blood tests were performed at the first visit of each patient. BMI was calculated as body weight in kilograms divided twice by body height in meters, which also was measured routinely at the first visit of each patient. Patients were divided into 4 groups by their BMI according to World Health Organization criteria[23]: underweight when the BMI is 18.5 kg/m² or less, normal when the BMI is 18.5 to 25 kg/m², overweight when the BMI is 25 to 30 kg/m², and obese when the BMI is greater than 30 kg/m². A diagnosis of diabetes mellitus was based on medical history or a 75-g oral glucose tolerance test.[24] Heavy alcohol consumption was defined as drinking alcohol equivalent to 100% ethanol of more than 80 g/day.[25,26] The amount of alcohol intake routinely was asked at the first visit in a questionnaire. Past history of blood transfusion before the diagnosis of chronic hepatitis also was examined. Human immunodeficiency virus antibody was not tested for routinely because the prevalence of human immunodeficiency virus co-infection among HCV-positive patients is very low in Japan.[27] We also examined the history of interferon therapies and responses during the follow-up period. A sustained virologic response (SVR) was defined as undetectable HCV RNA at least 24 weeks after the end of therapy.

Patient Follow-Up Evaluations and Diagnosis of Hepatocellular Carcinoma

Each patient was screened for HCC with ultrasonography at or immediately after the first visit and those in whom HCC was detected were not included in this study. Afterward, patients were followed up at the outpatient clinic with blood tests including tumor markers and ultrasonography every 3 to 6 months. Contrast-enhanced computerized tomography was performed when the serum α-fetoprotein (AFP) level showed an abnormal increase and/or tumors were detected as possible HCC in ultrasonography.[28] HCC was diagnosed by dynamic computerized tomography, considering hyperattenuation in the arterial phase with washout in the late phase as the definite sign of HCC.[29] When diagnosis of HCC was not clear, ultrasound-guided tumor biopsy was performed and pathologic diagnosis was made based on Edmondson and Steiner[30] criteria. Observations were censored on June 30, 2006.

Statistical Analysis

Data were expressed as the median and range (25th–75th percentiles) unless otherwise indicated. Continuous variables were compared among groups by analysis of variance (parametric) or the Kruskal–Wallis test (nonparametric). Trends in accordance with BMI were assessed with the exact trend test (proportion) or the Jonckheere–Terpstra test (continuous variables). A P value of less than .05 on a 2-tailed test was considered significant. The annual incidence of hepatocarcinogenesis in each group was assessed by person-year method. The cumulative incidence of HCC was estimated using the Kaplan–Meier method. In the analysis of risk factors for hepatocarcinogenesis, we tested the following variables obtained at the time of entry in univariate and multivariate Cox proportional hazard regression analyses: age, sex, BMI, heavy alcohol drinking, comorbidity with diabetes mellitus, serum albumin concentration, total bilirubin concentration, alanine aminotransferase (ALT) levels, prothrombin time activity, platelet counts, and α-fetoprotein (AFP) concentration. We also performed sensitivity analysis among those who did not receive interferon therapies during the follow-up period. Multichotomous categoric variables were represented by corresponding binary dummy variables. Data processing and analysis were performed by using the S-PLUS 2000 (MathSoft Inc., Seattle, WA).

Results

Patient Profiles

Baseline characteristics of patients, 727 men and 704 women, are shown in Table 1. There were 112 underweight patients, 1023 normal-range BMI patients, 265 overweight patients, and 31 obese patients. Heavy alcohol consumption was noted in 70 patients (4.9%) and diabetes mellitus was detected in 133 patients (9.3%). There were 271 patients whose AFP level exceeded 20 ng/mL (>100 ng/mL in 46 patients and >400 ng/mL in 8 patients).

We compared age, sex, heavy alcohol consumption, comorbidity with diabetes mellitus, serum albumin level, total biliru-

Table 1. Baseline Characteristics

Variable	Total (n = 1431)	Underweight (n = 112)	Normal BMI (n = 1023)	Overweight (n = 265)	Obese (n = 31)	*P*
Age, y[a]	60.1 (52.4–67.0)	64.3 (50.7–69.1)	60.3 (53.1–67.3)	59.1 (50.7–65.5)	55.9 (42.4–63.1)	.009[b]
Male, n (%)	727 (50.8)	44 (39.3)	528 (51.2)	141 (53.2)	14 (45.2)	.15[c]
Drinking >80 g/day, n (%)	70 (4.9)	5 (4.5)	51 (5.0)	13 (4.9)	1 (3.2)	.92[c]
Diabetes mellitus, n (%)	133 (9.3)	11 (9.8)	88 (8.6)	27 (10.2)	7 (22.6)	.11[c]
Serum albumin level, g/dL[a]	4.0 (3.8–4.3)	4.0 (3.8–4.3)	4.1 (3.8–4.3)	4.0 (3.7–4.2)	3.9 (3.6–4.1)	.99[d]
Total bilirubin level, mg/dL[a]	0.7 (0.6–0.9)	0.6 (0.5–0.8)	0.7 (0.5–0.9)	0.8 (0.6–1.0)	0.7 (0.5–0.8)	<.0001[d]
ALT level, IU/L[a]	61.0 (36.0–98.0)	51.0 (29.8–83.5)	61.0 (35.0–98.0)	69.0 (42.0–115)	80.0 (38.0–109)	.04[b]
Prothrombin time activity (%)[a]	84.8 (72.7–97.2)	88.9 (75.0–96.6)	85.3 (74.2–100)	80.6 (69.0–94.1)	85.2 (77.3–91.6)	.95[d]
Platelet count, $\times 10^3/\mu L$[a]	153 (108–201)	153 (115–203)	153 (110–203)	149 (92–194)	153 (122–189)	.95[b]
AFP level >20 ng/mL, n (%)	271 (18.9)	11 (9.8)	190 (18.6)	64 (24.1)	6 (19.4)	.0047[c]
Patients who received interferon, n (%)	209 (14.6)	8 (7.1)	151 (14.8)	48 (18.1)	2 (6.5)	.102[c]
Patients who achieved SVR, n (%)	69 (4.8)	1 (0.89)	55 (5.4)	13 (4.9)	0 (0)	.75[c]

[a]Expressed as median (25th–75th percentiles).
[b]Analysis of variance test.
[c]Exact trend test.
[d]Jonckheere test.

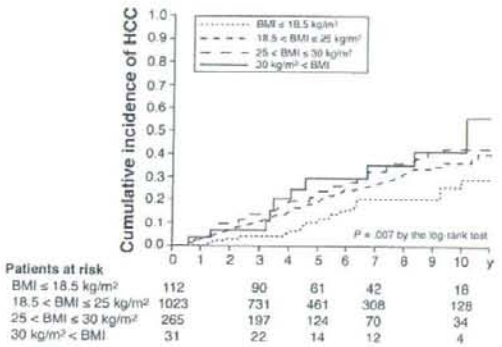

Figure 1. Cumulative incidence of HCC divided by BMI. Dotted line, BMI of 18.5 kg/m² or less; small-dash line, BMI of 18.5 to less than 25 kg/m²; long-dash line, BMI of 25 to less than 30 kg/m²; solid line, BMI of greater 30 kg/m². Overweight and obese patients were revealed to be at significantly higher risk (P = .022 and .005), respectively, as compared with underweight patients. P = .007 by the log-rank test.

bin level, ALT level, prothrombin time activity, platelet count, and AFP positivity (>20 ng/mL) among the 4 groups (Table 1). A total of 15 patients were excluded from this analysis because of warfarin administration affecting prothrombin time activity (2 patients in the underweight group, 11 in the normal BMI group, and 2 in the overweight group). There was a significant difference in the mean age among the BMI groups (P = .009 by analysis of variance), decreasing in the order of BMI, and the mean ALT level among the BMI groups (P = .04 by analysis of variance), increasing in the order of BMI. The increasing trend in total bilirubin level over BMI also was statistically significant (P < .0001 by Jonckheere test), whereas the trends in serum albumin level and prothrombin time activity were not. The proportion of patients positive for AFP (>20 ng/mL) significantly increased with BMI (P = .0047 by exact trend test).

The number (proportion) of patients who had a history of blood transfusion before the diagnosis of chronic hepatitis was 61 (55%), 471 (46%), 106 (40%), and 17 (55%) in the underweight, normal, overweight, and obese patients, respectively. The median duration of infection estimated from the year of blood transfusion was 35, 34, 35, and 30 years in the underweight, normal, overweight, and obese patients, respectively. There was no statistical significance on the proportion of those with blood transfusion and the duration of infection.

