Original Investigation

Nomogram for Preoperative Estimation of Microvascular Invasion Risk in Hepatitis B Virus–Related Hepatocellular Carcinoma Within the Milan Criteria

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IMPORTANCE The presence of microvascular invasion (MVI) decreases surgical outcomes of hepatocellular carcinoma (HCC). An accurate preoperative prediction of MVI can help surgeons to better choose surgical procedures, but accuracy is still difficult to achieve.

OBJECTIVE To develop a nomogram to predict MVI presence before liver resection for hepatitis B virus (HBV)–related HCC within the Milan criteria (solitary nodule ≤5 cm; ≤3 nodules, none >3 cm; and no macrovascular invasion).

DESIGN, SETTING, AND PARTICIPANTS Data on 1004 consecutive patients who underwent liver resection for HBV-related HCC within the Milan criteria at the Eastern Hepatobiliary Surgery Hospital between April 6, 2004, and February 22, 2011, were prospectively collected. Of these, patients who underwent surgery in an earlier period formed the training cohort (n = 707) for nomogram development, and those who underwent surgery thereafter formed the validation cohort (n = 297) to confirm the model's performance. Data analysis was conducted from August 1 to November 11, 2014.

EXPOSURES Liver resection for HCC.

MAIN OUTCOMES AND MEASURES Overall survival and time to recurrence after liver resection were measured. Multivariate logistic regression was used to identify the independent risk factors associated with MVI that then were incorporated into the nomogram.

RESULTS Histopathologically identified MVI was found in 211 of 707 patients (29.8%) and 89 of 297 patients (30.0%) in the training and validation cohorts, respectively. In the training cohort, the 5-year recurrence and overall survival rates were 78.5% and 46.9%, respectively, in patients with MVI and 58.4%, and 70.9%, respectively, in patients without MVI (both $P < .001$). The preoperative factors associated with MVI were large tumor diameter, multiple nodules, incomplete capsule, α-fetoprotein level greater than 20 ng/mL, platelet count less than 100×10^3/μL, hepatitis B virus DNA load greater than 10^4 IU/mL, and a typical dynamic pattern of tumors on contrast-enhanced magnetic resonance imaging. Incorporating these 7 factors, the nomogram achieved good concordance indexes of 0.81 (95% CI, 0.78-0.85) and 0.80 (95% CI, 0.75-0.86) in predicting MVI in the training and validation cohorts, respectively, and had well-fitted calibration curves. The positive and negative predictive values (95% CIs) of the nomogram were calculated, resulting in positive predictive values of 57.2% (52.0%-64.9%) and 57.9% (49.2%-68.5%) and negative predictive values of 87.2% (83.2%-89.4%) and 83.2% (76.0%-87.7%) for the training and validation cohorts, respectively. Patients who had a nomogram score of less than 200 or 200 or greater were considered to have low or high risks of MVI presence, respectively.

CONCLUSIONS AND RELEVANCE The nomogram achieved an optimal preoperative prediction of MVI in HBV-related HCC within the Milan criteria. Using the model, the risk for an individual patient to harbor MVI can be determined, which can lead to a rational therapeutic choice.

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Hepatitis B virus (HBV) infection is associated with 70% to 90% of patients with hepatocellular carcinoma (HCC) in the highly endemic Asia-Pacific regions, particularly China.[1] Liver resection and liver transplantation are potentially curative treatments in select patients.[2] Previous studies[3] showed the prevalence of microvascular invasion (MVI) in specimens obtained from liver resection or transplantation to be between 15.0% and 57.1%. The presence of MVI is a histopathologic feature that indicates aggressive behavior of the HCC and predicts worse prognosis after liver resection and transplantation.[4,5]

Currently, the diagnosis of MVI is determined on histologic examination of the surgical specimens obtained after liver resection or transplantation. Therefore, the influence of the diagnosis on preoperative decision making is limited.[3] An accurate preoperative estimation of MVI presence can help surgeons choose appropriate surgical procedures for patients based on risk-benefit assessment. If liver resection is considered for patients with a high risk of MVI, a wide resection margin might be the preferred procedure with curative intent.[6] For liver transplantation in patients with HCC, the absence of MVI has been included as an essential variable for the new inclusion criteria.[5] Furthermore, patients who were predicted to have MVI are suitable candidates for studies on neoadjuvant therapy. Thus, it is important that the presence of MVI can be estimated preoperatively. In addition, because MVI is more likely to occur in an advanced tumor stage (T-stage) HCC, formulating an estimate in early T-stage HCC has specific clinical significance because patients with the early T-stage are the main candidates for curative treatments such as liver resection or transplantation.[2]

Many efforts on preoperative estimation of MVI have been made over the past decade.[3] Some authors[7-9] reported that the "typical dynamical pattern"[8(p2509)] (ie, arterial enhancement and washout) on contrast-enhanced magnetic resonance imaging (MRI) was closely associated with MVI. However, other investigators[3] stated that these results required further prospective validation to avoid potential interobserver variability. The use of serum or tumor biomarkers to estimate MVI risk has also been proposed.[10] Unfortunately, these serum markers can also be abnormally high in patients with benign liver disease.[11] Although some gene signatures have been shown to be associated with tumor vascular invasion,[3] their clinical applicability in the preoperative risk estimation of MVI remains to be determined.

