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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Prognostic Value and Prediction of Extratumoral Microvascular Invasion for Hepatocellular Carcinoma

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ABSTRACT

Background. There are few reports on microvascular invasion (MVI) located intra- or extratumorally and prognosis of hepatocellular carcinoma (HCC).

Objective. The aim of this study was to evaluate patient outcome according to the location of MVI, and to build a nomogram predicting extratumoral MVI.

Methods. We included 681 consecutive patients who underwent hepatic resection (HR) or liver transplantation (LT) for HCC from January 1994 to June 2012, and evaluated patient outcome according to the degree of vascular invasion (VI). A nomogram for predicting extratumoral MVI was created using 637 patients, excluding 44 patients with macrovascular invasion, and was validated using an internal ($n = 273$) and external patient cohort ($n = 256$).

Results. The 681 patients were classified into four groups based on pathological examination (148 no VI, 33 intratumoral MVI, 84 extratumoral MVI, and 29 macrovascular invasion in patients who underwent HR; 238 no VI, 50 intratumoral MVI, 84 extratumoral MVI, and 15 macrovascular invasion in patients who underwent LT). Multivariate analysis revealed that extratumoral MVI was an independent risk factor for overall survival in patients who underwent HR (hazard ratio 2.62, $p < 0.0001$) or LT (hazard ratio 1.99, $p = 0.0005$). Multivariate logistic regression analysis identified six independent risk factors for extratumoral MVI: α-fetoprotein, tumor size, non-boundary type, alkaline phosphatase, neutrophil-to-lymphocyte ratio, and aspartate aminotransferase. The nomogram for predicting extratumoral MVI using these factors showed good concordance indices of 0.774 and 0.744 in the internal and external validation cohorts, respectively.

Conclusions. The prognostic value of MVI differs according to its invasiveness. The nomogram allows reliable prediction of extratumoral MVI in patients undergoing HR or LT.

Vascular invasion (VI) is one of the strongest risk factors for poor prognosis after both hepatic resection (HR) and liver transplantation (LT) for hepatocellular carcinoma (HCC) [1, 2]. Macrovascular invasion, which can be diagnosed by preoperative imaging, can cause widespread dissemination of cancer cells and portal hypertension. Therefore, the presence or absence of macrovascular invasion has a major impact on deciding upon treatment strategies, as mentioned in the Barcelona Clinic Liver Cancer (BCLC) staging system.

In contrast, microvascular invasion (MVI), which is also a poor prognostic factor [3, 4], is difficult to identify by preoperative imaging and it can only be reliably assessed in

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tumor specimens. In the process of cancer cell metastasis, tumor cells initially invade the nearest microvessels, such as small vascular intratumoral spaces, and then disseminate via these microvessels. This leads to the hypothesis that extratumoral MVI is a high risk factor for tumor recurrence. Although there is agreement on defining some forms of MVI, such as tumor emboli in portal radicle veins, large capsule vessels or vascular spaces lined by endothelial cells, the histological locations of MVI have not been clearly defined. Roayaie et al. suggested that MVI located more than 1 cm from tumor edge was associated with poor survival [5]. However, there are few reports on MVI located intra- or extratumorally and prognosis. We therefore studied the prognostic significance of the degree of intratumoral MVI, extratumoral MVI, and macrovascular invasion. We also established a nomogram for predicting extratumoral MVI and validated it with two different patient cohorts.

PATIENTS AND METHODS

The study cohort consisted of 1051 patients who were diagnosed preoperatively with HCC and underwent potentially curative HR (n = 490) or LT (n = 561) from January 1994 to June 2016 at Paul Brousse Hospital, Villejuif, France. Exclusion criteria were (1) not HCC at pathological examination (n = 74); (2) missing clinicopathological data (n = 14); and (3) R2 resection (n = 5). The remaining 958 patients were analyzed. Eligible patients who underwent HR (n = 294) or LT (n = 387) between January 1994 and June 2012 were assessed for prognosis according to the degree of VI. To make a nomogram for predicting extratumoral MVI using preoperative parameters, patients with macrovascular invasion (n = 44) were excluded and the remaining 637 were studied as a training cohort. To validate the model, patients who underwent surgery between July 2012 and June 2016, excluding four patients with macrovascular invasion, were enrolled as an internal validation cohort (n = 273). Patients who underwent HR for HCC without macrovascular invasion, between January 2007 and December 2012 at Kumamoto University, were enrolled as the external validation cohort (n = 256) [electronic supplementary Fig. 1].

