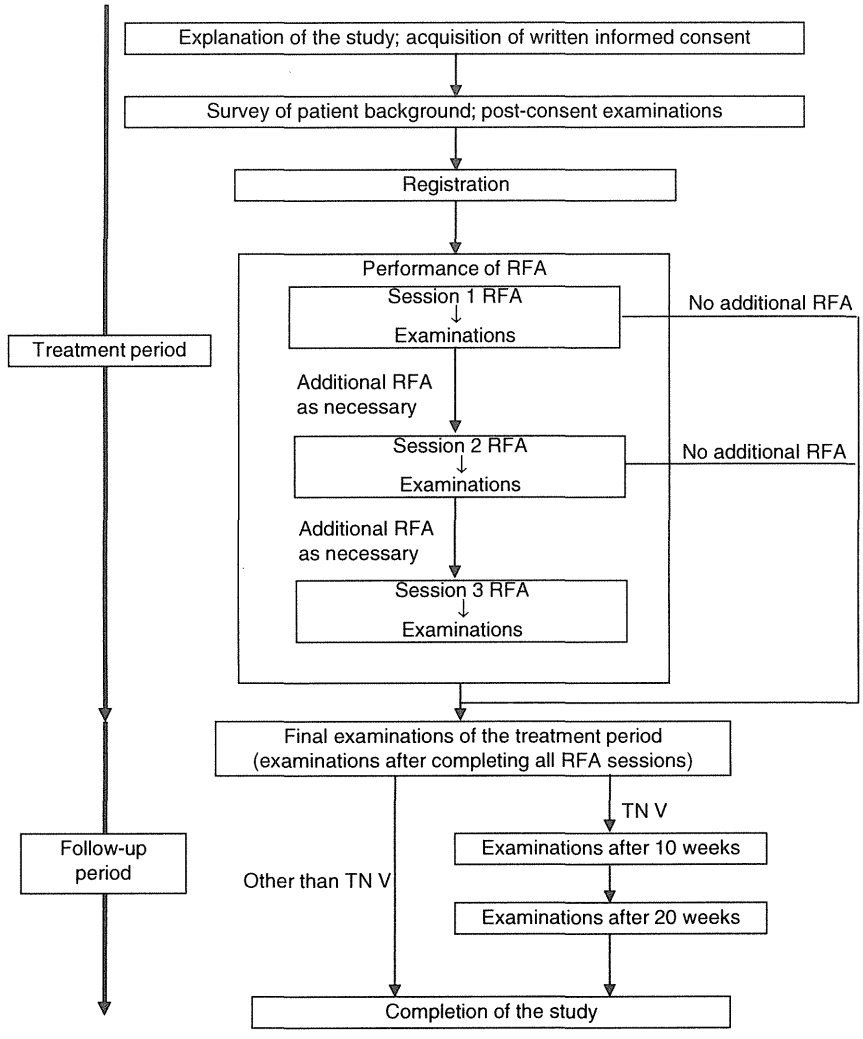

Fig. 4 Clinical study procedure. *TN* Tumor necrosis

Efficacy

Of the 90 patients who completed this clinical treatment study, 88 showed Class V TN (97.8 %). The 2 patients (2.2 %) who did not show 100 % TN both had primary liver cancers and were categorized as Class IV TN. The CNR was 100 % in patients with metastatic liver cancer (7/7 patients) and 97.6 % in patients with primary liver cancer (81/83 patients). The Japanese package insert for the Cool-tip RF System [21] states that the CNR obtained by that system was 86.2 % (50/58 patients). Assuming a 5 % non-inferiority margin, the lower limit of the confidence interval (one-sided 97.5 %) was 92.2 %, and the p value was <0.001 for the exact test based on binomial distribution.

The initial success rate (Class V TN after 1 session) was 77.8 % (70 of 90 patients), while Class V TN was seen in 16 (17.8 %) patients following a second session. The remaining 4 (4.4 %) patients underwent a third RFA session, and 2 were rated as Class V TN following that session.

We used 1 applicator in 20 patients, 2 simultaneously in 54 patients, and 3 simultaneously in 16 patients. We used 30-mm electrodes in all the patients, except in 3 of the 16 patients in whom 3 electrodes were used simultaneously; in these 3 patients we used 3 40-mm electrodes. A representative case in which 3 applicators were used is shown in Fig. 5.

Of the 88 patients who proceeded to the follow-up phase, excluding the single out-of-hospital fatality, examination at 24 weeks showed that CR was obtained in 94.3 % (82/87). The cumulative local recurrence rate at the end of 24 weeks in the follow-up period was 5.7 % (5/87 patients; ITT analysis) (Table 2).

Figure 6a, b shows a comparison of the treatment results of the Cool-tip RF System clinical trial [21] and the number of patients analyzed for the CNR and the efficacy

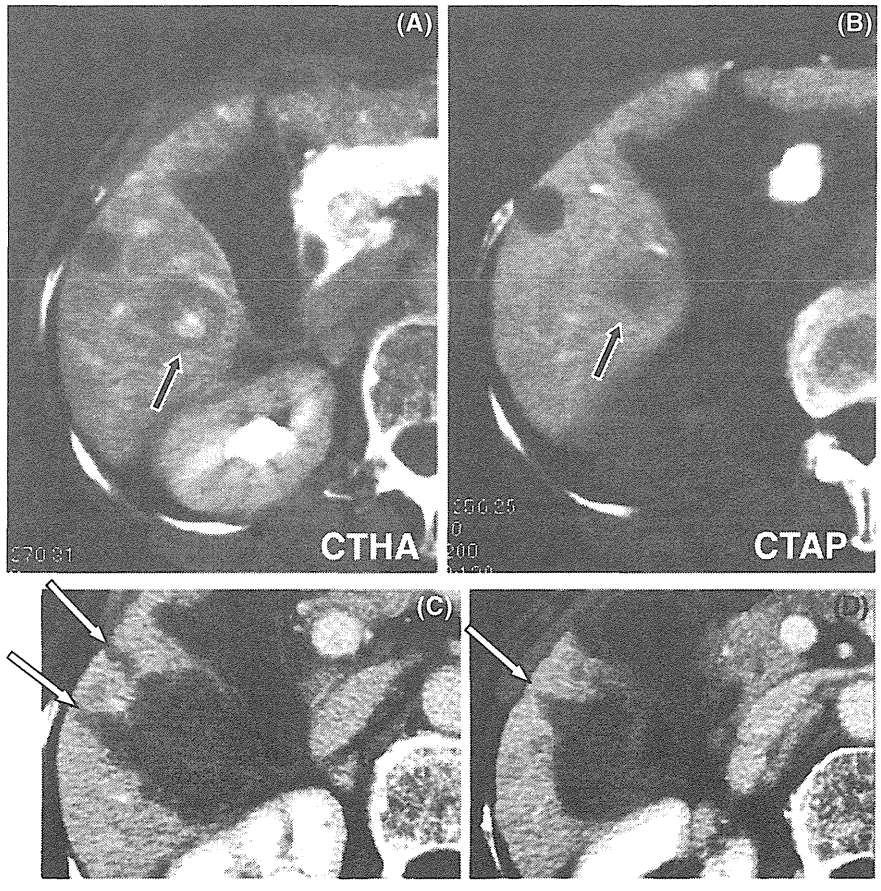

Fig. 5 Images in a female patient who had hepatocellular carcinoma (HCC) in segment VI. Before treatment, scans obtained on computed tomography during hepatic arteriography (CTHA) (**a**) and computed tomography during arterial portography (CTAP) (**b**) showed a nodular HCC (*arrow*) measuring 2.5 cm. Three applicators were placed in parallel in the HCC in liver segment VI, and then the tumor was ablated in one procedure (total ablation time 13 min 42 s, total applied energy 35.3 kJ). After the procedure, computed tomography (CT) images showed a necrotic area of 46 mm in diameter including the nodular HCC (**c** [*arrows* show applicator for insertion paths], **d** [*arrow* shows applicator for insertion paths])

Table 2 Maintenance of the therapeutic effect (TE) (overall assessment of the TE; intention-to-treat (ITT) analysis)

	This clinical study		Patients who underwent local therapy [24]	
	10 weeks	24 weeks[a]	3 months	6 months
Complete response (CR) (no. of patients)	85/88 (96.6 %)	82/87 (94.3 %)	4468/5394 (82.8 %)	4318/5378 (80.3 %)
Other (no. of patients)	3/88 (3.4 %)[b]	5/87 (5.7 %)[c]	926/5394 (17.2 %)	1060/5378 (19.7 %)

[a] One patient who died was omitted from the 24-week assessment

[b] Includes 3 patients who developed local recurrence within 10 weeks

[c] Includes 5 patients who developed local recurrence within 24 weeks

of each RFA session in the present clinical study. As shown in Fig. 6a, the complete necrosis (Class V TN) rate with the CelonPOWER System was 97.8 % (88/90 patients), which was higher than the rate of 86.2 % (50/58 patients) with the Cool-tip RF System. These results thus confirm the non-inferiority of the CelonPOWER System ($p < 0.001$; Fisher's exact test based on binomial distribution). As shown in Fig. 6b, the percentage of patients in whom treatment was completed in a single session was 77.8 % (70/90 patients) in the present study with the CelonPOWER System, compared with 51.7 % (31/60 patients) in the Cool-tip RF System study [10].