Owing to this lack of a specific and practical predictive method, development of a predictive model that incorporates factors associated with MVI based on preoperative clinicopathologic data becomes desirable. Of all the available models, a nomogram can provide an individualized, evidence-based, highly accurate risk estimation. Nomograms are easy to use and can facilitate management-related decision making. To our knowledge, we have established the first nomogram for preoperative MVI risk estimation in HCC.

Methods

Patients

Between April 6, 2004, and February 22, 2011, data on consecutive patients who had hepatitis B surface antigen positivity and had undergone liver resection for histologically confirmed HCC were prospectively collected at the Eastern Hepatobiliary Surgery Hospital. The study was approved by the Institutional Ethics Committee of the Eastern Hepatobiliary Surgery Hospital. Informed consent was obtained from all patients for their data to be used for research. Patients did not receive financial compensation.

The inclusion criteria were (1) HCC within the Milan criteria (solitary nodule ≤5 cm; ≤3 nodules, none >3 cm; and no macrovascular invasion)[5] according to imaging findings, (2) Child-Pugh A or B7 (score ≤7 [none to mild compromise]) liver function, (3) receipt of preoperative contrast-enhanced MRI of the abdomen, and (4) having undergone R0 tumor resection as defined in a previous report.[12] Patients who had concomitant positive hepatitis C virus antibody, received any preoperative anticancer treatments, had a history of other cancers, and had incomplete clinical data were excluded. Eligible patients who underwent surgery between April 6, 2004, and October 7, 2008, were included into the training cohort for development of the nomogram, and those who underwent surgery between October 15, 2008, and February 22, 2011, were entered into the validation cohort.

Preoperative Examination and Hepatectomy

Routine preoperative examination included liver and renal function tests, hepatitis B and C immunology, HBV DNA load, serum α-fetoprotein level, abdominal ultrasonography, contrast-enhanced MRI and computed tomographic scan of the abdomen, and radiograph or noncontrast computed tomographic scan of the chest. The preoperative diagnosis was based on criteria of the American Association for the Study of Liver Diseases.[13] Anatomical hepatectomy was the preferred method for tumors that were within a Couinaud segment, sector, or hemiliver. Nonanatomical hepatectomy was performed for tumors situated peripherally. After discharge, all patients were monitored regularly in the outpatient clinic and prospectively for recurrence by a standard protocol.[12] The diagnostic criteria for tumor recurrence were similar to those used for the initial HCC[13]; briefly, these criteria were the appearance of new lesions with typical radiologic features of HCC on 2 imaging studies. The end points of this study were overall survival and time to recurrence. Overall survival was measured from the date of liver resection to the date of the patient's death or the date of last follow-up visit. Time to recurrence was calculated from the date of liver resection to the date when tumor recurrence was diagnosed.

Clinicopathologic Variables

The clinicopathologic variables in this study are reported in **Table 1**. The imaging data included tumor number, diameter, capsule status, and location, and cirrhosis based on preoperative contrast-enhanced MRI.[14] In addition, the presence of arterial enhancement, washout, and the typical dynamic pattern of lesions on each of the dynamic imaging phases (before contrast, postcontrast arterial, portal venous, and delay phases) were recorded. *Arterial enhancement* was defined as a higher signal intensity of the lesions on arterial phase images than on precontrast images.[8,9] *Washout* was defined as

