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Microvascular Invasion in Small-sized Hepatocellular Carcinoma: Significance for Outcomes Following Hepatectomy and Radiofrequency Ablation

KATSUNORI IMAI, YO-ICHI YAMASHITA, TOSHIHIKO YUSA, YOSUKE NAKAO, RUMI ITOYAMA, SHIGEKI NAKAGAWA, HIROHISA OKABE, AKIRA CHIKAMOTO, TAKATOSHI ISHIKO and HIDEO BABA

Department of Gastroenterological Surgery, Graduate School of Life Sciences, Kumamoto University, Kumamoto, Japan

Abstract. *Background/Aim: The significance of microvascular invasion (MVI) of hepatocellular carcinoma (HCC) after radiofrequency ablation (RFA) is unknown. Patients and Methods: We studied 149 patients with solitary small-sized HCC (≤3 cm) who underwent hepatectomy, and developed a predictive model of MVI using independent factors related to the presence of MVI. The predictive model was applied to 159 patients who underwent RFA, and their outcomes were examined. Results: A multivariate analysis revealed that α-fetoprotein ≥15 ng/ml (relative risk (RR) 3.05, p=0.02), des-γ-carboxy prothrombin ≥100 mAU/ml (RR 4.19, p=0.003), and tumor size ≥2 cm (RR 3.37, p=0.03) were independent risk factors of MVI. Among the patients who underwent RFA, the survival in patients with risk factors 2-3 was significantly worse, and local recurrence was more frequently obserbed than those with 0-1. Conclusion: When an HCC tumor is expected to display MVI, RFA may not be suitable in terms of poorer survival and local disease-control rates.*

Hepatocellular carcinoma (HCC) is the sixth most common malignancy worldwide (1, 2). Although advances in imaging modalities, surgical techniques, and surveillance programs have improved the outcomes of patients with HCC, the long-term outcomes of HCC patients remain unsatisfactory because of the high frequency of recurrence (3).

Microvascular invasion (MVI) is a histological feature that indicates aggressive behavior of HCC. The presence of MVI has been reported to be a poor-prognosis factor of recurrence and long-term survival after liver resection or transplantation (4-14). Macrovascular invasion can often be detected before surgery by imaging modalities including ultrasonography (US), enhanced computed tomography (CT), and magnetic resonance (MR) imaging. However, the detection of MVI by preoperative imaging modalities is difficult, and preoperative prediction of MVI is thus an issue of great importance for planning the treatment of HCC. As a consequence, considerable efforts have been made to predict MVI before surgery (14-24).

Radiofrequency ablation (RFA) is a well-established local treatment designed to produce localized tumor destruction by heating the tumor tissue and the surrounding liver tissue. Because of its excellent efficacy, repeatability, safety, and low invasiveness, RFA is gradually becoming an alternative procedure for small-sized HCC. However, the significance of MVI in patients who underwent RFA is unclear, because the diagnosis of MVI is based on histological examination of surgical specimens.

The current study aimed to develop a simple predictive model of MVI in resected small-sized HCC, and to investigate the prognostic significance of MVI expectation in patients who underwent RFA based on the proposed predictive model.

Patients and Methods

Patients who underwent surgical treatment with curative intent for HCC between 2000 and 2015 at the Kumamoto University Hospital, Kumamoto, Japan were identified retrospectively from a prospectively maintained database. Among them, patients with solitary small-sized HCC (≤3 cm) who underwent either hepatectomy or RFA were identified and enrolled in this study. Patients who presented with HCC tumors with MVI on the preoperative imaging modalities were excluded from this study. Based on an analysis of clinicopathological variables in patients who underwent hepatectomy, a predictive model of MVI was

Correspondence to: Katsunori Imai, Department of Gastroenterological Surgery, Graduate School of Life Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan. Tel: +81 963735212, Fax: +81 963714378, e-mail: katsuimai@hotmail.com

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developed. Subsequently, this predictive model was applied to the cohort of patients who underwent RFA and the significance of MVI expectation for long-term outcome after RFA was examined.

