

Novel Nomograms Based on Gamma-Glutamyl Transpeptidase-to-Lymphocyte Ratio Predict Prognosis of Hepatocellular Carcinoma Patients After Hepatectomy

Cheng Ma^{1-4,*}, Yin Cao^{1,*}, Guang Zhang^{1-3,*}, Jiannan Qiu², Yan Zhou¹, Peng Wang², Shuo Wang², Dongliang Yan¹, Ding Ma², Chunping Jiang¹⁻³, Zhongxia Wang¹⁻³

¹Department of Hepatobiliary Surgery, Drum Tower Clinical College of Nanjing Medical University, Nanjing, People's Republic of China; ²Department of Hepatobiliary Surgery, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, People's Republic of China; ³Department of Tissue Engineering, Jinan Microecological Biomedicine Shandong Laboratory, Jinan, People's Republic of China; ⁴Department of Gastrointestinal Surgery, Xuzhou Central Hospital, Xuzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhongxia Wang; Chunping Jiang, Email freud_t@126.com; chunpingjiang@163.com

Background: The prediction of prognosis of hepatocellular carcinoma (HCC) is of great significance in improving disease outcome and optimizing clinical management, while reliable prognostic indicators are lacking. This study was conducted to develop readily-to-use nomograms for prognosis prediction of HCC after hepatectomy.

Materials and Methods: Data of eligible patients were collected and analyzed retrospectively. Independent prognostic factors were identified by Cox regression, and nomograms for the prediction of disease-free survival (DFS) and overall survival (OS) were developed. The performance of the nomograms was evaluated by receiver operating characteristics (ROC) curves, C-indexes and calibration curves and was verified by the validation cohort. The predictive value of the nomograms was also compared with the 8th edition of American Joint Committee on Cancer (AJCC) Tumor-Node-Metastasis (TNM) and the Barcelona Clinic Liver Cancer (BCLC) staging systems.

Results: In total, 599 patients were enrolled in the analysis: 420 in the training cohort and 179 in the validation cohort. The optimal cut-off value of Gamma-Glutamyl Transpeptidase-to-Lymphocyte Ratio (GLR) was 19.5. GLR contributed significantly to the nomograms with good predictive power. In ROC analyses, the areas under curve (AUCs) of the nomograms for 1-, 3- and 5-year DFS and OS prediction were 0.758, 0.756, 0.734 and 0.810, 0.799, 0.758, respectively. The C-indexes of the DFS nomogram were 0.697 (95% CI 0.665–0.729) in the training cohort and 0.710 (95% CI 0.664–0.756) in the validation cohort. For OS prediction, the C-indexes were 0.741 (95% CI 0.704–0.778) and 0.758 (95% CI 0.705–0.811) in the training and validation cohorts, respectively. The calibration curves demonstrated satisfactory agreement between nomogram predictions and actual observations. The nomograms demonstrated superior predictive performance to the TNM and the BCLC staging systems.

Conclusion: Our novel nomograms showed adequate performance in the prediction of HCC prognosis after hepatectomy, which may facilitate the risk stratification and individualized management of HCC patients.

Keywords: hepatocellular carcinoma, nomogram, gamma-glutamyl transferase, lymphocyte, hepatectomy

Introduction

Liver cancer ranks the sixth in the most prevalent cancers and is the third leading cause of cancer-related death around the world.¹ Hepatocellular carcinoma (HCC) represents the most common histological type, comprising 75–85% of liver cancer cases.^{1,2} As a leading high-risk area of HCC, China takes about half of the disease burden worldwide and Jiangsu, a province in Eastern China, is one of the regions with the highest incidence of HCC in China.³ To date, surgical

resection of HCC has become a safe treatment with a low mortality rate.⁴ Despite recent improvement of diagnosis and management of HCC, the prognosis after hepatectomy remains unsatisfactory due to high incidence of recurrence and metastasis.⁵

The Tumor-Node-Metastasis (TNM) staging and the Barcelona Clinic Liver Cancer (BCLC) classification are the most widely used systems for predicting the prognosis of HCC. However, the TNM system only depends on pathological factors and does not perform well in survival prediction.⁶ In tertiary centers, up to 50% of patients present deviations from BCLC therapeutic recommendations.⁷ For laboratory parameters, serum α -fetoprotein (AFP) is the most widely used and readily available method for the diagnosis and monitoring of HCC.⁸ Unfortunately, AFP is not an optimal prognostic indicator of HCC, especially for small tumors.⁹ Therefore, there is an urgent need for effective indicators of HCC prognosis.

Chronic inflammation is closely correlated with the occurrence and progression of HCC.¹⁰ HCC features dysregulated inflammatory mediators, aberrant immune response¹¹ and altered peripheral blood cell counts with prognostic significance.¹² Based on this background, multiple immune-inflammation-based prognostic indicators, developed with economical and non-invasive blood cell counts and biochemical parameters, have been proposed to predict the prognosis of HCC. As a classic indicator of liver inflammation, gamma-glutamyl transpeptidase (GGT) is induced during the development of multiple tumors and is correlated with poor survival.^{13,14} Lymphocytes play a central role in systemic and local immune-inflammatory response.¹⁵ Lymphocytopenia indicates a state of diminished immune function and may contribute to adverse survival of HCC patients.¹⁶ By combining GGT and lymphocyte count, GGT-to-lymphocyte ratio (GLR) is recently developed as an inflammation-based indicator with prognostic value in HCC patients and was validated in cohorts from Southern China.^{17,18} By combining multiple independent factors, nomograms could provide readily-to-use tools for risk estimation and decision-making with improved predictive performance. In this study, we developed and validated novel prognostic nomograms with good predictive efficacy by incorporating GLR and other clinically available objective clinicopathological characteristics in HCC patients receiving hepatectomy.

Materials and Methods

Study Population and Data Collection

The data of patients who underwent hepatectomy with curative intention at the Department of Hepatobiliary Surgery, Drum Tower Clinical College of Nanjing Medical University between July 2004 and August 2016 were retrieved from electronic medical record system and were analyzed retrospectively. To avoid potential bias brought by short follow-up period, patients who underwent surgery in a relatively early period (>5 years before the analysis) with complete follow-up data were included.^{19–21} Patients eligible for the following criteria were included in this study: (1) >18 years old; (2) with pathologically confirmed diagnosis of HCC; (3) underwent curative hepatectomy; (4) with complete laboratory, pathological and follow-up data. The exclusion criteria included (1) <18 years old; (2) cholangiocarcinoma, metastatic tumor and recurrent HCC; (3) resections with residual tumor; (4) incomplete laboratory, pathological or follow-up data; (5) perioperative mortality; (6) patients with diseases of blood, immune or lymphatic system; (7) evidence of infectious or inflammatory diseases. Data were collected by two independent investigators (MC and CY) and were cross-checked by the third investigator (ZG). The clinical parameters collected included general information (age, gender, history of diabetes and hypertension and history of tobacco or alcohol consumption), preoperative laboratory test results (blood cell counts, biochemical tests, hepatitis virus antigen and antibody tests and serum AFP levels), preoperative radiological and ultrasound data (location of tumor, size and number of tumor, signs of cirrhosis and ascites), operative information (portal occlusion, intraoperative bleeding and intraoperative blood transfusion) and histopathological data (tumor size and number, differentiation, vascular invasion, status of resection margin). The TNM stage was determined by the American Joint Committee on Cancer (AJCC) staging manual (8th edition). The BCLC stage was determined as previously described.²² The status of vascular invasion was subcategorized into microvascular invasion (MVI) and macrovascular invasion (MaVI). The definition of MVI was the presence of cancer cell nests with >50 cells in the endothelial vascular lumen under microscopy.²³ Tumor invading the main trunk or large branches of hepatic vein or portal vein was considered as macrovascular invasion. GLR was calculated as value of serum GGT (U/L)/lymphocyte

count ($10^9/L$).^{17,18} The study was approved by institutional ethics committee of the Drum Tower Clinical College of Nanjing Medical University and was in accordance with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards. The identities of patients included in this study were kept anonymous to the researchers by computer-generated ID numbers, and therefore consent from the patients was waived.

