Prognostic Nomograms Based on Microvascular Invasion Grade for Early-stage Hepatocellular Carcinoma Patients After Curative Hepatectomy

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Abstract

Background: Hepatocellular carcinoma (HCC) is among the most frequent causes of cancer-related deaths worldwide. Although predictive models for postoperative early recurrence in patients with HCC have been established, this is the first study to develop and evaluate a predictive model based on the microvascular invasion classification for early recurrence and survival after curative hepatectomy in patients with early-stage HCC.

Methods: The database of patients with early-stage HCC who underwent curative hepatectomy in the First Affiliated Hospital of Fujian Medical University and the First Affiliated Hospital of Xiamen University was retrospectively reviewed. Kaplan-Meier curves and Cox proportional hazards regression models were used to analyse disease-free survival (DFS) and overall survival (OS). Nomogram models were constructed on the datasets from the First Affiliated Hospital of Fujian Medical University, which were validated using bootstrap resampling with 30% samples as internal validation. Data of patients from the First Affiliated Hospital of Xiamen University were used for external validation.

Results: A total of 703 patients with early-stage HCC were included in our study. An eight-factor nomogram for predicting recurrence or metastasis and a six-factor nomogram for predicting survival were created. The concordance indexes were 0.775 (95% confidence interval [CI], 0.720-0.830) for the DFS nomogram and 0.812 for the OS nomogram (95% CI, 0.732-0.892) in the training cohort; 0.865 (95% CI, 0.806-0.924) and 0.839 (95% CI, 0.675-1.00), respectively, in the internal validation cohort; and 0.857 (95% CI, 0.763-0.951) and 0.842 (95% CI, 0.708-0.970), respectively, in the external validation cohort. The calibration curves showed optimal agreement between the predicted and observed DFS and OS rates. The predictive accuracy was significantly better than that of the classic HCC staging systems.

Conclusions: This study developed and validated nomograms for predicting recurrence, especially early recurrence, and overall survival in patients with early-stage HCC after curative resection with high predictive accuracy.

Background

Hepatocellular carcinoma (HCC) is among the most frequent causes of cancer-related deaths worldwide ^[1]. Despite remarkable improvements in comprehensive HCC treatment, radical surgical resection and liver transplantation are considered the only curative treatments for patients with HCC classified as early-stage (stages 0 and A) according to the Barcelona Clinic Liver Cancer (BCLC) staging system. However, postoperative recurrence and metastasis rates of patients with early HCC vary from 50–70% ^[2], resulting in poor overall survival (OS). Early recurrence after liver resection for HCC is the leading cause of death during the first 2 years ^[3]. Therefore, developing a model for predicting postoperative recurrence, especially early recurrence, for patients with early-stage HCC to guide risk stratification and treatment is urgently needed.

Microvascular invasion (MVI), a mass of cancer cells in the vascular cavity with adhesion to endothelial cells, and only visible under a microscope ^[4], has been reported by previous studies to be an indicator of early invasive manifestation of HCC. It is a crucial independent predictive factor for early recurrence and poor OS among patients with HCC who underwent hepatectomy or received liver transplantation. Most patients with BCLC early-stage HCC with early recurrence are pathologically verified as MVI positive ^[5–7]. Moreover, a previous study found that more invading tumor cells and multiple-invaded microvessels might be related to poor survival and recurrence rates ^[4]. These findings suggest that the BCLC staging system should reappraise HCC based on the presence or grade of MVI to distinguish the biological behavior of early-stage HCC.

MVI is graded according to the number of cancer cells and the distance of MVI to the tumor according to the Standard for Diagnosis and Treatment of Primary Liver Cancer ^[8]. Although predictive models for postoperative early recurrence in patients with HCC have been established, a predictive model for patients with early BCLC stage HCC patients according to the MVI grade has not been reported.

Therefore, we retrospectively investigated the clinical and histopathological characteristics of patients with early HCC after curative hepatectomy from multiple centres to establish a prognostic nomogram based on MVI grade to predict early recurrence and OS.

Methods

Patients and study design

The database was retrospectively derived from patients with HCC who underwent hepatectomy at the First Affiliated Hospital of Fujian Medical University (FHFU) and the First Affiliated Hospital of Xiamen University (FHXU) from March 2015 to March 2020.

The inclusion criteria for patients with HCC patients in this study were: (1) early-stage HCC (BCLC stage 0 or A) diagnosis that was confirmed by postoperative pathology; (2) Child-Pugh A or B liver function before surgery; (3) R0 surgical resection of tumor with curative intent; (4) all patients who survived for at least 30 days after surgery; (5) no preoperative anticancer treatments that could introduce any bias; and (6) clinicopathological data and follow-up information were available. Patients with the following criteria were excluded: (1) recurrent HCC, (2) combined hepatocellular cholangiocarcinoma, (3) previous history of malignancy, and (4) age < 18 years.

Nomogram models were constructed on the datasets from the FHFU, which were also validated using bootstrap resampling as internal validation, and the dataset from the FHXU was used for external validation. This study was approved by the Clinical Research Ethics Committee of the two centres. Written informed consent was obtained from all subjects before the operation. All procedures were performed in accordance with the Declaration of Helsinki.