Preoperative workups. The diagnosis of HCC was based on routine imaging modalities including US, dynamic CT, MR imaging, and CT angiography. Because of the specific complication of cancer cell seeding, liver biopsy prior to RFA was not suggested in our department, as described previously (25). Therefore, making a diagnosis of HCC without pathological evidence mainly depended on typical findings, *i.e.*, early-phase enhancement and late-phase contrast washout in at least two imaging techniques. Elevation of tumor markers including α-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), and des-γ-carboxy prothrombin (DCP), as well as the background of hepatitis virus infection, were also considered supplemental, as described previously (25, 26).

Before treatment, all patients underwent routine laboratory tests, including measurement of tumor markers such as AFP, AFP-L3, and DCP, liver function tests including indocyanine retention rate at 15 min (ICG-R15), and ^{99m}Tc-galactosyl human serum albumin (GSA) scintigraphy. The surgical procedure was selected based on the tumor location, extent of the tumor, liver functional reserve, and the patient's general condition, as described previously (25, 26). Briefly, hepatectomy was considered as the treatment of first choice for patients with good liver functional reserve, and anatomical resection was employed if the liver function allowed. RFA was selected for patients with a deeply located tumor requiring major hepatectomy leading to insufficient remnant liver volume, insufficient liver functional reserve, or high operative risk associated with their general condition (25, 26). This study was approved by the Institutional Ethics Committee of Kumamoto University Hospital and was performed in accordance with the 1975 Declaration of Helsinki. Written informed consent was obtained from all patients before treatment.

Surgical strategy. The type of hepatectomy was selected based on tumor location, extent of tumor invasion, parenchymal liver function, and the patient's general condition, as described previously (25-27). If the liver function allowed, anatomical resection was employed. In patients with insufficient liver functional reserve, limited resection was performed.

RFA was performed using a cooled-tip electrode with a length of 2-3 cm (Radionics, Burlington, MA, USA) and connected to a 500-kHz RF generator (Radionics, Burlington, MA, USA) under the programmed cyclic impedance control condition as described previously (25, 26). A 17-G electrode was inserted into the lesion under either US guidance or direct visual guidance, ablation was initiated, and the power was increased to 60 W in a 2-cm length needle and 80 W in a 3-cm length needle. The duration of maximum ablation was 8-10 min and the impedance was closely monitored. All ablations aimed to achieve at least a 0.5-cm margin of nontumorous liver parenchyma, if possible, in a single session.

Postoperative workups. All postoperative complications were graded according to the Dindo–Clavien classification (28). After treatment, all patients underwent regular follow-up to monitor serum AFP, AFP-L3 and DCP levels, and imaging studies, including US and dynamic CT every 2-4 months to detect any intrahepatic or distant recurrence. Recurrence was defined as the appearance of a lesion with radiological features typical of HCC, as confirmed by US, CT, or MR imaging. Local recurrence was defined as the reappearance of tumor progression either within the ablation site or in contact with the ablation site on contrast-enhanced CT or MR imaging (26, 29). When tumor recurrence was confined to the remnant liver, various treatment modalities were selected, including repeat hepatectomy, RFA, transcatheter arterial chemoembolization, chemotherapy with sorafenib, or a combination of these methods.

Histological study. All resected specimens were fixed in 10% formaldehyde solution and cut into 0.5-1.0-cm slices. After macroscopic examination, the slices were embedded in paraffin, and 5-mm sections were stained with hematoxylin–eosin. A histological examination of the resected specimens was performed by pathologists who did not know the outcome of the patients. Histological grading of tumor differentiation was made on the highest-grade areas of each patient. MVI was defined as a tumor cell within a vascular space lined by endothelium that was visible only on microscopy.

Statistical analysis. Continuous variables are expressed as median (range). Continuous and categorical variables were compared using the Mann–Whitney *U*-test and the χ^2 test, respectively. Survival analyses were performed using the Kaplan-Meier method, and the results were compared using the log-rank test. Overall survival (OS) was calculated from the date of treatment until death or the last follow-up examination. Disease-free survival (DFS) was defined as the period between treatment and the first postoperative recurrence or death. For the univariate analysis of the factors that predicted MVI, the optimal cut-off values of continuous variables for differentiation between the groups were determined based on receiver operating characteristics (ROC) analysis. Variables with a *p*-value of ≤0.10 in the univariate analysis were subjected to a multivariate logistic regression analysis using a stepwise backward elimination procedure. A predictive model was then developed based on the results of the multivariate logistic analysis, as described previously (30, 31). All statistical analyses were performed using the JMP (SAS Institute, Cary, North Carolina, USA) and R version 3.1.1 (http://www.r-project.org) software programs. *p*-Values of <0.05 were considered to indicate statistical significance.

