Development and Validation of Prognostic Nomograms Predicting Recurrence and Survival in Patients with Solitary Hepatocellular Carcinoma Following Curative Liver Resection

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Abstract

Background: This work was designed to establish and verify our nomograms integrating clinicopathological characteristics with hematological biomarkers to predict both disease-free survival (DFS) and overall survival (OS) in solitary hepatocellular carcinoma (HCC) patients following hepatectomy.

Methods: We scrutinized the data retrospectively from 414 patients with a clinicopathological diagnosis of solitary HCC from Guangxi Medical University Cancer Hospital (Nanning, China) between January 2004 and December 2012. Following the random separation of the samples in a 7:3 ratio into the training set and validation set, the former set was assessed by Cox regression analysis to develop two nomograms to predict the 1-year and 3-year DFS and OS (3-years and 5-years). This was followed by discrimination and calibration estimation employing Harrell’s C-index (C-index) and calibration curves, while the internal validation was also assessed.

Results: In the training cohort, the tumor diameter, tumor capsule, macrovascular invasion, and alpha-fetoprotein (AFP) were included in the DFS nomogram. Age, tumor diameter, tumor capsule, macrovascular invasion, microvascular invasion, and aspartate aminotransferase (AST) were included in the OS nomogram. The C-index was 0.691 (95% CI: 0.644-0.738) for the DFS-nomogram and 0.713 (95% CI: 0.670-0.756) for the OS-nomogram. The survival probability calibration curves displayed a fine agreement between the predicted and observed ranges in both data sets.

Conclusion: Our nomograms combined clinicopathological features with hematological biomarkers to emerge effective in predicting the DFS and OS in solitary HCC patients following curative liver resection. Therefore, the potential utility of our nomograms for guiding individualized treatment clinically and monitor the recurrence monitoring in these patients.

Introduction

According to the Global Cancer Statistics 2018, liver cancer is the sixth most ubiquitous type of cancer and occupies the fourth rank as a global predominant cause of death due to cancer worldwide. Hepatocellular carcinoma (HCC) makes up 75% to 85% of primary liver cancers diagnosed [1]. It is estimated that approximately 383,000 patients lose lives annually from liver cancer in China, accounting for 51% of global liver cancer-related deaths because of the elevated hepatitis B virus (HBV) infection trend [2]. Over the past few years, while sizeable progress has been made in HCC treatments, curative liver resection is still a major approach[3]. Unfortunately, the high recurrence (intra- and extrahepatic) remains a challenge in the long-term prognosis [4]. Hence, it becomes vital to further elucidate the prognostic factors of HCC patients following curative liver resection. Currently, the most widespread HCC staging system employed is the Barcelona Clinic Liver Cancer (BCLC) classification, which is the only staging system with robust prospective validation [5]. This system is valuable in prognostic prediction and guiding disease management. However, for single HCC tumors, clarity is yet to see the light of the day. In
1999, the BCLC staging system was first proposed and such single tumors were classified as BCLC stage A [6]. Bruix et al (2002) classified single HCC (>5 cm) as intermediate stage (B)[7]. Interestingly, in the flowchart of the latest updated version, only multinodular HCC was mentioned as intermediate-stage hepatocellular carcinoma (BCLC B)[8]. Mazzaferro et al and Torzilli et al presented a strong discussion in HCC staging in patients with a single tumor more than 5 cm in diameter[9]. Obviously, a consensus is yet to see the light of the day. Further, a specific staging system for solitary HCCs is yet to be unveiled. These concerns necessitate the urgent establishment of a consistent prognostic model to predict the prognoses of solitary HCC patients following hepatectomy.

