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ORIGINAL RESEARCH

Prognostic Role of Mitotic Index in Hepatocellular Carcinoma: Potential Clinical Implications for Very Early Stage Disease

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Background: Surgical resection and ablation therapy are both primary treatment options for very early stage hepatocellular carcinoma (HCC). Accurate risk stratification is important, since patients at higher risk of recurrence may derive greater benefit from curative resection than from ablation. We investigated whether the mitotic index is associated with early recurrence in HCC and may serve as a prognostic marker to refine risk assessment in very early stage disease.

Methods: The number of mitoses was counted in representative tumor slides from 942 cases of surgically resected HCC from Samsung Medical Center. A high mitotic index was defined as more than eight mitoses in 10 high-power fields. The relationship between mitotic index, clinicopathological characteristics, and prognosis were analyzed. External validation was performed using 112 HCC cases obtained from Hallym University Sacred Heart Hospital.

Results: High mitotic index was identified in 296 patients and was significantly associated with aggressive clinicopathological features including higher Edmondson grade, advanced American Joint Committee on Cancer T stage, and early tumor recurrence. Patients with a high mitotic index displayed a significantly shorter early recurrence-free survival (e-RFS). In subgroup analysis of patients with very early stage, the high mitotic index group showed unfavorable influences on e-RFS.

Conclusion: High mitotic index is a significant predictor of early recurrence in HCC patients and may provide useful prognostic information in very early stage disease. While its direct role in guiding primary treatment selection is limited, the mitotic index could contribute to risk stratification and postoperative management strategies.

Keywords: hepatocellular carcinoma, prognosis, recurrence, mitosis, mitotic index

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the fourth leading cause of cancer-related deaths worldwide.[1] Surgical resection is the treatment of choice for HCC, but patient prognosis after hepatectomy is unsatisfactory due to the high recurrence rate.[2] Thus, accurate prediction of tumor recurrence using appropriate treatment strategies is crucial for improving patient outcomes.[3]

Several predictive biomarkers for HCC recurrence after surgical resection have been investigated.[4,5] However, most of these biomarkers are not routinely analyzed or used in daily pathological examinations. Therefore, time-efficient, easily applicable, and reproducible predictive markers are urgently needed to stratify HCC patients who are likely to experience early tumor recurrence after surgery.

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High cell proliferation is a classic hallmark of cancer, and mitosis of tumor cells is a simple and useful method for evaluating cell proliferation under microscopic examination.[6,7] The prognostic role of mitotic index has been demonstrated in various types of tumors. Thus, a high mitotic index is now used as a key histologic factor for tumor grading systems in diverse organs, including the breast, ovary, adrenal gland, skin, soft tissue, and gastrointestinal tract.[8–13] However, the prognostic role of mitotic index in HCC as a predictive biomarker has not yet been fully elucidated.

There is still a clinically unmet need for the treatment of HCC patients at a very early stage (Barcelona Clinic Liver Cancer [BCLC] stage 0). Both resection and ablation therapy are recommended, but guidelines for the selection between the two treatments have not been clearly established.[14] Although many studies reported no significant prognostic difference between the two treatment options in HCC patients at a very early stage,[15–23] some studies have demonstrated that the surgical resection may provide superior survival outcomes compared with ablation.[24–29] Therefore, identifying reliable biomarkers of recurrence risk could be helpful in refining treatment selection.

We previously demonstrated that a high mitotic index of tumor cells was significantly associated with shorter disease-specific survival in 282 HCC patients.[7] In the present study, we sought to further evaluate the prognostic effect of mitotic index in a larger cohort (n = 942) and validate the findings in an independent external cohort (n = 112). Specifically, we investigated whether the mitotic index is associated with early tumor recurrence (within 24 months) and whether it could serve as a potential biomarker to inform clinical decision-making, particularly in very early stage HCC.

Methods

Patient Selection

From January 2008 to December 2012, 1297 patients underwent hepatic resection for HCC at the Samsung Medical Center (SMC), Seoul, Korea. Of the 1297 patients, those who received liver transplantation (n = 166) and other treatments including transarterial chemoembolization, radiofrequency ablation (RFA), and chemotherapy before surgery (n = 89) were excluded. Additionally, 2 patients were excluded because of intraoperative RFA and previous operative history. The remaining 1040 patients underwent curative resection as a first-line treatment for primary HCC, and 98 patients were excluded as follows: 5 patients died within 30 days after surgery, 3 patients displayed tumor involvement of the microscopic resection margin on pathologic examination, 24 patients had lymph node or distant metastasis, 57 patients had portal vein invasion, and 9 patients had unavailable slides. Finally, 942 cases that were appropriate for pathological slide review were considered in the retrospective analysis. Figure 1 shows the flowchart of patient selection.

An external cohort of 112 HCC patients were prepared from Hallym University Sacred Heart Hospital (HUMC), Anyang, Republic of Korea, for validation. All patients were treated with surgical resection as first-line treatment for primary HCC between January 2011 and December 2015, and no patients received previous treatment before surgery, exhibited positive tumor resection margin, lymph node or distant metastasis.

Patients' medical records were reviewed to obtain clinical information on age, sex, underlying etiology of chronic liver disease, serum α-fetoprotein (AFP) and albumin-bilirubin (ALBI) score. Histopathologic features including tumor differentiation, microvascular invasion, multicentric occurrence, intrahepatic metastasis, tumor necrosis, and background non-tumor liver pathology, were collected by reviewing pathology reports. Histological differentiation of HCC was determined using the Edmondson-Steiner grading system.[30] Multicentric occurrence and intrahepatic metastasis were determined according to the criteria of the Liver Cancer Study Group of Japan.[31] Two-year cutoffs were used to classify tumor recurrence as early or late.[32] Patients were staged according to the American Joint Committee on Cancer (AJCC) staging manual 8th edition and the BCLC staging system.[33]

After surgery, all patients underwent dynamic contrast-enhanced CT and serum AFP measurement every 3 months for surveillance. Dynamic contrast-enhanced MRI was additionally performed when tumor recurrence was suspected. Tumor recurrence was diagnosed based on radiologic evaluation of CT and/or MRI results.

The primary endpoint was early tumor recurrence within 24 months after surgical resection, which is primarily associated with intrahepatic metastasis, reflecting tumor aggressiveness.[32] This study was approved by the Institutional Review Board of the Samsung Medical Center (IRB No. 2019–08-018) and Hallym University Sacred Heart Hospital (IRB No. 2021–09-008).