Preoperative Diagnosis and Treatment Strategy

Preoperative parameters such as serum laboratory parameters and α-fetoprotein (AFP) were obtained within 2 weeks prior to the operation. A diagnosis of HCC was based on ultrasound and contrast-enhanced computed tomography (CE-CT) or magnetic resonance imaging (MRI). If it was difficult to achieve a diagnosis by imaging, percutaneous tumor biopsy was performed. For patients who underwent pretreatment, the clinical parameters and radiological images were obtained from the latest date prior to surgery after pretreatment. The types of tumor shape by CE-CT were classified by an experienced radiologist according to the gross classification by CT, as previously reported: [6, 7] single nodular type with a round or oval shape and clear boundary, with or without a fibrous pseudocapsule, was defined as boundary type, while other types, such as single nodular type with extranodular growth, confluent multinodular type, or infiltrative type, were defined as non-boundary type. If it was not possible to evaluate tumor shapes by CE-CT because of lipiodol deposition after transcatheter arterial chemoembolization (TACE), the tumor shapes were evaluated by MRI.

Treatment strategies were decided upon at a multidisciplinary meeting with liver surgeons, hepatologists, and radiologists. The policy for treatment strategy has been described previously [8].

Pathological Examination

Macrovascular invasion was diagnosed according to preoperative imaging, such as CE-CT or MRI, and confirmed by pathological examination. Sections were stained with hematoxylin–eosin-safran for microscopic examination, and specimens were cut into 5-mm-thick slices. For tumors < 2 cm in size, entire nodules were sampled. For larger lesions, nodules were sampled extensively from the center to the periphery, with the number of samples being at the discretion of the pathologist. Intra- and extratumoral MVI was evaluated by two pathologists (CG, MS), both experts in liver tumor pathology. Intratumoral MVI was defined when cancer cells were only present in the lumen of vessels within the nodules (electronic supplementary Fig. 2a). Cancer cells present in capsular vessels were also defined as intratumoral MVI (electronic supplementary Fig. 2b). Extratumoral MVI was defined when cancer cells were present in the lumen of veins in the parenchyma outside the nodules, regardless of the distance from the edge of the nodules to the cancer cells (electronic supplementary Fig. 2c). Tumors that had both intra- and extratumoral MVI were defined as having extratumoral MVI. If there was any uncertainty, immunohistochemistry using a vascular antibody (CD34, D240) was performed to confirm tumoral invasion.

Statistical Analysis

Most variables among the 681 patients had no missing data other than body mass index (n = 20), neutrophil-to-lymphocyte ratio (NLR; n = 40), platelet count (n = 8), albumin (n = 43), creatinine (n = 3), γ-