Table 1. Participant Characteristics

Variable	Cohort, No. (%)		*P* Value
	Training (n = 707)	Validation (n = 297)	
Age, mean (SD), y	52.1 (10.5)	52.6 (11.7)	.36
Sex			
Male	611 (86.4)	241 (81.1)	.03
Female	96 (13.6)	56 (18.9)	
Diabetes mellitus			
Yes	113 (16.0)	59 (19.9)	.14
No	594 (84.0)	238 (80.1)	
HBeAg			
Positive	237 (33.5)	91 (30.6)	.37
Negative	470 (66.5)	206 (69.4)	
HBV DNA load, IU/mL			
>10^4	294 (41.6)	118 (39.7)	.59
≤10^4	413 (58.4)	179 (60.3)	
Antiviral therapy[a]			
Yes	101 (14.3)	55 (18.5)	.09
No	606 (85.7)	242 (81.5)	
α-Fetoprotein, ng/mL			
≤20	330 (46.7)	134 (45.1)	.25
20-400	187 (26.4)	93 (31.3)	
≥400	190 (26.9)	70 (23.6)	
ALT, U/L			
>44	286 (40.5)	129 (43.4)	.38
≤44	421 (59.5)	168 (56.6)	
GGT, U/L			
>64	290 (41.0)	134 (45.1)	.23
≤64	417 (59.0)	163 (54.9)	
Total bilirubin, mg/dL			
>1.0	214 (30.3)	89 (30.0)	.92
≤1.0	493 (69.7)	208 (70.0)	
Albumin, g/dL			
≥3.5	668 (94.5)	282 (94.9)	.88
<3.5	39 (5.5)	15 (5.1)	
PT, seconds			
>13	150 (21.2)	65 (21.9)	.81
≤13	557 (78.8)	232 (78.1)	
Platelets, × 10^3/μL			
≥100	570 (80.6)	243 (81.8)	.66
<100	137 (19.4)	54 (18.2)	
Glucose, mg/dL			
>126	75 (10.6)	51 (17.2)	.004
≤126	632 (89.4)	246 (82.8)	
Creatinine, mg/dL			
>1.2	11 (1.6)	2 (0.7)	.37
≤1.2	696 (98.4)	295 (99.3)	

(continued)

Table 1. Participant Characteristics (continued)

Variable	Cohort, No. (%)		*P* Value
	Training (n = 707)	Validation (n = 297)	
WBCs, /μL			
≥4000	519 (73.4)	232 (78.1)	.12
<4000	188 (26.6)	65 (21.9)	
RBCs, mean (SD), × 10^6/μL	4.49 (0.54)	4.51 (0.61)	.59
Varices			
Absent	603 (85.3)	253 (85.2)	.97
Mild	104 (14.7)	44 (14.8)	
Child-Pugh class			
A	697 (98.6)	294 (99.0)	.77
B	10 (1.4)	3 (1.0)	
Imaging results			
Tumor diameter, mean (SD), cm[b]	3.27 (1.15)	3.15 (1.14)	.12
No. of tumors			
Solitary	659 (93.2)	275 (92.6)	.73
Multiple	48 (6.8)	22 (7.4)	
Tumor capsule			
Incomplete	338 (47.8)	140 (47.1)	.85
Complete	369 (52.2)	157 (52.9)	
Cirrhosis			
No	355 (50.2)	140 (47.1)	.43
Yes	352 (49.8)	157 (52.9)	
Typical dynamic pattern			
Absence	185 (26.2)	85 (28.6)	.42
Presence	522 (73.8)	212 (71.4)	
Tumor location			
Peripheral	571 (80.8)	228 (76.8)	.15
Central	136 (19.2)	69 (23.2)	
Tumor boundary[c]			
Not smooth	123 (17.4)	54 (18.2)	.77
Smooth	584 (82.6)	243 (81.8)	
Hepatectomy			
Anatomical	425 (60.1)	174 (58.6)	.65
Nonanatomical	282 (39.9)	123 (41.4)	
Blood transfusion			
Yes	35 (5.0)	13 (4.4)	.70
No	672 (95.0)	284 (95.6)	
MVI			
Presence	211 (29.8)	89 (30.0)	.97
Absence	496 (70.2)	208 (70.0)	
Edmondson-Steiner classification			
I-II	316 (44.7)	138 (46.5)	.61
III-IV	391 (55.3)	159 (53.5)	

(continued)

Table 1. Participant Characteristics (continued)

Variable	Cohort, No. (%)		P Value
	Training (n = 707)	Validation (n = 297)	
Pathologic examination			
Tumor diameter, mean (SD), cm	3.24 (1.14	3.15 (1.16)	.26
No. of tumors			
Solitary	656 (92.8)	276 (92.9)	.94
Multiple	51 (7.2)	21 (7.1)	
Tumor capsule			
Incomplete	365 (51.6)	158 (53.2)	.65
Complete	342 (48.4)	139 (46.8)	
Cirrhosis			
No	350 (49.5)	137 (46.1)	.33
Yes	357 (50.5)	160 (53.9)	

Abbreviations: ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; MVI, microvascular invasion; PT, prothrombin time; RBCs, red blood cells; WBCs, white blood cells.

SI conversion factors: To convert albumin to grams per liter, multiply by 10; α-fetoprotein to micrograms per milliliter, multiply by 1; ALT and GGT to microkatals per liter, multiply by 0.0167; creatinine to micromoles per liter, multiply by 88.4; glucose to millimoles per liter, multiply by 0.0555; platelets to $\times 10^9$/L, multiply by 1; red blood cells to $\times 10^{12}$/L, multiply by 1; total bilirubin to micromoles per liter, multiply by 17.104; white blood cells to $\times 10^9$/L , multiply by 0.001.