During the follow-up period, a total of 209 patients received interferon therapies and 69 patients achieved SVR (Table 1). The proportion of patients who received interferon therapies was the highest in the obese group but there was no significant trend over BMI (P = .102 by exact trend test). There was no significant trend of the proportion of patients who achieved SVR rates over BMI (P = .75 by exact trend test).

Incidence of Hepatocellular Carcinoma

The mean follow-up period was 6.1 years or 8729 person-years overall and 6.6 years, 6.0 years, 5.9 years, and 7.1 years for underweight, normal BMI, overweight, and obese groups, respectively. During the follow-up period, a total of 264 (18.4%) patients had been lost to follow-up evaluation: 21 (18.8%) patients in the underweight group, 197 (19.3%) in the normal BMI group, 40 (15.1%) in the overweight group, and 6 (19.4%) in the obesity group (P = .47 by the Fisher exact test). By the end of the follow-up period, HCC developed in 340 patients (3.9% per 1 person-year). The cumulative incidence rates of HCC at 3, 5, and 10 years estimated by the Kaplan-Meier method were 10.5%, 19.7%, and 36.8%, respectively. Cumulative incidence rates at 3, 5, and 10 years in each group were 3.8%, 10.4%, and 29.6% (3.0% per 1 person-year) in the underweight group; 10.5%, 19.6%, and 36.0% (5.1% per 1 person-year) in the normal BMI group; 13.8%, 23.0%, and 42.7% (5.8% per 1 person-year) in the overweight group; and 6.7%, 29.2%, and 41.2 % (7.2% per 1 person-year) in the obese group, respectively (Figure 1). The incidence rates differed significantly among the 4 groups (P = .007 by the log-rank test), increasing in accordance with BMI (Figure 1).

Risk Analyses

Univariate analyses showed that the normal, overweight, and obese patients had a higher risk of HCC, in this order of magnitude, than the underweight patients (Table 2). Other significant risk factors for HCC included older age, male sex, comorbidity with diabetes mellitus, heavy alcohol intake, lower serum albumin level, higher total bilirubin level, higher ALT level, lower prothrombin time activity, lower platelet counts, and AFP greater than 20 ng/mL.

These factors were assessed by using a multivariate proportional hazard regression model (Table 3). The overweight and obese patients were revealed to be at significantly higher risk with a hazard ratio of 1.86 (95% confidence interval [CI], 1.09–3.16; P = .022) and 3.10 (95% CI, 1.41–6.81; P = .005), respectively, as compared with the underweight patients. Patients with a normal BMI also showed higher risk but did not reach statistical significance (relative risk, 1.52; P = .094). Comorbidity with diabetes mellitus did not reach statistical significance either (relative risk, 1.26; P = .15). The other risk factors indicated to be significant were older age, male sex, heavy alcohol intake, AFP level greater than 20 ng/mL, and laboratory parameters indicative of more advanced liver diseases, such as serum albumin level. The ALT level did not reach statistical significance (relative risk, 1.001; P = .16).

Table 2. Risk Factors for HCC Development: Univariate Analysis

Variable	Hazard ratio (95% CI)	P
Age (per 1 year old)	1.07 (1.06–1.08)	<.001
Male sex	1.95 (1.56–2.43)	<.001
Diabetes mellitus	1.58 (1.17–2.13)	.005
Alcohol >80 g/day	2.04 (1.59–2.62)	<.001
BMI		
≤18.5 kg/m²	1.00	
>18.5 kg/m² to ≤25 kg/m²	1.65 (1.02–2.66)	.041
>25 kg/m² to ≤30 kg/m²	1.99 (1.19–3.33)	.009
>30 kg/m²	2.20 (1.02–4.77)	.045
Serum albumin level (per 1.0 g/dL)	0.20 (0.16–0.25)	<.001
Total bilirubin level (per 1.0 mg/dL)	1.90 (1.57–2.30)	<.001
ALT level (per 1 IU/L)	1.003 (1.002–1.005)	<.001
Prothrombin time activity (per 1%)	0.94 (0.94–0.95)	<.001
Platelet count (per $10^3/\mu L$)	0.98 (0.982–0.986)	<.001
AFP level >20 ng/mL	4.57 (3.67–5.69)	<.001

Table 3. Risk Factors for HCC Development: Multivariate Analysis

Variable	Hazard ratio (95% CI)	*P*
Age (per 1 year old)	1.07 (1.06–1.09)	<.001
Male sex	2.10 (1.64–2.69)	<.001
Diabetes mellitus	1.26 (0.92–1.71)	.15
Alcohol >80 g/day	1.41 (1.07–1.86)	.015
BMI		
≤18.5 kg/m^2	1.00	
>18.5 kg/m^2 to ≤25 kg/m^2	1.52 (0.93–2.47)	.094
>25 kg/m^2 to ≤30 kg/m^2	1.86 (1.09–3.16)	.022
>30 kg/m^2	3.10 (1.41–6.81)	.005
Serum albumin level (per 1.0 g/dL)	0.55 (0.39–0.77)	<.001
Total bilirubin level (per 1.0 mg/dL)	0.80 (0.59–1.08)	.016
ALT level (per 1 IU/L)	1.001 (1.000–1.003)	.16
Prothrombin time activity (per 1%)	0.97 (0.96–0.98)	<.001
Platelet count (per 10^3/μL)	0.92 (0.90–0.95)	<.001
AFP level >20 ng/mL	1.77 (1.37–2.30)	<.001

The increasing risk of HCC development in proportion to BMI also was shown in the subgroup analysis in patients who did not have a history of interferon therapies during the follow-up period (n = 1222). Among these interferon-untreated patients, overweight and obese patients were at significantly higher risk with a relative risk of 1.94 (95% CI, 1.14–3.30; P = .014) and 3.18 (95% CI, 1.44–7.04; P = .0043) as compared with the underweight patients, respectively.

Discussion

The increasing prevalence of obesity, which leads to various morbidities, is now a major concern in the health care system in developed countries.[16,17,31–33] It is well documented that obesity contributes to the development of various diseases including diabetes, hypertension, cardiovascular, and cerebrovascular with increased mortality. In addition, obesity is also known as a risk factor of cancers. Calle et al[17] reported that obesity was associated with increased mortality from various cancers including esophagus, colorectal, stomach, breast, ovary, and liver cancer. Our current study showed that obese patients with chronic hepatitis C were at a higher risk of HCC with an adjusted relative risk of 3.10 compared with underweight patients. N'Kontchou et al[35] also reported overweight as an independent risk factor of hepatocarcinogenesis in chronic hepatitis C patients. In the current study, we also found a tendency of higher risk of HCC even in normal BMI patients than in underweight ones. Thus, the risk of HCC appears to increase in proportion to BMI in a wide range of its values, from underweight to obese.

Because this study was observational, we cannot determine whether obesity directly enhances hepatocarcinogenesis. One possibility is that obesity affects hepatocarcinogenesis through changes in the levels of adipocytokines. Reportedly, the plasma level of adiponectin is correlated negatively with BMI and differs substantially even between underweight (≤18.5 kg/m^2) and normal-range (18.5–25 kg/m^2) BMI,[36] and a lower level of adiponectin, a major adipose cytokine, is associated with the risk of several cancers.[37,38] Another possible hypothesis may be that obesity promotes hepatocarcinogenesis through steatosis in the liver. The association between liver steatosis and HCC has been reported recently among HCV-positive patients.[18,39–41] Hepatic steatosis, which also is associated closely with obesity, causes hepatic inflammation in the liver without hepatitis viruses[32,34,42–44] and the underlying mechanisms are well investigated in nonalcoholic steatohepatitis. Although we did not assess fatty changes in each patient, the fact that a higher BMI correlated with a higher ALT level suggests that steatosis-related hepatic inflammation may play a role also in hepatocarcinogenesis in chronic hepatitis C patients. The mechanisms by which steatosis promotes fibrosis in chronic hepatitis C currently are under investigation. Some piece of evidence implicates enhanced oxidative stress, activation of subsinusoidal stellate cells, and increased susceptibility to apoptosis.[45]

There is obviously a strong relationship between diabetes and obesity. Several large-scale cohort studies reported that diabetes was a significant risk factor for primary liver cancer.[16,46] In the current study, however, the presence of diabetes mellitus was associated significantly with the risk of HCC in univariate analysis but did not retain significance in multivariate analysis controlling for BMI. The effect of insulin resistance on hepatocarcinogenesis is difficult to assess because HCV infection is reported to induce insulin resistance by itself.[47] Further studies with data on insulin resistance are required to elucidate the exact contribution of diabetes mellitus on hepatocarcinogenesis in chronic hepatitis C patients.

