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Predictive model for microvascular invasion of hepatocellular carcinoma among candidates for either hepatic resection or liver transplantation

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- 21 The authors declare no conflict of interest
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- 23

Abstract

Background: Microvascular invasion (MVI) is the strongest prognostic factor [of survival](#) in patients with hepatocellular carcinoma (HCC). We therefore developed a predictive model for MVI of HCC, to help guide treatment strategies in patients scheduled for either hepatic resection (HR) or liver transplantation (LT).

Methods: HCC patients who underwent HR or LT from 1994 to 2016 were divided into training and validation cohorts. A predictive model for MVI was developed based on MVI risk factors in the training cohort, and validated in the validation cohort.

Results: A total of 910 patients (425 HR, 485 LT) were included in the training (n=637) and validation (n=273) cohorts. Multivariate analysis identified alfa-fetoprotein ≥ 100 ng/ml (relative risk [RR] 3.05, $P < 0.0001$), tumor size ≥ 40 mm (RR 1.98, $P = 0.0002$), non-boundary HCC type (RR 1.91, $P = 0.001$), neutrophil-to-lymphocyte ratio (RR 1.86, $P = 0.002$), and aspartate aminotransferase (RR 1.53, $P = 0.02$) as associated with MVI.

The estimated probability of MVI ranged from 17.0% in patients with none of these factors to 86.9% in the presence of all factors. This model achieved a C-index of 0.732 in the validation cohort. The 5-year overall survival of patients with $\geq 50\%$ probability of MVI was poorer than that of patients with $< 50\%$ probability (HR; 39.1% vs 61.2%, $P < 0.0001$, LT; 5-year OS, 54.8% vs 79.0%, $P = 0.05$).

Conclusion: This model developed from preoperative data allows reliable prediction of

43 MVI and may help with preoperative decisions regarding the suitability of HR or LT in
44 patients with HCC. in candidates for either HR or LT.

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Introduction

Microvascular invasion (MVI) of hepatocellular carcinoma (HCC) is a significant poor risk prognostic factor of survival in patients undergoing hepatic resection (HR) and liver transplantation (LT)¹⁻⁴, and a better predictor of tumor recurrence and overall survival (OS) than the commonly used Milan criteria⁵. However, MVI is only diagnosed by pathological examination after HR or LT, and is impossible to predict preoperatively. Given that the probability of preoperative MVI may influence the choice of surgical strategy and allow the selection of patients likely to benefit from HR or LT, it is necessary to develop a more accurate predictive model for MVI.

Advanced [¹⁸F] fluorodeoxyglucose positron emission tomography or diffusion-weighted magnetic resonance imaging (MRI) images, as well as genetic markers, may help to predict MVI⁶⁻⁹. However, studies of these techniques have included limited numbers of patients, and furthermore, the costs and expertise required by these techniques may inhibit their routine use. A predictive model of MVI based on easily available factors and imaging techniques may thus offer a more practical and useful approach.

Treatment strategies take into consideration the patient's general condition, tumor status, and liver function^{10,11}; HR is generally considered when the tumor status and

liver function are favorable, while LT may be preferred when the liver function is unfavorable and tumor status less advanced. Most risk models for MVI are adapted for either HR or LT², but not all patients can be clearly assigned to one of these approaches because of their background, tumor status, liver function, and organ shortage. A predictive model for MVI that could be used to guide treatment strategies in patients scheduled for either HR or LT would therefore be a valuable tool. This study therefore aimed to identify independent predictors of MVI and establish a user-friendly model to predict MVI based on preoperative factors in candidates for either HR or LT. We then sought to assess the value of our model in a validation cohort.

Patients and Methods

Patient Population

We ~~enrolled~~ **included** 1051 consecutive patients diagnosed with HCC preoperatively, who underwent HR (n=490) or LT (n=561) at the Paul Brousse Hospital, Villejuif, France, between January 1994 and June 2016. We excluded patients who were diagnosed as not having HCC by pathological examination (n=74), underwent R2 resection (n=5), had macrovascular invasion (n=48), or who had insufficient data (n=14). The remaining 910 patients were included in the study. Patients diagnosed with HCC

preoperatively were included, even if they underwent pretreatment before HR or LT. Patients who underwent surgery between January 1994 and June 2012 were assigned to the training cohort (n=637) and those who underwent surgery between July 2012 and May 2016 were assigned to the validation cohort (n=273). The median follow-up time for the training cohort was 86.3 months (HR patients, 59.0 months; LT patients, 116.5 months).