Safety

The overall safety assessment was performed for the entire clinical study period, i.e., inclusive of the treatment period and the follow-up period. Of the 91 patients included in the safety analysis, no procedure was rated as unsafe, although 2 procedures (2.2 %) were rated as somewhat unsafe, one with an abdominal wall burn and one with biliary peritonitis owing to bile leakage; 78 procedures (85.7 %) were rated as safe overall and 11 procedures (12.1 %) were rated as safe. There was no device failure. In the patient with biliary peritonitis, three 30-mm electrodes had been

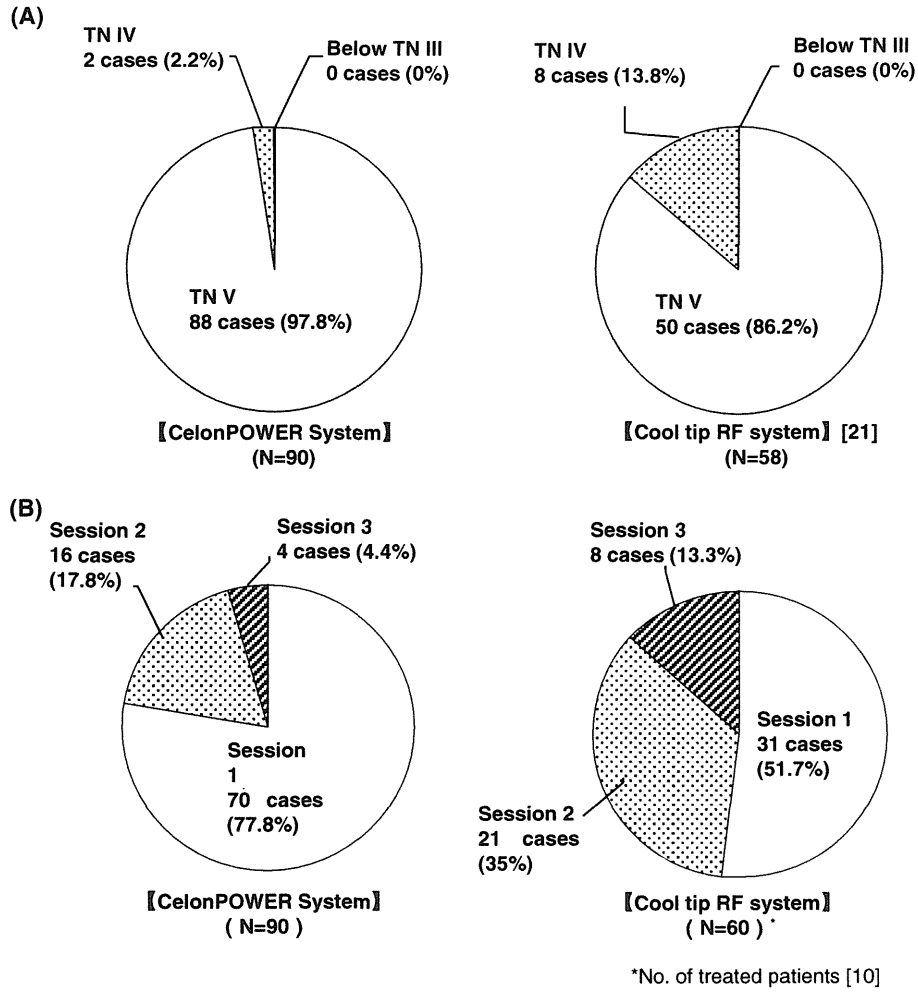

Fig. 6 Comparison of the present results obtained with the CelonPOWER System and the clinical study results reported for the Cool-tip RF System. The percentage of Class V tumor necrosis (*TN*) (TN 100 %) cases (**a**) and the number of patients in whom each RFA session was completed (**b**)

simultaneously inserted into an S8 tumor, and treatment was finished in a single ablation.

During the course of the entire clinical study period, serious adverse events (i.e., events for which a causal relationship with the CelonPOWER System could not be ruled out) were seen in 3 patients, consisting of abdominal wall burn, pleural effusion, and biliary peritonitis. Each of those events was judged to be serious because they required prolongation of hospitalization, and each required treatment. In addition, it was judged that each of these serious adverse events was a known adverse event that had been observed with similar, already-approved RFA devices [21–23]. Also, the single fatality, which occurred at home, had occurred in a patient who had been hospitalized for treatment on the suspicion of peritonitis based on the examinations performed after 10 weeks in the follow-up period. The patient's condition had improved and the patient had been discharged, and it was later confirmed that death had occurred at home. Autopsy revealed the cause of death to have been due to the progression of cirrhosis, and it was thus thought that the death was not related to the treatment with the CelonPOWER System. Table 3 shows the most common adverse effects (those observed in 5 % of patients or more) and all of these (pleural effusion, nausea, vomiting, postprocedural pain, and fever) have been known to occur with previously approved local therapeutic devices. Moreover, all the adverse events were easily controllable.

Discussion

We set out to prospectively determine whether a bipolar RFA device (CelonPOWER System) was safe and effective in the treatment of liver cancer and whether it could be demonstrated to be non-inferior to a monopolar RFA system currently approved and employed clinically in Japan (Cool-tip RF System).

Treatment was completed in a fewer number of sessions when using the CelonPOWER System than with the Cool-

Table 3 Frequently observed adverse effects (5 % or more) (adverse reactions at an incidence of >5 % in the overall study period)

Adverse event	No. of patients	%	No. of patients treated (%)	Treatments
Aspartate aminotransferase (AST) increase	72	79.1	0 (0)	–
Alanine aminotransferase (ALT) increase	69	75.8	0 (0)	–
Lactate dehydrogenase (LDH) increase	22	24.2	0 (0)	–
Total bilirubin increase	20	22.0	0 (0)	–
Pleural effusion	12	13.2	2 (2.20)	Human serum albumin, cefmetazole sodium, tazobactam piperacillin hydrate
Vomiting	12	13.2	7 (7.69)	Metoclopramide
Nausea	10	11.0	9 (9.89)	Metoclopramide, domperidone, diazepam
Postoperative pain	9	9.9	3 (3.30)	Pentazocine, loxoprofen sodium hydrate, acetaminophen, diclofenac sodium
White blood cell count increase	8	8.8	1 (1.10)	Sulbactam sodium–cefoperazone sodium
Platelet count decrease	6	6.6	0 (0)	–
Alkaline phosphatase (ALP) increase	5	5.5	0 (0)	–
Fever	5	5.5	5 (5.49)	Loxoprofen sodium hydrate, acetaminophen, cefmetazole sodium

tip RF System, suggesting that this new system yields efficacy that is at least equivalent to that achieved with the Cool-tip RF System, while causing less of a treatment burden on the patient.

We assessed the TE level, and its maintenance in ITT cases after 10 weeks and 24 weeks (6 months) in the follow-up period of this clinical study and found that the overall TE assessment was not inferior to that of the National Follow-up Survey Report on Primary Hepatic Carcinoma (2004–2005) [24] issued by the Liver Cancer Study Group of Japan (Table 2). Considering that the method for overall TE assessment in that report was the same as that employed in the present study, it is reasonable to conclude that the TE maintenance with the CelonPOWER System is not inferior to that of other local therapy.

Nishikawa et al. reported on local recurrence when using monopolar systems clinically. They found that, in 269 patients with solitary hypervascular HCCs who had undergone RFA, the 1- and 2-year cumulative local recurrence rates were 12.8 and 23.6 %, respectively [25]. We believe that our present results for the cumulative local recurrence rate (5.7 % for 6 months) with the CelonPOWER System are comparable to those reported results.

The introduction of a new device inevitably raises the question of its safety. In our series, there were 3 adverse events–one event of abdominal wall burn and one of pleural effusion during the treatment period, and one event of biliary peritonitis during the follow-up period. These adverse events were previously known to be possible adverse events that had been observed with the Cool-tip, RITA, and Boston monopolar RFA systems that have already been approved for clinical use in Japan [21–23]. Therefore, similar caution concerning internal adverse events is necessary when using the CelonPOWER System, although the problem of external burns does not exist with this system.

The high-incidence (≥5 %) device-related adverse event rate during the course of our clinical study was similar to the rates with the Cool-tip, RITA, and Boston monopolar RFA systems [21–23].

Therefore, these events are not unique to the CelonPOWER System, and the safety of the CelonPOWER System is not inferior to that of the existing approved RFA devices.

This study has several limitations. First of all, although it was a prospective study, it was not a randomized controlled clinical study. However, all consecutive patients who satisfied the enrollment criteria were offered the opportunity to participate and the study was performed in all those who provided informed consent and decided to receive the treatment. After providing informed consent, 5 patients decided not to participate and 1 ceased treatment after 1 session, due to the difficulty posed by the proximity of the lesion to the heart and lungs.

Although we were able to compare our own results immediately after treatment with those of the Cool-tip RF

System and other systems, we were not able to compare the results 6 months after treatment because of the lack of such data for the Cool-tip RF System, because of the different GCP guidelines in force at the time of the Cool-tip RF System study. However, the 6-month follow-up data of our study were very satisfactory. Furthermore, because there were no such data available in the reports on the Cool-tip RF System, we could not compare the levels of experience of the operators in the two studies.