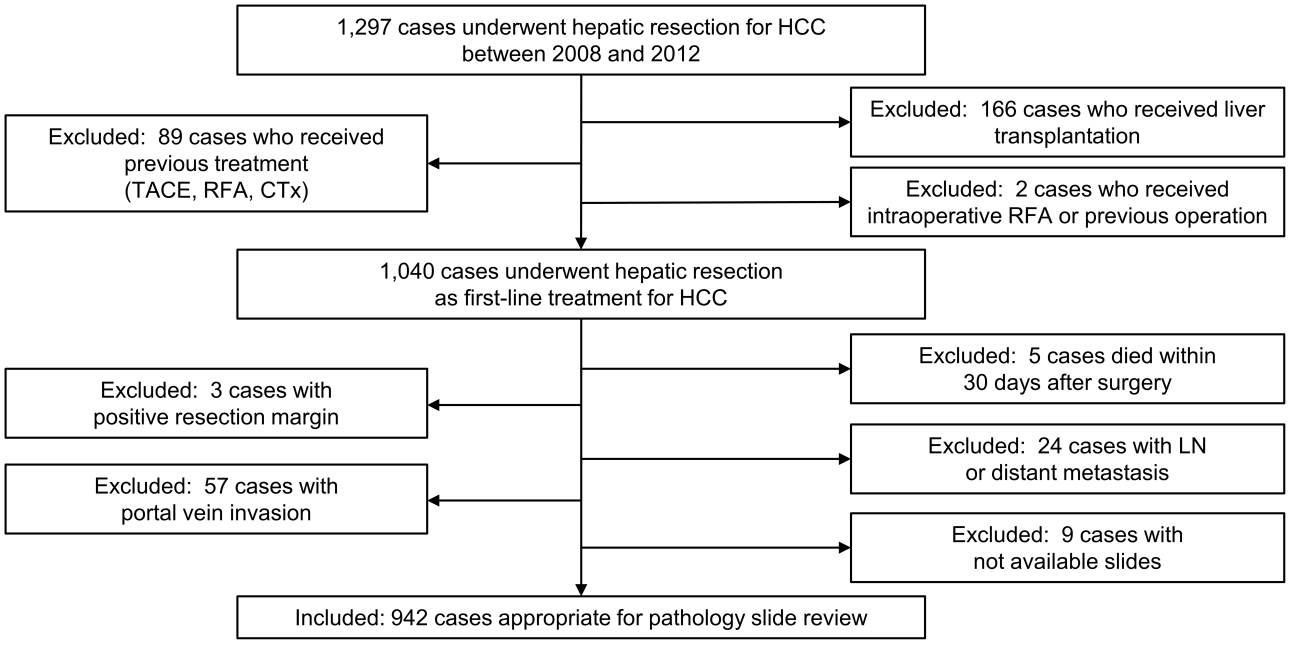

Figure 1 Flowchart of patient selection process.
Abbreviations: HCC, hepatocellular carcinoma; TACE, Transarterial chemoembolization; RFA, Radiofrequency ablation; CTx, chemotherapy; LN, lymph node.

Evaluation of Mitotic Index

The mitotic index of tumor cells was evaluated as described previously.[7] Briefly, two pathologists (SC and SP) examined the hematoxylin and eosin-stained slides and counted the number of mitoses in 10 high-power fields (HPFs). We first identified hotspot areas that contained the most mitotic figures. After counting the mitoses in the hotspot, the count was extended to nine adjacent non-overlapping fields. When mitoses were sparse and randomly distributed throughout the tumor and there was no identifiable hot spot, a representative mitosis was chosen, and the count began with that field. Mitotic figures were assessed based on the criteria of Baak,[34] and were included in the count only when a consensus was achieved between the two pathologists using a multi-head microscope. The mitotic index was determined as the highest mitotic count among the assessed fields. For validation in external cohort, one pathologist (YAC) determined the mitotic index in the same way.

Statistical Analysis

We used the X-Tile statistics package (Yale University, New Haven, CT, USA) to determine the optimal cutoff value of the high mitotic index with the best statistical significance related to patient survival,[35] and cases were dichotomized into low and high mitotic index groups based on the established cutoff value. The relationships between mitotic index and clinicopathological parameters were analyzed using the chi-squared test or Fisher's exact test. The Kaplan-Meier method was used to construct the survival curves. Cox regression analysis was performed to assess the factors independently associated with early tumor recurrence. Statistical analyses and visualization were performed using R version 4.2.2 (https://cran.r-project.org), and a p-value <0.05 (two sided) was considered statistically significant.

Results

Patients Characteristics

The clinicopathological characteristics of 942 SMC patients are summarized in Table 1.

The median age was 56 years (range, 20–83 years); 761 (80.8%) patients were male and 181 (19.2%) were female. The median tumor size was 3.1 cm, and 195 (20.7%) patients had tumors larger than 5 cm. Seven hundred and fifty-nine patients (80.6%) were infected with hepatitis B virus, and 43 (4.6%) were infected with hepatitis C virus. Combined

Table 1 The Association Between Mitotic Index and Clinicopathologic Parameters in All 942 Hepatocellular Carcinoma Patients

	Total n=942	Mitotic Index		p value
		Low (<8) n=646 (%)	High (≥8) n=296 (%)	
Age, year				
≤55	442	279 (43.2)	163 (55.1)	0.001
>55	500	367 (56.8)	133 (44.9)	
Gender				
Female	181	130 (20.1)	51 (17.2)	0.338
Male	761	516 (79.9)	245 (82.8)	
Tumor size, cm				
≤5.0	747	518 (80.2)	229 (77.4)	0.365
>5.0	195	128 (19.8)	67 (22.6)	
Edmondson grade				
1	61	56 (8.7)	5 (1.7)	<0.001*
2	824	574 (88.9)	250 (84.5)	
3	54	15 (2.3)	39 (13.2)	
4	3	1 (0.2)	2 (0.7)	
Microvascular invasion				
(-)	516	404 (62.5)	112 (37.8)	<0.001
(+)	426	242 (37.5)	184 (62.2)	
Intrahepatic metastasis				
(-)	867	597 (92.4)	270 (91.2)	0.616
(+)	75	49 (7.6)	26 (8.8)	
Multicentric occurrence				
(-)	885	601 (93.0)	284 (95.9)	0.111
(+)	57	45 (7.0)	12 (4.1)	
Necrosis				
(-)	583	445 (68.9)	138 (46.6)	<0.001
(+)	359	201 (31.1)	158 (53.4)	
AJCC T-stage				
1a	199	136 (21.1)	63 (21.3)	<0.001*
1b	312	251 (38.9)	61 (20.6)	
2	399	243 (37.6)	156 (52.7)	
3	27	16 (2.5)	11 (3.7)	
4	5	0 (0.0)	5 (1.7)	
BCLC stage				
0	199	136 (21.1)	63 (21.3)	0.754
A	263	176 (27.2)	87 (29.4)	
B	480	334 (51.7)	146 (49.3)	
C	0	0 (0.0)	0 (0.0)	
D	0	0 (0.0)	0 (0.0)	
ALBI Grade				
1	639	431 (66.7)	208 (70.3)	0.533
2	281	200 (31.0)	81 (27.4)	
3	22	15 (2.3)	7 (2.4)	
AFP level, ng/mL				
≤200	705	517 (80.0)	188 (63.5)	<0.001
>200	237	129 (20.0)	108 (36.5)	

(*Continued*)

Table 1 (Continued).