Preoperative Diagnosis and Treatment

HCC diagnosis was based on imaging data (ultrasound, contrast-enhanced computed tomography [CE-CT], and MRI), serum alfa-fetoprotein (AFP), and clinical parameters according to recent international guidelines¹². The optimal treatment strategies for HCC patients were determined at multidisciplinary meetings involving surgeons, oncologists, hepatologists, and radiologists. ~~Patients with single, peripheral tumors and compensated liver function were usually selected to undergo HR and R0 resection, whereas patients~~ [Patients](#) with multiple tumors with unpreserved liver function or severe portal hypertension were proposed for LT, as described previously¹³.

Clinical Parameters

All clinical parameters, including hematological and biochemical findings and tumor markers, were obtained within 1 month before surgery. The largest tumor size and tumor number preoperatively were based on preoperative CE-CT. For patients who underwent pretreatment, the clinical parameters and the radiological images were obtained at the latest date before surgery after pretreatment. The largest tumor gross shape was evaluated by experienced radiologists based on CE-CT, and classified into one of four types based on gross morphological appearance. Tumor shapes that were not evaluable by CE-CT due to lipiodol deposition after transcatheter arterial chemoembolization (TACE) were evaluated by MRI. The types were defined as follows: type 1, single nodular type with round or oval shape with a clear boundary, with or without fibrous pseudocapsule; type 2, single nodular type with extranodular growth roughly resembling type 1 but with varying degrees of local extranodular growth; type 3, confluent multinodular type comprising a cluster of small and confluent nodules, each with a clear margin or capsule, and the tumor lobulated as a whole; and type 4, infiltrative type with irregular shape and unclear border^{14,15}. We defined type 1 as boundary type and types 2–4 as non-boundary types.

Pathological Examination

All pathological specimens were routinely examined by two pathologists (CG, MS) who were experts in liver tumor pathology. Specimens were cut into 5-mm thick slices. For tumors <2 cm, nodules were sampled in their entirety; for larger lesions, nodules were sampled extensively from the center to the periphery, with the number of samples at the discretion of the pathologist. Sections were stained with hematoxylin-eosin-safran. MVI was defined when cancer cells were present within the lumens of veins and venules. In the event of any uncertainty, tumoral invasion was confirmed by immunohistochemistry using a vascular antibody (CD34, D240).

Statistical Analysis

Categorical variables were compared using χ^2 or Fisher's exact tests. Continuous variables were shown as mean $\pm$ standard deviation, and compared using Mann-Whitney U tests. Cut-off values for continuous factors were determined based on receiver-operating characteristic (ROC) curve analysis.

There were no missing data for most variables among the training cohort, except for hepatitis B virus-DNA (n=16, 2.5%), hepatitis C virus-RNA (n=78, 12.2%), body mass index (n=19, 3.0%), platelet count (n=8, 1.3%), albumin (n=37, 5.8%), neutrophil-to-lymphocyte ratio (NLR) (n=35, 5.5%), creatinine (n=6, 0.9%),

gamma-glutamyltransferase (n=11, 1.7%), alkaline phosphatase (ALP) (n=27, 4.2%),
alanine aminotransferase (ALT) (n=2, 0.3%), and boundary-type on CT (n=90, 14.1%).
The exclusion of patients with missing data might lead to biased risk estimates^{16,17} and
multiple imputation (N=20) was therefore performed for all multivariate analyses using
the R package (mice). There were no missing data in the validation cohort. Each of the
imputed datasets (n=20) was analyzed separately using a multivariable Cox regression
or logistic regression model and pooled to identify independent predictors of survival or
MVI, respectively. Variables with a *P* value of < 0.20 in univariate analysis were
subjected to multivariate analysis using Cox proportional hazards and logistic
regression models. All variables associated with prognosis and MVI were candidates
using a stepwise backward elimination procedure with a threshold of *P* < 0.05. The
level of significance for all tests was set at *P* < 0.05. The probabilities of MVI in the
predictive model were calculated based on the coefficients obtained in the multivariate
logistic regression model. The predictive performance of the model was measured by
the concordance index (C-index) and calibration with 400 bootstrap samples.
Cumulative ~~overall survival~~ (OS) and recurrence-free survival (RFS) curves were
obtained using the Kaplan–Meier method. The OS and RFS rates after surgery were
estimated by the log-rank test. Survival time was calculated from the date of HR or LT