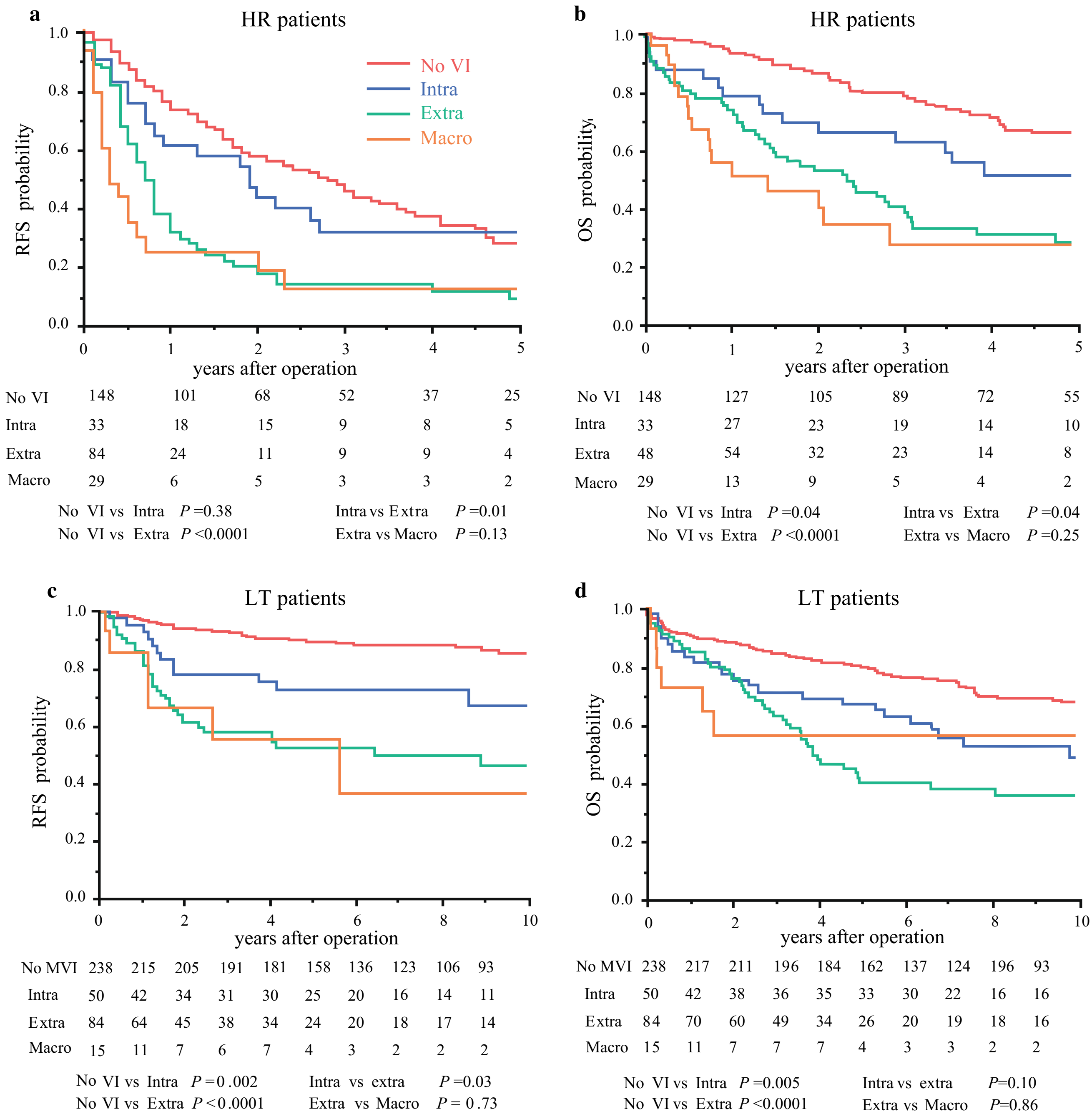


FIG. 1 Kaplan–Meier survival curves according to degree of vascular invasion. **a** RFS and **b** OS for patients who underwent HR. **c** RFS and **d** OS for patients who underwent LT. *Extra* extratumoral microvascular invasion, *Intra* intratumoral microvascular invasion, *Macro* macrovascular invasion, *HR* hepatic resection, *LT* liver transplantation, *MVI* microvascular invasion, *OS* overall survival, *RFS* recurrence-free survival, *VI* vascular invasion

glutamyltranspeptidase (GGT; n = 13), alkaline phosphatase (ALP; n = 29), alanine aminotransferase (ALT; n = 2), boundary type in CT (n = 90), erythrocyte transfusion (n = 40), pathological tumor size (n = 6), tumor differentiation (n = 48), satellite lesions (n = 9), and resection margins (n = 16, among the patients who underwent HR). Exclusion of patients with missing data might have led to biased risk estimates [9, 10], therefore multiple imputation was performed for all multivariate analyses on a dataset that included imputed predictive variables using the R library (mice). Each of the imputed datasets (n = 20) were analyzed separately and pooled using a multivariable Cox proportional hazard and logistic regression model to assess the prognosis and probability of extratumoral MVI, respectively.

Continuous variables are shown as mean ± standard deviation. The cut-off values of continuous variables were determined based on receiver operating characteristic (ROC) analysis. The Chi square test and Fisher's exact test were used for comparison of categorical factors between groups, and continuous variables were compared using the Mann–Whitney U test. Prognostic analysis between groups was performed by Kaplan–Meier curve and log-lank test. Survival time was calculated from the operation date to the date of the event of interest (death for overall survival [OS], relapse for recurrence-free survival [RFS]) or the date of last follow-up. To estimate RFS, patients without evidence of recurrence were censored at the time of last follow-up or death, whichever came first. Univariate and multivariate analysis of risk factors for prognosis was performed using a Cox proportional hazard model, and analysis of risk factors for extratumoral MVI was conducted using a logistic regression model. Variables with a p value < 0.20 in univariate analysis were applied to multivariate analysis, and significant factors were calculated using a stepwise backward elimination procedure with a threshold of $p < 0.05$. The logistic regression model, arbitrarily fitted to the first imputed dataset, was the basis for the nomogram. The predictive performance of the nomogram was assessed by evaluating the degree of discrimination with the C-statistic using 400 bootstrap re-samples. Statistical analyses were carried out using JMP version 12 (SAS Institute, Inc., Cary, NC, USA) and R version 3.1.1 (R Project for Statistical Computing, Vienna, Austria).

RESULTS

Patient Characteristics

The 681 patients who underwent HR (n = 294) or LT (n = 387) between January 1994 and June 2012 were divided into four groups according to the degree of VI. Seventy-one patients with tumors that had both intra- and extratumoral MVI were classified into the extratumoral MVI group. Patient backgrounds are shown in Table 1. There were significant differences between the four groups in pretreatment history, Model for End-Stage Liver Disease (MELD) score, number fulfilling the Milan criteria, Child–Pugh, AFP, largest tumor size, non-boundary type, and satellite lesions among the patients who underwent HR. Among the patients who underwent LT, there were significant differences between the four groups in pretreatment history, number fulfilling the Milan criteria, AFP, largest tumor size, non-boundary type, tumor number according to pathology, and satellite lesions.

Survivals and Recurrence Pattern According to the Degree of Vascular Invasion

The median follow-up time of the 681 patients was 81.6 months (HR patients, 57.7 months; LT patients, 109.7 months). OS and RFS were significantly different among the no VI, intratumoral MVI, and extratumoral groups based on the degree of VI (Fig. 1). In contrast, RFS and OS of extratumoral MVI did not differ significantly from those of macrovascular invasion among the patients who underwent HR and LT.