Clinical variables

Demographic, laboratory, and HCC pathological data were collected. The laboratory tests included various tests for routine blood parameters, full sets of tests for blood clotting, full sets of tests for blood biochemistry, and hepatitis virus markers. Imaging data included, but were not limited to, the number of tumours, presence of satellite nodules, diameter of the largest nodule, tumour capsule, and cirrhosis based on preoperative contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI). The diagnosis and classification of MVI was confirmed according to the Standard for Diagnosis and Treatment of Primary Liver Cancer [4].

Follow-up

During the follow-up, serum alpha-fetoprotein (AFP) levels were measured, and ultrasonography, CT, or MRI of the chest and abdomen was done once every 2 months for the first 2 years after surgery. For patients who were free of cancer recurrence 2 years after surgery, a 6-month interval surveillance was performed. Disease-free survival (DFS) was defined as the duration from the first surgery to the first recurrence, metastasis, or death. OS was defined as the duration from the first surgery to death or the last follow-up.

Statistical analysis

Continuous variables are expressed as mean $\pm$ standard deviation. Chi-squared or Fisher's exact tests were used to assess differences in categorical variables. The Wilcoxon rank-sum test was used to compare continuous variables between groups. The cut-off values were established using the X-tile software version 3.6.1 (Yale University School of Medicine, New Haven, Connecticut, United States). For DFS and OS curves during follow-up, Kaplan-Meier curves, log-rank Mantel-Cox test, and Cox proportional hazards regression analyses were used. Nomograms were generated using the rms package in R software version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria) [9]. The predictive accuracy and discriminative ability of the nomogram were assessed using concordance index (C-index) [7] and calibration curves. The larger the C-index, the more accurate the prognostic prediction is. A value of $P < 0.05$ was considered significant.

Results

Characteristics of patients in study and validation cohorts

Overall, 703 patients (490 from FHFU used as training cohort, and 213 from FHXU used as external validation cohort) with BCLC early-stage HCC were included. The baseline characteristics of the two cohorts are shown in Table 1. The average age of the entire cohort was 53.7 ± 10.9 years, with a male to female ratio of 4.72:1 (580/130). The average follow-up time for all patients was 18.8 ± 10.2 months. The 8-month, 1-, 2-, and 3-year recurrence or metastasis rates were 5.8%, 8.1%, 11.8%, and 12.7%, respectively. The 8-month, 1-, 2-, and 3-year survival rates were 1.7%, 2.4%, 3.9%, and 4.2%, respectively. Among them, M1 was observed in 173 cases (24.6%), while M2 was observed in 102 cases (14.5%). Between-group differences in sex, age, operative time, follow-up time, ASA scores, laboratory, and HCC pathological data were not significant (Table 1). The association of MVI grade relative to OS or DFS is shown in Fig. 1. The Kaplan-Meier curves of OS and DFS showed that the M2 group had significantly poorer outcomes than the M1 and M0 groups (both $P < 0.001$).

Table 1
The basic clinical characteristics of early-stage HCC patients

Clinical parameter	Total (n = 703)	FHFU cohort (n = 490)	FHXU cohort (n = 213)
Sex, male/female	580/123	404/86	175/38
Age, year	53.7 ± 10.9	53.4 ± 11.1	52.8 ± 10.5
BCLC staging system, (0/A)	45/658	31/459	13/200
WBC, 10 ⁹ /L	5.2 ± 1.6	5.0 ± 1.9	4.9 ± 1.5
PLT, 10 ⁹ /L	160.3 ± 62.5	158.6 ± 58.7	161.6 ± 65.3
Hb, g/L	142.4 ± 14.9	139.6 ± 15.1	141.6 ± 15.5
Hematocrit, %	41.5 ± 3.9	41.3 ± 2.7	40.4 ± 3.3
MCV, fL	90.7 ± 4.8	92.2 ± 5.1	89.9 ± 5.0
MCH, pg	31.1 ± 2.0	31.3 ± 1.9	31.2 ± 1.8
Neutrophil, 10 ⁹ /L	3.1 ± 1.2	3.0 ± 1.1	3.1 ± 1.2
Lymphocyte, 10 ⁹ /L	1.6 ± 0.6	1.6 ± 0.6	1.6 ± 0.6
Monocyte, 10 ⁹ /L	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
NR, %	58.3 ± 9.3	59.4 ± 9.8	55.4 ± 8.9
LR, %	31.8 ± 8.5	32.3 ± 8.2	32.1 ± 08.6
MR, %	7.0 ± 2.0	7.0 ± 1.9	7.0 ± 2.1
RDW, %	13.2 ± 0.9	13.1 ± 0.8	13.1 ± 0.8
RBC, 10 ⁹ /L	4.6 ± 0.6	4.6 ± 0.6	4.6 ± 0.5
AFP, µg/L	339.1 ± 484.8	319 ± 480	364.7 ± 491.4
ALT, U/L	35.1 ± 28.0	35.3 ± 27	36.4 ± 35.5
HBV DNA level, <10 ⁶ / ^{>} 10 ⁶ IU/mL	283/420	197/293	86/127
Albumin, g/L	42.2 ± 3.2	42.5 ± 3.1	42.1 ± 3.1
Scr, µmol/L	72.1 ± 15.6	76.9 ± 17.1	77.4 ± 16.5
γ-GGT, U/L	78.3 ± 102.9	107.1 ± 75.0	75.6 ± 97.2
ALP, U/L	86.5 ± 45.9	84.1 ± 37.4	84.4 ± 39.8
TBil, µmol/L	14.7 ± 6.1	13.5 ± 6.0	13.3 ± 6.7
DBil, µmol/L	5.5 ± 3.0	5.5 ± 3.0	5.3 ± 3.1
IBil, µmol/L	9.2 ± 3.8	9.0 ± 3.4	9.7 ± 3.3
TBA, µmol/L	9.9 ± 13.4	8.8 ± 17.1	9.2 ± 15.6