[a] Antiviral therapy was given before surgery.

[b] Preoperative imaging was based on contrast-enhanced magnetic resonance imaging.

[c] Tumor boundary on imaging was categorized as (1) smooth, presenting as a nodular-shaped tumor on all axial, coronary, and sagittal imaging or (2) not smooth, presenting as single nodule with no clear boundary.

hypointensity of the lesions compared with the surrounding liver on any late dynamic images other than the arterial phase images.[7-9] *Typical dynamic pattern* indicated the presence of both arterial enhancement and washout.[8,9,13,15] Two experienced radiologists independently evaluated all preoperative imaging data. Any controversies in imaging findings between the radiologists were settled by discussion, and a final standard radiologic report on each patient was generated. All surgical specimens were routinely examined histopathologically, especially looking for the presence of MVI. The examination was carried out independently by 3 pathologists. The definition of MVI was in line with that reported by Roayaie et al.[16] Briefly, *MVI* was defined as the presence of tumor in a portal vein, hepatic vein, or a large capsular vessel of the surrounding hepatic tissue lined by endothelium that was visible only on microscopy.

Statistical Analysis

Continuous variables are expressed as mean (SD) and compared using an unpaired, 2-tailed *t* test or Mann-Whitney test. Categorical variables were compared using the χ^2 test or Fisher exact test. To assess interobserver agreement in MRI signal interpretation, the κ statistics were calculated for the imaging variables recorded from the independent radiologists. Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. Multivariate Cox proportional hazards regression model was used to evaluate the independent prognostic factors of overall survival and tumor recurrence.

The significance of each variable in the training cohort was assessed by univariate logistic regression analysis for investigating the independent risk factors of presence of MVI. All variables associated with MVI at a significant level were candidates for stepwise multivariate analysis. A nomogram was formulated based on the results of multivariate logistic regression analysis and by using the *rms* package of R, version 3.0 (http://www.r-project.org/). The nomogram is based on proportionally converting each regression coefficient in multivariate logistic regression to a 0- to 100-point scale. The effect of the variable with the highest β coefficient (absolute value) is assigned 100 points. The points are added across independent variables to derive total points, which are converted to predicted probabilities. The predictive performance of the nomogram was measured by concordance index (C index) and calibration with 1000 bootstrap samples to decrease the overfit bias.[17]

For clinical use of the model, the total scores of each patient were calculated based on the nomogram. Receiver operating characteristic curve analysis was used to calculate the optimal cutoff values that were determined by maximizing the Youden index (ie, sensitivity + specificity − 1). Accuracy of the optimal cutoff value was assessed by the sensitivity, specificity, predictive values, and likelihood ratios.

In all analyses, $P < .05$ was considered to indicate statistical significance. All analyses were performed using SAS, version 9.1 (SAS Institute Inc) and R, version 3.0. Data analysis was conducted from August 1 to November 11, 2014.

Results

Clinicopathologic Characteristics

During the study period, 1119 consecutive patients who had HBV-related HCC within the Milan criteria based on preoperative imaging underwent liver resection. Of these, 1004 patients who met the inclusion criteria were enrolled, and 707 and 297 patients were divided into the training and validation cohorts, respectively (eFigure 1 in the Supplement).

The clinicopathologic characteristics of the patients are listed in Table 1. The baseline clinicopathologic data were similar between the training and validation cohorts. Histopathologically identified MVI was found in 211 (29.8%) and 89 (30.0%) patients in the 2 cohorts, respectively.

Postoperative Prognosis and Independent Prognostic Factors

The study was censored on June 28, 2014. The median follow-up time was 51.6 (range, 2.4-107.4) months and 45.1 (range, 2.1-66.7) months in the training and validation cohorts, respectively. In the training cohort, for patients with MVI, the 1-, 3-, and 5-year recurrence rates were 38.4%, 66.4%, and 78.5%, respectively; corresponding rates for overall survival were 83.4%, 63.7%, and 46.9%. The 1-, 3-, and 5-year recurrence rates for patients without MVI were 19.5%, 46.4%, and 58.4%, respectively; the cor-

Table 2. Univariate Logistic Regression Analysis of MVI Presence Based on Preoperative Data in the Training Cohort

