was scheduled every 3–4 months, and chest CT or bone scintigraphy was performed if extrahepatic recurrence was suspected. HCC recurrence was defined as the detection of an early enhanced lesion by dynamic CT scan concomitantly with late washout. Local tumor progression was defined as the appearance of viable cancer tissue touching the initially treated tumor and distant recurrence separated from the primary site. When intrahepatic HCC recurrence was detected, RFA was performed if the recurrence met the initial inclusion criteria. If there was no indication for RFA, we chose TACE, percutaneous ethanol injection therapy, surgical resection, systemic chemotherapy, or symptomatic therapy according to the guidelines established by the Liver Cancer Study Group of Japan (11) and AASLD (3). The end of follow-up was tumor progression beyond the Milan criteria, death, or latest medical attendance until March 31, 2012.

Statistical analysis

The primary endpoint of the present analysis was tumor progression beyond the Milan criteria, and the secondary endpoint was death. The cumulative incidences of recurrence exceeding the Milan criteria and survival after initial successful RFA were determined by the Kaplan–Meier method, and the risk factors associated with recurrence exceeding the Milan criteria and death were identified using the Cox

proportional hazards regression model independently for tumor progression and death. Survival analysis was performed on a per patient basis. The starting date of follow-up was defined as the completion date of the initial RFA session. Multivariate analysis was performed using the Cox proportional hazards model, including variables with a marginal p value of <0.05 by univariate analysis. All statistical analyses were performed using StatView 5.0 (SAS Inc., Cary, NC, USA).

RESULTS

The patient characteristics are shown in Table 1. The minimum follow-up period was 7 months, and the median follow-up period was 47.4 months (range, 7–147 months). During follow-up, HCC recurred in 270 of 323 patients (83.6%), and local tumor progression was observed in 47 patients (14.6%). Tumor progression beyond the Milan criteria was observed in 193 patients, of which 174 (90.1%) died because of tumor progression and 19 (9.8%) died without tumor progression. The cumulative survival rates at 1, 3, 5, 7, and 10 years were 96.2%, 84.4%, 69.9%, 52.7%, and 40.6%, respectively. The cumulative recurrence rate exceeding the Milan criteria at 1, 3, and 5 years was 15.1%, 46.0%, and 61.1%, respectively. Major complications were observed in

only 2 cases (0.6%): one was gastric penetration after ablation of segment 2 and the other was hemothorax after ablation of segment 7. Both cases recovered without surgery.

Risk factors for exceeding the Milan criteria and overall survival

The univariate analysis results showed that the higher AFP level (> 100 ng/mL), higher PIVKA-II level (>100 mAU/mL), larger tumor size (diameter > 20mm), and earlier recurrence of intrahepatic lesion (within 1 year after initial RFA) were significantly associated with the risk for recurrence exceeding the Milan criteria (Table 2). Multivariate analysis with the Cox proportional hazards model indicated that the higher AFP level (hazard ratio 1.59, $p = 0.005$), larger tumor size (hazard ratio 1.54, $p = 0.012$), and early recurrence within 1 year after initial RFA (hazard ratio 2.76, $p < 0.001$) were independent risk factors associated with recurrence exceeding the Milan criteria (Table 2). No association was observed between recurrence exceeding the Milan criteria and Child–Pugh score. Risk factors associated with overall survival are shown in Table 3. Multivariate analysis with the Cox proportional hazards model indicated that the initial higher AFP level (hazard ratio 2.03, $p = 0.0003$), Child–Pugh B (hazard ratio 2.42, $p < 0.0001$), and early recurrence within 1 year after initial RFA (hazard ratio

2.09, p = 0.0001) were independent risk factors associated with overall survival. There was no significant difference in overall survival and recurrence exceeding the Milan criteria between the patients (n = 11) whose imaging findings by mRECIST criteria at 3 months after RFA were non-complete response (non-CR) and the patients with complete response (CR) (n = 312).

Predictability of the long-term survival rate and recurrence exceeding the Milan criteria by risk group

To predict long-term survival and recurrence exceeding the Milan criteria, we formed risk groups on the basis of two relevant clinical predictors: the initial tumor marker (AFP, >100 ng/mL) and the presence of earlier recurrence. The cumulative incidence of recurrence exceeding the Milan criteria according to these predictors is shown in Figure 1, and the cumulative survival rate is shown in Figure 2. The 3- and 5-year survival rates of patients with both risk factors were 33.5% and 22.6%, respectively, although the patients were initially treated with RFA for early stage HCC. The cumulative recurrence rate for the low risk group (n = 203), who had no risk factor (initial AFP, early recurrence, tumor size), at 1, 3, 5 years were 3.7%. 66.5%, 74.7% and the cumulative survival rate for the low risk group at 1, 3, 5 , 7, 10 years were 98.5%, 93.1%,

78.0%, 56.5% and 46.6%.

