외과학 석사 학위논문

간세포암으로 간절제술을 시행받은 환자에서 Restricted Cubic Spline 모델을 이용한 절제 변연과 재발의 관계

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이우형의 석사학위논문을 인준함

2016년 7 월

위 원 장 _________________ (인)

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Abstract

Correlation Between Resection Margin and Disease Recurrence with a Restricted Cubic Spline Model in Patients with Resected Hepatocellular Carcinoma

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Background: Although the adequate resection margin (RM) is an important principle of oncologic surgery, the relationship between RM and recurrence of resected hepatocellular carcinoma (HCC) is unclear.

Methods: We retrospectively reviewed the clinical data for 419 patients who underwent liver resection for HCC between January 2004 and June 2015 in a tertiary hospital. The risk of recurrence was plotted against RM using the restricted cubic spline (RCS) model. The perioperative and oncologic outcomes were compared between two groups of patients classified according to the inflexion point of the RCS plot.

Results: Because the risk of recurrence decreased sharply until an RM of 1 cm, the patients were divided according to an RM of < 1 cm (*n* = 233; narrow RM group) or ≥ 1 cm (*n* = 186; wide RM group). The 5-year recurrence-free survival (RFS) rate was lower in the narrow RM group (34.8% vs 43.8%, *P* = 0.042) and recurrence near the resection site was more frequent in the narrow RM group (4.7% vs 0%, *P* = 0.010). In subgroup analysis, the patients with multiple lesions, and prior transarterial chemoembolization (TACE) or radiofrequency ablation (RFA) were excluded. In patients with 2-5cm sized single HCC, wide RM patients showed higher 5-year RFS than narrow RM (43.3% vs 26.7%, *P* = 0.037), and narrow RM (hazard ratio [HR] 1.750, 95% confidence interval [CI] 1.029–2.976, *P* = 0.039) was the independent prognostic factor in recurrence after surgical treatment as well as anatomical resection (HR 1.875, 95% CI 1.103-3.187, *P* = 0.020).

Conclusion: In 2-5cm sized single HCC without prior TACE/RFA, RM of ≥ 1 cm is associated with lower risk of recurrence after liver resection and is the independent prognostic factor for disease recurrence.

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keywords : *Hepatocellular carcinoma, recurrence, resection margin, restricted cubic spline model*

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I. Introduction

Although there are several curative treatment modalities for hepatocellular carcinoma (HCC), surgical resection is the mainstay approach in patients with a sufficient liver functional reserve (1). In prior reports, however, half of the patients who underwent curative resection for HCC experienced recurrence (2,3). Previous studies revealed that tumor, host, and surgical factors were associated with early recurrence after liver resection for HCC (4). Although it is hard to address the tumor and host factors, many surgical factors can be addressed by the surgeon, who can delay recurrence by performing anatomic resection or meticulous dissection in patients with reduced intraoperative transfusion (4).

However, the relationship between the resection margin (RM) and disease recurrence after HCC resection is unclear. Although some studies have shown that a wide RM is associated with better oncologic outcomes than is a narrow RM, other studies revealed no oncologic benefits of a wide versus narrow RM (5-7).

The differing results are related to the inclusion of heterogeneous patients, as well as confounding factors, which potentially influenced recurrence after surgery. Furthermore, most studies divided patients into narrow and wide RM groups by applying arbitrary values based on their clinical experience. We determined the adequate RM by using the restricted cubic spline (RCS) model. It characterizes a non-linear dose-response association between continuous variable and an outcome. Furthermore, researchers can check the assumption of linearity of the association visually in RCS plot. Using many advantages of RCS plot, previous study found optimal sodium excretion related with minimal cardiovascular events (8). We found RM related with stable recurrence after surgery according to the inflexion point of the RCS plot. Furthermore, we compared the perioperative and oncologic outcomes between patients divided into narrow and wide RM groups.

II. Materials and Methods

1. Patients and Study design

Between January 2004 and June 2015, 419 patients who underwent open or laparoscopic liver resection for HCC at Seoul National University Bundang Hospital were included in the present study. Patients with a pathologic finding of microscopic remnant tumor or major vascular invasion were excluded, because these increase the risk of recurrence in resected HCC (9,10).

Unlike previous studies, the RM was estimated using an RCS plot, which is the natural logarithm value of the relative risk for recurrence plotted against the RM (8,11). We classified the patients into two groups based on the inflexion point of the RCS plot and compared the perioperative and oncologic outcomes between the two groups of patients. Primary outcome was to find relevance of recurrence and RM in patients with resected HCC.

The following perioperative data were retrieved from electronic medical records: preoperative laboratory findings (total bilirubin, serum albumin, platelet count, prothrombin time, viral status, indocyanine green retention rate at 15 min, and α-fetoprotein), intraoperative data (extent of resection, operation time, estimated blood loss, use of Pringle maneuver, and transfusion), immediate postoperative outcomes (hospital stay, and type and severity of complications), and pathologic results (tumor size, the number of tumors, satellite nodule, microvascular invasion, Edmonson score, the presence of cirrhotic liver, and RM). RM was defined as the shortest distance from the viable tumor to the resected specimen. In patients with previous TACE, RM was measured from the tumor to the resected specimen because it is possible to contain viable tumor even after undergoing TACE. The patients were followed up twice per year by computed tomography, magnetic resonance imaging, and measurement of tumor markers. Recurrence and its pattern were evaluated in the imaging studies. Intrahepatic recurrence was classified as near the resection site within 1cm, adjacent to the resection site, same lobe, or another lobe. Informed consent was obtained from the patient before surgery. This study was approved by our Institutional Review Board.

2. Surgical techniques

The type of operation was selected according to the tumor's location and the patient's estimated liver function. Parenchymal-sparing liver resection was performed wherever possible. Major liver resection was defined as resection of more than three segments. The surgical techniques used in our institution have been described in more detail elsewhere (12). Open liver resection was performed with the patient in the supine position via an inverted L incision. Laparoscopic liver resection was performed with the patient in the lithotomy position and the reverse Trendelenburg position. The surgeon stood between the patient's legs or on the right side of the patient. The endoscopist and assistant stood on the left side of the patient. Pneumoperitoneum was established and maintained at < 13 mmHg. Usually, two main 12 mm working ports were inserted into the epigastrium and right upper quadrant of the abdomen along the subcostal area. Additional 5 mm ports were placed in the left subcostal area for the assistant. The position of the trocar varied according to the surgical type.

Intraoperative ultrasonography was performed to localize the tumor, identify adjacent vasculature, and maintain an appropriate RM. A laparoscopic Pringle maneuver was used in some patients to minimize bleeding during parenchymal transection. After controlling the corresponding pedicular structures, superficial hepatic parenchyma was transected using ultrasonic shears, and deeper parenchymal transection was performed using a laparoscopic Cavitron Ultrasonic Surgical Aspirator (Integra Lifesciences, Plainsboro, NJ, USA). Bleeding from small branches of the hepatic veins was controlled with endoclips and a sealing device. After irrigating the surgical field, a silastic drain was inserted and the wound was closed in layers.

3. Statistical Analysis

Data are expressed as the mean and standard deviation. The χ^2test was used to compare categorical variables and Student's *t* test was used to compare continuous variables between the two groups. We used RCS plots to determine the shape of the correlation between RM and risk of recurrence. The value of the Y axis was estimated as the natural logarithm of the relative risk of recurrence after liver resection according to the RM. The inflexion point represents the point of decreased risk of recurrence, and was used as the cutoff value for the narrow and wide RM.

