Original Article
Comparison of portal and capsular microscopic vascular invasion in the outcomes of early HCC after curative resection

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Abstract: Microscopic vascular invasion (MVI) is a strong risk factor associated with tumor recurrence and poor overall survival (OS) among hepatocellular carcinoma (HCC) patients after resection. Two types of MVI are identified: portal vein and capsular vein invasion. However, little is known about the impact of different types of MVI on HCC recurrence. The present study aimed to compare HCC recurrence and OS between the portal vein and capsule vein MVI. Patients with Barcelona Clinic Liver Cancer (BCLC) stage 0 or A HCC who underwent primary resection between January 2001 and June 2016 were consecutively recruited. Factors that influenced OS and recurrence-free survival (RFS) were analyzed using Cox proportional hazards models. Of the 857 eligible patients, 327 (38.2%) had MVI, and 530 (61.8%) were without MVI. Of the 327 patients with MVI, 85 (26.0%) were with portal vein, 178 (54.4%) with capsular vein, and 64 (19.6%) with both-MVI type. Patients with both-MVI type suffered from a higher proportion of BCLC stage A ($P < 0.001$), capsular invasion ($P = 0.002$), and satellite nodules ($P < 0.001$). Both-MVI type is an independent risk factor for HCC recurrence (hazard ratio [HR]: 1.69; 95% CI, 1.22-2.36, $P = 0.002$) and mortality (HR: 2.29; 95% CI, 1.59-3.29, $P < 0.001$) compared with non-MVI. We further found that both-MVI type was significantly associated with a higher risk of extrahepatic recurrence (EHR) (HR: 8.74; 95% CI, 2.38-32.03, $P = 0.001$). Among HCC patients after curative resection, concurrent portal and capsular MVI is a risk factor for HCC recurrence, especially for EHR, in comparison with non-MVI or only portal or capsular MVI alone.

Keywords: Hepatocellular carcinoma, microscopic vascular invasion, overall survival

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer occurrence and the third leading cause of cancer-related death in the world [1, 2]. Several international associations have proposed guidelines about the diagnosis, staging, and treatment of HCC [3-5]. Most of the HCC therapeutic strategies are based on the Barcelona Clinic Liver Cancer (BCLC) stage, which considers the tumor number, tumor size, patients' performance, and Child-Pugh grade.

The primary liver resection has been applied as one of the curative treatments for early-stage (BCLC stage 0 or A) HCC [3-7]. However, the 5-year tumor recurrence of approximately 54.8% remains a clinical problem [8]. Previous studies have proposed several risk factors and predictors of poor prognosis and tumor recurrence [9, 10], which include pathologic features.

Microscopic vascular invasion (MVI) is one of the pathologic features and has been demon-
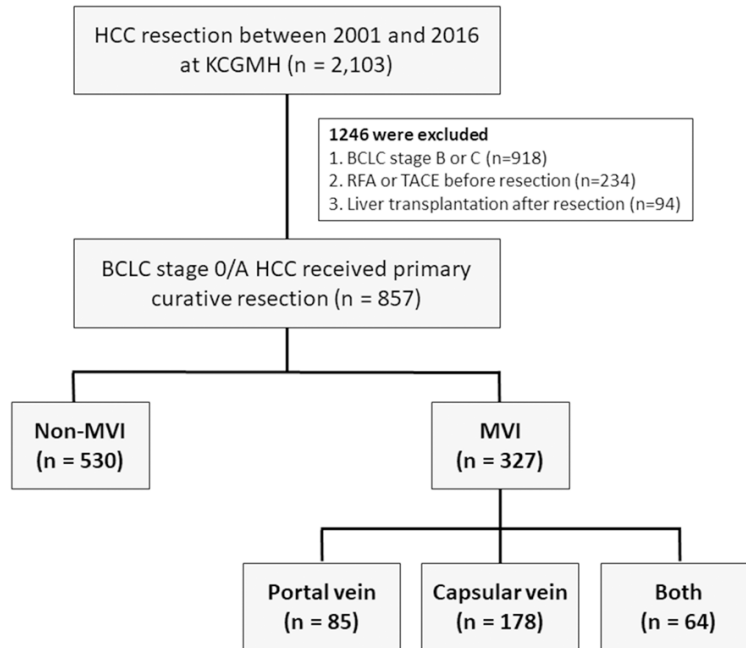
Figure 1. Flowchart of the study population.

strated as a strong risk factor for tumor recurrence and poor overall survival (OS) among patients after HCC resection [11-17]. Previous studies have proposed numerous classifications of MVI [18-20], which include those based on the number of vessels with MVI, the number of cancer cells within a vascular lumen, and the distance of MVI from the primary tumor. However, these classifications seem too complicated for daily practice, and no universal consensus about the MVI classification is currently put in place. Recently, a novel and simple MVI classification was reported that divided MVI into microscopic portal vein invasion and microvessel invasion [20]. This classification strengthened the importance of the microscopic portal vein invasion. Kuo et al. validated this MVI classification, in which the patients with microscopic portal vein invasion had worse 5-year OS than patients with microvessel invasion only or patients without MVI [13].

In our daily practice, two types of MVI are identified: portal vein and capsule vein. However, little is known about the impact of the different types of MVI on HCC recurrence. This study aimed to compare the HCC recurrence and OS between the portal vein and capsule vein MVI and provide the implication for further active surveillance or adjuvant therapies in MVI patients.

Patients and methods

Patient enrollment and methods

A total of 2103 patients who received HCC resection between 2001 and 2016 at Kaohsiung Chang Gung Memorial Hospital (KCGMH) were recruited. After excluding patients with (1) BCLC stage B or C, (2) patients who received radiofrequency ablation (RFA) or transarterial chemoembolization (TACE) before resection, and (3) patients who underwent liver transplantation after resection, 857 patients with BCLC stage 0 or A HCC who underwent primary resection were enrolled in this study (**Figure 1**).

The pathologic slides were reviewed by the experienced pathologist (Ting-Ting Liu, M.D.) and diagnosed as different types of MVI (portal vein and capsular vein invasion types) (**Figure 2**). The patients with MVI were stratified into portal vein type, capsule vein type, or both type (concurrent portal and capsule vein) MVI. The capsule vein type MVI is defined as an MVI in a tumor capsule or peri-tumoral areas. The portal vein type MVI is defined as MVI in the surrounding portal vein.