Variable	OR (95% CI)	*P* Value
Age, y	1.00 (0.98-1.01)	.66
Sex, male vs female	1.51 (0.91-2.50)	.11
Diabetes mellitus, yes vs no	1.07 (0.69-1.65)	.77
HBeAg, positive vs negative	1.21 (0.86-1.69)	.28
HBV DNA load, $>10^4$ vs $\leq 10^4$, IU/mL	2.31 (1.66-3.20)	<.001
Antiviral therapy, yes vs no[a]	0.58 (0.35-0.96)	.03
α-Fetoprotein, ng/mL		
20-400 vs ≤20	1.61 (1.06-2.42)	.02
≥400 vs ≤20	3.32 (2.25-4.91)	<.001
ALT, >44 vs ≤44 U/L	0.81 (0.58-1.13)	.22
GGT, >64 vs ≤64 U/L	1.13 (0.82-1.59)	.46
Total bilirubin, >1.0 vs ≤1.0 mg/dL	1.10 (0.78-1.56)	.58
Albumin, <3.5 vs ≥3.5 g/dL	0.92 (0.45-1.88)	.82
PT, >13 vs ≤13 s	1.01 (0.68-1.50)	.96
Platelets, <100 vs ≥100 × 10^3/μL	2.25 (1.53-3.30)	<.001
Glucose, >126 vs ≤126 mg/dL	0.78 (0.45-1.34)	.37
Creatinine, >1.2 vs ≤1.2 mg/dL	0.52 (0.11-2.42)	.40
WBCs, <4000 vs ≥4000/μL	1.49 (1.05-2.12)	.03
RBCs, 10^6/μL	0.93 (0.70-1.25)	.65
Varices, mild vs absent	0.85 (0.53-1.35)	.48
Imaging results[b]		
Tumor diameter	1.54 (1.32-1.79)	<.001
No. of tumors, multiple vs solitary	3.03 (1.67-5.48)	<.001
Tumor capsule, incomplete vs complete	3.15 (2.24-4.42)	<.001
Cirrhosis, yes vs no	1.24 (0.90-1.71)	.19
Typical dynamic pattern, presence vs absence	4.20 (2.60-6.79)	<.001
Tumor location, peripheral vs central	1.48 (0.96-2.29)	.07
Tumor boundary, not smooth vs smooth[c]	0.84 (0.54-1.29)	.42

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; MVI, microvascular invasion; OR, odds ratio; PT, prothrombin time; RBCs, red blood cells; WBCs, white blood cells.

SI conversion factors: See Table 1.

[a] Antiviral therapy was given before surgery.

[b] Preoperative imaging was based on contrast-enhanced magnetic resonance imaging.

[c] Tumor boundary on imaging was categorized as (1) smooth, presenting as a nodular-shaped tumor on all axial, coronary, and sagittal imaging, or (2) not smooth, presenting as a single nodule with no clear boundary.

responding rates for overall survival were 95.0%, 82.2%, and 70.9%. In the validation cohort, for patients with MVI, the 1-, 3-, and 5-year recurrence rates were 30.7%, 64.8%, and 74.4%, respectively; the corresponding rates for overall survival were 83.1%, 60.9%, and 46.5%. The 1-, 3-, and 5-year recurrence rates for patients without MVI were 18.1%, 38.0%, and 44.2%, respectively; the corresponding rates for overall survival were 95.2%, 79.5% and 72.4% (eFigure 2 in the Supplement) ($P < .001$ for recurrence and survival).

All variables listed in Table 1 were used for univariate and multivariate Cox regression analysis. The tumor-related variables were based on postoperative histopathologic data.

The results of univariate analysis of overall survival and recurrence are reported in eTable 1 in the Supplement. On multivariate analysis, the presence of MVI was one of the independent risk factors of both recurrence and overall survival (eTable 2 in the Supplement).

Development and Validation of an MVI-Predicting Nomogram

All variables used in this analysis were based on the data obtained preoperatively. The tumor-related variables, including diameter, number, status of capsule, boundary, location, typical dynamic pattern, were assessed by preoperative imaging studies.

The results of univariate logistic analysis are presented in **Table 2.** On multivariate analysis, with results reported as odds ratio (95% CI), large tumor diameter (1.70 [1.41-2.05]), multiple tumors (5.10 [2.47-10.52]), incomplete tumor capsule (3.63 [2.46-5.34]), presence of typical dynamic pattern (3.20 [1.88-5.45]), high serum α-fetoprotein level (for 20-400 vs ≤20 ng/mL, 1.65 [1.03-2.62]; for ≥400 vs ≤20 ng/mL, 3.46 [2.21-5.40]; to convert to micrograms per milliliter, multiply by 1), platelet count <100 × 10^3/μL (1.85 [1.19-2.88]; to convert to × 10^9/L, multiply by 1), and HBV DNA load $>10^4$ IU/mL (2.33 [1.59-3.42]) were independently associated with MVI (**Table 3**).