The survival rates were calculated using the Kaplan–Meier method and were compared between the two groups using the log-rank test. To reduce potential bias, a multivariable Cox proportional hazards model was used to determine prognostic factors for recurrence. The RCS plot was re-evaluated after adjusting for variables identified in the Cox proportional hazard model. In all analyses, a *P*-value of < 0.05 (two-sided) was considered statistically significant. R version 3.3 (R foundation, Vienna, Austria) and SPSS®version 22.0 (SPSS Corp., Chicago, IL, USA) were used for all analyses.

Ⅲ. Results

1. Preoperative Characteristics of the Patients (Figure 1, Table 1)

The RCS plot showed a steep decrease in the risk of recurrence before an RM of 1 cm and the curve was stable from 1 cm onwards (Figure 1). Based on the inflexion point of the RCS plot, we classified the patients according to RM of < 1 cm (narrow RM group; n = 233) or ≥ 1 cm (wide RM group; n = 186). There were 326 men and 93 women with a mean age of 58.4 years. Overall, 331 patients had viral hepatitis markers, including hepatitis B in 302 patients, hepatitis C in 28 patients, and both hepatitis B and C in 1 patient. Additionally, 29 and 12 patients were classified as Child class B and C, respectively. The mean indocyanine green retention rate at 15 min was 11.1%. There were no differences in the preoperative variables, including preoperative laboratory findings, between the narrow and wide RM groups (Table 1).

Figure 1. Restricted cubic spline plot for disease recurrence according to the resection margin in patients with hepatocellular carcinoma. The dotted line represents the 95% confidence interval.

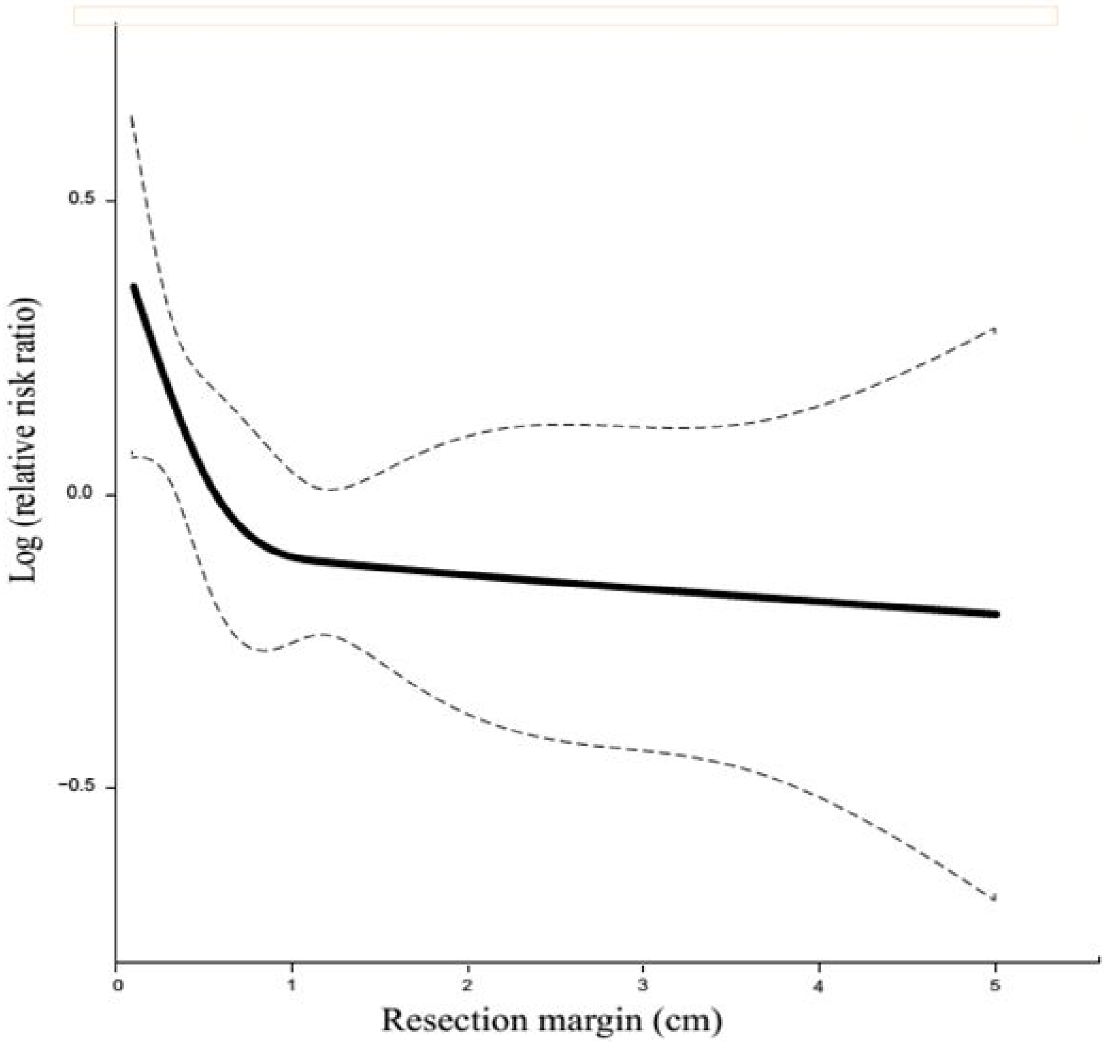


Table 1. Preoperative characteristics according to resection margin

	Narrow RM (< 1cm) (*n* = 233)	Wide RM (≥ 1 cm) (*n* = 186)	*P* value
Age (years)	59.1 ± 11.0	57.4 ± 12.6	0.138
Sex, male	182 (78.1)	144 (77.4)	0.906
BMI (kg/m^2)	23.8 ± 2.9	24.0 ± 3.8	0.572
HBsAg(+)/anti-HCV(+)/both	171/18/0	131/10/1	0.318
Child–Pugh class, A/B/C	207/18/8	171/11/4	0.587
ICG-R15 (%)	10.6 ± 8.6	11.8 ± 22.6	0.557
Previous TACE/RFA	66 (28.3)	52 (28.0)	1.000
Albumin (g/dL)	4.0 ± 0.4	4.1 ± 0.4	0.133
Total bilirubin (mg/dL)	0.8 ± 0.5	1.0 ± 2.4	0.419
Prothrombin time (INR)	1.0 ± 0.1	1.1 ± 0.2	0.524
Platelet count (×10^3/L)	165.0 ± 65.8	161.4 ± 65.1	0.571
AFP (IU/L)	814.8 ± 3845.2	717.3 ± 3599.2	0.792

Results are presented as the mean ± standard deviation, *n* (%), or *n.*

RM, resection margin; BMI, body mass index; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; ICG-R15, indocyanine green retention rate at 15 min; TACE, transarterial chemoembolization; RFA, radiofrequency ablation; INR, international normalized ratio; AFP, alpha fetoprotein.

2. Intraoperative Data (Figure 2, Table 2)

Tumor site based on Couinaud's classification is presented in Figure 2. Minor and major hepatectomy was performed in 117 and 302 patients, respectively. Operation time was longer in the narrow RM group than in the wide RM group (294.1 min vs 265.5 min, *P* = 0.048). The extent of liver resection, blood loss, use of the Pringle maneuver, and the intraoperative transfusion rate were not significantly different between the two groups (Table 2).