	Total n=942	Mitotic Index		p value
		Low (<8) n=646 (%)	High (≥8) n=296 (%)	
Etiology				
Non-viral	136	120 (18.6)	16 (5.4)	<0.001*
HBV	759	494 (76.5)	265 (89.5)	
HCV	43	29 (4.5)	14 (4.7)	
HBV & HCV	4	3 (0.5)	1 (0.3)	
Liver cirrhosis				
(-)	531	377 (58.4)	154 (52.0)	0.080
(+)	411	269 (41.6)	142 (48.0)	
Early recurrence				
(≤2 years)				
(-)†	683	494 (76.5)	189 (63.9)	<0.001
(+)	259	152 (23.5)	107 (36.1)	
Late recurrence				
(>2 years)				
(-)†	566	409 (82.8)	157 (83.1)	1.000
(+)	117	85 (17.2)	32 (16.9)	

Notes: *by Fisher's exact test, otherwise by chi-square test, † No early or late recurrence.

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; ALBI, albumin-bilirubin; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus.

hepatitis B and C virus infections were identified in 4 (0.4%) patients. Viral markers were not detected in 136 (14.4%) patients. Approximately 54% of patients had AJCC stage 1 disease. The most common BCLC stage was stage B (480/942, 51.0%), followed by stage A (263/942, 27.9%), and stage 0 (199/942, 21.1%). Microvascular invasion, intrahepatic metastasis, and multicentric occurrence were observed in 45.2%, 8.0%, and 6.1% of patients, respectively. Tumor necrosis was observed in 359 patients (38.1%). Approximately 44% of HCCs occur in the background of liver cirrhosis. Tumor recurrence developed in 376 (39.9%) patients, early recurrence in 259 (27.5%), and late recurrence in 117 (12.4%).

Mitotic Index in HCC and Its Association with Clinicopathological Characteristics

The overall mitotic index of 942 HCC is shown in Figure 2. The mean mitotic index was 7.0 (median, 3.5; range, 0–132). The mitotic index was regarded as high when eight or more mitoses were identified in 10 HPF, which was determined as the best cut-off value related to early recurrence-free survival (e-RFS) via the X-tile software. A high mitotic index was observed in 296 patients (31.4%). The association between high and low mitotic indices and clinicopathological characteristics is summarized in Table 1. A high mitotic index was significantly associated with younger age ($p = 0.001$), higher Edmondson grade ($p < 0.001$), microvascular invasion ($p < 0.001$), tumor necrosis ($p < 0.001$), advanced AJCC T stage ($p < 0.001$), increased AFP level ($p < 0.001$), hepatitis B virus infection ($p < 0.001$), and early tumor recurrence ($p < 0.001$).

Very early stage (BCLC stage 0) was identified in 21.1% (199/942) of patients. The correlation between mitotic index and clinicopathological parameters is shown in Table 2. The high mitotic index group was associated with higher Edmondson grade ($p = 0.010$), microvascular invasion ($p = 0.029$), tumor necrosis ($p = 0.012$), and early tumor recurrence ($p = 0.006$).

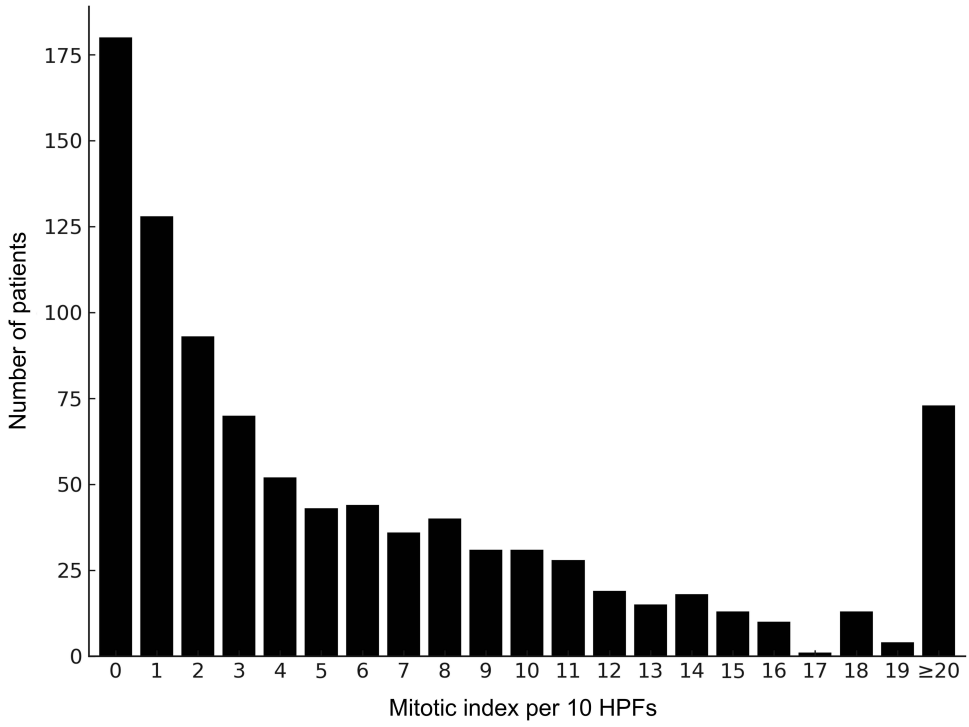

Figure 2 Overview of mitotic index in 942 hepatocellular carcinoma patients from Samsung Medical Center.

Effect of Mitotic Index on Early Recurrence of HCC

Patients in the high mitotic index group showed significantly greater e-RFS (2-year recurrence rate 37.5% vs 24.3%; $p < 0.001$) than those in the low mitotic index group (Figure 3A). Differences in e-RFS were statistically significant in specific subgroups of very early (Figure 3B) and early stage (BCLC stage A) tumors (Figure 3C and D).

Table 2 The Association Between Mitotic Index and Clinicopathologic Parameters in 199 Very Early Stage Hepatocelluar Carcinoma Patients

	Total n=199	Mitotic Index		p value
		Low (<8) n=136 (%)	High (≥8) n=63 (%)	
Age, year				
≤55	101	66 (48.5)	35 (55.6)	0.441
>55	98	70 (51.5)	28 (44.4)	
Gender				
Female	43	34 (25.0)	9 (14.3)	0.128
Male	156	102 (75.0)	54 (85.7)	
Edmondson				
1	26	22 (16.2)	4 (6.3)	0.010
2	165	112 (82.4)	53 (84.1)	
3	5	1 (0.7)	4 (6.3)	
4	3	1 (0.7)	2 (3.2)	
Microvascular invasion				
(-)	156	113 (83.1)	43 (68.3)	0.029
(+)	43	23 (16.9)	20 (31.7)	

(*Continued*)

Table 2 (Continued).