Shen et al developed an advancement over the current system as a solitary HCC postoperative prognosis model encompassing tumor size, microvascular invasion, and major vascular invasion to predict 3- and 5-year survival [10]. An earlier study showed that age, gender, HBV infection, lack of a tumor capsule, cirrhosis, and an alphafetal protein (AFP) level >400 μg/l were also independent risk factors influencing solitary HCC survival and recurrence in patients undergoing curative partial hepatectomy [11, 12]. In addition to clinicopathological factors and AFP, studies have unveiled that other blood biomarkers are related to HCC prognosis. These biomarkers are inclusive of the platelet count, serum albumin levels[13], neutrophil-to-lymphocyte ratio (NLR)[14], Aspartate aminotransferase-platelet ratio index (APRI)[15], platelet-to-lymphocyte ratio (PLR)[16] and the prognostic nutritional index (PNI)[17]. Therefore, such biomarkers related to inflammatory cells in the peripheral blood and liver biological functional aspects may be employed to check the prognosis or the survival rate of HCC patients. Nomograms, estimating an individual probability of a clinical event by integrating a slew of prognostic and determinant variables fit the bill for biologically and clinically integrated models while satisfying our quest for personalized medicine [18]. Studies have documented the widespread usage of nomograms in the prognosis of a slew of malignancies such as gastric cancer, pancreatic ductal adenocarcinoma, and hepatocellular carcinoma [19-21].

The present study aimed to develop and verify two nomograms integrating clinicopathological factors with hematologic markers to predict disease-free survival (DFS) and overall survival (OS) in solitary HCC patients following curative liver resection.

**Materials And Methods**

**Patient selection**

This entailed a retrospective analysis of 414 patients with Solitary HCC after curative liver resection between January 2004 and December 2012 at the Guangxi Medical University Cancer Hospital (Nanning, China). Randomly grouping of the patients was done into the training set (n=290) and the validation set (n=124) (7:3 ratio). The inclusion criteria included: (1) solitary HCC confirmed via pathology; (2) initial treatment HCC process; (3) without extrahepatic metastasis and any other malignancy. The exclusion
criteria included: (1) anti-inflammatory drug or immunosuppressive therapy administration in patients three months prior to the operation, or reports of chronic inflammatory diseases; (2) pregnancy; (3) patients with myocardial infarction or muscle damage; (4) incomplete clinicopathological data or lost to follow-up. This retrospective study received approval from the Ethics Committee of the aforementioned hospital and was conducted as per the Declaration of Helsinki protocols. No informed consent was needed as this work entailed the review of anonymous patient data, and did not involve using human tissue samples or patient intervention.

**Clinical Data Collection**

According to the electronic medical records (EMR) system from the Guangxi Medical University Cancer Hospital, the enlisted clinical-pathological data obtained were gender, age (in years), liver cirrhosis, Child-Pugh class, tumor diameter (cm), tumor capsule, macrovascular invasion, and microvascular invasion. Further, the hematological characteristics (measured within one week prior to surgery) were leukocyte, neutrophil, lymphocyte, platelet counts, along with aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, Prothrombin time (PT), Direct Bilirubin (DBIL), total bilirubin (TBLI) and alpha-fetoprotein (AFP) levels. Then, we computed the following aspects of inflammation such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), AST to platelet ratio index (APRI), systemic immune-inflammation index (SII), prognostic nutritional index (PNI), AST to neutrophil ratio index (ANRI), and AST to lymphocyte ratio index (ALRI).

**The Follow-up**

Follow-up of all patients as in accordance with the National Comprehensive Cancer Network (NCCN) guidelines after primary surgery. The follow-up was every three to six months in the initial two years. Patients displaying no signs of recurrence were followed up every six months in the third to fifth years until tumor recurrence was observed, after which it was on an annual basis. The assessments incorporated physical examinations, liver ultrasound, dynamic chest computed tomography, laboratory tests (encompassing blood routine examination, liver function test, and serum AFP analysis). June 2020 was the last follow-up time. The endpoint of our study was OS and DFS. While the time from surgery to death or the last follow-up was the OS, the time from surgery to local HCC recurrence or distant metastasis was the DFS.

