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

Jingpeng Ke  
First Affiliated Hospital of Xiamen University

Honghao Ye  
Fuzhou University

Fangzhou Lin  
Fuzhou University

Yingjun Shi  
Fuzhou University

Aoxue Zhong  
Fuzhou University

Hengkai Chen (✉️ 18259056002@163.com)  
First Affiliated Hospital of Fujian Medical University

Research Article

Keywords: nomogram, microvascular invasion grade, early-stage HCC

Posted Date: January 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1276658/v1

License: ☕️ ☀️ This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

**Background:** This is the first study to develop and evaluate a predictive model based on the microvascular invasion (MVI) classification for early recurrence and survival after curative hepatectomy in patients with early-stage hepatocellular carcinoma (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 were retrospectively reviewed. Kaplan-Meier curves and Cox proportional hazards regression models were used to analyze disease-free survival (DFS) and overall survival (OS). Nomogram models were constructed on the datasets from the First Affiliated Hospital of Fujian Medical University and the datasets were validated using bootstrap resampling with 30% samples as internal validation. Data of patients from 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 for predicting survival were created. The concordance indexes (C-index) 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), respectively, in the training cohort, and 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 early-stage HCC patients after curative resection with high predictive accuracy.

Introduction

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 HCC patients 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% to 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 early-stage HCC patients 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 HCC patients who underwent hepatectomy or received liver transplantation. Most BCLC early-stage HCC patients with early recurrence were pathologically verified as MVI positive (5-7). Moreover, a previous study found that more invading tumor cells and multiple-invaded micro vessels 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 even 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 HCC patients have been established, a predictive model for early BCLC stage HCC patients according to the MVI grade has not been reported.

Therefore, we retrospectively investigated the clinical and histopathological characteristics of early HCC patients after curative hepatectomy from multicenter to establish a prognostic nomogram based on MVI grade to predict early recurrence and OS.
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 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 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 parameters of blood routine, 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 tumors, presence of satellite nodules, diameter of the largest nodule, tumor 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. Overall survival (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 ± 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) 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 in the study. 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-year, 2-, and 3-year recurrence or metastasis rates were 5.8%, 8.1%, 11.8%, and 12.7%, respectively. The 8-month, 1-year, 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 Figure 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).

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

**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 generated (Figure 2). The results were showed in Table 3. The C-index of the nomogram for DFS was 0.775 with a 95% confidence interval (CI) of 0.720-0.830. The C-index for OS was 0.812 (95% CI, 0.732-0.892). The validation showed good consistency between the observed and predicted 8-month, 1-year, 2-year, 3-year DFS, 8-month, 1-year OS, 2-year and 3-year OS (Figure 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 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 external validation cohort (Table 3). Taken together, the nomogram models were able to accurately predict postoperative relapse and OS in BCLC early-stage HCC patients.

**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, Japan Integrated Staging Score (JIS) and Hong Kong Liver Cancer prognostic classification scheme (HKLC). The results were showed in Table 3. In the training cohort, the C-index of the nomogram for DFS and OS was 0.775 and 0.812, 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), HKLC (DFS: 0.595; OS: 0.612) staging systems. Correspondingly, in 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), which was also significantly higher than the AJCC (internal cohort: 0.622; external cohort: 0.586 for FDS and internal cohort: 0.615; external cohort: 0.578 for OS), BCLC (internal cohort: 0.602; external cohort: 0.574 for FDS and internal cohort: 0.608; external cohort: 0.571 for OS), JIS (internal cohort: 0.606; external cohort: 0.581 for FDS and internal cohort: 0.599; external cohort: 0.574 for OS), HKLC (internal cohort: 0.625; external cohort: 0.558 for FDS and internal cohort: 0.619; external cohort: 0.541 for OS) staging systems. Overall, the predictive accuracy of the nomogram models was superior to that of these classical staging systems for DFS and OS.

**Discussion**

Although BCLC early-stage HCC patients usually have a better prognosis than those 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, none include MVI as a criterion. A predictive model based on the classification of MVI for recurrence, especially early recurrence in patients with early-stage HCC, has not been reported. Therefore, we established nomograms based on the classification of MVI for recurrence and OS in early-stage HCC patients after curative resection, 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 HCC patients is mainly influenced by the following three factors: (1) patient factors, such as immune function, nutritional status, liver function, and status of hepatitis virus infection; (2) tumor factors, such as tumor diameter, MVI classification, and satellite nodules; and (3) factors of treatment, such as postoperative adjuvant treatment. 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, while two factors were tumor-related factors including tumor 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 types of MVI can be used as a morphological marker to evaluate the biology and progression of HCC\(^{4,14,15}\). However, the detection rate of MVI is low, varying from 12.4% to 33.1% in early-stage HCC patients, and the clinical value of MVI for early-stage HCC patients after curative resection remains controversial\(^{16-18}\). In our study, the detection rate of MVI was 39.1% (275/703), and it was an independent risk factor associated with DFS and OS (Figure 1, p<0.001). It is well known that tumor size is related to patient prognosis. The presence of tumor enlargement predicts poor prognosis in patients with HCC. The corresponding cutoff value of tumor size is used in different guidelines to predict prognosis because the correlation between tumor size and poor prognosis in patients is not linear. In this study, the cutoff values were set as 5 and 10 cm. Our study identified tumors with a diameter >10 cm as a significant risk factor for recurrence. Interestingly, although AFP is known as a common classical marker for the diagnosis and prognosis of HCC, it was not an independent factor associated with 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 associated 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 diagnosis, prognosis of early-stage HCC\(^{20}\).