FHFU, the first affiliated hospital of Fujian Medical University; FHXU, the first affiliated hospital of Xiamen University; BCLC staging system, Barcelona Clinic Liver Cancer staging system; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; NR, neutrophil ratio; LR, lymphocyte ratio; MR, monocyte ratio; RDW, red blood cell distribution width; RBC, red blood cell; AFP, α-fetoprotein; ALT, alanine aminotransferase; HBV DNA level, hepatitis B virus deoxyribonucleic acid level; Scr, Serum creatinine; γ-GTT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TBA, total bile acid; TP, total protein; ALB, albumin; GLB, globulin; PAB, prealbumin; AFU, α-fucosidase; ADA, adenosine deaminase; LDH, lactate dehydrogenase; GLU, Glucose; TCHO, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TT, thrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; PT, prothrombin time; MVI, microvascular invasion.

Clinical parameter	Total (n = 703)	FHFU cohort (n = 490)	FHXU cohort (n = 213)
TP, g/L	69.7 ± 5.0	74.9 ± 4.7	75.6 ± 4.8
ALB, g/L	42.2 ± 3.2	42.5 ± 3.1	42.1 ± 3.1
GLB, g/L	27.5 ± 4.2	29.2 ± 5.3	27.7 ± 4.8
ALB/GLB	1.6 ± 0.3	1.6 ± 0.3	1.6 ± 0.3
PAB, mg/L	233.0 ± 71.1	240.2 ± 70.4	235.4 ± 70.3
AFU, g/L	27.5 ± 11.5	27.3 ± 11.3	26.5 ± 9.8
ADA, U/L	6.7 ± 2.2	6.6 ± 2.2	6.6 ± 2.2
LDH, U/L	168.7 ± 63.8	164.6 ± 46.9	168 ± 64.2
Urea, mmol/L	5.5 ± 1.4	5.4 ± 1.4	5.6 ± 1.4
Uric acid, μmol/L	320 ± 78.5	325 ± 75.5	333 ± 79.9
GLU, mmol/L	5.5 ± 1.4	5.4 ± 1.3	5.4 ± 1.4
TCHO, mmol/L	4.2 ± 0.9	4.2 ± 0.9	4.2 ± 0.9
TG, mmol/L	1.2 ± 0.7	1.2 ± 0.7	1.2 ± 0.6
HDL, mmol/L	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3
LDL, mmol/L	2.8 ± 0.8	2.8 ± 0.8	2.6 ± 0.7
Apo-A1, g/L	119.2 ± 30.9	117.7 ± 29.8	121.5 ± 31.0
Apo-B, g/L	85.6 ± 22.0	86.7 ± 22.5	82.3 ± 20.5
Calcium, mmol/L	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1
Phosphorus, mmol/L	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
Magnesium, mmol/L	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Kalium, mmol/L	4.1 ± 0.3	4.1 ± 0.3	4.3 ± 0.3
Natrium, mmol/L	141 ± 2.4	141 ± 2.3	141 ± 2.4
Chlorine, mmol/L	103.0 ± 2.9	103.1 ± 2.7	103.0 ± 3.1
TT, second	20.0 ± 1.5	20.1 ± 1.5	20.0 ± 1.8
FIB, g/L	2.4 ± 0.8	2.4 ± 0.7	2.4 ± 0.8
APTT, second	28.0 ± 4.2	27.8 ± 4.1	28.0 ± 3.7
PT, second	11.7 ± 1.1	11.9 ± 1.0	12.3 ± 1.2
Tumor size, centimeter	5.4 ± 3.6	5.2 ± 3.3	5.4 ± 3.8
Tumor number, single/multiple	670/33	467/23	203/10
Satellite nodules, yes/no	389/314	276/214	113/100

FHFU, the first affiliated hospital of Fujian Medical University; FHXU, the first affiliated hospital of Xiamen University; BCLC staging system, Barcelona Clinic Liver Cancer staging system; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; NR, neutrophil ratio; LR, lymphocyte ratio; MR, monocyte ratio; RDW, red blood cell distribution width; RBC, red blood cell; AFP, α-fetoprotein; ALT, alanine aminotransferase; HBV DNA level, hepatitis B virus deoxyribonucleic acid level; Scr, Serum creatinine; γ-GTT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TBA, total bile acid; TP, total protein; ALB, albumin; GLB, globulin; PAB, prealbumin; AFU, α-fucosidase; ADA, adenosine deaminase; LDH, lactate dehydrogenase; GLU, Glucose; TCHO, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TT, thrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; PT, prothrombin time; MVI, microvascular invasion.