3. Immediate Postoperative Data and Pathologic Data (Table 2)

There were no intraoperative mortalities or morbidities requiring re-operation. Postoperative complications occurred in 67 patients (15.9%), and included bile leakage (6, 1.4%), fluid collection (31, 7.3%), wound infection (8, 1.9%), bleeding (4, 0.9%), ileus (4, 0.9%), and pulmonary complications (18, 4.2%). The rates of postoperative complications (21.2% vs 19.5%, *P* = 0.783) and major complications (50% vs 58.6%, *P* = 0.390), classified as grade ≥ III according to the Clavien–Dindo system, were not significantly different between the two groups. However, the frequency of postoperative bile leakage was significantly greater in the narrow RM group than in the wide RM group (15.7% vs 0%, *P* = 0.034). The duration of hospital stay was similar in both groups (10.9 days vs 10.2 days, *P* = 0.515).

Regarding pathologic findings, the mean tumor size and RM were 3.8 cm and 1.2 cm, respectively. More patients in the narrow RM group than patients in the wide RM group had liver cirrhosis (64.9% vs 53.2%, *P* = 0.016). However, the type of liver cirrhosis was not significantly different between the two groups (*P* = 0.944). There were no significant differences in other pathologic variables between the two groups (Table 2).

Figure 2. Tumor location according to Couinaud's classification.

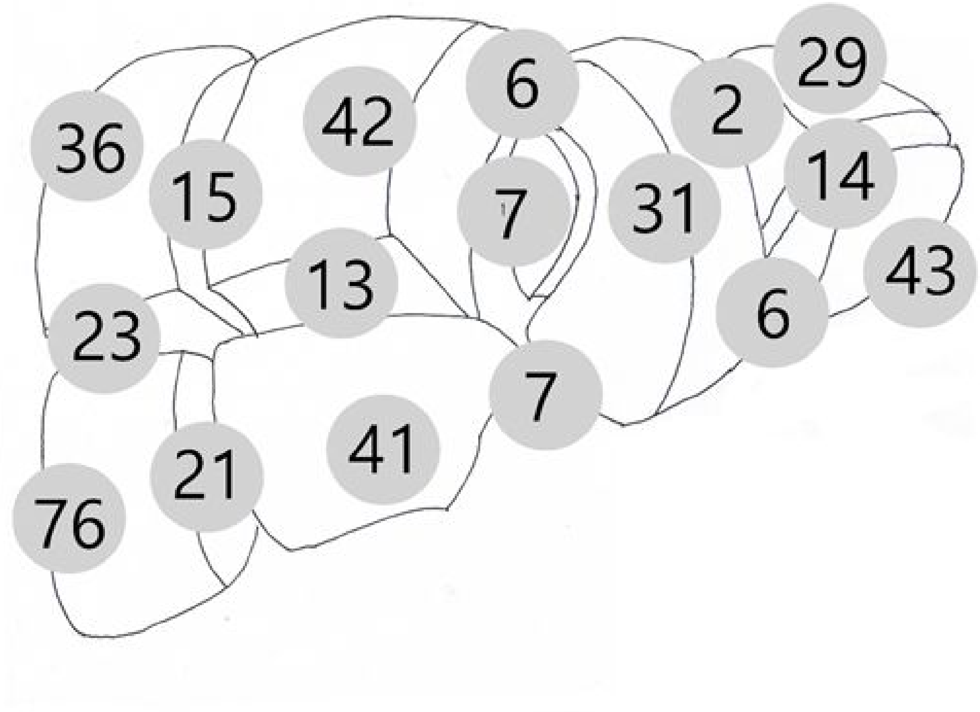


Table 2. Intraoperative, postoperative, and pathologic results according to resection margin

	Narrow RM (< 1 cm) (*n* = 233)	Wide RM (≥ 1 cm) (*n* = 186)	*P* value
Major resection	57 (24.5)	60 (32.3)	0.081
Anatomic resection	156 (67)	131 (70.4)	0.461
Operation time (min)	294.1 ± 153.4	265.5 ± 138.7	0.048
Blood loss (mL)	847.5 ± 1405.0	972.7 ± 1837.5	0.433
Pringle maneuver	67 (28.8)	42 (22.6)	0.167
Intraoperative transfusion	44 (18.9)	43 (23.2)	0.332
Postoperative complications	38 (21.2)	29 (19.5)	0.783
Bile leakage	6	0	0.034
Fluid collection	13	18	0.184
Wound infection	4	4	1.000
Bleeding	3	1	0.629
Ileus	3	1	0.629
Pulmonary complications	12	6	0.337
Major complications	19 (50)	17 (58.6)	0.390
Hospital stay (days)	10.2 ± 9.3	11.1 ± 13.9	0.451
Tumor size (cm)	3.8 ± 2.8	3.7 ± 2.6	0.789
Multiple lesions	27 (11.6)	16 (8.6)	0.336
RM (cm)	0.4 ± 0.2	2.1 ± 1.3	<0.001
Satellite nodules	25 (11)	14 (7.7)	0.310
Microvascular invasion	86 (37.1)	56 (30.3)	0.176
Edmonson score I+II/III+IV	104/75	100/49	0.109
Liver cirrhosis	150 (64.9)	99 (53.2)	0.016
Type of cirrhosis: micro/macro/mixed	15/52/84	10/35/51	0.944

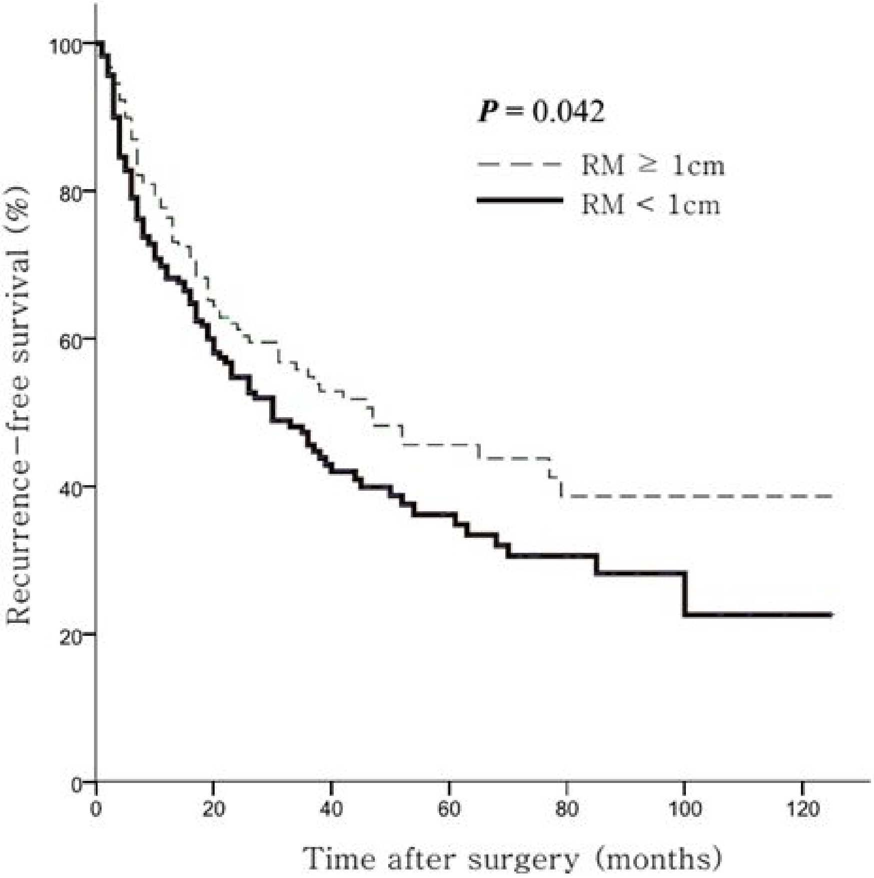
Results are presented as the mean ± standard deviation, *n* (%), or *n*.

RM, resection margin.