One of the major concerns is that a higher BMI may merely indicate the presence of ascites or edema, the status that reflects advanced liver disease. Thus, we excluded those with ascites evident at enrollment. Another confounder to be considered was a history of antiviral therapy because it was reported that antiviral therapy reduced the risk of hepatocarcinogenesis[18,19] and BMI also closely was related to SVR rate.[50] The results may be biased if either the indication for interferon therapies or its virologic response varied among BMI groups. Thus, we performed a subgroup analysis excluding those who received interferon therapies after entry. The results also showed that the risk of HCC development increased in proportion to BMI.

Cirrhosis is the most important risk factor for HCC.[15] However, because this study was based on outpatient care, liver biopsy rarely was performed. Thus, we cannot assess the relationship between BMI and fibrosis stage. Although it is not clear whether liver fibrosis plays a direct role in hepatocarcinogenesis, the degree of fibrosis may surrogate the accumulated DNA damages as a consequence of long-term necroinflammation and regeneration. Recently, it was reported that fibrosis in chronic hepatitis C correlates significantly with BMI.[51] BMI and steatosis probably are correlated because obesity may aggravate steatosis. Thus, even if accelerated fibrosis is a consequence of necroinflammation exacerbated by obesity-related liver steatosis, the relationship between BMI and HCC is not spurious, although possibly mediated by histology.

In summary, the current study has shown an association between BMI and the risk of hepatocarcinogenesis in chronic hepatitis C patients in a wide range of BMIs. Although the mechanism of this phenomenon remains to be investigated, patients may practically be advised to avoid obesity.

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Original article

Percutaneous radiofrequency ablation of liver cancer in the hepatic dome using the intrapleural fluid infusion technique

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Background: Intrapleural fluid infusion improves ultrasonographic visualization of tumours in the hepatic dome. The aim of this study was to assess the safety and long-term efficacy of ultrasonographically guided percutaneous radiofrequency ablation for tumours in the hepatic dome with intrapleural infusion.
Methods: Of 2575 patients with hepatocellular carcinoma or hepatic metastases treated with radiofrequency ablation, intrapleural fluid infusion was performed in 587 patients for tumours in the hepatic dome. After the tip of a 14-G metallic needle was positioned in the pleural cavity under ultrasonographic guidance, 500–1000 ml of 5 per cent glucose solution was infused in 5–15 min. Radiofrequency ablation was performed using an internally cooled electrode. Long-term results were evaluated in 347 patients with a single hepatocellular carcinoma who were naive to any treatment.
Results: Intrapleural fluid infusion was successfully performed in all 587 patients. The major complication rate on a per tumour basis was similar for patients treated with and without intrapleural infusion (1·6 *versus* 1·6 per cent; $P = 0·924$). The overall and recurrence-free survival were both similar for naive patients with a single hepatocellular carcinoma treated with and without intrapleural infusion ($P = 0·429$ and $P = 0·109$ respectively). Intrapleural infusion was not associated with lower overall survival in multivariable analysis.
Conclusion: With intrapleural fluid infusion, radiofrequency ablation for tumours in the hepatic dome was safe and effective, resulting in satisfactory overall and recurrence-free survival.

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Introduction

The incidence of hepatocellular carcinoma (HCC) is increasing worldwide, for which surgical resection is considered the first-line curative treatment. However, many patients with HCC are not eligible for resection because they have multiple tumour nodules or impaired liver function. For these patients, several less invasive local ablation therapies, such as ethanol injection, microwave coagulation and radiofrequency ablation (RFA), have been developed in the past two decades[1]. Among these, real-time ultrasonographically guided percutaneous RFA is now widely accepted for the treatment of unresectable small HCC because of its safety[2] and efficacy[3,4]. RFA seems preferable because of patients' quality of life and economic aspects, as complete tumour necrosis is achieved in fewer treatment sessions and therefore requires shorter hospitalization[4]. RFA has recently been accepted as a palliative or 'bridge-to-surgery' therapy for metastatic hepatic tumours[5].

One major problem of percutaneous RFA is that it is not considered applicable to tumours in the hepatic dome, just beneath the diaphragm, because of poor visualization on ultrasonography[6–9]. More complicated and invasive approaches, such as transthoracic ablation under computed tomography (CT) guidance[10] and ablation under laparoscopy or laparotomy[11–13], have been reported as suitable for such tumours, and the usefulness of the intrapleural fluid infusion technique has been proposed by some investigators[6–8]. The infused fluid (artificial pleural effusion) improves ultrasonographic tumour depiction by displacing the lung and acting as an acoustic window, enabling operators to treat tumours in the hepatic dome percutaneously under real-time ultrasonographic guidance. This technique seems promising because it is simple, safe and less invasive. However, studies so far have included

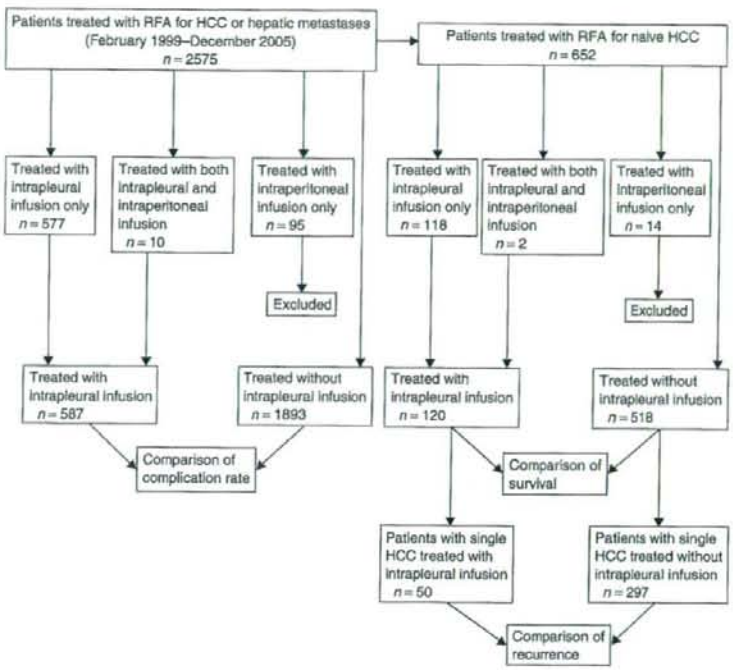


Fig. 1 Schematic flow chart of the study. RFA, radiofrequency ablation; HCC, hepatocellular carcinoma

relatively few patients. The safety and long-term results of RFA with intrapleural fluid infusion remained unclear.

The present authors introduced percutaneous RFA for hepatic tumours in February 1999 and have treated more than 2500 patients since then. In that time, they have used the intrapleural fluid infusion technique in 587 patients for RFA of tumours in the hepatic dome. The aim of this retrospective study was to assess safety and long-term results of RFA with intrapleural fluid infusion.

Methods

Between February 1999 and December 2005, percutaneous RFA was performed in 2575 patients with liver cancer (including hepatic metastasis) at the University of Tokyo Hospital. The inclusion criteria for RFA of HCC have been reported elsewhere[2], but briefly they were the absence of uncontrolled ascites, a platelet count of at least 50 000/mm^3 and prothrombin activity of at least 50 per cent, and no intravascular tumour invasion. In most patients with HCC, the number of tumours was three or fewer and tumour diameter was 3 cm or less, but some patients who did not meet the criteria were nevertheless treated with RFA after careful selection. The decision to use RFA for hepatic metastasis was made for each patient by author consensus based on the size and number of hepatic metastatic nodules, controllability of the primary lesion, distant metastases to organs other than the liver and associated medical complications.

