Accurate prediction of microvascular invasion occurrence and effective prognostic estimation for patients with hepatocellular carcinoma after radical surgical treatment

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Research Article

Keywords: Microvascular invasion, hepatocellular carcinoma, survival analysis, prediction model, therapeutic strategy

Posted Date: April 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1517313/v1

Abstract

Background

Hepatocellular carcinoma (HCC) is the third most common cause of cancer death worldwide with an overall 5-year survival rate was less than 18%, which may be related to the tumor microvascular invasion (MVI). This study aims to compare the clinical prognosis of HCC patients with or without MVI after radical surgical treatment, and further analyze the preoperative risk factors related to MVI to detect a new treatment strategy for HCC.

Methods

According to postoperative pathological diagnostic of MVI occurrence, 160 study patients undergoing radical hepatectomy were divided into MVI negative group (n = 68) and MVI positive group (n = 92). The clinical outcomes and prognosis of HCC patients was compared between the two groups, and then the clinical characteristic parameters were analyzed by multivariate Logistic regression to construct a MVI prediction model. Then, the practicability and validity of the model were evaluated, and the clinical prognosis of different MVI risk groups were subsequently compared.

Result

There were no significant differences between the MVI negative and MVI positive group in clinical baseline, haematological and imageological data. Also, the clinical outcome comparison between the two groups presented no significant differences excepted the pathological grading ($P = 0.002$) and survival and recurrence rate after surgery ($P < 0.001$). The MVI prediction model, based on preoperative AFP, tumor diameter and TNM stage, presented superior predictive efficacy (AUC = 0.7997) and good practicability (high H-L goodness of fit, $P = 0.231$). Compared the MVI high-risk group, the patients in the MVI low-risk group shown higher survival rate ($P = 0.002$) and lower recurrence rate ($P = 0.004$).

Conclusion

MVI is an independent risk factor of HCC for poor prognosis after radical resection. The MVI prediction model, consist of AFP, tumor diameter and TNM stage, exhibits a superior predictive efficacy and strong clinical practicability for MVI prediction and prognosis estimation, which provides a new therapeutic strategy for clinical standardized treatment of HCC patients.

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor and the third most common cause of cancer death worldwide[1, 2]. At present, comprehensive treatment based on radical surgery is

currently considered as the most effective treatment for HCC[3]. However, even radical excision is achieved, metastasis and recurrence are frequently occurred in about 70% within 5-years after resection, which resulting in HCC patients with a 5-years survival rate of less than 18%[4, 5]. Therefore, an accurate prediction tool is urgently demanded clinically to effectively predict the prognosis of HCC in the preoperative period, and to give appropriate treatment to HCC patients in order to improve the prognosis of HCC patients.

The risk prediction model based on clinical characteristic parameters plays an important role in predicting the prognosis of HCC, which demonstrating significant guidance for the comprehensive treatment of HCC patients[6, 7]. Several risk factors, including vascular invasion, maximum tumor diameter (MTD), tumor differentiation and et al, are associated with a high recurrence rate of HCC patients after radical resection[8, 9]. Microvascular invasion (MVI) mainly includes both macrovascular invasion and microvascular invasion, which is known as microvascular tumor thrombus, mainly refers to the nests of tumor cells in the vascular lumen lined by endothelial cells under the microscope, and more than 50 suspended tumor cells in the vascular lumen[10, 11]. Studies have confirmed that MVI is a predictor of poor prognosis after surgical resection or liver transplantation, and a turning point affecting the postoperative survival of HCC patients[12, 13]. However, the gold standard for clinically MVI detection is still the histopathological examination of postoperative specimens, results in the loss of the best treatment opportunity for most HCC patients before a definite diagnosis of MVI. Hence, to strengthen the screening and evaluation of independent risk factors for MVI can early monitor the recurrence and metastasis of tumor cells and create opportunity for re-treatment of HCC patients.

In this study, a retrospective study was conducted to analyze the difference between survival and recurrence in HCC patients with or without MVI undergoing radical surgical treatment, and then, an MVI prediction model was successfully constructed and validated by analyzing the clinical risk factors for MVI occurrence. Subsequently, in order to further verify the validity of this model, all patients were divided into high-risk and low-risk groups for survival analysis. The specific research process was presented in Fig. 1. The model demonstrated great clinical significance for preoperative MVI risk assessment and prognosis prediction of HCC, which played an important role in guiding treatment strategy and improving the prognosis of more HCC patients.

2. Patients And Methods

2.1 Study population

A total of 160 patients admitted to the Department of Hepatopancreatobiliary Surgery in the First Affiliated Hospital of University of South China from January 2016 to December 2018 for surgical treatment of liver cancer were enrolled as the study subjects. The patients in this study were all performed anatomic and radical resection by a technical surgeon group with more than 500 cases of anatomic and radical hepatectomy experience. The inclusion criteria for all patients were as follows: (1) Preoperative imaging examination was consistent with the image characteristics of liver malignant tumor; (2)

Preoperative child-Pugh grading standard of liver function was Grade A or B; (3) Postoperative pathological diagnosis of liver cancer; (4) Complete clinical data. Exclusion criteria mainly include: (1) Previous malignant tumor and surgical history; (2) No obvious surgical indications for advanced malignant tumors: (3) Pregnancy; (4) Complicated with other serious infectious diseases; (5) Postoperative pathological diagnosis of the primary lesion was benign liver lesion. The study was approved by the Ethics Committee of the First Affiliated Hospital of University of South China. The requirement for informed consent was waived due to the retrospective nature of this study.

2.2 MVI diagnostic criteria, grouping and clinical outcome comparison

MVI assessment of all study patients were based on postoperative pathology which was diagnosed by two experienced pathologists. MVI defined as[14]: nests of cancer cells can be observed under the light microscope and distributed in the vascular lumen lined by endothelial cells which is mainly including the intracapsular vessels of the tumor or the portal vein branches adjacent to the cancer surrounding liver tissue lined by the endothelium that was visible only on microscopy. All study patients were classified into MVI positive group and MVI negative group. The clinical outcomes between the two groups were compared, including intraoperative data (operation time, bleeding, blood transfusion and conversion to laparotomy) and postoperative data (haematological indexes, incidence of complications, hospital stays, pathological grading, overall survival/recurrence). The postoperative haematological indexes mainly included blood biochemistry tests in the postoperative 1, 3 and 5 days (POD1, POD3 and POD5).