Follow-Up

Postoperative follow-up was carried out by regular examination in the outpatient clinic and was supplemented by telephone communication when needed. Blood cell count, biochemical test, serum AFP and abdominal ultrasonography or contrast-enhanced computed tomography were performed during the follow-up in outpatient clinic visits. The tests were performed monthly during the first three months after surgery and every three months in the first two years. After that, patients were followed up every six months during the third to fifth years. Afterwards, the follow-up was carried out annually. The diagnosis of recurrence was established based on imaging and laboratory tests and was confirmed pathologically when possible. Disease-free survival (DFS) was calculated as the time between surgery and the date of cancer recurrence, metastasis or the last follow-up, while overall survival was determined by the interval between surgery and death or the last follow-up.

Statistical Analysis

Statistical analyses were performed by SPSS v25.0 (IBM Corporation, Armonk, NY, USA) and R software v3.6.2 (The R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics were compared by chi square test or Fisher's exact test when applicable. Univariate analyses were performed by the Cox proportional hazard model. Factors with P value <0.1 were entered as candidate variables into multivariate Cox regression analyses to identify independent prognostic factors. Nomograms were formulated based on the results of the multivariate Cox proportional hazards regression analysis. Receiver operating characteristic (ROC) curves were generated, and areas under curve (AUCs) were calculated to evaluate the predictive characteristics of the model. The concordance indexes (C-indexes) were calculated to assess the accuracy of nomograms, and the calibration curves were used to determine the consistency of the models. Time-dependent ROC curves were drawn to compare the efficacy of different staging systems. The optimal cut-off value of the GLR was obtained by X-tile software v3.6.1 (Yale University School of Medicine, New Haven, CT, USA).²⁴ P-values <0.05 were considered statistically significant.

Results

Patient Characteristics

A total of 599 patients with pathologically diagnosed HCC were included in this study. The patients were randomly divided into training cohort ($n = 420$) and validation cohort ($n = 179$) by the ratio of 7:3. This ratio was repeatedly employed in the previous similar nomogram-related studies because this ratio provided enough sample size for both model establishment and validation. Also, the random selection method also avoided potential bias from dividing the groups by time or other factors.^{25–27} The baseline demographics, clinicopathological and operative characteristics of both the training and validation cohorts are shown in Table 1. According to the follow-up data, 384 (64.1%) of the patients developed recurrent HCC and 237 (39.6%) of them died of HCC. The DFS rate at 1-, 3- and 5-year, were 63.2%, 43.3% and 32.6%, respectively. The OS rate at 1-, 3- and 5-year were 85.2%, 74.0% and 54.5%, respectively. The optimal cut-off value of GLR with the best discriminative efficacy of survival was determined by X-tile software as 19.5 (Chi-square value = 23.39, $P < 0.0001$, Figure 1).

Identification of Prognostic Factors for DFS and OS

To identify the prognostic factors for DFS and OS, univariate and multivariate Cox proportional hazard regression analyses were performed. The results showed that elevated serum-positive HBeAg (HR 1.453, 95% CI 1.081–1.951, $P = 0.013$), AFP level (HR = 1.287, 95% CI 1.004–1.649, $P = 0.047$), larger tumor size (5–10 cm, HR = 1.690, 95% CI 1.119–2.552, $P = 0.013$; >10 cm, HR = 3.211, 95% CI 1.941–5.312, $P < 0.001$), multiple tumors (HR = 1.820, 95%

Table I Baseline Characteristics of Patients

Characteristic	Training Cohort	Validation Cohort	P value
	n = 420 (%)	n = 179 (%)	
Gender			
Male	339 (80.7)	150 (83.8)	0.372
Female	81 (19.3)	29 (16.2)	
Age			
≤50	138 (32.9)	61 (34.1)	0.771
>50	282 (67.1)	118 (65.9)	
Hypertension			
No	324 (77.1)	138 (77.1)	0.990
Yes	96 (22.9)	41 (22.9)	
Diabetes			
No	369 (87.9)	160 (89.4)	0.594
Yes	51 (12.1)	19 (10.6)	
Smoking			
No	350 (83.3)	141 (78.8)	0.184
Yes	70 (16.7)	38 (21.2)	
Alcohol consumption			
No	369 (87.9)	157 (87.7)	0.960
Yes	51 (12.1)	22 (12.3)	
HBsAg			
Negative	83 (19.8)	42 (23.5)	0.307
Positive	337 (80.2)	137 (76.5)	
HBeAg			
Negative	330 (78.6)	138 (77.1)	0.689
Positive	90 (21.4)	41 (22.9)	
HCVAb			
Negative	413 (98.3)	176 (98.3)	0.616
Positive	7 (1.7)	3 (1.7)	
AFP			
≤200 ng/mL	241 (57.4)	107 (59.8)	0.586
>200 ng/mL	179 (42.6)	72 (40.2)	
Portal occlusion			
No	138 (32.9)	49 (27.4)	0.185
Yes	282 (67.1)	130 (72.6)	
Blood loss			
≤500 mL	267 (63.6)	112 (62.6)	0.816
>500 mL	153 (36.4)	67 (37.4)	
Transfusion			
No	299 (71.2)	129 (72.1)	0.828
Yes	121 (28.8)	50 (27.9)	
Cirrhosis			
No	97 (23.1)	47 (26.3)	0.407
Yes	323 (76.9)	132 (73.7)	
Tumor size			
≤5 cm	76 (18.1)	42 (23.5)	0.230
5–10 cm	294 (70.0)	113 (63.1)	
>10 cm	50 (11.9)	24 (13.4)	
Tumor number			
Single	308 (73.3)	135 (75.4)	0.594
Multiple	112 (26.7)	44 (24.6)	

(Continued)

Table I (Continued).

Characteristic	Training Cohort	Validation Cohort	P value
	n = 420 (%)	n = 179 (%)	
MVI			
No	252 (60.0)	116 (64.8)	0.269
Yes	168 (40.0)	63 (35.2)	
MaVI			
No	349 (83.1)	151 (84.4)	0.703
Yes	71 (16.9)	28 (15.6)	
Differentiation			
Well	64 (15.2)	36 (20.1)	0.007
Moderate	216 (51.4)	106 (59.2)	
Poor	140 (33.3)	37 (20.7)	
Surgical margin			
>1 cm	246 (58.6)	116 (64.8)	0.153
≤1 cm	174 (41.4)	63 (35.2)	
GLR			
≤19.5	102 (24.3)	44 (24.6)	0.939
>19.5	318 (75.7)	135 (75.4)	
TNM stage			
I/II	277 (66.0)	123 (68.7)	0.408
III/IV	143 (34.0)	56 (31.3)	
BCLC stage			
0/A	268 (63.8)	120 (67.0)	0.747
B	81 (19.3)	31 (17.3)	
C	71 (16.9)	28 (15.7)	

CI 1.389–2.384, $P < 0.001$), the presence of MVI (HR = 1.506, 95% CI 1.169–1.939, $P = 0.002$), tumor differentiation (moderate differentiation, HR = 1.958, 95% CI 1.293–2.968, $P = 0.002$; poor differentiation, HR = 2.562, 95% CI 1.670–3.930, $P < 0.001$) and high GLR (GLR >19.5, HR = 1.560, 95% CI 1.118–2.177, $P = 0.009$) were independent prognostic factors for DFS (Table 2).

