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A System of Classifying Microvascular Invasion to Predict Outcome after Resection in Patients with Hepatocellular Carcinoma

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Abstract

Background & Aims—Hepatocellular carcinoma (HCC) recurs in approximately 70% of cases after resection. Vascular invasion by tumor cells can be classified as gross or microscopic (mVI) and is a risk factor for recurrence. We examined a large cohort of patients with HCC that were treated by resection to identify features of microvascular invasion that correlated with recurrence and survival.

Methods—We reviewed the records of all HCC resections performed at the Mount Sinai School of Medicine between January 1990 and March 2006 to identify those with mVI, established by histological analysis. The numbers and sizes of vessels invaded, invasion of a vessel with a muscular wall, distance from the tumor, and satellite nodules were recorded.

Results—Of the 384 patients that underwent resection for HCC, 131 (34.1%) met the entry criteria. The median follow-up period was 28.9 mo. There were 68 recurrences and 54 deaths. In multivariate analysis, invasion of a vessel with a muscular wall predicted recurrence (hazard ratio 1.8, $P=0.02$); invasion of a vessel with a muscular wall (hazard ratio=2.2, $P=0.018$) and invasion of a vessel that was more than 1 cm from the tumor (hazard ratio=2.1, $P=0.015$) predicted survival. A risk score that assigned point for the presence of each variable was correlated with recurrence ($p=0.028$) and survival ($p<0.0001$).

Conclusion—A novel classification system that includes invasion of a vessel with a muscular wall and invasion of a vessel that is more than 1 cm from the tumor can accurately predict risk of recurrence and survival of patients with mVI after HCC resection.

Background & Aims

Resection of hepatocellular carcinoma (HCC) is an accepted first line treatment for patients with well preserved liver function and no evidence of portal hypertension.¹ Unfortunately, resection is associated with a significant chance of recurrence with many series reporting recurrence rates of 60% to 70% at 5 years.²⁻⁶ Vascular invasion has repeatedly been identified as a risk factor for recurrence and death after resection of HCC.^{3,5-10}

Vascular invasion is usually identified either as macroscopic, when the invasion of the vessel is visible on gross examination, or as microscopic, when the invasion is visible only on microscopy. Consequently, microscopic vascular invasion (mVI) can encompass a wide spectrum ranging anywhere from invasion of a single small vessel near the tumor capsule to just short of gross vascular invasion. As a result, one would also expect a wide range of outcomes in patients with mVI undergoing surgical resection. While staging systems have been proposed for patients with gross vascular invasion, there have been no robust studies stratifying patients with mVI in terms of recurrence and survival.¹¹

In the present study, we examine a large cohort of patients with HCC and mVI treated with surgical resection. We examine the factors that correlate with recurrence and survival in this group of patients. A classification system for vascular invasion based on a risk score linked with prognosis is proposed.

Methods

We analyzed the prospectively collected data of a cohort of patients undergoing hepatic resection for HCC between January 1990 and March 2007 after obtaining approval from the Institutional Review Board. In order to have the outcomes affected primarily by tumor related factors and not by the degree of underlying liver disease or portal hypertension, a homogenous study population was selected out. Consequently, the inclusion criteria required patients to have Child's-Pugh A liver function and platelet count $>100,000/\mu\text{l}$ with HCC and no evidence of extrahepatic disease on contrast enhanced CT scan of the chest and abdomen or contrast enhanced MRI of the abdomen and non-contrast CT of the chest. There were no strict exclusion criteria based on tumor size or number.

A minimum of 10 sections of non-tumoral hepatic parenchyma were examined microscopically by two expert pathologists (S.T and M.F.). At least one section of non-tumoral hepatic parenchyma 1cm away from the tumor was also examined. Patients without a margin of non-tumoral tissue $\geq 1\text{cm}$ were excluded. Microscopic vascular invasion (mVI) was defined as tumor within a vascular space lined by endothelium that was visible only on microscopy.