2.3 Analysis of clinical characteristic parameters

The clinical characteristic parameters mainly including the basic clinical data, haematological data and imageological data. The basic clinical data: Age, Gender, HBV infection, liver cirrhosis, diabetes, hypertension and smoking; the preoperative haematological data: A carcinoma embryonic antigen (CEA), alpha-fetoprotein (AFP), alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), albumin (ALB) and prothrombin time (PT); the preoperative imageological data: tumor diameter, number of tumors and TNM stage (tumor stage confirmed by imaging examination). According to the literature report, the classification criteria for each candidate index were determined: Age (< 60 years or ≥ 60 years), CEA (< 5 ng/mL or ≥ 5 ng/mL), AFP (< 400 ng/mL or ≥ 400 ng/mL), ALT (< 40 U/L or ≥ 40 U/L), AST (< 34 U/L or ≥ 34 U/L), TBIL (< 34 mmol/L or ≥ 34 mmol/L), ALB (< 33 g/L or ≥ 33 g/L), PT (< 13 s or ≥ 13 s), tumor diameter (< 5 cm or ≥ 5 cm), number of tumors (< 2 or ≥ 3), TNM stage (I-II, III-IV).

2.4 Follow-up evaluation

Follow-up was carried out mainly by telephone, supplemented by systematic inquiry of multiple admission records. The time of follow-up was defined as 36 months after discharge at the time of first admission. Follow-up was conducted to December 2021. The patients were followed up every 3 months during the first postoperative years, and every 6 months for the next two years. Follow-up mainly includes survival, tumor recurrence, whether there is relevant treatment and so on. The median follow-up duration was 22.8 months (range 2 to 36 months).

2.5 Statistical analysis

SPSS 25.0 software was used for statistical analysis. Measurement data were presented as follows: mean ± standard deviation, and independent sample *t*-test was performed for measurement data subject to normal distribution. Chi-square test and Fisher's exact test were used to analyze the statistical data. Univariate analysis and multivariate analysis with the Logistic regression proportional hazard model were performed to evaluate the MVI risk factors. The Hosmer-Lemeshow (H-L) test was used to evaluate the goodness of fit of the MVI prediction model. Overall survival and overall recurrence were defined as the time from operation until death or censoring, which were calculated by the Kaplan-Meier analysis, and the difference in groups was assessed by the Log-Rank test. All *P* values were two-sided, with statistical significance set at *P* values less than 0.05.

3. Results

3.1 Patient characteristics

All study patients were classified into MVI negative group (n = 68) and MVI positive group (n = 92), and the preoperative baseline data comparison between the two groups were shown in Table 1. There were no significant differences between the two groups in clinical baseline data including age, gender, HBV infection, liver cirrhosis, diabetes, hypertension, smoking ($P > 0.05$). The preoperative haematological data including CEA, ALT, AST, TBIL, ALB and PT between the two groups also presented no significant differences ($P > 0.05$), while the AFP shown significant difference (222.4 ± 678.7 ng/mL vs 1227.6 ± 3112.0 ng/mL, $P = 0.039$). There were no significant differences between the two groups in preoperative imageological data including tumor diameter, number of tumors and TNM stage ($P > 0.05$).

Table 1
Preoperative baseline data comparison between the two groups

Variable	MVI negative (n = 68)	MVI positive (n = 92)	*P*-value
Age (years)	57.5 ± 9.4	54.3 ± 12.8	0.194
Gender			
Female	62(91.2)	72(78.3)	0.140
Male	6(8.8)	20(21.7)	
HBV infection			
Present	18(26.5)	20(21.7)	0.159
Absent	50(73.5)	72(78.3)	
Liver cirrhosis			
Present	32(47.1)	36(39.1)	0.492
Absent	36(52.9)	56(60.9)	
Diabetes			
Present	15(22.1)	23(25)	0.605
Absent	53(77.9)	69(75)	
Hypertension			
Present	12(17.6)	23(25)	0.288
Absent	56(82.4)	69(75)	
Smoking			
Present	13(19.1)	20(21.7)	0.753
Absent	55(80.9)	72(78.3)	
CEA (ng/mL)	3.15 ± 0.89	3.22 ± 1.06	0.659
AFP (ng/mL)	222.4 ± 678.7	1227.6 ± 3112.0	**0.039**
ALT (U/L)	40.9 ± 32.1	54.5 ± 48.4	0.160
AST (U/L)	49.3 ± 39.2	54.3 ± 45.8	0.685
TBIL (mmol/L)	15.9 ± 7.1	16.7 ± 8.8	0.664
ALB (g/L)	37.4 ± 3.6	38.1 ± 4.6	0.433
PT (s)	13.5 ± 1.1	13.7 ± 1.0	0.232

Variable	MVI negative (n = 68)	MVI positive (n = 92)	*P*-value
Tumor diameter (cm)	4.6 ± 3.2	5.8 ± 3.3	0.095
Number of tumors	1.2 ± 0.3	1.6 ± 0.4	0.751
TNM stage	28(41.2)	25(27.8)	0.063
I-II	40(58.9)	67(72.2)	
III-IV			

3.2 The clinical outcome comparison between the two groups

The comparative analysis of clinical data between the two groups were shown in Table 2. There were no significant differences between the two groups in intraoperative data including operation time, intraoperative bleeding, intraoperative blood transfusion and the incidence of conversion to laparotomy ($P > 0.05$). The incidence of postoperative complications between the two groups including infection of incision, lung infection, pleural effusion, biliary fistula and seroperitoneum presented no significant differences ($P > 0.05$), and no patient in the two groups occurred postoperative abdominal bleeding and hepatic failure. No significant difference of the postoperative hospital stays was found between the two groups ($P > 0.05$). However, the difference of the pathological grading was observed between the two groups, 32 (47.1%) cases of poor differentiation were found in the MVI negative group and 76 (82.6%) cases in the MVI positive group, indicates significant difference ($P = 0.002$).

Table 2
Comparative analysis of clinical data between the two groups

Variable	MVI negative (n = 68)	MVI positive (n = 92)	*P*-value
Operation time (min)	127.9 ± 52.5	149.6 ± 46.0	0.054
Intraoperative bleeding (mL)	164.1 ± 155.9	190.9 ± 160.8	0.305
Intraoperative blood transfusion	4(5.9)	14(15.2)	0.288
Conversion to laparotomy	5(7.4)	12(13.0)	0.306
Postoperative complications			
Infection of incision	0(0.0)	2(2.17)	0.387
Lung infection	12(17.7)	14(15.2)	0.770
Pleural effusion	16(23.5)	20(21.7)	0.850
Biliary fistula	5(7.4)	12(13.0)	0.306
Seroperitoneum	4(5.9)	8(8.7)	0.560
Abdominal bleeding	0(0.0)	0(0.0)	N/A
Hepatic failure	0(0.0)	0(0.0)	N/A
Postoperative hospital stays (day)	11.2 ± 3.5	12.0 ± 4.7	0.439
Pathological grading	36(52.9)	16(17.4)	**0.002**
High/ moderate	32(47.1)	76(82.6)	
Poor			

The blood biochemistry comparison between the two groups 1, 3 and 5 days after operation was shown in Fig. 2. There were no significant differences between the two groups in the postoperative haematological indexes including ALT, TBIL, ALB and PT ($P > 0.05$). The overall survival and recurrence comparison between the two groups 36 months after surgery were presented in Fig. 3. Compared the MVI positive group, the patients in the MVI negative group presented a better survival rate and lower recurrence rate ($P < 0.001$), indicates better clinical outcomes.