**Statistical analysis**

R software (version 4.0.2; http://www.Rproject.org) was employed for this purpose. Except for age, tumor diameter, and AFP, all continuous variables were divided into three categories: ≤Q1, Q1-Q3, and ≥Q3 according to the first quartile (Q1) and the third quartile (Q3). The expression of categorical variables was as frequency and percentage subjected to analyses via the Chi-square test or Fisher's exact test.
Following the 7:3 assignment of the samples as described above, a Cox regression model was performed in the training set to probe the candidate variables by univariate and multivariate analyses. The latter analyses encompassed variables with \( P < 0.05 \) in the univariate test, and progressive candidate factors were implemented by backward stepwise selection. Then, two nomograms showing the DFS (1- and 3-years) and OS (3- and 5-year) were constructed using the training set's multivariate Cox regression analysis results (employing the rms package in R). The discrimination and calibration of the nomograms were scrutinized by Harrell's C-index (C-index) and calibration curves with the assessment of the internal verification. The maximum value of the C-index was 1.0 that was demonstrative of the strong predictive capacity of the model while a value of 0.5 demonstrated no discrimination[22]. Finally, employing the constructed Cox regression model, we calculated each patient's total scores. We classified patients into the following three prognostic risk subgroups based on the first quartile and third quartile as the high-risk group (≥Q3), medium-risk group(Q1-Q3) and low-risk group(≤Q1). We analyzed these risk subgroups compared Kaplan-Meier survival curves across the groups employing the log-rank test, and the statistical tests employed were two-tailed with statistical significance at \( P<0.05 \).

Results

Fundamental Patient Characteristics

The random separation of all the enrolled 414 patients with solitary HCC following curative liver resection was described above with 70% in the training cohort and 30% in the validation cohort. The baseline features of both groups are displayed in Table 1. In the training set, 255 patients (87.93%) were males, 216 patients (74.48%) were over 60 years old while 254 patients (87.59%) had positive HBV infection. Pathological examination corroborated that 240 cases (82.76%) had liver cirrhosis and 26 (8.97%) had a microvascular invasion. In terms of capsular infiltration, 104(35.86%) patients exhibited an incomplete tumor capsule and 48 (16.55%) of them lacked a tumor capsule. Moreover, more than 95% of cases exhibited Child-Pugh class A liver function, 61.38% had tumor diameter >5 cm, 14.48% of them suffered from the macrovascular invasion and 19.31% demonstrated serum AFP content >400 ng/ml. There were no conspicuous differences between the training cohort and validation cohort with regard to the distribution of these features. For all patients, the median DFS was 23 months (95% CI: 18-33.9 months), with 59.5% and 39.6% being the DFS at one and three years, respectively. The OS values at three years and five years were 55.3% and 39.2%, respectively, with a median survival of 43 months.