4. Long-term Outcomes (Table 3)

The median follow-up period was 37.5 months (range, 1–117 months). The 5-year overall survival (OS) and recurrence-free survival (RFS) rates were 73.0% and 39.8%, respectively. Although the 5-year OS was not significantly different between the narrow and wide RM groups (69.5% vs 72.9%, P = 0.690), the 5-year RFS was greater in the wide RM group than in the narrow RM group (43.8% vs 34.8%, P = 0.042) (Figure 3). Of 419 evaluable patients, 200 (47.7%) patients experienced recurrence after curative resection. Overall, 157 (78.5%) and 43 (21.5%) patients experienced intrahepatic or extrahepatic recurrence, respectively. Although the pattern of extrahepatic recurrence was comparable in both groups (P = 0.934), the pattern of intrahepatic recurrence was significantly different between the two groups. Recurrence near the RM was more frequent in the narrow RM group than in the wide RM group (4.7% vs 0%, P = 0.010). The treatment of recurrence comprised repeated liver resection alone in 8 patients, transarterial chemoembolization (TACE) or radiofrequency ablation (RFA) in 135 patients, liver resection combined with TACE or RFA in 14 patients, administration of sorafenib in 16 patients, and salvage liver transplantation in 3 patients. The management of disease recurrence was not significantly different between the two groups (P = 0.644) (Table 3).

Figure 3. Recurrence-free survival curves in patients with a narrow (< 1 cm) or wide (≥ 1 cm) resection margin. RM, resection margin.



No. at risk

RM ≥ 1cm	185	84	51	28	14	4	1
RM < 1cm	232	95	44	25	14	4	0

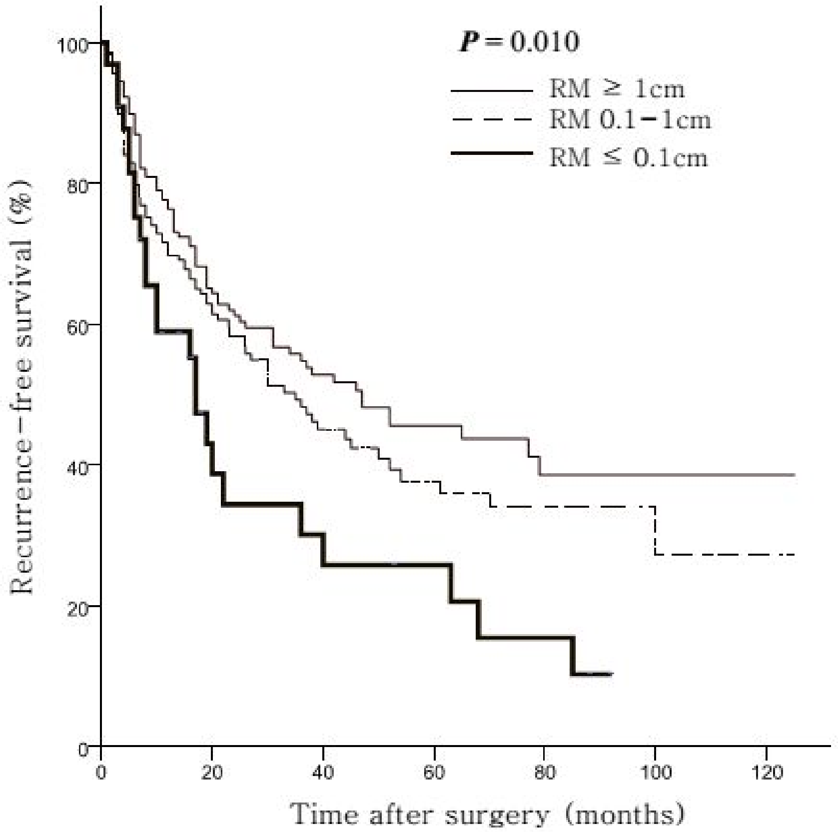
Table 3. Recurrence pattern according to resection margin

	Narrow RM (< 1 cm) (*n* = 233)	Wide RM (≥ 1 cm) (n = 186)	*P* value
Recurrence pattern			0.742
Intrahepatic recurrence	94 (78.3)	63 (78.8)	0.010
Near the RM	11 (4.7)	0	
Adjacent	38 (16.3)	37 (19.9)	
Same lobe	23 (9.9)	13 (7.0)	
Other lobe	20 (8.6)	12 (6.5)	
Extrahepatic recurrence	26 (21.7)	17 (21.3)	0.934
Lung	10 (4.3)	6 (3.2)	
Bone	3 (1.3)	4 (2.2)	
Brain	1 (0.4)	1 (0.5)	
Peritoneum	3 (1.3)	2 (1.1)	
Mutifocal	7 (3.0)	4 (2.2)	
Perihepatic mass	2 (0.9)	0 (0)	
Management, n (%)			0.644
Re-resection	4 (1.7)	4 (2.2)	
Re-resection with TACE/RFA	9 (2.6)	5 (4.2)	
TACE/RFA	79 (77.6)	56 (83.3)	
Sorafenib	11 (4.7)	5 (2.7)	
Transplantation	0	3 (1.6)	
Unknown	17 (16.5)	7 (9.5)	

Results are presented as the *n* (%). RM, resection margin; TACE, transarterial chemoembolization; RFA, radiofrequency ablation.

Although the inflexion point on the RCS plot was around 1 cm, we observed a steeply decreasing risk of recurrence within the group of patients with an RM of < 1 cm. This plot indicates that heterogeneous patients with different risks of recurrence were included in the narrow RM group. Therefore, we re-classified the RM as ≤ 0.1 cm (*n* = 33), 0.1–1 cm (*n* = 200), and ≥ 1 cm (*n* = 186) to evaluate the heterogeneity of risk within the narrow RM group. The Kaplan–Meier curves showed that the 5-year RFS was significantly different among these three groups (≤ 0.1 cm vs 0.1–1 cm vs ≥ 1 cm: 20.7% vs 35.9% vs 43.8%; *P* = 0.010; Figure 4). The 5-year RFS in the ≤ 0.1 cm group (20.7%) was significantly lower than that in the 0.1–1 cm group (35.9%, *P* = 0.034) and the ≥ 1 cm group (43.8%, *P* = 0.003), but was not significantly different between the 0.1–1 cm group and the ≥ 1 cm group (35.9% vs 43.8%, *P* = 0.161). Perioperative variables were compared among three RM groups. In pathologic results, microvascular invasion in RM ≤ 0.1 cm group was more frequent compared with other groups (≤ 0.1 cm vs 0.1–1 cm vs ≥ 1 cm: 53.1% vs 34.5% vs 30.3%, *P* = 0.031). Otherwise, there were no significant differences among three RM groups.

Figure 4. Recurrence-free survival curves for patients divided into three groups according to the resection margin of ≤ 0.1 cm, 0.1–1 cm, and ≥ 1 cm. RM, resection margin.