to the date of the event of interest, 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. All statistical analyses were performed using JMP ver.12 (SAS Institute, Cary, NC, USA), R version 3.1.1. and the R package (mice).

Results

Risk Factors for Prognosis in Training Cohort and Outcomes with or without

Microvascular Invasion

The backgrounds of the patients in the training and validation cohorts are shown in supplementary Table 1. We initially evaluated the risk factors for poor OS and RFS among the training cohort (Table 1). In patients who underwent HR, MVI was an independent risk factor for OS (~~relative risk [RR], 2.51; 95% confidence interval [CI],~~ ~~1.63–3.87; $P < 0.0001$~~ **hazard ratio, 2.63; 95% confidence interval [CI], 1.72–4.05; $P < 0.0001$**) and RFS (~~RR, 1.58; 95% CI, 1.03–2.43; $P = 0.04$~~ **hazard ratio, 1.98; 95% CI, 1.44–2.70; $P < 0.0001$**). MVI was also an independent risk factor for OS (~~RR, 2.01; 95% CI, 1.42–2.83; $P < 0.0001$~~ **hazard ratio, 2.35; 95% CI, 1.69–3.27; $P < 0.0001$**) and RFS (~~RR, 3.07; 95% CI, 1.63–5.79; $P = 0.0005$~~ **hazard ratio, 3.22; 95% CI, 1.73–6.17; $P = 0.0002$**) in patients who underwent LT. The Kaplan–Meier curve according to MVI is

shown in Fig 1. The 5-year OS and RFS in patients who underwent HR were significantly different with and without MVI (OS, 38.4% vs 66.1%, $P < 0.0001$; RFS, 15.8% vs 28.6%, $P < 0.0001$, respectively). The 5-year OS and RFS in patients who underwent LT with and without MVI were 51.4 vs 80.6%, and 60.9% vs 89.9%, respectively (OS, $P < 0.0001$; RFS, $P < 0.0001$).

Preoperative Risk Factors for MVI after HR or LT in the Training Cohort

All variables in this univariate analysis were based on data obtained preoperatively.

Univariate analysis identified eight preoperative factors associated with MVI after HR and LT: previous TACE, platelet count, NLR, ALP, AST, AFP, largest tumor size on preoperative imaging, tumor number on preoperative imaging >3 , and non-boundary type (Table 2). Multivariate analysis identified five independent predictors for MVI:

AFP ≥ 100 ng/ml (RR, 3.05; 95%CI, 1.98–4.70; $P < 0.0001$), largest tumor size in image ≥ 40 mm (RR, 1.98; 95%CI, 1.38–2.84; $P = 0.0002$), non-boundary type (RR, 1.91; 95%CI, 1.29–2.82; $P = 0.001$), NLR ≥ 3.2 (RR, 1.86; 95%CI, 1.26–2.75; $P = 0.002$), and AST ≥ 62 (RR, 1.53; 95%CI, 1.08–2.17; $P = 0.02$) (Table 2). In addition, the predictive factors of MVI for each group underwent HR or LT, or patients without cirrhosis were shown in supplementary Table 2.

Development and Validation of Predictive Model for MVI Using Training Cohort

The predictive model was then developed using the previous five factors based on logistic regression analysis (Table 3). The estimated probabilities (%) for MVI were thus weighted according to the RR for each of the five factors. The estimated probability of MVI was 17.0% in patients without any factors, and increased to 86.9% in the presence of all factors. This predictive model was validated internally in the training cohort using the bootstrap validation method, with a bootstrap-corrected C-index of 0.710. In addition, calibration plots for the probability of MVI showed good calibration for the presence of MVI between the risk estimation according to the model and histopathologic confirmation in surgical specimens (Fig. 2A).