Baseline characteristic data including demographic, serum biochemistry, and tumor characteristics were retrieved from the medical record. Histopathological diagnosis and the type of MVI were confirmed by the pathologists. Child-Pugh grade and BCLC stage were documented following current guidelines and international consensus. The diagnosis of HCC was based on the American Association for the Study of Liver Disease and the European Association for the Study of the Liver (EASL) guidelines [7, 21]. The diagnosis of liver cirrhosis was based on pathological reports and Ishak score 5-6 [22]. We adopted a new definition of metabolic-associated fatty liver disease
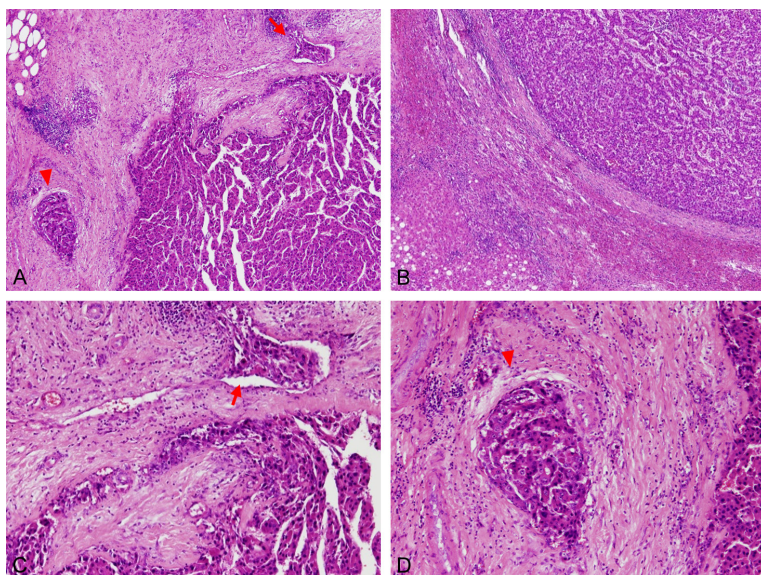
Figure 2. Pathological features of the portal and capsular MVI. Sections after tumor resection were reviewed under microscopy by experienced pathologists. (A) Both capsule vein MVI (arrow) and portal vein MVI (arrowhead) under 40× magnification, (B) No capsular vein or portal vein MVI surrounding the tumor bed under 40× magnification, (C) Capsular vein MVI under 100× magnification: MVI (arrow) in surrounding tumor capsule (D) Portal vein MVI under 100× magnification: MVI in the portal vein with surrounding bile duct and hepatic arteriole.

confirmed by contrast-induced computed tomography (CT) or magnetic resonance imaging (MRI). Bone scintigraphy, positron emission tomography-CT, brain CT, MRI, or pathological examination were applied as part of the investigation in some patients. Recurrence-free survival (RFS) was defined as the period from tumor resection to the detection of recurrence. The OS was defined as the period from tumor removal by resection to the death, to the last contact, or by December 31, 2020.

Statistical analysis

We performed statistical analysis using SPSS 23.0 statistical package (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean (± standard deviation (SD)) for quantitative variables with normal distribution or as the median for variables without normal distribution. The differences in continuous and categorical variables across the four groups were assessed using ANOVA and Chi-square. The RFS and OS in different types of MVI were analyzed using Kaplan-Meier survival curves and the log-rank test. Chi-square test and Fisher's exact test were applied in the analysis for categorical variables. A p-value < 0.05 was considered a statistical significance level.

Analysis of predictor factors for RFS and OS was performed by the Cox proportional hazards model. To reduce the probability of false positive in multivariate analysis, all potential risk factors ($P < 0.1$ in the univariate analysis) were required to be included in the multivariate analysis for the HCC recurrence, all-cause mortality, and HCC EHR. In this study, we demonstrated two models in multivariate analysis. In the multivariate model 1, all potential risk factors ($P < 0.1$ in the univariate analysis) were forced into the model. For the multivariate model 2, it used forward stepwise conditional LR method with $P < 0.05$ as selection criterion. As demonstrated in the separated tables for

(MAFLD) rather than nonalcoholic fatty liver disease based on our acknowledgment of the pathogenesis and its impact on HBV-related early-stage HCC after curative liver resection [23, 24]. The diagnosis of MAFLD following the 2020 international consensus of EASL is based on the presence of liver steatosis in addition to either overweight/obesity, presence of type 2 diabetes mellitus (DM), or presence of at least two risk factors of metabolic dysfunction [25].

The diagnosis of HCC recurrence was based on the typical imaging findings or continual elevation of serum alpha-fetoprotein (AFP) level. Biopsies were performed and histopathology evidence for the recurrence was assessed in the patients with controversial image or lab findings. Furthermore, recurrences were divided into either early recurrence (< 2 years) or late recurrence (≥ 2 years), which were from different pathogenesis [26]. Extrahepatic recurrence (EHR) was defined as situation that the patients who have EHR with or without concurrent intrahepatic recurrence as their first recurrence. Patients with previous local recurrence were excluded. The diagnosis of EHR was

Table 1. Patient Characteristics of the study population

	Total (n = 857)	MVI (n = 327)	Non-MVI (n = 530)	P value
Age (years), mean ± SD	58.6 ± 11.5	58.9 ± 11.4	58.5 ± 11.6	0.645
Male, n (%)	670 (78.2)	259 (79.2)	411 (77.5)	0.568
Diabetes mellitus, n (%)	222 (25.9)	89 (27.2)	133 (25.1)	0.491
HBV, n (%)	484 (56.5)	185 (56.5)	299 (56.4)	0.963
HCV, n (%)	300 (35.0)	129 (39.4)	171 (32.3)	0.032
AST > 40 U/L, n (%)	321 (37.5)	119 (36.4)	202 (38.1)	0.613
ALT > 40 U/L, n (%)	374 (43.6)	139 (42.5)	235 (44.3)	0.599
Platelets < 150 10^3/µL, n (%)	411 (48.0)	171 (52.3)	240 (45.3)	0.046
Albumin (g/dL); mean ± SD	3.6 ± 0.6	3.6 ± 0.6	3.7 ± 0.6	0.129
Liver cirrhosis, n (%)	400 (46.7)	157 (48.0)	243 (45.8)	0.537
Child-Pugh grade, n (%)				0.216
A	786 (91.7)	295 (90.2)	491 (92.6)	
B	71 (8.3)	32 (9.8)	39 (7.4)	
BCLC stage, n (%)				< 0.001
0	124 (14.5)	6 (1.8)	118 (22.3)	
A	733 (85.5)	321 (98.2)	412 (77.7)	
AFP > 10 ng/mL, n (%)	446 (52.5)	198 (60.6)	248 (46.8)	< 0.001
Tumor size (cm)[a]; mean ± SD	2.9 ± 1.0	3.0 ± 1.0	2.8 ± 1.0	< 0.001
Tumor number, n (%)				0.186
Single	780 (91.0)	303 (92.7)	477 (90.0)	
Multiple	77 (9.0)	24 (7.3)	53 (10.0)	
Histological grade n (%)				< 0.001
Well	111 (13.1)	13 (4.0)	98 (18.8)	
Moderate	715 (84.3)	302 (92.6)	413 (79.1)	
Poor	22 (2.6)	11 (3.4)	11 (2.1)	
Capsule invasion[b], n (%)	738 (86.3)	283 (86.5)	455 (86.2)	0.878
Satellite nodule, n (%)	21 (2.5)	19 (5.8)	2 (0.4)	< 0.001
MAFLD, n (%)	105 (23.3)	43 (22.1)	62 (24.2)	0.589

Data are expressed as mean ± standard deviation or n (%). Abbreviations: MVI, microvascular invasion; CHB, chronic hepatitis B; CHC, chronic hepatitis C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; BCLC, Barcelona clinical liver cancer; MAFLD, metabolic-associated fatty liver disease. [a]Diameter of the largest tumor nodule. [b]Including partial and totally capsule invasion, and no well capsule.

different prognosis, the multivariate model 2 was better for the interpretation. All the statistical results in this article referred to the multivariate model 2.