No. at risk

RM ≥ 1 cm	185	83	51	28	14	4	1
RM 0.1-1 cm	199	85	37	20	11	4	0
RM ≤ 0.1 cm	32	9	6	4	2	0	0

To reduce possible confounding factors, we excluded the patients with multiple lesions (*n* = 43). In patients with a single HCC (*n* = 376), the 5-year RFS rate in wide RM group was higher than narrow RM group. (45.8% vs 34.6%, *P* = 0.027). Furthermore, we excluded patients with prior TACE/RFA to evaluate recurrence after first line surgical treatment (Figure 5). In patients who underwent surgical treatment for single HCC, there were no significant difference in 5-year RFS between wide and narrow RM groups (43.3% vs 35.4%, *P* = 0.147). The patients were classified into 3 groups (tumor size ≤ 2 cm, 2-5 cm, and > 5 cm ; figure 5) according to the tumor size. In very early stage (tumor size ≤ 2cm, *n* = 75), the 5-year RFS was not different between wide and narrow RM (53.5 % vs 46.8 %, *P* = 0.755). However, in patients with 2-5 cm sized single HCC, wide RM group showed higher 5-year RFS rate than narrow RM significantly (54.4 % vs 32.5 %, *P* = 0.036; figure 6). In tumor >5cm group, there were no significant difference in RFS rate between wide and narrow RM (21.3% vs 26.3%, *P* = 0.881)

Figure 5. Flowchart for subgroup analysis in patients with single hepatocellular carcinoma without prior transarterial embolization or radiofrequency ablation.

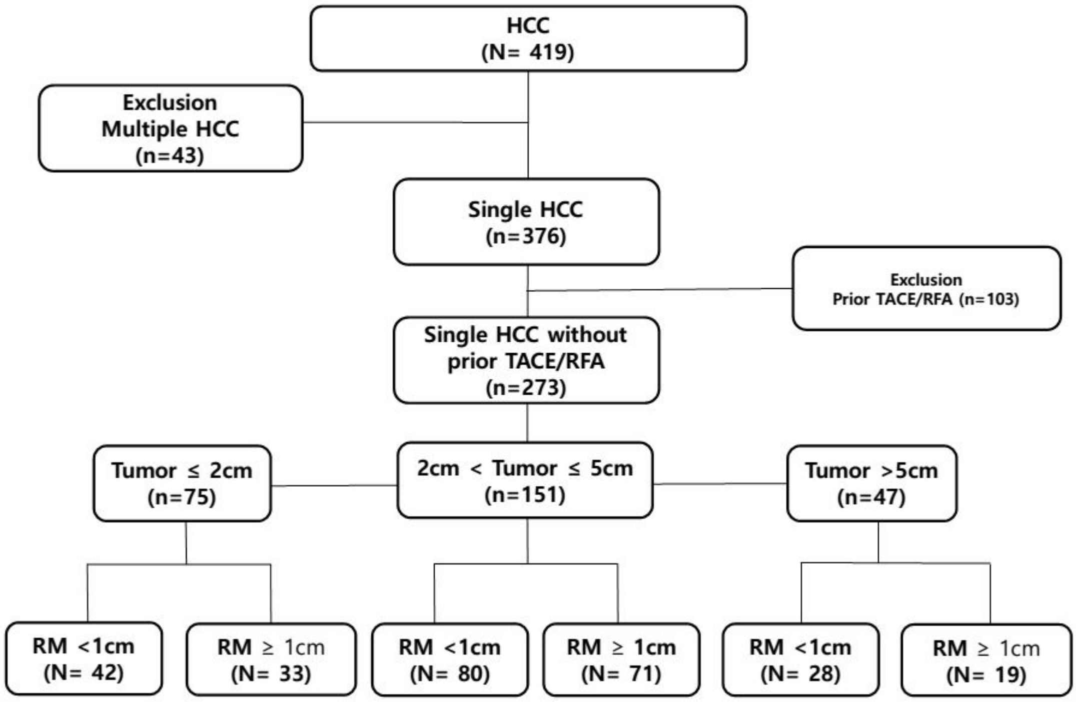
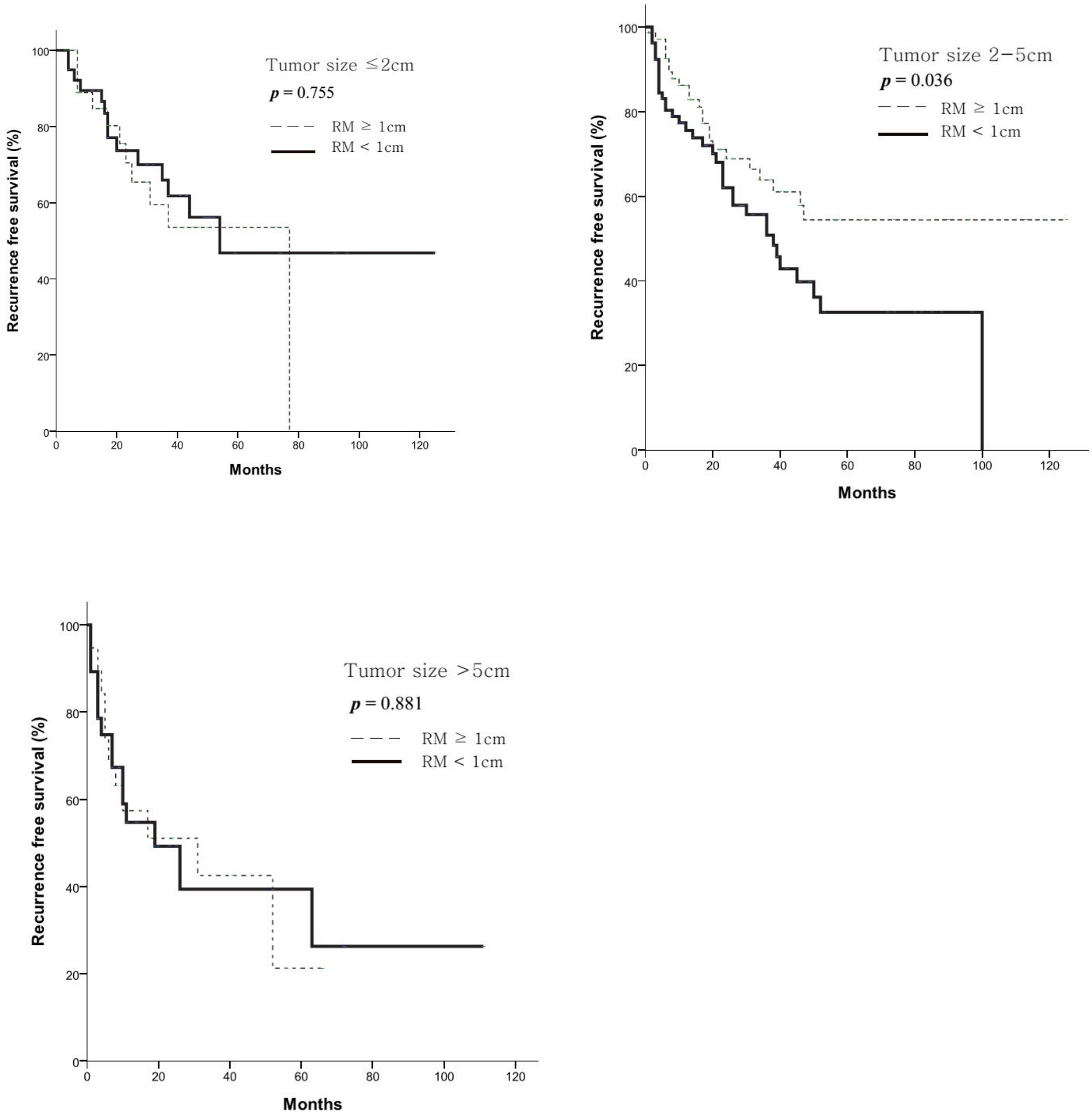


Figure 6. Recurrence-free survival curves for patients between wide and narrow RM in tumor < 2 cm, 2-5 cm, and >5cm subgroups. RM, resection margin.