Validation of the Predictive Model for MVI Using the Validation Cohort

The model was subsequently validated using the validation cohort. A calibration plot for the probability of MVI showed good calibration between the model's predicted and actual observations (unadjusted C-index 0.750, 95%CI, 0.683–0.805; bootstrap-corrected C-index 0.732) (Fig. 2B). When the validation cohort was divided into patients who underwent HR and those who underwent LT, the bootstrap-corrected

C-indexes were 0.691 and 0.798, respectively, indicating good concordances in both groups (Fig. 2C, D). Moreover, when the validation cohort was divided into patients within and beyond the Milan criteria, the bootstrap-corrected C-indexes were 0.749 and 0.796, respectively (supplementary Fig 1A, B). The C-index of Child–Pugh A, and B or C were 0.759 and 0.760, respectively (supplementary Fig 1C, D).

The relationships between MVI probabilities and actual MVI rates confirmed by pathological examination are summarized in Table 4. The MVI probabilities corresponded closely with the actual MVI rates.

Outcomes of Patients with High or Low MVI Probabilities

We evaluated the outcomes of HCC patients according to MVI probability in the training cohort. The optimal cut-off value for MVI probability was 49.0% according to ROC analysis (area under the curve, 0.721). We therefore compared the prognoses of patients with MVI probabilities $<50\%$ and $\geq 50\%$. Among patients who underwent HR, the 5-year OS and RFS rates in patients with MVI probability $\geq 50\%$ were significantly lower than those of patients with MVI probability $<50\%$. (5-year OS, 39.1% vs 61.2%, $P < 0.0001$; 5-year RFS, 20.1% vs 24.7%, $P < 0.0001$) (Fig. 3A, B). Among patients who underwent LT, there was no significant difference in OS between patients with MVI

probabilities $\geq 50\%$ and $< 50\%$ (5-year OS, 54.8 % vs 79.0%, $P = 0.05$), but the 5-year RFS in patients with an MVI probability $\geq 50\%$ was significantly poorer than in patients with an MVI probability $< 50\%$ (5-year RFS, 61.2% vs 88.2%, $P < 0.0001$) (Fig. 3C, D). When the training cohort was divided into patients within and beyond the Milan criteria, the outcomes of patients with an MVI probability $\geq 50\%$ was worse than that of patients with MVI probability $< 50\%$ (supplementary Fig 2).

Comparison of predictive powers

The predictive powers of MVI in our predictive model, the other predicting model (artificial neural network¹⁸), and the LT criteria (Milan criteria, UCSF criteria, and AFP model¹⁹) were compared by ROC curve analysis in the validation cohort. The area under ROC curve of our model was significantly better than those of the other models among either patients who underwent HR or patients who underwent LT (Supplementary Fig. 3).

Discussion

The results of this study showed that the risk of death or recurrence in patients with MVI, which accounted for about 40% of all patients, was two to three times higher than in patients without MVI who underwent HR or LT. Preoperative evaluation of the probability of MVI could thus influence the choice of surgical strategy. Multivariate analysis identified five preoperative factors that were significantly associated with the presence of MVI: AFP ≥ 100 ng/ml, no-boundary type in CE-CT, NLR ≥ 3.2 , AST ≥ 62 , and largest tumor size in CE-CT ≥ 40 mm. The predictive model suggested that the probability of MVI varied from 17.0%–86.9%, depending on the combinations of these five factors. The performance of this model was supported by C-index values of 0.710 and 0.732 in the training and validation cohorts, respectively, and by optimal calibration curves demonstrating agreement between the predicted and actual observations. Moreover, the predictive power for MVI of the model was significantly better than those of the other MVI predicting model and LT criteria.