Table 1: Baseline features
| Variable               | Training cohort (N=290) | validation cohort (N=124) | χ²  | P     |
|-----------------------|-------------------------|---------------------------|-----|-------|
|                       | NO (%)                  | NO (%)                    |     |       |
| **Sex**               |                         |                           | 0.06| 0.813 |
| Female                | 35(12.07)               | 16(12.9)                  |     |       |
| Male                  | 255(87.93)              | 108(87.1)                 |     |       |
| **Age (year)**        |                         |                           | 0.05| 0.8156|
| ≤60                   | 216(74.48)              | 91(73.39)                 |     |       |
| >60                   | 74(25.52)               | 33(26.61)                 |     |       |
| **Cirrhosis**         |                         |                           | 0.02| 0.902 |
| No                    | 50(17.24)               | 22(17.74)                 |     |       |
| Yes                   | 240(82.76)              | 102(82.26)                |     |       |
| **ChildPugh**         |                         |                           | 0.03| 0.874 |
| A                     | 277(95.52)              | 118(95.16)                |     |       |
| B                     | 13(4.48)                | 6(4.84)                   |     |       |
| **Tumordiameter (cm)**|                         |                           | 0.23| 0.6323|
| ≤5                    | 112(38.62)              | 51(41.13)                 |     |       |
| >5                    | 178(61.38)              | 73(58.87)                 |     |       |
| **Tumorcapsule**      |                         |                           | 1.35| 0.5079|
| Complete              | 138(47.59)              | 63(50.81)                 |     |       |
| incomplete            | 104(35.86)              | 46(37.1)                  |     |       |
| Absence               | 48(16.55)               | 15(12.1)                  |     |       |
| **Macrovascular invasion** |                     |                           | 0.42| 0.5187|
| No                    | 248(85.52)              | 109(87.9)                 |     |       |
| Yes                   | 42(14.48)               | 15(12.1)                  |     |       |
| **Microvascular invasion** |                     |                           | 0.54| 0.4633|
| No                    | 264(91.03)              | 110(88.71)                |     |       |
| Yes                   | 26(8.97)                | 14(11.29)                 |     |       |
| **HBV**               |                         |                           | 0.34| 0.5607|
| Variable          | Training cohort (N=290) | validation cohort (N=124) | \( \chi^2 \) | \( P \) |
|-------------------|-------------------------|---------------------------|--------------|------|
| No (%)            | 36(12.41)               | 18(14.52)                 | 2.28         | 0.32 |
| Yes (%)           | 254(87.59)              | 106(85.48)                |              |      |
| Leukocyte \(10^9/\text{L} \) |                      |                           |              |      |
| \( \leq 5.28 \)  | 67(23.1)                | 37(29.84)                 | 0.42         | 0.8104 |
| 5.28~8.1         | 148(51.03)              | 60(48.39)                 |              |      |
| \( >8.1 \)       | 75(25.86)               | 27(21.77)                 |              |      |
| Lymphocyte \(10^9/\text{L} \) |                      |                           |              |      |
| \( \leq 1.3 \)   | 79(27.24)               | 30(24.19)                 | 0.43         | 0.8083 |
| 1.3~2.08         | 140(48.28)              | 62(50)                    |              |      |
| \( >2.08 \)      | 71(24.48)               | 32(25.81)                 |              |      |
| Neutrophil \(10^9/\text{L} \) |                      |                           |              |      |
| \( \leq 3 \)     | 75(25.86)               | 29(23.39)                 | 0.52         | 0.7728 |
| 3~5.79           | 145(50)                 | 62(50)                    |              |      |
| \( >5.79 \)      | 70(24.14)               | 33(26.61)                 |              |      |
| Platelet \(10^9/\text{L} \) |                      |                           |              |      |
| \( \leq 134 \)   | 74(25.52)               | 31(25)                    | 0.21         | 0.8998 |
| 134~239.5        | 146(50.34)              | 59(47.58)                 |              |      |
| \( >239.5 \)     | 70(24.14)               | 34(27.42)                 |              |      |
| NLR               |                         |                           |              |      |
| \( \leq 1.79 \)  | 73(25.17)               | 32(25.81)                 |              |      |
