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Original Article

Value of Non-tumoral Liver Volume in the Prognosis of Large Hepatocellular Carcinoma Patients After R0 Resection

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Abstract

Background and Aims: Hepatectomy is an effective treatment for selected patients with large hepatocellular carcinoma (HCC). This study aimed to develop a nomogram incorporating non-tumoral liver volume (non-TLV) and liver function markers to predict the patients' overall survival (OS) and disease-free survival (DFS). ***Methods:*** Data of 198 consecutive large HCC patients who underwent hepatectomy at the Zhongshan Hospital Xiamen University were collected. Another 68 patients from the Mengchao Hepatobiliary Surgery Hospital served as an external validation cohort. The nomograms were developed based on the independent prognostic factors screened by multivariate Cox regression analyses. Concordance index (C-index), calibration curves, and time-dependent receiver operating characteristic (ROC) curves were used to measure the discrimination and predictive accuracy of the models. ***Results:*** High HBV DNA level, low non-TLV/ICG, vascular invasion, and a poorly differentiated tumor were confirmed as independent risk factors for both OS and DFS. The model established in this study predicted 5-year post-operative survival and DFS in good agreement with the actual observation confirmed by the calibration curves. The C-indexes of the nomograms in predicting OS and DFS were 0.812 and 0.823 in the training cohort, 0.821 and 0.846 in the internal validation cohort, and 0.724 and 0.755 in the external validation cohort. The areas under the ROC curves (AUCs) of nomograms for predicted OS and DFS at 1, 3, and 5 year were 0.85, 0.86, 0.83 and 0.76, 0.76, 0.63, respectively. ***Conclusions:*** Nomograms with non-TLV/ICG predicted the prognosis of single large HCC patients accurately and effectively.

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Introduction

Hepatocellular carcinoma (HCC), the sixth most malignant tumor worldwide, is the most common primary malignant tumor of the liver and the second leading cause of cancer-related death.[1,2] There are various treatment methods for liver cancer, including surgical resection and radiofrequency ablation. However, the prognosis of HCC patients after surgical resection is poor, and the survival rate is not optimistic. Besides, patients are in the terminal stages of HCC when diagnosed, unsuitable for surgical resection, and often have other liver diseases, resulting in a poor prognosis.[3] Currently, new antitumor therapies accompanied by various (neo)adjuvant treatments have significantly improved patient survival.[4,5] However, the 5-year recurrence rate of HCC patients undergoing radical surgery has remained as high as 65–70%, and the post-operative 5-year survival rate is less than 40%.[6,7] Therefore, the prognosis of liver cancer has become an important clinical issue.

The prognosis of HCC patients is influenced by many factors, the most important of which lie in the tumor itself and the underlying liver condition.[8] Understanding tumor occurrence and development is of great help in determining their prognosis. As reported by previous studies, poor liver function and high tumor invasiveness lead to the rapid growth of HCC.[9] Imaging findings also showed that poorly differentiated HCC had a shorter tumor volume doubling time,[10] suggesting that the growth rate before surgery can be used to determine the biological characteristics of HCC. However, in clinical practice, images are often collected at a specific time,

Keywords: Hepatocellular carcinoma; Hepatectomy; Prognosis; Nomogram.

Abbreviations: AFP, alpha fetoprotein; ALPPS, associated liver partition and portal vein ligation for staged hepatectomy; BCLC, Barcelona Clinic Liver Cancer; C-index, concordance index; CLIP, Cancer of the Liver Italian Program; CNLC, China Liver Cancer; DFS, disease-free survival; EMT, epithelial-mesenchymal transition; FLR, future liver remnant; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HCC, hepatocellular carcinoma; ICG-R15, 15-m retention rate of indocyanine green; MVI, microvascular invasion; non-TLV, non-tumoral liver volume; OS, overall survival; PVE, portal venous embolization; PVTT, portal vein tumor thrombus; RCTs, randomized controlled trials; ROC, receiver operating characteristic; SLV, standard liver volume; TACE, transarterial chemoembolization.

Contributed equally to this work.

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so it is impossible to measure the time since the beginning of hepatocyte cancer transformation, making it difficult to determine the HCC growth rate.

Clinically, it has been observed that patients with poor liver condition and/or a high degree of pathologic malignancy have smaller non-tumoral liver volumes (non-TLVs) and shorter survival times, whereas patients with better liver condition and/or a low degree of pathologic malignancy have larger non-TLVs and longer survival times., We therefore studied the pre-operative standardized non-TLV, combined with the 15 m retention rate of indocyanine green (ICG-R15),[11,12] a method recommended by many guidelines to assess the functional reserve of the liver, to identify a comprehensive indicator. We also used the indicator to evaluate the tumor as well as the potential liver function and to further verify its prognostic value in HCC patients.

Methods

Patients and study design

Data of consecutive patients who underwent hepatectomy for large HCC at the Zhongshan Hospital Xiamen University between January 2015 and December 2020 and the Mengchao Hepatobiliary Surgery Hospital between January 2016 and April 2019 were retrospectively reviewed. Patients were required to meet the following inclusion criteria: (1) histopathologically confirmed solitary HCC with a diameter ≥5 cm; (2) without any history of other malignancies; (3) without distant metastasis; or (4) without any pre-operative antitumor therapy. Patients were excluded if they met the following exclusion criteria: (1) non-R0 liver resection; (2) perioperative death; or (3) missing data. Qualified Zhongshan Hospital patients were randomly assigned to the training or internal validation cohort in a 2:1 ratio. Qualified Mengchao Hepatobiliary Surgery Hospital patients were the external validation cohort.

Surgical procedure and follow-up

Anatomical hepatectomy was the first choice for surgical treatment, but when the tumor was located at the junction of several segments, at the periphery of the liver, or when the patient could not tolerate anatomical resection, nonanatomical hepatectomy was performed. Patients with portal vein invasion up to the second branch were eligible for surgery. For hepatic inflow occlusion, intermittent occlusion in cycles of 15 m clamped and 5 m unclamped. R0 resection was defined as no cancer cells found on the surgical margin by microscopy. Perioperative mortality was monitored for 60 days after surgery.

After discharge, patients were followed-up at least every 2–3 months for the first 2 years and every 3–6 months thereafter. Follow-up included a detailed history, physical examination, and hematological and imaging evaluation. Recurrence was diagnosed by more than two chief physicians during follow-up. Overall survival (OS) and disease-free survival (DFS) were the study endpoints. The study was performed following the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Zhongshan Hospital, Xiamen University (reference number 2015036). Informed consent was obtained from all participants or their families.