Some previous studies attempted to predict MVI, but most had some limitations such as small sample sizes, and being limited to patients with early HCC or hepatitis B antigen-related HCC^{2,20}, thus limiting the general use of these models in all HCC patients. In contrast, the current study ~~enrolled~~ included all patients diagnosed with

261 HCC preoperatively, and thus included patients with various tumor characteristics and
262 liver functions. The current study analyzed common datasets for patients who
263 underwent HR or LT, ~~with the aim of establishing a predictive model appropriate for~~
264 ~~candidates for either HR or LT, given that~~ because not all patients with HCC can be
265 clearly assigned to one of these approaches. The resulting predictive model
266 demonstrated high accuracy in patients with Child–Pugh A (C-index, 0.759), B or C
267 (C-index, 0.760) among the validation cohort, suggesting that it could be adopted for
268 patients with or without cirrhosis. Moreover, the high C-indexes for patients within
269 (0.749) and beyond the Milan criteria (0.796) also suggested that the predictive model
270 was suitable for both groups. Furthermore, the high C-indexes in patients who
271 underwent LT (0.798) and HR (0.691) suggested that the model may be useful for
272 helping to determine the optimal treatment strategy in both groups of patients.

273 In addition to tumor size and number, MVI should also be considered when choosing a
274 surgical strategy, because this is one of the strongest factors predicting poor prognosis
275 among patients undergoing HR or LT, even within the Milan criteria^{5,21,22}. The
276 prognosis of patients with MVI who underwent LT was extremely poor (5-y OS, 51.4%;
277 5-y RFS, 60.9%). ~~In particular, among the patients with MVI who underwent LT and~~
278 ~~could be followed up for >5 years, the recurrence rate was as high as 52% (47/90) (data~~

279 ~~not shown~~). Therefore, it may be better to avoid upfront LT for patients who are more
280 likely to have MVI, not only because of the poor prognosis but also because of the
281 ~~current organ shortage~~. The cut-off value based on MVI probability was 50%, according
282 to ROC analysis. This cut-off value could be an important clinical indicator for deciding
283 on the treatment strategy for HCC. The outcomes of LT in patients with high MVI
284 probability ($\geq 50\%$) were ~~extremely~~ poor (5-year OS: 49.7%; 5-year RFS: 48.0%),
285 suggesting that these patients should not be considered for upfront LT, ~~and neoadjuvant~~
286 ~~therapies such as TACE or sorafenib may be needed to reduce the probability of MVI.~~
287 ~~Jeong et al. reported that even in patients with concurrent HCC and MVI, TACE and~~
288 ~~radiotherapy prior to LT showed acceptable oncologic outcomes²³.~~ AFP was the
289 strongest risk factor for MVI in our study. If locoregional therapy can reduce the AFP
290 value to less than 100 ng/ml without exacerbating other factors (tumor size, tumor shape,
291 NLR, and AST), the probability of MVI will be reduced as shown in Table 3. ~~Halazun et~~
292 ~~al. reported that the AFP response to locoregional therapies prior to LT was an~~
293 ~~independent predictor of RFS^{24,23}.~~ ~~Therefore, locoregional therapy prior to LT may be~~
294 ~~needed to reduce the probability of MVI and improve the prognosis for patients with~~
295 ~~HCC suspected to have MVI.~~ Therefore, for patients with a high MVI probability, the
296 decrease in the MVI probability after locoregional therapy prior to LT may be important

297 in selecting good candidates for LT, and this may lead to an improved prognosis.

298 Patients with a $\geq 50\%$ probability of MVI among patients who underwent HR showed

299 significantly poorer prognoses than those with $< 50\%$ probability (5-year OS: 42.4% vs

300 56.7%, $P = 0.001$). ~~If liver function is preserved in patients with $\geq 50\%$ probability,~~

301 ~~anatomical HR or ensuring wide surgical margins is recommended, because of its~~

302 ~~reported beneficial effect on the prognosis of patients with vascular invasion~~²⁵⁻²⁸. There

303 is no consensus on how to undergo HR for HCC with MVI. However, in this study, the

304 rate of microsatellite lesions in patients with MVI was 47% (118/251), which was

305 significantly higher than for patients without MVI (23%, 89/386) ($P < 0.0001$, data not

306 shown). Zhou et al. reported that the required minimal length of the resection margin is

307 6 mm to achieve 100% micrometastasis clearance in the surrounding liver tissue for

308 HCC²⁴. Yamashita et al. reported that the disadvantage with the DFS rate for patients

309 with HCC with MVI could be removed by hepatic resection with a wide tumor margin

310 of ≥ 5 mm²⁵. Although surgical margin is controversial, a wide surgical margin might be

311 one effective therapy for removing the main tumor with these satellite lesions or tumor

312 thrombi. We suggest that these concepts may even be adopted in patients within the

313 Milan criteria, because patients with a $\geq 50\%$ probability had a poorer prognosis than

314 those with a $< 50\%$ probability, among patients meeting the Milan criteria. This model

could therefore be used to calculate the MVI probability preoperatively, thus guiding the choice of surgical strategy.