For OS, the independent prognostic factors (Table 3) included elevated AFP (HR = 1.711, 95% CI 1.225–2.390, $P = 0.002$), larger tumor size (>10 cm, HR = 2.271, 95% CI 1.180–4.373, $P = 0.014$), multiple tumors (HR = 1.976, 95% CI 1.420–2.749, $P < 0.001$), the presence of MVI (HR = 1.587, 95% CI 1.146–2.197, $P = 0.005$), MaVI

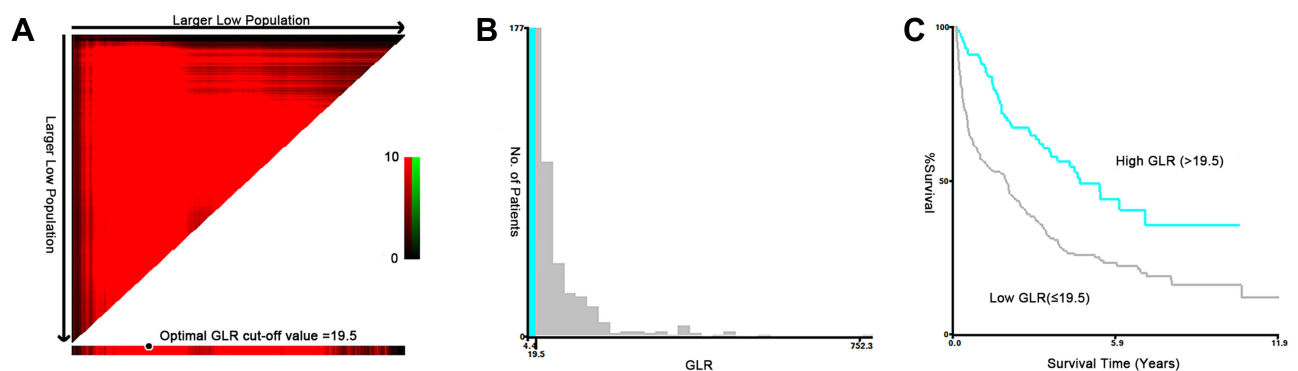


Figure 1 Determination of the optimal cut-off value of GLR by X-tile analysis. **(A)** X-tile plot generated by GLR and survival data of the patients. The black point on the horizontal bar highlighted the optimal outcome-based cut-off value. **(B)** The histogram of the cohort. The cohort was divided into two groups based on the GLR cut-off value. **(C)** Kaplan–Meier curve displayed the difference of survival between high GLR and low GLR groups.

Abbreviation: GLR, gamma-glutamyl transpeptidase-to-lymphocyte ratio.

Table 2 Univariate and Multivariate Cox Proportional Hazard Regression Analyses of DFS

Characteristics	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	P value	HR	95% CI	P value
Gender (Male vs Female)	0.871	0.615–1.234	0.436			
Age (>50 vs ≤50)	0.939	0.700–1.260	0.675			
Hypertension (Yes vs No)	0.990	0.711–1.377	0.950			
Diabetes (Yes vs No)	1.245	0.840–1.845	0.275			
Smoking (Yes vs No)	0.913	0.628–1.327	0.632			
Alcohol consumption (Yes vs No)	1.351	0.866–2.108	0.184			
HBsAg (Positive vs Negative)	0.831	0.585–1.179	0.299			
HBeAg (Positive vs Negative)	1.505	1.094–2.072	0.012	1.453	1.081–1.951	0.013
HCVAb (Positive vs Negative)	1.425	0.578–3.513	0.441			
AFP, ng/mL (>200 vs ≤200)	1.306	0.994–1.716	0.055	1.287	1.004–1.649	0.047
Portal occlusion (Yes vs No)	1.007	0.763–1.328	0.962			
Blood loss, mL (>500 vs ≤500)	0.972	0.780–1.332	0.860			
Transfusion (Yes vs No)	1.107	0.797–1.537	0.545			
Cirrhosis (Yes vs No)	1.405	1.010–1.953	0.044	1.367	0.998–1.874	0.052
Tumor size, cm						
≤5 cm	Reference			Reference		
5–10 cm	1.676	1.099–2.555	0.016	1.690	1.119–2.552	0.013
>10 cm	3.295	1.937–5.607	<0.001	3.211	1.941–5.312	<0.001
Tumor number (Multiple vs Single)	1.765	1.339–2.326	<0.001	1.820	1.389–2.384	<0.001
MVI (Yes vs No)	1.446	1.110–1.883	0.006	1.506	1.169–1.939	0.002
MaVI (Yes vs No)	1.306	0.935–1.824	0.118			
Differentiation						
Well	Reference			Reference		
Moderate	1.899	1.245–2.897	0.003	1.958	1.293–2.968	0.002
Poor	2.555	1.650–3.957	<0.001	2.562	1.670–3.930	<0.001
Surgical margin, cm (≤1 vs >1)	1.245	0.963–1.612	0.094	1.231	0.962–1.583	0.098
GLR (>19.5 vs ≤19.5)	1.477	1.043–2.093	0.028	1.560	1.118–2.177	0.009

(HR = 1.775, 95% CI 1.224–2.575, $P = 0.003$), tumor differentiation (moderate differentiation, HR = 2.662, 95% CI 1.406–5.043, $P = 0.003$; poor differentiation, HR = 3.299, 95% CI 1.722–6.320, $P < 0.001$) and high GLR (GLR >19.5, HR = 2.012, 95% CI 1.246–3.248, $P = 0.004$).

Development and Validation of Nomograms for the Prediction of DFS and OS

Based on the results of Cox regression analyses, nomograms were developed to predict the probabilities of 1-, 3- and 5-year DFS (Figure 2A) and OS (Figure 2B). The risk points of each risk factor could be generated by drawing an upward line to the point axis. The probability of survival could be read by the corresponding position of the accumulated points on the total point axis.

To evaluate the performance of the nomograms, the ROC curves were generated and the areas under curve (AUCs) were calculated. As indicated in Figure 3A and B, the novel nomograms showed good predictive value on both DFS and OS. The AUCs for 1-, 3- and 5-year DFS prediction were 0.758, 0.756 and 0.734, respectively. For the prediction of 1-, 3- and 5-year OS prediction, the AUCs were 0.810, 0.799, and 0.758, respectively. To assess the predictive accuracy and consistency of the nomograms, C-indexes were calculated and calibration curves were generated. The C-index was 0.697 (95% CI 0.665–0.729) for DFS prediction while 0.741 (95% CI 0.704–0.778) for OS prediction. The calibration curves for both DFS and OS (Figure 3C and D) showed good agreement between nomogram predictions and the actual observation results.

The developed novel nomograms were further validated by an internal validation cohort. The ROC curves in the validation cohort are shown in Figure 4A and B. The AUCs were 0.784, 0.766 and 0.755 for 1-, 3- and 5-year DFS

Table 3 Univariate and Multivariate Cox Proportional Hazard Regression Analyses of OS

Characteristics	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	P value	HR	95% CI	P value
Gender (Male vs Female)	0.971	0.619–1.521	0.896	1.297	0.913–1.843	0.146
Age (>50 vs ≤50)	1.433	0.970–2.116	0.071			
Hypertension (Yes vs No)	0.947	0.615–1.460	0.806			
Diabetes (Yes vs No)	1.204	0.735–1.973	0.461			
Smoking (Yes vs No)	1.035	0.644–1.663	0.887			
Alcohol consumption (Yes vs No)	1.272	0.700–2.309	0.43			
HBsAg (Positive vs Negative)	0.874	0.563–1.356	0.548			
HBeAg (Positive vs Negative)	1.375	0.908–2.082	0.133			
HCVAb (Positive vs Negative)	2.499	0.934–6.689	0.068			
AFP, ng/mL (>200 vs ≤200)	1.821	1.277–2.598	0.001			
Portal occlusion (Yes vs No)	0.881	0.628–1.240	0.468			
Blood loss, mL (>500 vs ≤500)	1.363	0.903–2.059	0.141			
Transfusion (Yes vs No)	1.114	0.732–1.693	0.615			
Cirrhosis (Yes vs No)	1.275	0.821–1.978	0.279			
Tumor size, cm				Reference	Reference	Reference
≤5 cm	Reference					
5–10 cm	1.368	0.780–2.397	0.274			
>10 cm	2.07	1.054–4.061	0.034			
Tumor number (Multiple vs Single)	1.834	1.302–2.584	0.001			
MVI (Yes vs No)	1.572	1.120–2.207	0.009			
MaVI (Yes vs No)	1.593	1.074–2.364	0.021			
Differentiation						
Well	Reference					
Moderate	2.529	1.320–4.845	0.005			
Poor	3.204	1.648–6.230	0.001			
Surgical margin, cm (≤1 vs >1)	1.154	0.827–1.609	0.399			
GLR (>19.5 vs ≤19.5)	1.826	1.111–3.000	0.018			

prediction and 0.810, 0.805 and 0.810 for 1-, 3- and 5-year OS prediction, respectively, which were in consistent with the results of the training cohort. The C-indexes of the nomograms for predicting DFS and OS were 0.710 (95% CI 0.664–0.756) and 0.758 (95% CI 0.705–0.811). Similar to the training cohort, the calibration curves plotted in the validation cohort also revealed a good consistency between nomogram predictions and actual observations (Figure 4C and D).