In conclusion, the present clinical study confirmed that the CelonPOWER System is a very safe and highly effective RFA system for liver cancer in Japanese patients. In addition, because this system is a bipolar device, it operates with high energy efficiency, and because multiple multipolar applicators can be employed simultaneously, coagulation necrosis of an extensive tumor tissue volume can be achieved in a short treatment time. Moreover, throughout the course of this clinical study, most of the patients did not experience hot flushes or perspiration. It is therefore anticipated that the CelonPOWER System will become used as a next-generation RFA system that is not only safer than existing systems, but is highly effective and places less physical burden on the patient.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Radiofrequency ablation for hepatocellular carcinoma: the relationship between a new grading system for the ablative margin and clinical outcomes

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Abstract
Background In our previous study, we classified the radicality (R grading) of percutaneous radiofrequency ablation (RFA) therapy for single hepatocellular carcinoma (HCC) according to the extent of the ablated margin, and demonstrated that this grading system was useful for predicting local tumor progression (LTP) after RFA. The aim of this study was to measure the overall survival (OS), the recurrence free survival (RFS), and the distant recurrence (DR) rate for each R grade (A–D), and to examine the relationship between clinical outcome and R grading.
Methods This study involved 368 patients with solitary HCC who had undergone RFA. The mean tumor diameter was 2.0 ± 0.7 cm. We calculated the post-RFA cumulative OS, RFS, and DR rate for each R grade and analyzed the factors contributing to clinical outcomes.
Results In the multivariate analysis, significant factors were as follows: tumor size >2 cm, serum albumin >3.5 g/dL, prothrombin time >70 %, HCC recurrence within 1 year, and R grading (grade A) in OS; cause of liver disease (hepatitis B), gamma glutamyl transpeptidase (GGT) >80 IU/L, platelet count >10 × 10^4/mm^3, and R grading (grade A or B) in RFS; GGT >80 IU/L, platelet count >10 × 10^4/mm^3, and R grading (grade A or B) in DR. In patients with sufficient Lipiodol accumulation (n = 219), very similar results were obtained. However, in patients with grade A and B (n = 232), R grade was not a significant independent factor linked to OS, although grade A patients had lower LTP rate.
Conclusions Our proposed R grading system appears to be useful for predicting clinical outcomes after RFA.

Keywords Hepatocellular carcinoma · Radiofrequency ablation · Ablative margin · Clinical outcome

Introduction

Hepatocellular carcinoma (HCC) ranks fifth among the most prevalent cancers in the world and is the third most common cause of cancer-related death [1–4]. The poor prognosis of HCC has shown some improvement due to advances in therapeutic options including surgical resection, percutaneous ablation therapy such as radiofrequency thermal ablation (RFA) and percutaneous ethanol injection therapy (PEI), transcatheter arterial chemoembolization (TACE), liver transplantation, radioembolization and molecular-targeted drugs, and imaging modalities for HCC [5–11]. However, the heterogeneity of HCC patients is still a big challenge. The choice of the treatment modality for HCC mainly depends on tumor size, the number of tumors, liver function, the presence or absence of vascular invasion, and the causative factors of liver disease [5–11].

In most HCC patients, successful treatment of HCC is followed by recurrence, leading to high mortality rates. In 68–96 % of patients recurrence only occurs at intrahepatic sites [12]. Therefore, the prediction of HCC recurrence and the performance of appropriate therapy for this recurrence after initial treatment are essential for the optimization of clinical outcome [5].

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Image-guided tumor ablation therapy is recommended in early-stage HCC patients when surgical interventions are precluded because of insufficient hepatic functional reserve [13]. RFA therapy, which was introduced in Japan in 1999, has demonstrated superior local tumor control effects and greater survival benefit over PEI in several randomized controlled trials and is currently established as the standard therapy for early-stage HCC [5, 13–16]. More recently, several investigators have used RFA to treat selected patients with resectable HCC with favorable clinical outcomes, and RFA is gradually being used to treat resectable HCC in many countries, in addition to Japan [17].

Although many studies related to prognostic predictors after the surgical resection of early-stage HCC have been reported [18, 19], to the best of our knowledge there have been few studies on treatment response to RFA as a prognostic predictor of survival [20, 21]. In 2004, Sala et al. reported that Child–Pugh classification and initial treatment response could be used as prognostic factors for predicting the survival. Of those patients who had received percutaneous ablation therapy, however, most were treated with PEI [22].

We have routinely classified cases treated with RFA into four groups based on the extent of the ablative margin, a novel classification system, which we have referred to as radicality grading (R grades: A, B, C, and D). This classification system was useful for predicting local tumor progression (LTP) after RFA [23]. The objective of the present study was to examine the usefulness of the R grading system as a predictor not only of LTP, but also overall survival (OS), recurrence free survival (RFS), and distant recurrence (DR) using a larger cohort than in our previous study [23].

Patients and methods

Patients and HCC diagnosis

We performed RFA therapy in 395 treatment-naive patients diagnosed with solitary HCC in the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, between January 2004 and January 2012. Of these patients, 27 were lost to follow-up and were excluded from the study. Thus, 368 patients with single HCC were analyzed in the present study. Prior to RFA, written informed consent was obtained from all patients. The ethics committee of our department approved the protocol for RFA therapy (approval number 412). The current study comprised a retrospective analysis of patient records and all treatments were conducted in an open-label manner. The study protocol complied with all of the provisions of the Declaration of Helsinki.

Hypervascular HCC was diagnosed using abdominal ultrasound and dynamic computed tomography (CT) scans (hyperattenuation during the arterial phase in all or some part of the tumor and hypoattenuation in the portal-venous phase). Arterial and portal phase dynamic CT images were obtained at approximately 30 and 120 s, respectively, after the injection of contrast material. In all cases of hypovascular HCC, percutaneous tumor biopsy was performed according to the diagnostic criteria for HCC proposed by the Japan Society of Hepatology [8]. All of the patient histological diagnoses were well-differentiated HCCs. There were 336 hypervascular HCCs and 32 hypovascular HCCs in the current study. For all patients, abdominal angiography was performed before RFA. We confirmed solitary HCC with no vascular invasion and no satellite nodules using CT during hepatic arteriography (CTHA) and arterial-portography (CTAP) [24].

Assessment of treatment efficacy

To assess treatment efficacy, we performed dynamic 16-column multi-detector CT (MDCT) using 3-mm-slice scans within 1 week after RFA. The patients were then classified into four groups as follows: grade A (absolutely curative), a 5 mm or larger ablative margin around the entire tumor; grade B (relatively curative), an ablative margin around the tumor but less than 5 mm in diameter in some places; grade C (relatively non-curative), only an incomplete ablative margin around the tumor although no residual tumor was apparent; grade D (absolutely non-curative), the tumor was not completely ablated [23] (Fig. 1). Using this method to assess treatment efficacy, we calculated not only the LTP rate but also the OS, RFS and DR rates for all 368 cases. In other words, we used the R grading method and examined its usefulness in assessing clinical outcomes. We defined LTP as the presence of a recurrent nodule adjacent to the ablated area after RFA using dynamic CT scanning. Recurrence that occurred distantly from ablated area in the same segment was included as DR. Extrahepatic recurrence was determined using chest CT scan, whole abdominal CT scan and bone scintigraphy. LTP and DR were determined by three radiologists experienced in liver imaging as described previously [23]. Decisions with regard to R grading were made by the three radiologists experienced in the liver imaging modalities mentioned above.

In our department, during abdominal angiography, we routinely perform arterial infusion of iodized oil (Lipiodol Ultra-Fluid, Schering Japan, Osaka, Japan). Lipiodol was injected to intensify the radiologic visibility of the target tumor [23]. In patients where the tumor location could be determined owing to the dense accumulation of Lipiodol, we assessed treatment efficacy using dynamic CT scans.

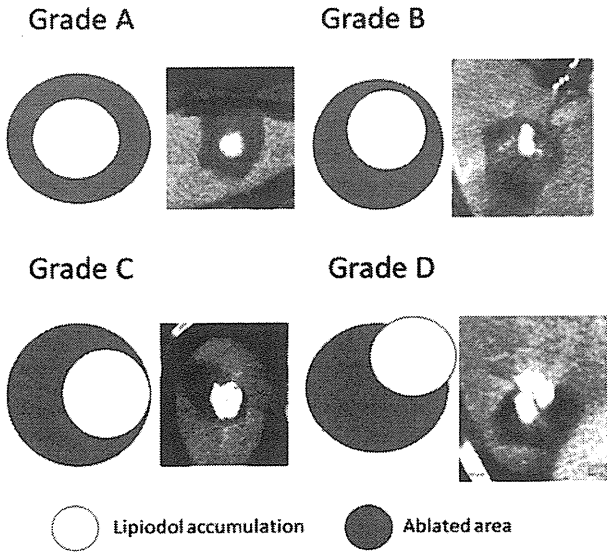

Fig. 1 Diagrammatic representation of the proposed R grading method (reproduced from [23], with permission). *Grade A* (absolutely curative), an ablative margin of at least 5 mm is achieved around the entire tumor; *grade B* (relatively curative), an ablative margin extends around the entire tumor but is less than 5 mm in diameter in some places; *grade C* (relatively non-curative), a complete ablative margin is not formed although no residual tumor is apparent; *grade D* (absolutely non-curative), the tumor has not been entirely ablated

However, in patients in whom it was difficult to determine the exact location of the tumor because of insufficient Lipiodol accumulation (e.g., hypovascular HCC) and for those where Lipiodol had only accumulated in part of the tumor (e.g., nodule-in-nodule HCC), we measured the ablative margin using CTHA with a CTAP image as the reference image. Follow-up consisted of periodic blood tests and monitoring of tumor markers, including des-γ-carboxy prothrombin (DCP), which was measured using a chemiluminescent enzyme immunoassay (Lumipulse PIV-KAII Eisai, Eisai, Tokyo, Japan). Dynamic CT scans and/or magnetic resonance imaging (MRI) was obtained every 3–4 months after RFA.

In addition to the R grade, a total of 20 factors including patient characteristics, clinical biochemical data, and tumor markers were retrospectively examined for their contribution to OS, RFS, DR, and LTP using univariate and multivariate analyses.