Of those patients, 577 who had tumours in the subphrenic hepatic dome had intrapleural fluid infusion, ten had both intrapleural and intraperitoneal fluid infusion[14], and 95 had intraperitoneal fluid infusion alone and were excluded from the study (*Fig. 1*). Two categories of patients received intrapleural fluid infusion. The first was those whose tumour was completely invisible or only partly visible on ultrasonography because of air in the lung (invisible tumours), where RFA was not feasible without intrapleural fluid or other assistance. The second was those whose whole tumour was visible but who were considered at high risk of pneumothorax on needle insertion without artificial pleural fluid (visible tumours),

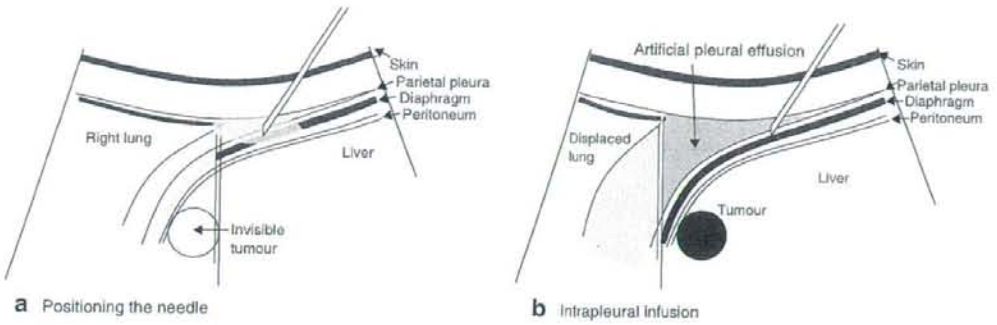


Fig. 2 The intrapleural fluid infusion technique. **a** A small amount of 5 per cent glucose solution is injected to determine the position for the metallic needle. The needle is positioned in the pleural cavity, with the tip just on the diaphragm. **b** Intrapleural infusion of 500 ml of 5 per cent glucose solution improves tumour visualization at ultrasonography

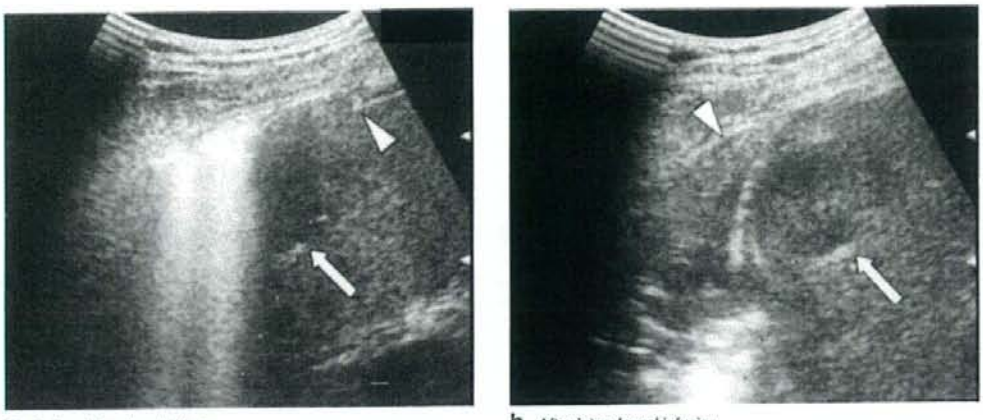
where RFA would not have been safe without intrapleural fluid. The second group included patients with tumours in the liver dome that were seen only under deep expiration, and those in whom the needle insertion tract considered to be most appropriate was interrupted by the lung. The safety of RFA with intrapleural fluid infusion was assessed on both per patient and per tumour bases in patients with HCC or hepatic metastases. All major early and late complications were recorded for each patient treated with RFA.

The survival rate was compared on a per patient basis in naive patients with HCC receiving RFA between those treated with and without intrapleural fluid infusion. During the observation period, 652 naive patients with HCC received RFA as an initial treatment. Of these, 120 had tumours in the hepatic dome and received RFA with intrapleural fluid infusion, including two treated with both intrapleural and intraperitoneal fluid infusion. Meanwhile, 14 naive patients with HCC received RFA using intraperitoneal fluid infusion only and were excluded from the analysis. Therefore, 120 patients treated with intrapleural fluid infusion and 518 patients treated without were compared. As the recurrence of HCC is dependent on the number of nodules, the recurrence-free survival after RFA was assessed exclusively in 347 naive patients with a single HCC, of whom 50 received RFA with intrapleural fluid infusion. Patients treated with and without intrapleural fluid infusion were compared at three distinct levels: complications of RFA among all patients with HCC or metastatic tumours, overall survival among naive patients with HCC and recurrence-free survival among naive patients with single HCC. Follow-up continued to the end of December 2005. Each patient gave written informed consent before RFA treatment. This study was approved by the local ethics committee.

Table 1 Characteristics of patients with and without intrapleural fluid infusion

	RFA with intrapleural infusion ($n = 587$)	RFA without intrapleural infusion ($n = 1893$)†	P‡
Age (years)*	67·8	67·9	0·280
Sex ratio (M : F)	420 : 167	1238 : 655	0·006§
Hepatocellular carcinoma	563	1695	< 0·001§
Metastatic tumour	24	198	
Visible on ultrasonography	38		
Invisible on ultrasonography	549		
Maximum tumour diameter (mm)*	24·5	24·1	0·440
Tumour number*	2·37	2·02	< 0·001

*Values are means. †The 95 patients treated with intraperitoneal fluid infusion were excluded. RFA, radiofrequency ablation. ‡Student's t test unless otherwise indicated; §Fisher's exact probability test.



a Before intrapleural infusion **b** After intrapleural infusion

Fig. 3 Intrapleural fluid infusion performed in a patient with hepatocellular carcinoma located in segment 8, 3·5 cm in diameter. **a** Before intrapleural fluid infusion, the cranial side of the tumour (arrow) is invisible because of the gas in the lung. The tip of the 14-G metallic needle (arrowhead) is placed just on the diaphragm. **b** After infusing 500 ml of 5 per cent glucose solution the entire tumour (arrow) is clearly visible (position for artificial pleural effusion is shown by the arrowhead)

Table 2 Major complications after radiofrequency ablation with and without intrapleural fluid infusion

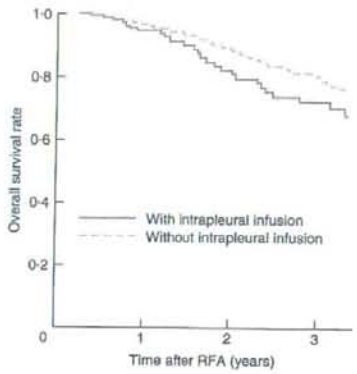
	RFA with intrapleural infusion ($n = 587$)	RFA without intrapleural infusion ($n = 1893$)*	P‡
Massive pleural effusion (requiring thoracic drainage)	5	2	
Intrapleural bleeding (requiring blood transfusion)	2	0	
Pneumothorax	1	0	
Liver abscess	4	6	
Extrahepatic tumour seeding	3	29	
Intraperitoneal bleeding (requiring blood transfusion)	2	2	
Gastrointestinal tract perforation	2	7	
Liver infarction	1	7	
Skin burn	1	0	
Others	1	10	
Total	22 (3·7)	63 (3·3)	0·605
Total complication rate per tumour (%)	1·6	1·6	0·924

Values in parentheses are percentages. *The 95 patients treated with intraperitoneal fluid infusion were excluded. RFA, radiofrequency ablation. ‡Fisher's exact probability test.

Intrapleural fluid infusion technique

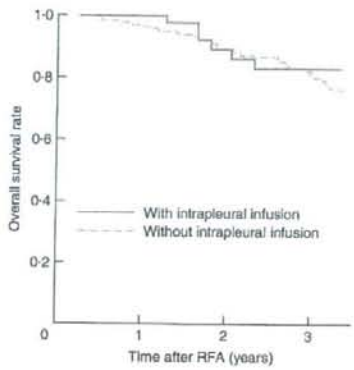
A 5 per cent glucose solution kept at room temperature and a 14-G metallic needle consisting of a trocar and an inner stylet were used for intrapleural infusion. Before receiving intrapleural fluid infusion and RFA, patients were sedated with 30 mg intravenous pentazocine and 25 mg hydroxyzine pamoate. After giving local anaesthesia, about 20 ml of 5 per cent glucose solution was injected with a 20-G needle between the chest wall and the diaphragm to find the best place for the tip of the 14-G metallic needle while avoiding liver injury in the following procedure.