Comparison of Predictive Performance Between the Nomograms and Conventional Staging Systems

The predictive value of the novel nomograms was compared with conventional staging systems including the TNM staging system (AJCC 8th edition) and the BCLC staging system. The comparison of C-indexes demonstrated that our current nomograms were superior to the conventional staging systems. Specifically, the C-indexes of the TNM staging and the BCLC staging for DFS prediction were 0.629 (95% CI 0.586–0.672) and 0.641 (95% CI 0.596–0.686), both of which were significantly smaller than the C-index of the nomogram. Similarly, the C-index of the nomogram for OS prediction also showed advantage over C-indexes of the TNM (0.650, 95% CI 0.595–0.705) and the BCLC staging (0.675, 95% CI 0.618–0.732).

Time-dependent ROC curves of the nomograms, the TNM and the BCLC staging system were generated for the comparison of prognostic values. As shown in Figure 5A and B, the time-dependent AUCs of the nomogram showed consistent superiority over both the TNM and the BCLC staging systems throughout the observation period, indicating the adequate discriminative efficacy and satisfactory stability of the novel nomograms for the prediction of both DFS and OS in HCC patients.

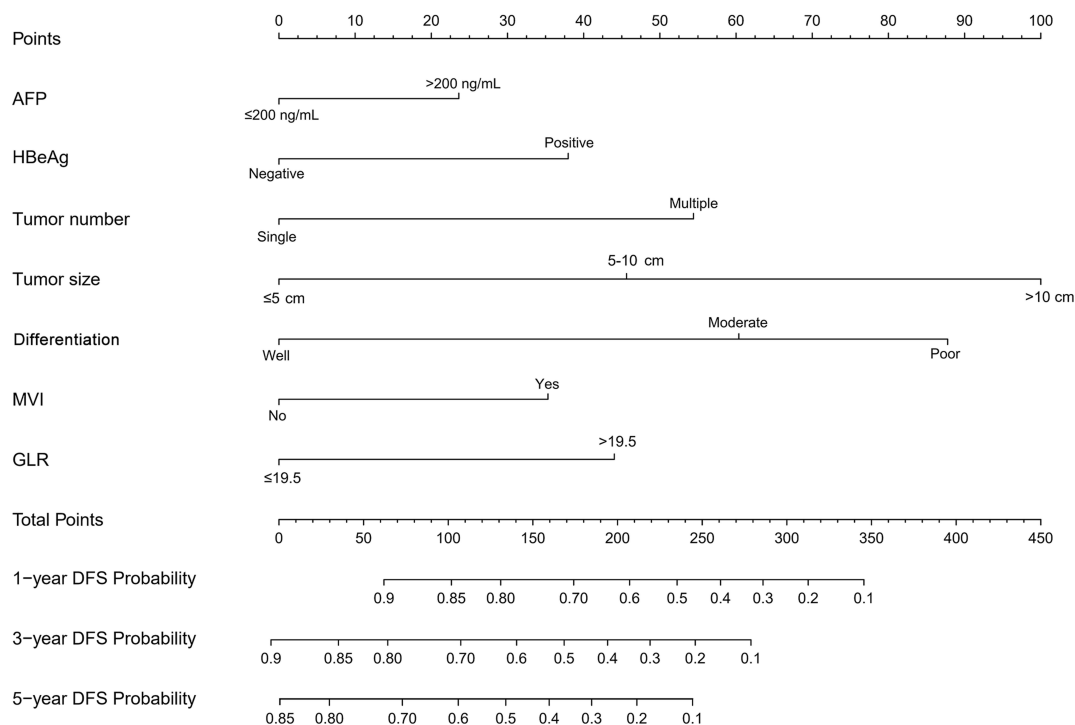
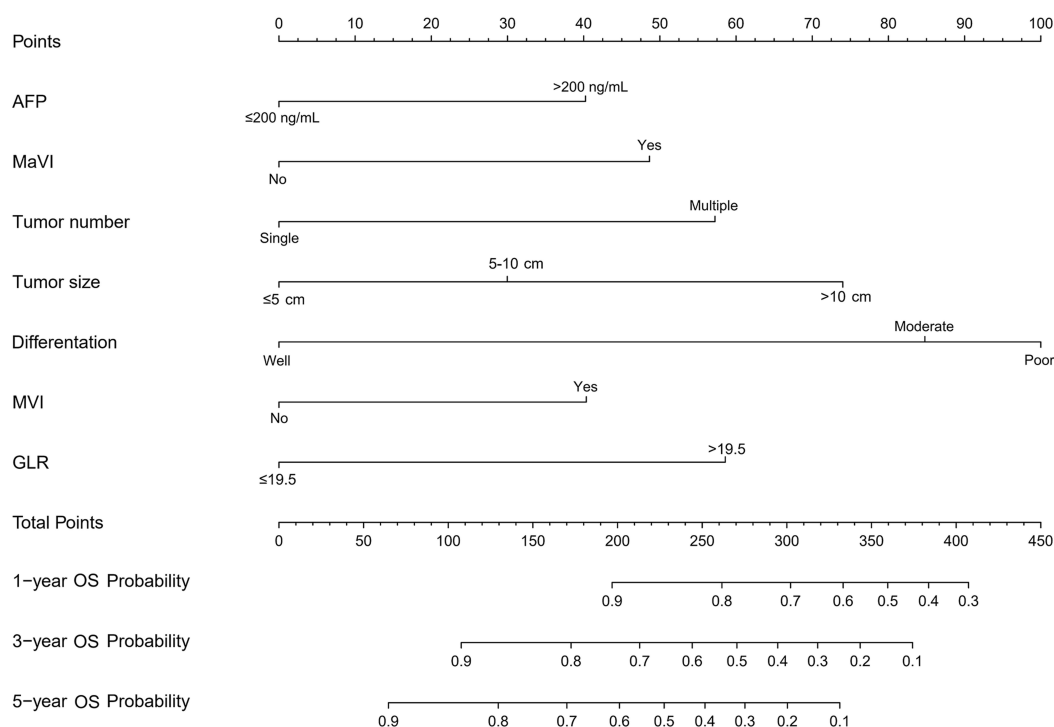
A**B**

Figure 2 Nomograms for the prediction of 1-, 3- and 5-year DFS and OS. Each independent prognostic factor identified in the Cox regression was assigned a point. The total points could be obtained by calculating the sum of all factors. With the total points, the probabilities of 1-, 3- and 5-year DFS (**A**) and OS (**B**) could be predicted. **Abbreviations:** AFP, serum α -fetoprotein; HBeAg, Hepatitis Be Antigen; MVI, microvascular invasion; MaVI, macrovascular invasion; GLR, gamma-glutamyl transpeptidase-to-lymphocyte ratio; DFS, disease-free survival; OS, overall survival.