Results

Between 2000 and 2015, a total of 308 patients underwent either hepatectomy (n=149) or RFA (n=159) for solitary small-sized HCC (≤3 cm) as an initial treatment at our institution. The background characteristics of these two cohorts are summarized in Table I. Compared to the hepatectomy group, the patients in the RFA group were characterized primarily by increased serum concentration of total bilirubin; decreased serum concentration of albumin, platelet count, and prothrombin activity; and impaired ICG-R15 and uptake ratio of the liver to the liver plus heart at 15 min (LHL15) as determined by ^{99m}Tc-GSA scintigraphy. These findings suggested that liver function was impaired in the RFA group. On the contrary, the patients in the hepatectomy group had larger tumor size and higher levels of tumor markers, suggesting that their tumors were more advanced.

In the hepatectomy group, anatomical resection was performed in 64 patients (43%). In the RFA group, approaches for ablation were as follows: percutaneous, 88 patients; laparoscopy, 45; thoracoscopy, 21; and laparotomy, five. The operating time was significantly shorter, and the amount of blood loss was smaller in the RFA group than in the hepatectomy group. Complication (Clavien-Dindo ≥II) was observed more frequently in the hepatectomy group than in the RFA group.

Median follow-up was 55.6 months after hepatectomy and 45.1 months after RFA. The OS rates at 1, 3, and 5 years were 96.5, 91.0, and 86.7%, and the DFS rates at these times were 86.7, 59.0, and 48.4% after hepatectomy, respectively (Figure 1a). After RFA, in contrast, the OS rates at 1, 3, and 5 years were 99.3, 90.7, and 73.3%, and the DFS rates at these times were 61.0, 25.8, and 11.3%, respectively (Figure 1b).

Predictive factors and predictive model of MVI in resected cases. In the hepatectomy cohort, MVI was observed in 27 of 149 patients (18.1%, Table I). The OS and DFS in patients who underwent hepatectomy were comparable between patients with and without MVI (p=0.21 and p=0.09, respectively, Figure 2), suggesting that MVI did not affect the long-term outcome in patients with solitary small-sized HCC if hepatectomy was performed.

Univariate and multivariate analyses of factors related to MVI are shown in Table II. A univariate analysis revealed that age <70 (p=0.042), AFP ≥15 ng/ml (p=0.007), AFP-L3 ≥10% (p=0.034), DCP ≥100 mAU/ml (p<0.0001), and tumor size ≥2 cm (p=0.0054) were significantly associated with MVI. A multivariate analysis revealed that AFP ≥15 ng/ml (relative risk (RR) 3.05, p=0.02), DCP ≥100 mAU/ml (RR 4.19, p=0.003), and tumor size ≥2 cm (RR 3.37, p=0.03) were independent predictive factors of MVI.

Subsequently, a predictive model for estimating the probability of MVI was developed using the three independent predictive factors shown in Table III. For patients without any factors, the probability of MVI was 3.3%. The addition of subsequent factors increased the probability of MVI to 12.5% for 1 factor, 32.6% for 2 factors, and 59.6% for 3 factors. The c-index, a measure of model discrimination represented by the area under the ROC curve, was 0.782.

Significance of risk3 factors for MVI on outcomes after hepatectomy and RFA. The OS according to the number of risk factors for MVI (0-1 *vs*. 2-3) was similar after hepatectomy (5-year OS; 0-1: 89.3%, 2-3: 81.3%, p=0.50, Figure 3a). Likewise, the DFS was also comparable between the groups with the number of risk factors 0-1 and 2-3 (5-year DFS; 0-1: 47.1%, 2-3: 51.5%, p=0.89, Figure 3b). In contrast, among the patients who underwent RFA, the OS in patients with risk factors 2-3 was significantly worse than in those with 0-1 (5-year OS; 0-1: 80.0%, 2-3: 55.8%, p=0.0037, Figure 3c), although the DFS did not show a significant difference (5-year DFS; 0-1: 11.6%, 2-3: 6.8%, p=0.20, Figure 3d). Local recurrence after RFA was more frequent in patients with risk factors 2-3 than in those with 0-1 (3-year local recurrence rate; 0-1: 8.4%, 2-3: 30.7%, p=0.012, Figure 4).