| 1.79~3.73        | 143(49.31)              | 63(50.81)                 |              |      |
| \( >3.73 \)      | 74(25.52)               | 29(23.39)                 |              |      |
| PLR               |                         |                           |              |      |
| \( \leq 79.59 \) | 73(25.17)               | 31(25)                    |              |      |
| 79.59~155.32     | 144(49.66)              | 62(50)                    |              |      |
| \( >155.32 \)    | 73(25.17)               | 31(25)                    |              |      |
| Variable | Training cohort (N=290) | Validation cohort (N=124) | $\chi^2$ | $P$ |
|----------|-------------------------|---------------------------|--------|----|
|          | NO (%)                  | NO (%)                    |        |    |
| ALBI     |                         |                           | 0.03   | 0.9869 |
| $\leq-2.9$ | 73(25.17) | 31(25)       |        |    |
| -2.9~2.39 | 145(50)   | 63(50.81)     |        |    |
| $>2.39$  | 72(24.83)  | 30(24.19)     |        |    |
| APRI     |                         |                           | 1.79   | 0.4084 |
| $\leq0.42$ | 69(23.79) | 35(28.23)     |        |    |
| 0.42~1.02 | 144(49.66) | 63(50.81)    |        |    |
| $>1.02$  | 77(26.55)  | 26(20.97)     |        |    |
| SII      |                         |                           | 1.59   | 0.452 |
| $\leq274.86$ | 69(23.79) | 35(28.23)     |        |    |
| 274.86~810.49 | 150(51.72) | 56(45.16)   |        |    |
| $>810.49$ | 71(24.48)  | 33(26.61)     |        |    |
| ANRI     |                         |                           | 2.64   | 0.2671 |
| $\leq6.7$ | 67(23.1)  | 37(29.84)     |        |    |
| 6.7~17.69 | 151(52.07) | 55(44.35)    |        |    |
| $>17.69$ | 72(24.83)  | 32(25.81)     |        |    |
| ALRI     |                         |                           | 0.55   | 0.7581 |
| $\leq18.59$ | 70(24.14) | 34(27.42)     |        |    |
| 18.59~44.38 | 146(50.34) | 61(49.19)   |        |    |
| $>44.38$ | 74(25.52)  | 29(23.39)     |        |    |
| PNI      |                         |                           | 0.31   | 0.8577 |
| $\leq45.17$ | 73(25.17) | 31(25)       |        |    |
| 45.17~51.2 | 147(50.69) | 60(48.39)   |        |    |
| $>51.2$  | 70(24.14)  | 33(26.61)     |        |    |
| PTsã      |                         |                           | 2.02   | 0.365 |
| $\leq11.9$ | 78(26.9)  | 29(23.39)     |        |    |
| Variable     | Training cohort (N=290) | validation cohort (N=124) | $\chi^2$ | $P$  |
|--------------|-------------------------|---------------------------|---------|------|
|              | NO (%)                  | NO (%)                    |         |      |
| 11.9~13.9    | 137(47.24)              | 68(54.84)                 |         |      |
| >13.9        | 75(25.86)               | 27(21.77)                 |         |      |
| **Albumin (g/L)** &nbsp; |                      |                           | 0.1     | 0.9534 |
| ≤36.9        | 74(25.52)               | 33(26.61)                 |         |      |
| 36.9~42.5    | 145(50)                 | 60(48.39)                 |         |      |
| >42.5        | 71(24.48)               | 31(25)                    |         |      |
| **AST (U/L)** &nbsp; |                      |                           | 0.51    | 0.7749 |
| ≤33.25       | 71(24.48)               | 33(26.61)                 |         |      |
| 33.25~66     | 147(50.69)              | 64(51.61)                 |         |      |
| >66          | 72(24.83)               | 27(21.77)                 |         |      |
| **ALT (U/L)** &nbsp; |                      |                           | 0.3     | 0.8619 |
| ≤28          | 75(25.86)               | 33(26.61)                 |         |      |
| 28~59        | 146(50.34)              | 59(47.58)                 |         |      |
| >59          | 69(23.79)               | 32(25.81)                 |         |      |
| **DBIL (umol/L)** &nbsp; |                     |                            | 2.29    | 0.3179 |
| ≤3.1         | 77(26.55)               | 31(25)                    |         |      |
| 3.1~6.27     | 135(46.55)              | 67(54.03)                 |         |      |
| >6.27        | 78(26.9)                | 26(20.97)                 |         |      |
| **TBIL (umol/L)** &nbsp; |                     |                            | 2.55    | 0.2797 |
| ≤9.5         | 78(26.9)                | 30(24.19)                 |         |      |
| 9.5~17       | 135(46.55)              | 68(54.84)                 |         |      |
| >17          | 77(26.55)               | 26(20.97)                 |         |      |
| **AFP (ng/ml)** &nbsp; |                     |                            | 1.36    | 0.2435 |
| ≤400         | 234(80.69)              | 106(85.48)                |         |      |
| >400         | 56(19.31)               | 18(14.52)                 |         |      |