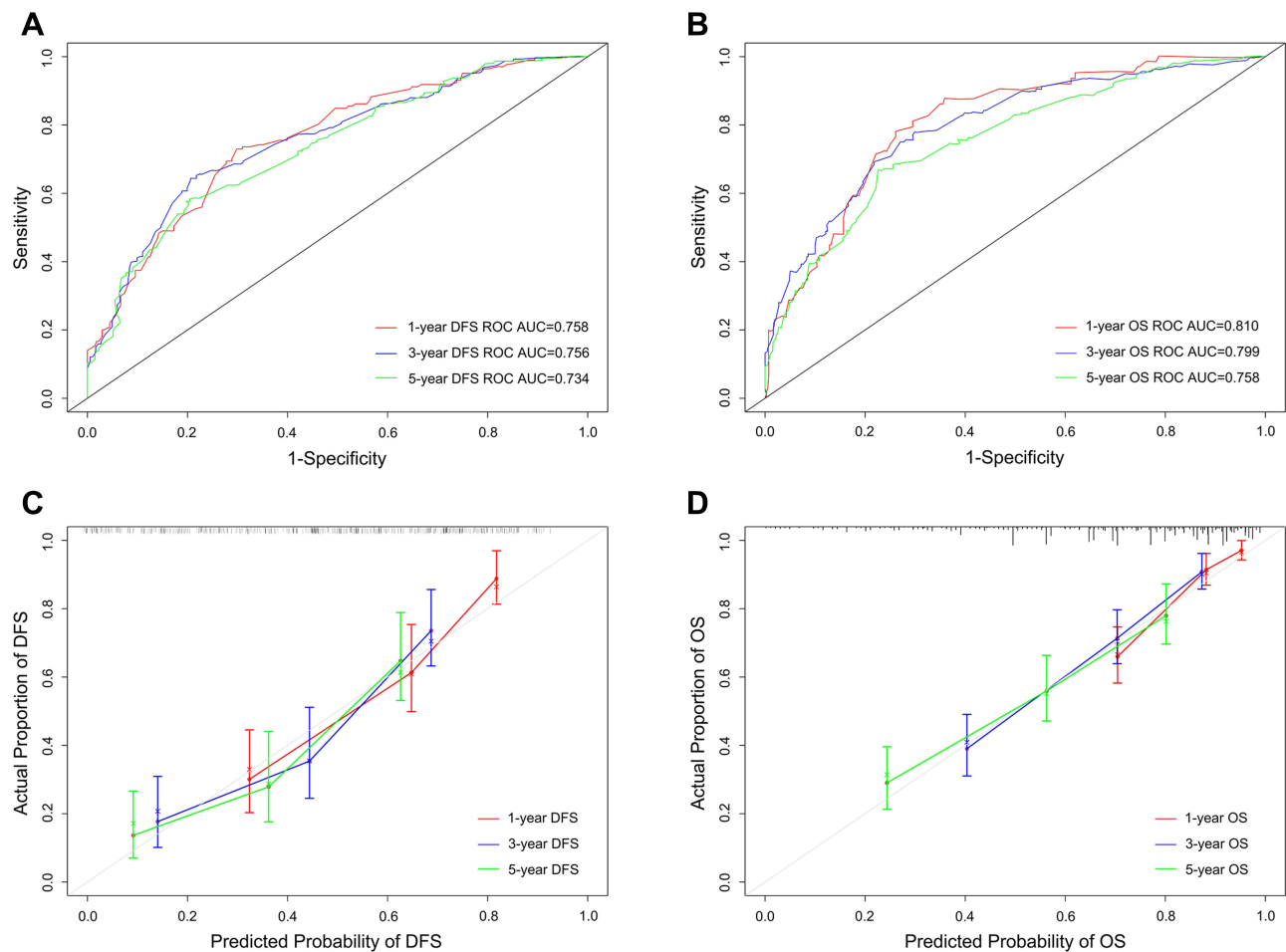


Figure 3 Evaluation of the performance of the nomograms in the training cohort. (A) ROC curves of the nomogram predicting 1-, 3- and 5-year DFS in the training cohort. (B) ROC curves of the nomogram predicting 1-, 3- and 5-year OS in the training cohort. (C) Calibration curves of the nomogram predicting 1-, 3- and 5-year DFS in the training cohort. (D) Calibration curves of the nomogram predicting 1-, 3- and 5-year OS in the training cohort.

Abbreviations: DFS, disease-free survival; OS, overall survival; ROC, receiver operating characteristic; AUCs, the areas under curve.

Discussion

For resectable HCC, hepatectomy remains the best treatment option. However, high incidence of postoperative recurrence limited the prognosis after surgery.² It is indicated that up to 70% of HCC patients developed recurrent disease after resection and only about 50–60% of them survived longer than 5 years.^{2,28,29} In this study, 67.4% of the patients suffered from recurrence of HCC and about a half of them died in 5 years, which is similar with the reported data. Therefore, the prediction and stratification of postoperative prognosis are of great significance so that early intervention for high-risk patients could be applied. Currently, the 8th edition of AJCC-TNM and the BCLC systems are the most widely used staging tools for HCC. The TNM staging system relies solely on tumor pathological factors, which significantly limited its prognostic value. As another widely used system, the BCLC staging incorporated tumor burden, liver function and performance status to stratify HCC patients and to guide clinical management.²² Unfortunately, the performance of BCLC system in prognosis stratification is also unsatisfactory and deviations from recommended treatment are common.^{7,30–32} Therefore, novel tools with improved performance in the prediction of HCC prognosis are urgently needed.

Nomograms provide illustrated and readily-to-use tools for personalized risk estimation and decision-making through integrating multiple prognostic factors. Recently, numerous nomograms with promising predictive values are developed in various cancers, including HCC.^{33,34} In this study, we established prognostic nomograms based on a novel immune-inflammation-based indicator GLR with good performance in the prediction of prognosis in HCC patients receiving hepatectomy. The novel nomograms were developed based on seven independent risk factors identified in multivariate

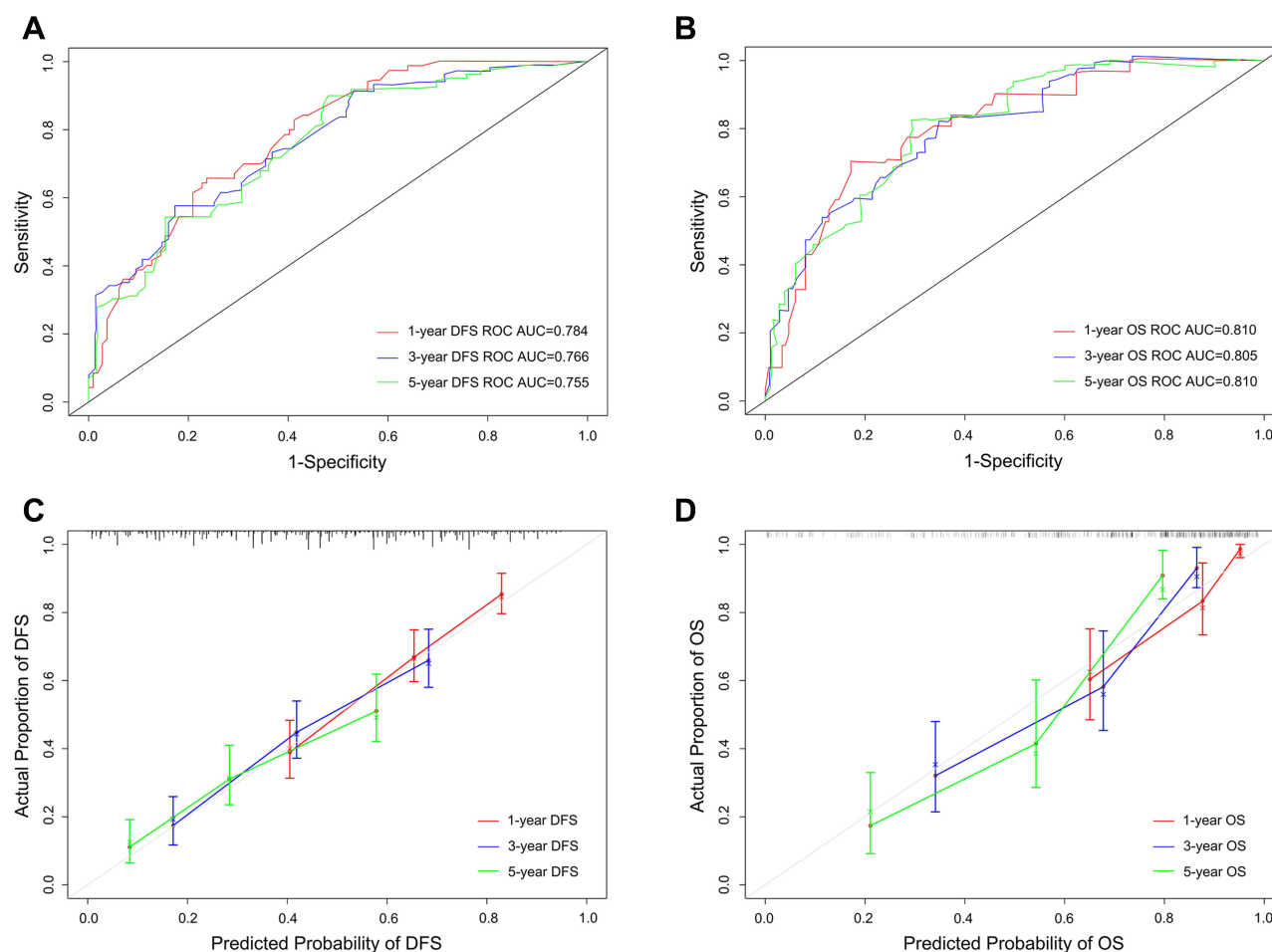


Figure 4 Performance of the nomograms in the validation cohort. **(A)** ROC curves of the nomogram predicting 1-, 3- and 5-year DFS in the validation cohort. **(B)** ROC curves of the nomogram predicting 1-, 3- and 5-year OS in the validation cohort. **(C)** Calibration curves of the nomogram predicting 1-, 3- and 5-year DFS in the validation cohort. **(D)** Calibration curves of the nomogram predicting 1-, 3- and 5-year OS in the validation cohort.