Main measures

Demographic data, past histories, serological indicators, pre-operative imaging data, and pathological data were recorded. Standard liver volume (SLV), pre-operative non-TLV and non-TLV/ICG were calculated. Three-dimensional (3D) reconstruction used Iqqa-Liver software (EDDA Technology, Princeton, NJ, USA) was based on enhanced CT scans. Pre-operative functional liver volume was represented by non-TLV. We standardized the non-TLV and then calculated non-TLV/ICG.

$$\text{Body surface area } (m^2) = 0.0061 \times \text{height (cm)} + 0.0128 \times \text{weight (kg)} - 0.1529$$

$$\text{SLV } (m^3) = 706.2 \times \text{body surface area } (m^2) + 2.4$$

$$\text{non-TLV/ICG} = \text{non-TLV/SLV/ICG-R15}$$

Statistical analysis

Differences in categorical variables were compared using χ^2 or Fisher's exact tests. Continuous variables with a normal distribution were compared using the independent samples t-test, and that did not conform were examined using the Mann-Whitney U test. Independent prognostic factors, identified by Uni- and multivariate Cox regression analysis, were used to formulate the prognostic nomogram. The model's performance was measured by the concordance index (C-index) using 1,000-times bootstrapping, and calibration curves comparing the predicted versus the observed Kaplan–Meier estimates of survival probability. ROC curves were used to reflect the prognostic value of the nomogram model at 1, 3, and 5 years. The statistical analysis was performed with SPSS 26.0 and R 4.1.2. *P*-values <0.05 were considered statistically significant.

Results

Clinicopathologic features

Between January 2015 and December 2020, 252 consecutive patients underwent partial hepatectomy for a single large HCC at the Zhongshan Hospital Xiamen University (Fig. 1). Thirty-two did not meet the inclusion criteria, two with a history of malignant tumor, 10 with distant metastasis, and 20 who had received pre-operative antitumor therapy. Twenty-two of the remaining 220 patients who met the exclusion criteria were excluded from the study, three because of surgical mortality, two had received non-R0 resection, and 17 patients had incomplete information or lost to follow-up. The baseline clinicopathologic features of the 198 patients who were included in the prognostic analysis are shown in Table 1. No patients were infected with hepatitis C virus, 51.5% had alpha fetal protein (AFP) >200 μg/L, 77.8% were hepatitis B surface antigen (HBsAg) positive, 65.2% had hepatitis B virus deoxyribonucleic acid (HBV DNA) levels ≤10^4 IU/mL, 21.7% had portal vein tumor thrombus (PVTT), and 59.6% had microvascular invasion (MVI) on pathological examination. The median tumor diameter was 8.5 (interquartile range 6.03–10.95) cm, the median ICG-R15 was 4 (2.82–6.38)%, and the median standardized non-TLV was 63.82% (41.42–82.14%). The 198 patients were randomly divided into a training cohort of 132 and an internal validation cohort of 66. Between January 2016 and April 2019, 68 consecutive patients underwent partial hepatectomy for a single large HCC and met the inclusion criteria at the Mengchao Hepatobiliary Surgery Hospital. Of those, 64.7% patients had an AFP >200 μg/L, 85.3% were HBsAg positive, 58.8% had an HBV DNA level ≤10^4 IU/mL, 38.2% had PVTT, and 75.0% had MVI on pathological examination. These 68 patients were the external validation cohort. There were no significant differences in baseline clinicopathological features between the training and internal validation cohorts. Compared with the patients in Zhongshan Hospital Xiamen University, the patients in the

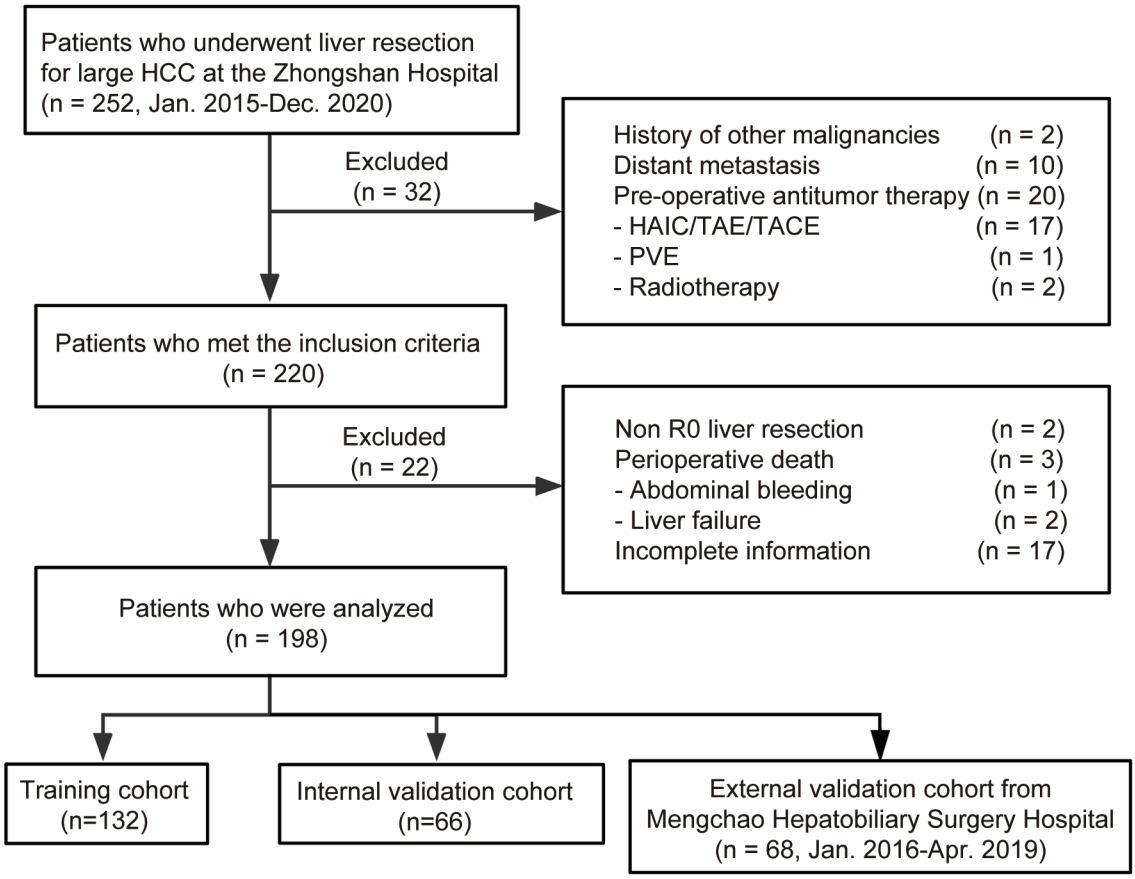

Fig. 1. Flowchart of patients identified in this study. HCC, hepatocellular carcinoma; HAIC, hepatic arterial infusion chemotherapy; TAE, transcatheter arterial embolization; TACE, transcatheter arterial chemoembolization; PVE, portal venous embolization.

external validation cohort were younger and more had an AFP >200 μg/L, an Edmonson–Steiner classification of III/IV, and had a PVTT (Table 1).