Previous studies have reported that immune function and nutritional status are related to HCC patient prognosis\(^{21-25}\). 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 tumor microcirculation, will undoubtedly affect 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 tumors, but also make HCC patients 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 tumor 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 dataset 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 and meaningful information including postoperative adjuvant treatment collected in the future may improve accuracy of our results. Third, hepatitis B virus (HBV) infection, known to be associated with a poor prognosis of HCC patients, 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.
Conclusion

In summary, we developed and validated nomograms for predicting recurrence, especially early recurrence, and OS in early-stage HCC patients after curative resection. Their predictive performances were better than the common classical HCC staging systems, and they can help clinicians achieve better outcomes in this group of patients.

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 hemoglobin; 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

Conflicts of interest

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

Acknowledgements

This study was supported by the Startup Fund for Scientific Research, Fujian Medical University (Grant Number: 2019QH2032). In addition, Hengkai Chen would like to thank his family, especially his wife Dan Lin, children Shuen Chen and Shuhan Chen for providing him with complete spiritual support over the past years.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30.
2. Dhir M, Melin AA, Douaiher J, Lin C, Zhen WK, Hussain SM, et al. A Review and Update of Treatment Options and Controversies in the Management of Hepatocellular Carcinoma. Ann Surg. 2016;263(6):1112-25.
3. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. Cancer. 2000;89(3):500-7.
4. Janssen KJ, Donders AR, Harrell FE, Jr., Vergouwe Y, Chen Q, Grobbee DE, et al. Missing covariate data in medical research: to impute is better than to ignore. J Clin Epidemiol. 2010;63(7):721-7.
5. Adam R, Bhangui P, Vibert E, Azoulay D, Pelletier G, Duclos-Vallée JC, et al. Resection or transplantation for early hepatocellular carcinoma in a cirrhotic liver: does size define the best oncological strategy? Ann Surg. 2012;256(6):883-91.
6. Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15(4):361-87.
7. (2019). 2020.
8. Iguchi T, Shirabe K, Aishima S, Wang H, Fujita N, Ninomiya M, et al. New Pathologic Stratification of Microvascular Invasion in Hepatocellular Carcinoma: Predicting Prognosis After Living-donor Liver Transplantation. Transplantation. 2015;99(6):1236-42.
9. Tsilimigras DI, Mehta R, Moris D, Sahara K, Bagante F, Paredes AZ, et al. Utilizing Machine Learning for Pre- and Postoperative Assessment of Patients Undergoing Resection for BCLC-0, A and B Hepatocellular Carcinoma: Implications for Resection Beyond the BCLC Guidelines. Ann Surg Oncol. 2020;27(3):866-74.
10. Banerjee S, Wang DS, Kim HJ, Sirlin CB, Chan MG, Korn RL, et al. A computed tomography radiogenomic biomarker predicts microvascular invasion and clinical outcomes in hepatocellular carcinoma. Hepatology. 2015;62(3):792-800.

11. Chan AWH, Zhong J, Berhane S, Toyoda H, Cucchetti A, Shi K, et al. Development of pre and post-operative models to predict early recurrence of hepatocellular carcinoma after surgical resection. J Hepatol. 2018;69(6):1284-93.

12. Erstad DJ, Tanabe KK. Prognostic and Therapeutic Implications of Microvascular Invasion in Hepatocellular Carcinoma. Ann Surg Oncol. 2019;26(5):1474-93.

13. Roayaie S, Blume IN, Thung SN, Guido M, Fiel MI, Hiotis S, et al. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. Gastroenterology. 2009;137(3):850-5.

14. Erstad DJ, Tanabe KK. Prognostic and Therapeutic Implications of Microvascular Invasion in Hepatocellular Carcinoma. Ann Surg Oncol. 2019;26(5):1474-93.

15. Roayaie S, Blume IN, Thung SN, Guido M, Fiel MI, Hiotis S, et al. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. Gastroenterology. 2009;137(3):850-5.