The 14-G needle was carefully inserted through an intercostal space until the tip just reached the diaphragm, aiming the tip of the needle between the costal and diaphragmatic parts of parietal pleura (*Figs 2* and *3*). If the needle had been inserted deeper beyond the diaphragm, the infusion may have become intraperitoneal. The site of



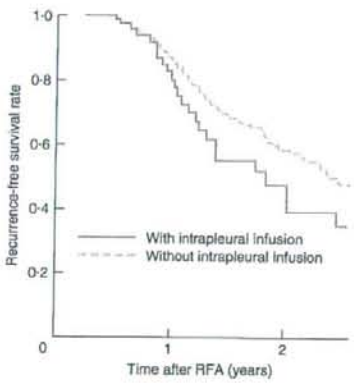
No. at risk				
With intrapleural infusion	120	97	57	32
Without intrapleural infusion	518	433	319	222

a Survival of naive patients with HCC



No. at risk				
With intrapleural infusion	50	41	26	14
Without intrapleural infusion	297	254	198	140

b Survival of naive patients with single HCC



No. at risk			
With intrapleural infusion	50	24	8
Without intrapleural infusion	297	186	99

c Recurrence-free survival of naive patients with single HCC

Fig. 4 **a** Survival of naive patients with hepatocellular carcinoma (HCC) treated with radiofrequency ablation (RFA) with (120 patients) and without (518 patients) intrapleural infusion ($P = 0{\cdot}113$, log rank test). **b** Survival of naive patients with a single HCC treated with RFA with (50 patients) and without (297 patients) intrapleural infusion ($P = 0{\cdot}429$, log rank test). **c** Recurrence-free survival of naive patients with a single HCC treated with RFA with (50 patients) and without (297 patients) intrapleural fluid infusion ($P = 0{\cdot}109$, log rank test)

Table 3 Characteristics of 638 naive patients with hepatocellular carcinoma treated with radiofrequency ablation with and without intrapleural fluid infusion

	RFA with intrapleural infusion ($n = 120$)	RFA without intrapleural infusion ($n = 518$)*	P†
Age (years)	67·9	68·5	0·370
Sex ratio (M : F)	74 (61·7) : 46 (38·3)	344 (66·4) : 174 (33·6)	0·338‡
Curative	112 (93·3)	496 (95·8)	0·240‡
Palliative	8 (6·7)	22 (4·2)	
Visible on ultrasonography	9 (7·5)		
Invisible on ultrasonography	111 (92·5)		
Maximum tumour diameter (mm)	28·0	26·2	0·109
Tumour number	2·3	1·7	< 0·001
α-Fetoprotein (ng/ml)	611	356	0·374
Lectin-reactive α-fetoprotein (%)	10·6	8·4	0·442
Des-γ-carboxy prothrombin (mega arbitrary units/l)	212	485	0·221
Alanine aminotransferase (units/l)	62·8	58·4	0·271
Albumin (g/dl)	3·64	3·61	0·616
Bilirubin (mg/dl)	0·93	0·93	0·982
Ascites (present)	13 (10·8)	67 (12·9)	0·647‡
Platelet count ($\times 10^4/\mu l$)	12·6	12·2	0·475

Values in parentheses are percentages. *The 14 patients treated with intraperitoneal fluid infusion were excluded. RFA, radiofrequency ablation. †Student's t test unless otherwise indicated; ‡Fisher's exact probability test.

needle insertion was to be inferior to the edge of the lung to avoid lung injury (pneumothorax).

The inner stylet was then removed and 500 ml of 5 per cent glucose solution was infused. If the needle had been positioned appropriately, the infusion was generally completed in 5–10 min. If the visualization of the tumour had not improved enough, another 500 ml of 5 per cent glucose solution was infused. RFA was performed after confirming the visualization of the targeted tumour. Oxygen saturation was routinely monitored and patients received supplemental oxygen (2–4 l/min) through nasal cannulas when oxygen saturation fell below 95 per cent. Infused solution was not intentionally drained after RFA.

Radiofrequency ablation

RFA was performed using a 17-G internally cooled electrode with a 2-cm or 3-cm exposed tip connected to a 500-kHz radiofrequency generator (Radionics, Burlington, Massachusetts, USA). An electrode was inserted percutaneously under real-time ultrasonographic guidance and positioned precisely within the target tumour. The temperature of the tip was maintained at 10–20°C by internal circulation of chilled water. Each ablation lasted 12 min for the 3-cm electrode and 6 min for the 2-cm electrode. Additional pentazocine 30 mg and hydroxyzine pamoate 25 mg were administered if there was strong pain during ablation. Multiple ablations were performed in a session when the target tumour diameter was roughly more than 2 cm. An additional session was held several days later when required.

One day after the last session, the efficacy was evaluated by dynamic CT. Complete tumour necrosis was defined as hypoattenuation of the entire lesion with a surrounding margin of at least 5 mm. Patients received additional RFA until complete necrosis was confirmed. Each patient was followed up at the outpatient clinic, with ultrasonography and dynamic CT every 3–4 months. The pattern of recurrence in naive patients with a single HCC was classified as local tumour progression, intrahepatic recurrence in the same subsegment but distant from the primary tumour, or intrahepatic recurrence distant from the primary subsegment. Simultaneous multinodular recurrence located in both the primary and distant subsegments was also recorded as recurrence distant from the primary subsegment.

Statistical analysis

Difference in means between groups was assessed by Student's t test and frequency distribution by Fisher's exact probability test. Overall and recurrence-free survival rates were calculated by the Kaplan–Meier method, and the difference between groups was assessed by the log rank test. Survival curves were truncated at 3 years when the number of patients at risk became small. Independent factors for overall survival were assessed with Cox proportional hazards regression analysis. The

factors included in the analysis were: intrapleural fluid infusion, tumour size, tumour number, tumour biomarkers (α-fetoprotein, des-γ-carboxy prothrombin and lectin-reactive α-fetoprotein), age, sex, alanine aminotransferase, albumin, bilirubin, platelet count and presence of ascites. Ascites was regarded as present even if dissipated by diuretics (patients with intractable ascites were contraindicated for RFA). Intrapleural fluid infusion was included in multivariable analysis, together with other factors significant in univariable analysis, to elucidate the influence of intrapleural fluid infusion on overall and recurrence-free survival. $P < 0.050$ was considered significant. Statistical analyses in this study were performed using StatView® software version 5.0 (SAS Institute, Cary, North Carolina, USA).

Results

The clinical characteristics of all patients who had RFA treatment appear in *Table 1*. RFA was performed with intrapleural fluid infusion in 587 patients. Of these, the tumour was not visible before intrapleural fluid infusion in 549 patients and was visible in the remaining 38, when intrapleural infusion was performed to assure safe needle insertion. There were no significant differences in age, or maximum tumour diameter. The proportion of males and HCC were significantly higher in the intrapleural infusion group. Tumour number was significantly larger in the intrapleural effusion group. This was probably because the more tumours there were, the more likely it was that at least one of them would require intrapleural fluid infusion.

Safety

There were no complications associated specifically with the intrapleural fluid infusion procedure, such as pneumothorax or haemothorax. About one-third of patients received supplemental oxygen because of lowered oxygen saturation. No patient developed further respiratory distress due to intrapleural fluid infusion and oxygen supplementation was discontinued by the next morning.

Major complications associated with RFA with intrapleural fluid infusion appear in *Table 2*. The overall major complication rate was no different between patients with and without intrapleural infusion (3·7 *versus* 3·2 per cent, $P = 0.605$). On per tumour basis, similarly, no difference was found in overall complication rates between patients with and without intrapleural infusion (1·6 *versus* 1·6 per cent; $P = 0.924$). The frequency of each complication was not significantly different between the two groups.

Massive pleural effusion requiring drainage occurred in five patients, but was not directly associated with the infusion technique itself. The effusion was exudative in all five patients.

Long-term results

The clinical characteristics of all 638 naive patients with HCC treated with RFA as the initial treatment are presented in *Table 3*. Tumour numbers were significantly greater in the intrapleural infusion group ($P < 0.001$). The mean maximum tumour diameter was also larger in that group, although not significantly. There were no significant differences in other factors such as age, sex, alanine aminotransferase, platelet count and tumour markers.

The mean follow-up was 2·79 (range 0·07–6·88) years. The overall survival curves of patients treated with and without intrapleural fluid infusion appear in *Fig. 4a*. Overall survival was not significantly different between the two groups ($P = 0.109$). The difference was further decreased ($P = 0.429$) when the analysis was limited to naive patients with a single HCC (*Fig. 4b*).

Table 4 Factors immediately before the initial treatment associated with death

Factors	Hazard ratio	P*
Univariable analysis		
Ascites (present)	2·63 (1·81, 3·80)	< 0·001
DCP (> 40 mega arbitrary units/l)	2·32 (1·70, 3·17)	< 0·001
Albumin (< 3·5 g/dl)	2·24 (1·63, 3·07)	< 0·001
Lectin-reactive α-fetoprotein (> 10%)	2·18 (1·56, 3·04)	< 0·001
Tumour size (> 3 cm)	2·04 (1·50, 2·79)	< 0·001
Bilirubin (> 1·0 mg/dl)	1·83 (1·33, 2·51)	< 0·001
Age (> 65 years)	1·93 (1·33, 2·81)	< 0·001
α-Fetoprotein (> 100 ng/ml)	1·69 (1·22, 2·35)	0·002
Platelet count (< 10^4/μl)	1·51 (1·11, 2·05)	0·009
Tumour number (≥ 2)	1·35 (0·99, 1·83)	0·057
Intrapleural fluid infusion	1·37 (0·93, 2·02)	0·112
Alanine aminotransferase (> 60 units/l)	0·84 (0·61, 1·16)	0·279
Sex (male)	0·95 (0·69, 1·32)	0·772
Multivariable analysis		
Ascites (present)	2·00 (1·34, 2·98)	< 0·001
Age (> 65 years)	1·94 (1·94, 2·84)	< 0·001
DCP (> 40 mega arbitrary units/l)	1·76 (1·25, 2·47)	0·001
Albumin (< 3·5 g/dl)	1·57 (1·11, 2·22)	0·011
Tumour size (> 3 cm)	1·65 (1·18, 2·29)	0·032
Lectin-reactive α-fetoprotein (> 10%)	1·53 (0·98, 2·39)	0·061
Platelet count (< 10^4/μl)	1·33 (0·94, 1·87)	0·108
Intrapleural fluid infusion	1·36 (0·91, 2·02)	0·137
Bilirubin (> 1·0 mg/dl)	1·29 (0·92, 1·80)	0·141
α-fetoprotein (> 100 ng/ml)	1·17 (0·77, 1·79)	0·465

Values in parentheses are 95 per cent confidence intervals. DCP, des-γ-carboxy prothrombin. *Cox proportional hazards model.