Post-operative OS and DFS

The study was censored on December 31, 2021. The median survival of patients at the Zhongshan Hospital Xiamen University was 48.2 [95% confidence interval (CI): 35.4–61.0] months. The 1, 3, and 5-year OS rates were 82.3%, 59.1%, and 44.2%, and the 1, 3, and 5 year DFS rates were 61.3%, 38.3%, and 29.1%, respectively. The median survival of patients at the Mengchao Hepatobiliary Surgery Hospital was 53.2 (95% CI: 22.0–85.1) months. The 1, 3, and, 5 year OS rates were 79.8%, 51.7%, and 43.1%, and the 1, 3, and 5 year DFS rates were 60.0%, 30.6%, and 11.5%, respectively (Supplementary Fig. 1).

The median survival of patients in the training and internal validation cohort was 44.2 (95% CI: 31.6–56.8) months and 60.3 (95% CI: 21.3–99.2) months, respectively. The mean survival of patients in the training and validation cohort was 51.7 (95% CI: 43.4–60.0) months and 46.3 (95% CI: 38.2–54.3) months, respectively. The 1, 3, and 5 year OS rates of the training and internal validation cohort were 82.8%, 60.2%, 41.1%, and 81.2%,57.0%, and 42.2%, respectively. The 1, 3, and 5 year DFS rates of the training and internal validation cohort were 62.8%, 40.5%, 30.4% and 58.3%, 35.5, 26.7%, respectively. There were no significant differences in the OS and DFS rates between the training cohort, internal validation cohort, and external validation cohort after hepatectomy.

Prognostic prediction of the value of non-TLV/ICG

The value of non-TLV/ICG was used alone to predict patient outcomes. Time-dependent ROC curves showed that the AUC values of that factor for prediction of OS and DFS at 1, 3, and 5 years were 0.717, 0.723, 0.788 and 0.606, 0.662, 0.741, respectively (Supplementary Fig. 2). The pre-operative predictive value of non-TLV/ICG value alone was good.

Univariable and multivariable analysis of OS and DFS in the training cohort

Univariable and multivariable analysis were performed using clinicopathological data and non-TLV/ICG. Multivariable analysis was performed on factors that significantly affected OS and DFS on univariable analysis (Table 2). The independent risk factors of OS were high HBV DNA levels, low non-TLV/ICG, vascular invasion, and poorly differentiated tumors. Independent risk factors of DFS were age ≥60 years, AFP level >200 μg/L, high HBV DNA level, low non-TLV/ICG, vascular invasion, and poorly differentiated tumors (Table 3).

Nomogram for predicting prognosis of large HCC patients

The independent risk factors of OS and DFS based on non-TLV/ICG and clinicopathological features were incorporated into the nomograms for the prediction of OS and DFS (Figs.

Table 1. Clinicopathological features

Variable	Number (%)/Median (interquartile range)			*P**	*P***
	Training (n=132)	Internal validation (n=66)	External validation (n=68)		
Age (years)	55.0 (46.0, 66.0)	51.5 (43.2, 61.0)	50.5 (43.7, 59.2)	0.105	0.031
Gender				0.949	0.678
male	106 (80.3)	54 (81.8)	57 (83.8)		
female	26 (19.7)	12 (18.2)	11 (16.2)		
Smoking				0.631	0.402
yes	42 (31.8)	24 (36.4)	17 (25.0)		
no	90 (68.2)	42 (63.6)	51 (75.0)		
Alcohol abuse				0.953	0.279
yes	30 (22.7)	16 (24.2)	21 (30.9)		
no	102 (77.3)	50 (75.8)	47 (69.1)		
Hypertension				0.775	0.273
yes	36 (27.3)	16 (24.2)	13 (19.1)		
no	96 (72.7)	50 (75.8)	55 (80.9)		
Diabetes				1.000	0.625
yes	14 (10.6)	7 (10.6)	5 (7.4)		
no	118 (89.4)	59 (89.4)	63 (92.6)		
AFP (μg/L)				0.291	0.042
≤200	68 (51.5)	28 (42.4)	24 (35.3)		
>200	64 (48.5)	38 (57.6)	44 (64.7)		
WBC ($\times 10^9$/L)	6.6 (5.2, 7.8)	6.3 (5.0, 7.6)	6.0 (4.7, 7.3)	0.444	0.121
HGB (g/L)	131.0 (118.7, 151.0)	142.0 (121.2, 152.7)	131.0 (118.0, 149.5)	0.324	0.768
PLT ($\times 10^9$/L)	218.5 (177.7, 282.5)	213.5 (164.2, 283.5)	206.5 (151.7, 282.5)	0.695	0.201
ALB (g/L)	39.8 (36.2, 43.2)	39.7 (37.9, 43.9)	40.6 (38.1, 43.6)	0.503	0.357
TBIL (μmol/L)	11.7 (8.8, 17.0)	12.4 (8.6, 16.9)	13.2 (10.1, 16.9)	0.828	0.107
DBIL (μmol/L)	4.4 (3.1, 6.2)	4.5 (3.0, 6.2)	5.1 (3.9, 7.3)	0.891	0.059
ALT (μ/L)	30.1 (21.1, 43.1)	29.6 (21.0, 40.9)	26.9 (18.3, 44.4)	0.792	0.52
AST (μ/L)	37.0 (25.5, 58.9)	38.4 (25.7, 60.5)	41.0 (27.9, 61.7)	0.767	0.331
GGT (μ/L)	83.7 (49.9, 146.2)	88.2 (57.5, 133.5)	94.2 (53.9, 166.6)	0.804	0.492
ALP (μ/L)	91.8 (76.0, 115.2)	87.1 (73.4, 103.6)	89.7 (70.2, 127.3)	0.406	0.848
Cr (μmol/L)	67.9 (59.4, 79.7)	67.5 (59.7, 80.2)	68.0 (60.0, 77.2)	0.782	0.579
PT (s)	12.5 (11.6, 13.3)	12.4 (11.7, 13.2)	12.8 (11.9, 13.5)	0.730	0.154
HBsAg				0.672	0.203
positive	101 (76.5)	53 (80.3)	58 (85.3)		
negative	31 (23.5)	13 (19.7)	110 (14.7)		
HBsAb				0.603	0.222
positive	19 (14.4)	7 (10.6)	5 (7.4)		
negative	113 (85.6)	59 (89.4)	63 (92.6)		
HBeAg				0.793	0.398
positive	11 (8.3)	7 (10.6)	9 (13.2)		
negative	121 (91.7)	59 (89.4)	59 (86.8)		
HBeAb				0.866	0.453
positive	97 (73.5)	47 (71.2)	54 (79.4)		

(continued)

