Value of Serological Indicators Combined with Tumor Diameter in Constructing a Nomogram for Predicting Microvascular Invasion in Hepatocellular Carcinoma: A Postprint

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Abstract

Background Microvascular invasion (MVI) is an aggressive behavior of hepatocellular carcinoma (HCC) and an independent predictor of tumor recurrence after hepatectomy or liver transplantation. Preoperative prediction of MVI holds important clinical significance.

Objective To construct a nomogram for predicting MVI using non-invasive imaging and serological indicators to provide clinical reference.

Methods A retrospective analysis was performed on relevant clinical data from 284 patients who underwent curative HCC surgery at the Second Affiliated Hospital of Kunming Medical University from 2016 to 2021. Based on admission date, HCC patients admitted between 2016 and 2020 were assigned to the model group (n=208), while those admitted in 2021 were assigned to the validation group (n=76). LASSO regression and multivariate Logistic regression analysis were conducted to identify independent risk factors for MVI in HCC. R software was utilized to develop a nomogram model for preoperative prediction of MVI risk in HCC. The Bootstrap method was employed for internal validation, while the validation group was used for external validation. The concordance index, calibration curve, and decision curve analysis (DCA) were used to assess the discrimination, calibration, and clinical utility of the nomogram.

Results Multivariate Logistic regression analysis demonstrated that white blood cell count (WBC) >7.1$\times10^{9}$/L [OR=3.144, 95%CI (1.301, 7.598), P=0.011], tumor diameter >7.05 cm [OR=4.179, 95%CI (1.980, 8.818), P<0.001], S-Index>0.097 [OR=4.142, 95%CI (1.221, 14.046), P=0.023], AAR>0.879 [OR=2.191, 95%CI (1.078, 4.454), P=0.03], and ANRI>24.074 [OR=2.449, 95%CI (1.055, 5.688), P=0.037] were independent predictors of MVI in HCC. The nomogram model established using these five variables combined with alpha-fetoprotein (AFP) exhibited concordance indices of 0.800 [95%CI (0.739, 0.861)] and 0.755 [95%CI (0.641, 0.868)] in the model and validation groups, respectively. The model showed good agreement with the calibration prediction curve. The optimal cutoff value for the nomogram was determined to be 174 points using the Youden index. At this cutoff, the sensitivity, specificity, positive predictive value, and negative predictive value were 90%, 61%, 71%, and 85% in the model group, and 78%, 71%, 76%, and 74% in the validation group, respectively.

Conclusion The nomogram constructed with AFP>45 ng/mL, WBC>7.1$\times10^{9}$/L, tumor diameter >7.05 cm, S-Index>0.097, AAR>0.879, and ANRI>24.074 can effectively predict the risk of MVI in HCC preoperatively. Utilization of this nomogram can conveniently guide clinical management of HCC patients through routine examination and laboratory parameters.

Full Text

The Value of a Nomogram Established by Serological Indicators and Tumor Diameter for Predicting Microvascular Invasion in Hepatocellular Carcinoma

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Abstract

Background: Microvascular invasion (MVI) represents an aggressive biological behavior of hepatocellular carcinoma (HCC) and serves as an independent predictor of tumor recurrence following hepatectomy or liver transplantation. Preoperative prediction of MVI holds significant clinical importance. **Objective:** To construct a nomogram using non-invasive imaging and serological indicators for predicting MVI and to provide a clinical reference tool. **Methods:** We retrospectively analyzed clinical data from 284 patients who underwent curative resection for HCC at the Second Affiliated Hospital of Kunming Medical University between 2016 and 2021. Patients admitted from 2016 to 2020 were assigned to the model group (n=208), while those admitted in 2021 formed the validation group (n=76). LASSO regression and multivariate logistic regression analyses were performed to identify independent risk factors for MVI in HCC. A preoperative nomogram model for predicting MVI risk was developed using R software. Internal validation was conducted via the bootstrap method, and external validation was performed using the validation group. The model's discrimination, calibration, and clinical utility were evaluated using the concordance index, calibration curves, and decision curve analysis (DCA). **Results:** Multivariate logistic regression analysis revealed that white blood cell count (WBC) >7.1$×10$^{\{9\}/L[OR = 3.144, 95×10}${9}$/L, tumor diameter >7.05 cm, S-Index>0.097, AAR>0.879, and ANRI>24.074 can effectively predict preoperative MVI risk in HCC patients. This nomogram can conveniently guide clinical management of HCC patients using routine laboratory and imaging parameters.