	Total n=199	Mitotic Index		p value
		Low (<8) n=136 (%)	High (≥8) n=63 (%)	
Intrahepatic metastasis				
(-)	197	134 (98.5)	63 (100.0)	1.000
(+)	2	2 (1.5)	0 (0.0)	
Bile duct invasion				
(-)	195	134 (98.5)	61 (96.8)	0.592
(+)	4	2 (1.5)	2 (3.2)	
Multicentric occurrence*				
(-)	194	133 (97.8)	61 (96.8)	0.653
(+)	5	3 (2.2)	2 (3.2)	
Necrosis				
(-)	161	117 (86.0)	44 (69.8)	0.012
(+)	38	19 (14.0)	19 (30.2)	
ALBI Grade				
1	133	90 (66.2)	43 (68.3)	0.936
2	63	44 (32.4)	19 (30.2)	
3	3	2 (1.5)	1 (1.6)	
AFP level, ng/mL				
≤200	166	115 (84.6)	51 (81.0)	0.666
>200	33	21 (15.4)	12 (19.0)	
Etiology*				
Non-viral	13	12 (8.8)	1 (1.6)	0.069
HBV	178	120 (88.2)	58 (92.1)	
HCV	7	3 (2.2)	4 (6.3)	
HBV & HCV	1	1 (0.7)	0 (0.0)	
Liver cirrhosis				
(-)	77	54 (39.7)	23 (36.5)	0.784
(+)	122	82 (60.3)	40 (63.5)	
Early recurrence (≤2 years)				
(-)†	165	120 (88.2)	45 (71.4)	0.006
(+)	34	16 (11.8)	18 (28.6)	
Late recurrence (>2 years)				
(-)†	136	101 (84.2)	35 (77.8)	0.465
(+)	29	19 (15.8)	10 (22.2)	

Notes: *by Fisher's exact test, otherwise by chi-square test, † No early or late recurrence.
Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; ALBI, albumin-bilirubin; AFP, α-fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus.

We performed univariate and multivariate analyses twice, including all patients and patients with very early stage HCC, respectively. On univariate analysis of all patients, early recurrence of HCC was significantly correlated with male sex ($p = 0.038$), larger tumor size ($p < 0.001$), higher Edmondson grade ($p < 0.001$), microvascular invasion ($p < 0.001$), intrahepatic metastasis ($p < 0.001$), multicentric occurrence ($p = 0.005$), advanced AJCC T stage ($p < 0.001$), higher BCLC stage ($p < 0.001$), and a high mitotic index ($p < 0.001$). Multivariable analysis showed that larger tumor size ($p = 0.016$), microvascular invasion ($p < 0.001$), intrahepatic metastasis ($p < 0.001$), multicentric occurrence ($p = 0.002$), and a high mitotic index ($p = 0.009$) were independent predictors of early recurrence (Table 3). The discrimination of this multivariable model exhibited C-index of 0.714 (Standard error[SE], 0.016).

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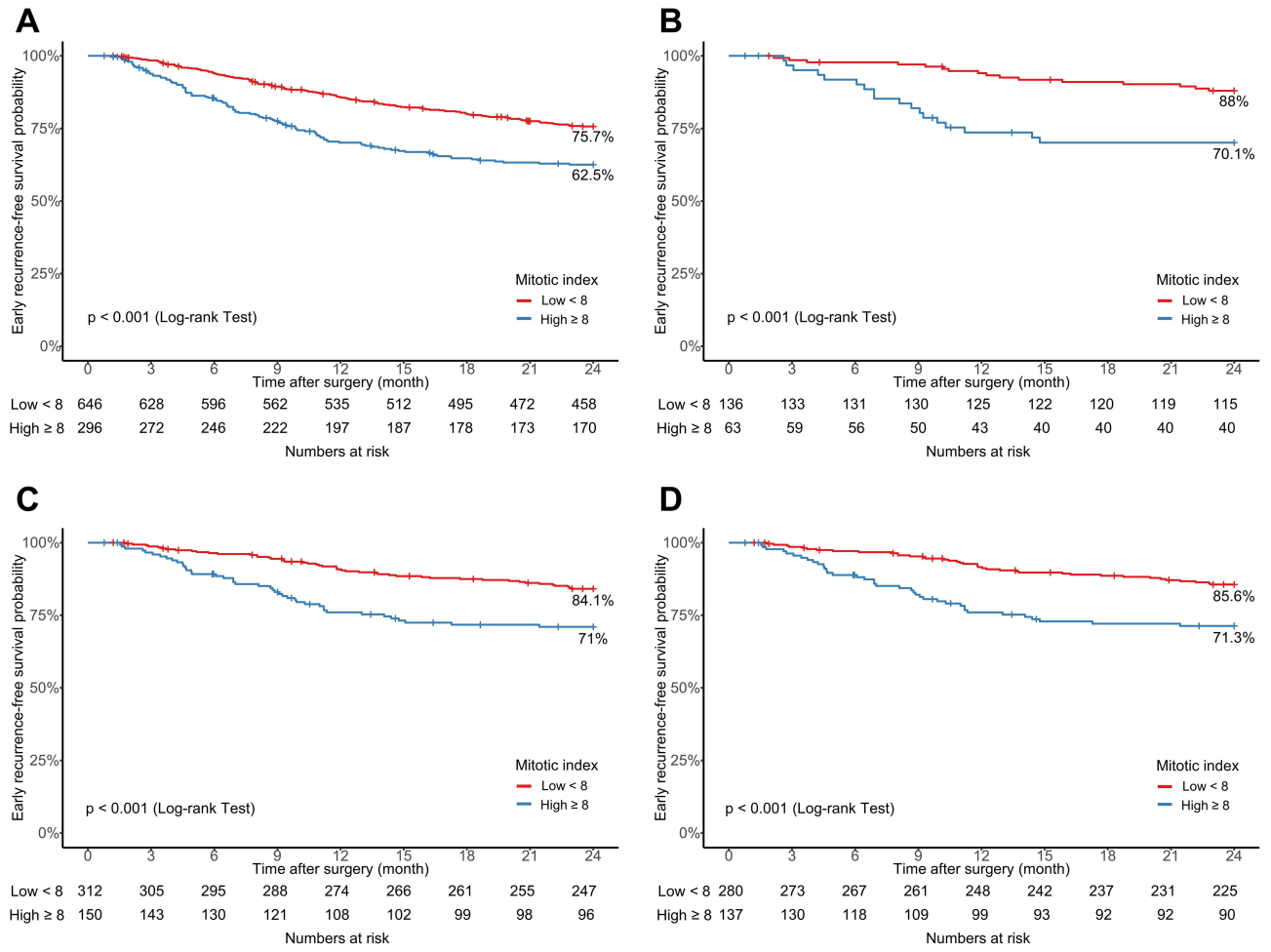

Figure 3 Kaplan-Meier survival curves for early recurrence-free survival in 942 hepatocellular carcinoma patients from Samsung Medical Center. (**A**) All patients. (**B**) very early stage patients. (**C**) very early and early stage (single or multiple tumors) patients. (**D**) very early and early stage (single tumor) patients.