Table 1. *(continued)*

Variable	Number (%)/Median (interquartile range)			*P**	*P***
	Training (n=132)	Internal validation (n=66)	External validation (n=68)		
negative	35 (26.5)	19 (28.8)	14 (20.6)		
HBcAb				0.940	0.881
positive	116 (87.9)	57 (86.4)	61 (89.7)		
negative	16 (12.1)	9 (13.6)	7 (10.3)		
HBV DNA level (IU/mL)				0.874	0.406
≤10^4	87 (65.9)	42 (63.6)	40 (58.8)		
u10^4	45 (34.1)	24 (36.4)	28 (41.2)		
ICG-R15 (%)	4.2 (2.9, 6.9)	3.7 (2.7, 5.3)	3.7 (3.1, 7.5)	0.058	0.692
Standardized non-TLV (%)	64.0 (41.8, 81.0)	63.3 (40.8, 82.6)	65.2 (45.3, 81.1)	0.875	0.808
non-TLV/ICG	22.5 (13.9, 36.7)	27.1 (15.7, 37.6)	21.2 (12.4, 31.4)	0.237	0.340
Cirrhosis				0.451	1.000
yes	63 (47.7)	36 (54.5)	33 (48.5)		
no	69 (52.3)	30 (45.5)	35 (51.5)		
Tumor diameter (cm)	8.5 (6.0, 11.0)	8.6 (6.4, 10.8)	8.0 (6.0, 9.6)	0.928	0.350
PVTT				0.951	0.016
yes	28 (21.2)	15 (22.7)	26 (38.2)		
no	104 (78.8)	51 (77.3)	42 (61.8)		
MVI				0.798	0.061
yes	80 (60.6)	38 (57.6)	51 (75.0)		
no	52 (39.4)	28 (42.4)	17 (25.0)		
Capsule				0.577	0.177
complete	59 (44.7)	22 (39.4)	30 (44.1)		
incomplete	73 (55.3)	40 (60.6)	38 (55.9)		
Edmonson–Steiner Classification				1.000	0.053
I/II	102 (77.3)	50 (75.8)	43 (63.2)		
III/IV	30 (22.7)	16 (24.2)	25 (36.8)		
Blood transfusion				0.807	0.123
yes	15 (11.4)	6 (9.1)	14 (20.6)		
no	117 (88.6)	60 (90.9)	54 (79.4)		
Clamping time (m)				0.296	0.722
>10, ≤20	29 (22.0)	8 (12.1)	13 (19.1)		
>20	39 (29.5)	23 (34.8)	18 (26.5)		
≤10	26 (19.7)	11 (16.7)	12 (17.6)		
0	38 (28.8)	24 (36.4)	25 (36.8)		
Anatomical liver resection				0.243	0.184
yes	71 (53.8)	42 (63.6)	44 (64.7)		
no	61 (46.2)	24 (36.4)	24 (35.3)		

*Comparison between the training cohort and the internal validation cohort. **Comparison between the training cohort and the external validation cohort. AFP, alpha fetal protein; ALB, albumin; ALT, alanine transaminase; Cr, creatinine; DBIL, direct bilirubin; HBcAb, hepatitis B core antibody; HBeAb, hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HCC, hepatocellular carcinoma; ICG-R15, 15-m retention rate of indocyanine green; MVI, microvascular invasion; non-TLV: non-tumoral liver volume; PLT, platelet; PT, prothrombin time; PVTT, portal vein tumor thrombus; TBIL, total bilirubin; WBC, white blood cell.

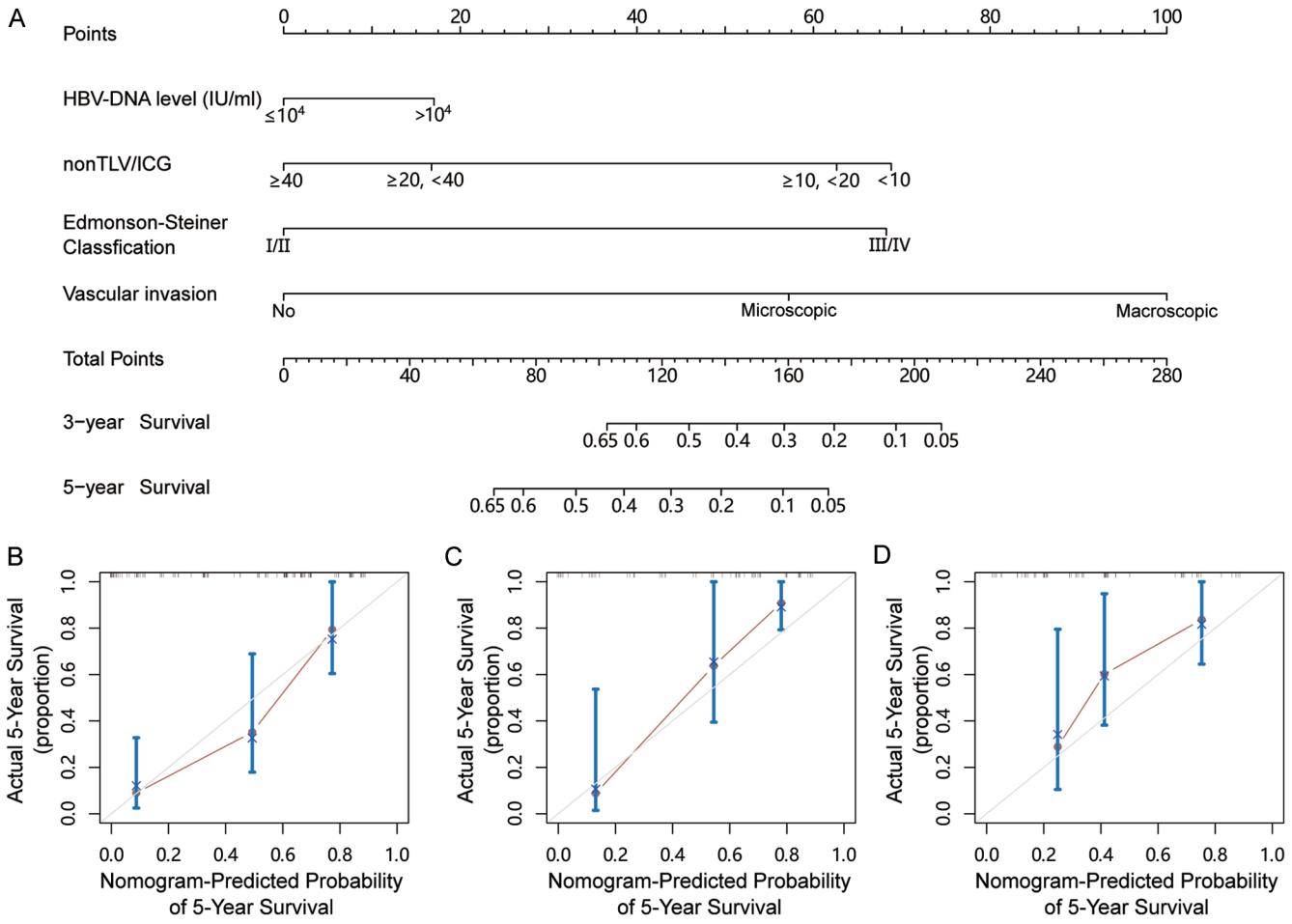

Fig. 2. Establishment and calibration of the nomogram predicting OS for HCC patients. (A) Nomogram for overall survival prediction. (B-D) Calibration curves for the probability of the post-operative 5-year overall survival in training (B), internal validation (C) and external cohort (D). HCC, hepatocellular carcinoma.