RFA procedure

The details of our RFA procedure have been described previously [23]. Briefly, we routinely used a cool-tip needle (Radionics Corp., Burlington, MA, USA) while performing RFA. Using the intercostal or subcostal approach a 17-gauge, 2- or 3-cm cooled-tip electrode was inserted under real-time ultrasound guidance. The duration of a single ablation session using RFA was 12 min for the 3-cm electrode and 6 min for the 2-cm electrode. All procedures were performed under ultrasound guidance by one of five operators who had at least 3 years of experience performing RFA. We used the artificial ascites technique to prevent collateral thermal injury when the anticipated RFA zone was in contact with a critical organ, such as the hepatic flexure of the colon. We also used this technique to improve visibility when the index tumor was located in the hepatic dome area.

Complete ablation of HCC was defined as hypoattenuation of the lesion including the surrounding liver parenchyma. Therefore, we routinely performed additional RFA treatment until we had confirmed that the ablative margin surrounded the entire circumference of the tumor (R grade: grade A or B), provided that patient consent had been obtained.

Statistical analysis

Data were analyzed using univariate and multivariate analysis. Continuous variables were compared using the unpaired t test, and categorical variables were compared using Fisher's exact test. Time to recurrence was defined as the interval between initial RFA and first confirmed recurrence. For analysis of RFS, DR, and LTP, follow-up ended at the time of first recurrence; other patients were censored at their last follow-up visit and the time of death from any cause without recurrence. For analysis of OS, follow-up ended at the time of death from any cause, censoring the remaining patients at the last follow-up visit. The cumulative OS, RFS, DR, and LTP rates were calculated using the Kaplan–Meier method, and tested using the log-rank test. The Cox proportional hazard model was used for multivariate analysis of factors that were considered significant in univariate analysis. These statistical methods were used to estimate the interval from initial RFA treatment. Data were analyzed using SPSS software, version 9.0 (SPSS Inc., Chicago, IL, USA) for Microsoft Windows. Data are expressed as mean ± standard deviation (SD). Values of $P < 0.05$ were considered to be statistically significant.

Results

Clinical characteristics

The clinical characteristics of the patients with HCC are shown in Table 1. The mean tumor diameter, observation period, and number of treatment sessions were 2.0 ± 0.7 cm, 3.0 ± 2.0 years, and 1.25 ± 0.48, respectively. Three hundred and thirty-six patients (91.3 %) had

Table 1 Baseline characteristics of patients with hepatocellular carcinoma ($n = 368$)

Variables	Number of patients or mean ± SD
Gender	
Male/female	217/151
Age (years)	69.9 ± 9.0
Tumor size (cm)	2.0 ± 0.7
Tumor location	
S1/S2/S3/S4/S5/S6/S7/S8	1/18/40/34/44/37/46/148
Tumor vascularity	
Hyper/hypo	336/32
Observation period (years)	3.0 ± 2.0
Number of RFA sessions	1.25 ± 0.48
Cause of liver disease	
Hepatitis C/hepatitis B/non B, non C	289/29/50
Child–Pugh classification	
Chronic hepatitis/Child–Pugh A/B/C	80/232/52/4
R factor	
Grade A/B/C/D	70/162/100/36
HCC recurrence	
Yes/no	214/154
Local tumor progression	
Yes/no	104/264
Body mass index (kg/m^2)	
>25/<25	111/257
Diabetes mellitus	
Yes/no	119/249
Post-RFA antiviral therapy	
Yes/no	48/320
Biochemical analysis	
AST (IU/L)	57.6 ± 30.3
ALT (IU/L)	49.8 ± 40.3
ALP (IU/L)	357.8 ± 201.8
GGT (IU/L)	73.2 ± 71.3
Albumin (g/dL)	3.79 ± 0.51
Total bilirubin (mg/dL)	0.95 ± 0.54
Prothrombin time (%)	85.3 ± 15.6
Platelets (×10^4/mm^3)	11.0 ± 5.0
AFP (ng/mL)	150.4 ± 829.8
DCP (mAU/mL)	354.4 ± 2391

SD standard deviation, *S* segment of the liver, *RFA* radiofrequency thermal ablation, *R* radicality, *HCC* hepatocelluar carcinoma, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transpeptidase, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

hypervascular HCCs and 32 (8.7 %) had hypovascular HCCs. We confirmed 104 cases of LTP (28.3 %) and 214 cases of HCC recurrence (58.2 %). Using the proposed R grading system, we classified 70 patients as grade A (19.0 %), 162 as grade B (44.0 %), 100 as grade C (27.2 %), and 36 as grade D (9.8 %).

Reason for not attempting to perform additional RFA in patients with grade C and D cases

It was considered that grade C and D cases (total 136 cases) should receive additional RFA because a sufficient ablative margin was not obtained. The reasons we did not attempt to perform additional RFA and the number of cases were as follows: (1) cases in which additional RFA was abandoned owing to a doctor's decision, because it was extremely difficult to perform additional RFA at sites such as directly under the hepatic dome, or the heart (43 cases); (2) cases in which additional RFA was considered to be difficult to perform because of poor visibility under ultrasonography owing to extreme obesity and inability to hold the breath during the performance of RFA (19 cases); (3) cases in which high rates of complications were expected, such as when tumors at the site of the hepatic hilar lesion were treated by RFA (27 cases); (4) cases in which additional RFA was difficult to perform because ascites appeared after the first session of RFA owing to poor hepatic function before RFA (22 cases); (5) cases whose informed consent could not be obtained for additional RFA for reasons such as physical burden (15 cases); and (6) cases involving extremely elderly patients (aged 80 years and over) with a perceived minimal survival advantage after additional RFA (10 cases).

OS and RFS rates for all cases

In terms of OS for all cases, the 1-, 3-, and 5-year OS rates after initial RFA were 95.5, 77.4, and 59.8 %, respectively (Fig. 2a). In terms of RFS for all cases, the 1-, 3-, and 5-year RFS rates after initial RFA were 74.5, 34.1, and 18.0 %, respectively (Fig. 2b).

OS, RFS, and DR rates analyzed using R grading

We also determined the rates of OS, RFS, and DR for each of the four grades of the R grading system. The cumulative rates of OS at 1, 3, and 5 years were as follows: 98.6, 90.3, and 79.5 %, respectively, for grade A; 95.6, 75.3, and 66.7 %, respectively, for grade B; 95.8, 73.3, and 44.8 %, respectively, for grade C; and 88.5, 63.8, and 35.0 %, respectively, for grade D. These differences between individual grades reached statistical significance, indicating that a more complete and a larger ablation margin was associated with a favorable OS rate (grade A vs. grade B, $P = 0.046$; grade B vs. grade C, $P = 0.324$; grade C vs. grade D, $P = 0.173$; grade A vs. grade C, $P = 0.010$; grade A vs. grade D, $P = 0.001$; grade B vs. grade D,

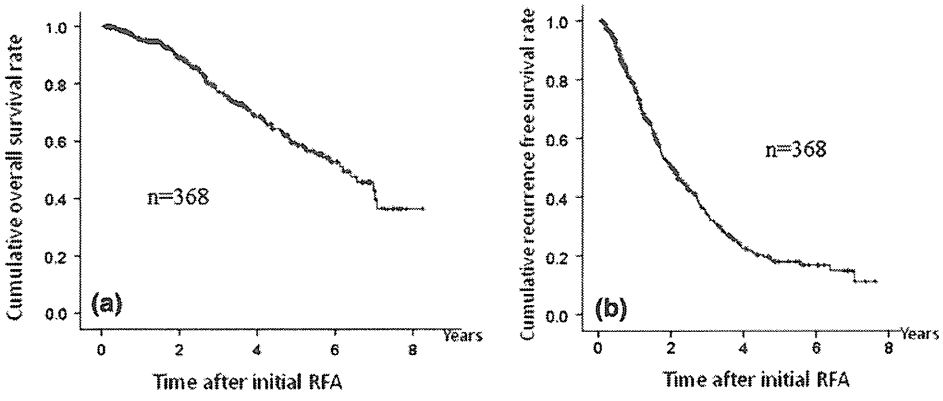

Fig. 2 **a** Cumulative overall survival (OS) rates for all cases ($n = 368$). The 1-, 3-, and 5-year OS rates after initial RFA were 95.5, 77.4 and 59.8 %, respectively. **b** Cumulative recurrence free survival (RFS) rates for all cases ($n = 368$). The 1-, 3-, and 5-year RFS rates after initial RFA were 74.5, 34.1, and 18.0 %, respectively

$P = 0.026$; and overall significance, $P = 0.008$) (Fig. 3a). The cumulative rates of RFS at 1, 3, and 5 years were as follows: 88.0, 44.2, and 25.9 %, respectively, for grade A; 88.0, 44.2, and 23.5 %, respectively, for grade B; 69.0, 21.9, and 13.3 %, respectively, for grade C; and 49.1, 8.2, and 4.5 %, respectively, for grade D. These differences between individual grades also reached statistical significance, indicating that a more complete and larger ablation margin was associated with a lower RFS rate (grade A vs. grade B, $P = 0.839$; grade B vs. grade C, $P < 0.001$; grade C vs. grade D, $P = 0.033$; grade A vs. grade C, $P < 0.001$; grade A vs. grade D, $P < 0.001$; grade B vs. grade D, $P < 0.001$; and overall significance, $P < 0.001$), although for grades A and B very similar Kaplan–Meier curves were obtained (Fig. 3b). The cumulative rates of DR at 1, 3 and 5 years were as follows: 11.0, 52.7 and 71.0 %, respectively, for grade A; 11.0, 54.1 and 72.9 %, respectively, for grade B; 26.0, 72.5 and 83.2 %, respectively, for grade C; and 34.1, 76.0 and 88.0 %, respectively, for grade D. These differences between individual grades also reached statistical significance, indicating that a more complete and larger ablation margin was associated with a lower DR rate (grade A vs. grade B, $P = 0.981$; grade B vs. grade C, $P = 0.001$; grade C vs. grade D, $P = 0.322$; grade A vs. grade C, $P = 0.009$; grade A vs. grade D, $P = 0.002$; grade B vs. grade D, $P < 0.001$; and overall significance, $P < 0.001$), although almost identical Kaplan–Meier curves were obtained for grades A and B (Fig. 3c).