Clinical parameter	Total (n = 703)	FHFU cohort (n = 490)	FHXU cohort (n = 213)
MVI, M0/M1/M2	428/173/102	294/121/75	134/52/27
Tumor capsule, yes/no	328/375	228/262	100/113
Cirrhosis, yes/no	208/495	142/348	66/147
Follow-up time (months)	18.8 ± 10.2	19.0 ± 10.2	18.3 ± 10.3
Recurrence/metastasis rates (%)	5.8/8.1/	6.5/9.2/	4.2/5.6/
(8-month/1-year/2-year/3-year)	11.8/12.7	11.7/12.5	9.5/10.4
Survival rate (%)	1.7/2.4/	1.9/2.5/	1.8/2.2/
(8-month/1-year/2-year/3-year)	3.9/4.2	4.1/4.4	3.6/4.0
FHFU, the first affiliated hospital of Fujian Medical University; FHXU, the first affiliated hospital of Xiamen University; BCLC staging system, Barcelona Clinic Liver Cancer staging system; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; NR, neutrophil ratio; LR, lymphocyte ratio; MR, monocyte ratio; RDW, red blood cell distribution width; RBC, red blood cell; AFP, α-fetoprotein; ALT, alanine aminotransferase; HBV DNA level, hepatitis B virus deoxyribonucleic acid level; Scr, Serum creatinine; γ-GTT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TBA, total bile acid; TP, total protein; ALB, albumin; GLB, globulin; PAB, prealbumin; AFU, α-fucosidase; ADA, adenosine deaminase; LDH, lactate dehydrogenase; GLU, Glucose; TCHO, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TT, thrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; PT, prothrombin time; MVI, microvascular invasion.			

The univariate analysis results for DFS and OS in the study cohort are shown in Table 2. Multivariate analysis revealed that eight factors including neutrophils, alkaline phosphatase (ALP), urea, low-density lipoprotein (LDL), apolipoprotein A1 (Apo-A1), thrombin time (TT), tumour size, and MVI grade were independent prognostic factors for DFS, while six factors including TT, MVI grade, mean corpuscular haemoglobin (MCH), monocyte, prealbumin (PAB) and α-fucosidase (AFU) were prognostic factors for OS (Table 2). Therefore, these variables were included in the subsequent analysis to establish predictive models.

Table 2

Univariate and multivariate of clinical parameters associated with DFS and OS in early-stage HCC patients after R0 resection

Clinical parameter	DFS	OS		
	HR (95% CI)	p-value	HR (95% CI)	p-value
Univariate analysis				
Age, year	0.25(0.08–0.77)	0.015	0.98 (0.95-1)	0.091
Sex, male/female	0.81(0.45–1.46)	0.998	0.88 (0.41–1.9)	0.741
BCLC staging system, (0/A)	3.04(0.75–12.33)	0.122	2.74 (0.68–11.29)	0.995
WBC, $\leq 3.7/>3.7 \times 10^9/L$	2.41(0.98–6.02)	0.055	1.21(1.06–1.5.2)	0.013
PLT, $\leq 160/>160 \times 10^9/L$	1.76(1.12–2.77)	0.018	1.00(1.00–1.00)	0.027
Hb, $\leq 125/>125 \times 10^9/L$	2.05(0.81–5.06)	0.133	0.99(0.97–1.12)	0.312
Hematocrit, $\leq 42.2/>42.2\%$	1.56(0.67–2.32)	0.073	0.98(0.91–1.12)	0.692
MCV, $\leq 87/>87fL$	0.66(0.42–1.03)	0.061	0.95(0.90–1.01)	0.055
MCH, $\leq 31.5/>31.5pg$	0.45(0.28–0.72)	< 0.001	0.85(0.76–0.96)	0.007
Lymphocyte, $\leq 1.5/>1.5 \times 10^9/L$	0.93(0.60–1.45)	0.732	0.86(0.52–1.55)	0.585
Neutrophil, $\leq 3.3/>3.3 \times 10^9/L$	2.02(1.01–3.80)	0.045	1.43(1.14–1.72)	0.002
Monocyte, $\leq 0.5/>0.5 \times 10^9/L$	1.54(0.89–2.60)	0.126	7.51(1.63–15.86)	0.012
LR, $\leq 41.6/>41.6\%$	0.56(0.33–0.96)	0.036	0.95(0.92–0.99)	0.011
NR, $\leq 47.8/>47.8\%$	1.60(0.97–2.52)	0.068	1.05(1.00-1.10)	0.033
RDW, $\leq 13.6/>13.6\%$	1.81(1.12–3.01)	0.016	1.12(0.89–1.56)	0.292
RBC, $\leq 4.5/>4.5 \times 10^9/L$	1.35(0.86–2.63)	0.152	1.22(0.66–2.24)	0.533
AFP, < 400/ $\geq 400\mu g/L$	2.01(1.20–3.26)	0.005	1.00(1.00–1.00)	< 0.001
ALT, < 61/ ≥ 61 U/L	13.12(1.85–5.40)	< 0.001	1.00(0.99-1.00)	1.000
HBV DNA, < 50/ $\geq 50 \times 10^9$ IU/mL	1.74(1.12–2.96)	0.016	1.00(1.00–1.00)	0.971
Albumin, < 50/ $\geq 50g/L$	0.47(0.23–0.93)	0.031	0.89(0.82–0.97)	0.008
Scr, < 76/ $\geq 76\mu mol/L$	0.62(0.34–1.12)	0.123	0.98(0.96–1.02)	0.240
γ -GGT, < 34/ $\geq 34U/L$	2.10(1.12–3.95)	0.021	1.00(1.00–1.00)	0.541
ALP, < 76/ ≥ 76 &<117 $\geq 117U/L$	4.75(2.61–8.56)	< 0.001	1.00(1.00–1.00)	0.182
TBil, < 16.4/ $\geq 16.4\mu mol/L$	1.44(0.88–2.12)	0.169	0.97 (0.93–1.24)	0.313
DBil, < 5.6/ $\geq 5.6\mu mol/L$	1.52(0.97–2.30)	0.065	0.95 (0.83–1.10)	0.415
IBil, < 6.6/ $\geq 6.6\mu mol/L$	1.55(0.83–2.70)	0.178	0.94 (0.86-1.00)	0.182