The results of Cox regression analysis on the overall survival of naive patients with HCC appear in *Table 4*. Intrapleural fluid infusion was included in the multivariable analysis along with the other factors significantly associated with survival in the univariable analysis. Larger maximum tumour size, greater age, higher des-γ-carboxy prothrombin, lower albumin and the presence of ascites were significantly associated with a low survival rate. However, intrapleural infusion was not significantly associated with survival ($P = 0{\cdot}137$).

Recurrence was found in 38 (76 per cent) of the naive patients with a single HCC treated with RFA with intraperitoneal infusion and 207 (69·7 per cent) of those treated without infusion ($P = 0{\cdot}410$). The recurrence-free survival did not differ significantly between naive patients with a single HCC treated with intrapleural infusion and those treated without (*Fig. 4c*).

Discussion

This large-scale study has demonstrated that the intrapleural fluid infusion technique allows safe and effective treatment of tumours located in the subphrenic hepatic dome by percutaneous ablation. RFA with intrapleural infusion resulted in successful ablation of all tumours in the hepatic dome, confirmed by contrast-enhanced CT. Long-term efficacy of RFA, further assessed by comparing overall and recurrence-free survival, was similar between patients treated with and without intrapleural infusion, suggesting that the treatment of these tumours was not compromised.

Major complications noted in this study included pneumothorax treated with thoracic drainage in one patient, haemothorax requiring blood transfusion in two and massive pleural effusion treated with thoracic drainage in five. In these patients, prolongation of hospitalization (2–4 weeks) was necessary, although surgical therapy was not required in any patient. Therefore, the intrapleural infusion technique does not guarantee complete safety of RFA.

In all five of the patients who developed massive pleural effusion after RFA with intrapleural infusion, the pleural effusion was exudative. The exudative effusion itself was probably a result of inflammation of the diaphragm caused by RFA, and in addition the infused intrapleural fluid may have increased the effusion volume. If so, drainage of the infused fluid after treatment may have alleviated respiratory symptoms and shortened hospital stay.

The overall complication rate (3·7 per cent) and complication rate per tumour (1·6 per cent) of patients treated with intrapleural infusion were similar to those of patients treated without. This complication rate appears acceptable considering that most tumours in the first group could not have been visualized on ultrasonography without the intrapleural infusion technique.

Perforation of the diaphragm was reported by Koda and colleagues[15], although this did not occur in the present study. Massive pleural effusion presumably caused by thermal injury of the diaphragm was the most frequent complication found in the intrapleural infusion group. Although infused fluid may have reduced thermal energy reaching the diaphragm, apparently it was not effective enough to prevent inflammation. Separation of the liver and the diaphragm seems to be necessary for this purpose. The intraperitoneal fluid infusion method, originally developed by Ohmoto and colleagues[9] to visualize the tumour in the hepatic dome for percutaneous microwave coagulation therapy, may be more effective in preventing thermal injury to the diaphragm when the target tumour is close. The present authors reported the safety of RFA with intraperitoneal fluid infusion to prevent gastrointestinal tract perforation when the target tumour was adjacent to the gastrointestinal tract[14]. Effects of intraperitoneal fluid infusion for RFA of tumours in the hepatic dome, possibly in combination with intrapleural fluid infusion, are to be investigated in future studies.

Although tumours located in the hepatic dome have sometimes been considered unsuitable for percutaneous ultrasonographically guided percutaneous tumour ablation therapies because of their poor visualization[6–9], the intrapleural fluid infusion technique allowed treatment of these tumours by percutaneous RFA safely and completely, resulting in satisfactory overall and recurrence-free survival rates. Complications related to electrode insertion or thermal injury to the diaphragm were infrequent and readily treated conservatively.

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Neoplastic Seeding After Radiofrequency Ablation for Hepatocellular Carcinoma

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BACKGROUND: Neoplastic seeding reportedly occurs in up to 12.5% of patients treated with radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC). The aim of this study is to assess the incidence, risk factors, and prognosis of neoplastic seeding after RFA among a large number of patients with a long-term follow-up.

METHOD: From February 1999 to December 2004, 1,031 patients underwent a total of 1,845 treatments with RFA for a total of 3,837 HCC nodules. The following variables were assessed to elucidate the risk factors of neoplastic seeding: age, sex, positivity for viral markers, tumor size, number of tumor nodules, number of RFA sessions, tumor location, percutaneous biopsy prior to RFA, alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP) and *lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) levels, and the degree of tumor differentiation.

RESULTS: Neoplastic seeding was detected in 33 patients (3.2% per patient) at intervals of 4.8–63.8 (median, 15.2) months after RFA. On multivariate logistic regression analysis, only the poor differentiation degree was associated with the risk of neoplastic seeding ($P = 0.012$). Of tumor factors, tumor size, and AFP, DCP, and AFP-L3 levels were significantly associated with the poor differentiation degree. The cumulative survival rates 1 and 2 yr after the detection of neoplastic seeding were 86% and 47%, respectively.

CONCLUSION: Poor differentiation degree was the risk factor of neoplastic seeding after RFA for HCC. The surrogate markers for poor differentiation degree were larger tumor size and elevated tumor marker levels. Indication for RFA should be carefully considered for HCC patients under these conditions.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy worldwide, showing increasing incidence in the United States and elsewhere (1, 2). Current options for the treatment of this cancer consist of surgical resection, transcatheter arterial chemoembolization (TACE), and percutaneous ablation therapy. Although surgical resection is usually considered as the first-choice treatment, few patients affected by HCC are the ideal candidates for surgical resection because of impaired background liver function as a result of chronic hepatitis B or C (3). TACE is feasible even with impaired liver function reservoir, but complete elimination of whole lesions is difficult to attain with TACE only. Orthotopic liver transplantation (OLT) is a strategy that can treat both cancer and liver dysfunction, and indeed has shown excellent survival in patients at an early stage of the cancer (*e.g.*, single nodule of $\leq$5 cm in diameter or less than three nodules of $\leq$3 cm in diameter) (4). However, with increasing demand for donor tissue but a limited supply, the waiting time now exceeds 1 yr in Europe and the United States (5, 6).

Radiofrequency ablation (RFA) was introduced as an alternative locoregional therapy to ethanol injection, which can achieve high local cure without deteriorating background liver function (7–10). RFA has been shown to be superior to ethanol injection in terms of local cure, and it can achieve better overall survival (11–14). However, various complications associated with RFA have been reported, although infrequently, as the number of treated patients has been increased (10, 15, 16). Among them, neoplastic seeding is one of the most unfavorable complications that can be critical especially for those who are waiting for liver transplantation (17).

Thus, we conducted this study to elucidate the incidence of neoplastic seeding after RFA, assess its characteristics, and evaluate the risk factors as experienced in a single high-volume center.

PATIENTS AND METHODS

Patients

Between February 1999 and December 2004, 1,031 patients with HCC were treated with RFA at the authors' department. All of these patients were consecutively included in this study. Inclusion criteria for RFA were as follows: total bilirubin concentration lower than 3 mg/dL, platelet count no less than $50 \times 10^3/mm^3$, and prothrombin activity no less than 50% (approximately equal to 1.5 international normalized ratio [INR]). Ascites should be controlled beforehand by diuretics. Patients with portal vein tumor thrombosis or extrahepatic metastasis were excluded. We also excluded patients who had a history of bilioenteric anastomosis or sphincterotomy, which are considered as a high risk for hepatic abscess formation. In general, we performed RFA on patients with three or fewer lesions, all of which were 3 cm or smaller in diameter. However, we performed ablation also on patients who did not meet these conditions when complete ablation could be anticipated in all tumors without deteriorating liver function (18). Written informed consent was obtained from each enrolled patient, and the protocol was approved by the institutional review board.