LTP in the total patient population

In the present study, we used a larger cohort ($n = 368$) than in our previous study ($n = 269$) [23]. All 269 patients analyzed in our previous study overlapped with our current study. In terms of LTP, the findings were almost identical to those reported in our previous study [23], namely that a more complete and a larger ablation margin was associated with a lower rate of LTP (grade A vs. grade B, $P = 0.008$; grade B vs. grade C, $P < 0.001$; grade C vs. grade D, $P = 0.010$; grade A vs. grade C, $P < 0.001$; grade A vs. grade D, $P < 0.001$; grade B vs. grade D, $P < 0.001$; and overall significance, $P < 0.001$) (Supplemental Fig. 1).

Univariate analysis of factors contributing to OS, RFS, DR, and LTP

Using univariate analysis, we found the following factors to be significantly associated with OS: tumor size >2 cm ($P = 0.040$), tumor vascularity ($P = 0.037$), cause of liver disease ($P = 0.010$), serum albumin >3.5 g/dL ($P < 0.001$), total bilirubin >1 mg/dL ($P = 0.046$), prothrombin time >70 % ($P < 0.001$), platelet count $>10 \times 10^4/mm^3$ ($P < 0.001$), post-RFA antiviral therapy ($P = 0.014$), HCC recurrence within 1 year after RFA ($P < 0.001$), and R grading score ($P = 0.008$) (Table 2). Significant factors associated with RFS were cause of liver disease ($P = 0.032$), AST >40 IU/L ($P = 0.013$), GGT >80 IU/L ($P = 0.019$), serum albumin >3.5 g/dL ($P = 0.004$), total bilirubin >1 mg/dL ($P = 0.019$), platelets $>10 \times 10^4/mm^3$ ($P < 0.001$), and R grading score ($P < 0.001$) (Table 2). Significant factors associated with DR were cause of liver disease ($P = 0.047$), AST >40 IU/L ($P < 0.001$), GGT >80 IU/L ($P = 0.008$), serum albumin >3.5 g/dL ($P = 0.008$), total bilirubin >1 mg/dL ($P = 0.016$), prothrombin time >70 % ($P = 0.014$), platelets $>10 \times 10^4/mm^3$ ($P < 0.001$), and R grading score ($P < 0.001$) (Table 2). Significant factors associated with LTP were tumor size >2 cm ($P = 0.001$), tumor vascularity ($P = 0.010$), DCP >200 mAU/L ($P = 0.017$), and R grading score ($P < 0.001$) (Table 2).

Multivariate analysis of factors contributing toOS, RFS, DR, and LTP

The hazard ratios (HRs) calculated using multivariate analysis for the 10 factors found to be significantly associated with OS using univariate analysis are detailed in Supplemental Table 1. Tumor size >2 cm, serum albumin

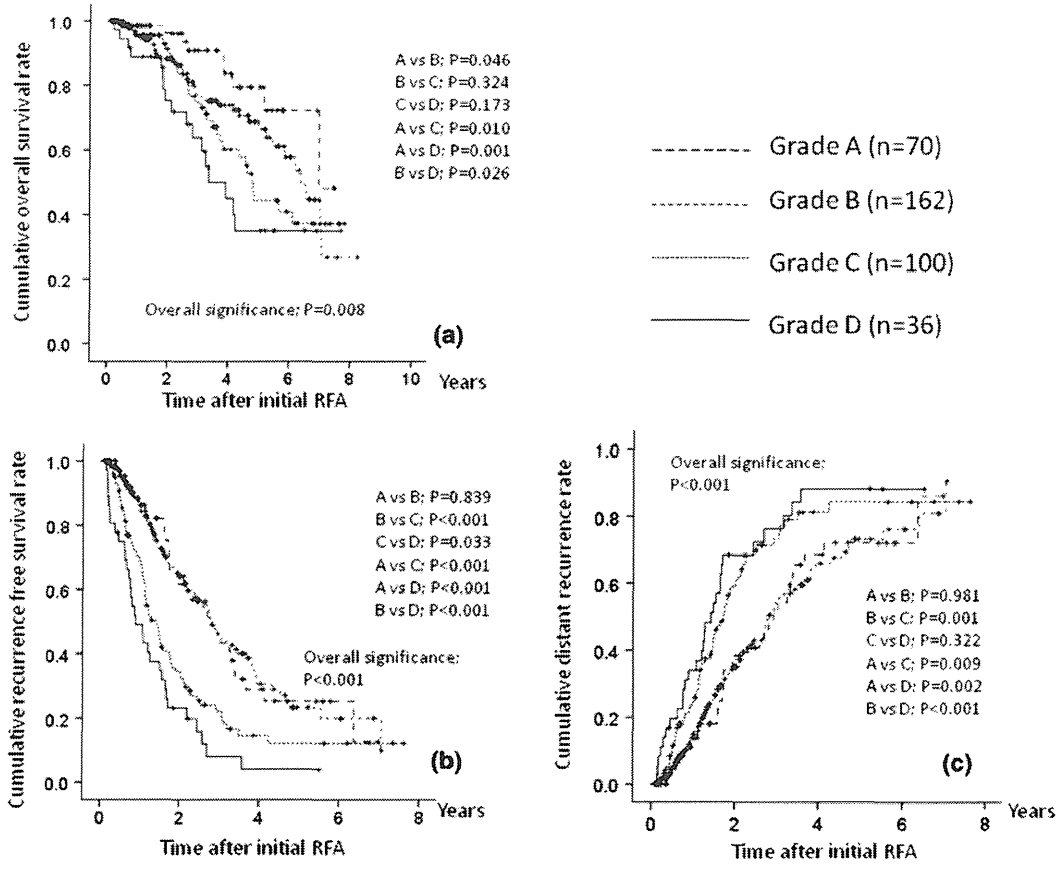

Fig. 3 **a** Cumulative overall survival (OS) rates and **b** cumulative recurrence free survival (RFS) rates according to R grading. See text for details

>3.5 g/dL, prothrombin time >70 %, HCC recurrence within 1 year after initial RFA, and the R grading score (grade A) were found to be significant independent factors linked to OS. The HRs calculated using multivariate analysis of the seven factors that were found to be significantly associated with RFS using univariate analysis are detailed in Supplemental Table 1. Cause of liver disease (hepatitis B), platelet count >10 × 10^4/mm^3, GGT >80 IU/L, and the R grading score (grade A or B) were found to be significant independent factors linked to RFS. The HRs calculated using multivariate analysis of the eight factors that were found to be significantly associated with DR using univariate analysis are detailed in Supplemental Table 1. Platelet count >10 × 10^4/mm^3, GGT >80 IU/L, and the R grading score (grade A or B) were found to be significant independent factors linked to DR. The HRs calculated using multivariate analysis of the four factors that were found to be significantly associated with LTP using univariate analysis are detailed in Supplemental Table 1. Only the R grading score was found to be a significant independent factor linked to LTP.

HCC recurrence

During the follow-up period, 214 patients (58.2 %) had HCC recurrence in the present study. In these patients, LTP alone was found in 12 patients, LTP with DR was found in 92 patients, and DR alone was found in the other 110 patients. Patterns of HCC recurrence were as follows: single HCC recurrence in the liver in 124 patients, single HCC recurrence with invasion of the inferior vena cava in one patient, multiple HCC recurrences in the liver in 84 patients, multiple HCC recurrences in the liver with lung metastases in one patient, multiple HCC recurrences in the liver with peritoneal dissemination in one patient, and single lymph node metastasis in three patients. There were 76 patients who had HCC recurrence within 1 year after initial RFA. In no patient was neoplastic seeding identified. Treatment methods for the first recurrence were as follows: surgical resection in eight patients, RFA in 149 patients, TACE in 26 patients, PEI in eight patients, systemic chemotherapy in three patients, and no specific treatment in 20 patients owing to liver failure or other reasons.

Table 2 Univariate analyses contributing to OS, RFS, DR, and LTP after initial RFA for all cases ($n = 368$)

Variables	n	P value[a]			
		OS	RFS	DR	LTP
Age (>65 years), yes/no	260/108	0.436	0.253	0.612	0.054
Gender (male), yes/no	217/151	0.786	0.884	0.617	0.907
Tumor size (>2 cm), yes/no	139/229	0.040	0.065	0.170	0.001
Tumor vascularity (hyper), yes/no	336/32	0.037	0.289	0.197	0.010
Tumor location (segment 8), yes/no	148/220	0.458	0.844	0.731	0.140
Cause of liver disease					
Hepatitis B/hepatitis C/non B non C	29/289/50	0.010	0.032	0.047	0.357
AST (>40 IU/L), yes/no	256/112	0.103	0.013	<0.001	0.961
ALT (>40 IU/L), yes/no	185/183	0.200	0.695	0.395	0.311
ALP (>340 IU/L), yes/no	146/222	0.863	0.124	0.051	0.608
GGT (>80 IU/L), yes/no	106/262	0.920	0.019	0.008	0.851
Albumin (>3.5 g/dL), yes/no	214/154	<0.001	0.004	0.008	0.157
Total bilirubin (>1 mg/dL), yes/no	107/261	0.046	0.019	0.016	0.094
Prothrombin time (>70 %), yes/no	307/61	<0.001	0.059	0.014	0.134
Platelets (>10 × 10^4/mm^3), yes/no	192/176	<0.001	<0.001	<0.001	0.055
AFP (>100 ng/mL), yes/no	64/304	0.720	0.635	0.356	0.308
DCP (>200 mAU/L), yes/no	46/322	0.127	0.296	0.486	0.017
Post-RFA antiviral therapy, yes/no	47/321	0.014	0.689	0.584	0.110
Body mass index (>25 kg/m^2), yes/no	111/257	0.616	0.604	0.593	0.462
Diabetes mellitus, yes/no	119/249	0.261	0.155	0.052	0.194
HCC recurrence within 1 year after RFA, yes/no	76/292	<0.001			
R grade, A/B/C/D	70/162/100/36	0.008	<0.001	<0.001	<0.001

RFA radiofrequency thermal ablation, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transpeptidase, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin, *HCC* hepatocellular carcinoma, *R* radicality, *OS* overall survival, *RFS* recurrence free survival, *DR* distant recurrence, *LTP* local tumor progression

[a] Log-rank test

Analysis of patients with sufficient Lipiodol accumulation

We also performed additional analysis in patients with sufficient Lipiodol accumulation (219 out of 368 patients; 59.5 %) using dynamic CT scans after RFA, because the most important advantage of the current study was the accurate assessment of the R grade using Lipiodol accumulation. Using the proposed R grading system, we classified 45 patients as grade A (20.5 %), 100 as grade B (45.7 %), 53 as grade C (24.2 %), and 21 as grade D (9.6 %).