BCLC staging system, Barcelona Clinic Liver Cancer staging system; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; LR, lymphocyte ratio; NR, neutrophil ratio; MR, monocyte ratio; RDW, red blood cell distribution width; MPC, Mean platelet volume; RBC, red blood cell; AFP, α -fetoprotein; ALT, alanine aminotransferase; HBV DNA level, hepatitis B virus deoxyribonucleic acid level; Scr, Serum creatinine; γ -GTT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TBA, total bile acid; TP, total protein; ALB, albumin; GLB, globulin; PAB, prealbumin; AFU, α -fucosidase; ADA, adenosine deaminase; LDH, lactate dehydrogenase; GLU, Glucose; TCHO, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TT, thrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; PT, prothrombin time; MVI, microvascular invasion.

Clinical parameter	DFS	OS		
	HR (95% CI)	p-value	HR (95% CI)	p-value
TBA, < 7.2/≥7.2μmol/L	2.33(1.20–4.32)	0.011	0.95 (0.89–1.01)	0.061
TP, < 77 /≥77g/L	0.26(0.07–1.01)	0.049	1.00 (0.95–1.10)	0.706
ALB, < 37/≥37g/L	1.40(0.23–0.93)	0.031	0.89 (0.82–0.97)	0.008
GLB, < 28.2/≥28.2g/L	1.42(0.88–2.10)	0.168	1.00(0.96–1.06)	0.727
ALB/GLB < 2.2/≥2.2	0.39(0.19–0.82)	0.013	1.73 (0.26–1.30)	0.186
PAB, ≤ 200/>200mg/L	0.32(0.14–0.73)	0.007	0.99 (0.99-1.00)	0.0014
AFU, < 38/≥38g/L	4.12(1.62–10.04)	0.003	1.02 (1.00-1.03)	0.032
ADA, < 7/≥7U/L	1.92(1.25–3.04)	0.008	1.12 (0.99–1.32)	0.063
LDH, < 212/≥212U/L	2.02(1.12–3.76)	0.033	1.00 (1.00–1.00)	< 0.001
Urea, < 4/≥4&<6.9/≥6.9mmol/L	0.22 (0.08–0.59)	0.003	0.95 (0.76–1.22)	0.623
Uric acid, < 379/≥379μmol/L	1.64(0.99–2.62)	0.053	1.01(0.99–1.03)	0.192
GLU, < 4.9/≥4.9mmol/L	0.60(0.38–0.94)	0.025	0.97(0.78–1.22)	0.821
TCHO, < 3.4/≥3.4mmol/L	1.95(0.74–4.78)	0.184	1.02(0.75–1.40)	0.990
TG, < 1.1/≥1.1mmol/L	0.60(0.37–0.98)	0.043	0.51(0.24–1.18)	0.081
HDL, < 1/≥1mmol/L	0.70(0.43–1.12)	0.156	1.25(0.48–3.05)	0.702
LDL, < 3/≥3mmol/L	2.08(1.22–3.16)	0.005	0.98(0.65–1.52)	0.905
Apo-A1, < 83/≥83g/L	0.45(0.22–0.91)	0.028	1.00(0.99–1.01)	0.760
Apo-B, < 113/≥113g/L	2.01(1.14–3.66)	0.027	1.00(0.99–1.01)	0.708
Calcium, < 2.5/≥2.5mmol/L	1.75(0.24–12.4)	0.590	2.01(0.09–4.01)	0.660
Phosphorus, < 1.1/≥1.1mmol/L	1.72(1.03–3.01)	0.043	10.01(1.82–20.14)	0.008
Magnesium, < 0.8/≥0.8mmol/L	0.70(0.38–1.25)	0.212	2.1(0.02–4.24)	0.751
Kalium, < 4.5/≥4.5mmol/L	1.84(1.01–3.23)	0.044	3.72(1.65–8.62)	0.003
Natrium, < 141/≥141mmol/L	0.57(0.36–0.89)	0.014	0.99(0.87–1.15)	0.860
Chlorine, < 102/≥102mmol/L	0.46(0.29–0.73)	< 0.001	0.93(0.84–1.02)	0.151
TT, < 20/≥20second	0.46(0.22–0.95)	0.035	0.75(0.59–0.96)	0.025
FIB, < 2.8/≥2.8g/L	2.10(1.34–3.37)	0.002	1.81(1.40–2.34)	< 0.001
APTT, < 25.7/≥25.7second	0.59(0.37–0.92)	0.020	1.01(0.93–1.15)	0.960
PT, < 11.3/≥11.3second	1.52(0.94–2.57)	0.092	1.00(0.76–1.41)	0.933
Tumor size, < 5/≥5&<10/≥10centimeter	3.82(2.12–6.84)	< 0.001	1.22 (1.14–1.35)	< 0.001

BCLC staging system, Barcelona Clinic Liver Cancer staging system; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; LR, lymphocyte ratio; NR, neutrophil ratio; MR, monocyte ratio; RDW, red blood cell distribution width; MPC, Mean platelet volume; RBC, red blood cell; AFP, α-fetoprotein; ALT, alanine aminotransferase; HBV DNA level, hepatitis B virus deoxyribonucleic acid level; Scr, Serum creatinine; γ-GTT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TBA, total bile acid; TP, total protein; ALB, albumin; GLB, globulin; PAB, prealbumin; AFU, α-fucosidase; ADA, adenosine deaminase; LDH, lactate dehydrogenase; GLU, Glucose; TCHO, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TT, thrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; PT, prothrombin time; MVI, microvascular invasion.