Abbreviations: DFS, disease-free survival; OS, overall survival; ROC, receiver operating characteristic; AUCs, the areas under curve.

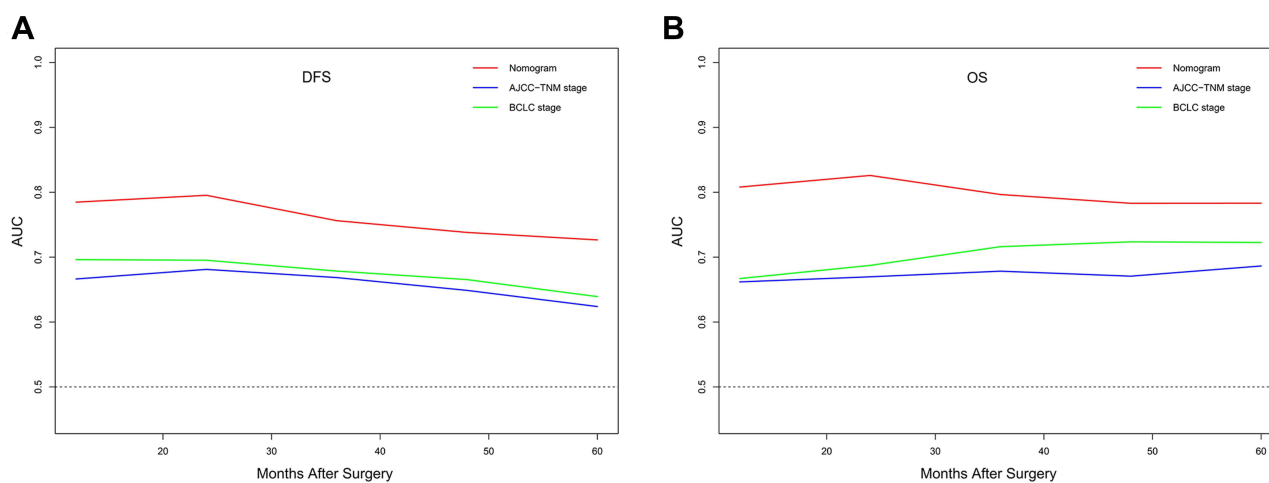


Figure 5 Time-dependent ROC curves of the nomograms, the TNM and the BCLC staging systems. **(A)** Time-dependent ROC curves of DFS predicting. **(B)** Time-dependent ROC curves of OS predicting.

Abbreviations: DFS, disease-free survival; OS, overall survival; AUCs, the areas under curve; AJCC, American Joint Committee on Cancer; TNM, tumor node metastasis; BCLC, Barcelona Clinic Liver Cancer.

Cox regression analyses for DFS and OS, respectively. The combination of cancer-related biological markers with tumor characteristics is a practical strategy to improve prognosis prediction efficacy. Our nomograms incorporated well-established tumor characteristics with prognostic significance including multiple tumors, tumor size, tumor differentiation, MVI and MaVI with biological markers including AFP, HBeAg and GLR.^{35–38}

To date, AFP remains the most widely used serum tumor marker for the diagnosis, surveillance and prognosis prediction of HCC.³⁹ The level of AFP may reflect the growth, differentiation, invasion and metastasis of HCC and could serve as an indicator of prognosis.^{40,41} In this study, as expected, elevated AFP level was an independent risk factor for both DFS and OS and contributed to the construction of nomograms. Considering the fact that most Chinese HCC patients have viral hepatitis background,³ markers of virus were also included in the analyses. Our study showed that positive HBeAg was an independent risk factor for DFS, which is consistent with previous reports.^{42–44} This result further emphasized the importance of antiviral therapy since HBeAg was demonstrated to have a negative impact on prognosis and seroconversion of HBeAg may be beneficial for reducing recurrence of HCC.⁴³

Dysregulation of inflammation and immune reaction are considered as the hallmarks of cancer.⁴⁵ For HCC, the disturbance of inflammation and immune response plays an especially critical role since it underlies both the tumor-promoting environment and the pathogenesis of background liver diseases. This led to the proposal of multiple immune-inflammation-based prognostic markers including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), gamma-glutamyl transpeptidase-to-platelet ratio (GPR), etc.^{46–48} Derived from economical and readily available clinical blood and biochemical tests, these indicators provided useful tools for clinicians to predict the prognosis of HCC. Among the source parameters to derive the prognostic markers, GGT is a classic enzymatic marker of hepatic inflammation and is routinely tested for liver function evaluation. Elevation of GGT level reflects inflammation in liver microenvironment, which may contribute to the development and recurrence of HCC.⁴⁹ On the other hand, GGT expression could also facilitate tumor formation, progression, metastasis and drug resistance through multiple mechanisms.^{50–52} Importantly, multiple studies revealed that elevated GGT correlated with poor prognosis of HCC, supporting the value of GGT as a prognostic indicator.^{52–55} Lymphocyte plays an essential and central role in systemic and local immune-inflammatory reaction as well as anti-tumor response.^{15,56} The level of tumor infiltrating lymphocyte correlated with the survival of HCC,⁵⁷ while lymphocytopenia predicted poor prognosis in HCC patients.^{16,58} By combining GGT and lymphocyte, GLR was recently proposed to be a novel promising inflammation-based prognostic indicator in HCC.^{17,18} In our HCC cohort from Eastern China, the optimal cut-off value of GLR was determined as 19.5 based on the outcome-based analysis by X-tile software. GLR exhibited adequate prognostic value in the following univariate and multivariate Cox regression and was incorporated in our nomograms as an independent predictive factor. As shown in the nomograms, GLR contributed significantly to the performance of the model with similar or even stronger power as the well-established risk factors including AFP, HBeAg, multiple tumors, MVI and MaVI.

The current nomograms demonstrated good predictive efficacy as indicated by ROC curves, C-indexes and calibration curves. The performance remained stable in an internal validation cohort. The nomograms also exhibited superior prognosis predictive ability to conventional tools including the TNM and the BCLC staging systems. These novel nomograms integrating tumor characteristics, viral and tumor biomarkers and immune-inflammation-based indicators, may contribute to the risk stratification after surgery and facilitate the individualized interventions in high-risk patients. Active interventions could include intensive postoperative surveillance; antiviral therapy for viral hepatitis;⁴³ adjuvant therapies for patients with tumor-related risk factors such as multiple tumors, large tumor size, poor differentiation and vascular invasions;⁵⁹ anti-inflammation and immune-regulatory interventions,^{60,61} which might improve the prognosis of HCC after curative hepatectomy.

Nevertheless, limitations of this study should be noted. Although the nomograms were validated with an internal cohort and displayed stable performance, the results generated from our single-center data need further external or multicenter verification. The nomograms have been developed in a selected patient group of HCC and predict survivals of the patients treated by only surgical resection, so it is not applicable for all HCC patients. Due to the retrospective nature of the current study, future prospective investigations are also required to verify the predictive performance of the nomograms.

Conclusion

In this study, we developed novel nomograms incorporating tumor characteristics, viral and tumor biomarkers and immune-inflammation-based prognostic indicator GLR. The nomograms demonstrated adequate performance in the prediction of prognosis of HCC patients after curative hepatectomy and showed advantage over the traditional TNM and BCLC staging systems. These easy-accessible tools may contribute to the risk stratification after surgery and might facilitate the individualized interventions in high-risk patients.

Data Sharing Statement

The raw data supporting the conclusions of this article will be made available by the author Cheng Ma (Email: xzmacheng@163.com) upon reasonable request.

Ethics Statement

The study was approved by the institutional ethics committee of the Drum Tower Clinical College of Nanjing Medical University and was in accordance with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards. The identities of patients included in this study were kept anonymous to the researchers by computer-generated ID numbers, and therefore consent from the patients was waived.

Acknowledgments

The authors thank Dr. Weibo Chen for technical assistance in this study.