Technical Terms

We defined a session as a single intervention episode that consists of one or more ablations performed on one or more tumors, and a treatment as the completed effort to ablate one or more tumors that consists of one or more sessions according to the working party report on the image-guided tumor ablation (19). To assess the depth of tumor location as a possible risk factor for neoplastic seeding, we categorized the location into the following two groups: *direct subcapsular insertion* when the tumor was located just under the surface of the liver and the ablation needle was directly inserted to the tumor, and *deep* when otherwise. When a tumor was located just under the liver surface but the needle was approached from the opposite direction through nontumorous tissue, the location was defined as deep. Subcapsular location of tumors, regardless of the route of needle insertion, was evaluated in a distinct analysis.

Diagnosis of HCC

HCC was diagnosed based on typical findings on ultrasonography and computed tomography (CT) (hyperattenuation in the arterial phase and hypoattenuation in the portal-venous phase) (20). The diagnosis of HCC was also confirmed histopathologically with ultrasound-guided biopsy in the majority of patients. Until 2004, we performed tumor biopsy before almost every RFA treatment. Afterward we restricted biopsy to cases where definite diagnosis could not be made on dynamic CT. Specimens for histological evaluation were obtained by ultrasound-guided core needle biopsy using a 20-gauge needle (Bard Monopty, C.R. Bard Inc., Covington, GA). Histopathological grading of tumor differentiation was done according to the criteria of Edmondson *et al.* (21).

Radiofrequency Ablation

A 17-gauge cooled-tip electrode with a 2- or 3-cm exposed tip was inserted under real-time ultrasound guidance. The electrode was connected to a 500 kHz RF Generator (Radionics, Burlington, MA) (22, 23). A tip temperature of 10–20°C was maintained by a peristaltic pump infusing chilled saline solution. After insertion of the electrode into the lesion, we started ablation at 60 W for the 3-cm exposed tip and 40 W for the 2-cm exposed tip. The power was increased to 140 W at a rate of 20 W/min. When a rapid increase in impedance was observed during thermal ablation, we minimized the output for 15 s and restarted the emission at a lower output. The duration of a single ablation was 12 min for the 3-cm electrode and 6 min for the 2-cm electrode. After RF exposure, the pump was stopped and the temperature of the needle tip was measured. When the temperature was below 65 °C, additional ablation was performed. When the target nodule was larger than 2 cm in diameter, we performed multiple ablations. When the total ablation time in a treatment took more than 60 min, we divided a treatment into two or more sessions in consideration (10). After 1 to 2 sessions of RFA, dynamic CT was performed 1 to 3 days after the last session with a slice thickness of 5 mm to evaluate treatment efficacy. The interval between the initiation of contrast material infusion and CT image recording was 30 s and 120 s for single detector-row spiral CT (Highspeed Advantage; GE Medical Systems, Milwaukee, WI) and 25 s, 40 s, and 120 s for multidetector-row CT (LightSpeed QX/I; GE Medical Systems). The images were presented after axial reconstruction with a slice thickness of 5 mm. Complete ablation was defined on the CT findings as nonenhancement in the entire lesion with a safety margin in the surrounding liver parenchyma. Patients received additional sessions of ablation until complete ablation was confirmed in each nodule.

The follow-up consisted of bimonthly blood tests and monitoring of tumor markers at the outpatient clinic, and ultrasonography and dynamic CT scan were performed every 4 months. The observation up to December 31, 2006 was used in this analysis.

Neoplastic Seeding

The diagnosis of neoplastic seeding was made by imaging modalities, which was usually performed as surveillance for intrahepatic recurrence. A newly detected tumor attached to the peritoneum or the pleura was considered as neoplastic seeding. The incidence of neoplastic seeding was assessed on the basis of the number of patients. Survival after the diagnosis of tumor seeding was assessed according to the Kaplan-Meier method.

Statistical Analysis

Risk factors for neoplastic seeding were analyzed on treatment basis. To elucidate the risk factors of neoplastic seeding, the following variables were assessed: age, sex, positivity for hepatitis B surface antigen (HBsAg), positivity for anti-hepatitis C antibody (anti-HCVAb), tumor size,

number of tumor nodules, number of RFA sessions, number of insertions of the RFA needle, tumor location, percutaneous biopsy prior to RFA, the level of alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) and *lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), and the degree of tumor differentiation. Continuous variables were compared between those with and without neoplastic seeding by Student's *t*-test. χ^2 test or Fisher's exact test was applied to compare nominal valuables between the groups. Factors showing *P* value of less than 0.1 as a predictor were further analyzed with a multivariate logistic regression model using stepwise selection of variables based on Akaike information criterion (AIC) (24). To find surrogate markers for differentiation of tumor, which requires percutaneous biopsy, we applied multivariate logistic regression analysis with poor differentiation (Edmondson grade 3 or greater) as the dependent variable and the following factors as independent variables: size and number of tumor nodules and AFP, DCP, and AFP-L3 values. The differences with a *P* value of less than 0.05 were considered statistically significant. All statistical analyses were performed with S-plus 2000 (Insightful Co., Seattle, WA).

RESULTS

As 459 of 1,031 patients received two or more treatments during the study period because of tumor recurrence, the total number of treatments was 1,845, consisting of 3,585 sessions of RFA to 3,837 nodules. The baseline characteristics of patients were summarized in Table 1. When one patient received more than one treatment, the data on the initial treatment were adopted. The mean age of the patients was 67.3 yr (range 42.3–91.5). The patients were male dominant (68.0%) and the majority was HCV positive (80.3%). The median tumor size was 2.5 (range 0.8–9.7) cm.

During the follow-up period (median 35.3 months; range 0.6–95.8 months), neoplastic seeding was diagnosed in 33 patients (3.2% per patient, or 1.8% per treatment), with an interval time of 4.8–63.8 (median 15.2) months after RFA. The number of seeding nodule was 1 in 22 cases, 2 or 3 in 2 cases, and 4 or more in 9 cases. Twenty-one cases of seeding occurred on the line of previous RFA needle insertion, and 12 cases occurred distantly. Seeding was located on the peritoneum in 25 patients, on the pleura in 7 patients, and in the abdominal wall in 1 patient. In the 25 patients with seeding on the peritoneum, the seeding was located adjacent to the surface of the liver except one patient who showed seeding in the pelvic cavity.

We analyzed risk factors for neoplastic seeding not on patient basis but on treatment basis because most variables differed on each treatment. Univariate analysis showed that the following factors were associated with neoplastic seeding: younger age ($P = 0.02$), HBsAg positivity ($P = 0.003$), the absence of anti-HCVAb ($P = 0.046$), and poorly differentiated tumor ($P = 0.003$), whereas sex, tumor size, tumor number, number of sessions, number of insertions of RFA needle, tumor location, and biopsy prior RFA showed no significant association (Table 2). Multivariate analysis with stepwise variable selection revealed that the best model for the prediction of neoplastic seeding included poorly differentiated tumor (odds ratio [OR], 3.18; 95% confidence interval [CI] 1.3–7.8) and AFP-L3 value higher than 15% (OR 1.77; 95% CI 0.723–4.35) (Table 3). The logistic regression analysis to find surrogate marker for poor differentiation showed that larger tumor and higher value of tumor markers were significantly associated with the poor differentiation (Table 4).

Of the 33 patients with neoplastic seeding, 32 patients were treated for the tumor seeding: resection in 16, RFA in 12, ethanol injection in 2, radiation in 5, and chemotherapy in 3 (6 patients received a combination therapy of resection, RFA, radiation, or chemotherapy). By the end of the follow-up, 21 patients with neoplastic seeding died. The causes of death were cancer progression in 18, liver failure in 1, and malignancy unrelated to liver in 2 patients. Among them, 6 patients died from the growth of seeding nodules. Detailed causes of death were peritonitis carcinomatosa in 3, tumor rupture in 1, and cachexia because of HCC progression in 2 patients. In the remaining 15 patients with neoplastic seeding, seeding nodules were under control (curatively resected

Table 1. Baseline Characteristics of the Patients (N = 1,031)

Variables (%)	
Mean age (yr)*	67.3 ± 8.1
Male gender	701 (68.0%)
Etiology	
HBs-Ag positive only	103 (10.0%)
HCV-Ab positive only	810 (78.6%)
Both positive	18 (1.7%)
Both negative	100 (9.7%)
Alcoholic consumption >80 g/day	162 (15.7%)
Child-Pugh score	
Class A	700 (67.9%)
Class B	316 (30.6%)
Class C	15 (1.5%)
Tumor size (cm)†	2.5 (1.9–3.2)
Tumor number	
Single	514 (49.9%)
2–3	385 (37.3%)
>3	132 (12.8%)
Number of sessions	
Single	399 (38.7%)
2	335 (32.5%)
>2	297 (28.8%)
Tumor location	
Direct subcapsular insertion	163 (15.8%)
Deep	868 (84.2%)
Tumor marker	
AFP>100 ng/mL	234 (22.7%)
DCP>100 mAU/mL	152 (14.7%)
AFP-L3 >15%	231 (22.4%)

*Values are expressed as mean ± standard deviations.
†Values are expressed as median, first, and third quartile.