Clinical parameter	DFS		OS	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Tumor number, single/multiple	0.62(0.20–12.0)	0.415	1.55(0.55–4.36)	0.408
Satellite nodules, yes/no	1.72(1.13–2.61)	0.022	1.2 (0.69–2.1)	0.525
MVI, M0/M1/M2	2.60(1.51–4.45)	< 0.001	2.20(1.61–3.05)	< 0.001
Tumor capsule, yes/no	0.87(0.67–1.15)	0.308	0.62(0.20–2.01)	0.415
Cirrhosis, yes/no	0.77(0.46–1.32)	0.313	0.54(0.25–1.15)	0.107
Multivariate analysis				
Neutrophil	0.34(0.19–0.60)	< 0.001		
ALP	4.41(2.05–9.62)	< 0.001	-	-
Urea	0.46(0.26–0.80)	0.007	-	-
LDL	2.15(1.34–3.67)	0.003	-	-
Apo-A1	0.32(0.16–0.66)	0.002	-	-
TT	0.34(0.16–0.70)	0.003	0.92(0.87–0.97)	0.003
Tumor size	2.20(1.29–3.82)	0.008	-	-
MVI grade	2.31(1.25–4.16)	0.009	0.80(0.64–0.99)	0.023
MCH	-	-	0.67(0.54–0.83)	< 0.001
Monocyte	-	-	4.67(2.37–9.68)	< 0.001
PAB	-	-	0.56(0.38–0.84)	0.005
AFU	-	-	0.71(0.61–0.82)	< 0.001
BCLC staging system, Barcelona Clinic Liver Cancer staging system; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; LR, lymphocyte ratio; NR, neutrophil ratio; MR, monocyte ratio; RDW, red blood cell distribution width; MPC, Mean platelet volume; RBC, red blood cell; AFP, α -fetoprotein; ALT, alanine aminotransferase; HBV DNA level, hepatitis B virus deoxyribonucleic acid level; Scr, Serum creatinine; γ -GTT, γ -glutamyl transpeptidase; ALP, alkaline phosphatase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TBA, total bile acid; TP, total protein; ALB, albumin; GLB, globulin; PAB, prealbumin; AFU, α -fucosidase; ADA, adenosine deaminase; LDH, lactate dehydrogenase; GLU, Glucose; TCHO, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TT, thrombin time; FIB, fibrinogen; APTT, activated partial thromboplastin time; PT, prothrombin time; MVI, microvascular invasion.				

Establishment of nomogram model for postoperative early-relapse and evaluation of its discriminability and calibration

Based on the independent prognostic factors, nomograms for DFS and OS in the study cohort were established (Fig. 2). The results are shown in Table 3. The C-index of the nomogram for DFS was 0.775 (95% confidence interval [CI], 0.720–0.830). The C-index for OS was 0.812 (95% CI, 0.732–0.892). The validation showed excellent consistency between the observed and predicted 8-month, 1-, 2- and 3-year DFS, and 8-month, 1-, 2- and 3-year OS (Fig. 2); with a C-index of 0.865 (95% CI, 0.806–0.924) for DFS and a C-index of 0.839 for OS (95% CI, 0.675–1.00) in the internal validation cohort, and with a C-index of 0.857 (95% CI, 0.763–0.951) for DFS and a C-index of 0.842 (95% CI, 0.708–0.970) for OS in the external validation cohort (Table 3). Calibration curves of internal verification and external verification showed good consistency between the observed and predicted events (Fig. 3). Taken together, the nomogram models were able to accurately predict postoperative relapse and OS in patients with BCLC early-stage HCC.

Table 3
The C-index of the nomograms and classical staging systems

Prognostic system	Training cohort				Internal validation cohort				External validation cohort			
	DFS		OS		DFS		OS		DFS		OS	
	C-index	95%CI	C-index	95%CI	C-index	95%CI	C-index	95%CI	C-index	95%CI	C-index	95%CI
Nomograms	0.775	0.720–0.830	0.812	0.732–0.892	0.865	0.806–0.924	0.839	0.675–1.00	0.957	0.763–0.951	0.842	0.708–0.970
AJCC	0.591	0.558–0.628	0.588	0.546–0.611	0.622	0.581–0.662	0.615	0.572–0.649	0.586	0.544–0.607	0.578	0.533–0.599
BCLC	0.601	0.563–0.648	0.599	0.550–0.641	0.602	0.568–0.651	0.608	0.575–0.655	0.574	0.534–0.622	0.571	0.530–0.619
JIS	0.589	0.543–0.632	0.592	0.548–0.637	0.606	0.552–0.639	0.599	0.554–0.643	0.581	0.535–0.622	0.574	0.528–0.616
HKLC	0.595	0.562–0.629	0.612	0.568–0.632	0.625	0.581–0.649	0.619	0.577–0.638	0.558	0.528–0.580	0.541	0.512–0.568
C-index, concordance index; DFS, disease-free survival; OS, overall survival; CI, confidence interval; AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer staging system; JIS, the Japan Integrated Staging Score; HKLC, the Hong Kong Liver Cancer prognostic classification scheme.												