2A and 3A). The C-indexes of the nomogram for OS prediction in the training, internal validation, and external validation cohorts were 0.812 (95% CI: 0.747–0.877), 0.821 (95% CI: 0.739–0.903), and 0.724 (95% CI: 0.782–0.666), respectively. The C-indexes of the nomogram for DFS prediction in the training, internal validation, and external validation cohorts were 0.823 (95% CI: 0.883–0.763), 0.846 (95% CI: 0.912–0.780), and 0.755 (95% CI: 0.810–0.700), respectively. The calibration curves for the probability of post-operative 5 year survival and disease-free survival (DFS) showed good agreements between the prediction made by the two nomograms and actual observation (Figs. 2B–D and 3B–D).

Compared with the usual HCC staging systems, the nomograms improved the prediction accuracy. Time-dependent ROC curves showed that the AUC values of the nomogram for predicted OS at 1, 3, and 5 years were 0.85, 0.86, and 0.83, respectively (Fig. 4A–C). Time-dependent ROC curves showed that the AUC values of the nomogram for predicted DFS at 1, 3, and 5 years were 0.76, 0.76, and 0.63, respectively (Fig. 4D–F).

Prognostic discrimination of the nomogram

The prognostic discrimination of the nomograms was further identified by dividing the predicted survival probabilities into tertiles. The models were able to accurately stratify patients into three prognostic subgroups with significantly different prognoses. The resulting Kaplan–Meier curves revealed significant prognostic differences between any two adjacent groups, with a median OS of 77.6, 45.1, and 23.9 months for tertiles 1, 2, and 3, respectively (p<0.001). The 5 year survival rates for tertiles 1, 2, and 3 were 75.4%, 43.6%, and 8.4%, respectively (Fig. 5A). We also used the DFS prediction scores to group all the patients enrolled in the study into tertiles, with median disease-free survival of 45.9, 33.9, and 11.5 months for tertiles 1, 2, and 3, respectively (p<0.001). The 5 year DFS for tertiles 1, 2, and 3 was 40.3%, 22.0%, and 8.5%, respectively (Fig. 5B).

Discussion

Post-operative recurrence is the main cause of low long-term survival of HCC patients.[13,14] Consequently, the prediction of post-operative recurrence and survival has a significant role in guiding the choice of treatment. Studies have identified factors affecting the prognosis of HCC, including tumor capsule, tumor number, surgical resection margin, PVTT, HBV DNA, AFP, and others.[15,16] Traditional HCC staging systems and predictive models based on the currently known risk factors cannot fully meet the needs of clinical practice. Therefore, it is particularly critical to identify new influencing fac-

Table 2. Univariable analysis of OS and DFS in the training cohort

Variable	OS			DFS		
	P	**HR**	**95%CI**	***P***	**HR**	**95%CI**
Age (years)	0.097	0.984	0.966–1.003	0.000	0.973	0.958–0.988
Sex: male vs. female (ref)	0.660	0.950	0.412–1.754	0.067	0.722	0.509–1.023
Smoking: yes vs. no (ref)	0.987	0.995	0.547–1.811	0.944	0.991	0.777–1.265
Alcohol abuse: yes vs. no (ref)	0.231	0.679	0.359–1.284	0.290	0.867	0.667–1.129
Hypertension: yes vs. no (ref)	0.266	1.480	0.738–2.969	0.061	1.313	0.988–1.744
Diabetes: yes vs. no (ref)	0.407	7.536	0.552–4.278	0.230	1.292	0.850–1.962
AFP (μg/L): >200 vs. ≤200 (ref)	0.006	2.245	1.243–4.052	0.001	1.464	1.159–1.849
WBC ($\times10^9$/L)	0.175	0.912	0.800–1.041	0.525	0.996	0.870–1.074
HGB (g/L)	0.067	1.014	0.999–1.028	0.094	1.010	0.998–1.021
PLT ($\times10^9$/L)	0.311	0.998	0.995–1.002	0.444	0.999	0.996–1.002
ALB (g/L)	0.364	1.023	0.974–1.074	0.454	0.984	0.945–1.026
TBIL (μmol/L)	0.319	1.022	0.979–1.068	0.040	1.038	1.002–1.076
DBIL (μmol/L)	0.460	1.035	0.944–1.135	0.278	1.042	0.967–1.124
ALT (μ/L)	0.212	1.004	0.998–1.010	0.059	1.004	1.000–1.009
AST (μ/L)	0.097	1.003	0.999–1.006	0.281	1.002	0.999–1.005
GGT (μ/L)	0.430	0.999	0.996–1.002	0.264	1.001	0.999–1.003
ALP (μ/L)	0.470	0.998	0.993–1.003	0.520	0.999	0.997–1.002
Cr (μmol/L)	0.665	0.998	0.988–1.008	0.474	1.002	0.996–1.009
PT (s)	0.129	1.205	0.947–1.533	0.008	1.289	1.070–1.553
HBsAg: negative vs. positive (ref)	0.046	0.442	0.198–0.985	0.013	0.666	0.483–0.918
HBsAb: negative vs. positive (ref)	0.079	1.559	0.934–2.602	0.174	1.290	0.849–1.863
HBeAg: negative vs. positive (ref)	0.758	0.934	0.605–1.442	0.400	0.860	0.606–1.221
HBeAb: negative vs. positive (ref)	0.149	0.782	0.558–1.096	0.116	0.804	0.612–1.056
HBcAb: negative vs. positive (ref)	0.149	0.690	0.412–1.155	0.120	0.718	0.472–1.090
HBV DNA level (IU/mL): $>10^4$ vs. $\leq10^4$ (ref)	0.019	1.411	1.058–1.881	0.001	1.482	1.177–1.865
ICG-R15 (%)	0.130	1.056	0.984–1.133	0.057	1.057	0.998–1.119
Standardized non-TLV (%)	0.043	0.341	0.121–0.966	0.058	0.456	0.203–1.026
Non-TLV/ICG	0.007	0.975	0.956–0.993	0.025	0.986	0.974–0.998
Cirrhosis: yes vs. no (reference)	0.203	0.831	0.625–1.105	0.604	0.941	0.749–1.183
Tumor diameter (cm)	0.056	1.068	0.998–1.142	0.012	1.076	1.016–1.140
Vascular invasion						
None (reference)						
microscopic	0.004	3.378	1.465–7.788	0.131	1.530	0.881–2.655
macroscopic	<0.001	7.237	2.956–17.718	<0.001	3.538	1.909–6.557
Capsule: incomplete vs. complete (reference)	0.397	1.129	0.852–1.496	0.230	1.150	0.915–1.446
Edmonson–Steiner classification: I/II vs. III/IV (reference)	<0.001	0.448	0.335–0.598	<0.001	0.601	0.472–0.767
Blood transfusion: yes vs. no (reference)	0.651	0.916	0.625–1.341	0.929	0.984	0.694–1.397
Clamping time (min)	0.480	1.006	0.990–1.023	0.379	1.006	0.993–1.019
Anatomical liver resection: yes vs. no (reference)	0.103	0.787	0.590–1.050	0.582	0.938	0.746–1.179