5. Prognostic Factors for Recurrence (Table 4,5, Figure 6)

To exclude potential confounding factors for recurrence in resected HCC, Cox proportional hazard model was performed using known risk factors. Presence of multiple tumors (*P* = 0.002), presence of microvascular invasion (*P* = 0.004), tumor size > 5 cm (*P* = 0.008), and RM < 1 cm (*P* = 0.042) were significant risk factors for recurrence. In multivariable analysis, multiple tumor (hazard ratio [HR] 1.813, 95% confidence interval [CI] 1.193–2.753, *P* = 0.005), prior TACE/RFA (HR 1.556, 95% CI 1.083–1.971, *P* = 0.005) and the presence of microvascular invasion (HR 1.499, 95% CI 1.112–2.022, *P* = 0.009) were independent prognostic factors for recurrence after HCC resection (Table 4).

In patients with 2-5cm sized single HCC without prior TACE/RFA, wide RM group showed better oncologic outcome compared with narrow RM group. We searched prognostic factors for recurrence in these 151 patients. Univariate analysis found non-anatomical resection (*P* = 0.024), and RM < 1cm (*P* = 0.036) as a significant factors. In multivariate analysis, non-anatomical resection (HR 1.875, 95% CI 1.103–3.187, *P* = 0.020) and RM < 1cm (HR 1.750, 95% CI 1.029–2.976, *P* = 0.039) were independent prognostic factors for disease recurrence in pateints with single HCC, which was measured as 2-5cm and underwent no TACE/RFA (Table 5).

The adjusted RCS plot showed that the risk of recurrence was decreased at an RM of about 1 cm. This analysis was adjusted for the presence of satellite nodules, presence of microvascular invasion, Edmonson score, presence of multiple tumors, and pathologic T stage based on the American Joint Committee on Cancer (AJCC), 7th edition, because these were significant risk factors for disease recurrence after HCC resection (Figure 7).

Table 4. Univariate and multivariable analyses of prognostic factors for recurrence-free survival in all hepatocellularcarcinoma patients

	Univariate analysis			Multivariable analysis	
Variable	Subgroup (*n*)	MST (months)	*P*-value	OR (95% CI)	*P*-value
Age	< 70 years (343)	37	0.876		
	≥ 70 years (76)	36			
Sex	Male (326)	31	0.181		
	Female (93)	45			
AFP (ng/dL)	≤ 400 (347)	36	0.921		
	> 400 (66)	39			
Child class	A (378)	36	0.743		
	B (29)	30			
	C (12)				
HBV	Yes (303)	38	0.990		
	No (117)	36			
HCV	Yes (29)	30	0.139		
	No (391)	38			
Prior TACE/RFA	Yes (117)	45.2	0.006	1.556 (1.083–1.971)	0.005
	No (302)	60			
Anatomic resection	Yes (287)	40	0.292		
	No (132)	30			
Transfusion	Yes (87)	35	0.816		
	No (331)	36			
Multiple tumors	Yes (43)	13	0.002	1.813 (1.193–2.753)	0.005
	No (376)	38			
Tumor size > 5 cm	Yes (98)	19	0.008	1.333 (0.973–1.826)	0.074
	No (321)	39			
Microvascular	Yes (142)	43	0.004	1.499 (1.112-2.022)	0.009

invasion					
	No (275)	62			
Cirrhotic liver	Yes (168)	38	0.098		
	No (349)	34			
Complications	Yes (90)	20	0.069		
	No (286)	37			
RM < 1 cm	Yes (233)	47	0.042	1.276 (0.953–1.708)	0.101
	No (186)	30			

MST, median survival time; OR, odds ratio; CI, confidence interval; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; RM, resection margin.

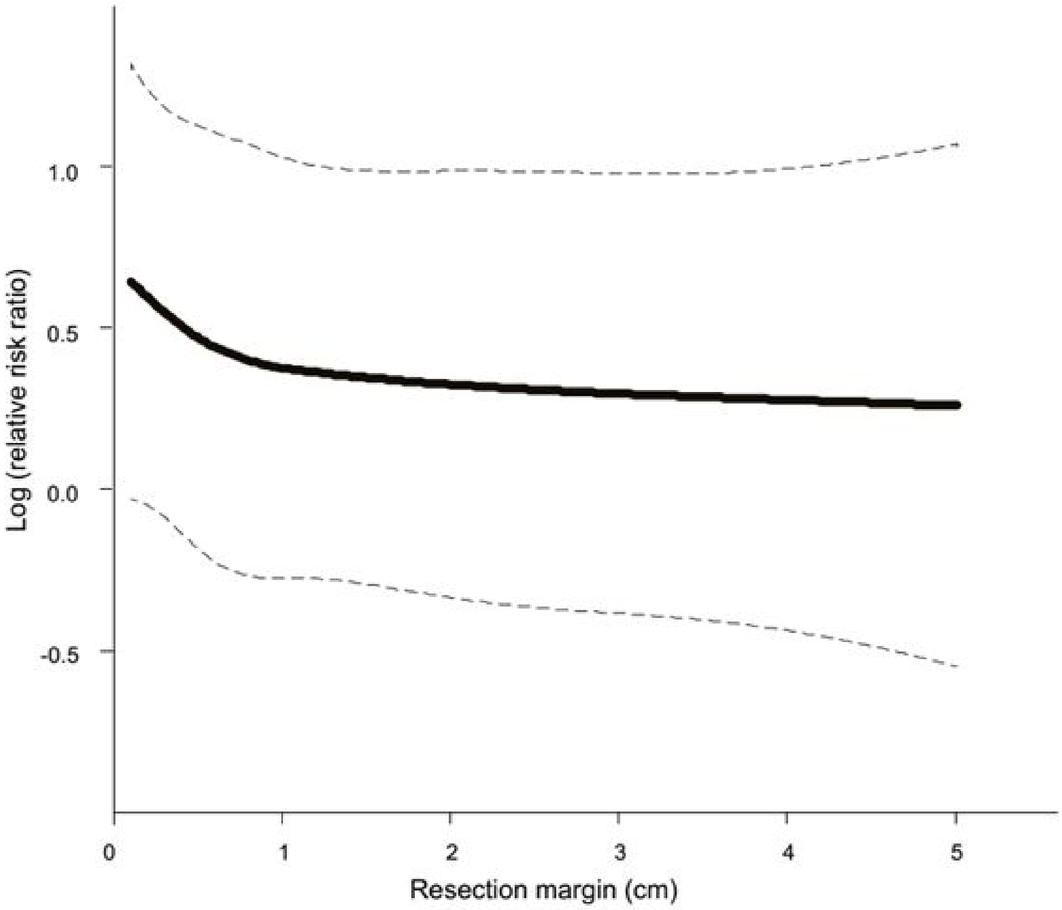
Table 5. Univariate and multivariable analyses of prognostic factors for recurrence-free survival in patients with 2-5 cm sized singe hepatocellular carcinoma without prior transarterial chemoembolization or radiofrequency ablation

	Univariate analysis			Multivariable analysis	
Variable	Subgroup (*n*)	MST (months)	*P*-value	OR (95% CI)	*P*-value
Age	< 70 years (121)	62	0.318		
	≥ 70 years (30)	69			
Sex	Male (115)	59.9	0.119		
	Female (36)	72.5			
AFP (ng/dL)	≤ 400 (134)	66.2	0.239		
	> 400 (16)	33			
Child class	A (142)	56	0.661		
	B (7)	30			
	C (2)				
HBV	Yes (110)	63.2	0.774		
	No (41)	64.8			
HCV	Yes (7)	40.0	0.229		
	No (144)	65.8			
Microvascular invasion	Yes (54)	49.2	0.079	1.527 (1.897–2.599)	0.118
	No (97)	71.1			
Anatomical resection	Yes (83)	70	0.024	1.875 (1.103–3.187)	0.020
	No (38)	42			
Transfusion	Yes (21)	64.7	0.419		
	No (130)	64.7			
Edmonson ≥ III	Yes (83)	65.5	0.599		
	No (38)	55.7			
Cirrhotic liver	Yes (87)	56.3	0.073	1.299 (0.738–2.287)	0.364

	No (63)	76.4			
Complications	Yes (18)	61.7	0.972		
	No (103)	63.2			
RM < 1 cm	Yes (80)	48.1	0.036	1.750 (1.029–2.976)	0.039
	No (71)	77.7			

MST, median survival time; OR, odds ratio; CI, confidence interval; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; RM, resection margin.