In a subgroup analysis among patients with very early stage, microvascular invasion ($p < 0.001$), and high mitotic index ($p = 0.002$) were significantly associated with early recurrence on univariate analysis (Table 4). In multivariable analysis of very early stage patients, microvascular invasion ($p = 0.007$) and high mitotic index ($p = 0.013$) were independent predictors of early recurrence. The corresponding model yielded a C-index of 0.707 (SE, 0.044), indicating modest discriminatory performance.

Table 3 Univariable and Multivariable Analysis for Early Recurrence-Free Survival in All 942 Hepatocellular Carcinoma Patients

	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Age, year				
>55 vs ≤55	0.92 (0.72–1.17)	0.478	1.01 (0.78–1.31)	0.938
Gender				
Male vs Female	1.44 (1.02–2.03)	0.038	1.41 (0.99–2.01)	0.054
Tumor size, cm				
>5.0 vs ≤5.0	2.52 (1.95–3.25)	< 0.001	1.49 (1.08–2.05)	0.016

(*Continued*)

Table 3 (Continued).

	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Edmondson grade				
3,4 vs 1,2	2.26 (1.51–3.36)	< 0.001	1.39 (0.91–2.13)	0.124
Microvascular invasion				
(+) vs (-)	3.09 (2.38–4)	< 0.001	1.87 (1.37–2.55)	< 0.001
Necrosis				
(+) vs (-)	1.93 (1.51–2.47)	< 0.001	1.25 (0.95–1.64)	0.112
Intrahepatic metastasis				
(+) vs (-)	4.07 (2.98–5.57)	< 0.001	2.64 (1.87–3.72)	< 0.001
Multicentric occurrence				
(+) vs (-)	1.81 (1.2–2.74)	0.005	1.96 (1.28–2.99)	0.002
AJCC T-stage				
2,3,4 vs 1	1.94 (1.35–2.78)	< 0.001	0.90 (0.58–1.4)	0.649
BCLC stage				
B vs 0,A	2.01 (1.55–2.59)	< 0.001	1.38 (0.98–1.96)	0.066
ALBI grade				
2 vs 0.1	0.93 (0.38–2.25)	0.873	1.08 (0.44–2.65)	0.867
AFP level, ng/mL				
>200 vs ≤200	1.29 (0.98–1.69)	0.069	1.00 (0.75–1.34)	0.998
Etiology				
Viral vs Non-viral	1.01 (0.71–1.42)	0.978	1.17 (0.81–1.71)	0.402
Liver cirrhosis				
(+) vs (-)	0.99 (0.78–1.27)	0.953	1.11 (0.85–1.43)	0.445
Mitotic index				
High vs Low	1.79 (1.39–2.29)	< 0.001	1.44 (1.1–1.89)	0.009
Model performance: C-index (SE)	0.714 (0.016)			

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; ALBI, albumin-bilirubin; AFP, α-fetoprotein; SE, standard error.

Table 4 Univariable and Multivariable Analyses for Early Recurrence-Free Survival in 199 Very Early Stage Hepatocellular Patients

	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Age, year				
>55 vs ≤55	1.23 (0.63–2.43)	0.544	1.38 (0.64–2.95)	0.413
Gender				
Male vs Female	1.12 (0.49–2.57)	0.791	1.13 (0.46–2.80)	0.790
Edmondson grade				
3,4 vs 1,2	1.76 (0.42–7.36)	0.436	0.96 (0.21–4.32)	0.955
Microvascular invasion				
(+) vs (-)	3.08 (1.56–6.11)	0.001	2.73 (1.32–5.64)	0.007
Necrosis				
(+) vs (-)	1.74 (0.81–3.72)	0.156	0.98 (0.42–2.27)	0.959
ALBI grade				
2 vs 0.1	0 (0-Inf)	0.997	0 (0-Inf)	0.997
AFP level, ng/mL				
>200 vs ≤200	0.98 (0.38–2.53)	0.968	0.93 (0.34–2.56)	0.893

(*Continued*)

Table 4 (Continued).

	Univariable Analysis		Multivariable Analysis	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Etiology				
Viral vs Non-viral	2.26 (0.31–16.52)	0.422	2.00 (0.26–15.4)	0.506
Liver cirrhosis				
(+) vs (-)	1.24 (0.61–2.55)	0.550	1.06 (0.50–2.27)	0.880
Mitotic index				
High vs Low	2.95 (1.5–5.79)	0.002	2.48 (1.21–5.08)	0.013
Model performance: C-index (SE)	0.707 (0.044)			

Abbreviations: AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; ALBI, albumin-bilirubin; AFP, α-fetoprotein; SE, standard error.

In the main SMC cohort, time-dependent receiver operating characteristic analysis at 24 months revealed that mitotic index ≥8 showed modest predictive ability for early recurrence. The area under the curve was 0.571 for all HCC patients and 0.636 for those with very early HCC (Supplementary Figure 1).

External Validation of Mitotic Index in Independent HUMC Cohort

For 112 HUMC patients, the median age was 59 years (range, 30–95 years); 96 (85.7%) patients were male and 16 (14.3%) were female. The median tumor size was 3.0 cm, and 26 (23.2%) patients had tumors larger than 5 cm. Seventy-three patients (65.2%) were infected with hepatitis B virus, and 7 (6.3%) were infected with hepatitis C virus. Combined hepatitis B and C virus infection were observed in 1 patient (8.9%). Viral markers were not identified in 31 patients. Sixty patients (53.6%) had AJCC stage 1 disease. BCLC stage A (97/112, 86.6%) was most common, followed by stage B (10/112, 8.9%), 0 (4/112, 3.6%), and D (1/112, 0.9%). Microvascular invasion, intrahepatic metastasis, and multicentric occurrence were observed in 39 (34.8%), 6 (5.4%), and 11 (9.8%) patients, respectively. Sixty-four patients (57.1%) had background liver cirrhosis. Early and late tumor reccurences was developed in 29 (25.9%) and 22 (19.6%) patients, respectively. (Supplementary Table 1).

High mitotic index was observed in 27 patients (24.1%) of HUMC cohort, and showed significant association with higher Edmondson grade ($p = 0.010$), tumor necrosis ($p = 0.021$), advanced AJCC T stage ($p = 0.010$), increased AFP level ($p = 0.014$), and early tumor recurrence ($p = 0.005$) (Supplementary Table 1). Very early and early stage (BCLC stage 0 and A) was identified in 3.6% (4/112) and 86.6% (97/112) of patients, respectively (Supplementary Table 2). The high mitotic index group showed significant association with higher Edmondson grade ($p = 0.007$), tumor necrosis ($p = 0.042$) increased AFP level ($p = 0.017$) in a subgroup analysis among patients with very early or early stage HCC.

