used to evaluate viable tumor burden irrespective of the presence of tumor necrosis which can compromise radiographic evaluation. This approach may be applicable to the evaluation of responses to chemotherapy, particularly to cytostatic agents (ClinicalTrials.gov number, NCT00784290).

Keywords Doubling time · RECIST · AFP · PIVKA-II · HCC · TSU-68

Abbreviations

AFP	Alpha-fetoprotein
CEA	Carcinoembryonic antigen
CR	Complete response
CT	Computed tomography
DCP	Des-gamma-carboxyprothrombin
DT	Doubling time
FGFR	Fibroblast growth factor receptor
HCC	Hepatocellular carcinoma
PD	Progressive disease
PDGFR	Platelet-derived growth factor receptor
PR	Partial response
PSA	Prostate-specific antigen
RECIST	Response Evaluation Criteria In Solid Tumors
SD	Stable disease
TACE	Transcatheter arterial chemoembolization
VEGFR-2	Vascular endothelial growth factor receptor-2

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Introduction

Phase II trials of chemotherapeutic agents for solid tumors usually adopt an objective tumor response as the primary endpoint, the rationale being that the tumor response will be a surrogate for the effects of a particular agent on survival outcomes [1–4]. In evaluating a tumor response to a cancer drug, the Response Evaluation Criteria In Solid Tumors (RECIST) guidelines are usually adopted. However, the total tumor volume thus determined is not necessarily proportional to the number of viable tumor cells, e.g., in cases of massive tumor necrosis without tumor shrinkage [5–9].

With the progress in molecular targeted cancer drugs, concerns about the appropriate design of clinical trials of such agents have emerged [10, 11]. In contrast to conventional cytotoxic agents, molecular targeted agents often show cytostatic effects, i.e., a slowing of tumor growth. The effects of such agents upon the tumor growth rate may be better evaluated not by the changes in tumor burden but by the rate of changes for which RECIST may not be particularly suitable.

Most solid malignant tumors show an exponentially increasing volume in the natural course of their growth. The tumor volume doubling time (DT) is the parameter that defines the speed of the increase. Serum levels of several tumor markers, including prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP), have been reported to correlate with tumor volume in an individual patient [12–15]. The rate of changes in tumor volume may be calculated on the basis of repeated measurements of the serum tumor marker levels. The DT of serum PSA levels has also been proposed as a biological parameter that can be used to predict the prognosis of prostate cancer, and PSA determination has now become an integral part of the disease management [16–18].

The aim of the present study was to elucidate the usefulness of tumor marker DT to evaluate the efficacy of TSU-68 against hepatocellular carcinoma (HCC). TSU-68 is an orally administered, small-molecule inhibitor of multiple receptor tyrosine kinases, vascular endothelial growth factor receptor-2 (VEGFR-2), platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR) [19]. As a potent antiangiogenic agent, TSU-68 is expected to be effective against HCC [20], and a phase I/II study has been recently conducted in Japan [21]. In that clinical trial, the serum levels of AFP and des-gamma-carboxyprothrombin (DCP) were also scheduled to be periodically determined. Although the effect was assessed by radiologic examinations, the effect of TSU-68 may be more accurately evaluated by changes in tumor growth speed based on specific tumor marker levels [22–26].

Methods

Clinical trial

This study was conducted according to the ethical guidelines for epidemiologic research designed by the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare of Japan. The study design was approved by the institutional review board of the University of Tokyo Hospital.

An open-label phase I/II trial of TSU-68 for the treatment of HCC was performed between September 2003 and February 2007 at three institutions in Japan [21]. Twenty-five of the participating patients were enrolled from the University of Tokyo Hospital. In the present study, clinical data for these 25 patients, including analyses conducted before and after the trial, were further evaluated. Briefly, histologically confirmed HCC patients without indication or response to resection, ablation, or transcatheter arterial chemoembolization (TACE) were deemed eligible. The eligibility criteria also included a World Health Organization (WHO) performance status of 2 or better, a life expectancy of not less than 90 days, and a liver function of Child–Pugh class A or B. Patients were not eligible if they had received ablation, TACE, chemotherapy, or irradiation within 4 weeks, or surgery within 6 weeks, of the commencement of the trial (washout phase).

The phase I study began with a 400 mg bid oral dose of TSU-68. Because of dose-limiting toxicities, however, this was reduced to 200 mg bid in the subsequent phase II study. At the end of each 4-week cycle, dynamic contrast-enhanced computed tomography (CT) consisting of early and late arterial, and portal venous phases was performed, and contiguous transverse sections with a thickness of 5 mm were obtained. Responses were assessed on the basis on the RECIST evaluations in predetermined target lesions. The serum levels of HCC-specific tumor markers, AFP and DCP, were scheduled to be determined every 2 weeks. TSU-68 administration was discontinued when progressive disease (PD) was observed by RECIST.