The cumulative rates of OS at 1, 3, and 5 years were as follows: 100, 96.0, and 90.6 %, respectively, for grade A; 94.1, 77.6, and 69.6 %, respectively, for grade B; 98.2, 69.3, and 41.8 %, respectively, for grade C; and 85.0, 56.7, and 29.1 %, respectively, for grade D. These differences between individual grades reached statistical significance (grade A vs. grade B, $P = 0.041$; grade B vs. grade C, $P = 0.217$; grade C vs. grade D, $P = 0.147$; grade A vs. grade C, $P = 0.004$; grade A vs. grade D, $P < 0.001$; grade B vs. grade D, $P = 0.005$; and overall significance, $P = 0.001$) (Fig. 4a). The cumulative rates of RFS at 1, 3, and 5 years were as follows: 84.0, 50.9, and 27.3 %, respectively, for grade A; 84.0, 45.4, and 23.3 %, respectively, for grade B; 66.7, 20.0, and 8.9 %, respectively, for grade C; and 41.8, 11.3, and 5.4 %, respectively, for grade D. These differences between individual grades reached statistical significance, indicating that a more complete and a larger ablation margin was associated with a favorable RFS rate (grade A vs. grade B, $P = 0.953$; grade B vs. grade C, $P < 0.001$; grade C vs. grade D, $P = 0.293$; grade A vs. grade C, $P = 0.002$; grade A vs. grade D, $P < 0.001$; grade B vs. grade D, $P < 0.001$; and overall significance; $P < 0.001$), althoughvery similar Kaplan–Meier curves were obtained for grades A and B (Fig. 4b). The cumulative rates of DR at 1, 3, and 5 years were as follows: 16.0, 44.0, and 67.7 %, respectively, for grade A; 14.0, 53.2, and 72.0 %, respectively, for grade B; 29.5, 78.7, and 89.3 %, respectively, for grade C; and 48.0, 85.0, and 93.1 %, respectively, for grade D. These differences between individual grades reached statistical significance, indicating that a more complete and a larger ablation margin was

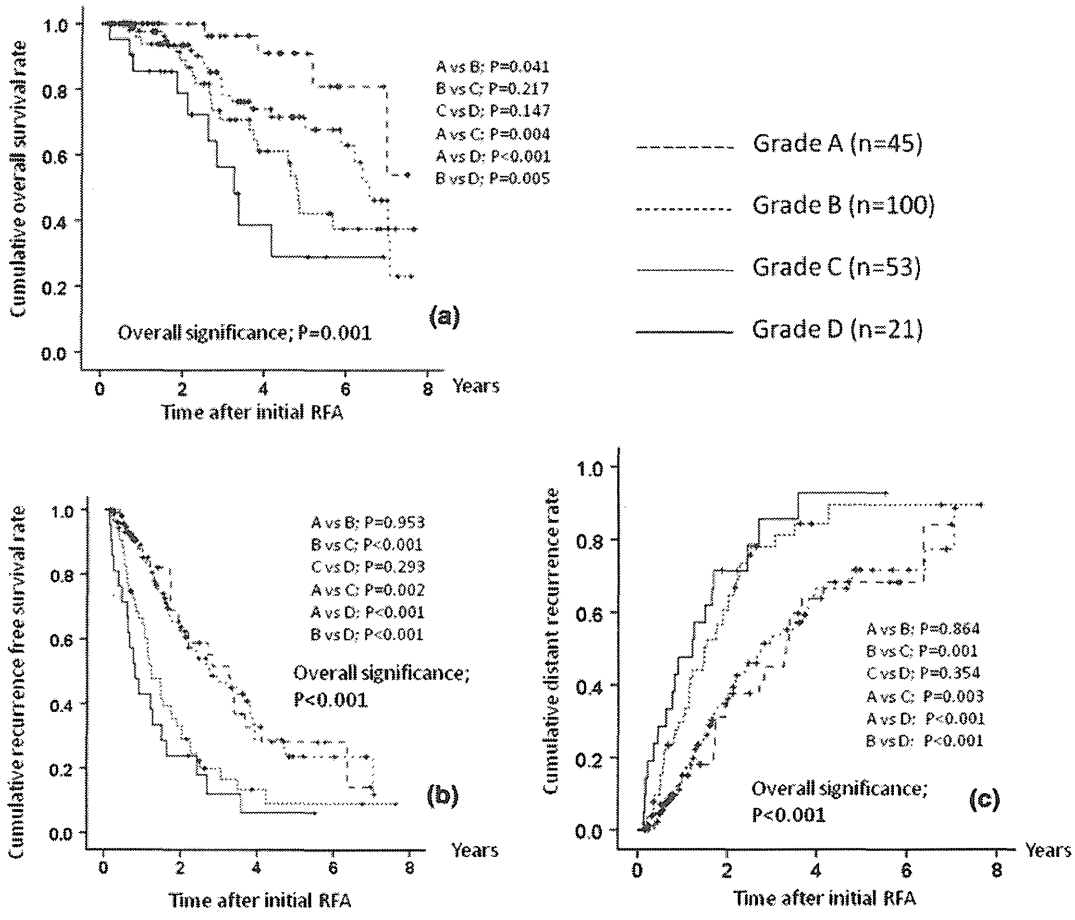

Fig. 4 **a** Cumulative overall survival (OS) rates **b** cumulative recurrence free survival (RFS) rates according to R grading in patients with sufficient Lipiodol accumulation. See text for details

associated with a favorable DR rate (grade A vs. grade B, $P = 0.864$; grade B vs. grade C, $P = 0.001$; grade C vs. grade D, $P = 0.354$; grade A vs. grade C, $P = 0.003$; grade A vs. grade D, $P < 0.001$; grade B vs. grade D, $P < 0.001$; and overall significance, $P < 0.001$), althoughthe Kaplan–Meier curves obtained for grades A and B were almost identical (Fig. 4c).

LTP in patients with sufficient Lipiodol accumulation

In patients with sufficient Lipiodol accumulation, in terms of LTP, differences between individual grades reached statistical significance indicating that a more complete and a larger ablation margin was associated with a favorable LTP rate (grade A vs. grade B, $P = 0.039$; grade B vs. grade C, $P < 0.001$; grade C vs. grade D, $P = 0.117$; grade A vs. grade C, $P < 0.001$; grade A vs. grade D, $P < 0.001$; grade B vs. grade D, $P < 0.001$; and overall significance, $P < 0.001$) (Supplemental Fig. 2).

Univariate analysis of factors contributing to OS, RFS, DR, and LTP in patients with sufficient Lipiodol accumulation

Significant factors contributing to OS, RFS, DR, and LTP evaluated using univariate analysis in patients with sufficient Lipiodol accumulation were as follows: cause of liver disease ($P = 0.017$), AST >40 IU/L ($P = 0.020$), serum albumin >3.5 g/dL ($P < 0.001$), total bilirubin >1 mg/dL ($P = 0.030$), prothrombin time >70 % ($P = 0.001$), platelets $>10 \times 10^4/mm^3$ ($P < 0.001$), HCC recurrence within 1 year after RFA ($P < 0.001$), and R grade ($P = 0.001$) in OS; cause of liver disease ($P = 0.018$), AST >40 IU/L ($P = 0.007$), GGT >80 IU/L ($P = 0.018$), platelets $>10 \times 10^4/mm^3$ ($P = 0.002$), and R grade ($P < 0.001$) in RFS; cause of liver disease ($P = 0.020$), AST >40 IU/L ($P < 0.001$), GGT >80 IU/L ($P = 0.029$), serum albumin >3.5 g/dL ($P = 0.045$), platelets $>10 \times 10^4/mm^3$ ($P = 0.001$), and R grade ($P < 0.001$) in DR; tumor size >2 cm

($P = 0.024$), DCP >200 mAU/L ($P = 0.036$), and R grade ($P < 0.001$) in LTP (Table 3).