Funding

This research was supported by Research Project of Jinan Microecological Biomedicine Shandong Laboratory (JNL202204A, JNL202219B); the National Natural Science Foundation of China (No. 81572393 and 81602093); the Natural Science Foundation of Jiangsu Province (No. BK20160118); the Key Project supported by the Medical Science and Technology Development Foundation, Nanjing Municipality Health Bureau (No. ZKX15020 and ZKX17022); the Fundamental Research Funds for the Central Universities (No. 021414380215, 021414380242 and 021414380258) and the Chen Xiao-Ping Foundation for the Development of Science and Technology of Hubei Province (No. CXPJJH12000001-2020318).

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209–249. doi:10.3322/caac.21660
2. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet.* 2018;391(10127):1301–1314. doi:10.1016/S0140-6736(18)30010-2
3. Zhou J, Sun H, Wang Z, et al. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (2019 edition). *Liver Cancer.* 2020;9(6):682–720. doi:10.1159/000509424
4. Fan ST, Lo CM, Liu CL, et al. Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg.* 1999;229(3):322–330. doi:10.1097/00000658-199903000-00004
5. Shah SA, Cleary SP, Wei AC, et al. Recurrence after liver resection for hepatocellular carcinoma: risk factors, treatment, and outcomes. *Surgery.* 2007;141(3):330–339. doi:10.1016/j.surg.2006.06.028
6. Chen ZH, Hong YF, Lin J, et al. Validation and ranking of seven staging systems of hepatocellular carcinoma. *Oncol Lett.* 2017;14(1):705–714. doi:10.3892/ol.2017.6222
7. Golfieri R, Bargellini I, Spreafico C, Trevisani F. Patients with Barcelona Clinic Liver Cancer stages B and C hepatocellular carcinoma: time for a subclassification. *Liver Cancer.* 2019;8(2):78–91. doi:10.1159/000489791
8. Lim J, Singal AG. Surveillance and diagnosis of hepatocellular carcinoma. *Clin Liver Dis.* 2019;13(1):2–5. doi:10.1002/cld.761
9. Giannini EG, Marengo S, Borgonovo G, et al. Alpha-fetoprotein has no prognostic role in small hepatocellular carcinoma identified during surveillance in compensated cirrhosis. *Hepatology.* 2012;56(4):1371–1379. doi:10.1002/hep.25814
10. Cheng Y, Ma XL, Wei YQ, Wei XW. Potential roles and targeted therapy of the CXCLs/CXCR2 axis in cancer and inflammatory diseases. *Biochim Biophys Acta Rev Cancer.* 2019;1871(2):289–312. doi:10.1016/j.bbcan.2019.01.005

11. Sun B, Karin M. Inflammation and liver tumorigenesis. *Front Med*. 2013;7(2):242–254. doi:10.1007/s11684-013-0256-4
12. Hong YM, Yoon KT, Hwang TH, Cho M. Pretreatment peripheral neutrophils, lymphocytes and monocytes predict long-term survival in hepatocellular carcinoma. *BMC Cancer*. 2020;20(1):937. doi:10.1186/s12885-020-07105-8
13. Milito A, Brancaccio M, Lisurek M, Masullo M, Palumbo A, Castellano I. Probing the interactions of sulfur-containing histidine compounds with human gamma-glutamyl transpeptidase. *Mar Drugs*. 2019;17(12):650. doi:10.3390/md17120650
14. Jarcuska P, Janicko M, Drazilova S, et al. Gamma-glutamyl transpeptidase level associated with metabolic syndrome and proinflammatory parameters in the young Roma population in eastern Slovakia: a population-based study. *Cent Eur J Public Health*. 2014;22(Suppl):S43–S50. doi:10.21101/cejph.a3901
15. Salman T, Kazaz SN, Varol U, et al. Prognostic value of the pretreatment neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio for patients with neuroendocrine tumors: an Izmir oncology group study. *Chemotherapy*. 2016;61(6):281–286. doi:10.1159/000445045
16. Mossanen JC, Tacke F. Role of lymphocytes in liver cancer. *Oncoimmunology*. 2013;2(11):e26468. doi:10.4161/onci.26468
17. Li S, Xu W, Liao M, et al. The significance of gamma-glutamyl transpeptidase to lymphocyte count ratio in the early postoperative recurrence monitoring and prognosis prediction of AFP-negative hepatocellular carcinoma. *J Hepatocell Carcinoma*. 2021;8:23–33. doi:10.2147/JHC.S286213
18. Liao M, Qin W, Liao Y, Yao R, Yu J, Liao W. Prognostic value of gamma-glutamyl transpeptidase to lymphocyte count ratio in patients with single tumor size ≤ 5 cm hepatocellular carcinoma after radical resection. *Front Oncol*. 2019;9:347. doi:10.3389/fonc.2019.00347
19. Agopian VG, Harlander-Locke M, Zarrinpar A, et al. A novel prognostic nomogram accurately predicts hepatocellular carcinoma recurrence after liver transplantation: analysis of 865 consecutive liver transplant recipients. *J Am Coll Surg*. 2015;220(4):416–427. doi:10.1016/j.jamcollsurg.2014.12.025
20. Ma L, Deng K, Zhang C, et al. Nomograms for predicting hepatocellular carcinoma recurrence and overall postoperative patient survival. *Front Oncol*. 2022;12:843589. doi:10.3389/fonc.2022.843589
21. Qin YL, Wang S, Chen F, et al. Prediction of outcomes by diffusion kurtosis imaging in patients with large (≥ 5 cm) hepatocellular carcinoma after liver resection: a retrospective study. *Front Oncol*. 2022;12:939358. doi:10.3389/fonc.2022.939358
22. Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis*. 2010;30(1):61–74. doi:10.1055/s-0030-1247133
23. Cong WM, Bu H, Chen J, et al. Practice guidelines for the pathological diagnosis of primary liver cancer: 2015 update. *World J Gastroenterol*. 2016;22(42):9279–9287. doi:10.3748/wjg.v22.i42.9279
24. Camp RL, Dolled-Fillhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res*. 2004;10(21):7252–7259. doi:10.1158/1078-0432.CCR-04-0713
25. Jia Z, Yan Y, Wang J, et al. Development and validation of prognostic nomogram in ependymoma: a retrospective analysis of the SEER database. *Cancer Med*. 2021;10(17):6140–6148. doi:10.1002/cam4.4151
26. Tang X, Zhou X, Li Y, et al. A novel nomogram and risk classification system predicting the cancer-specific survival of patients with initially diagnosed metastatic esophageal cancer: a SEER-based study. *Ann Surg Oncol*. 2019;26(2):321–328. doi:10.1245/s10434-018-6929-0
27. Yu Y, Tan Y, Xie C, et al. Development and validation of a preoperative magnetic resonance imaging radiomics-based signature to predict axillary lymph node metastasis and disease-free survival in patients with early-stage breast cancer. *JAMA Netw Open*. 2020;3(12):e2028086. doi:10.1001/jamanetworkopen.2020.28086
28. Ishizawa T, Hasegawa K, Aoki T, et al. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology*. 2008;134(7):1908–1916. doi:10.1053/j.gastro.2008.02.091
29. Franssen B, Alshebeeb K, Tabrizian P, et al. Differences in surgical outcomes between hepatitis B- and hepatitis C-related hepatocellular carcinoma: a retrospective analysis of a single North American center. *Ann Surg*. 2014;260(4):650–6;discussion 656–8. doi:10.1097/SLA.0000000000000917
30. Huitzil-Melendez FD, Capanu M, O'Reilly EM, et al. Advanced hepatocellular carcinoma: which staging systems best predict prognosis? *J Clin Oncol*. 2010;28(17):2889–2895. doi:10.1200/JCO.2009.25.9895
31. Chun YH, Kim SU, Park JY, et al. Prognostic value of the 7th edition of the AJCC staging system as a clinical staging system in patients with hepatocellular carcinoma. *Eur J Cancer*. 2011;47(17):2568–2575. doi:10.1016/j.ejca.2011.07.002
32. Satala CB, Jung I, Kobori L, et al. Benefits of the 8th American joint committee on cancer system for hepatocellular carcinoma staging. *J Gastrointest Cancer*. 2021;52(1):243–248. doi:10.1007/s12029-020-00394-z
33. Fu YP, Yi Y, Huang JL, et al. Prognostic nomograms stratify survival of patients with hepatocellular carcinoma without portal vein tumor thrombosis after curative resection. *Oncologist*. 2017;22(5):561–569. doi:10.1634/theoncologist.2016-0231
34. Peng J, Zhang J, Zhang Q, Xu Y, Zhou J, Liu L. A radiomics nomogram for preoperative prediction of microvascular invasion risk in hepatitis B virus-related hepatocellular carcinoma. *Diagn Interv Radiol*. 2018;24(3):121–127. doi:10.5152/dir.2018.17467
35. Erstad DJ, Tanabe KK. Prognostic and therapeutic implications of microvascular invasion in hepatocellular carcinoma. *Ann Surg Oncol*. 2019;26(5):1474–1493. doi:10.1245/s10434-019-07227-9
36. Fukami Y, Kaneoka Y, Maeda A, et al. Liver resection for multiple hepatocellular carcinomas: a Japanese nationwide survey. *Ann Surg*. 2020;272(1):145–154. doi:10.1097/SLA.0000000000003192
37. Huang WJ, Jeng YM, Lai HS, Sheu FY, Lai PL, Yuan RH. Tumor size is a major determinant of prognosis of resected stage I hepatocellular carcinoma. *Langenbecks Arch Surg*. 2015;400(6):725–734. doi:10.1007/s00423-015-1329-4
38. Lei Z, Li J, Wu D, et al. Nomogram for preoperative estimation of microvascular invasion risk in hepatitis B virus-related hepatocellular carcinoma within the Milan criteria. *JAMA Surg*. 2016;151(4):356–363. doi:10.1001/jamasurg.2015.4257
39. Song P, Tobe RG, Inagaki Y, et al. The management of hepatocellular carcinoma around the world: a comparison of guidelines from 2001 to 2011. *Liver Int*. 2012;32(7):1053–1063. doi:10.1111/j.1478-3231.2012.02792.x
40. Ma WJ, Wang HY, Teng LS. Correlation analysis of preoperative serum alpha-fetoprotein (AFP) level and prognosis of hepatocellular carcinoma (HCC) after hepatectomy. *World J Surg Oncol*. 2013;11:212. doi:10.1186/1477-7819-11-212
41. Kang SH, Kim DY, Jeon SM, et al. Clinical characteristics and prognosis of hepatocellular carcinoma with different sets of serum AFP and PIVKA-II levels. *Eur J Gastroenterol Hepatol*. 2012;24(7):849–856. doi:10.1097/MEG.0b013e3283535c34
42. Liu Y, Wang ZX, Cao Y, Zhang G, Chen WB, Jiang CP. Preoperative inflammation-based markers predict early and late recurrence of hepatocellular carcinoma after curative hepatectomy. *Hepatobiliary Pancreat Dis Int*. 2016;15(3):266–274. doi:10.1016/S1499-3872(16)60094-2