Patients

Among the patients who had participated in the aforementioned trial, those who met the following criteria were included in the present study: (1) tumor growth prior to TSU-68 administration could be evaluated with two CT examinations performed 1–3 months before the trial and upon enrollment; (2) serum tumor marker levels could be determined at least three times during the washout phase, and a linear regression of the logarithmic transformation of marker levels over time showed an r^2 greater than

0.80; and (3) TSU-68 had been administered for at least 4 weeks.

Radiological evaluation of tumor volume

Radiological evaluations were performed according to RECIST guidelines version 1.0 [27]. Not more than 10 lesions, including intrahepatic tumors and extrahepatic metastases, were selected as target lesions prior to TSU-68 administration. In addition to RECIST, we also in our present analyses estimated the volume of each target lesion as a sphere taking the average of its major and minor axes as the diameter [28], and thereby calculating the radiological tumor volume DT as

$$\mathrm{DT} = \log(2) \times \frac{t_2 - t_1}{\log(V_2) - \log(V_1)}$$

where V_1 and V_2 are the volumes at times t_1 and t_2 [29].

Tumor markers

The HCC-specific tumor markers, AFP and DCP, were measured every 2 weeks for each patient registered in the trial. The serum AFP levels were measured via an enzyme immunoassay (ST AIA-PACK AFP, Tosoh, Tokyo, Japan) and DCP was measured using a chemiluminescent enzyme immunoassay (LUMIPULSE PIVKA-II, Eisai, Tokyo, Japan). These markers were also assayed after the termination of TSU-68 treatment, usually with a longer interval.

Tumor marker doubling time

In the present analyses, we assumed that the serum levels of tumor marker are proportional to the viable tumor volume with a fixed coefficient intrinsic to an individual case, when the tumor was producing the marker in question. Independently of the coefficient, the DT values can be calculated from two data points as

$$\mathrm{DT} = \log(2) \times \frac{t_2 - t_1}{\log(C_2) - \log(C_1)}$$

where C_1 and C_2 are the serum concentrations of tumor marker at times t_1 and t_2.

When data were available at more than two points, we first performed linear regression analysis of log-transformed tumor marker levels over time to determine the slope, and the DT was then calculated as

$$\mathrm{DT} = \frac{\log(2)}{\mathrm{slope}}.$$

Note in this case that the DT becomes negative when the tumor marker levels decrease following treatment.

Tumor volume and tumor marker levels during the washout phase

The total volume of target lesions was measured via two CT examinations during the washout phase: one at 4–12 weeks before and another immediately prior to the commencement of TSU-68 treatment. The tumor volume DT was then calculated as described above. The tumor marker DT was also calculated, and the relationship between the two sets of DT values was analyzed.

Changes in the DTs during TSU-68 treatment

The serum tumor marker DT during the first 8 weeks of TSU-68 administration was similarly calculated and compared with the DT measured before the drug therapy. If TSU-68 administration had been effective, the DT should be elongated, or yield a negative value. The evaluation of drug responses based on tumor marker DT was then compared with that by the RECIST method.

Tumor marker DT after the cessation of TSU-68 treatment

When a patient was observed without any anticancer treatment for more than 4 weeks after the cessation of TSU-68 treatment and tumor marker levels were determined more than once during this period, tumor marker DTs after the cessation of TSU-68 were similarly calculated and compared with those measured at 4 weeks and immediately before the cessation of treatment. The study design we used for estimating tumor marker DT before, during, and after TSU-68 administration is summarized in Fig. 1.

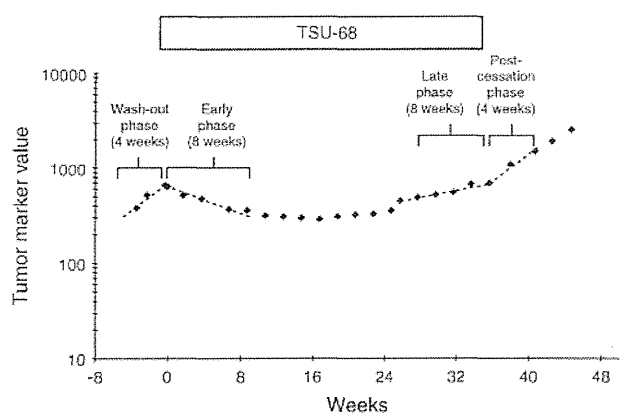

Fig. 1 Linear regression representation of the log tumor marker levels over time where DT = log(2)/slope. The slope can be calculated using a least-squares regression or two log-transformed tumor marker values: DT = log(2) × ($t_2 - t_1$)/[log(TM2) − log(TM1)], where t = time and TM = tumor marker level