Multivariate analysis of factors contributing to OS, RFS, DR, and LTP in patients with sufficient Lipiodol accumulation

The HRs calculated using multivariate analysis for the eight factors found to be significantly associated with OS using univariate analysis are detailed in Supplemental Table 2. Prothrombin time >70 %, platelets >10 × $10^4/mm^3$, HCC recurrence within 1 year after initial RFA, and the R grading score (grade A or B) were found to be significant independent factors linked to OS. The HRs calculated using multivariate analysis of the five factors that were found to be significantly associated with RFS using univariate analysis are detailed in Supplemental Table 2. Cause of liver disease (hepatitis B), platelet count >10 × $10^4/mm^3$, GGT >80 IU/L, and the R grading score (grade A or B) were found to be significant independent factors linked to RFS. The HRs calculated using multivariate analysis of the six factors that were found to be significantly associated with DR using univariate analysis are detailed in Supplemental Table 2. Cause of liver disease (hepatitis B), platelet count >10 × $10^4/mm^3$, and the R grading score (grade A or B) were found to be significant independent factors linked to DR. The HRs calculated using multivariate analysis of the three factors that were found to be significantly associated with LTP using univariate analysis are detailed in Supplemental Table 2. Only the R grading (grade A or B) score was found to be a significant independent factor linked to LTP.

Univariate analysis of factors contributing to OS, RFS, DR, and LTP in patient grade A and B groups

We also analyzed grade A and B patients ($n = 232$), because grade C and D patients were considered to be non-curative; it is obvious that patients categorized into these two grades had a worse prognosis relative to patients who had curative therapy and were categorized as being grades A and B.

Table 3 Univariate analyses contributing to OS, RFS, DR, and LTP after initial RFA in patients with sufficient Lipiodol accumulation ($n = 219$)

Variables	n	P value[a]			
		OS	RFS	DR	LTP
Age (>65 years), yes/no	153/66	0.945	0.214	0.331	0.181
Gender (male), yes/no	131/88	0.673	0.829	0.599	0.498
Tumor size (>2 cm), yes/no	96/123	0.687	0.167	0.311	0.024
Tumor location (segment 8), yes/no	90/129	0.352	0.688	0.599	0.531
Cause of liver disease					
Hepatitis B/hepatitis C/non B non C	20/168/31	0.017	0.018	0.020	0.365
AST (>40 IU/L), yes/no	147/72	0.020	0.007	<0.001	0.263
ALT (>40 IU/L), yes/no	106/113	0.469	0.873	0.460	0.679
ALP (>340 IU/L), yes/no	80/139	0.358	0.208	0.097	0.789
GGT (>80 IU/L), yes/no	63/156	0.775	0.018	0.029	0.085
Albumin (>3.5 g/dL), yes/no	133/86	<0.001	0.092	0.045	0.889
Total bilirubin (>1 mg/dL), yes/no	74/145	0.030	0.163	0.106	0.054
Prothrombin time (>70 %), yes/no	181/38	0.001	0.185	0.080	0.134
Platelets (>10 × $10^4/mm^3$), yes/no	121/98	<0.001	0.002	0.001	0.303
AFP (>100 ng/mL), yes/no	41/178	0.287	0.384	0.145	0.478
DCP (>200 mAU/L), yes/no	31/188	0.432	0.162	0.563	0.036
Post-RFA antiviral therapy, yes/no	29/190	0.054	0.867	0.920	0.223
Body mass index (>25 kg/m^2), yes/no	65/154	0.733	0.146	0.155	0.672
Diabetes mellitus, yes/no	73/146	0.567	0.446	0.231	0.346
HCC recurrence within 1 year after RFA, yes/no	48/171	<0.001			
R grade, A/B/C/D	45/100/53/21	0.001	<0.001	<0.001	<0.001

RFA radiofrequency thermal ablation, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transpeptidase, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin, *HCC* hepatocellular carcinoma, *R* radicality, *OS* overall survival, *RFS* recurrence free survival, *DR* distant recurrence, *LTP* local tumor progression

[a] Log-rank test

Significant factors contributing to OS, RFS, DR, and LTP using univariate analysis in grade A and B patients were as follows: serum albumin >3.5 g/dL ($P < 0.001$), platelets >10 × 10^4/mm^3 ($P = 0.024$), HCC recurrence within 1 year after RFA ($P = 0.002$), and R grade ($P = 0.046$) in OS; cause of liver disease ($P = 0.013$), AST >40 IU/L ($P = 0.002$), GGT >80 IU/L ($P = 0.023$), serum albumin >3.5 g/dL ($P = 0.038$), platelets >10 × 10^4/mm^3 ($P = 0.004$), and body mass index >25 kg/m^2 ($P = 0.048$) in RFS, cause of liver disease ($P = 0.007$), AST >40 IU/L ($P = 0.001$), GGT >80 IU/L ($P = 0.021$), serum albumin >3.5 g/dL ($P = 0.032$), platelets >10 × 10^4/mm^3 ($P = 0.003$), and body mass index >25 kg/m^2 ($P = 0.044$) in DR; tumor size >2 cm ($P = 0.049$) and R grade ($P = 0.008$) in LTP (Table 4).

Multivariate analysis of factors contributing to OS, RFS, DR, and LTP in grade A and B patients

Multivariate analysis indicated that significant factors contributing to OS, RFS, DR, and LTP that were also considered significant in univariate analyses in grade A and B patients were as follows: serum albumin >3.5 g/dL ($P < 0.001$) and HCC recurrence within 1 year after RFA ($P = 0.015$) in OS; cause of liver disease (hepatitis B) ($P = 0.020$), platelets >10 × 10^4/mm^3 ($P = 0.049$), and GGT >80 IU/L ($P = 0.045$) in RFS; cause of liver disease (hepatitis B) ($P = 0.013$) and platelets >10 × 10^4/mm^3 ($P = 0.046$) in DR; and R grade ($P = 0.026$) in LTP. The HRs and 95 % CI for these factors are detailed in Supplemental Table 3.

Baseline characteristics between grades A and B

In terms of OS and LTP, there was a significant difference between grades A and B in the current study; however, in terms of RFS and DR, there was no significant difference between grades A and B. Hence, we compared the baseline characteristics in grade A patients with those of grade B patients. Baseline characteristics between the two groups are shown in Supplemental Table 4. In terms of tumor size

Table 4 Univariate analyses contributing to OS, RFS, DR, and LTP after initial RFA in patients with grade A and grade B ($n = 232$)

Variables	n	P value[a]			
		OS	RFS	DR	LTP
Age (>65 years), yes/no	160/72	0.811	0.720	0.662	0.932
Gender (male), yes/no	131/101	0.607	0.776	0.998	0.415
Tumor size (>2 cm), yes/no	76/156	0.388	0.821	0.618	0.049
Tumor vascularity (hyper), yes/no	212/20	0.573	0.863	0.996	0.245
Tumor location (segment 8), yes/no	88/144	0.651	0.796	0.994	0.272
Cause of liver disease					
Hepatitis B/hepatitis C/non B non C	17/185/30	0.110	0.013	0.007	0.517
AST (>40 IU/L), yes/no	159/73	0.053	0.002	0.001	0.442
ALT (>40 IU/L), yes/no	124/108	0.793	0.295	0.177	0.410
ALP (>340 IU/L), yes/no	96/136	0.408	0.129	0.080	0.805
GGT (>80 IU/L), yes/no	66/166	0.349	0.023	0.021	0.471
Albumin (>3.5 g/dL), yes/no	135/97	<0.001	0.038	0.032	0.307
Total bilirubin (>1 mg/dL), yes/no	60/172	0.330	0.928	0.761	0.557
Prothrombin time (>70 %), yes/no	199/33	0.103	0.810	0.651	0.774
Platelets (>10 × 10^4/mm^3), yes/no	123/109	0.024	0.004	0.003	0.795
AFP (>100 ng/mL), yes/no	37/195	0.730	0.511	0.197	0.132
DCP (>200 mAU/L), yes/no	23/209	0.979	0.672	0.378	0.644
Post-RFA antiviral therapy, yes/no	33/199	0.078	0.860	0.752	0.107
Body mass index (>25 kg/m^2), yes/no	69/163	0.667	0.048	0.044	0.645
Diabetes mellitus, yes/no	69/163	0.784	0.785	0.566	0.663
HCC recurrence within 1 year after RFA, yes/no	29/203	0.002			
R grade, A vs. B	70/162	0.046	0.839	0.981	0.008

RFA radiofrequency thermal ablation, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transpeptidase, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin, *HCC* hepatocellular carcinoma, *R* radicality, *OS* overall survival, *RFS* recurrence free survival, *DR* distant recurrence, *LTP* local tumor progression

[a] Log-rank test

($P = 0.003$) and Child–Pugh classification ($P = 0.033$), there were significant differences between the two groups.

Causes of death according to the R grading score

Ninety-eight patients (26.6 %) died during the follow-up period. There were nine deaths (12.9 %) in grade A patients, 40 deaths (24.7 %) in grade B patients, 32 deaths (32.0 %) in grade C patients, and 17 deaths (47.2 %) in grade D patients. The causes of death according to R grading were: HCC recurrence (four patients), liver failure (three patients), and miscellaneous (two patients) in the grade A group; HCC recurrence (12 patients), liver failure (19 patients), and miscellaneous (nine patients) in the grade B group; HCC recurrence (15 patients), liver failure (nine patients), and miscellaneous (eight patients) in the grade C group; and HCC recurrence (10 patients), liver failure (six patients), and miscellaneous (one patient) in the grade D group.

Major adverse events related to the initial RFA according to the R grading

Major adverse events related to the initial RFA treatment according to the R grading system as defined by current guidelines [25] were as follows: intra-abdominal bleeding (one patient) and biloma (one patient) in the grade A cases, intra-abdominal bleeding (one patient) in the grade B cases, pneumothorax (one patient) and liver abscess (one patient) in the grade C cases, and refractory ascites (one patient) in the grade D cases. These serious adverse events improved during the same hospitalization. There was no needle tract seeding or deaths related to complications associated with RFA, and consequently the mortality rate was 0 %.