In similar to SMC cohort, high mitotic index group exhibited significantly greater e-RFS (2-year recurrence rate 50.6% vs 20.3%; $p < 0.001$) than those in the low mitotic index group (Figure 4A). Statistical significance was also observed when very early and early stage patients were analyzed (Figure 4B and C).

Univariate analysis revealed that early tumor recurrence was significantly associated with larger tumor size (p = 0.013), advanced AJCC T stage (p = 0.036), and a high mitotic index (p = 0.001). In multivariate analysis, only a high mitotic index (p = 0.011) was independent predictor of early recurrence (Supplementary Table 3). In univariate and multivariate analysis for HUMC patients with very early or early stage, only a high mitotic index showed significant association ($p = 0.017$) and was an independent predictor ($p = 0.029$) of early tumor recurrence. (Supplementary Table 4) The discrimination of these multivariable models showed C-indices of 0.744 (SE, 0.050) for all HCC patients and 0.730 (SE, 0.049) for the BCLC 0/A subgroup, comparable to the values observed in the main cohort.

In the external validation HUMC cohort, the time-dependent receiver operating characteristic analysis at 24 months demonstrated that mitotic index ≥8 had modest discriminatory power for predicting early recurrence. The area under the curve was 0.655 for all HCC patients and 0.625 for the subgroup with BCLC stage 0/A (Supplementary Figure 2).

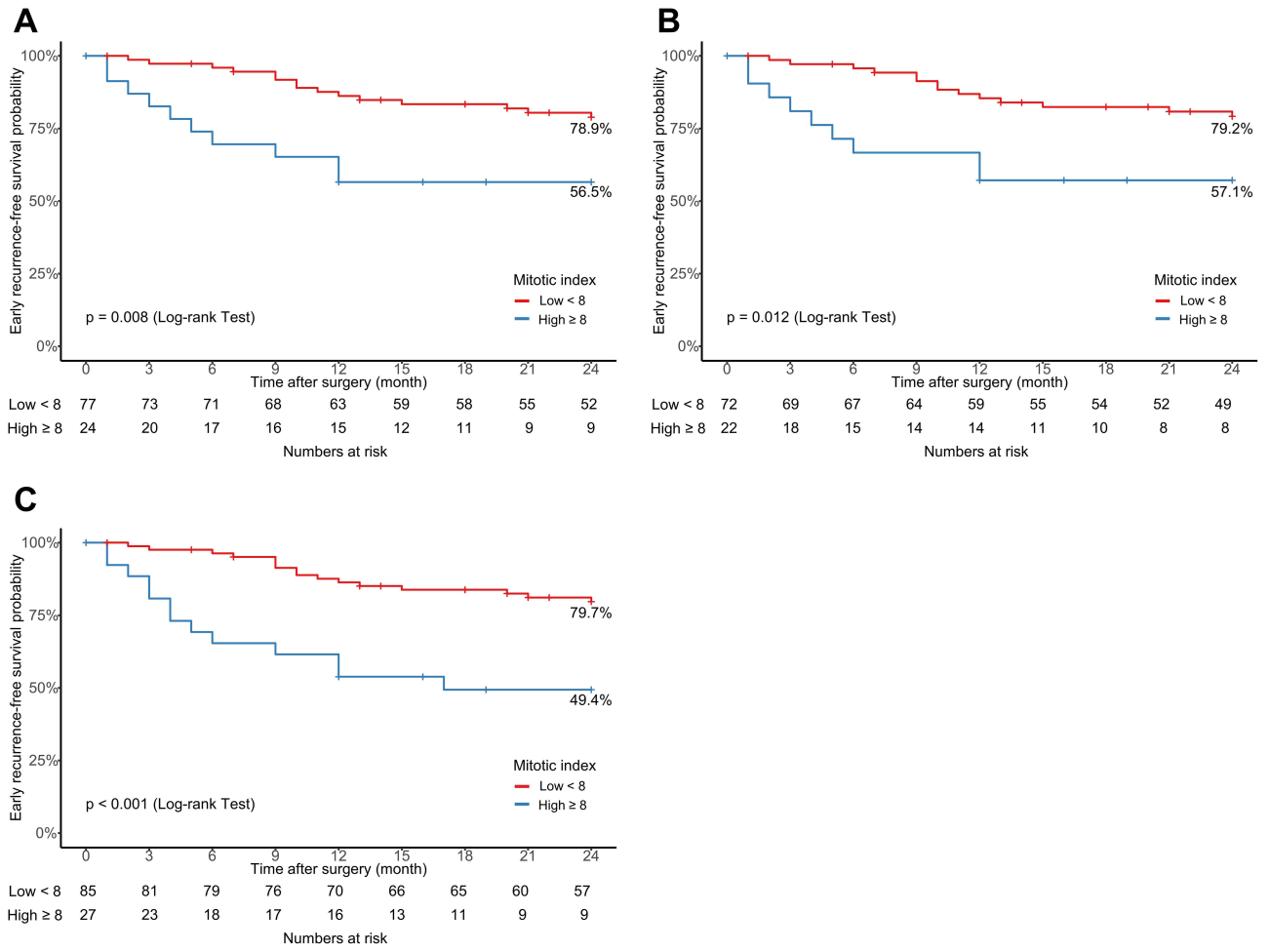

Figure 4 Kaplan-Meier survival curves for early recurrence-free survival in 112 hepatocellular carcinoma patients from Hallym University Medical Center. (**A**) All patients. (**B**) very early and early stage (single or multiple tumors) patients. (**C**) very early and early stage (single tumor) patients.

Discussion

In the present study, we evaluated the prognostic significance of the mitotic index in a large cohort of HCC patients who underwent surgical resection as a primary treatment from two independent institutes. We established an optimal cut-off value for a high mitotic index showing the most significant RFS. In particular, we assessed whether a high mitotic index could accurately predict early tumor recurrence in very early stage HCC patients after surgery.

Several studies have investigated the relationship between the mitotic index and clinicopathological factors in HCC. Ouchi et al reported that a high mitotic index was associated with multicentric occurrence and advanced tumor stage and was also an independent prognostic factor for OS.[36] Nanashima et al demonstrated that a high mitotic index was significantly correlated with vascular invasion, poorly differentiated histology, and tumor recurrence.[37] They also noted that a high mitotic index was associated with a shorter OS in univariate analysis. In our previous study, we also found that a high mitotic index was an independent predictor of shorter disease-specific survival.[7] However, none of the studies have described an association between the mitotic index and early tumor recurrence of HCC. To the best of our knowledge, this is the first report to demonstrate that a high mitotic index is an independent predictor of early recurrence, especially in very early stage HCC.