These independently associated risk factors were used to form an MVI risk estimation nomogram (**Figure**, A). The resulting model was internally validated using the bootstrap validation method. The nomogram demonstrated good accuracy in estimating the risk of MVI, with an unadjusted C index of 0.81 (95% CI, 0.78-0.85) and a bootstrap-corrected C index of 0.81. In addition, calibration plots graphically showed good

Table 3. Multivariate Logistic Regression Analysis of MVI Presence Based on Preoperative Data in the Training Cohort

Variable	β[a]	OR (95% CI)	*P* Value
HBV DNA load, IU/mL, $>10^4$ vs $\leq 10^4$	0.85	2.33 (1.59-3.42)	<.001
α-Fetoprotein, ng/mL			
20-400 vs ≤20	0.5	1.65 (1.03-2.62)	.04
≥400 vs ≤20	1.24	3.46 (2.21-5.40)	<.001
Platelets, <100 vs ≥100 × 10^3/μL	0.61	1.85 (1.19-2.88)	.007
Imaging results[b]			
Tumor diameter	0.53	1.70 (1.41-2.05)	<.001
No. of tumors multiple vs solitary	1.63	5.10 (2.47-10.52)	<.001
Tumor capsule, incomplete vs complete	1.29	3.63 (2.46-5.34)	<.001
Typical dynamic pattern, presence vs absence	1.16	3.20 (1.88-5.45)	<.001

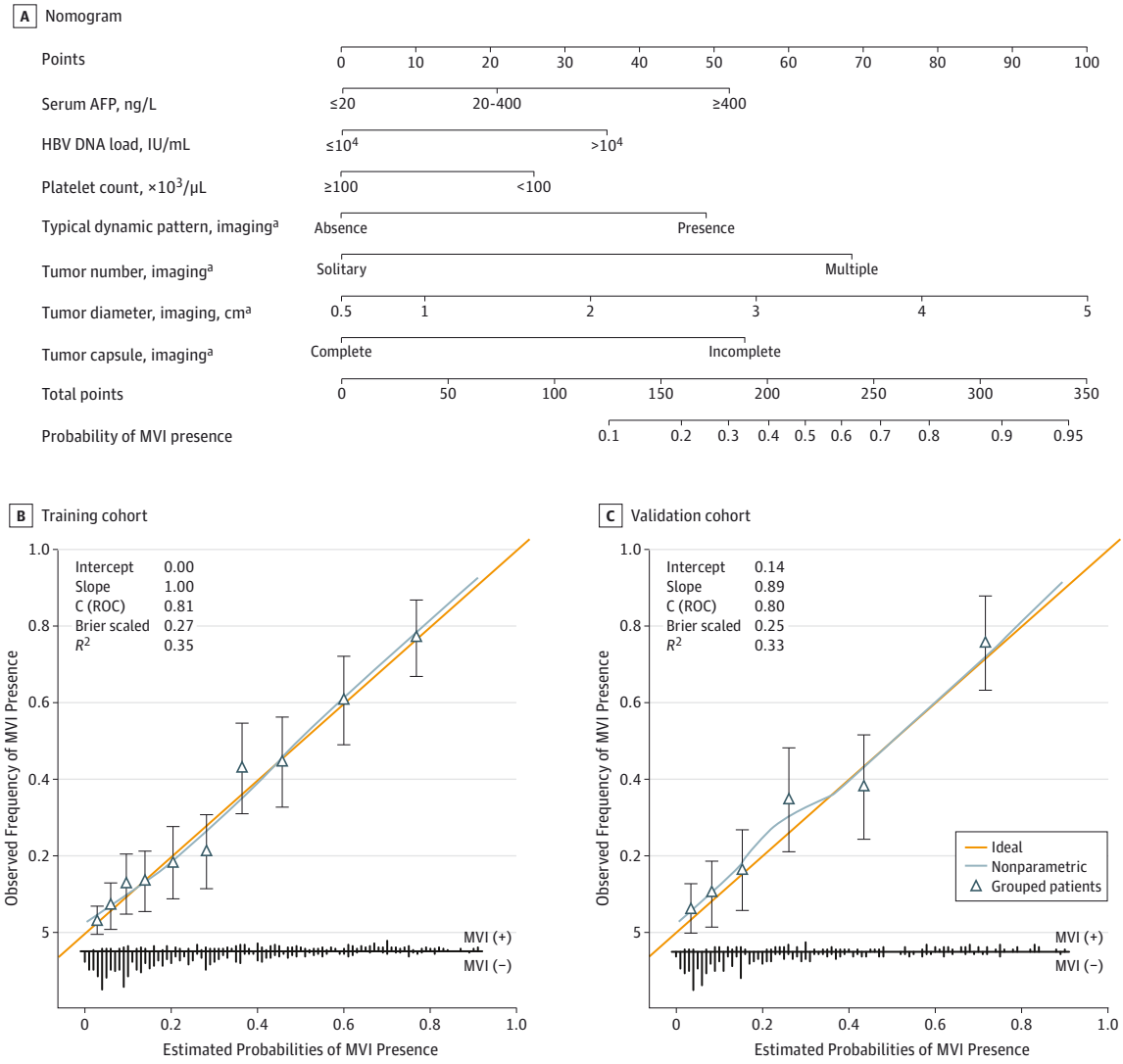
Abbreviations: HBV, hepatitis B virus; OR, odds ratio; MVI, microvascular invasion.