Results

Characteristics of the study population

Patients' baseline characteristics are shown in **Table 1**. A total of 670 (78.2%) men and 187 (21.8%) women were recruited, with a mean age of 58-year-old at enrollment. A total of 222 (25.9%) patients had DM; 484 patients (56.5%) had chronic hepatitis B (CHB), and 300 (35.0%) had chronic hepatitis C (CHC), similar to the contributions of CHB and CHC in Taiwan HCC cohort [27]. A total of 400 pati-

ents (46.7%) suffered from liver cirrhosis, and 446 patients (52.5%) had high preoperative AFP levels (> 10 ng/mL). The majority in this population consisted of Child-Pugh grade A (91.7%) and BCLC stage A (85.5%). The mean tumor size was 2.9 ± 1.0 cm, and 780 patients (91.0%) had a single tumor. Most of the patients (84.3%) had a tumor in moderate differentiation and (86.3%) with capsular invasion. The mean follow-up duration was 87.2 ± 44.5 months.

Patients with MVI were 327 (38.2%) in this population. In comparison with non-MVI group, MVI group had higher proportion of BCLC stage A (P < 0.001), higher serum AFP level (P < 0.001), larger tumor size (P < 0.001), more sat-

Table 2. Clinicopathological characteristics of HCC patients with different types of MVI

	MVI (n = 327)	MVI-portal vein (n = 85)	MVI-capsular vein (n = 178)	MVI-both (n = 64)	P value
Age (years), mean ± SD	58.9 ± 11.4	59.4 ± 11.5	58.7 ± 10.9	58.7 ± 11.6	0.889
Male, n (%)	259 (79.2)	61 (71.8)	143 (80.3)	55 (85.9)	0.093
Diabetes mellitus, n (%)	89 (27.2)	26 (30.6)	39 (21.9)	24 (37.5)	0.040
HBV, n (%)	185 (56.6)	47 (55.3)	101 (56.7)	37 (57.8)	0.952
HCV, n (%)	129 (39.4)	40 (47.1)	61 (34.3)	28 (43.8)	0.103
AST > 40 U/L, n (%)	119 (36.4)	31 (36.5)	65 (36.5)	23 (35.9)	0.996
ALT > 40 U/L, n (%)	139 (42.5)	29 (34.1)	80 (44.9)	30 (46.9)	0.185
Platelets < 150 10^3/μL, n (%)	171 (52.3)	47 (55.3)	92 (51.7)	32 (50.0)	0.791
Albumin (g/dL); mean ± SD	3.6 ± 0.6	3.6 ± 0.6	3.6 ± 0.6	3.4 ± 0.7	0.225
Liver cirrhosis, n (%)	157 (48.0)	43 (50.6)	82 (46.1)	32 (50.0)	0.742
Child-Pugh grade, n (%)					0.678
A	295 (90.2)	78 (91.8)	161 (90.4)	56 (87.5)	
B	32 (9.8)	7 (8.2)	17 (9.6)	8 (12.5)	
BCLC stage, n (%)					< 0.001
0	6 (1.8)	4 (4.7)	2 (1.1)	0 (0)	
A	321 (98.2)	81 (95.3)	176 (98.9)	64 (100)	
AFP > 10 ng/mL, n (%)	198 (60.6)	52 (61.2)	102 (57.3)	44 (68.8)	0.272
Tumor size (cm)[a]; mean ± SD	3.0 ± 1.0	2.9 ± 0.9	3.0 ± 1.1	3.2 ± 0.9	0.320
Tumor number, n (%)					0.065
Single	303 (92.7)	81 (95.3)	167 (93.8)	55 (95.9)	
Multiple	24 (7.3)	4 (4.7)	11 (6.2)	9 (4.1)	
Histological grade n (%)					0.939
Well	13 (4.0)	3 (3.6)	7 (3.9)	3 (4.7)	
Moderate	302 (92.6)	77 (91.7)	166 (93.3)	59 (92.2)	
Poor	11 (3.4)	4 (4.8)	5 (2.8)	2 (3.1)	
Capsule invasion[b], n (%)	283 (86.5)	65 (76.5)	164 (92.1)	54 (84.4)	0.002
Satellite nodule, n (%)	19 (5.8)	11 (2.9)	1 (0.6)	7 (10.9)	< 0.001
MAFLD, n (%)	43 (22.1)	14 (26.9)	19 (19.0)	10 (23.3)	0.523

Data are expressed as mean ± standard deviation or n (%). Abbreviations: MVI, microvascular invasion; CHB, chronic hepatitis B; CHC, chronic hepatitis C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; BCLC, Barcelona clinical liver cancer; MAFLD, metabolic-associated fatty liver disease. [a]Diameter of the largest tumor nodule. [b]Including partial and totally capsule invasion, and no well capsule.

ellite nodule (P < 0.001), and less well differentiated tumors (P < 0.001). However, no significant difference was observed in the Child-Pugh grade. The presence of liver cirrhosis between the two groups did not differ significantly. The demographics (age and sex category), serum biochemistry (AST, ALT, platelet, and albumin), underlying liver disease (CHB, CHC, and MAFLD), and the proportion of DM comorbid were comparable between the two groups.

Clinicopathologic characteristics of HCC patients according to different MVI subgroups

In the MVI group, 327 patients were further divided into three subgroups. A total of 85 (26.0%) patients were classified into the MVI-portal vein group, 178 (54.4%) patients into the MVI-capsule vein group, and 64 (19.6%) patients into the MVI-both type group based on the pathologic diagnosis. The patient characteristics in different MVI subgroups are shown in **Table 2**.