Discussion

OS, RFS, DR, and LTP rates are the parameters most widely used to evaluate the efficacy of treatments for HCC [26]. A number of studies have reported on the association between the ablative margin after RFA and LTP [1, 14, 15, 23, 27–32]. Investigations related to the factors that could contribute to the long-term survival of HCC patients undergoing percutaneous ablation therapy have also been carried out; however, most of them involved treatment with PEI therapy [22, 33, 34]. To the best of our knowledge there have been few reports regarding the relationship between the ablative margin after RFA and OS, RFS, and DR [20, 21]. Therefore, in the present study, our objective was to examine the relationship between our previously proposed grading system related to the ablative margin after RFA and clinical outcome.

In general, the prognosis of HCC patients treated by RFA is highly dependent on tumor characteristics and liver function [30]. However, in our study, R grading was found to be a significant independent favorable factor associated with OS (grade A), RFS (grade A or B), and DR (grade A or B). Moreover, in patients with sufficient Lipiodol accumulation, in terms of OS, RFS, and DR, almost the same results were obtained in the multivariate analysis, although in grade A and B patients, the difference of R grade did not reach statistical significance in terms of OS in the multivariate analysis. Our results suggested that our proposed R grading system for RFA seemed to be useful for predicting not only LTP, but also clinical outcome.

Tumor size was a significant independent factor linked to OS for all cases, although it was not a significant factor linked to OS in patients with sufficient Lipiodol accumulation in our multivariate analysis. Peng et al. [35] reported that survival of HCC patients treated with RFA was dependent on tumor size. Indeed, in the current study, most of the patients whose tumor size was larger than 3 cm developed LTP after RFA [23], and this might have been related to poor prognosis. In general, complete ablation of HCC tumors larger than 3 cm in size can be achieved using RFA [9, 10]. Therefore, for HCC tumors larger than 3 cm, RFA combined with another therapy such as TACE may be effective in reducing LTP and optimizing clinical outcome [36].

Serum albumin, prothrombin time, and platelet count reflect liver functional reserve. In our multivariate analysis, serum albumin and prothrombin time for all patients, and prothrombin time and platelet count for patients with sufficient Lipiodol accumulation, were demonstrated to be significant independent factors associated with OS. HCC patients with cirrhosis and low levels of serum albumin, prothrombin time, and platelet count can develop protein-energy malnutrition (PEM) with increased catabolism [37]. PEM is associated with a high morbidity and mortality due to an increased risk of life-threatening complications, resulting in poor survival and a reduced quality of life [38]. In these patients with underlying PEM, RFA therapy may further worsen their nutritional condition and even occasionally lead to the development of ascites and jaundice, resulting in an irreversible outcome [39]. Therefore, in these patients, branched chain amino acid treatment to improve their liver function and nutritional condition may be effective [39].

In our study, HCC recurrence within 1 year after RFA was found to be a significant independent factor linked to OS in all cases, in patients with sufficient Lipiodol accumulation and in grade A and B patients. In 76 patients with HCC recurrence within 1 year after RFA, DR from the

ablated area was confirmed in 72 patients (94.7 %). The most frequent event observed during the follow-up period of curatively treated HCC patients was intrahepatic recurrence distant from the ablated area [20, 21, 26, 30]. It is generally believed that recurrences after curative treatment for HCC in the early post-treatment period are not due to incomplete treatment of the primary tumor, but arise from pre-existing microscopic tumor foci that are not detected by imaging modalities; in addition, HCC recurrence can be caused by malignant cells that have been disseminated during the RFA procedure [40]. OS has been demonstrated to be significantly related to early HCC recurrence [26, 40]. Careful monitoring using imaging modalities is required, especially in the first year after initial RFA. In 214 patients who developed HCC recurrence, 149 patients (69.6 %) received RFA therapy for this recurrence in the present study. The fact that RFA can be repeated multiple times makes it particularly valuable for controlling intrahepatic recurrences and is a major advantage of this modality [26].

In the present study using multivariate analysis, cause of liver disease (hepatitis B) was shown to be a significant favorable factor associated with RFS in all cases, in patients with sufficient Lipiodol accumulation and in patients with grade A and B. Of 29 patients with hepatitis B in our study, 23 patients (79.3 %) were treated with nucleotide analogues during the follow-up period and all of their HBV viral loads were less than 3.0 log IU/mL. The other six patients (20.7 %) were not treated with nucleotide analogues owing to the low HBV viral load (<3.0 log IU/mL). Goto et al. [41] reported that serum HBV viral load was associated with the risk of recurrence of HBV-related HCC after RFA, and Chuma et al. [42] reported that the HBV DNA level and antiviral therapy were associated with HCC recurrence. Our findings were similar to those of these two reports; obtaining a sustained low hepatitis B viral load seems to be essential in reducing the incidence of HCC recurrence after RFA. On the other hand, of 289 HCV-related HCC patients in the present study, only 25 (8.7 %) had received interferon (IFN) therapy after initial RFA. In the current study, there were no significant differences between patients who received IFN after initial RFA and those who did not in terms of OS ($P = 0.102$) and RFS ($P = 0.748$). However, several previous studies have reported that IFN therapy after tumor ablation improved prognosis in HCV-related HCC [43–45]. One possible reason for this discrepancy is that the size of our IFN group was very small relative to the non-IFN group. In this regard, a larger prospective study will be needed in the future.

Serum GGT level was a significant factor contributing to RFS in the multivariate analysis in all cases, in patients with sufficient Lipiodol accumulation and in patients with grade A and B. Several studies reported that a high level of GGT was related to a higher incidence of HCC development and recurrence [46, 47]. Ju et al. [48] reported that a high GGT level was associated with tumor characteristics such as tumor size and lower serum albumin level. In HCC patients with a high level of GGT before RFA, careful follow-up examination after RFA is required.

Amano et al. [49] reported that low platelet count was an independent prognostic factor contributing to adverse OS and RFS after the treatment for HCC. In our study, platelet count was shown to be an independent factor for RFS using multivariate analysis in all cases, in patients with sufficient Lipiodol accumulation and in patients with grade A and B. Our results were similar to their report [49]; in HCC patients with low platelet count before RFA, as well as in those with a high GGT level before RFA, we concluded that close observation for HCC recurrence is required.

We also examined the relationship between each R grade in terms of DR. Considered overall there was a significant difference ($P < 0.001$) between the four grades, although no significant differences were observed between grades A and B and between grades C and D, both in all cases and in patients with sufficient Lipiodol accumulation. One possible reason for these results is that our proposed R grading system was closely associated with LTP after RFA; and LTP, meaning a lack of local disease control, can lead to the presence of microscopic vascular invasion or satellite lesions, which is probably an accurate predictor of DR [50, 51]. In patients with incomplete ablation of RFA such as grades C and D, if they are feasible for surgical resection, additional resection may be required to optimize their clinical outcome [52, 53].

In the present study, sufficient Lipiodol accumulation was observed on dynamic CT after RFA in 219 patients (59.5 %), and there were no hypovascular HCCs with sufficient Lipiodol accumulation. Our proposed R grading system does not seem to be useful in hypovascular HCCs. As we previously reported, exactly how we can measure the ablative margin in patients with insufficient Lipiodol accumulation will be a challenge that will be met in a future study [23], although there have been several reports regarding the usefulness of MRI in evaluating the ablative margin after RFA [54, 55].

Despite the fact that no significant difference was observed in terms of RFS between the grade A and B groups, there was a significant difference in terms of OS between these two groups; however, multivariate analysis of patients in the grade A and B groups indicated that R grading did not differ significantly in terms of OS. One possible reason for this was that, as shown in Supplemental Table 4, the Child–Pugh classification was significantly better in the grade A group and tumor size was significantly larger in the grade B group. Shiina et al. [56] reported that the Child–Pugh classification and tumor size were significantly related to survival. In patients with a sufficient

ablative margin, liver function and tumor characteristics may be important for survival prognosis [30, 56].

Between grades A and B, in terms of both RFS and DR, very similar Kaplan–Meier curves were obtained. Nevertheless, there was a significant difference between these two groups in terms of LTP. One possible reason for this was that the number of patients with LTP in the grade A and B groups was considerably smaller than the number of patients with DR in grade A and B groups (one patient with LTP alone, one patient with both LTP and DR, and 31 patients with DR alone, respectively, in the grade A group and two patients with LTP alone, 23 patients with both LTP and DR, and 52 patients with DR alone, respectively, in the grade B group). It is considered that DR rather than LTP significantly affected RFS in patients in the grade A and B groups.

In many institutions in Japan, Lipiodol chemolization with or without embolic agents before RFA had been performed for small HCCs. In the current study, in some cases, Lipiodol chemolization with or without embolic agents was performed before RFA, and in the other cases Lipiodol infusion was performed before RFA; the dose of chemotherapeutic agents such as epirubicin was determined by the attending physicians and the procedures of Lipiodol chemolization with or without embolic agents differed between individual patients (data not shown). In our study, we did not focus on the chemotherapeutic effects, but rather on tumor visibility due to Lipiodol accumulation. Moreover, Shibata et al. [57] reported in their prospective comparative study that combined RFA plus TACE and RFA alone had equivalent effectiveness in the treatment of small HCCs, so combination treatment might not be necessary. In addition, Wang et al. [58] reported in their meta-analysis that TACE plus RFA did not result in a survival benefit over RFA alone for small HCCs. Hence, we did not include Lipiodol chemolization with or without embolization as one of the variables in our analysis.

The present study had several limitations. First, it was a retrospective study. Second, patients who were lost to follow-up were excluded from the study, leading to bias. Third, the mean observation period was not long enough for analysis of survival. Fourth, in patients with insufficient Lipiodol accumulation, accurate assessment of R grading could not be made. However, using our proposed grading system we demonstrated that initial treatment response to RFA reflected clinical outcome.

In conclusion, our proposed R grading system was demonstrated to be useful for predicting not only LTP, but also clinical outcome.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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