Table 2. Characteristics of Patients With/Without Neoplastic Seeding

Variable	Seeding Was Identified (N = 33)	Seeding Was Not Identified (N = 1,812)	*P* Value
Age, yr	65.0 ± 9.5	68.2 ± 7.9	0.02
Male gender	26 (78.8%)	1,256 (69.3%)	0.39
HBs-Ag positive only	8 (24.2%)	165 (9.1%)	0.003
HCV-Ab positive only	22 (66.7%)	1,459 (80.5%)	0.046
Both positive	0 (0%)	30 (1.7%)	0.46
Both negative	3 (9.1%)	158 (8.7%)	0.94
Tumor size, cm	2.5 ± 1.0	2.4 ± 1.1	0.81
Number of tumors	2.1 ± 1.4	2.1 ± 1.4	0.96
Number of sessions	1.9 ± 1.1	1.9 ± 1.1	0.73
Number of insertions	5.3 ± 2.8	4.4 ± 2.2	0.29
Direct subcapsular insertion	8 (24.2%)	328 (18.1%)	0.23
Subcapsular location	21 (63.4%)	1,087 (60.0%)	0.72
Biopsy performed	22 (66.7%)	1,091 (60.2%)	0.28
Poorly differentiated tumor*	8 (36.4%)	143 (13.1%)	0.003
AFP >100 ng/mL	7 (21.2%)	424 (23.4%)	0.77
DCP >100 mAU/mL	7 (21.2%)	266 (14.7%)	0.29
AFP-L3 >15%	11 (33.3%)	374 (20.6%)	0.08

*Among 1,113 treatments (on 774 patients) in which biopsy was performed.

or did not affect survival) throughout the follow-up period. The cumulative survival rates of the 33 patients after the diagnosis of neoplastic seeding at 1 and 2 yr were 81% and 45%, respectively (Fig. 1).

DISCUSSION

RFA has been proposed as a promising alternative to conventional ethanol injection and shown to be more effective in terms of local efficacy (11, 12, 14). The safety of RFA, however, was questioned following the reported occurrence of neoplastic seeding in 12.5% of patients with HCC (4 in 32 patients) (17). Llovet *et al.* concluded that RFA with a cooled-tip needle should not be considered as a curative treatment for HCC or an adjuvant therapy before liver transplantation. However, this extremely high incidence of neoplastic seeding was contradicted by several investigators, who reported lower incidence from 0% to 0.9% (15, 25, 26). Livraghi *et al.* reported that seeding was identified in 12 patients (0.9%) among a total of 1,314 treated cases during the median follow-up period of 37 months (26). The incidence of 33 events in 1,031 patients (3.2%) in this study was rather high as compared with the latter reports. The difference may be because of more advanced tumor stage (21% patients had nodules larger than 3 cm in diameter) in this study, where we often employed strategy of multiple electrode insertion to achieve complete response (10). Alternatively, the rate of complications may have been underestimated in multicenter survey with questionnaire. In contrast, all complications analyzed in this study had been recorded in the database immediately after their identification (27).

Table 3. Multivariate Logistic Regression

Variable	Odds Ratio (95% CI)	*P* Value
Poorly differentiated tumor	3.18 (1.3–7.8)	0.012
AFP-L3 >15%	1.77 (0.723–4.35)	0.21

Table 4. Factors Correlated With Poorly Differentiated HCC

Variable	Odds Ratio (95% CI)	*P* Value
Tumor size		0.018
2.1–5.0 cm	1.57 (1.08–2.28)	
>5.0 cm	2.37 (1.10–5.08)	
Number of tumors >3	1.30 (0.84–2.00)	0.25
AFP >100 ng/ml	2.54 (1.77–3.65)	<0.001
DCP > 100 mAU/ml	1.76 (1.16–2.66)	0.01
aAFP-L3 >15%	2.96 (2.05–4.26)	<0.001

Among the 1,113 cases on which biopsy were performed, 151 cases had poorly differentiated HCC.

According to the previous reports, subcapsular tumor location, poor degree of differentiation, high baseline AFP levels, and tumor biopsy prior to RFA were associated with neoplastic seeding (17, 26). Also in this study, poor differentiation degree was significantly associated with neoplastic seeding in multivariate analysis. It is possible that poorly differentiated HCC grows more rapidly, and thus the seeding could be identified earlier. Another possibility is that cancer cells may be prone to migrate through needle tract into the peritoneal cavity as poorly differentiated HCC lacks cohesiveness (21).

Llovet *et al.* reported that subcapsular location of tumor was also a risk factor for neoplastic seeding (17). Intratumoral pressure increased by heat during RFA may facilitate dissemination of viable cancer cells into the peritoneal cavity, especially when the tumor is located subcapsular (28). Thus, we have adopted gradual elevation of radiofrequency power to avoid rapid increase in intratumoral pressure (10).

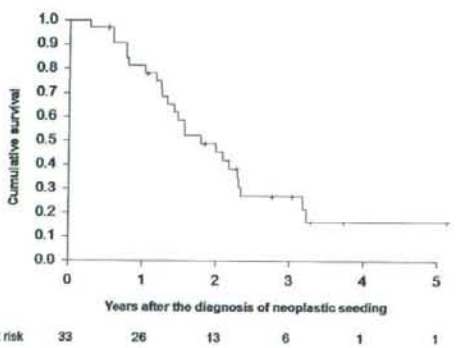

Figure 1. Cumulative survival of patients with neoplastic seeding. Cumulative survival rates of 33 patients at 1, 2, and 3 yr after the diagnosis of neoplastic seeding were 81.3%, 45.4%, and 26.9%, respectively.

This may explain why the location of tumor did not affect the incidence of neoplastic seeding in this study.

The same authors also reported that tumor biopsy prior to RFA was a risk factor of neoplastic seeding after RFA (26). A possible explanation of neoplastic seeding related to tumor biopsy is that viable cancer cells are extruded into the peritoneal cavity during RFA through the tract perforated by the biopsy needle. Another possibility is that cancer cells attached to the biopsy needle drop during needle withdrawal (29, 30). In this study, there also was a tendency of higher incidence of neoplastic seeding in patients who had received biopsy. It would be safer to avoid tumor biopsy when the diagnosis of HCC can be confirmed by dynamic CT or magnetic resonance imaging (20).

Poor differentiation degree was the only risk factor of neoplastic seeding found in this study. To find surrogate markers for differentiation of tumor, we applied univariate logistic regression analysis. Of the tumor factors, tumor size, AFP, AFP-L3, and DCP showed a significant association with poor differentiation degree. The application of RFA should be conservative for patients with large tumors (*e.g.*, >5 cm) or elevated tumor marker levels especially in candidates for surgical resection or liver transplantation.

Although the presence of neoplastic seeding may indicate aggressive tumor character, the survival rate was not extremely low in the patients with neoplastic seeding (the cumulative survival rates at 1 and 2 yr were 81% and 45%, respectively). In approximately 80% of patients with neoplastic seeding in this study, seeding nodules themselves did not affect directly the patients' survival. Taking into consideration the relatively low incidence of neoplastic seeding, 3.2% per patient, or 1.8% per treatment, the risk of neoplastic seeding after RFA would be considered acceptable in general.

In conclusion, poor differentiation degree was the risk factor of neoplastic seeding after RFA for HCC. Larger tumor size and elevated tumor marker levels surrogate poor differentiation. The indication for RFA should be carefully considered for HCC patients under these conditions.

STUDY HIGHLIGHTS

What Is Current Knowledge

- Neoplastic seeding is one of the most unfavorable complications after radiofrequency ablation (RFA).
- Subcapsular tumor location, poor degree of differentiation, high baseline alpha-fetoprotein (AFP) levels, and tumor biopsy prior to RFA were reported to be associated with seeding.

What Is New Here

- Seeding occurred in 33 (3.2%) of 1,031 patients treated with RFA in this study.
- Only poor differentiation degree was the risk factor of seeding after RFA.

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CONFLICT OF INTEREST

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