In patients with mVI, the size of the largest vessel invaded ($<5\text{mm}$, $5\text{-}10\text{mm}$, $>10\text{mm}$), number of vessels invaded (1, 2-5, >5), and furthest distance on invasion from the tumor capsule ($\leq 1\text{cm}$, $>1\text{cm}$) were recorded as categorical variables. The presence of a muscular wall in any vessel with invasion as well as adherence of the intravascular tumor to the vessel wall were also noted. The presence of muscular wall was confirmed by trichrome stain, in which the muscle cells stained red. The presence of muscle in the wall was further assessed by immunoreactivity for anti-smooth muscle actin antibody (Figure 1). In addition to histological characteristics assessing extent of mVI listed above, other clinical variables such as tumor size, number, and grade, as well as presence of satellites were included in the analysis. Satellites were defined as tumors $\leq 2\text{cm}$ in size and located $\leq 2\text{cm}$ from the main tumor. Several clinical variables including liver function, platelet count, and need for transfusion were also recorded and analyzed.

The patients were followed with serial contrast enhanced CT scan of the chest and abdomen or contrast enhanced MRI of the abdomen and non-contrast CT of the chest every 3 months for the first year, every 4 months for the second year, and subsequently every 6 months. Solitary pulmonary recurrences were treated with lung resection. Patients with a solitary liver recurrence and Child's-Pugh A liver disease and no evidence of portal hypertension underwent a second hepatic resection. Patients with multiple intrahepatic recurrences or compromised hepatic function were treated with radiofrequency ablation and/or transarterial chemoembolization. Patients with recurrence confined to the liver and without significant comorbidities were also referred for liver transplantation. Patients undergoing liver transplantation were censored at the time of transplant for the purposes of this study.

Statistical analysis

Categorical data were compared using χ^2 or Fisher's exact test as indicated. Continuous variables were compared using Student's t test or Man-Whitney test for variables with an abnormal distribution. Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. All variables found to be significant on univariate analysis ($P \leq .05$) were entered into a step-down Cox proportional hazard regression analysis. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-offs of continuous variables. SPSS version 16.0 for Windows was used.

Results

During the study period, 385 patients underwent hepatic resection for treatment of HCC. Of these, 14 had either platelet count $<100,000/\mu\text{l}$ or Child's B liver function and they were excluded from analysis (Figure 2). No vascular invasion was detected in 109 (29%) of patients, while 151 (40%) had mVI and 111 (30%) had gross vascular invasion on pathologic examination. The patient demographics, liver function and tumor characteristics are provided in table 1. Survival and recurrence were significantly different based on presence and degree of vascular invasion (Figure 3).

Of the 151 patients with mVI found on pathology, 20 did not have adequate margin to assess non-tumoral tissue at 1cm from the tumor. These patients were excluded leaving 131 patients as the final mVI study population. Median follow-up of these patients was 29 months. During the study period, there were 54 deaths, including 4 (3%) perioperative deaths, and 68 recurrences. Fifty five (81%) of the recurrences were limited to the remaining liver.

Among the histological variables assessing extent of mVI, invasion of a vessel with a muscular wall, invasion of a vessel $\geq 1\text{cm}$ from the tumor capsule and invasion of >5 vessels were significantly associated with recurrence (Table 2). Multivariate analysis including these three variables found only invasion of a vessel with a muscular wall as a significant and independent predictor of recurrence (Table 2).

Invasion of a vessel with a muscular wall and invasion of a vessel $\geq 1\text{cm}$ from the tumor capsule were significant predictors of survival on univariate analysis (Table 2). On multivariate analysis, both variables were significant and independent predictors of survival and had similar hazard ratios and 95% confidence intervals (Table 2). A risk score assigning one point for invasion of a vessel with a muscular wall and one point for invasion of a vessel $\geq 1\text{cm}$ from the tumor capsule was calculated for each patient. This risk score was found to significantly and independently correlate with risk of recurrence and death (Figure 4).