AFP, alpha fetal protein; ALB, albumin; ALT, alanine transaminase; CI, confidence interval; Cr, creatinine; DBIL, direct bilirubin; DFS, disease-free survival; HBcAb, hepatitis B core antibody; HBeAb, hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; HCC, hepatocellular carcinoma; HR, hazard ratio; ICG-R15, 15-m retention rate of indocyanine green; non-TLV, non-tumoral liver volume; OS, overall survival; PLT, platelet; PT, prothrombin time; TBIL, total bilirubin; WBC, white blood cell.

Table 3. Multivariable analysis of OS and DFS in the training cohort

Variable	OS			DFS		
	P	**HR**	**95% CI**	***P***	**HR**	**95% CI**
Age (years): <60 vs. ≥60 (ref)	–	–	–	0.047	1.729	1.007–2.970
AFP (μg/L): >200 vs. ≤200 (ref)	0.161	1.605	0.828–3.111	0.046	1.688	1.010–2.821
TBIL (μmol/L)	–	–	–	0.184	1.027	0.987–1.068
PT (s)	–	–	–	0.336	1.110	0.898–1.373
HBV DNA level (IU/mL): $>10^4$ vs. $\leq 10^4$ (reference)	0.047	1.922	1.009–3.660	0.020	1.836	1.102–3.059
non-TLV/ICG						
<10 (reference)						
≥10, <20	0.776	0.888	0.392–2.013	0.577	0.820	0.409–1.645
≥20, <40	0.010	0.342	0.150–0.778	0.003	0.326	0.156–0.680
≥40	0.011	0.235	0.077–0.721	0.004	0.249	0.098–0.635
Tumor diameter (cm)	–	–	–	0.361	0.966	0.896–1.041
Vascular invasion						
None (reference)						
Microscopic	0.034	2.562	1.075–6.107	0.109	1.624	0.897–2.939
Macroscopic	0.001	5.276	2.062–13.495	0.002	2.991	1.517–5.898
Edmonson–Steiner classification: I/II vs. III/IV (reference)	<0.001	0.299	0.159–0.563	0.002	0.443	0.262–0.749

AFP, alpha fetal protein; CI, confidence interval; ref, reference variable; DFS, disease-free survival; HBV DNA, hepatitis B virus deoxyribonucleic acid; HR, hazard ratio; non-TLV, non-tumoral liver volume; OS, overall survival; PT, prothrombin time; TBIL, total bilirubin.

tors. In this study, we established a model using non-TLV/ICG, which is a new pre-operative predictive indicator, and found that combined with other indicators, including vascular invasion, HBV DNA, age, AFP, and Edmonson–Steiner classification, the model predicted the prognosis of patients with a single HCC more accurately and efficiently.

MVI frequently influenced post-operative recurrence of HCC patients, but unlike PVTT, no accurate method is available for pre-operative evaluation of MVI.[17–19] The first step of tumor metastasis is usually epithelial-mesenchymal transition (EMT), which is an important biological process in which tumor cells acquire the ability to migrate and invade the stroma. Activation of the EMT transcription program in HCC usually leads to MVI, which in turn promotes intrahepatic and distant tumor metastasis.[20,21] At present, the diagnosis of MVI depends on post-operative pathology despite the development of pre-operative molecular and imaging techniques, undermining its potential assistance in selecting personalized pre-operative treatment.[22,23] Regarding its clinical importance, many teams have established predictive models of MVI based on pre-operative data to accurately predict MVI preoperatively and select the most appropriate treatment from radiofrequency ablation, hepatectomy, or liver transplantation, and even (neo)adjuvant chemotherapy.[24–26]

HBV DNA was also a significant independent risk factor for the prognosis of HCC patients. About 70–90% of HCC patients in China have associated hepatitis B virus (HBV) infection. HBV genes in the nuclei of human hepatocytes covalently form closed circular DNA that cannot be eradicated, making treatment with the current nucleos(t)ide analogs and interferon difficult,[27,28] and resulting in long-term chronic damage of liver tissue and increased risk of HCC.[29] Integration of HBV DNA into the host genome occurs early in clonal tumor expansion and induces both genomic instability and direct insertional mutagenesis of diverse cancer-related genes. HBx protein is produced by post-coding translation of the HBV genome. It stimulates cell proliferation, invasion, metastasis, and angiogenesis by activating signaling pathways related to the cell cycle and cell transformation.[30–32] In patients with chronic HBV infection, the risk of HCC increases with active HBV replication,[33] and HBV infection increases the risk of HCC recurrence after radical hepatectomy.[34,35] In addition, active HBV infection induces MVI through chronic inflammation and the expression of metastatic-associated protein 1 to inhibit local immune monitoring,[36,37] promoting the recurrence of HCC patients. Therefore, antiviral therapy that inhibits HBV activity has an essential role in the course of HBV-associated HCC as well as the entire course of HBV infection.

In this study, non-TLV/ICG was an independent risk factor for prognosis in HCC patients. We found that standardized non-TLV correlated with Edmonson–Steiner Classification, vascular invasion, and capsular invasion (Supplementary Fig. 3). Interestingly, standardized non-TLV was not correlated with tumor diameter, indicating that the time of liver hyperplasia was different in similarly sized tumors because of the difference in tumors growth rates. That suggests that non-TLV indirectly reflected the degree of pathologic tumor malignancy.[38] Previous studies of associated liver partition and portal vein ligation for staged hepatectomy (ALPPS) showed that the proliferative ability of the future liver remnant (FLR) varied greatly with different liver conditions. Therefore, we believe that non-TLV reflected individual differences in liver conditions through different liver hyperplasia abilities. In a previous study, Kang and Schadde[39] found a non-proportional relationship between liver volume and ICG, indicating that neither liver volume nor ICG alone fully reflected liver condition.[39] Fortunately, non-TLV/ICG combined the charac-