Comparison of predictive accuracy between the nomogram models and the classical staging systems

The predictive value of the constructed model, in terms of clinical practicability, was compared with that of the 8th edition American Joint Committee on Cancer (AJCC) staging system, the BCLC staging system, the Japan Integrated Staging Score (JIS) and the Hong Kong Liver Cancer prognostic classification scheme (HKLC). The results are shown in Table 3. In the training cohort, the C-index of the nomogram for DFS and OS was 0.775 and 0.812, respectively, which was significantly higher than the AJCC (DFS: 0.591; OS: 0.588), BCLC (DFS: 0.601; OS: 0.599), JIS (DFS: 0.589; OS: 0.592), and HKLC (DFS: 0.595; OS: 0.612) staging systems. Similarly, in the validation cohort, the C-index of the nomogram for DFS (internal cohort: 0.865; external cohort: 0.857) and OS (internal cohort: 0.839; external cohort: 0.842), was also significantly higher than the AJCC (internal cohort: 0.622, external cohort: 0.586 for DFS; and internal cohort: 0.615, external cohort: 0.578 for OS), BCLC (internal cohort: 0.602, external cohort: 0.574 for DFS; and internal cohort: 0.608, external cohort: 0.571 for OS), JIS (internal cohort: 0.606, external cohort: 0.581 for DFS; and internal cohort: 0.599, external cohort: 0.574 for OS), HKLC (internal cohort: 0.625, external cohort: 0.558 for DFS; and internal cohort: 0.619, external cohort: 0.541 for OS) staging systems. Overall, the nomogram models exhibited superior predictive accuracy to that of these authoritative staging systems for DFS and OS.

Discussion

Despite patients with BCLC early-stage HCC generally present better prognosis relative to patients with late-stage HCC, a considerable number of patients still suffer from recurrence and metastasis. The presence of MVI is accepted worldwide as one of the most powerful predictors of poor prognosis in patients with early-stage HCC [4, 8, 9]. Furthermore, recent studies have found that the grade of MVI is closely related to postoperative recurrence, especially early recurrence [8, 10–13]. Of the two most used pathological staging systems for HCC, neither includes MVI as a criterion. A predictive model based on the MVI grading system for recurrence, especially early recurrence in patients with early-stage HCC, has not been reported. Therefore, we established nomograms based on the MVI grading system for recurrence and OS in patients with early-stage HCC after curative surgery, and further validation showed good agreement between the nomogram predictions and actual observations in terms of the predictive probability. In addition, our nomograms had greater predictive performance than the two classical staging systems, the BCLC and AJCC staging systems.

The prognosis of patients with HCC is mainly affected by: (1) patient factors, such as immune function, nutritional state, liver function, and status of hepatitis virus infection; (2) tumour factors, such as tumour diameter, MVI classification, and satellite nodules; and (3) factors of treatment, in particularly adjuvant treatment after surgery. In our study, nine of the twelve risk factors associated with recurrence or OS were patient factors, including neutrophil, monocyte, ALP, PAB, MCH, Urea, LDL, Apo-A1, and TT levels, while three

factors were tumour-related factors including tumour size, MVI classification, and AFU. These results indicate that the prognosis of HCC is a multifactorial and complex process.

As the histopathological types and grades of MVI represent the histopathological changes that occur when a cancer embolus in a vessel evolves to become a satellite lesion or a metastatic site, the histopathological type of MVI can be used as a morphological marker to evaluate the biology and progression of HCC [4, 14, 15]. Whereas, the detectability rate of MVI is low, ranging from 12.4–33.1% in patients with early-stage HCC, and the prognostic value of MVI for patients with early-stage HCC after curative surgery remains disputable [16–18]. In our study, MVI was an independent risk factor related to DFS and OS (Fig. 1, $P < 0.001$) with a detection rate of 39.1% (275/703). It is generally known that tumour size is related to patient prognosis; the presence of tumour enlargement predicts poor prognosis in patients with HCC. The cut-off value of tumour size is widely used in different guidelines to predict prognosis as the relationship between tumour size and poor prognosis in patients is not linear. In this study, the cut-off values were set as 5 and 10 cm. Our study identified tumours with a diameter > 10 cm as a significant risk factor for recurrence. Interestingly, although AFP is known as a typical clinical marker for the diagnosis and prognosis of patients with HCC, it was not an independent factor related to prognosis in early-stage HCC after curative hepatectomy in our study. This may be due to the low sensitivity of AFP in predicting the prognosis of early-stage HCC. It has been reported that AFP cannot be detected in 30–35% of patients with primary HCC, while an increased AFP level is also found in those with normal health [19]. Of note, AFU was a significantly independent factor correlated with OS in early-stage HCC. It is reported that AFU is a specific marker for HCC, which exhibits higher sensitivity and specificity than AFP in diagnosing HCC. In particular, AFU is highly and accurately discriminative of AFP-negative and early-stage HCC. Therefore, dynamic monitoring of AFU is of great significance for the diagnosis and prognosis of early-stage HCC [20].