Univariate analysis revealed that extratumoral MVI was a risk factor for OS and RFS among patients who underwent HR or LT (electronic supplementary Tables 1 and 2). Multivariate analysis using imputed data revealed that extratumoral MVI was a common independent poor prognostic factor in patients who underwent HR or LT (OS in HR, hazard ratio 2.62; RFS in HR, hazard ratio 2.07; OS in LT, hazard ratio 1.99; RFS in LT, hazard ratio 2.66) (Table 2). Intratumoral MVI was an independent risk factor of OS among LT patients, but did not remain significantly associated with OS and RFS after HR, and to RFS after LT, in multivariate analysis.

Risk Factors for Extratumoral Microvascular Invasion (MVI)

Preoperative prediction of extratumoral MVI could be important in deciding on the treatment strategy. We were able to diagnose macrovascular invasion by preoperative imaging in 44 patients; therefore, these patients were excluded and we evaluated the preoperative risk factors for extratumoral MVI in the remaining 637 patients (the training cohort). Patient backgrounds are shown in electronic supplementary Table 3.

Univariate analysis of risk factors for extratumoral MVI is shown in electronic supplementary Table 4. Multivariate analysis identified that NLR $\geq$ 3.0, AST $\geq$ 62 U/l, ALP $\geq$ 160 U/l, AFP 100–1000 ng/ml or $>$ 1000 ng/ml, largest tumor size 40–60 mm or $>$ 60 mm, and non-boundary type were independent risk factors for extratumoral MVI (Table 3).

Establishment of a Predictive Nomogram for Extratumoral MVI

We constructed a predictive nomogram for extratumoral MVI using the six factors listed above, based on the results of multivariate analysis (Fig. 2). Electronic supplementary Fig. 3a shows the calibration plot for the nomogram. The c-index, based on the data containing the missing data, was 0.746, and the mean c-index, based on 20 imputed data, was 0.727 (black lines in electronic supplementary Fig. 3a). These demonstrated good agreement between prediction by the nomogram and the actual observation.

TABLE 1 Baseline characteristics according to degree of vascular invasion

	Hepatic resection group				p value	Liver transplantation group				p value
	No VI [n = 148 (%)]	Intra [n = 33 (%)]	Extra [n = 84 (%)]	Macro [n = 29 (%)]		No VI [n = 238 (%)]	Intra [n = 50 (%)]	Extra [n = 84 (%)]	Macro [n = 15 %)]	
Age, years[a]	64.0 ± 11	62.9 ± 12	60.2 ± 15	61.3 ± 11	0.40	56.7 ± 8	54.7 ± 11	57.1 ± 9	53.4 ± 15	0.62
Sex, male/female	120/28	31/2	68/16	20/9	0.22	200/38	43/7	74/10	13/2	0.83
HBsAg+	18 (12.2)	5 (15.2)	16 (19.1)	8 (27.6)	0.19	42 (17.7)	12 (24.0)	9 (10.7)	3 (20.0)	0.22
HCVAb+	44 (29.7)	13 (39.4)	31 (36.9)	7 (24.1)	0.40	98 (41.2)	18 (36.0)	38 (45.2)	9 (60.0)	0.37
BMI[a]	25.7 ± 5	24.2 ± 4	24.8 ± 5	25.1 ± 4	0.42	26.2 ± 5	27.5 ± 5	25.4 ± 5	26.3 ± 6	0.08
Diabetes	41 (27.7)	7 (21.2)	18 (21.4)	6 (20.7)	0.64	60 (25.2)	20 (40.0)	29 (34.5)	2 (13.3)	0.05
Pretreatment history	100 (61.0)	19 (57.6)	36 (42.9)	9 (31.0)	< 0.0001	200 (84.0)	32 (64.0)	68 (81.0)	9 (60.0)	0.03
MELD score[a]	7.3 ± 2	7.7 ± 2	7.6 ± 3	7.7 ± 3	0.04	11.6 ± 6	13.3 ± 7	11.6 ± 6	10.8 ± 7	0.21
Within the Milan criteria	73 (49.3)	17 (51.5)	24 (28.6)	0 (0)	< 0.0001	188 (79.0)	36 (72.0)	48 (57.1)	0 (0)	< 0.0001
Child–Pugh A/B/C	118/22/0	20/10/0	58/16/0	26/2/1	0.006	95/112/30	16/27/7	35/43/6	9/5/1	0.42
AFP ng/ml[a]	448 ± 3259	6788 ± 26,528	12,873 ± 49,131	4138 ± 10,121	< 0.0001	96 ± 432	1882 ± 8029	2026 ± 16,039	66,746 ± 258,177	0.0003
AFP ≥ 100 (ng/ml)	21 (14.3)	11 (33.3)	35 (42.7)	17 (58.6)	< 0.0001	24 (10.4)	12 (24.0)	24 (28.9)	2 (13.3)	0.0008
Largest tumor size in image (mm)[a]	55 ± 37	64 ± 44	85 ± 51	68 ± 31	< 0.0001	27 ± 18	32 ± 24	38 ± 26	45 ± 42	< 0.0001
Tumor number in image[a]	1.4 ± 1	1.8 ± 2	1.8 ± 2	1.5 ± 1	0.98	2.0 ± 2	2.4 ± 2	2.6 ± 3	2.5 ± 3	0.48
Non-boundary type in image	54 (36.5)	19 (57.6)	58 (69.1)	28 (96.6)	< 0.0001	44 (23.8)	10 (26.3)	25 (42.4)	12 (80.0)	< 0.0001
Largest tumor size in pathology (mm)[a]	53 ± 40	71 ± 55	98 ± 65	83 ± 41	< 0.0001	27 ± 18	33 ± 23	41 ± 30	53 ± 39	< 0.0001
Tumor number in pathology[a]	1.5 ± 3	2.1 ± 4	1.6 ± 2	1.5 ± 1	0.78	2.5 ± 3	3.1 ± 3	4.5 ± 6	2.7 ± 4	0.0001
Satellite lesions	29 (19.6)	9 (27.3)	44 (53.0)	19 (65.5)	< 0.0001	60 (26.0)	17 (34.0)	48 (57.1)	6 (40.0)	< 0.0001