Of the other clinical variables analyzed, presence of satellite nodules and tumor size, with a cut-off of 10cm as determined by ROC curve analysis, were also found to be significant predictors of recurrence on univariate analysis (Table 3). The presence of satellites, presence of >1 tumor nodule, tumor size $>10\text{cm}$, and need for blood transfusion were found to be significantly associated with survival on univariate analysis (Table 3). Variables including liver function, platelet count, patient age, AFP, tumor grade, and underlying liver disease were not significantly associated with either survival or recurrence in patients with mVI on univariate analysis in this cohort of patients.

In order to better understand the overall relevance of the risk score, multivariate analyses of recurrence and survival including the histological risk score as well as the other clinical variables found to be significant on univariate analysis were conducted. The risk score was found to be an independent predictor of both recurrence and survival after including these clinical variables (Table 3).

Finally, we aimed to further classify survival of the overall cohort according to a novel classification based on the absence of vascular invasion (class A), presence of microscopic vascular invasion (class B), which was further subdivided in B1-2 and 3 according to the results of the study, and presence of macroscopic vascular invasion (class C). (Table 4)

Discussion

Hepatic resection for HCC can result in excellent long-term survival in properly selected patients. Nevertheless, resection continues to be plagued by a high rate of tumor recurrence.

As our current study demonstrates, the large majority of recurrences are within the remaining liver. This is primarily due to intrahepatic dissemination of the tumor via the portal circulation. Consequently, it is not surprising that vascular invasion has been repeatedly identified as a predictor of recurrence and survival in series examining the outcomes of hepatic resection for HCC.

Microscopic vascular invasion (mVI), as demonstrated by our results, encompasses a heterogeneous population of patients with a wide range of potential outcomes. We have shown that a risk score based on histological features of mVI that includes invasion of a vessel with a muscular wall and invasion of vessels ≥ 1 cm from the tumor capsule, is able to stratify patients into 3 distinct groups with significantly different risks of recurrence and death. Interestingly, the patients with mVI and no risk factors had outcomes similar to patients with no vascular invasion while patients with mVI and both risk factors behaved like patients with gross vascular invasion in terms of both recurrence and survival (Table 4).

In addition to vascular invasion, liver function and degree of portal hypertension have also been identified as predictors of survival after liver resection for HCC.^{1,12,13} The fact that clinical variables associated with liver function and platelet count (which can serve as a surrogate marker for portal hypertension) did not correlate with survival in our series is not surprising, as only patients with well preserved liver function and no portal hypertension were selected for the study. As a result, only tumor related variables influenced the outcomes.

Some investigators have proposed referring all patients found to have mVI after hepatic resection for liver transplantation due to the significant risk of recurrence.¹⁴ An alternate approach would be to selectively refer patients for transplantation based on their likelihood of recurrence as determined by the classification system proposed in this study.

The success of sorafenib in the treatment of advanced HCC has opened the door for the use of such small targeted molecules in the adjuvant setting (¹⁵). As more and more of such small molecular therapies are developed, there will be a growing demand to study their benefits in an adjuvant setting. The ability to accurately stratify patients found to have mVI after resection of HCC can help to ensure a homogeneous study population for such adjuvant trials.

The proposed classification system clearly requires external validation. However, if the results of this study are confirmed in an independent cohort of patients, the ability to stratify patients with mVI after resection can have obvious and significant advantages.

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Abbreviations

HCC	hepatocellular carcinoma
mVI	microvascular invasion
CT	computed tomography
MRI	magnetic resonance imaging
ROC	receiver operating characteristic

Figure 1.
Vascular invasion of vessel with a muscular wall at 40× magnification with antibody to smooth muscle actin.

Figure 2.
Flow diagram of patients included in study.

Figure 3A

Figure 3B

Figure 3.
Overall survival (A) and recurrence (B) based on extent of vascular invasion.

Figure 4A

Figure 4B

Figure 4.
Overall survival (A) and recurrence (B) in patients with microvascular invasion based on histological risk score.