MVI-both type group had a higher proportion of BCLC stage A (P < 0.001) and satellite nodules (P < 0.001) than the others. The demography, underlying liver disease, biochemistry data, Child-Pugh grade, AFP level, tumor size, number, or differentiation were comparable among groups.
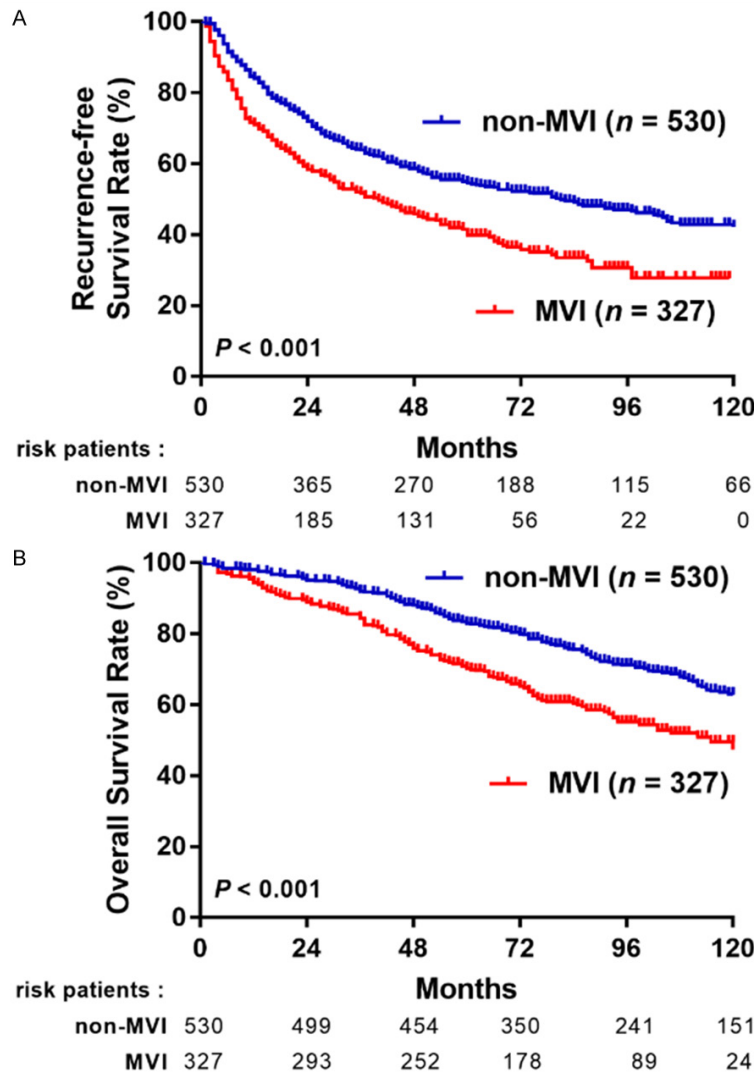
Figure 3. RFS (A) and OS (B) of HCC patients with or without MVI.

*Recurrence and survival analysis stratified by different types of MVI*

Of the 327 HCC patients with MVI, 85 (26.0%) were portal vein; 178 (54.4%) were capsular vein, and 64 (19.6%) were with MVI-both types. In the subgroup analysis, no significant differences between each group were observed (**Figure 4A**). However, in the OS analysis, patients with MVI-capsule type were significantly associated with higher survival outcomes than those with MVI-portal type (P = 0.024) and those with MVI-both type (P = 0.001) (**Figure 4B**).

In the multivariate analysis for HCC recurrence, MVI-both type is one of the strongest predictive factors for HCC recurrence (hazard ratio [HR]: 1.69; 95% CI, 1.22-2.36, P = 0.002). MVI-portal vein (HR: 1.55; 95% CI, 1.15-2.09, P = 0.004) and MVI-capsular vein (HR: 1.43; 95% CI, 1.14-1.80, P = 0.002) are associated with HCC recurrence. In addition to MVI, comorbidity related to DM (HR: 1.27; 95% CI, 1.04-1.56, P = 0.021), CHC (HR: 1.33; 95% CI, 1.10-1.62, P = 0.003), presence of liver cirrhosis (HR: 1.67; 95% CI, 1.39-2.01, P < 0.001), multiple tumor numbers (HR: 1.83; 95% CI, 1.38-2.44, P < 0.001), larger tumor size (> 2 cm) (HR: 1.48; 95% CI, 1.18-1.86, P = 0.001) and poor differentiated histological grade (HR: 2.37; 95% CI, 1.47-3.82, P < 0.001) are also predictors of HCC recurrence (**Table 3**).

Recurrence and survival analysis in HCC patients with and without MVI

A total of 471 (55.0%) patients experienced an HCC recurrence after 83 month-mean follow-ups, in which 202 (61.8%) were in the MVI group and 269 (50.8%) in the non-MVI group. In the MVI group, the 1-, 3-, 5-year RFS rates were 71.5%, 51.4%, and 41.5%, respectively. However, RFS rates were 84%, 63.5%, and 54.6%, respectively, in the non-MVI group (P < 0.001, **Figure 3A**). A total of 321 (37.5%) patients died during the follow-up period: 141 (43.1%) in the MVI group and 180 (34%) in the non-MVI group. The 1-, 3-, 5-year OS rates were 94.8%, 85.3%, and 70.9%, respectively in the MVI group. However, it was 98.1%, 92.4%, and 83.4%, respectively in the non-MVI group (P < 0.001, **Figure 3B**).

In the multivariate analysis for all-cause mortality, most of the associated predictors are similar for HCC recurrence, which include DM (HR, 1.72; 95% CI, 1.36-2.18, P < 0.001), Liver cirrhosis (HR: 1.81; 95% CI 1.44-2.27, P < 0.001), larger tumor size (> 2 cm) (HR: 1.51; 95% CI, 1.14-2.00, P = 0.004), MVI-portal vein type (HR: 1.85; 95% CI 1.31-2.62, P < 0.001) and MVI-both type (HR: 2.29; 95% CI 1.59-
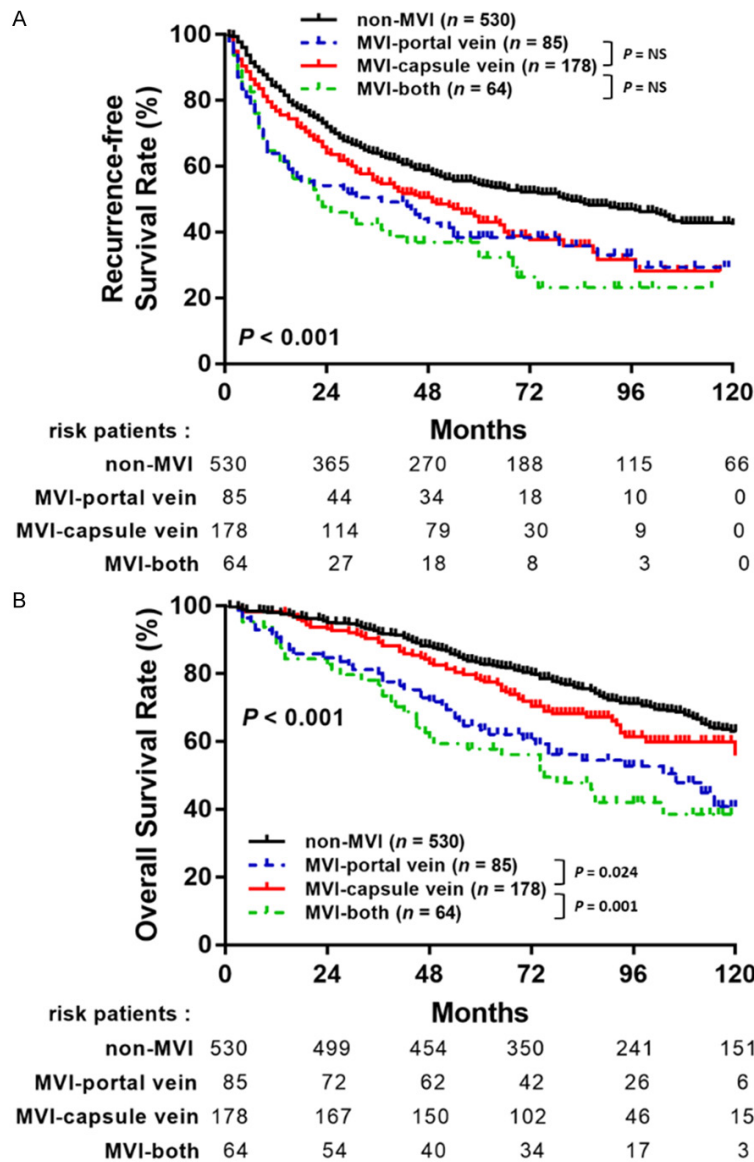
Figure 4. The comparison of RFS (A) and OS (B) stratified by different types of MVI.