16. Huang C, Zhu XD, Ji Y, Ding GY, Shi GM, Shen YH, et al. Microvascular invasion has limited clinical values in hepatocellular carcinoma patients at Barcelona Clinic Liver Cancer (BCLC) stages 0 or B. BMC Cancer. 2017;17(1):58.

17. Shindoh J, Andreou A, Aloia TA, Zimmitti G, Lauwers GY, Laurent A, et al. Microvascular invasion does not predict long-term survival in hepatocellular carcinoma up to 2 cm: reappraisal of the staging system for solitary tumors. Ann Surg Oncol. 2013;20(4):1223-9.

18. Huang C, Zhu XD, Ji Y, Ding GY, Shi GM, Shen YH, et al. Microvascular invasion has limited clinical values in hepatocellular carcinoma patients at Barcelona Clinic Liver Cancer (BCLC) stages 0 or B. BMC Cancer. 2017;17(1):58.

19. Li J, Liao Y, Suo L, Zhu P, Chen X, Dang W, et al. A novel prognostic index-neutrophil times y-glutamyl transpeptidase to lymphocyte ratio (NγLR) predicts outcome for patients with hepatocellular carcinoma. Sci Rep. 2017;7(1):9229.

20. Wang H, Wu MC, Cong WM. Microvascular invasion predicts a poor prognosis of solitary hepatocellular carcinoma up to 2 cm based on propensity score matching analysis. Hepatol Res. 2019;49(3):344-54.

21. Huang PY, Wang CC, Lin CC, Lu SN, Wang JH, Hung CH, et al. Predictive Effects of Inflammatory Scores in Patients with BCLC 0-A Hepatocellular Carcinoma after Hepatectomy. J Clin Med. 2019;8(10).

22. Fan W, Zhang Y, Wang Y, Yao X, Yang J, Li J. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios as predictors of survival and metastasis for recurrent hepatocellular carcinoma after transarterial chemoembolization. PLoS One. 2015;10(3):e0119312.

23. Goh BK, Kam JH, Lee SY, Chan CY, Allen JC, Jeyaraj P, et al. Significance of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and prognostic nutrition index as preoperative predictors of early mortality after liver resection for huge (≥10 cm) hepatocellular carcinoma. J Surg Oncol. 2016;113(6):621-7.

24. Liao W, Zhang J, Zhu Q, Qin L, Yao W, Lei B, et al. Preoperative Neutrophil-to-Lymphocyte Ratio as a New Prognostic Marker in Hepatocellular Carcinoma after Curative Resection. Transl Oncol. 2014;7(2):248-55.

25. Lin ZX, Ruan DY, Li Y, Wu DH, Ma XK, Chen J, et al. Lymphocyte-to-monocyte ratio predicts survival of patients with hepatocellular carcinoma after curative resection. World J Gastroenterol. 2015;21(38):10898-906.

26. Galizia G, Auricchio A, de Vita F, Cardella F, Mabilia A, Basile N, et al. Inflammatory and nutritional status is a predictor of long-term outcome in patients undergoing surgery for gastric cancer. Validation of the Naples prognostic score. Ann Ital Chir. 2019;90:404-16.

27. Kosuga T, Konishi T, Kubota T, Shoda K, Konishi H, Shiozaki A, et al. Value of Prognostic Nutritional Index as a Predictor of Lymph Node Metastasis in Gastric Cancer. Anticancer Res. 2019;39(12):6843-9.

28. Bai X, Feng L. Correlation between Prognostic Nutritional Index, Glasgow Prognostic Score, Systemic Inflammatory Response, and TNM Staging in Colorectal Cancer Patients. Nutr Cancer. 2020;72(7):1170-7.

29. Chen K, Ma J, Jia X, Ai W, Ma Z, Pan Q. Advancing the understanding of NAFLD to hepatocellular carcinoma development: From experimental models to humans. Biochim Biophys Acta Rev Cancer. 2019;1871(1):117-25.
30. Shi C, Xue W, Han B, Yang F, Yin Y, Hu C. Acetaminophen aggravates fat accumulation in NAFLD by inhibiting autophagy via the AMPK/mTOR pathway. Eur J Pharmacol. 2019;850:15-22.

31. Dou X, Li S, Hu L, Ding L, Ma Y, Ma W, et al. Glutathione disulfide sensitizes hepatocytes to TNFα-mediated cytotoxicity via IKK-β S-glutathionylation: a potential mechanism underlying non-alcoholic fatty liver disease. Exp Mol Med. 2018;50(4):1-16.

32. Bai Y, Pei W, Zhang X, Zheng H, Hua C, Min J, et al. ApoM is an important potential protective factor in the pathogenesis of primary liver cancer. J Cancer. 2021;12(15):4661-71.