3.3 The construction MVI prediction model

The clinical characteristic parameters including the basic clinical data, haematological data and imageological data were screened by univariable and multivariate Logistic regression analysis, which were shown in Table 3. These risk factors including AFP ≥ 400 kU/L (OR: 0.276, $P = 0.003$), TBIL ≥ 34mmol/L (OR: 0.422, $P = 0.015$), tumor diameter (TD) ≥ 5cm (OR: 0.423, $P = 0.022$) and TNM stage (OR:

0.238, $P < 0.001$) were screened by univariable Logistic regression analysis. After multivariate Logistic regression analysis, the risk factors including AFP ≥ 400 kU/L (OR: 0.072, $P < 0.001$), tumor diameter (TD) ≥ 5cm (OR: 0.400, $P = 0.041$) and TNM stage (OR: 0.094, $P < 0.001$) were selected to construct a logistic regression model (Table 4). Obtained by adding the total number of points scored in each of the three risk factors, the MVI prediction model is: 4–3⊆AFP – 1⊆TD – 2⊆PD. In order to distinguish the MVI low-risk group and high-risk group from all study patients, according to a best Youden index of 0.624, we obtained an optimal cut-off value of 2.5.

Table 3
Univariable and multivariate Logistic regression analyses of risk factors for presence of MVI

Variable	Univariate analysis			Multivariate analysis		
	OR	95%CI	P-value	OR	95%CI	P-value
Age ≥ 60	1.516	0.802–2.868	0.200			
Gender	0.675	0.357–1.278	0.228			
HBV infection	1.447	0.755–2.773	0.266			
Liver cirrhosis	1.383	0.733–2.607	0.317			
Diabetes	1.134	0.571–2.252	0.719			
Hypertension	1.209	0.645–2.264	0.554			
Smoking	0.879	0.449–1.722	0.707			
CEA ≥ 5ng/mL	0.614	0.326–1.157	0.131			
AFP ≥ 400kU/L	0.276	0.117–0.649	**0.003**	.0.074	0.027–0.207	**< 0.001**
ALT ≥ 40U/L	0.579	0.260–1.288	0.180			
AST ≥ 34U/L	0.574	0.265–1.246	0.160			
TBIL ≥ 34mmol/L	0.422	0.211–0.844	**0.015**	0.481	0.209–1.107	0.085
ALB ≥ 33g/L	1.850	0.930–3.678	0.079			
PT ≥ 13s	0.764	0.406–1.438	0.404			
Tumor diameter ≥ 5cm	0.423	0.202–0.885	**0.022**	0.395	0.163–0.955	**0.039**
Number of tumors ≥ 3pcs.	1.021	0.518–2.015	0.952			
TNM stage (III-IV stage)	0.238	0.116–0.486	**< 0.001**	0.099	0.042–0.236	**< 0.001**

Table 4
Multivariable analysis of risk factors of MVI and measurement of the risk score

Variable	Multivariate analysis			B coefficient	
	OR	95%CI	P-value		Points
AFP ≥ 400kU/L	0.072	0.026–0.198	**< 0.001**	2.630	3
TD ≥ 5cm	0.400	0.166–0.963	**0.041**	0.917	1
TNM (III-IV stage)	0.094	0.040–0.223	**< 0.001**	2.360	2

3.4 Model validation

The validation of MVI prediction model mainly contained the assessment of the area under the curve (AUC) and the calibration curve which were presented in Fig. 4. The AUC of the MVI prediction model was 0.7997 with a sensitivity of 0.685 and specificity of 0.847, indicates good predictive ability. Moreover, the calibration curve of MVI prediction model presented a good H-L goodness of fit (P = 0.231 > 0.05), and shown high coherence between the observed risk and the predicted risk, indicates that the model has a superior predictive performance.

3.5 The overall survival and recurrence comparison between the two groups

According to the cut-off value of 2.5, the patients were classified into MVI low-risk group (n = 60) and high-risk group (n = 100), and the overall survival and recurrence comparison between the two groups was shown in Fig. 5. Compared the MVI high-risk group, the patients in the MVI low-risk group shown a higher survival rate (P = 0.002) and lower recurrence rate (P = 0.004), indicates good clinical prognosis.

4. Discussion

The comprehensive treatment based on radical surgery is currently considered as the preferred and most effective method for HCC treatment[15, 16]. By eradicating the microscopic metastatic lesions around tumor to a certain extent, the promotion of the concept of anatomic hepatectomy has brought a very ideal surgical mean for the radical surgical treatment of HCC[17, 18]. Nevertheless, for the MVI positive patients, even after anatomic hepatectomy, the 5-year recurrence rate is still as high as 60–70%[19]. Therefore, preoperative prediction of MVI is particularly important. In our study, first, the clinical outcomes and prognosis of clinical HCC patients with or without MVI were compared and analyzed; then, based on preoperative AFP, tumor diameter and TNM stage, a MVI prediction model with superior predictive efficacy was constructed and validated, which provided a new therapeutic strategy for clinical comprehensive and standardized treatment of HCC patients.

AFP is an important indicator of HCC screening, which can reflect the occurrence and development of HCC, which is positively correlated with the pathological HCC process[20, 21]. AFP was proved to be an

independent risk factor for early recurrence and poor overall survival of HCC patients after hepatectomy, the association between MVI and AFP was always been concerned by researchers[22]. Patients with AFP ≥ 400 kU/L were usually considered the possibility of diagnosis of HCC, and it was also an important risk factor for the early occurrence of HCC[23, 24]. However, A single haematological index is generally not highly specific and its clinical application is limited. Several studies have confirmed that in the growth process of tumors, the poorly differentiated areas will gradually replace the well-differentiated areas, thus increasing the degree of malignancy and invasiveness of tumors[25]. Furthermore, large HCC tumor size stimulates invasive behavior, such as local, lymphatic and distant metastases[26]. Anatomic hepatectomy has been shown to be significantly beneficial for long-term survival in patients with tumors > 5 cm in diameter, which also suggested that the occurrence of MVI was higher in tumors with larger diameter[27, 28]. The tumor diameter and TNM stage based on the imageological data also were independent risk factors of for postoperative MVI[29, 30]. The greater tumor size might be associated with capsular invasion, satellite nodules, and tumor thrombus, and the poor TNM stage might be relevant to the degree of aggressiveness and malignancy of the tumor[31, 32]. In our MVI prediction model, consist of the preoperative AFP ≥ 400 kU/L, TD ≥ 5cm and III-IV TNM stages, demonstrates reasonable composition structure.

