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Title

Risk factors for exceeding the Milan criteria after successful radiofrequency ablation in patients with early stage hepatocellular carcinoma

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Abbreviations

AASLD, American Association for the Study of Liver Diseases; AFP, α-fetoprotein; CT, computed tomography; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation; MRI, magnetic resonance imaging; PIVKA-II, protein induced by vitamin K absence or antagonist II; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization

ABSTRACT

Background

Radiofrequency ablation (RFA) is an effective and safe noninvasive treatment for hepatocellular carcinoma (HCC) and may be useful as a bridging therapy in liver transplantation. Prognosis after liver transplantation in patients within the Milan criteria is excellent. The study aimed to identify risk factors associated with exceeding the Milan criteria after initial locally curative RFA therapy.

Methods

Among 554 primary HCC patients, 323 with early stage HCC following RFA were analyzed (mean age, 66 years; HCV/HBV/others, 249/33/41; Child–Pugh A/B/C, 256/67/0). The cumulative overall survival and recurrence rate exceeding the Milan criteria were analyzed by Kaplan–Meier analysis, and factors associated with overall survival were determined by Cox proportional hazards analysis.

Results

The overall cumulative survival rates at 1, 3, 5, and 10 years were 96%, 84%, 70%, and 41%, respectively, without liver transplantation. The cumulative recurrence rate exceeding the Milan criteria at 1, 3, and 5 years were 15%, 46%, and 61%, respectively. α-Fetoprotein (AFP) >100 ng/mL and recurrence within 1 year after initial ablation

were independently associated with earlier recurrence exceeding the Milan criteria and overall survival. 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.

Conclusions

Higher AFP and HCC recurrence within 1 year after RFA are risk factors for exceeding the Milan criteria and overall survival. Early liver transplantation or adjuvant therapy should be considered for patients with both risk factors.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, accounting for 70%–85% of all cases, and a major cause of mortality; it is the fifth most frequently diagnosed cancer and the second most frequent cause of cancer death in men. In women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death (1, 2). At present, the major curative treatments for HCC consist of hepatic resection, ablation therapy, and liver transplantation (3). Although hepatic resection and ablation therapy often show excellent effects in HCC, they cannot prevent recurrence in the remnant liver or eliminate other complications caused by concurrent liver cirrhosis. On the other hand, liver transplantation has become a favored option for HCC treatment because it provides not only local cure but also decreases the risks for recurrence and progressive liver disease. Liver transplantation for cirrhotic HCC patients who meet the Milan criteria (4) [solitary tumor ≤ 50mm or three or fewer lesions (none > 30mm)] offers long-term survival similar to that observed in patients transplanted for nonmalignant liver disease (5, 6). Some recent studies (7-9) reported that radiofrequency ablation (RFA) is an effective and safe noninvasive treatment for HCC, enabling complete ablation of an area up to 3 cm in diameter and is superior to microwave coagulation and percutaneous ethanol injection therapy. In a recent study

(10), for recurrent HCC within the Milan criteria, the 1-, 3-, and 5-year tumor-free survival rates for salvage liver transplantation were all 60%; the corresponding rates were 70.2%, 48.0%, and 48.0% for hepatic resection and 41.0%, 20.3%, and 10.9% for RFA (P=0.004). The patients in this study underwent either hepatic resection or RFA as an initial treatment for HCC within the Milan criteria. Therefore, it is very important to know when patients exceed the Milan criteria after initial RFA as a locally curative therapy for HCC. Hence, the aims of the present study were to identify the risk factors associated with recurrence exceeding the Milan criteria and clarify prognostic factors for overall survival in early stage HCC patients who received RFA as an initial therapy.

MATERIALS and METHODS

Patients

Between July 1999 and July 2005, 554 primary HCC patients were admitted to the Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital (Tokyo, Japan). The patients received the following appropriate therapies according to the appropriate guidelines released during study period by the Liver Cancer Study Group of Japan and BCLC staging system (11): 323 were treated by RFA, 35 by surgical resection,

158 by transcatheter arterial chemoembolization (TACE), 10 by systemic cytotoxic chemotherapy, 2 by percutaneous microwave coagulation, 4 by percutaneous ethanol injection therapy, 2 by radiation therapy, and 20 by best supportive care. There were no patients who underwent liver transplantation. Of these 554 patients, 323 were treated by RFA as an initial curative therapy for primary HCC and included in the following analyses. Inclusion criteria for RFA were as follows: HCC with solitary tumor ≤ 50mm or three or fewer lesions (none > 30mm), three or fewer lesions without major vascular or biliary invasion, total bilirubin concentration <2.5 mg/dL, platelet count >3 × $10^4/mm^3$, and prothrombin activity >50%. Some patients refused hepatic resection and chose RFA voluntarily on the basis of concerns about complications or physician recommendations, which took into account impairment of liver function, HCC location, and cardiopulmonary dysfunction. Patients with ascites uncontrolled by diuretics and/or extrahepatic metastasis were excluded. The reasons why the patients were selected for RFA instead of being offered liver transplantation were a Child-Pugh A classification (n = 256, 79.2%), age >65 years (n = 198, 61.3%), or heart or lung disease complications (n = 6, 1.9%). The number of patients who were classified as Child-Pugh B and who were younger than 66 years of age were 28 (8.7%). In these patients, there was 1 patient who had severe heart disease, and the remaining 27 patients did not have any

living donors. Written informed consent was obtained from all patients, and this study was approved by the ethics committee of Musashino Red Cross Hospital and conducted in accordance with the Declaration of Helsinki.

HCC diagnosis

HCC diagnosis was confirmed by typical radiographic findings on dynamic computed tomography (CT) with or without hepatic arterial and portal angiography and magnetic resonance imaging (MRI) or by needle biopsy. For triple-phase dynamic CT scans, arterial, portal, and equivalent phases were set at 35, 70, and 150 s, respectively, after injection of contrast agent. Spiral CT scans were obtained from 5-mm-thick sections. Board-certified radiologists diagnosed HCC on the basis of typical patterns, such as an early-phase hyperattenuation area or late-phase hypoattenuation on dynamic CT or MRI. Liver biopsy was performed when a definite diagnosis was not proved by imaging techniques, and the final diagnosis was confirmed by certified pathologists who were unaware of the patient's clinical data.

RFA procedure

RFA was performed under local anesthesia using the percutaneous approach (n = 279)

or general anesthesia using the laparoscopic approach (n = 44), both under real-time ultrasound guidance. The laparoscopic approach was selected for patients with HCC located on or near the liver surface (12). We used an internally water-cooled 17-gauge cooled-tip electrode with an impedance-controlled generator (Cosman generator, Cool-tip System; Radionics, Burlington, MA, USA). Ultrasonography was performed with a 3.0–6.0 MHz convex probe using Aloka SSD-5500 (Aloka, Tokyo, Japan), Sonoline Elegra (Siemens, Erlangen, Germany), and Aplio XV (Toshiba Medical Systems, Tokyo, Japan) systems. When the target nodule was > 20mm in diameter, we performed multiple needle insertions and multiple ablations of one nodule.

Assessment of treatment efficacy and follow-up

A dynamic CT scan with a section thickness of 5 mm was performed to evaluate the efficacy of ablation 1–3 days after RFA. Complete HCC ablation was defined as hypoattenuation of the entire tumor. Patients who were judged as incomplete ablated received additional therapy 1 week after the first ablation, which was continued until the treatment was judged completely effective. Blood was sampled every 2–3 months and tested for indicators of liver function and the markers α-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II). A dynamic CT scan

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