[a]Data are expressed as mean ± standard deviation

VI vascular invasion, *Intra* intratumoral microvascular invasion, *Extra* extratumoral microvascular invasion, *Macro* macrovascular invasion, *HBsAg* hepatitis B surface antigen, *HCVAb* hepatitis C virus antibody, *MELD* Model for End-Stage Liver Disease, *AFP* α-fetoprotein, *BMI* body mass index

TABLE 2 Multivariable analysis of risk factors for OS and RFS in patients who underwent hepatic resection or liver transplantation

		Hazard ratio	95% CI low	95% CI high	*p* value
HR OS	Macrovascular invasion	3.56	1.81	7.00	0.0002
	Extratumoral MVI	2.62	1.69	4.07	< 0.0001
	Albumin < 35 g/l	1.86	1.26	2.77	0.002
	Surgical margin < 1 mm	1.75	1.17	2.63	0.007
	Platelet count < 17 × 10^4/μl	1.63	1.08	2.46	0.02
	ALT ≥ 44 U/l	1.57	1.05	2.33	0.03
HR RFS	Macrovascular invasion	3.14	1.73	5.71	0.0002
	Extratumoral MVI	2.07	1.45	2.95	< 0.0001
	Tumor number ≥ 2	1.70	1.22	2.37	0.002
	Surgical margin < 1 mm	1.64	1.19	2.27	0.003
	NLR ≥ 3.2	1.58	1.11	2.24	0.01
	Platelet count < 17 × 10^4/μl	1.55	1.12	2.16	0.009
	ALP ≥ 151 U/l	1.51	1.07	2.12	0.02
LT OS	Poor differentiation	2.16	1.27	3.68	0.005
	Extratumoral MVI	1.99	1.35	2.93	0.0005
	Tumor size ≥ 20 mm	1.86	1.24	2.78	0.003
	Intratumoral MVI	1.72	1.08	2.74	0.02
	Satellite lesions	1.42	1.00	2.01	0.05
LT RFS	Poor differentiation	3.27	1.50	7.12	0.003
	Extratumoral MVI	2.66	1.61	4.39	0.0001
	Satellite lesions	2.64	1.58	4.41	0.0002
	AFP ≥ 100 ng/ml	2.14	1.27	3.60	0.004
	Platelet count ≥ 13 × 10^4/μl	2.04	1.20	3.46	0.008
	GGT ≥ 230 U/l	1.99	1.16	3.42	0.01
	Tumor size ≥ 20 mm	1.96	1.02	3.76	0.04

CI confidence interval, *OS* overall survival, *RFS* recurrence-free survival, *HR* hepatic resection. *LT* liver transplantation, *MVI* microvascular invasion, *NLR* neutrophil-to-lymphocyte ratio, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *AFP* α-fetoprotein, *GGT* γ-glutamyltransferase

glutamyltranspeptidase

TABLE 3 Multivariable analysis of risk factors for extratumoral microvascular invasion