According to recent treatment strategies used in the management of HCC, two options are recommended as the primary treatment for very early stage HCC: resection or ablation therapy.[14] However, due to conflicting data across studies, there are no standardized criteria for the selection of these two options because it is still controversial which treatment provides

better survival outcomes in very early stage HCC patients. Many studies have reported that there was no significant difference in DFS and OS between patients who underwent surgical resection and ablation therapy.[15–23] In contrast, several other studies have reported that patients with very early stage HCC who underwent surgical resection showed better OS and DFS than those who received ablation therapy.[24,26–29,38] Combining the previous results, it can be estimated that the surgical resection group had a better, or at least similar, prognosis to RFA in terms of RFS. Thus, surgical resection should be considered as the first treatment option to achieve better RFS for HCC with a high risk of recurrence.

As pathological examination was not routinely performed before HCC treatment, none of the previous studies evaluated histopathological findings as independent variables, which might have affected the conflicting results among previous studies. Tumor differentiation, Edmondson grade, microvascular invasion, necrosis, and mitotic index can be included as variables for predicting RFS and may offer individualized risk stratification for recurrence after RFA or surgical resection.

In the present study, we demonstrated that a high mitotic index was an independent predictor of early recurrence in a large cohort of HCC patients and independent external cohort from other institute. Moreover, subgroup analysis among patients with very early stage HCC showed that a high mitotic index was associated with unfavorable early recurrence. Importantly, the discriminatory performance of mitotic index was reproducible across both cohorts, with time-dependent AUC values ranging from 0.57 to 0.66 and C-indices around 0.71–0.74. Although these values indicate only modest predictive ability, they strengthen the robustness of our findings and suggest that mitotic index may provide clinically meaningful information when used in conjunction with other pathological or imaging factors. These findings support the role of mitotic index in early recurrence risk estimation and suggest its potential value in providing additional guidance for treatment decision-making or postoperative surveillance in very early stage HCC.

We acknowledge, however, that the immediate clinical applicability of mitotic index is limited, since it is most commonly evaluated in resection specimens and not routinely available prior to treatment. Nevertheless, mitotic figures can also be assessed in liver biopsy samples when performed, which indicates that mitotic index is not entirely restricted to postoperative assessment. Although liver biopsy is not performed in all very early HCC patients, preoperative assessment in selected cases may provide additional prognostic information. Furthermore, as counting mitoses is a simple, inexpensive, and widely available histologic method, mitotic index could contribute to histological staging systems and postoperative surveillance strategies.

While direct histologic assessment of mitotic index is not routinely available before surgery, non-invasive imaging approaches may provide complementary information. In particular,[18] F-fluorodeoxyglucose (FDG) uptake on positron emission transmission (PET)/CT has been widely studied as a prognostic marker in HCC, and several reports have shown that higher FDG uptake correlates with unfavorable tumor biology and poorer postoperative outcomes.[39–41] Moreover, FDG uptake has been positively associated with Ki-67 expression in HCC,[42] and mitotic index and Ki-67 show a strong correlation.[36] Taken together, these findings raise the possibility that FDG uptake may indirectly reflect mitotic activity and thus serve as a surrogate marker for tumor proliferative potential. Although FDG PET/CT is not universally applied in all very early HCC patients, its integration with histologic factors such as mitotic index could enhance risk stratification and provide additional guidance for surveillance or adjuvant strategies in selected cases. While further validation is required, the convergence of histologic and metabolic imaging markers represents a promising direction for improving individualized prognostic assessment in HCC.

This study has several limitations. First, its retrospective design may be subject to inherent selection bias, and both cohorts were derived from institutions within a single country, which may limit the generalizability of our findings to other populations. Second, although mitotic figures were assessed by two pathologists with consensus, some degree of interobserver variability cannot be excluded, and the cut-off value used in this study has not yet been universally validated. Third, while we attempted external validation, the relatively small sample size of the external cohort reduced statistical power, particularly for subgroup analyses. Finally, the clinical applicability of mitotic index remains limited, since it is usually assessed in surgical specimens and not routinely available preoperatively; future studies incorporating biopsy assessment or integration with non-invasive imaging markers will be necessary to establish its role in guiding treatment decisions.

Conclusion

In conclusion, we demonstrated that a high mitotic index of HCC is associated with aggressive clinicopathological parameters, including a higher risk of early recurrence, particularly in very early stage disease. Mitotic index may serve as a useful predictive and prognostic biomarker, contributing to risk stratification in clinical practice. While its direct role in primary treatment decision-making is limited, it could provide additional information to support individualized management strategies and postoperative surveillance in patients with very early HCC.

Ethics Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Institutional Review Board of the Samsung Medical Center (IRB No. 2019-08-018) and Hallym University Sacred Heart Hospital (IRB No. 2021-09-008). The requirement for informed consent was waived because this was a retrospective study using de-identified medical records, which posed minimal risk to participants. All data were anonymized prior to analysis to ensure patient confidentiality.

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Disclosure

The authors declare that they have no conflict of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca A Cancer J Clinicians*. 2018;68(6):394–424. doi:10.3322/caac.21492
2. Sherman M. Recurrence of hepatocellular carcinoma. *N Engl J Med*. 2008;359(19):2045–2047. doi:10.1056/NEJMe0807581
3. Portolani N, Coniglio A, Ghidoni S, et al. Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. *Ann Surg*. 2006;243(2):229–235. doi:10.1097/01.sla.0000197706.21803.a1
4. Chaiteerakij R, Zhang X, Addissie BD, et al. Combinations of biomarkers and Milan criteria for predicting hepatocellular carcinoma recurrence after liver transplantation. *Liver Transpl*. 2015;21(5):599–606. doi:10.1002/lt.24117
5. Zacharakis G, Aleid A, Aldossari KK. New and old biomarkers of hepatocellular carcinoma. *Hepatoma Res*. 2018;4(10). doi:10.20517/2394-5079.2018.76
6. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100(1):57–70. doi:10.1016/s0092-8674(00)81683-9
7. Ha SY, Choi M, Lee T, Park CK. The prognostic role of mitotic index in hepatocellular carcinoma patients after curative hepatectomy. *Cancer Res Treat*. 2016;48(1):180–189. doi:10.4143/crt.2014.321
8. Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer*. 1957;11(3):359–377. doi:10.1038/bjc.1957.43
9. Costa J, Wesley RA, Glatstein E, Rosenberg SA. The grading of soft tissue sarcomas. Results of a clinicohistopathologic correlation in a series of 163 cases. *Cancer*. 1984;53(3):530–541. doi:10.1002/1097-0142(19840201)53:3<530::aid-cncr2820530327>3.0.co;2-d
10. Silverberg SG. Histopathologic grading of ovarian carcinoma: a review and proposal. *Int J Gynecol Pathol*. 2000;19(1):7–15. doi:10.1097/00004347-200001000-00003
11. Aubert S, Wacrenier A, Leroy X, et al. Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. *Am J Surg Pathol*. 2002;26(12):1612–1619. doi:10.1097/00000478-200212000-00009
12. Fletcher CDM, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum Pathol*. 2002;33(5):459–465. doi:10.1053/hupa.2002.123545
13. Azzola MF, Shaw HM, Thompson JF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: an analysis of 3661 patients from a single center. *Cancer*. 2003;97(6):1488–1498. doi:10.1002/cncr.11196
14. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021;7(1):6. doi:10.1038/s41572-020-00240-3
15. Li YC, Chen PH, Yeh JH, et al. Clinical outcomes of surgical resection versus radiofrequency ablation in very-early-stage hepatocellular carcinoma: a propensity score matching analysis. *BMC Gastroenterol*. 2021;21(1):418. doi:10.1186/s12876-021-01995-z
16. Tohme S, Geller DA, Cardinal JS, et al. Radiofrequency ablation compared to resection in early-stage hepatocellular carcinoma. *HPB*. 2013;15(3):210–217. doi:10.1111/j.1477-2574.2012.00541.x
17. Pompili M, Saviano A, de Matthaeis N, et al. Long-term effectiveness of resection and radiofrequency ablation for single hepatocellular carcinoma </=3 cm. Results of a multicenter Italian survey. *J Hepatol*. 2013;59(1):89–97. doi:10.1016/j.jhep.2013.03.009
18. Feng K, Yan J, Li X, et al. A randomized controlled trial of radiofrequency ablation and surgical resection in the treatment of small hepatocellular carcinoma. *J Hepatol*. 2012;57(4):794–802. doi:10.1016/j.jhep.2012.05.007