Tumor type has been classified using CE-CT^{14,15,26,27}. In this study, we classified tumor shape (boundary or non-boundary type) using CE-CT, because this method was less ambiguous and a good predictor of gross classification. However, the Japanese gross classification has also been used as a prognostic factor [of survival](#) and to predict MVI²⁸. Wu et al. reported that tumor shape reflected the incidence of MVI, using various imaging techniques²⁹, while He et al. also typed tumors according to shape using CE-CT and associated them with MVI, with a similar accuracy to gross classification of 65.3%¹⁴.

There were some limitations to the current study. First, it was a retrospective, single-center study. Second, numerous imaging modalities are now available for predicting MVI preoperatively, and contrast-enhanced ultrasound shows a significantly higher correlation with gross classification than CE-CT (86.9% vs 65.6%)³⁰. Tumor shape evaluated by gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced MRI, or apparent diffusion coefficients evaluated by diffusion-weighted MRI, could also be useful for predicting MVI^{7,31}. The use of these imaging techniques may improve the predictive accuracy for MVI, but more experience and skill may be

required to implement them.

Conclusion

We developed a predictive model for MVI in patients with HCC, suitable for use in potential candidates for either HR or LT, based on tumor size, AST, NLR, tumor boundary, and AFP. This model could be a valuable tool in helping to decide treatment strategies in HCC patients.

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450

Table 1 Multivariate analysis of prognostic factors for overall and recurrence-free survival among patients who underwent hepatic resection or transplantation (Cox proportional hazard model)

		RR	95%CI	p-value
Hepatic resection OS	MVI	2.51	1.63-3.87	<0.0001
	Platelet <10×10 ⁴ /μl	1.90	1.10-3.29	0.02
	Creatinine ≥90 μmol/l	1.77	1.10-2.86	0.02
	Albumin <35 g/l	1.63	1.07-2.49	0.02
	Total bilirubin ≥20 μmol/l	1.63	1.02-2.62	0.04
Hepatic resection RFS	Total bilirubin >20 μmol/l	2.18	1.35-3.51	0.001
	MVI	1.58	1.03-2.43	0.04
Transplantation OS	MVI	2.01	1.42-2.83	<0.0001
	Non-boundary type in CT	1.80	1.20-2.72	0.005
	GGT ≥230 U/l	1.70	1.12-2.59	0.01
	Tumor number >3	1.59	1.11-2.28	0.01
	Creatinine >80 μmol/l	1.54	1.10-2.15	0.01
	Tumor size ≥30mm	1.52	1.09-2.13	0.01
	Age >60	1.46	1.04-2.04	0.03
Transplantation DFS	Tumor size ≥30mm	3.83	1.96-7.51	<0.0001
	MVI	3.07	1.63-5.79	0.0005
	GGT ≥230 U/l	2.66	1.35-5.25	0.005
	AFP ≥100 ng/ml	2.29	1.17-4.50	0.02
	Non-boundary type	2.11	1.14-3.90	0.02

		Hazard ratio	95% CI	P-value
Hepatic resection OS	Tumor number >3	2.73	1.14-5.52	0.03
	MVI	2.63	1.72-4.05	<0.0001
	Albumin <35g/l	1.82	1.21-2.76	0.004
Hepatic resection RFS	Tumor number >3	2.77	1.35-5.01	0.007
	MVI	1.98	1.44-2.70	<0.0001
Transplantation OS	MVI	2.35	1.69-3.27	<0.0001
	Age ≥52	1.67	1.13-2.56	0.01
	Tumor size ≥40mm	1.52	1.06-2.16	0.02
Transplantation RFS	Tumor size ≥40mm	4.43	2.40-8.17	<0.0001
	MVI	3.22	1.73-6.17	0.0002
	Non-boundary type	1.77	1.02-3.25	0.04