Tables

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            |
| 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          |
|                          | 1st Month | 2nd Month | 3rd Month |
|--------------------------|-----------|-----------|-----------|
| **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  |
| **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
Table 2. Univariate 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, ≤3.7/>3.7×10⁹/L                    | 2.41 (0.98-6.02)      | 0.055                 | 1.21 (1.06-1.52)      | 0.013                 |
| PLT, ≤160/>160×10⁹/L                    | 1.76(1.12-2.77)       | 0.018                 | 1.00 (1.00-1.00)      | 0.027                 |
| Hb, ≤125/>125×10⁹/L                    | 2.05 (0.81-5.06)      | 0.133                 | 0.99 (0.97-1.12)      | 0.312                 |
| Hematocrit, ≤42.2/>42.2%               | 1.56 (0.67-2.32)      | 0.073                 | 0.98 (0.91-1.12)      | 0.692                 |
| MCV, ≤87/>87fL                          | 0.66 (0.42-1.03)      | 0.061                 | 0.95 (0.90-1.01)      | 0.055                 |
| MCH, ≤31.5/>31.5pg                      | 0.45 (0.28-0.72)      | <0.001                | 0.85 (0.76-0.96)      | 0.007                 |
| Lymphocyte, ≤1.5/>1.5×10⁹/L            | 0.93(0.60-1.45)       | 0.732                 | 0.86 (0.52-1.55)      | 0.585                 |
| Neutrophil, ≤3.3/>3.3×10⁹/L             | 2.02(1.01-3.80)       | 0.045                 | 1.43 (1.14-1.72)      | 0.002                 |
| Monocyte, ≤0.5/>0.5×10⁹/L               | 1.54(0.89-2.60)       | 0.126                 | 7.51 (1.63-15.86)     | 0.012                 |
| LR, ≤41.6/>41.6%                        | 0.56 (0.33-0.96)      | 0.036                 | 0.95 (0.92-0.99)      | 0.011                 |
| NR, ≤47.8/>47.8%                        | 1.60 (0.97-2.52)      | 0.068                 | 1.05 (1.00-1.10)      | 0.033                 |
| RDW, ≤13.6/>13.6%                       | 1.81 (1.12-3.01)      | 0.016                 | 1.12 (0.89-1.56)      | 0.292                 |
| RBC, ≤4.5/>4.5×10⁹/L                    | 1.35 (0.86-2.63)      | 0.152                 | 1.22 (0.66-2.24)      | 0.533                 |
| AFP, <400/>≥400μg/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/>≥50×10⁹ IU/mL             | 1.74 (1.12-2.96)      | 0.016                 | 1.00 (1.00-1.00)      | 0.971                 |
| Albumin, <50/>≥50g/L                    | 0.47 (0.23-0.93)      | 0.031                 | 0.89 (0.82-0.97)      | 0.008                 |
| Scr, <76/>≥76μmol/L                     | 0.62 (0.34-1.12)      | 0.123                 | 0.98 (0.96-1.02)      | 0.240                 |
| γ-GGT, <34/>≥34U/L                      | 2.10 (1.12-3.95)      | 0.021                 | 1.00 (1.00-1.00)      | 0.541                 |
| ALP, <76/>≥76 & <117/>≥117U/L           | 4.75 (2.61-8.56)      | <0.001                | 1.00 (1.00-1.00)      | 0.182                 |
| TBil, <16.4/>≥16.4μmol/L                | 1.44 (0.88-2.12)      | 0.169                 | 0.97 (0.93-1.24)      | 0.313                 |
| DBil, <5.6/>≥5.6μmol/L                  | 1.52 (0.97-2.30)      | 0.065                 | 0.95 (0.83-1.10)      | 0.415                 |
| IBil, <6.6/>≥6.6μmol/L                  | 1.55 (0.83-2.70)      | 0.178                 | 0.94 (0.86-1.00)      | 0.182                 |
| 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                 |
| Parameter                  | Reference | Hazard Ratio | 95% CI       | P-value |
|---------------------------|-----------|--------------|--------------|---------|
| 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(1.34-3.7) | 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 |
| 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

| Parameter  | Hazard Ratio | 95% CI       | P-value |
|------------|--------------|--------------|---------|
| 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       | -       |
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-GT, γ-glutamyl transpeptidase; ALP, alkaline phophatase; TBil, total bilirubin; DBil, direct bilirubin; IBil, indirect bilirubin; TBA, total bile acid; TP, total protein; ALB, albumin; GLB, globumin; 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.

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                        |
|                   | 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       |
| 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      |
| 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      |
| 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      |
| 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      |

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.

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.

p<0.001
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; (A) 8-month, 1, 2, and 3-year OS in the external validation cohort;