Results

Patient characteristics

Among the 25 patients enrolled at the University of Tokyo Hospital, 15 met all of the inclusion criteria. The reasons for exclusion included insufficient tumor marker determination prior to TSU-68 administration (seven patients), results of CT prior to enrollment unavailable (one patient), no tumor marker elevation (one patient), and termination of TSU-68 administration at week 2 as a result of gastrointestinal bleeding (one patient). The baseline characteristics of the 15 patients included in the current study cohort are summarized in Table 1.

Table 1 Patient characteristics

Variable	$n = 15$
Age (years)	66.7 ± 6.3
Sex [no. (%)]	
Male	12 (80)
Female	3 (20)
Viral markers [no. (%)]	
HBs Ag+, HCV Ab−	2 (13)
HBs Ag−, HCV Ab+	13 (87)
Prior treatments[a] [no. (%)]	
TACE	13 (87)
Ablation	12 (80)
Surgery	5 (33)
Radiation	2 (13)
Systemic chemotherapy	1 (7)
Tumor stage [no. (%)[b]]	
I	0 (0)
II	0 (0)
III	7 (47)
IVa	4 (27)
IVb	4 (27)
Extrahepatic metastasis [no. (%)]	8 (53)
Portal vein thrombosis [no. (%)]	1 (7)

Plus-minus values represent the mean and standard deviation

HBs Ag hepatitis B surface antigen, *HCV Ab* hepatitis C antibody

[a] Number of pretreatments by surgery, radiofrequency ablation, transcatheter arterial chemoembolization, chemotherapy, or radiotherapy

[b] Based on the International Union Against Cancer (UICC) *TNM Classification of Malignant Tumors*, 6th edition, 2002

Tumor volume and tumor marker prior to TSU-68 administration

The relationship between the tumor volume and tumor marker DTs is shown in Fig. 2, where each point represents data from one patient. With the least-square method, the relationship was regressed to

$$y = 1.063x - 2.941$$

where x is the tumor volume DT and y is the tumor marker DT in days for both. The slope of regression was close to 1.0 with an r^2 value of 0.948, indicating that these two DTs were almost identical in each patient.

Changes in tumor marker DT during TSU-68 treatment

TSU-68 treatment was discontinued at week 4 in four patients because of the appearance of new lesions or a substantial increase in the volume of non-target lesions. The remaining 11 patients received this drug for at least 8 weeks and the response of the target lesions was evaluated in these patients as a stable disease (SD) by RECIST at week 4. Changes in the tumor marker DT before and after the commencement of TSU-68 administration are summarized in Table 2, together with the corresponding RECIST evaluation. When the tumor marker DT was increased following TSU-68 therapy, or became negative, this was considered to be an indication of at least partial drug efficacy. On the other hand, no beneficial effects were assigned to TSU-68 when the tumor marker DT was shortened following treatment. Such tumor marker-based evaluations were found to be compatible with RECIST, as a complete or partial response (CR, PR), or SD versus PD, in 12 of 15 patients. In the remaining three patients, a RECIST-based evaluation of PD was obtained in spite of an elongated tumor marker DT. In case 9, the RECIST-based evaluation became PD after cycle 2 because of the appearance of a new lesion, although the tumor marker DT was still elongated in this patient. Lymph node necrosis was observed by contrast-enhanced CT in this patient (Fig. 3), suggesting that TSU-68 remained effective. In case 10, a RECIST-based evaluation of PD was obtained because of an increase in the size of adrenal metastasis (a target lesion) although the hepatic lesions were decreased in size and the tumor marker DT was elongated. After the cessation of TSU-68 in patient 10, the left adrenal gland was excised and found to contain multiple necrotic lesions. Case 12 showed SD for the target lesion and an elongated tumor marker DT but was deemed to be a PD because of the appearance of new lesions.

In two of the other cases (nos. 1 and 2), a RECIST-based evaluation of SD was found but a negative tumor marker DT was also obtained. In case 1, the tumor marker DT became −21.1 days upon TSU-86 administration, which indicated an 84% decrease in tumor volume and 46% reduction in diameter by 8 weeks. Using RECIST parameters, a greater than 30% decrease in diameter typically corresponds to PR but case 1 was nevertheless evaluated as