Table I. *Background characteristics of the two cohorts*.

	Hepatectomy (n=149)	RFA (n=159)	p-Value
Age	68 (34-88)	68 (43-88)	0.64
Gender (Male/Female)	107/42	96/63	0.039
HBs-Ag-positive	41 (27.5%)	16 (10.1%)	<0.0001
HCV-Ab-positive	79 (53.0%)	120 (75.5%)	<0.0001
Total bilirubin (mg/dl)†	0.8 (0.2-2.1)	0.9 (0.1-2.5)	0.0004
Albumin (g/dl)†	4.1 (3.0-5.1)	3.6 (2.2-4.8)	<0.0001
Prothrombin activity (%)†	96 (43-140)	83 (14-125)	<0.0001
Platelet count (×104)†	13.7 (2.9-46.8)	9.2 (2.9-3.09)	<0.0001
ICG-R15 (%)†	12.7 (1.1-65.4)	27.7 (6.6-70.9)	<0.0001
^{99m}Tc-GSA LHL15†	0.89 (0.72-0.99)	0.85 (0.63-0.95)	<0.0001
Child-Pugh classification			<0.0001
5	118	65	
6	26	38	
7	4	44	
8	1	11	
9	0	0	
10	0	1	
Tumor size (cm)†	2.2 (0.8-3.0)	1.9 (0.5-3.0)	0.0051
AFP (ng/ml)†	8.2 (1.0-4588)	16.5 (1.5-864)	0.0058
AFP-L3 >10%	26 (18.3%)	13 (8.3%)	0.0097
DCP (mAU/ml)†	30 (3.6-17505)	25 (3.0-1142)	0.025
Anatomical resection	64 (43.0%)		
Approach for RFA ablation			
Percutaneous		88	
Laparoscopy		45	
Thoracoscopy		21	
Laparotomy		5	
Operating time (min)†	335 (144-745)	120 (10-375)	<0.0001
Blood loss (g)†	300 (0-3200)	5 (0-609)	<0.0001
Complication (Clavien-Dindo ≥II)	27 (18.1%)	5 (3.1%)	<0.0001
Red blood cell transfusion	4 (2.3%)	0 (0%)	0.015
Microvascular invasion	27 (18.1%)		

†Median. RFA: Radiofrequency ablation; HBs-Ag: hepatitis B surface antigen; HCV-Ab: anti-hepatitis C antibody; ICG-R15: indocyanine green retention rate at 15 min; ^{99m}Tc-GSA: ^{99m}Tc-galactosyl human serum albumin; AFP: α-fetoprotein; AFP-L3: *Lens culinaris* agglutinin-reactive fraction of AFP; DCP: des-γ-carboxy prothrombin.

Discussion

In the present study, we proposed a predictive model of MVI based on three independent predictive factors identified from a multivariate analysis in patients with solitary small-sized (≤3 cm) HCC after hepatectomy. The presence of MVI and