43. Shen J, Liu J, Li C, Wen T, Yan L, Yang J. The prognostic significance of serum HBeAg on the recurrence and long-term survival after hepatectomy for hepatocellular carcinoma: a propensity score matching analysis. *J Viral Hepat.* **2018**;25(9):1057–1065. doi:10.1111/jvh.12911
44. Kim SH, Choi SB, Lee JG, et al. Prognostic factors and 10-year survival in patients with hepatocellular carcinoma after curative hepatectomy. *J Gastrointest Surg.* **2011**;15(4):598–607. doi:10.1007/s11605-011-1452-7
45. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* **2011**;144(5):646–674. doi:10.1016/j.cell.2011.02.013
46. Wang D, Bai N, Hu X, et al. Preoperative inflammatory markers of NLR and PLR as indicators of poor prognosis in resectable HCC. *PeerJ.* **2019**;7:e7132. doi:10.7717/peerj.7132
47. Hu B, Yang XR, Xu Y, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. *Clin Cancer Res.* **2014**;20(23):6212–6222. doi:10.1158/1078-0432.CCR-14-0442
48. Luo D, Li H, Hu J, et al. Development and validation of nomograms based on gamma-glutamyl transpeptidase to platelet ratio for hepatocellular carcinoma patients reveal novel prognostic value and the ratio is negatively correlated with P38MAPK expression. *Front Oncol.* **2020**;10:548744. doi:10.3389/fonc.2020.548744
49. Tang ZY. Liver transplantation: a simple inflammation marker predicts liver cancer prognosis. *Nat Rev Gastroenterol Hepatol.* **2011**;8(7):367–368. doi:10.1038/nrgastro.2011.105
50. Hanigan MH. Gamma-glutamyl transpeptidase: redox regulation and drug resistance. *Adv Cancer Res.* **2014**;122:103–141. doi:10.1016/B978-0-12-420117-0.00003-7
51. Kunutsor SK. Gamma-glutamyltransferase-friend or foe within? *Liver Int.* **2016**;36(12):1723–1734. doi:10.1111/liv.13221
52. Xia J, Song P, Sun Z, Sawakami T, Jia M, Wang Z. Advances of diagnostic and mechanistic studies of gamma-glutamyl transpeptidase in hepatocellular carcinoma. *Drug Discov Ther.* **2016**;10(4):181–187. doi:10.5582/ddt.2016.01052
53. Zhang CH, Ni XC, Chen BY, Qiu SJ, Zhu YM, Luo M. Combined preoperative albumin-bilirubin (ALBI) and serum gamma-glutamyl transpeptidase (GGT) predicts the outcome of hepatocellular carcinoma patients following hepatic resection. *J Cancer.* **2019**;10(20):4836–4845. doi:10.7150/jca.33877
54. Yang Z, Ye P, Xu Q, et al. Elevation of serum GGT and LDH levels, together with higher BCLC staging are associated with poor overall survival from hepatocellular carcinoma: a retrospective analysis. *Discov Med.* **2015**;19(107):409–418.
55. Wu SJ, Lin YX, Ye H, Xiong XZ, Li FY, Cheng NS. Prognostic value of alkaline phosphatase, gamma-glutamyl transpeptidase and lactate dehydrogenase in hepatocellular carcinoma patients treated with liver resection. *Int J Surg.* **2016**;36(Pt A):143–151. doi:10.1016/j.ijssu.2016.10.033
56. Ostroumov D, Fekete-Drimusz N, Saborowski M, Kuhnel F, Woller N. CD4 and CD8 T lymphocyte interplay in controlling tumor growth. *Cell Mol Life Sci.* **2018**;75(4):689–713. doi:10.1007/s00018-017-2686-7
57. Atanasov G, Dino K, Schierle K, et al. Immunologic cellular characteristics of the tumour microenvironment of hepatocellular carcinoma drive patient outcomes. *World J Surg Oncol.* **2019**;17(1):97. doi:10.1186/s12957-019-1635-3
58. Nagai S, Abouljoud MS, Kazimi M, Brown KA, Moonka D, Yoshida A. Peritransplant lymphopenia is a novel prognostic factor in recurrence of hepatocellular carcinoma after liver transplantation. *Transplantation.* **2014**;97(6):694–701. doi:10.1097/01.TP.0000437426.15890.1d
59. Akateh C, Black SM, Conteh L, et al. Neoadjuvant and adjuvant treatment strategies for hepatocellular carcinoma. *World J Gastroenterol.* **2019**;25(28):3704–3721. doi:10.3748/wjg.v25.i28.3704
60. Casadei-Gardini A, Rovesti G, Dadduzio V, et al. Impact of aspirin on clinical outcome in advanced HCC patients receiving sorafenib and regorafenib. *HPB.* **2021**;23(6):915–920. doi:10.1016/j.hpb.2020.09.024
61. Zhu XD, Li KS, Sun HC. Adjuvant therapies after curative treatments for hepatocellular carcinoma: current status and prospects. *Genes Dis.* **2020**;7(3):359–369. doi:10.1016/j.gendis.2020.02.002

Publish your work in this journal

The Journal of Hepatocellular Carcinoma is an international, peer-reviewed, open access journal that offers a platform for the dissemination and study of clinical, translational and basic research findings in this rapidly developing field. Development in areas including, but not limited to, epidemiology, vaccination, hepatitis therapy, pathology and molecular tumor classification and prognostication are all considered for publication. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/journal-of-hepatocellular-carcinoma-journal>