SI conversion factors: See Table 1.

[a] Unstandardized β coefficients were calculated from the multivariate logistic regression model.

[b] Preoperative imaging was based on contrast-enhanced magnetic resonance imaging.

Figure. Nomogram for Preoperative Estimation of Microvascular Invasion (MVI) Risk and Its Predictive Performance



A, Nomogram to estimate the risk of MVI presence preoperatively in hepatitis B virus (HBV)–related hepatocellular carcinoma within the Milan criteria. To use the nomogram, find the position of each variable on the corresponding axis, draw a line to the points axis for the number of points, add the points from all of the variables, and draw a line from the total points axis to determine the MVI probabilities at the lower line of the nomogram. B, Validity of the predictive performance of the nomogram in estimating the risk of MVI presence in the training cohort (n = 707). C, Validity of the predictive performance of the nomogram in estimating the risk of MVI presence in the validation cohort (n = 297). The distribution of the predicted probabilities of MVI presence is shown at the bottom of the graphs, separating those with (+) and without (−) MVI. The triangles indicate the observed frequencies of MVI presence by the deciles of the predicted probability. AFP indicates α-fetoprotein; C index, concordance index; and ROC, receiver operating characteristic.

[a] Preoperative imaging was based on contrast-enhanced magnetic resonance imaging.

agreement on the presence of MVI between the risk estimation by the nomogram and histopathologic confirmation on surgical specimens (Figure, B).

In the validation cohort, the nomogram displayed a C index of 0.80 (95% CI, 0.75-0.86) for the estimation of MVI risk. There was also a good calibration curve for the risk estimation (Figure, C).

Risk of MVI Based on the Nomogram Scores

The optimal cutoff value of the total nomogram scores was determined to be 200. The sensitivity, specificity, positive predictive value, and negative predictive value when used in differentiating the presence from absence of MVI were 73.5%, 76.6%, 57.2%, and 87.2% in the training cohort, and 61.8%, 80.8%, 57.9%, and 83.2% in the validation cohort, respectively (**Table 4**).

Discussion

The presence of MVI significantly worsens the surgical outcomes of early HCC. In the present study, approximately 30%

Table 4. Accuracy of the Prediction Score of the Nomogram for Estimating the Risk of MVI Presence

Variable	Value (95% CI)	
	Training Cohort	Validation Cohort
Area under ROC curve, concordance index	0.81 (0.78-0.85)	0.80 (0.75-0.86)
Cutoff score	200	200
Sensitivity, %	73.5 (67.0-79.3)	61.8 (50.9-71.9)
Specificity, %	76.6 (72.6-80.3)	80.8 (74.7-85.9)
Positive predictive value, %	57.2 (52.0-64.9)	57.9 (49.2-68.5)
Negative predictive value, %	87.2 (83.2-89.4)	83.2 (76.0-87.7)
Positive likelihood ratio	3.1 (2.6-3.8)	3.2 (2.3-4.4)
Negative likelihood ratio	0.35 (0.28-0.44)	0.47 (0.36-0.62)

Abbreviations: MVI, microvascular invasion; ROC, receiver operating characteristic.

of patients with HBV-related HCC within the Milan criteria harbored MVI, which was an independent risk factor of tumor recurrence and overall survival. Our study also suggests that preoperative factors, including multiple tumors, large tumor diameter, incomplete tumor capsule, higher serum α-fetoprotein level, HBV DNA load greater than 10^4 IU/mL, platelet count less than $100 \times 10^3/\mu L$, and presence of a typical dynamic pattern on contrast-enhanced MRI, are significantly associated with MVI.

Previous studies[3] have attempted to use preoperative imaging as well as serum and tumorous biomarkers to predict MVI, but further clinical validation is required. In addition, because these studies did not focus on early T-stage HCC, the positive results are of limited clinical relevance since MVI is a common event in advanced HCC.[3] One study[18] reported an artificial neural network model that incorporated 3 factors (number of tumors, diameter, and serum α-fetoprotein level) in the preoperative risk estimation of MVI. However, other factors that have been recognized to be important for MVI formation were not included in the model.[3] Furthermore, the use of this model requires specific computer software, and it cannot be implemented using software for handheld devices, thus limiting its wide use. Of the currently available prediction tools, a nomogram has high accuracy and good discrimination characteristics in predicting outcomes and is easy to use.[19] In the present study, the proposed nomogram, which incorporated 7 comprehensive and easily available preoperative variables, performed well as supported by the C index values of 0.81 and 0.80 in the training and validation cohorts, respectively, and the optimal calibration curves demonstrating the agreements between prediction and actual observation.