Nowadays, radiometrics technology is used to extract predictive MVI model from CT images and preoperative MVI prediction of hematological indicators[33, 34]. Although some studies have found that special features of MRI can be used as typical features of MVI imaging diagnosis, such as image cystic insufficiency, coronary enhancement in arterial phase, and peritumoral low signal, etc[35, 36]. However, the process is quite complex, because radiomic feature extraction requires algorithms developed by scientists and engineers, and many algorithms are difficult to be recognized and put into clinical practice due to their overlapping or inadequate imaging. MVI status can also be reflected by specific clinical hematologic indicators including des-gamma carboxy prothrombin (DCP) and peripheral neutrophil to lymphocyte ratio (NLR), etc[37, 38]. Unfortunately, the predicted effect of preoperative hematological indexes is poor. In our prediction model, based on the risk factors of haematological and imageological data, with a high AUC of 0.7997 and a good H-L goodness of fit (P = 0.231), by successfully predicting the poor prognosis in high-risk patients, the prediction model can comprehensively screen tumor occurrence and invasiveness, so as to effectively preoperatively predict MVI for HCC patients.

In addition, our study proved that the HCC patients with poor differentiation resulted in a higher incidence of MVI compared with the patients with high/moderate differentiation, also leaded to a poor prognosis. Consistent with previous studies, poorly differentiated tumors were more aggressive than well-differentiated ones, so the prognosis of patients with poorly differentiated HCC after resection was worse than that of patients with highly differentiated, and the possibility of postoperative recurrence was increased[39, 40]. Although MVI positive has shown no significant difference in intraoperative and postoperative clinical indicators, while the difference of clinical prognosis can effectively guide the further comprehensive treatment strategy. MVI was an independent risk factor of HCC for poor prognosis after radical resection[41, 42]. Therefore, through accurate prediction of preoperative MVI, more reasonable and timely treatment options can be selected clinically, including radiofrequency ablation,

anatomic hepatectomy or liver transplantation, and even the choice between neoadjuvant and adjuvant therapy can be extended. In addition, although targeted therapy for MVI is the first choice in the traditional sense, targeted therapy should also be an important systematic treatment method for MVI, so as to truly achieve personalized treatment for patients based on tumor biological behavior[43–45].

This study also has some limitations, some studies have found that the margin of the tumor to the surgical margin plane can significantly affect postoperative outcomes, but this study was not further discussed in the survival analysis. Although the sample size included in this study reached the standard of statistical analysis, it was necessary to carried out a randomized controlled study with large sample for analysis and research on the complex etiology of HCC recurrence. In addition, the follow-up time of this study was 36 months, so it is necessary to carry out longer follow-up of patients and analyze the mortality and recurrence rates at different stages, so as to further study the relationship between MVI and the prognosis of HCC patients.

5. Conclusion

In this study, we have carried out a retrospective study to verify that the HCC patients with pathologic poor differentiation resulted in a higher incidence of MVI and also related to a poor prognosis after radical surgery. Then, we have constructed and validated a MIV prediction model for HCC patients based on preoperative AFP, tumor diameter and TNM stage, which presented superior predictive efficacy and strong clinical practicability. We concluded that MVI was an independent risk factor of HCC for poor prognosis after radical resection, and the HCC patients with high-risk of MVI incidence should receive more reasonable and timely comprehensive treatment due to early recurrence and poor survival after radical surgery. This study provided a new therapeutic strategy for clinical accurate prognostic judgment and standardized treatment of HCC patients.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of University of South China in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Consent for publication

We have obtained the consent of all patients to use clinical data for journal presentation.

Availability of data and materials

Due to privacy and ethical restrictions, the data are not publicly available.

Competing interests

All authors have declared no conflict of interest.

Funding

The study was funded by Scientific research Project of Health Commission of Hunan Province (No.202204014556, 20201064, 20201919), Scientific research key project of Hunan Education Department (No.21A0258), Natural Science Foundation of Hunan Province (Joint project of Science and Health) (No.2021JJ70039), Hunan Provincial Clinical Medical Technology Innovation Guide Project (No.2020SK51817), and The Medical clinical research "4310" program of University of South China.

Authors' contributions

YLX, SQ and GDC contributed to the conception of the study. PC, WPT, LXH and DCM contributed to data collection and audit. SQ and YLX performed the data analyses and wrote the manuscript. All authors contributed to the manuscript and approved the submitted version.

Acknowledgements

Not applicable.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries**. CA Cancer J Clin 2021, **71**:209–249.
2. Villanueva A: **Hepatocellular Carcinoma**. N Engl J Med 2019, **380**:1450–1462.
3. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK: **Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases**. Hepatology 2018, **68**:723–750.
4. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR: **A global view of hepatocellular carcinoma: trends, risk, prevention and management**. Nat Rev Gastroenterol Hepatol 2019, **16**:589–604.
5. Bruix J, Reig M, Sherman M: **Evidence-Based Diagnosis, Staging, and Treatment of Patients With Hepatocellular Carcinoma**. Gastroenterology 2016, **150**:835–853.
6. Pan YX, Chen JC, Fang AP, Wang XH, Chen JB, Wang JC, He W, Fu YZ, Xu L, Chen MS, et al: **A nomogram predicting the recurrence of hepatocellular carcinoma in patients after laparoscopic hepatectomy**. Cancer Commun (Lond) 2019, **39**:55.
7. Lei Z, Li J, Wu D, Xia Y, Wang Q, Si A, Wang K, Wan X, Lau WY, Wu M, Shen F: **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.