Figure 7. Restricted cubic spline plot with adjustment for the presence of satellite nodules, microvascular invasion, and multiple tumors, Edmonson score, and pathologic T stage. The gray shading represents the 95% confidence interval.



IV. Discussion

HCC is the fifth most common cancer, accounting for 7%–8% of all cancers (1,13). It was ranked as the third most common cause of cancer-related death worldwide, and is the main cause of cancer-related death in Korean men in their 40s or 50s (2,13). Liver resection is the mainstay curative treatment (1,13,14). However, the recurrence rate after hepatectomy is still high, approximately 50% at 2 years (15). Furthermore, early recurrence after curative resection is associated with poor prognosis (16,17). There are multiple risk factors for recurrence after HCC resection. In a prior report, the host factors for recurrence included hepatitis C, alcoholic abuse, and active hepatitis activity; the tumor factors included vascular invasion, satellite nodule, tumor size, and advanced TNM stage; and surgical factors included the extent of resection, RM, perioperative transfusion, adequate remnant liver function after surgery, and postoperative complications (4).

Among the known surgical factors associated with recurrence after HCC resection, some could be addressed by the surgeon. In particular, patients who underwent anatomic resection experienced better long-term outcomes than patients who underwent non-anatomic resection. Anatomic resection includes the relevant Glissonean pedicles and prevents dissemination of micrometastases along the portal branches (18). However, most patients with HCC have chronic liver disease or cirrhosis. These patients need a large remnant liver to prevent postoperative liver failure compared with patients without liver cirrhosis. Therefore, patients with liver cirrhosis need another surgical strategy aimed at obtaining an adequate surgical margin with a sufficient remnant liver volume. However, the relationship between the surgical margin and the oncologic outcome is unclear in patients with HCC (5).

Several surgeons have reported that the surgical margin is not associated with the prognosis of patients with resected HCC. A previous study showed no significant difference in the oncologic outcomes among patients divided by RM, but patients with a wide RM experienced more frequent postoperative complications compared with patients with a narrow RM (7). Likewise, Matsui et al. found no differences in the oncologic outcomes or recurrence patterns between the tumor exposure at the cut surface and non-exposure groups in their study, and they proposed that intrahepatic metastasis is based on portal venous dissemination or multicentric carcinogenesis (19). A recent retrospective study using propensity score analysis also showed no differences in OS, RFS, or the recurrence pattern, and the authors emphasized the importance of anatomic resection rather than a sufficient RM (20).

By contrast, other surgeons have reported that patients with a wide surgical margin experienced better oncologic outcomes. For example, Hu et al. reported that a large surgical margin was associated with better RFS among patients who satisfied the Milan criteria than patients who did not satisfy these criteria (21). Shimada et al. reported that patients with an RM of ≥ 1 cm had a better RFS in patients negative for hepatitis C virus and tumor size > 2.5 cm. They insisted that HCC with hepatitis C is associated with continued carcinogenesis in the remnant liver rather than microsatellite spreading (22). Sasaki et al. reported that RFS was better in patients with an RM of > 0.5 cm than in patients with an RM of ≤ 0.5 cm among patients with a tumor size of > 3 cm. They reported that large tumors had microsatellite nodules around the main tumor (23). Shi et al. reported that an RM of > 2 cm was associated with decreased recurrence among patients with a tumor size of ≤ 2 cm who were enrolled in a prospective randomized controlled study (24). They found that, after resection of the primary HCC, remnant micrometastases spread through the portal venous system and the number of micrometastases was correlated with the RM (15).

In this study, we evaluated the association between RM and disease recurrence after HCC resection. Because the association was not linear, the RCS model was used. RCS plots are useful for characterizing a dose–response association between a continuous exposure and an outcome (8,11). Furthermore, RCS plots allow researchers to make visual assumptions on the association. In this study, the RCS plot showed a steep decrease in the risk of recurrence until an RM of 1 cm. However, after the inflexion point of about 1 cm, the risk of recurrence stabilized. The pattern of risk was similar even after adjustment for other factors associated with recurrence. This adjusted model included the presence of satellite nodules, microvascular invasion, and multiple tumors, high Edmonson score, and T stage determined using the AJCC, 7th version. In previous studies, the patients were divided into two or three groups based on an arbitrary cut off value for RM. Here, were vealed a continuous association between RM and disease recurrence.

Nevertheless, there are several factors, other than an adequate RM, that are associated with recurrence after HCC resection (5,26). In this study, other factors associated with recurrence were presence of microvascular invasion, multiple tumors, prior TACE/RFA, and large tumor. We excluded patients with multiple tumor and prior TACE/RFA in subgroup analysis. In 2-5cm sized singe HCC without prior TACE/RFA, wide RM group showed higher 5-year RFS compared with narrow RM. Furthermore, narrow RM was the independent prognostic factor for recurrence in the 2-5cm sized single HCC.

The most important cause of postoperative recurrence was reported to be micrometastasis around the HCC, based on previous studies. Shi et al. reported that micrometastases after resection of the primary HCC are spread through the portal venous system and the number of micrometastases was correlated with the distance from the primary tumor (15). Zhou et al. determined the distribution of micrometastases around the primary HCC. Micrometastases were found 0.6 cm from the primary tumor in patients without macroscopic thrombi or satellite nodules, and the authors stated that the minimum RM should be 0.6 cm for micrometastasis-free resection (25). In this study, the frequency of recurrence near the RM was greater in the narrow RM group than in the wide RM group. The RM in 11 patients who experienced recurrence near the RM ranged from 0.03 to 0.8 cm. There were no significant differences in perioperative variables between patients with recurrence near the RM or patients with recurrence in other regions of the liver. This higher incidence of recurrence near the RM is likely due to micrometastases in the remnant liver parenchyma. Microscopic tumor emboli are may be present in microvessels extending beyond the resection plane. Accordingly, it is important to achieve an adequate RM, which was 1 cm based on the results in our study.

This study has several limitations. The retrospective study design introduces potential confounders. Furthermore, the patients were heterogeneous in terms of the proportions of patients with liver cirrhosis, multiple tumors or large tumors, and these factors may affect disease recurrence after HCC resection. A well-designed study is needed to confirm the impact of an adequate RM on disease recurrence.

In conclusion, the RCS plot showed that a wide RM of ≥ 1 cm is associated with a lower risk of recurrence after liver resection than a narrow RM of < 1 cm in patients with HCC. Furthermore, the results indicate that the surgical strategy should include achieving an RM of ≥ 1 cm to reduce the risk of disease recurrence after liver resection in 2-5cm sized single HCC without prior TACE/RFA.