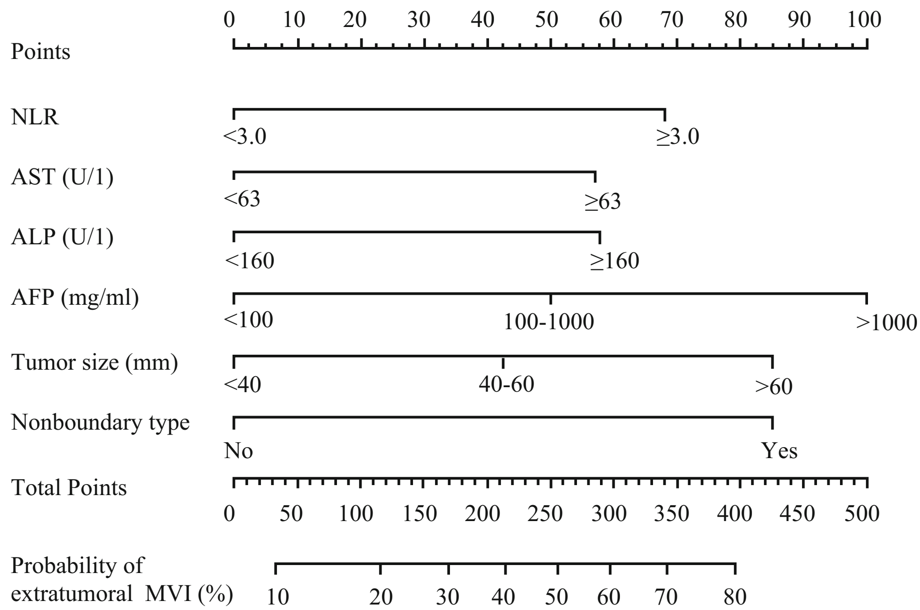
		RR	95% CI low	95% CI high	*p* value
NLR	≥ 3.0	1.82	1.22	2.73	0.007
AST (U/l)	≥ 62	1.76	1.18	2.61	0.005
ALP (U/l)	≥ 160	1.77	1.15	2.71	0.01
AFP (ng/ml)	100–1000	2.21	1.29	3.76	0.004
	> 1000	2.30	1.18	4.48	0.02
Tumor size (mm)	40–60	2.39	1.41	3.99	0.001
	> 60	2.34	1.43	3.83	0.001
Non-boundary type		2.10	1.38	3.20	0.002

RR relative risk, *CI* confidence interval, *NLR* neutrophil-to-lymphocyte ratio, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *AFP* α-fetoprotein

Validation of the Nomogram

To validate whether the nomogram would be applicable to other datasets, we conducted a validation study using data from the 273 and 256 HCC patients in the internal and external validation groups, respectively. Patient backgrounds are shown in electronic supplementary Table 3. Unadjusted c-indices of the internal and external validation groups were 0.800 (95% CI 0.738–0.861) and 0.765 (95% CI 0.701–0.829), respectively, and bootstrap-corrected c-indices of the internal and external validation groups were 0.774 and 0.744, respectively. These results demonstrated that the

FIG. 2 Nomogram for predicting extratumoral microvascular invasion in patients who underwent hepatectomy or liver transplantation for HCC. *AFP* α-fetoprotein, *ALP* alkaline phosphatase, *AST* aspartate aminotransferase, *MVI* microvascular invasion, *NLR* neutrophil-to-lymphocyte ratio



nomogram also showed good prediction in both validation patient cohorts (electronic supplementary Figs. 3b, c). When the internal validation cohort was divided into the HR (n = 160) and LT (n = 113) groups, the unadjusted C-indexes were 0.736 (95% CI 0.646–0.828) and 0.926 (95% CI 0.863–0.989), respectively, indicating good concordance in both groups. Relationships between probabilities of extratumoral MVI according to the nomogram and actual extratumoral MVI rates confirmed by pathological examination were found to correspond closely and are summarized in electronic supplementary Table 5.

Comparison of Predictive Powers

The predictive powers of extratumoral MVI in our nomogram, the other MVI predicting models (Cucchetti et al. [11]. and Lei et al. [12].), and the LT criteria (Milan criteria and AFP model [13]) among the training cohort were compared by ROC curve analysis. The area under ROC curve of our model was significantly better than those of the other models (electronic supplementary Fig. 4).

DISCUSSION

MVI of HCC is a significant poor prognostic factor of survival in patients undergoing HR and LT [4, 14–16] and a better predictor of tumor recurrence and OS than the commonly used Milan criteria [3]. Therefore, predicting it preoperatively is helpful in guiding treatment strategy. Although intratumoral MVI was not identified by multivariate analysis as an independent risk factor for survival, except for OS of patients who underwent LT, OS of the intratumoral MVI group among both HR and LT patients was significantly worse than that of the no VI group. Therefore, the possibility of tumor emboli within nodules should be considered. In contrast, extratumoral MVI was a common independent prognostic factor for both the HR and LT cohorts. In the patients with extratumoral MVI who underwent HR or LT, 5-year OS was only 29.2% or 40.7%, respectively. The prognosis of patients with extratumoral MVI did not differ from those in patients with macrovascular invasion. According to the BCLC staging system, patients with macrovascular invasion were staged as BCLC-C, meaning no criteria for LT and HR. Therefore, it becomes critical to diagnose the location of MVI (intra- or extratumor) since preoperative prediction of extratumoral MVI has a similar impact as the detection of macrovascular invasion, and is a major factor in deciding on treatment strategy.