8. Hu H, Qi S, Zeng S, Zhang P, He L, Wen S, Zeng N, Yang J, Zhang W, Zhu W, et al: **Importance of Microvascular Invasion Risk and Tumor Size on Recurrence and Survival of Hepatocellular Carcinoma After Anatomical Resection and Non-anatomical Resection**. Front Oncol 2021, **11**:621622.
9. Feng LH, Dong H, Lau WY, Yu H, Zhu YY, Zhao Y, Lin YX, Chen J, Wu MC, Cong WM: **Novel microvascular invasion-based prognostic nomograms to predict survival outcomes in patients after R0 resection for hepatocellular carcinoma**. J Cancer Res Clin Oncol 2017, **143**:293–303.
10. Renzulli M, Brocchi S, Cucchetti A, Mazzotti F, Mosconi C, Sportoletti C, Brandi G, Pinna AD, Golfieri R: **Can Current Preoperative Imaging Be Used to Detect Microvascular Invasion of Hepatocellular Carcinoma?** *Radiology* 2016, **279**:432–442.
11. Lee S, Kim SH, Lee JE, Sinn DH, Park CK: **Preoperative gadoxetic acid-enhanced MRI for predicting microvascular invasion in patients with single hepatocellular carcinoma**. J Hepatol 2017, **67**:526–534.
12. Roayaie S, Blume IN, Thung SN, Guido M, Fiel MI, Hiotis S, Labow DM, Llovet JM, Schwartz ME: **A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma**. Gastroenterology 2009, **137**:850–855.
13. Zheng J, Chakraborty J, Chapman WC, Gerst S, Gonen M, Pak LM, Jarnagin WR, DeMatteo RP, Do R, Simpson AL: **Preoperative Prediction of Microvascular Invasion in Hepatocellular Carcinoma Using Quantitative Image Analysis**. J Am Coll Surg 2017, **225**:778–788.
14. Lim KC, Chow PK, Allen JC, Chia GS, Lim M, Cheow PC, Chung AY, Ooi LL, Tan SB: **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.
15. Aoki T, Kubota K, Hasegawa K, Kubo S, Izumi N, Kokudo N, Sakamoto M, Shiina S, Takayama T, Nakashima O, et al: **Significance of the surgical hepatic resection margin in patients with a single hepatocellular carcinoma**. Br J Surg 2020, **107**:113–120.
16. Shindoh J, Makuuchi M, Matsuyama Y, Mise Y, Arita J, Sakamoto Y, Hasegawa K, Kokudo N: **Complete removal of the tumor-bearing portal territory decreases local tumor recurrence and improves disease-specific survival of patients with hepatocellular carcinoma**. J Hepatol 2016, **64**:594–600.
17. Li SQ, Huang T, Shen SL, Hua YP, Hu WJ, Kuang M, Peng BG, Liang LJ: **Anatomical versus non-anatomical liver resection for hepatocellular carcinoma exceeding Milan criteria**. Br J Surg 2017, **104**:118–127.
18. Inoue Y, Arita J, Sakamoto T, Ono Y, Takahashi M, Takahashi Y, Kokudo N, Saiura A: **Anatomical Liver Resections Guided by 3-Dimensional Parenchymal Staining Using Fusion Indocyanine Green Fluorescence Imaging**. Ann Surg 2015, **262**:105–111.
19. **EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma**. J Hepatol 2018, **69**:182–236.

20. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, Waljee AK, Singal AG: **Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis**. Gastroenterology 2018, **154**:1706–1718.
21. Akbulut S, Sahin TT, Yilmaz S: **Letter to the Editor: Comment on Alpha-Fetoprotein Decrease From > 1,000 to < 500 ng/mL in Patients With Hepatocellular Carcinoma Leads to Improved Posttransplant Outcomes**. Hepatology 2020, **72**:2242–2243.
22. Chen T, Dai X, Dai J, Ding C, Zhang Z, Lin Z, Hu J, Lu M, Wang Z, Qi Y, et al: **AFP promotes HCC progression by suppressing the HuR-mediated Fas/FADD apoptotic pathway**. Cell Death Dis 2020, **11**:822.
23. Gross-Goupil M, Saffroy R, Azoulay D, Precetti S, Emile JF, Delvart V, Tindiliere F, Laurent A, Bellin MF, Bismuth H, et al: **Real-time quantification of AFP mRNA to assess hematogenous dissemination after transarterial chemoembolization of hepatocellular carcinoma**. Ann Surg 2003, **238**:241–248.
24. Matsuda M, Asakawa M, Amemiya H, Fujii H: **Lens culinaris agglutinin-reactive fraction of AFP is a useful prognostic biomarker for survival after repeat hepatic resection for HCC**. J Gastroenterol Hepatol 2011, **26**:731–738.
25. Park VY, Choi JY, Chung YE, Kim H, Park MS, Lim JS, Kim KW, Kim MJ: **Dynamic enhancement pattern of HCC smaller than 3 cm in diameter on gadoxetic acid-enhanced MRI: comparison with multiphasic MDCT**. Liver Int 2014, **34**:1593–1602.
26. Ruff SM, Rothermel LD, Diggs LP, Wach MM, Ayabe RI, Martin SP, Boulware D, Anaya D, Davis JL, Mullinax JE, Hernandez JM: **Tumor grade may be used to select patients with multifocal hepatocellular carcinoma for resection**. HPB (Oxford) 2020, **22**:1004–1010.
27. Wang H, Yu H, Qian YW, Cao ZY, Wu MC, Cong WM: **Impact of Surgical Margin on the Prognosis of Early Hepatocellular Carcinoma (</=5 cm): A Propensity Score Matching Analysis**. Front Med (Lausanne) 2020, **7**:139.
28. Fukami Y, Kaneoka Y, Maeda A, Kumada T, Tanaka J, Akita T, Kubo S, Izumi N, Kadoya M, Sakamoto M, et al: **Liver Resection for Multiple Hepatocellular Carcinomas: A Japanese Nationwide Survey**. Ann Surg 2020, **272**:145–154.
29. Shinkawa H, Tanaka S, Kabata D, Takemura S, Amano R, Kimura K, Kinoshita M, Kubo S: **The Prognostic Impact of Tumor Differentiation on Recurrence and Survival after Resection of Hepatocellular Carcinoma Is Dependent on Tumor Size**. Liver Cancer 2021, **10**:461–472.
30. Zeng J, Zeng J, Liu J, Zeng J: **Development of pre and post-operative nomograms to predict individual survival for ideal liver resection candidates with hepatocellular carcinoma**. Liver Int 2021, **41**:2974–2985.
31. Lee YJ, Lee YR, Seo CG, Goh HG, Kim TH, Yim SY, Han NY, Lee JM, Choi HS, Kim ES, et al: **How Should We Assign Large Infiltrative Hepatocellular Carcinomas for Staging?** *Cancers (Basel)* 2020, **12**.
32. Ivanics T, Murillo PC, Claasen M, Patel MS, Morgenshtern G, Erdman L, Shwaartz C, Rajendran L, O'Kane GM, Hansen BE, et al: **Dynamic risk profiling of HCC recurrence after curative intent liver**

resection. *Hepatology* 2022.