DISCUSSION

In the present study, long-term survival after RFA was similar to that of patients receiving hepatic resection (13-17), especially in those with early stage HCC. Moreover, major complications were observed only in 0.6% of patients, indicating that RFA has considerable merit regarding both effectiveness and safety. The overall outcomes were similar to those in a report by Tateishi et al. (8) in which the 5-year survival rate was 54.3% and the rate of major complications was 1.9%/session. Ogihara et al. (17) reported that RFA was less invasive and associated with a lower complication rate and lower cost compared with resection. Their data also indicated that RFA was effective in ensuring local control of stage T1 HCC and was associated with survival rates similar to those obtained by surgical resection. Cucchetti et al (18) verified RFA was more cost-effective than resection for patients with very early HCC and in the presence of two or three nodules ≤30 mm and for patients with single larger early stage HCCs, surgical resection remained the best strategy to adopt as a result of better survival rates at an acceptable increase in cost.

Llovet et al. (19) reported that RFA was a useful bridging therapy for liver transplantation because a higher dropout rate (38%/year) was reported in patients without adequate adjuvant therapy for HCC. In a recent study of recurrent HCC within the Milan criteria (10), the 1-, 3-, and 5-year tumor-free survival rates for salvage liver transplantation were all 60% and the excellent 10-year survival would be expected for these patients. Therefore, it is very important to clarify the risk factors associated with exceeding the Milan criteria after locally curative RFA. We determined the probability and risk factors for tumor progression beyond the Milan criteria after successful locally curative RFA for primary HCC. Our results showed a recurrence rate exceeding the Milan criteria of 15.1% at 1 year to 46.0% at 3 years and patients who had a larger tumor size (diameter, > 20mm) and/or a higher AFP level (>100 ng/mL) at initial presentation and early recurrence after initial RFA were at a high risk for recurrence exceeding the Milan criteria. Therefore, in such high-risk patients, RFA should be carefully considered as a bridging therapy for liver transplantation and the physician should follow these patients carefully for tumor progression even after successful initial RFA.

We have reported that K19 expression was related to a high recurrence of HCC after RFA in 249 patients (20), and Ziol M, et al. (21) have reported that Endothelial

cell-specific molecule-1 (ESM-1) in stromal cells was predictive of recurrence after RFA in early HCC in 150 patients. However, there is no HCC-specific biomarker that can be measured to link post-RFA biology to recurrence and outcome and that is better than serum AFP. Tateishi, et al. (22) have reported on the prediction of the recurrence of HCC after RFA in 416 patients. Tumor marker levels were determined immediately before and 2 months after the treatment. The timing and frequency of measuring AFP would be 2 months after the RFA and then every 2–3 months.

There was no significant difference in the overall survival and recurrence exceeding the Milan criteria among patients with HCV, HBV, and NBNC. In patients with HCV (n = 248), larger tumor size (diameter, >20mm), AFP >100 ng/mL, and recurrence within 1 year after the initial ablation were independently associated with earlier recurrence exceeding the Milan criteria. AFP >100 ng/mL, and recurrence within 1 year after the initial ablation were independently associated with overall survival. In patients with HBV (n = 31), AFP >100 ng/mL was the only independent factor that was associated with overall survival. In patients with NBNC (n = 41), recurrence within 1 year after the initial ablation was the only independent factor that was associated with earlier recurrence exceeding the Milan criteria. The patients with both positive HBs antigen and HCV antibody (n = 3) were excluded from this analysis. However, the

number of patients with positive HBs antigen or with negativity of both HBs antigen and HCV antibody were too small to clarify the differences based on the underlying cause of liver disease.

In the initial study population of 554 primary HCCs, The 35 patients who received surgical resection were Child-Pugh A or non-cirrhotic patients, so they could not submit liver transplantation. The 158 patients who received TACE, 10 patients who received systemic cytotoxic chemotherapy, 20 patients who received best supportive care and 2 patients who received radiation therapy were exceeding for the Milan criteria. The remaining 6 patients were over 65 years old and could not submit liver transplantation. We did not include the patients who received TACE as an initial therapy in this study, because they already exceeded the Milan criteria. The number of the patients who received other therapies (Resection, MCT, PEI) was too small to analyze the recurrence and prognosis.