HCC recurrence. As shown in **Figure 5A**, the Kaplan-Meier method showed that the MVI-both type group was significantly associated with a higher risk of early recurrence than the MVI-capsule vein group (P = 0.017). Moreover, the MVI-portal vein group had a borderline significant trend than the MVI-capsule vein group (P = 0.068). In late recurrence, no significant difference was observed between each group (**Figure 5B**). Similarly, the multivariate analysis revealed that MVI-both type (HR, 1.81; 95% CI, 1.22-2.70, P = 0.003) and MVI-portal vein type (HR, 1.79; 95% CI, 1.24-2.58, P = 0.002) were independent risk factors of early recurrence when compared with a non-MVI group (**Table 5**). Other factors that were significantly associated with a higher risk of early recurrence were comorbidity related to CHC (HR, 1.34; 95% CI, 1.05-1.71, P = 0.019), higher AFP level (> 10 ng/mL) (HR, 1.39; 95% CI, 1.08-1.79, P = 0.010), presence of liver cirrhosis (HR: 1.89; 95% CI, 1.47-2.42, P < 0.001), multiple tumor number (HR, 1.79; 95% CI, 1.24-2.57, P = 0.002), larger tumor size (> 2 cm) (HR: 1.61; 95% CI, 1.18-2.19, P = 0.003) and poor differentiated histological grade (HR, 2.63; 95% CI, 1.53-4.52, P < 0.001).

Extrahepatic recurrence analysis stratified by different types of MVI

A higher prevalence of EHR in MVI patients had been demonstrated in a previous study [28]. However, less is known about the effect of MVI types on EHR. We analyzed stratified EHR based on different MVI types to explore the effect of MVI on EHR in HCC. Among 857 patients, only 15 patients (1.75%) developed EHR during the follow-up period, 5 patients

3.29, P < 0.001). Other predictors for all-cause mortality include old age (> 60-year-old) (HR: 1.34; 95% CI, 1.06-1.69, P = 0.013), Child-Pugh grade B vs. A (HR 1.78; 95% CI, 1.27-2.52, P = 0.001). However, MVI-capsular vein type failed to reach the statistically significant level for all causes of mortality (**Table 4**).

Early and late recurrence analysis stratified by different types of MVI

Among the total of 327 patients with MVI, 133 (40.7%) had early (< 2-year follow-up period), and 69 (59.3%) patients had late (≥ 2 years)

Table 3. Predict factors associated with HCC recurrence

Variable	Comparison	Univariate		Multivariate 1[a]		Multivariate 2[b]	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years)	> 60 vs. ≤ 60	1.33 (1.11-1.60)	0.002	1.21 (1.00-1.47)	0.052		
Sex	Male vs. Female	1.01 (0.81-1.26)	0.913				
Diabetes mellitus	Yes vs. No	1.45 (1.19-1.77)	< 0.001	1.25 (1.02-1.54)	0.031	1.27 (1.04-1.56)	0.021
CHB	Yes vs. No	0.87 (0.73-1.05)	0.136				
CHC	Yes vs. No	1.42 (1.18-1.71)	< 0.001	1.26 (1.03-1.54)	0.022	1.33 (1.10-1.62)	0.003
MAFLD	Yes vs. No	1.11 (0.83-1.47)	0.482				
AFP (ng/mL)	> 10 vs. ≤ 10	1.28 (1.07-1.54)	0.007	1.18 (0.98-1.43)	0.084		
Liver cirrhosis	Yes vs. No	1.67 (1.40-2.01)	< 0.001	1.63 (1.35-1.96)	< 0.001	1.67 (1.39-2.01)	< 0.001
Child-Pugh grade	B vs. A	1.41 (1.02-1.94)	0.036	1.15 (0.83-1.59)	0.411		
BCLC stage	A vs. 0	1.71 (1.28-2.29)	< 0.001	1.17 (0.76-1.79)	0.486		
Tumor number	Multiple vs. Single	1.73 (1.31-2.28)	< 0.001	1.80 (1.35-2.41)	< 0.001	1.83 (1.38-2.44)	< 0.001
Tumor size (cm)	> 2 vs. ≤ 2	1.45 (1.16-1.81)	0.001	1.36 (0.99-1.86)	0.059	1.48 (1.18-1.86)	0.001
Histological grade	Poor vs. well + moderate	2.56 (1.60-4.10)	< 0.001	2.30 (1.43-3.72)	0.001	2.37 (1.47-3.82)	< 0.001
Capsule invasion	Yes vs. No	1.10 (0.84-1.44)	0.508				
Satellite nodule	Yes vs. No	1.53 (0.88-2.66)	0.130				
Microvascular invasion	None	Reference					
	Capsule vein	1.39 (1.11-1.74)	0.004	1.38 (1.08-1.75)	0.01	1.43 (1.14-1.80)	0.002
	Portal vein	1.63 (1.21-2.19)	0.001	1.49 (1.09-2.02)	0.012	1.55 (1.15-2.09)	0.004
	Both	1.97 (1.43-2.73)	< 0.001	1.59 (1.13-2.23)	0.007	1.69 (1.22-2.36)	0.002

Abbreviations: HR, hazard ratio; CI, confidence interval; MVI, microvascular invasion; CHB, chronic hepatitis B; CHC, chronic hepatitis C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; BCLC, Barcelona clinical liver cancer; MAFLD, metabolic-associated fatty liver disease. [a]Multivariate 1: multivariable analysis based on all potential risk factors (P < 0.1 in the univariate Cox model) forced into the model. [b]Multivariate 2: multivariable analysis with variables (P < 0.1 in the univariate Cox model) using forward stepwise conditional LR method with P < 0.05 as selection criterion.