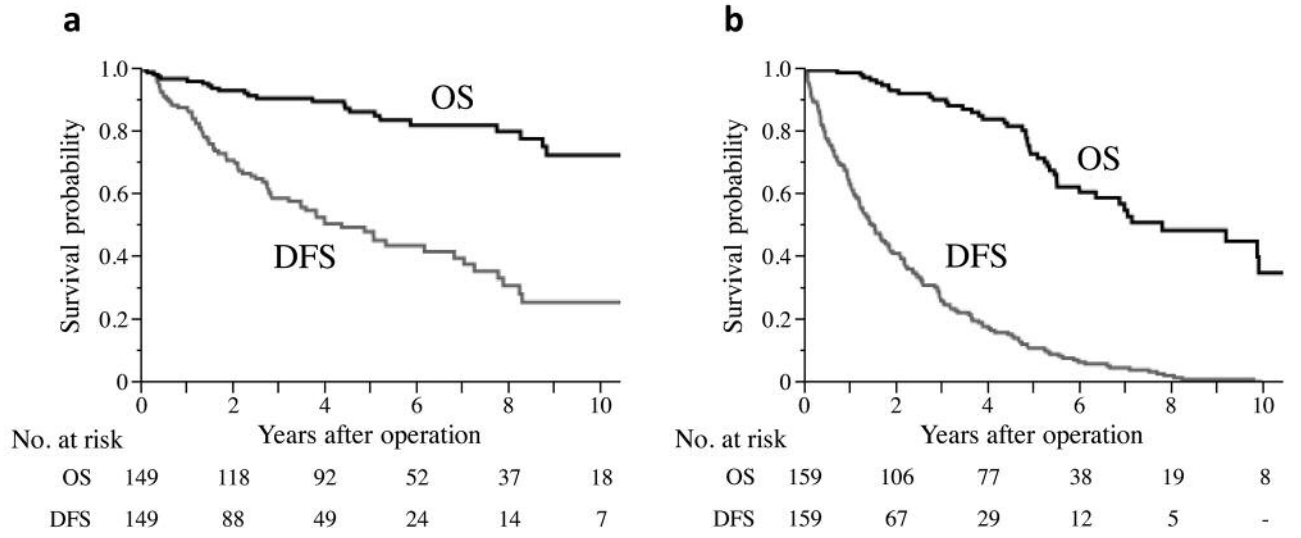

Figure 1. *Overall survival (OS) and disease-free survival (DFS) in patients who underwent hepatectomy (a) and radiofrequency ablation (b).*

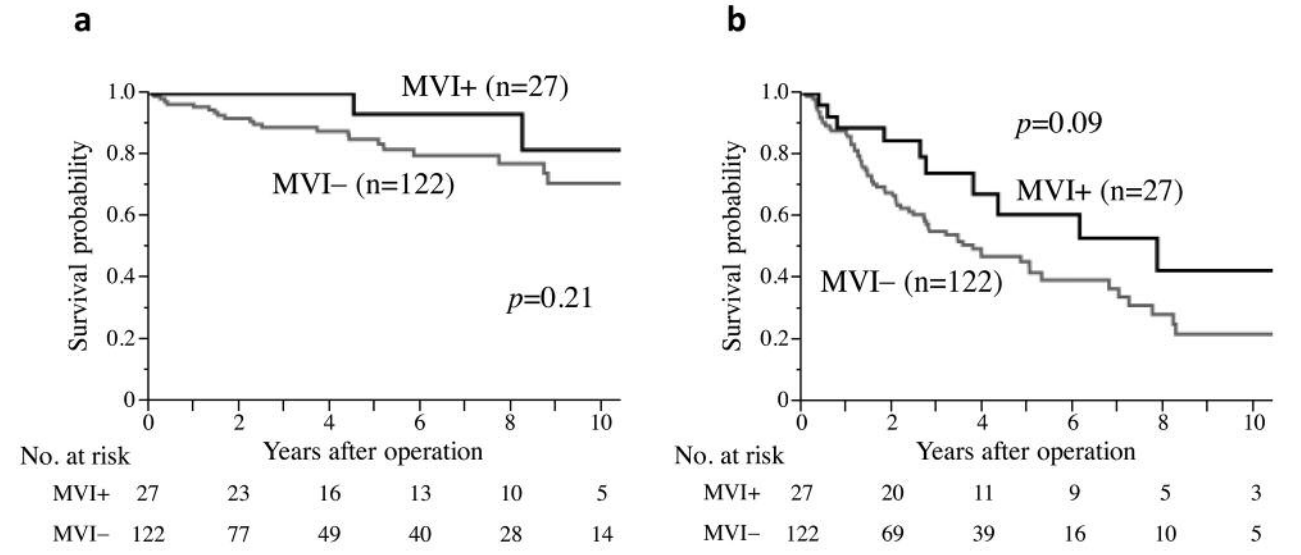

Figure 2. *Overall survival (a) and disease-free survival (b) in patients who underwent hepatectomy according to the presence or absence of microvascular invasion (MVI).*

the number of risk factor for MVI did not affect the OS or DFS after hepatectomy for such tumors. However, the patients with MVI-expected HCC (the number of risk factor 2-3) had a worse OS than those with MVI-unexpected HCC (the number of risk factor 0-1) after RFA. Furthermore, local recurrence after RFA was more frequent in patients with MVI-expected HCC (the number of risk factor 2-3).