457 ~~RR, relative risk; CI, confidence interval; OS, overall survival; RFS, recurrence-free~~
458 ~~survival; MVI, microvascular invasion; GGT, gamma-glutamyltransferase; AFP,~~
459 ~~alfa-fetoprotein.~~

460 Variables (age, gender, body mass index, diabetes, alcohol, HBs-Ag, HBV-DNA,
461 HCV-Ab, HCV-RNA, Child-Pugh, MELD score, platelet count, albumin, total bilirubin,
462 prothrombin time-international normalized ratio, creatinine, AST, ALT, ~~GGT-~~
463 ~~gamma-glutamyltransferase~~, ALP, NLR, ~~AFP~~ ~~alfa-fetoprotein~~, tumor size, tumor
464 number, transfusion, non-boundary type, tumor differentiation, MVI, satellite lesion,
465 tumor capsulation and surgical margin) were subjected to univariate analysis.

466

467 **Table 2** Univariate and multivariate analyses of preoperative risk factors for

468 microvascular invasion in the training cohort

		Univariate analysis			Multivariate analysis		
		No MVI (n=386) No, (%)	MVI (n=251) No, (%)	<i>P</i> value	RR	95%CI	<i>P</i> value
Gender	Male	320 (82.9)	216 (86.1)	0.24			
	Female	66 (17.1)	35 (13.9)				
Age	≥52	309 (80.0)	188 (74.9)	0.13	n.s.		
HBs-Ag	+	59 (15.8)	40 (16.3)	0.82			
HBV-DNA	≥4 log	5 (1.3)	9 (3.7)	0.06	n.s.		
HCV-Ab	+	142 (36.8)	100 (39.8)	0.44			
HCV-RNA	Positive	59 (18.1)	52 (22.3)	0.22			
Cirrhosis	+	60 (15.5)	43 (17.1)	0.60			
Child Pugh	A	213 (56.5)	129 (54.2)	0.31			
	B	134 (35.5)	96 (40.3)				
	C	30 (8.0)	13 (5.5)				
MELD score	≥10	133 (34.7)	84 (33.6)	0.77			
Body mass index	≥25	202 (54.2)	122 (49.8)	0.30			
Alcohol	+	100 (25.9)	53 (21.1)	0.16	n.s.		
Diabetes	+	101 (26.2)	74 (29.4)	0.36			
Previous TACE	+	256 (66.3)	146 (58.2)	0.04	n.s.		
Previous LAT	+	56 (14.5)	30 (12.0)	0.35			
Previous HR	+	19 (4.9)	18 (7.2)	0.24			
Platelet (×10 ⁴ /μl)	>23	50 (13.1)	62 (25.0)	0.0002	n.s.		
Total-Bilirubin (μmol/l)	≥18	212 (55.1)	133 (53.0)	0.61			
Albumin (g/l)	≥35	159 (43.4)	83 (35.4)	0.05	n.s.		
PT-INR	≥1.30	181 (46.9)	102 (40.6)	0.12	n.s.		
NLR	≥3.2	89 (24.3)	91 (38.7)	0.0002	1.86	1.26-2.75	0.002
Creatinine (μmol/l)	≥113	37 (9.6)	31 (12.3)	0.27			
GGT (U/l)	≥79	225 (59.1)	159 (64.9)	0.14	n.s.		
ALP (U/l)	≥122	128 (35.0)	108 (44.3)	0.02	n.s.		

AST (U/l)	≥62	148 (38.3)	130 (51.8)	0.0008	1.53	1.08-2.17	0.02
ALT (U/l)	≥40	194 (50.5)	146 (58.2)	0.06	n.s.		
AFP (ng/ml)	≥100	45 (11.9)	82 (33.1)	<0.0001	3.05	1.98-4.70	<0.0001
Image results							
Largest Tumor size in image (mm)	≥40	116 (30.0)	133 (53.0)	<0.0001	1.98	1.38-2.84	0.0002
Tumor number in image	≥2	168 (43.5)	106 (42.2)	0.75			
Tumor number in image	>3	28 (7.2)	33 (13.1)	0.01	n.s.		
Non-boundary type	Yes	98 (29.4)	112 (52.3)	<0.0001	1.91	1.29-2.82	0.001

469 MVI, microvascular invasion; RR, relative risk; CI, confidence interval; HBs-Ag,
470 hepatitis B surface antigen; HCV-ab, hepatitis C virus antibody; TACE, transcatheter
471 arterial chemoembolization; LAT, local ablation therapy; HR, hepatic resection;
472 PT-INR, prothrombin time–international normalized ratio; NLR,
473 neutrophil-to-lymphocyte ratio; GGT, gamma-glutamyltransferase; ALP, alkaline
474 phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP,
475 alfa-fetoprotein.