Table 1
Patient demographics, liver function and tumor characteristics

	No VI	mVI	Gross VI	p
n	109	131	111	
Age (y) *	60±12	61±12	58±12	N.S. [†]
Gender (M:F)	90:19	90:41	87:24	0.041
Liver disease (n)				N.S.
HBV	43	49	40	
HCV	29	49	46	
Alcohol	7	6	4	
other	8	6	6	
none	22	21	15	
Platelet (/μl) *	172±69	198±109	212±122	0.013
INR ^{*‡}	1.05±0.17	1.10±0.31	1.11±0.23	N.S.
Total bilirubin (mg/dl) *	0.91±0.46	0.89±0.81	1.12±0.96	N.S.
Albumin (g/dl) *	4.1±0.6	4.0±0.5	3.7±0.6	<0.001
AFP (ng/ml) *	1,217±3,884	4,976±17,364	22,971±86,709	0.003
# tumors *	1.2±0.5	1.4±0.8	1.5±0.8	N.S.
Diameter (cm) *	5.1±3.5	6.4±4.0	9.5±5.7	<0.0001
Grade (n)				<0.0001
Well	49	27	23	
Moderate	49	75	40	
Poor	11	29	39	

* mean ± standard deviation,

[†] not significant,

[‡] international normalization ratio

Table 2
Univariate and multivariate analyses of histologic features of mVI associated with recurrence and survival

UNIVARIATE ANALYSIS			
	n	Median time to event (mo.) [*]	p
RECURRENCE			
Vessel w/muscular wall			0.018
yes	73	31.8±6.1	
no	58	80.8±12.6	
Distance of invasion			0.0378
≤1cm from tumor	115	53.9±10.3	
> 1cm from tumor	16	25.6±8.5	
Number of vessels			0.0491
≤5 vessels	63	48.2±7.1	
> 5 vessels	68	41.3±10.0	
SURVIVAL			
Vessel w/muscular wall			0.002
yes	73	47.6±7.6	
no	58	88.3±13.5	
Distance of invasion			0.001
≤1cm from tumor	115	79.3±9.8	
> 1cm from tumor	16	27.9±8.0	
MULTIVARIATE ANALYSIS			
	HR [†]	95% Confidence Interval	p
RECURRENCE			
Vessel with muscular wall	1.8	1.2-2.9	0.020
SURVIVAL			
Vessel with muscular wall	2.2	1.1-4.2	0.018
Invasion >1cm from tumor	2.1	1.2-3.7	0.015

^{*} Median ± standard error,

[†] Hazard ratio

Table 3
Univariate and multivariate analyses of clinical variables associated with recurrence and survival

UNIVARIATE ANALYSIS			
	n	Median time to event (mo.) [*]	p
RECURRENCE			
Satellites			0.004
yes	38	28.0±25.0	
no	93	70.1±21.0	
Diameter of tumor			0.026
≤10cm	116	47.3±12.1	
> 10cm	15	21.7±8.4	
SURVIVAL			
Satellites			0.004
yes	38	33.1±5.3	
no	93	71.5±8.7	
Number of tumors			0.010
single	91	68.1±8.2	
multiple	40	34.6±6.0	
Blood transfusion			<0.0001
yes	44	36.8±6.4	
no	87	96.8±12.5	
Diameter of tumor			0.016
≤10cm	116	63.4±7.2	
> 10cm	15	28.8±12.1	
MULTIVARIATE ANALYSIS			
	HR [†]	95% Confidence Interval	p
RECURRENCE			
Risk score	1.8	1.1-3.0	0.027
Tumor diameter >10cm	2.3	1.2-4.6	0.017
SURVIVAL			
Risk score	2.2	1.5-3.2	<0.0001
Transfusion	2.1	1.3-3.6	0.011

^{*} Median ± standard error,

[†] Hazard ratio

Table 4
Novel classification system for resected HCC based on degree of vascular invasion

Class	Extent of vascular invasion	N	Survival (mo.) [*]	Time to recurrence (mo) [*]
A	None	109	87.7±15.7	29.8±7.1
B1	Microscopic invasion with no risk factors	58	72.1±22	27.1±5.1
B2	Microscopic invasion with 1 risk factor	58	36.9±16	19.0±7.7
B3	Microscopic invasion with 2 risk factors	15	8.2±1.2	7.1±1.9
C	Gross invasion	111	12.2±1.9	6.7±0.9

* median ± standard error