It is known that MVI is the beginning of intrahepatic dissemination and metastasis of tumor cells in HCC (32). Many previous studies reported that MVI was significantly associated with poor survival after hepatectomy and liver transplantation for HCC (4-14). However, some authors reported that MVI was not a prognostic factor for all HCC patients (33-35). Particularly in those with small-sized HCC

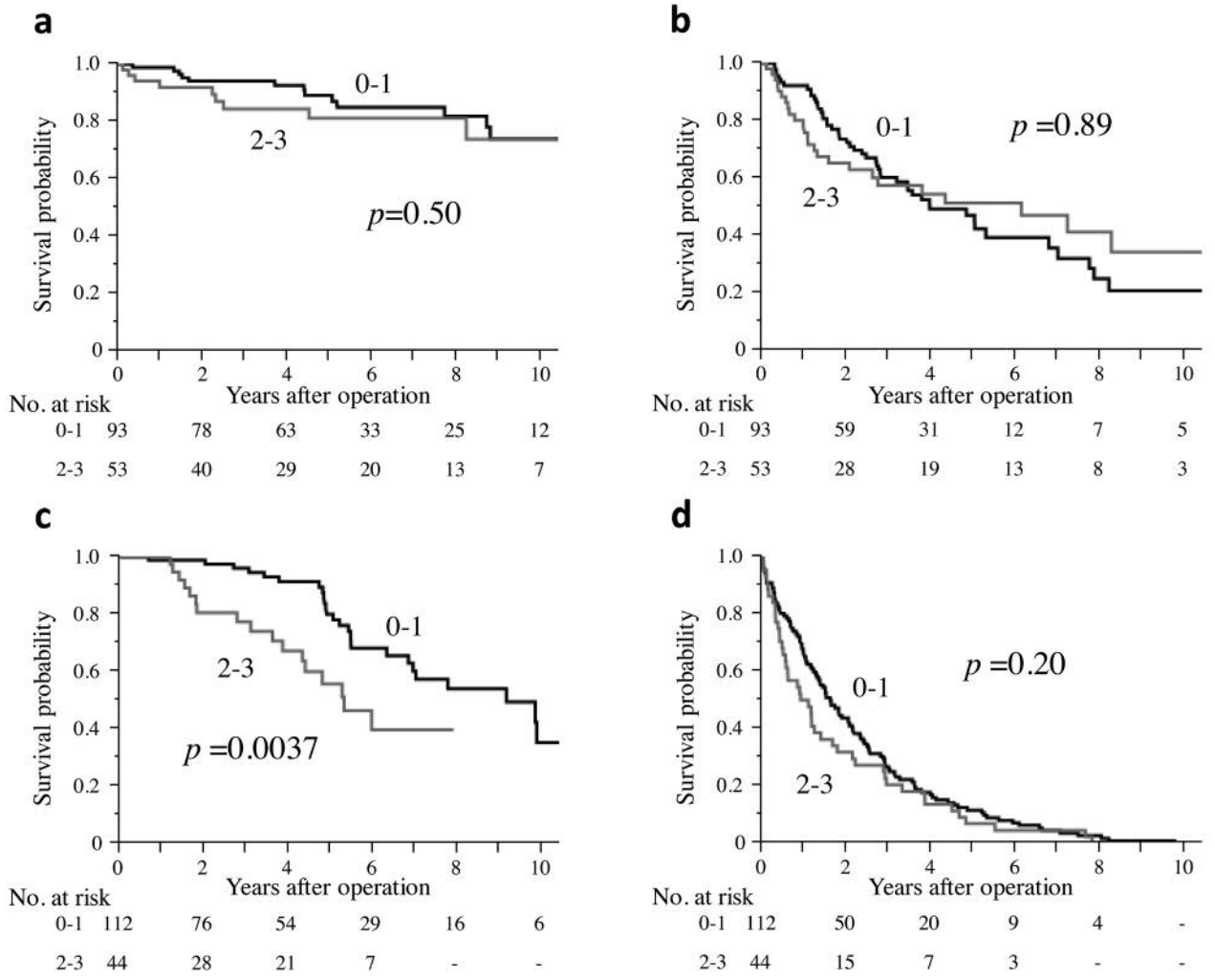

Figure 3. *Overall survival (a) and disease-free survival (b) in patients who underwent hepatectomy according to the number of risk factors for microvascular invasion (0-1 vs. 2-3). Overall survival (c) and disease-free survival (d) in patients who underwent radiofrequency ablation according to the number of risk factors for microvascular invasion (0-1 vs. 2-3).*

≤2 cm, MVI had a limited clinical value for prognosis. In the present study, The OS and DFS were comparable between patients with and without MVI after hepatectomy (Figure 2). Because the present study included only those patients with a solitary small-sized HCC, the HCC tumors were presumed to be less invasive than large or multiple tumors. In addition, anatomical resection was performed in approximately half of the patients. Although the effect of anatomical resection on tumor recurrence was unclear because of the limited number of patients, these findings suggest that micrometastasis-infiltrating peritumoral vasculatures, so-called MVI, might be removed together with the tumor by hepatectomy in patients with solitary small-sized HCC ≤3 cm.

Numerous researchers have attempted to identify possible predictive factors of MVI. Such factors include age (6), tumor size (10, 13, 14, 18, 20), multiple nodules (18), gross type (10, 24), tumor markers such as AFP (13, 18), AFP-L3 (22) and DCP (19, 20, 22, 24), typical dynamic pattern in enhanced CT (18), maximum standardized uptake value (SUVmax) in positron emission tomography (20), and apparent diffusion coefficient (ADC) value in MR imaging (23). In the present study, we identified three predictors of MVI: AFP ≥15 (ng/ml), DCP ≥100 (mAU/ml), and tumor size ≥2 (cm) (Table III). Based on these predictive factors, a predictive model for MVI was created. According to this model, the presence of these three factors was associated with an increasing probability of MVI up to 59.6% (Table III), thus confirming their clinical utility for patient selection on a daily practice.

Because the diagnosis of MVI is determined based on histological examination of surgical specimens after

Table II. *Univariate and multivariate analyses of factors for predicting microvascular invasion.*

			Univariate	Multivariate		
	MVI-positive	MVI-negative	*p*-Value	RR	95%CI	*p*-Value
Age <70	21 (77.8%)	70 (57.4%)	0.042	NS		
Gender (Male)	18 (66.7%)	89 (73.0%)	0.52			
HBs-Ag-positive	10 (37.0%)	31 (25.4%)	0.23			
HCV-Ab-positive	13 (48.2%)	66 (54.1%)	0.58			
Total bilirubin >1 (mg/dl)	4 (14.8%)	25 (20.5%)	0.49			
Albumin >3.5 (g/dl)	26 (96.3%)	112 (91.8%)	0.38			
Prothrombin activity >75 (%)	24 (88.9%)	114 (93.4%)	0.44			
Platelet count >15 ($\times 10^4$)	10 (37.0%)	57 (46.7%)	0.36			
ICG-R15 >10 (%)	18 (66.7%)	80 (69.0%)	0.82			
^{99m}Tc-GSA LHL15 <0.92	16 (59.3%)	56 (47.5%)	0.27			
AFP ≥15 (ng/ml)	15 (55.6%)	34 (27.9%)	0.007	3.05	1.20-7.99	0.02
AFP-L3 ≥10%	9 (33.3%)	17 (14.8%)	0.034	NS		
DCP ≥100 (mAU/ml)	14 (53.9%)	20 (16.7%)	<0.0001	4.19	1.63-11.0	0.003
Tumor size ≥2 (cm)	23 (85.2%)	71 (58.2%)	0.0054	3.37	1.10-12.8	0.03

MVI: Microvascular invasion; RR: relative risk; 95%CI: 95% confidence interval; HBs-Ag: hepatitis B surface antigen; HCV-Ab: anti-hepatitis C antibody; ICG-R15: indocyanine green retention rate at 15 min; ^{99m}Tc-GSA: ^{99m}Tc-galactosyl human serum albumin; AFP, α-fetoprotein; AFP-L3: *Lens culinaris* agglutinin-reactive fraction of AFP; DCP: des-γ-carboxy prothrombin; NS: not significant.