33. Xu T, Ren L, Liao M, Zhao B, Wei R, Zhou Z, He Y, Zhang H, Chen D, Chen H, Liao W: **Preoperative Radiomics Analysis of Contrast-Enhanced CT for Microvascular Invasion and Prognosis Stratification in Hepatocellular Carcinoma**. J Hepatocell Carcinoma 2022, **9**:189–201.
34. Lee IC, Huang JY, Chen TC, Yen CH, Chiu NC, Hwang HE, Huang JG, Liu CA, Chau GY, Lee RC, et al: **Evolutionary Learning-Derived Clinical-Radiomic Models for Predicting Early Recurrence of Hepatocellular Carcinoma after Resection**. Liver Cancer 2021, **10**:572–582.
35. Yang Y, Fan W, Gu T, Yu L, Chen H, Lv Y, Liu H, Wang G, Zhang D: **Radiomic Features of Multi-ROI and Multi-Phase MRI for the Prediction of Microvascular Invasion in Solitary Hepatocellular Carcinoma**. Front Oncol 2021, **11**:756216.
36. Wang W, Gu D, Wei J, Ding Y, Yang L, Zhu K, Luo R, Rao SX, Tian J, Zeng M: **A radiomics-based biomarker for cytokeratin 19 status of hepatocellular carcinoma with gadoxetic acid-enhanced MRI**. Eur Radiol 2020, **30**:3004–3014.
37. Ryu T, Takami Y, Wada Y, Tateishi M, Hara T, Yoshitomi M, Momosaki S, Yasumori K, Saitsu H, Okuda K: **A Clinical Scoring System for Predicting Microvascular Invasion in Patients with Hepatocellular Carcinoma Within the Milan Criteria**. J Gastrointest Surg 2019, **23**:779–787.
38. Zheng J, Seier K, Gonen M, Balachandran VP, Kingham TP, D'Angelica MI, Allen PJ, Jarnagin WR, DeMatteo RP: **Utility of Serum Inflammatory Markers for Predicting Microvascular Invasion and Survival for Patients with Hepatocellular Carcinoma**. Ann Surg Oncol 2017, **24**:3706–3714.
39. Agopian VG, Harlander-Locke MP, Markovic D, Zarrinpar A, Kaldas FM, Cheng EY, Yersiz H, Farmer DG, Hiatt JR, Busuttil RW: **Evaluation of Patients With Hepatocellular Carcinomas That Do Not Produce alpha-Fetoprotein**. Jama Surg 2017, **152**:55–64.
40. Zavaglia C, De Carlis L, Alberti AB, Minola E, Belli LS, Slim AO, Airoldi A, Giacomoni A, Rondinara G, Tinelli C, et al: **Predictors of long-term survival after liver transplantation for hepatocellular carcinoma**. Am J Gastroenterol 2005, **100**:2708–2716.
41. Lee S, Kang TW, Song KD, Lee MW, Rhim H, Lim HK, Kim SY, Sinn DH, Kim JM, Kim K, Ha SY: **Effect of Microvascular Invasion Risk on Early Recurrence of Hepatocellular Carcinoma After Surgery and Radiofrequency Ablation**. Ann Surg 2021, **273**:564–571.
42. Banerjee S, Wang DS, Kim HJ, Sirlin CB, Chan MG, Korn RL, Rutman AM, Siripongsakun S, Lu D, Imanbayev G, Kuo MD: **A computed tomography radiogenomic biomarker predicts microvascular invasion and clinical outcomes in hepatocellular carcinoma**. Hepatology 2015, **62**:792–800.
43. Lim H, Ramjeesingh R, Liu D, Tam VC, Knox JJ, Card PB, Meyers BM: **Optimizing Survival and the Changing Landscape of Targeted Therapy for Intermediate and Advanced Hepatocellular Carcinoma: A Systematic Review**. J Natl Cancer Inst 2021, **113**:123–136.
44. Wang H, Lu Z, Zhao X: **Tumorigenesis, diagnosis, and therapeutic potential of exosomes in liver cancer**. J Hematol Oncol 2019, **12**:133.
45. Rebouissou S, Nault JC: **Advances in molecular classification and precision oncology in hepatocellular carcinoma**. J Hepatol 2020, **72**:215–229.

Figures

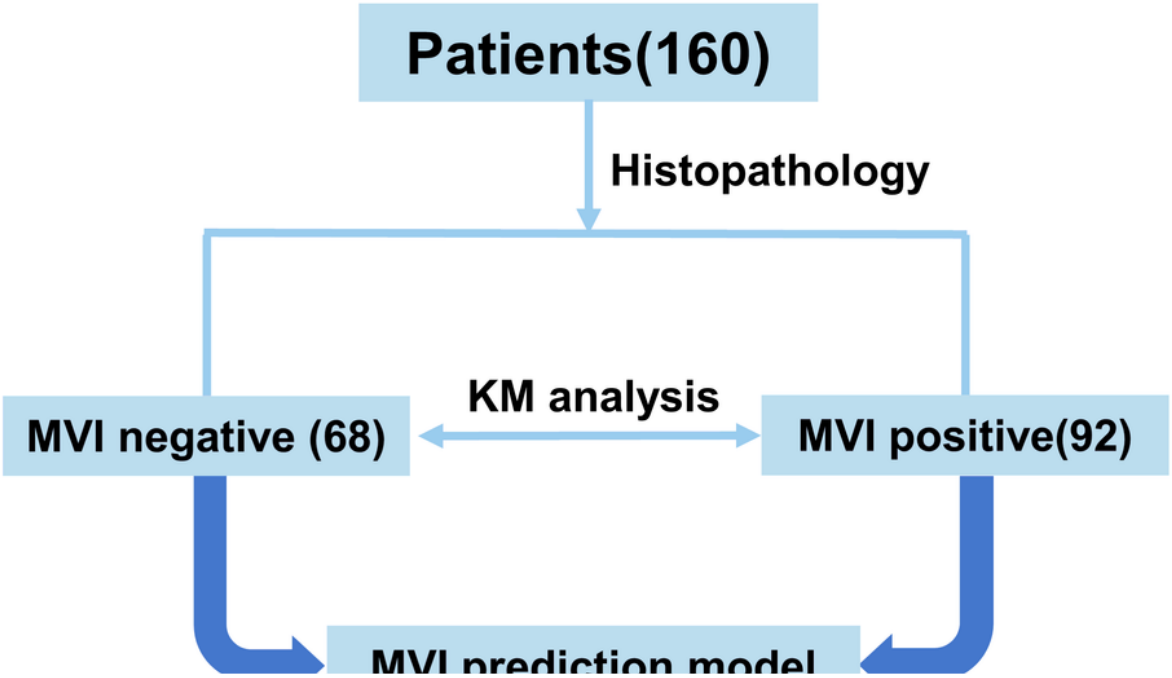

Figure 1

The specific research process of the study.