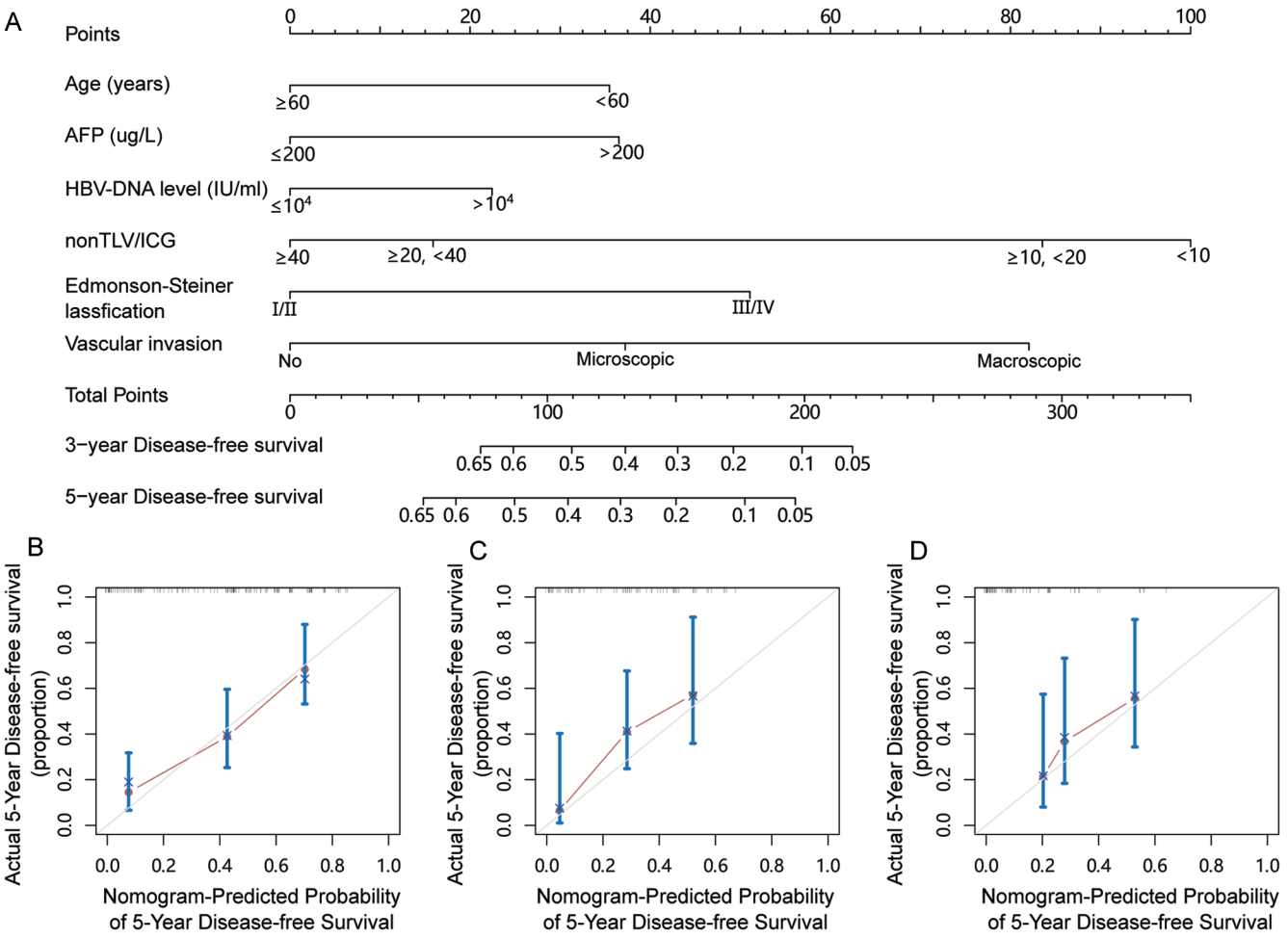

Fig. 3. Establishment and calibration of the nomogram predicting DFS for HCC patients. (A) Nomogram for disease-free survival prediction. (B-D) Calibration curves for the probability of the post-operative 5 year disease-free survival in the training (B), internal validation (C) and external cohorts (D). HCC, hepatocellular carcinoma.

teristics of the two indicators to assess the overall condition of the liver comprehensively. We also demonstrated that non-TLV/ICG had a good prediction ability in HCC patients in this study, and those with non-TLV/ICG ≥20 had a good prognosis (Supplementary Fig. 4). Based on the results of the model, we speculated that portal venous embolization (PVE), a minimal invasive pre-operative strategy to increase FLR to facilitate extended hemihepatectomy,[40,41] helped to speed normal liver growth and increase non-TLV, and may have improved the long-term prognosis of patients, especially those with non-TLV/ICG <20. Moreover, translational medicine therapies, such as interventional therapy, targeted therapy, and immunotherapy, could limit tumor progression, ensure compensatory hepatic hyperplasia, and further increase non-TLV to improve long-term prognosis. In summary, those strategies may increase the post-operative FLR, ensure the safety of the operations; and more importantly, improve the long-term prognosis of HCC patients.

In patients with a single HCC, the accuracy of our predictive model was superior to HCC staging systems, including Barcelona Clinic Liver Cancer (BCLC), China Liver Cancer (CNLN), and Cancer of the Liver Italian Program (CLIP) staging. Thus, more active measures are expected to prevent the post-operative recurrence of HCC patients with poor overall survival (OS) and DFS predicted by our model. For high-risk HCC patients, transarterial chemoembolization (TACE) adjuvant therapy is the most commonly used diagnosis and treatment method to prevent HCC recurrence after hepatectomy.[42] Nonetheless, the post-operative recurrence rate remains high after adjuvant TACE. Recently, with the development of new therapies in advanced HCC,[43] more researchers have focused on the anti-recurrent effects of targeted therapy and immunotherapy in patients with resectable HCC. Several randomized controlled trials (RCTs) are ongoing, thus our model may provide the basis for population selection for RCTs.

Our study has some limitations. Firstly, the patients included in this study had single, large HCCs and as the clinical manifestations of HCC are very complex, whether the study results apply to other types of HCC patients needs further investigation and verification. Secondly, most of the HCC patients in the model had underlying HBV infection, so the model results may not apply to HCC patients with other underlying liver diseases.