In the MVI risk estimation nomogram, multifocal lesions, large tumor size, incomplete tumor capsule, and high serum level of α-fetoprotein have been reported[3] to increase the possibility of vascular invasion in advanced HCCs. Our study demonstrated that these factors were also significantly associated with MVI in HBV-related early T-stage HCC. In addition, we demonstrated that a high serum HBV DNA load (>10^4 IU/mL), low platelet count (<$100 \times 10^3/\mu L$), and typical dynamic pattern of HCC noted on MRI were associated with an increased probability of MVI formation in early T-stage HCC.

Although there was no report on the association between viral level and MVI presence, Chen et al[20] reported that overexpression of hepatitis B spliced protein in HCC cells increased cell invasion and motility. Recently, Yang et al[21] reported HBV infection status to be strongly associated with elevated activity of the transforming growth factor β-miR-34a-CCL22 pathway, which renders an immune-subversive microenvironment to favor vascular dissemination of HCC cells. These studies support our clinical findings that viral load is an important factor associated with the risk of MVI. Antiviral therapy can effectively suppress HBV replication. In this study, most patients (92 of 101 [91.1%] and 50 of 55 [90.9%] in the training and validation cohorts, respectively) who received this treatment had viral loads of 10^4 IU/mL or less, which might contribute partially to a decreased rate of MVI among patients with a lower viral load.

In addition, a low platelet count was found to be a preoperative risk factor for MVI in this study. Because cirrhosis leads to hypersplenism with a low platelet count, and as portal blood flow to the liver slows, formation of MVI is facilitated. Studies[22] have suggested that an increased serum level of von Willebrand factor and decreased levels of anticoagulants (especially antithrombin) in patients with cirrhosis played a significant role in provoking thrombosis and vascular invasion. In addition, a typical dynamic pattern of HCC suggested that there was sufficient tumor blood supply and good vascular connection between tumor vessels and branches of portal venous system, which facilitates cancer cell invasion and MVI formation. However, hypovascular HCC has been recognized to be biologically less aggressive, and it gradually develops with stepwise differentiation and transformation to become hypervascular as the tumor grows.[15] Kim et al[8] reported that an HCC nodule less than 2 cm with MVI showed a typical dynamic pattern and hyperintensity on T2-weighted and diffusion-weighted images on contrast-enhanced MRI. Disagreement between radiologists might affect the results. However, using κ statistics, we obtained good agreement between the 2 radiologists for typical dynamic pattern (0.87) and also for tumor number (0.89) and encapsulation (0.84).

For clinical use of the model, we summarized the sensitivity, specificity, negative predictive value, and positive predictive value in estimating the risk of MVI using 200 as the cutoff value (Table 4). Patients with a score of 200 or more (210 of 1004, [20.9%]) are a high-risk subgroup of MVI (positive predictive value, 57.4%). Based on these preoperative predictions, the nomogram might serve as a tool to select patients for randomized clinical trials for evaluating the efficacy of liver resection in patients with early HCC and different risks of MVI. In addition, the suitability of liver transplantation can be assessed because absence of MVI is an essential variable in the new criteria for this treatment.[5] The preoperatively estimated MVI risk status can be used in recruiting patients into studies on neoadjuvant therapy for HCC.

The use of the nomogram in estimating the risk of a patient harboring MVI to direct clinical treatment is a new concept. Because MVI status is not the only factor in deciding on therapeutic procedures for HCC, other factors not included in the model, such as the patients' general performance, liver functional reserve, and tumor location, should also be considered.

Our study had some limitations. First, this analysis was based on data from a single institution; it is necessary to validate the results from other centers. Second, a prospective study is required to further confirm the reliability of the nomogram. Third, although the nomogram achieved good predictive accuracy, with a cutoff point of 200, it had 23.4% and 26.5% false-positive and false-negative rates in the training cohort, and 19.2% and 38.2% in the validation cohort, respectively, for predicting MVI presence, which remains high if major clinical decisions are needed. Finally, because the model was based on clinicopathologic data, specific markers to estimate MVI might further improve the accuracy.

Conclusions

By combining 7 preoperative risk factors of MVI, a nomogram was constructed. The model provides an optimal preoperative estimation of MVI risk in patients with HBV-related HCC within the Milan criteria.

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