The nomogram predicting extratumoral MVI demonstrated good prediction among both the internal and external validation cohorts. Moreover, the predictive power for extratumoral MVI of the nomogram was significantly better than that of the other MVI predicting models and LT criteria. If all six factors are negative, the probability of extratumoral MVI based on the nomogram is 7%. In contrast, the probability is 84% if all factors are present. In this study, we established a nomogram for extratumoral MVI from the common datasets of patients who underwent HR or LT. The purpose of building the nomogram from the common datasets of patients who underwent HR or LT was to establish a nomogram that is appropriate for patients who are candidates for either HR or LT. Not all patients with HCC can be clearly assigned to one of these approaches, i.e. HR and LT. Moreover, this nomogram incorporates only preoperative factors and its accuracy is good both for patients who underwent HR (c-index 0.736) or LT (c-index 0.926) in the internal validation cohort, and will therefore help with preoperative decisions on strategies for the treatment of HCC in patients who are candidates for either HR or LT. In a clinical setting, patients in the fourth quartile (nomogram points $> 195 =$ 37% probability, $n = 35$) who underwent LT showed extremely poor RFS in the training cohort (5-year RFS 58.5%; data not shown). Therefore, patients with high nomogram points should not be considered for LT upfront, meaning that some neoadjuvant therapy such as TACE may be needed to reduce the probability of extratumoral MVI [17]. With regard to HR, patients in the fourth quartile (nomogram points > 195, $n = 89$) showed significantly poorer prognoses than patients in quartiles 1–3 (5-year OS 45.4% vs. 57.5%, $p = 0.02$; data not shown). If liver function is preserved in patients with a high nomogram score, anatomical liver resection or ensuring wide surgical margins are recommended because of their reported beneficial effect on the prognosis of patients with VI [18–21].

Overall, 17 patients had occult macrovascular invasion, i.e. those who were diagnosed with macrovascular invasion by pathological examination only. All these patients had extratumoral MVI. The mean probability of extratumoral MVI for these 17 patients using the nomogram was 59% (31–78) and 16 patients (94%) exhibited a probability of $> 37\%$, which corresponds to the fourth quartile (data not shown). Therefore, this nomogram may be useful in distinguishing extratumoral MVI as well as occult macrovascular invasion.

Non-boundary type in image was significantly correlated with extratumoral MVI. In particular, infiltrative HCCs have a higher MVI rate, with poorer prognosis, than other types [22, 23]. In this study, the rate of extratumoral MVI was significantly higher in the infiltrative type than other types (57.7% vs. 23.6%, $p < 0.0001$; data not shown) and the relative risk in the infiltrative type was 2.45 (data not shown). Therefore, there was a strong correlation between infiltrative type and extratumoral MVI.

Recently, ALP was found to be correlated with cells with epithelial mesenchymal transition (EMT) phenotypes [24–27] EMT is considered as the first step in MVI by epithelial HCC cells [28]. Therefore, ALP may be useful not only as a marker of cholestasis but also as a biomarker for reflecting cancer cell proliferation or invasion. The addition of ALP to the predictive model for MVI could reflect invasive ability of tumor cells with more precision.

This study had several limitations. First, the nomogram was created from data obtained at a single institution. Second, the patient cohort included only patients who underwent HR or LT, and not those treated only with local ablation therapy or TACE. Third, the accuracy of the nomogram was high but less than perfect. Therefore, there is potential to have extratumoral MVI confirmed by postoperative pathological examination even if patients show a low probability of extratumoral MVI preoperatively. Nonetheless, some adjuvant chemotherapy may be needed to reduce the risk of recurrence in such cases since the prognosis of patients with extratumoral MVI is severe [29, 30].

CONCLUSIONS

Extratumoral MVI was an independent poor prognostic factor for patients who underwent HR or LT, and showed similar prognosis as patients with macrovascular invasion. The nomogram allowed reliable prediction of extratumoral MVI in patients undergoing HR or LT, and could therefore be valuable when deciding, preoperatively, on resection or LT, with or without neoadjuvant treatment, as the best strategy.

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REFERENCES

1. Otto G, Heuschen U, Hofmann WJ, Krumm G, Hinz U, Herfarth C. Survival and recurrence after liver transplantation versus liver resection for hepatocellular carcinoma: a retrospective analysis. Ann Surg 1998;227:424–432.
2. Jonas S, Bechstein WO, Steinmüller T, et al. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. Hepatology 2001;33:1080–1086.
3. Lim KC, Chow PK, Allen JC, et al. Microvascular invasion is a better predictor of tumor recurrence and overall survival following surgical resection for hepatocellular carcinoma compared to the Milan criteria. Ann Surg 2011;254:108–113.
4. Mazzaferro V, Llovet JM, Miceli R, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet Oncol 2009;10:35–43.
5. Roayaie S, Blume IN, Thung SN, et al. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. Gastroenterology 2009;137:850–855.
6. He J, Shi J, Fu X, et al. The clinicopathologic and prognostic significance of gross classification on solitary hepatocellular carcinoma after hepatectomy. Medicine 2015;94:e1331.
7. Fu X, Mao L, Tang M, et al. Gross classification of solitary small hepatocellular carcinoma on preoperative computed tomography: prognostic significance after radiofrequency ablation. Hepatol Res 2016;46:298–305.
8. Adam R, Bhangui P, Vibert E, et al. Resection or transplantation for early hepatocellular carcinoma in a cirrhotic liver: does size define the best oncological strategy? Ann Surg 2012;256:883–891.
9. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: Issues in developing models, evaluating asumptions and