**Cox Analysis of the Training Cohort**
The univariate and multivariate regression analysis of clinicopathological characteristics and blood-based biomarkers of 229 solitary HCC patients are shown (Table 2 and Table 3). In the univariate analysis, tumor diameter, tumor capsule, macrovascular invasion, microvascular invasion, ALRI, albumin, and AFP were associated with DFS, while age, tumor diameter, tumor capsule, macrovascular invasion, microvascular invasion, AST, NLR, APRI, ALRI, PNI, albumin, and AFP were associated with OS ($p<0.05$). Further independent prognostic factors were probed by backward stepwise selection. Tumor diameter, tumor capsule, macrovascular invasion, and AFP emerged as independent prognostic factors for DFS, whereas for OS, age, tumor diameter, tumor capsule, macrovascular invasion, microvascular invasion, and AST were the factors unveiled.

Table 2: Regression Analysis of DFS in the Training Cohort
|                                | Univariate Analysis |          | Multivariate Analysis |          |
|--------------------------------|---------------------|----------|-----------------------|----------|
|                                | HR(95%CI)           | P        | HR(95%CI)             | P        |
| Sex                            |                     |          |                       |          |
| female                         | Ref                 |          |                       |          |
| male                           | 0.97(0.59-1.62)     | 0.9191   |                       |          |
| Age (year)                     |                     |          |                       |          |
| ≤60                            | Ref                 |          |                       |          |
| >60                            | 1.19(0.85-1.68)     | 0.3095   |                       |          |
| Cirrhosis                      |                     |          |                       |          |
| no                             | Ref                 |          |                       |          |
| yes                            | 0.93(0.62-1.4)      | 0.7269   |                       |          |
| Child Pugh                     |                     |          |                       |          |
| A                              | Ref                 |          |                       |          |
| B                              | 1.13(0.55-2.3)      | 0.7388   |                       |          |
| Tumor diameter (cm)            |                     |          |                       |          |
| ≤5                             | Ref                 |          | Ref                   |          |
| >5                             | 2.09(1.48-2.94)     | <0.0001  | 1.63(1.14-2.34)       | 0.0071   |
| Tumor capsule                  |                     |          |                       |          |
| complete                       | Ref                 |          | Ref                   |          |
| incomplete                     | 2(1.41-2.84)        | 0.0001   | 1.67(1.16-2.39)       | 0.0059   |
| absence                        | 1.85(1.17-2.91)     | 0.0079   | 1.67(1.06-2.64)       | 0.0272   |
| Macrovascular invasion         |                     |          |                       |          |
| no                             | Ref                 |          | Ref                   |          |
| yes                            | 3.6(2.37-5.48)      | <0.0001  | 2.49(1.6-3.87)        | 0.0001   |
| Microvascular invasion         |                     |          |                       |          |