476

Table 3 Predictive model for microvascular invasion based on multivariate logistic

regression analysis

Number of positive factors	AFP $\geq$ 100	Tumor size $\geq$ 40	Non-boundary type	NLR $\geq$ 3.2	AST $\geq$ 62	MVI probability (%)
5	+	+	+	+	+	86.9
4	+	+	+	+	-	81.4
	+	+	+	-	+	78.3
	+	+	-	+	+	77.7
	+	-	+	+	+	77.1
	-	+	+	+	+	68.3
3	+	+	+	-	-	70.4
	+	+	-	+	-	69.6
	+	-	+	+	-	68.9
	+	+	-	-	+	65.5
	+	-	+	-	+	64.7
	+	-	-	+	+	63.8
	-	+	+	+	-	58.7
	-	+	+	-	+	54.0
	-	+	-	+	+	53.0
	-	-	+	+	+	52.3
2	+	+	-	-	-	55.5
	+	-	+	-	-	54.7
	+	-	-	+	-	53.7
	+	-	-	-	+	49.0
	-	+	+	-	-	43.6
	-	+	-	+	-	42.6
	-	-	+	+	-	41.9
	-	+	-	-	+	38.1
	-	-	+	-	+	37.3
	-	-	-	+	+	36.4
1	+	-	-	-	-	38.7
	-	+	-	-	-	28.8
	-	-	+	-	-	28.2
	-	-	-	+	-	27.4
	-	-	-	-	+	23.8
0	-	-	-	-	-	17.0

AFP, alfa-fetoprotein; NLR, neutrophil-to-lymphocyte ratio; AST, aspartate

aminotransferase; MVI, microvascular invasion.

Table 4

Relationship between MVI probability based on the model and actual MVI rate

MVI probability, %	Training cohort		Validation cohort		Total cohort	
	Actual MVI rate, %	<i>p</i> value	Actual MVI rate, %	<i>p</i> value	Actual MVI rate, %	<i>p</i> value
≤30	22.4 (56/250)	0.001 [†]	24.5 (37/151)	0.0009 [†]	23.2 (93/401)	<0.0001 [†]
>30-50	37.8 (51/135)	0.0002 [‡]	47.1 (32/68)	0.30 [‡]	40.9 (83/203)	0.0001 [‡]
>50-70	63.6 (63/99)	0.17 [¶]	57.5 (23/40)	0.02 [¶]	61.9 (86/139)	0.02 [¶]
>70	75.0 (36/48)		92.9 (13/14)		79.0 (49/62)	

[†]≤30 vs >30-50, [‡]>30-50 vs >50-70, [¶]>50-70 vs >70

MVI, microvascular invasion.

Figure legends

Figure 1. Overall survival curves for HCC patients who underwent HR (A) or LT (C), stratified according to the presence or absence of MVI. Recurrence-free survival curves for HCC patients who underwent HR (B) or LT (D), stratified according to the presence or absence of MVI.

Figure 2. Calibration plots comparing predicted and actual MVI probabilities among the training cohort (A), the validation cohort (B), HR patients in the training cohort (C), and transplantation patients in the training cohort (D). The dotted line indicates the ideal prediction and the black line is the bias-corrected prediction with 400 bootstraps.

Figure 3. Overall (A) and recurrence-free survival (B) curves in HCC patients who underwent HR according to MVI probability ($<50\%$ vs $\geq 50\%$) based on the model. Overall (C) and recurrence-free survival (D) curves in HCC patients who underwent LT according to MVI probability ($<50\%$ vs $\geq 50\%$) based on the model.

A

The training cohort

B

The validation cohort

C

HR patients among the validation cohort

D

LT patients among the validation cohort

A **B** **C** **D**