Keywords: Liver neoplasms; Microvascular invasion; Serology; Tumor diameter; Risk factors; Nomograms

Introduction

Hepatocellular carcinoma (HCC) ranks as the sixth most common malignancy and the third leading cause of cancer-related mortality worldwide. Microvascular invasion (MVI) in HCC is a critical risk factor for early recurrence following hepatectomy or liver transplantation. Accurate preoperative prediction of MVI carries substantial clinical value for treatment decision-making, neoadjuvant therapy planning, adjuvant therapy selection, and prognostic assessment. Currently, MVI can only be diagnosed through postoperative pathology. While previous studies have demonstrated that serum tumor markers, inflammatory indices, microRNAs, CT, MRI, and radiomics can predict MVI preoperatively, most have focused on only one or two serological indicators, and imaging modalities suffer from variability and operator-dependency. This study aims to analyze risk factors for MVI and develop a combined serological and imaging nomogram model for preoperative prediction of MVI in HCC to guide clinical practice.

1. Materials and Methods

1.1 Clinical Data

We retrospectively analyzed clinical data from 284 patients who underwent curative resection for HCC at the Second Affiliated Hospital of Kunming Medical University between 2016 and 2021. The cohort comprised 241 males (84.8%) and 43 females (15.2%). Based on admission date, patients treated from 2016 to 2020 were assigned to the model group (n=208), while those admitted in 2021 formed the validation group (n=76). Inclusion criteria were: (1) pathological diagnosis of HCC after liver resection; (2) no preoperative HCC-related treatments such as prior liver surgery, interventional therapy, radiotherapy, chemotherapy, or targeted therapy; (3) preoperative Child-Pugh classification A or B; and (4) complete clinicopathological data. Exclusion criteria included: (1) concurrent non-HCC malignancies; (2) recurrent HCC or metastatic liver cancer; (3) significant bleeding history; and (4) trauma, fever, acute infection, or hematological disorders within one week before surgery.

1.2 Observation Parameters

The following parameters were collected: (1) General information: sex, age, liver cirrhosis, hepatitis B history; (2) Preoperative laboratory tests: alpha-fetoprotein (AFP), total bilirubin (TB), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), international normalized ratio (INR), prothrombin time (PT), fibrinogen (FIB), white blood cell count (WBC), absolute neutrophil count (NE), absolute lymphocyte count (LY), absolute monocyte count (MO), and platelet count (PLT); (3) Inflammatory ratio indices: 15 serologically derived indicators including AST/PLT (APRI), FIB/ALB (FAR), NE/LY (NLR), PLT/LY (PLR), systemic inflammatory response index (SIRI), systemic immune-inflammation index (SII), prognostic nutritional index (PNI), and others detailed in Table 1 ; and (4) Preoperative imaging: tumor number and maximum tumor diameter measured on CT or MRI.

1.3 Statistical Analysis

Data were processed using SPSS 26.0 and R 4.1.3 software. Categorical variables were compared using the ^{2} test. Receiver operating characteristic (ROC) curves were used to determine optimal cutoff values for each parameter based on maximum Youden index, with continuous variables subsequently converted to binary variables. Given the potential collinearity among the 36 included serological and derived indices, we employed Least Absolute Shrinkage and Selection Operator (LASSO) regression with ten-fold cross-validation for variable screening to avoid multicollinearity and overfitting. The selected variables were then subjected to multivariate logistic regression analysis to identify independent predictors of MVI. A preoperative nomogram for predicting MVI was developed based on these independent factors. The Hosmer-Lemeshow test assessed model fit, while the concordance index (C-index) measured discrimination ability. Calibration curves with 1,000 bootstrap samples and external validation were used to evaluate accuracy. Decision curve analysis (DCA) assessed clinical net benefit across different threshold probabilities. The optimal cutoff value for the nomogram was determined using the Youden index, with corresponding sensitivity, specificity, positive predictive value, and negative predictive value calculated. Statistical significance was defined as P<0.05.