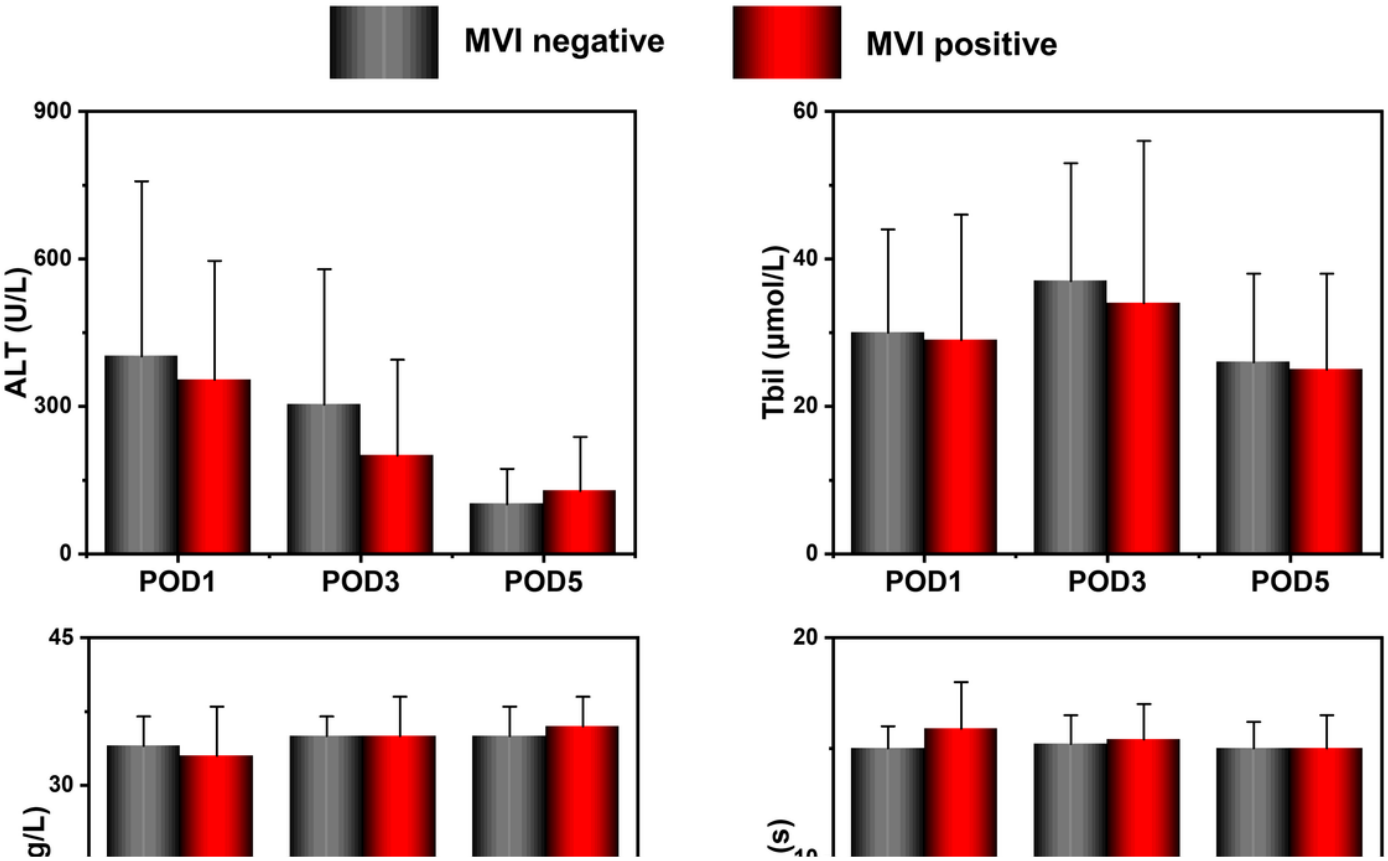

Figure 2

The blood biochemistry comparison between the MVI negative group and MVI positive group 1, 3 and 5 days after surgery.

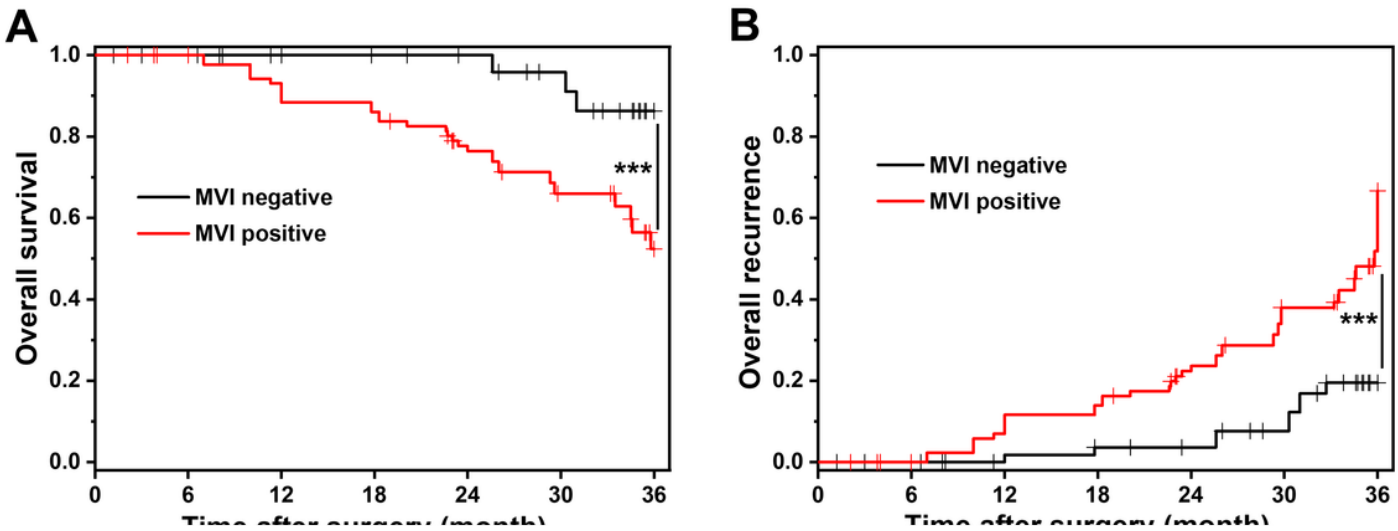

Figure 3

The overall survival (A) and recurrence(B) comparison between MVI negative group and MVI positive group 36 months after surgery. $^{***}P<0.001$