Previous studies have reported that immune function and nutritional status are related to the prognosis of patients with HCC [21–25]. In our nomogram models, neutrophil, monocyte, MCH, PAB, and urea (the final product of protein metabolism) are powerful immune and nutritional indices that can be used to predict prognosis. The prognosis of patients with low neutrophil and urea levels (indicating insufficient protein intake) is poor. The tumor microenvironment plays an important role in tumorigenesis. Immune and nutritional status, being part of tumour microcirculation, undoubtedly affects the prognosis of patients with HCC. Increasing evidence shows that basic nutritional status and systemic inflammation are related to the long-term prognosis of cancer patients [21, 26–28]. Malnutrition and low immune function not only affect the treatment effect in patients with malignant tumours, but also make patients with HCC more prone to relapse and metastasis [21].

In recent years, metabolic disorders, especially lipid metabolism disorders, have emerged as an important microenvironment for HCC pathogenesis [29, 30]. LDL and Apo-A1, as indices of liver lipid metabolism, served as significant predictors for the prognosis of early-stage HCC in this study. It is known that changes in the metabolism of liver lipids are closely related to the occurrence of liver cancer, and in the future, non-alcoholic fatty liver disease may be identified as one of the main causes of primary liver cancer [31]. Moreover, previous studies have also shown that lipid metabolism disorders can promote tumour cell proliferation by inhibiting the apoptosis of liver cancer cells, resulting in a poor prognosis [32].

Yet, there is still room for further improvement. First, our model is primarily based on retrospectively collected datasets from two Chinese institutions. Although the models performed well, the inclusion of additional cohorts from other institutions may improve the predictive accuracy of our model. Second, though the sample size in this study is adequate, a larger sample size in conjunction with meaningful information including postoperative adjuvant treatment collected in the future may improve the accuracy of our results. Third, hepatitis B virus (HBV) infection, known to be associated with a poor prognosis of HCC, showed limited prognostic value in our study. This may have been due to some patients receiving non-standardized anti-HBV treatments, which may have affected the statistical results.

Conclusions

In summary, we developed and validated nomograms for predicting recurrence, especially early recurrence, and OS in patients with early-stage HCC after curative surgery. The predictive performances were superior to the common typical HCC staging systems, and they can provide a reference for clinicians to improve better outcomes in this group of patients.

List Of Abbreviations

HCC, Hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; OS, overall survival; MVI, Microvascular invasion; FHFU, the First Affiliated Hospital of Fujian Medical University; FHXU, the First Affiliated Hospital of Xiamen University; CT, computed tomography; MRI, magnetic resonance imaging; AFP, α -fetoprotein; DFS, disease-free survival; C-index, concordance index; ALP, alkaline phosphatase; LDL, low-density lipoprotein; Apo-A1, apolipoprotein A1; TT, thrombin time; MCH, mean corpuscular haemoglobin; PAB, prealbumin; AFU, α -fucosidase; CI, confidence interval; AJCC, American Joint Committee on Cancer staging system; JIS, Japan Integrated Staging Score; HKLC, Hong Kong Liver Cancer prognostic classification scheme; HBV, hepatitis B virus.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Review Committee of The First Affiliated Hospital of Fujian Medical University and the Ethics Review Committee of The First Affiliated Hospital of Xiamen University. Written informed consent was obtained from all subjects before the operation. All procedures were performed in accordance with the Declaration of Helsinki.

Consent for publication

Written informed consent was obtained from every patient with HCC to perform tumour resection for analysis and publication.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conception and design: Ke, J and Chen, H. Development of methodology: Ye, H and Lin, F. Acquisition of Data: Ke, J, Ye, H, Shi, Y, Lin, F and Zhong A. Analysis and interpretation of data: Yang, H, Shi, Y and Zhong A. Writing, review, and/or revision of the manuscript: Ke, J, Ye, H and Chen, H. Study supervision: Chen, H.

Conflicts of interest

All the authors do not have any possible conflicts of interest.

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Figures

Figure 1

Kaplan-Meier estimates of the prognosis of patients with early-stage HCC according to microvascular invasion (MVI) grade. (A) The MVI grade satisfactorily determined the disease-free survival (DFS) in the whole cohort; (B) The MVI grade satisfactorily determined the overall survival (OS) in the whole cohort.

Figure 2

Nomograms for predicting disease-free survival (DFS) and overall survival (OS) in patients with early-stage HCC after curative hepatectomy. (A) DFS; (B) OS. MCH, mean corpuscular hemoglobin; ALP, alkaline phosphatase; PAB, prealbumin; AFU, α -fucosidase; Apo-A1, apolipoprotein A1; TT, thrombin time; MVI, microvascular invasion.

Figure 3

Calibration curves for predicting disease-free survival (DFS) and overall survival (OS) using the nomograms. (A) 8-month, 1, 2, and 3-year DFS in the training cohort; (B) 8-month, 1, 2, and 3-year DFS in the internal validation cohort; (C) 8-month, 1, 2, and 3-year DFS in the external validation cohort; (D) 8-month, 1, 2, and 3-year OS in the training cohort; (E) 8-month, 1, 2, and 3-year OS in the internal validation cohort; (F) 8-month, 1, 2, and 3-year OS in the external validation cohort;