| no                             | Ref                 |          |                       |          |
| yes                            | 2.55(1.54-4.22)     | 0.0003   |                       |          |
| AST (U/L)                      |                     |          |                       |          |
| ≤33.25                         | Ref                 |          |                       |          |
|                | 33.25~66 | 1.17(0.69-1.96) | 0.5602 |
|----------------|----------|-----------------|--------|
| >66            |          |                 |        |
| **HBV**        | Ref      |                 |        |
| no             | 1.25(0.84-1.87) | 0.2699       |
| yes            | 1.42(0.89-2.25) | 0.1386       |
| **Leukocyte** 10^9/L |        |                 |        |
| ≤5.28         | Ref      |                 |        |
| 5.28~8.1      | 0.76(0.52-1.09) | 0.1351       |
| >8.1          | 0.67(0.43-1.04) | 0.0727       |
| **Lymphocyte** 10^9/L |        |                 |        |
| ≤1.3          | Ref      |                 |        |
| 1.3~2.08      | 1.38(0.94-2.04) | 0.1036       |
| >2.08         | 0.94(0.58-1.5)  | 0.7878       |
| **Neutrophil** 10^9/L |        |                 |        |
| ≤3            | Ref      |                 |        |
| 3~5.79        | 1.25(0.84-1.85) | 0.2704       |
| >5.79         | 1.23(0.78-1.94) | 0.3722       |
| **Platelet** 10^9/L |        |                 |        |
| ≤134          | Ref      |                 |        |
| 134~239.5     | 1.13(0.78-1.64) | 0.521        |
| >239.5        | 1.14(0.73-1.77) | 0.5766       |
| **NLR**       |          |                 |        |
| ≤1.79         | Ref      |                 |        |
| 1.79~3.73     | 1.48(0.99-2.21) | 0.0591       |
| >3.73         | 1.49(0.94-2.36) | 0.0924       |
| **PLR**       |          |                 |        |
| ≤79.59        | Ref      |                 |        |
| 79.59~155.32  | 1.06(0.72-1.57) | 0.7627       |
| >155.32       | 1.23(0.79-1.91) | 0.3653       |
|        |        |        |        |
|--------|--------|--------|--------|
| ALBI   |        |        |        |
| ≤-2.9  | Ref    |        |        |
| -2.9~ -2.39 | 1.28(0.86-1.92) | 0.2227 |
| >-2.39 | 1.2(0.75-1.92) | 0.4423 |
| APRI   |        |        |        |
| ≤0.42  | Ref    |        |        |
| 0.42~1.02 | 1.25(0.85-1.85) | 0.251  |
| >1.02  | 1.24(0.79-1.96) | 0.3442 |
| SII    |        |        |        |
| ≤274.86 | Ref    |        |        |
| 274.86~810.49 | 1.48(0.97-2.26) | 0.0663 |
| >810.49 | 1.5(0.92-2.44) | 0.1039 |
| ANRI   |        |        |        |
| ≤6.7   | Ref    |        |        |
| 6.7~17.69 | 0.9(0.61-1.34) | 0.6182 |
| >17.69 | 1.48(0.96-2.28) | 0.0726 |
| ALRI   |        |        |        |
| ≤18.59 | Ref    |        |        |
| 18.59~44.38 | 0.86(0.6-1.25) | 0.4318 |
| >44.38 | 0.6(0.38-0.95) | 0.0278 |
| PNI    |        |        |        |
| ≤45.17 | Ref    |        |        |
| 45.17~51.2 | 1.12(0.76-1.64) | 0.5657 |
| >51.2  | 1.21(0.77-1.91) | 0.3994 |
| PT (s) |        |        |        |
| ≤11.9  | Ref    |        |        |
| 11.9~13.9 | 0.82(0.57-1.19) | 0.2891 |
| >13.9  | 0.71(0.45-1.12) | 0.1368 |
| Albumin (g/L) |        |        |        |
| Value  | Hazard Ratio | 95% CI      | P-value |
|--------|--------------|-------------|---------|
| ≤36.9  | Ref          |             |         |
| 36.9~42.5 | 1.18(0.79-1.79) | 0.4204     |         |
| >42.5  | 1.63(1.03-2.58) | 0.0358     |         |