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2. Results

2.1 Optimal Cutoff Values for Model Group Parameters

ROC curve analysis determined the following cutoff values: AFP 45 ng/mL, INR 1.185, FIB 3.03 g/L, WBC 7.1$\times 10^{9}$/L, NE 4.735$\times 10^{9}$/L, LY 1.132$\times 10^{9}$/L, MO 0.545$\times 10^{9}$/L, PLT 155.5$\times 10^{}$ APRI 1.555, FIB-4 4.851, GPR 0.410, S-Index 0.097, NLR 1.858, PLR 152.215, FAR 0.078, tumor diameter 7.05 cm, LMR 0.680, NrLR 256.734, SIRI 0.962,

SII 358.363, PNI 42.025, ALRI 38.247, AAR 0.879, and ANRI 24.074. For TB, ALB, ALT, AST, ALP, and GGT, cutoff values were based on the upper limits of normal from our institutional laboratory. All parameters were converted to binary variables for LASSO and multivariate logistic regression analyses.

2.2 Baseline Patient Characteristics

The study included 284 patients: 208 in the model group (175 males, 33 females; age 31-75 years) with 108 MVI-positive (51.9%) and 100 MVI-negative cases (48.1%); and 76 patients in the validation group with 41 MVI-positive (53.9%) and 35 MVI-negative cases (46.1%). In the model group, no significant differences were observed between MVI-positive and MVI-negative patients regarding sex, age \$ $60 years, hepatitisBhistory, livercirrhosis, TB > 20.5, ALB < 35, ALT > 40, GGT > 50, INR > 1.185, prolongedPT, FIB > 3.03, orMO > 0.545 (allP > 0.05). However, significantdifferenceswerefoundforAFP > 45 ng/mL, ALP > 125 U/L, WBC > 7.1\times10^{\{9\}/L, NE > 4.735\times10}\{9\}/L, PLT >$ 155.5×10^{9}\$/L, tumor diameter >7.05 cm, and tumor number >1 (all P<0.05, Table 2).

2.3 Nomogram Model Development

LASSO regression with ten-fold cross-validation selected nine variables from the 36 initial parameters: AFP, WBC, tumor diameter, tumor number, S-Index, NLR, SII, AAR, and ANRI (Figure 1 [Figure 1: see original paper]). Multivariate logistic regression analysis, with MVI as the dependent variable (1=yes, 0=no) and the selected variables as binary predictors based on their cutoff values, identified WBC>7.1\$×10^{9}\$/L, tumor diameter >7.05 cm, S-Index>0.097, AAR>0.879, and ANRI>24.074 as independent predictors of MVI (P<0.05, Table 3). A preoperative nomogram for predicting MVI was constructed using AFP, WBC, tumor diameter, S-Index, AAR, and ANRI (Figure 2 [Figure 2: see original paper]), where the total score corresponds to the predicted probability of MVI.

2.4 Calibration Curves for Model and Validation Groups

The Hosmer-Lemeshow test yielded ^{2}=7.944 (P=0.439), indicating good model fit. Internal validation using bootstrap resampling with 1,000 samples and external validation with the validation group demonstrated close agreement between the standard curve and calibration prediction curve (Figure 3 [Figure 3: see original paper]), confirming excellent consistency between predicted and actual MVI.

2.5 Decision Curve Analysis

The DCA curve based on net benefit and threshold probability showed that the nomogram provided net clinical benefit across a wide threshold probability range (0-0.6) (Figure 4 [Figure 4: see original paper]).

2.6 ROC Curve Analysis of Nomogram Predictive Value

The concordance index was 0.800 [95%CI (0.739, 0.861)] in the model group and 0.755 [95%CI (0.641, 0.868)] in the validation group (Figure 5 [Figure 5: see original paper]). Using the Youden index, the optimal total score cutoff was 174 points, with $ $174 points classified as high MVI risk and <174 points as low risk. At this threshold, sensitivity, specificity, positive predictive value, and negative predictive value were 90%, 61%, 71%, and 85% respectively in the model group, and 78%, 71%, 76%, and 74% respectively in the validation group (Table 4).