In our study, the incidence rate of exceeding the Milan criteria was similar to the data reported by Yamashiki et al. (23) in which the overall recurrence rate exceeding the Milan criteria was 9.0% and 32.8% at 1 and 3 years, respectively. Similar to us, they found that a high serum level of AFP or PIVKA-II and a tumor diameter of > 30mm affected the recurrence exceeding the Milan criteria as a result of tumor progression. An

elevated AFP level may be related to the histological grading. Parfitt et al. (24) reported that the histological grade of tumor differentiation and macroscopic vascular invasion were independent predictors of long-term survival after liver transplantation. However, the most significant risk factor in our cohort was early recurrence after initial RFA, suggesting that careful surveillance for recurrence is necessary even after complete local ablation, and if early recurrence occurs within 1 year, liver transplantation should be considered as soon as possible to avoid loss of the indication, even in patients in whom initial tumor size and number are small. Importantly, liver function tests, such as albumin level and prothrombin activity, were not identified as risk factors for recurrence exceeding the Milan criteria in our cohort, suggesting that preserved liver function itself does not necessarily indicate that there has been an adequate waiting time.

We here calculated the risk score from two simple factors: the initial tumor marker and early recurrence after initial complete RFA. The 3- and 5-year survival rates of patients with both risk factors were 33.5% and 22.6%, respectively, in spite of early stage at initial ablation. Conversely, the 3-and 5-year survival rates of patients with neither risk factor were 93.1% and 78.0%, respectively. The number of patients with both risk factors was small (12.1%); however, new therapeutic strategies (early transplantation or

repeated adjuvant therapy) were necessary to achieve long-term survival.

Takada et al. (25) reported that repeated nontransplant treatment for recurrent HCC, such as RFA and transluminal arterial embolization, prior to living donor liver transplantation (LDLT) might increase the risk of recurrence and impair the survival advantage conferred by LDLT. As our study focused mainly on recurrence exceeding the Milan criteria, we did not assess whether RFA performed prior to liver transplantation affected the final outcome of patients who actually received liver transplantation. Therefore, further controlled studies are warranted to confirm whether bridging therapy with RFA actually leads to better survival after transplantation. Nevertheless, liver transplantation should be considered before the patient exceeds the Milan criteria in order to achieve excellent survival after liver transplantation.

In conclusion, RFA presents a promising bridging therapy for liver transplantation in patients who are at low risk of tumor progression. However, patients with a higher AFP level at initial RFA and earlier recurrence even after successful RFA should be considered for timely liver transplantation or new adjuvant therapy. In these patients, the 3- and 5-year survival rates were below 50% although they were classified as early stage at initial therapy.

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Figure Legends

Figure 1. The cumulative recurrence rate exceeding the Milan criteria stratified by the number of risk factors. The cumulative recurrence rate exceeding the Milan criteria in patients with more risk factors was significantly higher than that in patients with fewer risk factors ($p < 0.0001$)

Figure 2. The cumulative survival rate stratified by the number of risk factors. The cumulative survival rate in patients with more risk factors was significantly lower than that in patients with fewer risk factors ($p < 0.0001$)

Table 1. Patient characteristics

Characteristics	Value
Patients, n	323
Age, years	66 ± 9
Duration of follow-up, years	4.0 (0.6-12.2)
Gender, male/female (%)	191 (59)/132 (41)
Clinical and laboratory data	
AFP, median (range), ng/mL	25.6 (1.2–76600)
PIVKA-II, median (range), mAU/mL	25 (7–10600)
Child–Pugh score, A/B (%)	256 (79)/67 (21)
Pathology	
Maximum diameter of HCC, mm	
≤20/21–30/31–50 (%)	117 (36)/158 (49)/48 (15)
Number of HCC nodules, n	
Single/multiple (%)	226 (70)/97 (30)
CLIP score, 0/1/2/3 (%)	173 (52)/114 (37)/32 (10)/3 (1)
Lymph node involvement	0
Metastasis	0

Major associated liver diseases	
HCV/HBV/HCV+HBV/others (%)	248 (76.8)/31 (9.6)/3 (0.9)/41 (12.7)