V. References

1. European Association For The Study Of The L, European Organisation For R, Treatment Of C. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol.* 2012;56:908-943.
2. Attwa MH, El-Etreby SA. Guide for diagnosis and treatment of hepatocellular carcinoma. *World J Hepatol.* 2015;7:1632-1651.
3. Torzilli G, Donadon M, Montorsi M. The surgical margin in liver resection for hepatocellular carcinoma: a real problem or not? *Ann Surg.* 2007;246:690-691; author reply 691-692.
4. Tung-Ping Poon R, Fan ST, Wong J. Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. *Ann Surg.* 2000;232:10-24.
5. Lafaro K, Grandhi MS, Herman JM, et al. The importance of surgical margins in primary malignancies of the liver. *J Surg Oncol.* 2015.
6. Shi M, Guo RP, Lin XJ, et al. Partial hepatectomy with wide versus narrow resection margin for solitary hepatocellular carcinoma: a prospective randomized trial. *Ann Surg.* 2007;245:36-43.
7. Lee KT, Wang SN, Su RW, et al. Is wider surgical margin justified for better clinical outcomes in patients with resectable hepatocellular carcinoma? *J Formos Med Assoc.* 2012;111:160-170.
8. Desquilbet L, Mariotti F. Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med.* 2010;29:1037-1057.
9. Matsumoto T, Kubota K, Aoki T, et al. Clinical Impact of Anatomical Liver Resection for Hepatocellular Carcinoma with Pathologically Proven Portal Vein Invasion. *World J Surg.* 2016;40:402-411.
10. Nara S, Shimada K, Sakamoto Y, et al. Prognostic impact of marginal resection for patients with solitary hepatocellular carcinoma: evidence from

570 hepatectomies. *Surgery.* 2012;151:526-536.
11. O'Donnell M, Mente A, Rangarajan S, et al. Urinary sodium and potassium excretion, mortality, and cardiovascular events. *N Engl J Med.* 2014;371:612-623.
12. Han HS, Shehta A, Ahn S, et al. Laparoscopic versus open liver resection for hepatocellular carcinoma: Case-matched study with propensity score matching. *J Hepatol.* 2015;63:643-650.
13. Korean Liver Cancer Study G, National Cancer Center K. 2014 KLCSG-NCC Korea Practice Guideline for the Management of Hepatocellular Carcinoma. *Gut Liver.* 2015;9:267-317.
14. Yu SJ. A concise review of updated guidelines regarding the management of hepatocellular carcinoma around the world: 2010-2016. *Clin Mol Hepatol.* 2016;22:7-17.
15. Shi M, Zhang CQ, Zhang YQ, et al. Micrometastases of solitary hepatocellular carcinoma and appropriate resection margin. *World J Surg.* 2004;28:376-381.
16. Hirokawa F, Hayashi M, Asakuma M, et al. Risk factors and patterns of early recurrence after curative hepatectomy for hepatocellular carcinoma. *Surg Oncol.* 2016;25:24-29.
17. Hayashi M, Shimizu T, Hirokawa F, et al. Clinicopathological risk factors for recurrence within one year after initial hepatectomy for hepatocellular carcinoma. *Am Surg.* 2011;77:572-578.
18. Kobayashi A, Miyagawa S, Miwa S, et al. Prognostic impact of anatomical resection on early and late intrahepatic recurrence in patients with hepatocellular carcinoma. *J Hepatobiliary Pancreat Surg.* 2008;15:515-521.
19. Matsui Y, Terakawa N, Satoi S, et al. Postoperative outcomes in patients

with hepatocellular carcinomas resected with exposure of the tumor surface: clinical role of the no-margin resection. *Arch Surg.* 2007;142:596-602; discussion 603.

20. Lee JW, Lee YJ, Park KM, et al. Anatomical Resection But Not Surgical Margin Width Influence Survival Following Resection for HCC, A Propensity Score Analysis. *World J Surg.* 2016.
21. Hu W, Pang X, Guo W, et al. Relationship of different surgical margins with recurrence-free survival in patients with hepatocellular carcinoma. *Int J Clin Exp Pathol.* 2015;8:3404-3409.
22. Shimada K, Sakamoto Y, Esaki M, et al. Role of the width of the surgical margin in a hepatectomy for small hepatocellular carcinomas eligible for percutaneous local ablative therapy. *Am J Surg.* 2008;195:775-781.
23. Sasaki K, Matsuda M, Ohkura Y, et al. Minimum resection margin should be based on tumor size in hepatectomy for hepatocellular carcinoma in hepatoviral infection patients. *Hepatol Res.* 2013;43:1295-1303.
24. Shi J, Lai EC, Li N, et al. Surgical treatment of hepatocellular carcinoma with portal vein tumor thrombus. *Ann Surg Oncol.* 2010;17:2073-2080.
25. Zhou XP, Quan ZW, Cong WM, et al. Micrometastasis in surrounding liver and the minimal length of resection margin of primary liver cancer. *World J Gastroenterol.* 2007;13:4498-4503.
26. Zhang TT, Zhao XQ, Liu Z, et al. Factors affecting the recurrence and survival of hepatocellular carcinoma after hepatectomy: a retrospective study of 601 Chinese patients. *Clin Transl Oncol.* 2015.
27. Shehta A, Han HS, Yoon YS, et al. Laparoscopic liver resection for

hepatocellular carcinoma in cirrhotic patients: 10-year single-center experience. *Surg Endosc.* 2016;30:638-648.

28. Zhou L, Rui JA, Wang SB, et al. Clinicopathological predictors of poor survival and recurrence after curative resection in hepatocellular carcinoma without portal vein tumor thrombosis. *Pathol Oncol Res.* 2015;21:131-138.

요약(국문초록)
간세포암으로 간절제술을 시행받은 환자에서 Restricted Cubic Spline 모델을 이용한 절제 변연과 재발의 관계

배경: 암을 절제하는 가운데에 적절한 절제 변연을 확보하는 것은 암수술의 중요한 원칙이지만 간세포암에서 절제 변연의 길이와 재발의 관계는 아직 논쟁이 많다. 본 연구는 restricted cubic spline 모델을 통하여서 절제변연의 길이와 재발의 관계를 밝히고자 하였다.

방법: 2004년 1월부터 2015년 1월까지 간세포암으로 간절제술을 시행받은 419명의 환자를 대상으로 하였으며, restricted cubic spline 모델을 통해 절제변연 1cm을 기준으로 하여 두 군을 나누었다.

결과: 절제 변연이 1cm 미만인 군이 수술 시간이 길었고 수술 후 담즙 누출이 많았으며, 수술 후 병리 결과에서는 간경변이 더 많았다. 그 이외에는 특별한 차이를 보이지 않았다. 5년 동안 재발이 되지 않은 비율을 비교해 보니 절제변연이 1cm 이상인 군이 그렇지 않은 군에 비해서 더 나은 성적을 보였다. 재발에 관계된 여러 요인을 고려한 risk adjusted resctricted spline model에서도 마찬가지로 1cm 이상의 변연을 확보하는 것이 낮은 재발율과 관련이 있었다. 재발에 영향을 미치는 다발성 간세포암이나 수술 전 간동맥색전술이나 고주파열치료를 받은 환자를 제외한 2-5cm 크기의 하나의 간세포암에서는 1cm이상의 절제 변연을 확보한 환자군이 그렇지 못한 환자군보다 5년 재발율이 더 낮았다. 다변량 분석에서도 위의 환자군에서는 비해부학적인 수술과 1cm 미만의 절제 변연이 간세포암 수술 후 재발에 관련된 독립적인 예후인자로 밝혀졌다.

결론: restricted cubic spline model을 통해 간세포암 수술에 있어서 적절한 변연의 길이는 1cm임을 밝혔으나 이는 독립적인 예후인자는 아니었다. 하지만, 2-5cm의 하나의 간세포암에서 첫 치료로 수술을 고려할 때에는 1cm 이상의 변연을 확보하는 것이 간절제술 후 낮은 재발과 관련이 있음을 본 연구를 통해 밝혔다.

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