Conclusions

In summary, the model with non-TLV/ICG predicted the prog-

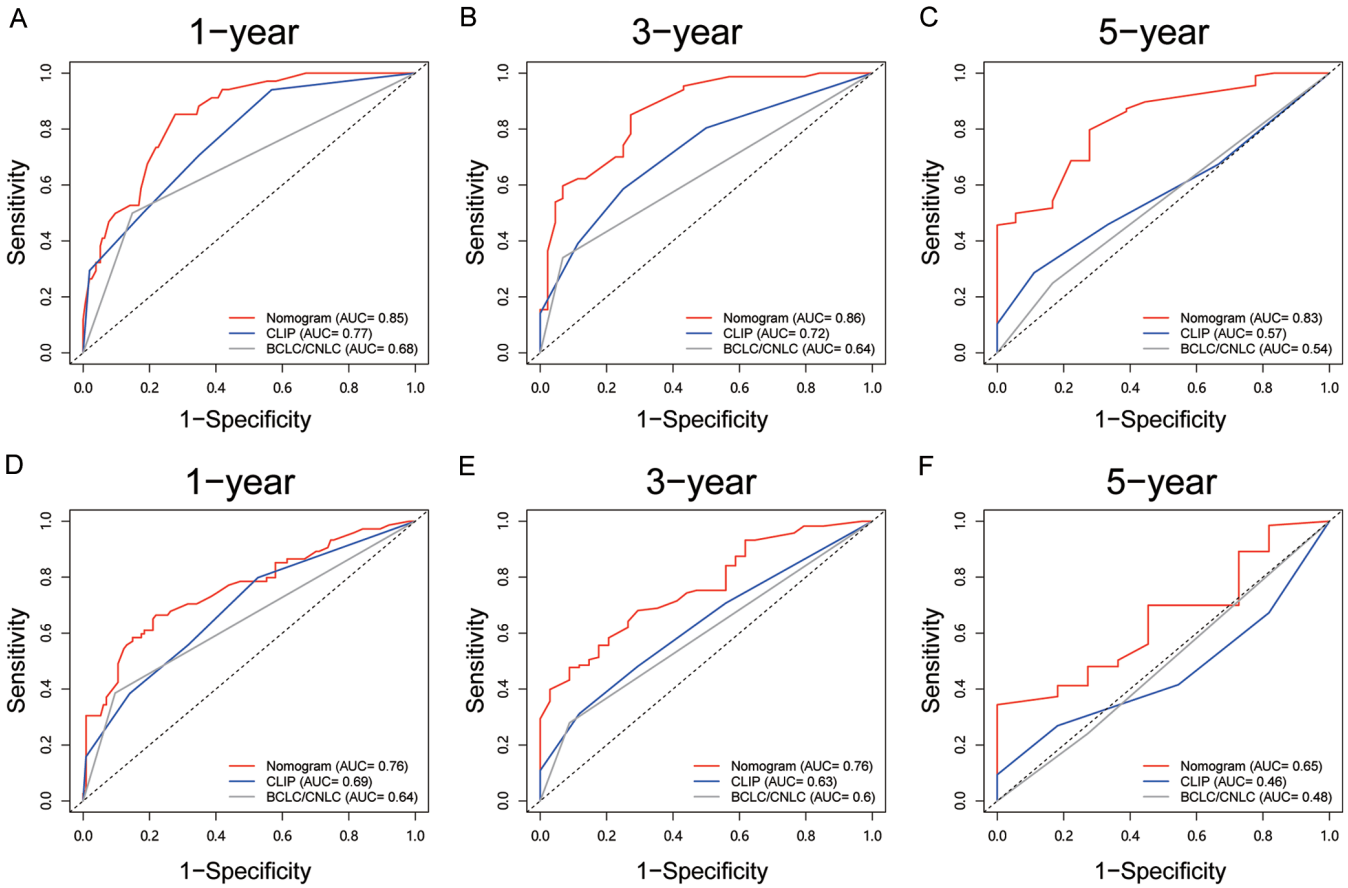

Fig. 4. Time-dependent receiver operating characteristic (ROC) curves of nomogram and HCC staging systems. (A–C) 1 (A), 3 (B), and 5 years (C) for evaluating the specificity and sensitivity of overall survival prediction in all included patients. Time-dependent ROC curves of nomogram and HCC staging systems at 1 (D), 3 (E), and 5 years (F) for evaluating the specificity and sensitivity of disease-free survival prediction in all included patients. CLIP, Cancer of the Liver Italian Program; BCLC, Barcelona Clinic Liver Cancer; CNLC, China Liver Cancer.

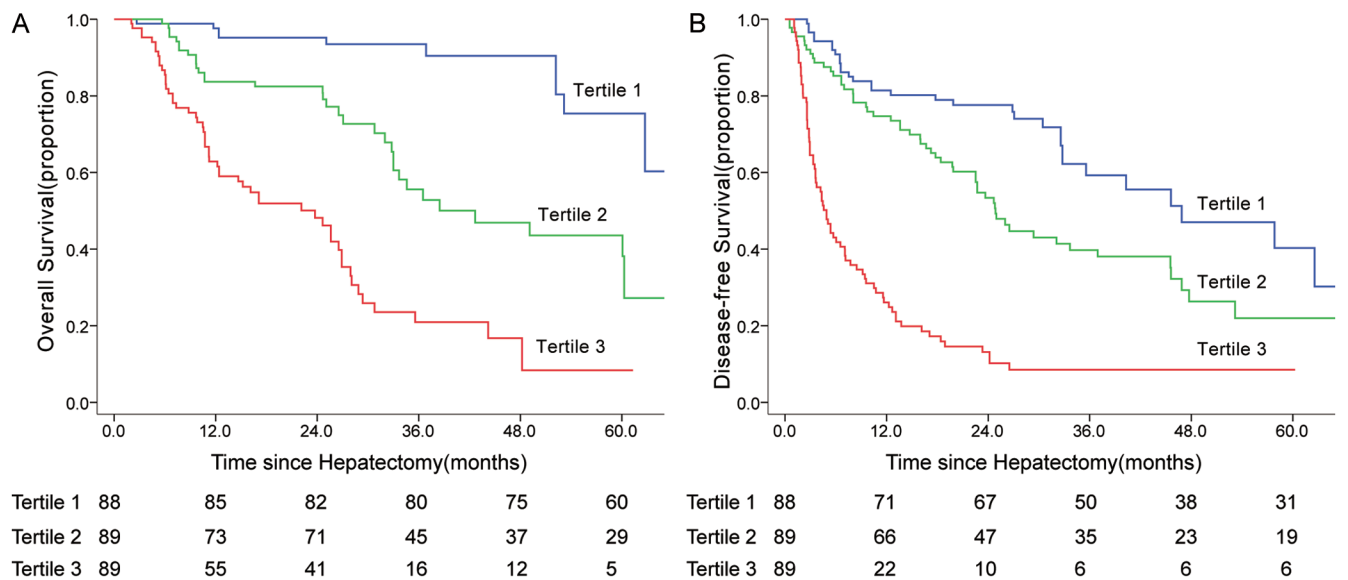

Fig. 5. Kaplan-Meier analysis for prognostic discrimination of the nomograms. (A–B) Kaplan-Meier survival curves for tertiles of the nomograms predicting overall survival (A) and disease-free survival (B). The scoring range of each tertile in panel A: tertile 1: 0–73; tertile 2: 74–143; tertile 3: >143. The scoring range of each tertile in panel B: tertile 1: 0–111; tertile 2: 112–186; tertile 3: >186.

nosis of single large HCC patients accurately and effectively, providing a basis for selecting follow-up patient treatment

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (PgL, YT), acquisition of data (DZ, HL, QF, DL, PtL, LT), analysis and interpretation of data (YT, DZ, YX, YZ, PgL), drafting of the manuscript (YT, DZ), obtained funding and critical revision (YX, PgL).

Ethical statement

The study was performed following the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Zhongshan Hospital, Xiamen University (reference number 2015036). Informed consent was obtained from all participants or their families.

Data sharing statement

All data are available upon request.

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