adequacy, and measuring and reducing error. Stat Med 1996;15:361–387.
10. Janssen KJM, Donders ART, Harrell FE, et al. Missing covariate data in medical research: To impute is better than to ignore. J Clin Epidemiol 2010;63:721–727.
11. Cucchetti A, Piscaglia F, Grigioni AD, et al. Preoperative prediction of hepatocellular carcinoma tumour grade and microvascular invasion by means of artificial neural network: a pilot study. J Hepatol 2010;52:880–888.
12. Lei Z, Li J, Wu D, et al. Nomogram for Preoperative Estimation of Microvascular Invasion Risk in Hepatitis B Virus-Related Hepatocellular Carcinoma Within the Milan Criteria. JAMA Surg 2016;151:356–363.
13. Duvoux C, Roudot-Thoraval F, Decaens T, et al. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. Gastroenterology 2012;143:986–994.
14. Vauthey JN, Lauwers GY, Esnaola NF, et al. Simplified staging for hepatocellular carcinoma. J Clin Oncol 2002;20:1527–1536.
15. Rodríguez-Perálvarez M, Luong TV, Andreana L, et al. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. Ann Surg Oncol 2013;20:325–339.
16. Shim JH, Jun MJ, Han S, et al. Prognostic nomograms for prediction of recurrence and survival after curative liver resection for hepatocellular carcinoma. Ann Surg 2015;261:939–946.
17. Allard MA, Sebagh M, Ruiz A, et al. Does pathological response after transarterial chemoembolization for hepatocellular carcinoma in cirrhotic patients with cirrhosis predict outcome after liver resection or transplantation? J Hepatol 2015;63:83–92.
18. Imamura H, Matsuyama Y, Miyagawa Y, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. Br J Surg 1999;86:1032–1038.
19. Yamashita YI, Imai D, Bekki Y, et al. Surgical outcomes of anatomical resection for solitary recurrent hepatocellular carcinoma. Anticancer Res 2014;34:4421–4426.
20. Zhao H, Chen C, Fu X, Yan X, Jia W, Mao L. Prognostic value of a novel risk classification of microvascular invasion in patients with hepatocellular carcinoma after resection. Oncotarget 2017;8:5474–5486.
21. Yamashita YI, Tsuijita E, Takeishi K, et al. Predictors for microinvasion of small hepatocellular carcinoma $\leq$ 2 cm. Ann Surg Oncol 2012;19:2027–2034.
22. Wang WT, Yang L, Yang ZX, et al. Assessment of Microvascular Invasion of Hepatocellular Carcinoma with Diffusion Kurtosis Imaging. Radiology 2018;286:571–580.
23. Yan X, Fu X, Deng M, et al. Infiltrative hepatocellular carcinoma: assessment of factors associated with outcomes in patients undergoing hepatectomy. Medicine (Baltimore) 2016;95:e3589.
24. Sobiesiak M, Sivasubramaniyan K, Hermann C, et al. The mesenchymal stem cell antigen MSCA-1 is identical to tissue non-specific alkaline phosphatase. Stem Cells Dev 2010;19:669–677.
25. Yamamoto K, Awogi T, Okuyama K, Takahashi N. Nuclear localization of alkaline phosphatase in cultured human cancer cells. Med Electron Microsc 2003;36:47–51.
26. Iwadate Y, Matsutani T, Hirono S, Shinozaki N, Saeki N. Transforming growth factor-beta and stem cell markers are highly expressed around necrotic areas in glioblastoma. J Neurooncol 2016;129:101–107.
27. Yi JK, Mehrazarin S, Oh JE, et al. Osteo-/odontogenic differentiation of induced mesenchymal stem cells generated through epithelial-mesenchyme transition of cultured human keratinocytes. J Endod 2014;40:1796–1801.
28. Chung KY, Cheng IKC, Ching AKK, Chu JH, Lai PBS, Wong N. Block of proliferation 1 (BOP1) plays an oncogenic role in hepatocellular carcinoma by promoting epithelial-to-mesenchymal transition. Hepatology 2011;54:307–318.
29. Zhu GQ, Shi KQ, Yu HJ, et al. Optimal adjuvant therapy for resected hepatocellular carcinoma: a systematic review with network meta-analysis. Oncotarget 2015;6:18151–18161.
30. Nitta H, Beppu T, Imai K, et al. Adjuvant hepatic arterial infusion chemotherapy after hepatic resection of hepatocellular carcinoma with macroscopic vascular invasion. World J Surg 2013;37:1034–1042.