Table 4. Predict factors associated with all-caused mortality

| Variable	Comparison	Univariate		Multivariate 1[a]		Multivariate 2[b]	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years)	> 60 vs. ≤ 60	1.46 (1.17-1.82)	0.001	1.32 (1.04-1.69)	0.024	1.34 (1.06-1.69)	0.013
Sex	Male vs. Female	0.97 (0.74-1.26)	0.807				
Diabetes mellitus	Yes vs. No	1.98 (1.57-2.50)	< 0.001	1.75 (1.37-2.22)	< 0.001	1.72 (1.36-2.18)	< 0.001
CHB	Yes vs. No	0.77 (0.62-0.96)	0.021	0.83 (0.61-1.11)	0.209		
CHC	Yes vs. No	1.29 (1.03-1.61)	0.027	0.92 (0.68-1.25)	0.586		
MAFLD	Yes vs. No	1.01 (0.72-1.42)	0.956				
AFP (ng/mL)	> 10 vs. ≤ 10	1.29 (1.03-1.60)	0.025	1.24 (0.98-1.56)	0.071		
Liver cirrhosis	Yes vs. No	1.84 (1.47-2.30)	< 0.001	1.77 (1.41-2.22)	< 0.001	1.81 (1.44-2.27)	< 0.001
Child-Pugh grade	B vs. A	2.06 (1.47-2.88)	< 0.001	1.76 (1.25-2.50)	0.001	1.78 (1.27-2.52)	0.001
BCLC stage	A vs. 0	1.57 (1.11-2.22)	0.011	0.85 (0.49-1.46)	0.544		
Tumor number	Multiple vs. Single	1.41 (1.00-2.00)	0.052	1.44 (1.00-2.08)	0.05		
Tumor size (cm)	> 2 vs. ≤ 2	1.50 (1.14-1.98)	0.004	1.66 (1.10-2.53)	0.017	1.51 (1.14-2.00)	0.004
Histological grade	Poor vs. well + moderate	2.34 (1.31-4.17)	0.004	1.85 (1.02-3.34)	0.041		
Capsule invasion	Yes vs. No	1.20 (0.86-1.69)	0.289				
Satellite nodule	Yes vs. No	2.37 (1.36-4.12)	0.002	1.51 (0.82-2.81)	0.189		
Microvascular invasion	None	Reference					
	Capsule vein	1.34 (1.00-1.80)	0.05	1.27 (0.94-1.73)	0.126		
	Portal vein	2.07 (1.47-2.91)	< 0.001	1.83 (1.27-2.63)	0.001	1.85 (1.31-2.62)	< 0.001
	Both	2.59 (1.81-3.70)	< 0.001	2.11 (1.45-3.09)	< 0.001	2.29 (1.59-3.29)	< 0.001

Abbreviations: HR, hazard ratio; CI, confidence interval; MVI, microvascular invasion; CHB, chronic hepatitis B; CHC, chronic hepatitis C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; BCLC, Barcelona clinical liver cancer; MAFLD, metabolic-associated fatty liver disease. [a]Multivariate 1: multivariable analysis based on all potential risk factors (P < 0.1 in the univariate Cox model) forced into the model. [b]Multivariate 2: multivariable analysis with variables (P < 0.1 in the univariate Cox model) using forward stepwise conditional LR method with P < 0.05 as selection criterion.

(0.94%) in the non-MVI group, 2 patients (2.35%) in the MVI-portal vein group, 3 pati- ents (1.69%) in MVI-capsule vein group, and 5 patients (7.81%) in MVI-both type groups. The
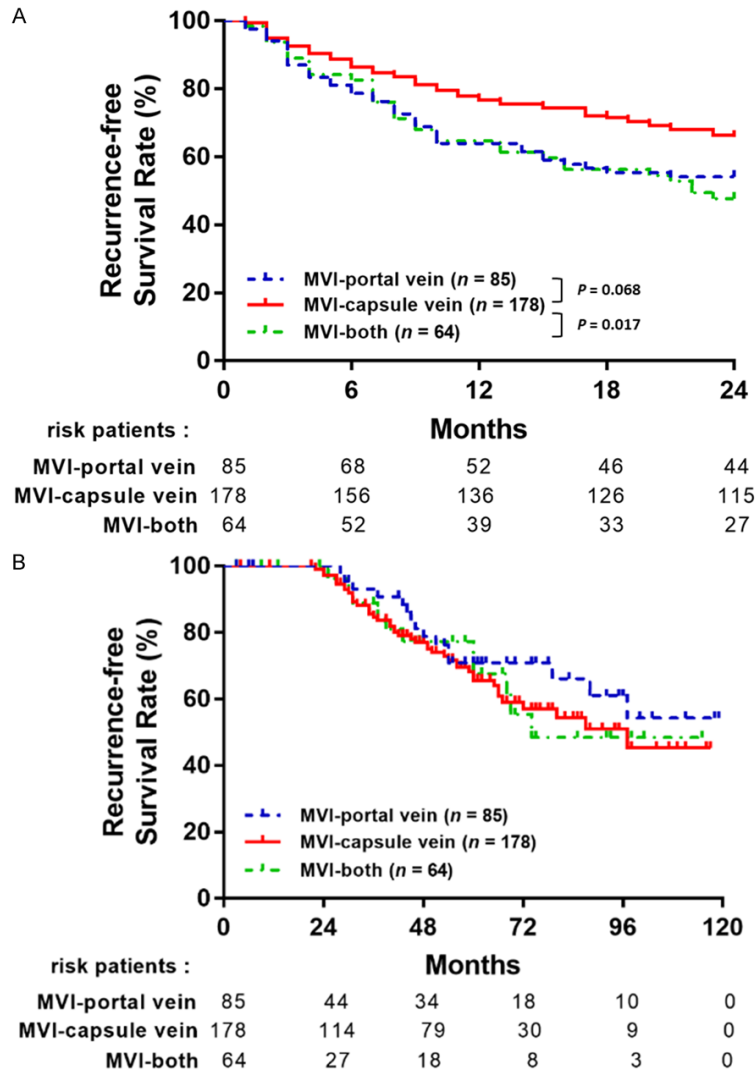
Figure 5. The comparison of (A) early RFS and (B) late RFS stratified by different types of MVI.

MVI-both type was significantly associated with a higher risk of EHR than other groups (P = 0.001, **Figure 6**). When considering the advanced analysis of EHR, only MVI-both type (HR, 8.74; 95% CI, 2.38-32.03, P = 0.001) and poor tumor differentiation (HR, 18.29; 95% CI, 3.56-93.88, P < 0.001) are strong predictors for EHR (**Table 6**).