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3. Discussion

Preoperative prediction of MVI in HCC patients is crucial for guiding surgical margin determination, liver transplantation decisions, and neoadjuvant therapy selection, and represents an independent factor affecting postoperative recurrence and overall survival. Identifying MVI preoperatively enables clinicians to stratify high-risk patients, improve treatment decisions, and prevent recurrence. This study developed a nomogram combining tumor diameter with five serological indicators to predict MVI.

AFP is associated with HCC growth and progression, promoting invasion and metastasis by upregulating metastasis-related proteins. Numerous studies have identified AFP level as a predictor of MVI suitable for inclusion in predictive models. Although multivariate analysis in this study showed AFP>45 ng/mL [OR=1.957, 95%CI (0.987, 3.879), P=0.054] was not statistically significant, we retained it as a model component given its established clinical relevance. The optimal AFP cutoff value varies across studies, necessitating larger sample sizes to determine appropriate thresholds.

Inflammation contributes to cancer development and progression. Inflammatory stimuli impair immune response, compromise immune cell recognition of "non-self" antigens, and promote tumor progression and immune evasion. Leukocytes release cytokines, chemokines, and enzymes that degrade extracellular matrix, facilitating tumor cell-endothelial cell adhesion and creating favorable conditions for invasion and metastasis. Research has demonstrated that leukocytes can form clusters with circulating tumor cells, protecting them and secreting interleukin-1 and interleukin-6 to enhance tumor cell proliferation and accelerate metastasis. Our study found WBC>7.1$\times10^{9}$/L [OR=3.144, 95%CI (1.301, 7.598), P=0.011] was closely associated with MVI, with higher WBC levels indicating increased MVI risk.

Maximum tumor diameter reflects tumor aggressiveness and MVI potential. Larger tumors have richer blood supply and higher MVI likelihood, as confirmed by multiple studies. Our study identified tumor diameter >7.05 cm as an independent risk factor for MVI, though the optimal cutoff varies across studies and requires validation in large multicenter cohorts.

S-Index, proposed by Chinese scholar ZHOU et al., combines GGT, PLT, and ALB to predict liver fibrosis and cirrhosis with ROC areas of 0.812 and 0.890 respectively. Tumor aggressiveness induces persistent oxidative stress, activating hepatic stellate cells that promote fibrosis and early postoperative recurrence. Fibrotic microenvironments alter hepatic blood flow, increase VEGF expression in hypoxic regions, and elevate IL-6 levels, enhancing tumor angiogenesis and anti-apoptotic capacity. Our study found S-Index>0.097 was an independent MVI risk factor.

ALT and AST are sensitive markers of hepatocellular injury. AST elevation reflects more severe damage, and AST/ALT ratio correlates with necrosis severity. Tumor invasion damages normal hepatocytes, elevating transaminase levels. High AST/ALT ratios have been associated with poor prognosis in primary liver cancer. Our study identified AAR>0.879 as an independent MVI predictor, though this relationship requires further validation.

ANRI, the AST-to-neutrophil ratio, reflects oxidative stress-induced hepatocyte apoptosis and inflammatory status. Neutrophils suppress anti-tumor immune responses and affect prognosis. Studies have linked high ANRI to shorter disease-free and overall survival in HCC. Our study found ANRI>24.074 was an independent MVI predictor.

In summary, single indicators often have limited sensitivity and specificity for MVI prediction. This nomogram, based on readily available laboratory and imaging parameters (AFP, WBC, tumor diameter, S-Index, AAR, ANRI), provides a practical tool for preoperative MVI risk assessment. However, the relatively small sample size and single-center design may introduce statistical bias, requiring further validation.

Author Contributions: TANG Can contributed to study design, data collection, and manuscript writing. LI Xiangyang, QIN Haoran, and LI Jing contributed to case data collection and organization. ZHU Hong participated in study design, manuscript revision, and final approval.

Conflict of Interest: The authors declare no conflicts of interest.

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Note: Figure translations are in progress. See original paper for figures.