19. Hung HH, Chiou YY, Hsia CY, et al. Survival rates are comparable after radiofrequency ablation or surgery in patients with small hepatocellular carcinomas. *Clin Gastroenterol Hepatol*. 2011;9(1):79–86. doi:10.1016/j.cgh.2010.08.018
20. Cho YK, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: a Markov model analysis. *Hepatology*. 2010;51(4):1284–1290. doi:10.1002/hep.23466
21. Livraghi T, Meloni F, Di Stasi M, et al. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: is resection still the treatment of choice? *Hepatology*. 2008;47(1):82–89. doi:10.1002/hep.21933
22. Choi D, Lim HK, Rhim H, et al. Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. *Eur Radiol*. 2007;17(3):684–692. doi:10.1007/s00330-006-0461-5
23. Chen MS, Li JQ, Zheng Y, et al. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg*. 2006;243(3):321–328. doi:10.1097/01.sla.0000201480.65519.b8
24. Chu HH, Kim JH, Kim PN, et al. Surgical resection versus radiofrequency ablation very early-stage HCC (</=2 cm Single HCC): a propensity score analysis. *Liver Int*. 2019;39(12):2397–2407. doi:10.1111/liv.14258
25. Liu PH, Hsu CY, Lee YH, et al. When to perform surgical resection or radiofrequency ablation for early hepatocellular carcinoma?: a nomogram-guided treatment strategy. *Medicine*. 2015;94(43):e1808. doi:10.1097/MD.0000000000001808
26. Cucchetti A, Piscaglia F, Cescon M, et al. Cost-effectiveness of hepatic resection versus percutaneous radiofrequency ablation for early hepatocellular carcinoma. *J Hepatol*. 2013;59(2):300–307. doi:10.1016/j.jhep.2013.04.009
27. Wang JH, Wang CC, Hung CH, Chen CL, Lu SN. Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. *J Hepatol*. 2012;56(2):412–418. doi:10.1016/j.jhep.2011.05.020
28. Li L, Zhang J, Liu X, Li X, Jiao B, Kang T. Clinical outcomes of radiofrequency ablation and surgical resection for small hepatocellular carcinoma: a meta-analysis. *J Gastroenterol Hepatol*. 2012;27(1):51–58. doi:10.1111/j.1440-1746.2011.06947.x
29. Huang J, Yan L, Cheng Z, et al. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg*. 2010;252(6):903–912. doi:10.1097/SLA.0b013e3181efc656
30. Edmondson H, Steiner P. Primary carcinoma of the liver: a study of 100 cases among 48,900 necropsies. *Cancer*. 1954;7:462–503.
31. Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*. 2nd ed. 2003.
32. Imamura H, Matsuyama Y, Tanaka E, et al. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. Research Support, Non-U.S. Gov't. *J Hepatol*. 2003;38(2):200–207.
33. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Research Support, Non-U.S. Gov't. *Semin Liver Disease*. 1999;19(3):329–338. doi:10.1055/s-2007-1007122
34. Baak JP. Mitosis counting in tumors. *Hum Pathol*. 1990;21(7):683–685. doi:10.1016/0046-8177(90)90026-2
35. Camp RL, Dolled-Filhart M, Rimm DL. X-Tile. A new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res*. 2004;10(21):7252–7259. doi:10.1158/1078-0432.Ccr-04-0713
36. Ouchi K, Sugawara T, Ono H, et al. Mitotic index is the best predictive factor for survival of patients with resected hepatocellular carcinoma. *Dig Surg*. 2000;17(1):42–48. doi:10.1159/000018799
37. Nanashima A, Tanaka K, Yamaguchi H, et al. Fibrosis and inflammatory activity in noncancerous tissue and mitotic index of cancer tissue in patients with hepatocellular carcinoma: relationship to clinicopathological factors and prognosis after hepatic resection. *Dig Dis Sci*. 2003;48(8):1517–1522. doi:10.1023/a:1024759606402
38. Liu PH, Hsu CY, Hsia CY, et al. Surgical resection versus radiofrequency ablation for single hepatocellular carcinoma </= 2 cm in a propensity score model. *Ann Surg*. 2016;263(3):538–545. doi:10.1097/SLA.0000000000001178
39. Detry O, Govaerts L, Deroover A, et al. Prognostic value of (18)F-FDG PET/CT in liver transplantation for hepatocarcinoma. *World J Gastroenterol*. 2015;21(10):3049–3054. doi:10.3748/wjg.v21.i10.3049
40. Hwang SH, Lee JW, Cho HJ, Kim KS, Choi GH, Yun M. Prognostic value of metabolic tumor volume and total lesion glycolysis on preoperative 18F-FDG PET/CT in patients with very early and early hepatocellular carcinoma. *Clin Nucl Med*. 2017;42(1):34–39. doi:10.1097/RLU.0000000000001449
41. Ida Y, Tamai H, Shingaki N, et al. Prognostic value of (18)F-fluorodeoxyglucose positron emission tomography in patients with small hepatocellular carcinoma treated by radiofrequency ablation. *Cancer Imaging*. 2020;20(1):74. doi:10.1186/s40644-020-00356-5
42. Yin Y, Liu J, Sun R, et al. Exploring the efficacy of (18)F-FDG PET/CT in hepatocellular carcinoma diagnosis: role of Ki-67 index and tumor differentiation. *Abdom Radiol*. 2023;48(11):3408–3419. doi:10.1007/s00261-023-04027-4