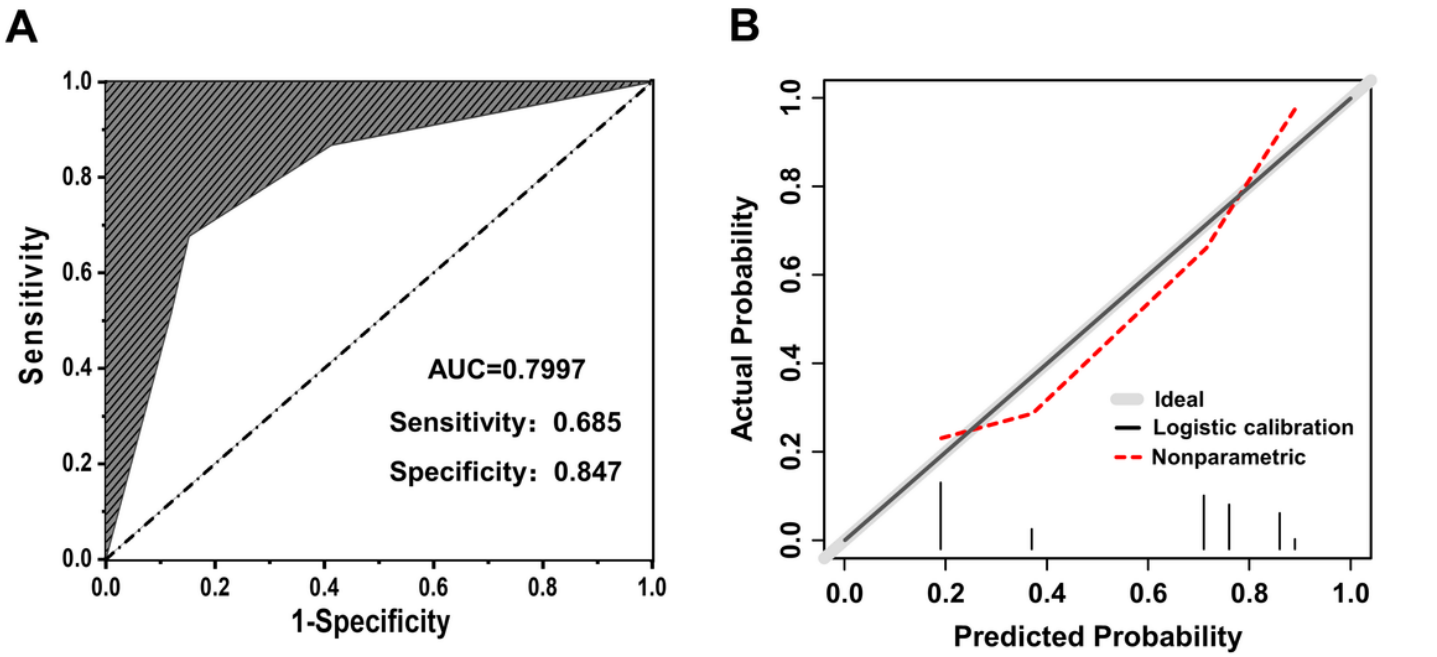

Figure 4

The validation of MVI prediction model. A: The area under the curve (AUC) for the MVI prediction model, B: The calibration curve of MVI prediction model.

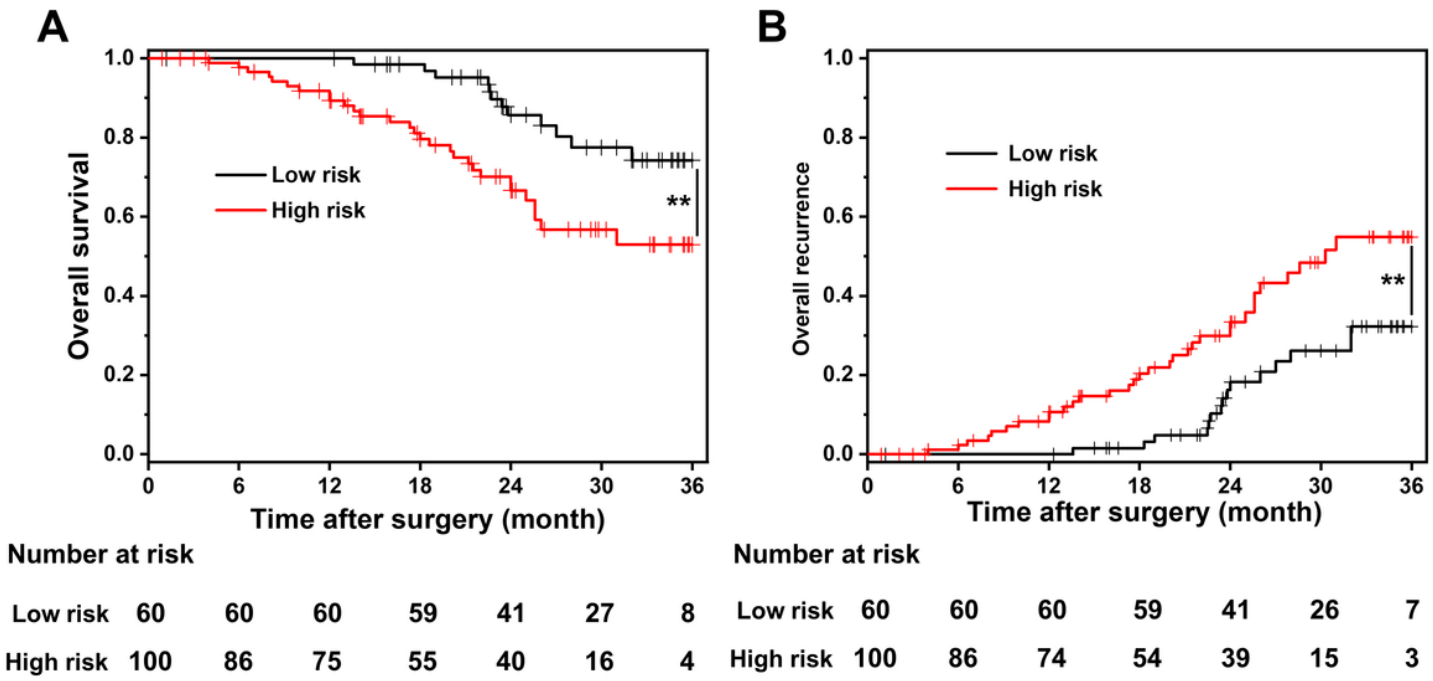

Figure 5

The overall survival and recurrence comparison between the MVI low-risk group and high-risk group. $^{**}P<0.01$