Discussion

HCC represents one of the most common cancers in the world, especially in the high hepatitis B prevalent area, such as Taiwan and other East Asia countries. Although surveillance of HCC in high-risk populations may improve early tumor diagnosis, improve treatment, and re-duce mortality rates, the clinical outcomes of patients receiving curative treatments remain unsatisfactory owing to the high rate of recurrence. MVI is a recent proposed pathological feature that has a strong association and predicted value for the recurrence and the poor prognosis in HCC patients receiving curative liver resection. A thorough search of the literature reveals that this is the first large-scale cohort study that focuses on RFS and OS of HCC after liver resection in different MVI subtypes. The results of this study showed the importance of MVI on the HCC recurrence after curative liver resection and OS. The results also reveal that the concurrent portal and capsular MVI is the most risk factor for HCC recurrence compared with the only portal or capsular MVI, which is a novel finding without previously published data.

Previous studies proposed that the patients with MVI had a worse 5-year disease-free survival (DFS) rate. Shuji Sumie et al. reported 5-year DFS in their cohort, revealing 20.8% and 52.6% in the MVI group and non-MVI group, respectively [11]. Kuo et al. demonstrated the importance of microscopic portal vein invasion. The 5-year cumulative recurrence rate was 26.6% in the microscopic portal vein invasion group. However, 5-year cumulative recurrence rate was 13.8% in non-vascular invasion group [13]. In the present study, the RFS and OS in MVI group were worse than non-MVI groups (5-year RFS: MVI: 41.5% vs. non-MVI: 54.6%, P < 0.05; 5-year OS: MVI: 70.9% vs. non-MVI: 83.4%, P < 0.05). The findings are consistent with that of previous studies [11, 13].

It is agreed that the blood flowing into the HCC is mainly fed via neovascularization from arterial vessels and drained mostly via portal vein around the tumor. The spread of cancer cells

Table 5. Predict factors associated with HCC early recurrence

Variable	Comparison	Univariate		Multivariate 1[a]		Multivariate 2[b]	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years)	> 60 vs. ≤ 60	1.29 (1.01-1.63)	0.04	1.15 (0.89-1.48)	0.303		
Sex	Male vs. Female	0.89 (0.67-1.18)	0.414				
Diabetes mellitus	Yes vs. No	1.31 (1.01-1.69)	0.043	1.16 (0.89-1.52)	0.263		
CHB	Yes vs. No	0.88 (0.69-1.12)	0.292				
CHC	Yes vs. No	1.42 (1.12-1.80)	0.004	1.26 (0.97-1.63)	0.086	1.34 (1.05-1.71)	0.019
MAFLD	Yes vs. No	1.16 (0.82-1.64)	0.409				
AFP (ng/mL)	> 10 vs. ≤ 10	1.62 (1.27-2.07)	< 0.001	1.42 (1.10-1.82)	0.007	1.39 (1.08-1.79)	0.01
Liver cirrhosis	Yes vs. No	1.93 (1.52-2.46)	< 0.001	1.87 (1.46-2.39)	< 0.001	1.89 (1.47-2.42)	< 0.001
Child-Pugh grade	B vs. A	1.49 (1.01-2.21)	0.044	1.14 (0.76-1.71)	0.526		
BCLC stage	A vs. 0	2.04 (1.33-3.13)	0.001	1.29 (0.71-2.33)	0.402		
Tumor number	Multiple vs. Single	1.67 (1.17-2.38)	0.005	1.72 (1.19-2.49)	0.004	1.79 (1.24-2.57)	0.002
Tumor size (cm)	> 2 vs. ≤ 2	1.59 (1.17-2.15)	0.003	1.40 (0.93-2.10)	0.112	1.61 (1.18-2.19)	0.003
Histological grade	Poor vs. well + moderate	2.94 (1.71-5.03)	< 0.001	2.46 (1.42-4.26)	0.001	2.63 (1.53-4.52)	< 0.001
Capsule invasion	Yes vs. No	0.83 (0.60-1.14)	0.25				
Satellite nodule	Yes vs. No	2.17 (1.19-3.97)	0.012	1.15 (0.59-2.24)	0.686		
Microvascular invasion	None	Reference					
	Capsule vein	1.33 (0.98-1.79)	0.064	1.25 (0.91-1.72)	0.161		
	Portal vein	2.02 (1.41-2.88)	< 0.001	1.71 (1.17-2.51)	0.006	1.79 (1.24-2.58)	0.002
	Both	2.31 (1.57-3.39)	< 0.001	1.72 (1.114-2.59)	0.009	1.81 (1.22-2.70)	0.003

Abbreviations: HR, hazard ratio; CI, confidence interval; MVI, microvascular invasion; CHB, chronic hepatitis B; CHC, chronic hepatitis C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; BCLC, Barcelona clinical liver cancer; MAFLD, metabolic-associated fatty liver disease. [a]Multivariate 1: multivariable analysis based on all potential risk factors ($P < 0.1$ in the univariate Cox model) forced into the model. [b]Multivariate 2: multivariable analysis with variables ($p < 0.1$ in the univariate Cox model) using forward stepwise conditional LR method with $P < 0.05$ as selection criterion.
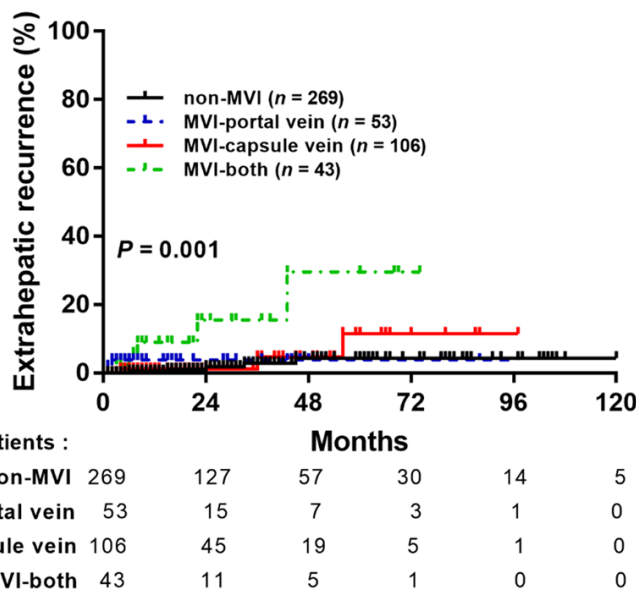
Figure 6. The comparison of extrahepatic recurrence of HCC stratified by different types of MVI.

mechanism, we assume that the progression of MVI commonly starts from the capsular vein to the surrounding small portal vein. MVI-both type is considered to have rapid growing and vascular invasive features. These characteristics represent the worst prognostic factors for the recurrence, early recurrence, OS, and EHR, which is compatible with the results of our present study.