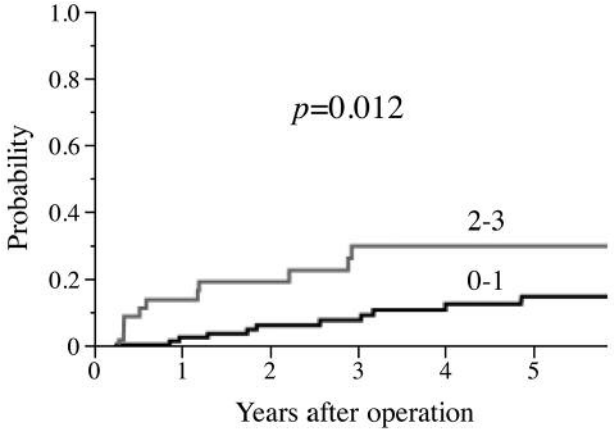

Figure 4. *Local recurrence rate in patients who underwent radiofrequency ablation according to the number of risk factors for microvascular invasion (0-1 vs. 2-3).*

Table III. *Predictive model estimating the probability of microvascular invasion.*

Factors	AFP ≥15 (ng/ml)	DCP ≥100 (mAU/ml)	Tumor size ≥2 (cm)	Probability (%)
0	–	–	–	3.3
1	+	–	–	9.4
	–	+	–	12.5
	–	–	+	10.3
2	+	+	–	30.4
	–	+	+	32.6
	+	–	+	26.0
3	+	+	+	59.6

AFP: α-Fetoprotein; DCP: des-γ-carboxy prothrombin.

hepatectomy or liver transplantation, the significance of MVI for patient outcome after RFA is uncertain. The only possible alternative is preoperative needle biopsy. However, we considered that needle biopsy prior to RFA is highly undesirable because it carries a risk of tumor dissemination or seeding (36). Therefore, in the present study, to address the significance of MVI for patient outcome, this predictive model was applied to the RFA cohort. As a result, in contrast to the hepatectomy cohort, the OS in patients with an MVI-expected tumor (the number of risk factor 2-3) was significantly worse than those with an MVI-unexpected tumor (the number of risk factor 0-1) (Figure 3). Furthermore, in those patients, local recurrence at the ablated site was more frequent (Figure 4). These findings suggest that the presence of MVI is a poor-prognosis factor after RFA, and thus that RFA should be contraindicated for patients with an MVI-expected tumor.

Local recurrence after RFA remains a serious problem; its rates have been reported to range from 3.2-26% (37-39). Local recurrence after RFA may be attributable to insufficient margin and/or the presence of vascular invasion of the tumor in the

adjacent liver tissue. In the current study, the factors related to the presence of MVI in the resected specimens were elevated AFP and DCP, and larger tumor size (Table II), which were all reported to be risk factors for local recurrence after RFA (38-42). From the viewpoint of the risk of local recurrence, RFA may not be suitable for patients with the three factors identified in this study, namely those patients with MVI-expected HCC, even though their tumors are small.

The retrospective data analysis and small sample size from a single institution are the main limitations of the present study. In addition, survival analysis in the RFA cohort was based on the estimated MVI from a predictive model, and not on actual histological assessment. However, since tumor biopsy should be avoided because of the issues of tumor dissemination or seeding, it is difficult to investigate the role of MVI in long-term outcome following RFA. Finally, a validation study using an external cohort is required to confirm the results of the present study.

In conclusion, a predictive model of MVI was developed using three independent factors that were available preoperatively. MVI had limited prognostic value in solitary small-sized HCC when hepatectomy was performed. On the other hand, MVI expected by the proposed predictive model has significant roles in terms of survival and local recurrence following RFA for solitary small-sized HCC. When the HCC tumor is expected to be accompanied with MVI, RFA may not be suitable in terms of poorer survival and local disease control rates.

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