EHR has a worse prognosis, most likely from their presentation at multiple sites with aggressive features. Patients with EHR are not amenable to resection, even for liver transplantation, revealing that the patient with a higher risk of EHR should consider more aggressive treatment. Thus, we focus on the first recurrence in EHR. In our analysis, patients with MVI-both type have the highest EHR, and MVI-both type is the only MVI subtype to predict EHR. This

via a portal vein in HCC is the main mechanism of intrahepatic metastasis [29]. Some studies mentioned that the interaction between HCC and the microenvironment may precede the vascular invasion of MVI [14]. Based on this

Table 6. Predict factors associated with HCC extrahepatic recurrence

Variable	Comparison	Univariate		Multivariate 1[a]		Multivariate 2[b]	
		HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age (years)	> 60 vs. ≤ 60	0.36 (0.12-1.14)	0.083	0.55 (0.14-2.16)	0.387		
Sex	Male vs. Female	0.54 (0.19-1.59)	0.265				
Diabetes mellitus	Yes vs. No	1.21 (0.38-3.80)	0.747				
CHB	Yes vs. No	2.01 (0.64-6.32)	0.232				
CHC	Yes vs. No	0.76 (0.24-2.39)	0.638				
MAFLD	Yes vs. No	0.43 (0.05-3.46)	0.429				
AFP (ng/mL)	> 10 vs. ≤ 10	14.89 (1.96-113.24)	0.009	6.69 (0.81-55.12)	0.077		
Liver cirrhosis	Yes vs. No	2.05 (0.73-5.78)	0.173				
Child-Pugh grade	B vs. A	0.96 (0.13-7.32)	0.969				
Tumor number	Multiple vs. Single	0.85 (0.11-6.49)	0.877				
Tumor size (cm)	> 2 vs. ≤ 2	1.53 (0.43-5.41)	0.514				
Histological grade	Poor vs. well + moderate	15.01 (4.20-53.68)	< 0.001	16.279 (3.09-85.75)	0.001	18.29 (3.56-93.88)	< 0.001
Capsule invasion	Yes vs. No	2.03 (0.27-15.53)	0.495				
Microvascular invasion	None	Reference					
	Capsule vein	1.92 (0.46-8.04)	0.371				
	Portal vein	3.01 (0.58-15.55)	0.188				
	Both	10.94 (3.16-37.96)	< 0.001	8.54 (2.34-31.22)	0.001	8.74 (2.38-32.03)	0.001

Abbreviations: HR, hazard ratio; CI, confidence interval; MVI, microvascular invasion; CHB, chronic hepatitis B; CHC, chronic hepatitis C; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha fetoprotein; BCLC, Barcelona clinical liver cancer; MAFLD, metabolic-associated fatty liver disease. [a]Multivariate 1: multivariable analysis based on all potential risk factors (P < 0.1 in the univariate Cox model) forced into the model. [b]Multivariate 2: multivariable analysis with variables (P < 0.1 in the univariate Cox model) using forward stepwise conditional LR method with P < 0.05 as selection criterion.

finding indicates that patients with MVI-both type should be monitored closely and consider further treatment, such as liver transplantation or adjuvant immune or target therapy before the development of EHR.

Unlike macroscopic vascular invasion, MVI is a pathological finding after curative HCC resection, which shows a lack of detection of MVI before operation. Therefore, we could only discuss further treatment based on the pathology diagnosis after the operation. This study revealed that the effect of MVI and related subtypes on outcome could be important evidence to make share decisions with the patient regarding the further plan. Unfortunately, histological information about the presence of MVI is not always available for HCC patients who receive non-surgical approaches, such as RFA, TACE, or radiotherapy. Hence, there is a pressing demand for an accurate and non-invasive method to detect MVI in preoperative risk-stratify patients. To date, many studies evaluate clinical risk factors that could be used to predict MVI in HCC, which include AFP, des-γ-carboxy prothrombin, tumor number, tumor size, and thrombocytopenia [30, 31], as shown in the presentation of the present study (**Table 1**). However, the performance evaluation of these clinical markers is not yet part of current

clinical practice owing to poor sensitivity and specificity. Recently, Krishnan *et al.* identified that fibronectin was a promising predictor of MVI in HCC [32]. They found that combining AFP and fibronectin improved Area under the Curve by up to 0.93, revealing a better prediction effect of MVI than either fibronectin or AFP alone. In the future, a combination of clinical and molecular serological markers for early and accurate prediction of different types of MVI can improve patients' outcomes.

In further clinical practice, getting precision medicine into the mainstream of clinical practice can promote patient management, since precision medicine can be customized to the whole person instead of the disease stage [33]. This approach considers many elements, both prognosis and the therapeutic decision are unfeasible to be included in the present practice guidelines. Currently, there is no established treatment in the adjuvant setting post HCC resection. Many neoadjuvant and adjuvant treatments have been tested unsuccessfully, including STORM trial [34]. Recently, systemic therapies, such as immune checkpoint inhibitors, tyrosine kinase inhibitors, and monoclonal antibodies offer encouraging results in the treatment of advanced HCC. The combination of Atezolizumab (anti-PDL1 anti-

body) and Bevacizumab (anti-VEGF antibody) has been demonstrated to offer better OS than Sorafenib [35, 36]. Thus, the combination of atezolizumab and bevacizumab has become the standard of care in first-line therapies for advanced HCC. Consequently, several clinical trials incorporate such agents in reducing the risk of recurrence. For the adjuvant trial design, identifying higher risk characteristics is crucial for the appropriate selection of target populations since complementary treatment strategies are beneficial. The results of the present study provide more information on MVI extensively, demonstrating it as an independent risk factor for adverse outcomes in HCC patients.

Several limitations are identified in our study design. First, this is a single-center, retrospective study, in which different surgeons performed the operations, and different pathologists reported the pathological findings. Second, the number of different MVI subtype groups is not even, which is based on the pathological findings and is inevitable in the study design. Third, future studies should validate the classification of MVI subtypes adopted, especially the underestimation of MVI-both type group. Our HCC registry only records etiologies that include hepatitis B virus, hepatitis C virus, and MAFLD. Data regarding postoperative treatment, other severe comorbidities, anti-viral therapy, and the abstinence from alcohol, which might affect prognosis, were not available.

Conclusions

Among early-stage HCC patients after curative resection, concurrent portal and capsular MVI is a risk factor for HCC recurrence (HR: 1.69; P = 0.002), especially the EHR (HR: 8.74; P = 0.001), in comparison with non-MVI, only portal or capsular MVI. This finding discloses the different risks associated with the MVI subtype. Thus, an individualized strategy for the MVI subtype should be con-sidered. A further large-scale cohort study is needed for further validation.

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Disclosure of conflict of interest

None.

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