**ALT (U/L)**

| Value  | Hazard Ratio | 95% CI      | P-value |
|--------|--------------|-------------|---------|
| ≤28    | Ref          |             |         |
| 28~59  | 1.08(0.73-1.6) | 0.6908     |         |
| >59    | 1.07(0.68-1.69) | 0.7654     |         |

**DBIL (umol/L)**

| Value   | Hazard Ratio | 95% CI      | P-value |
|---------|--------------|-------------|---------|
| ≤3.1    | Ref          |             |         |
| 3.1~6.27 | 1.11(0.74-1.65) | 0.6213     |         |
| >6.27   | 1.3(0.84-2.02) | 0.2348     |         |

**TBIL (umol/L)**

| Value   | Hazard Ratio | 95% CI      | P-value |
|---------|--------------|-------------|---------|
| ≤9.5    | Ref          |             |         |
| 9.5~17  | 1.02(0.69-1.51) | 0.9199     |         |
| >17     | 1.16(0.75-1.8) | 0.5165     |         |

**AFP (ng/ml)**

| Value  | Hazard Ratio | 95% CI      | P-value |
|--------|--------------|-------------|---------|
| ≤400   | Ref          |             |         |
| >400   | 1.98(1.36-2.9) | 0.0004     |         |

Table 3: Regression Analysis of OS in the Training Cohort (HR: Hazard Ratio)
| Variable          | Univariate Analysis |             |             | Multivariate Analysis |             |             |
|-------------------|---------------------|-------------|-------------|-----------------------|-------------|-------------|
|                   | HR (95%CI)          | P           | HR (95%CI)  | P                     |
| **Sex**           |                     |             |             |                       |             |             |
| Female            | Ref                 |             |             |                       |             |             |
| Male              | 1.11(0.66-1.86)     | 0.7022      |             |                       |             |             |
| **Age(year)**     |                     |             |             |                       |             |             |
| ≤60               | Ref                 |             |             |                       |             |             |
| >60               | 1.73(1.25-2.39)     | 0.0009      | 1.59(1.14-2.23)| 0.0066               |             |             |
| **Cirrhosis**     |                     |             |             |                       |             |             |
| No                | Ref                 |             |             |                       |             |             |
| Yes               | 0.94(0.62-1.41)     | 0.7564      |             |                       |             |             |
| **Child Pugh**    |                     |             |             |                       |             |             |
| A                 | Ref                 |             |             |                       |             |             |
| B                 | 1.01(0.5-2.06)      | 0.9757      |             |                       |             |             |
| **Tumordiameter (cm)** |             |             |             |                       |             |             |
| ≤5                | Ref                 |             |             |                       |             |             |
| >5                | 1.91(1.37-2.66)     | 0.0001      | 1.46(1.02-2.1) | 0.0382               |             |             |
| **Tumorcapsule**  |                     |             |             |                       |             |             |
| Complete          | Ref                 |             |             |                       |             |             |
| Incomplete        | 2.01(1.42-2.83)     | 0.0001      | 1.67(1.16-2.41)| 0.0062               |             |             |
| Absence           | 2.01(1.28-3.15)     | 0.0024      | 1.92(1.21-3.05)| 0.0053               |             |             |
| **Macrovascular invasion** |             |             |             |                       |             |             |
| No                | Ref                 |             |             |                       |             |             |
| Yes               | 4.63(3.11-6.89)     | <0.001      | 5.33(2.84-10.02)| <0.001              |             |             |
| **Microvascular invasion** |             |             |             |                       |             |             |
| No                | Ref                 |             |             |                       |             |             |
| Yes               | 3.15(1.96-5.07)     | <0.001      | 0.46(0.22-0.98) | 0.0436               |             |             |
| **AST (U/L)**     |                     |             |             |                       |             |             |
| ≤33.25            | Ref                 |             |             |                       |             |             |
|   | 33.25~66 | >66 | HBV   | No  | Yes  |
|---|---------|-----|-------|-----|------|
|   | 0.99(0.66-1.51) | 2.24(1.45-3.47) | 0.99(0.66-1.51) | 2.24(1.45-3.47) |
|   | 0.9758 | 0.0003 | 0.1174 | 0.1174 |
|   | 0.88(0.57-1.35) | 1.46(0.91-2.34) | 0.5621 | 0.5621 |
| Leukocyte $10^9/L$ |   |   |   |   |
| ≤ 5.28 | Ref | 1.11(0.75-1.64) | 0.5945 | 0.5945 |
| 5.28~8.1 | 1.11(0.75-1.64) | 0.5945 | 0.5945 |
| >8.1 | 1.25(0.8-1.95) | 0.3374 | 0.3374 |
| Lymphocyte $10^9/L$ |   |   |   |   |
| ≤ 1.3 | Ref | 1.05(0.72-1.53) | 0.8049 | 0.8049 |
| 1.3~2.08 | 1.05(0.72-1.53) | 0.8049 | 0.8049 |
| >2.08 | 0.7(0.45-1.09) | 0.118 | 0.118 |
| Neutrophil $10^9/L$ |   |   |   |   |
| ≤ 3 | Ref | 1.09(0.75-1.6) | 0.6528 | 0.6528 |
| 3~5.79 | 1.09(0.75-1.6) | 0.6528 | 0.6528 |
| >5.79 | 1.23(0.8-1.91) | 0.3463 | 0.3463 |
| Platelet $10^9/L$ |   |   |   |   |
| ≤ 134 | Ref | 0.7(0.49-1.02) | 0.0609 | 0.0609 |
| 134~239.5 | 0.7(0.49-1.02) | 0.0609 | 0.0609 |
| >239.5 | 0.75(0.49-1.14) | 0.1797 | 0.1797 |
| NLR |   |   |   |   |
| ≤ 1.79 | Ref | 1.16(0.79-1.69) | 0.4578 | 0.4578 |
| 1.79~3.73 | 1.16(0.79-1.69) | 0.4578 | 0.4578 |
| >3.73 | 1.6(1.03-2.47) | 0.0348 | 0.0348 |
| PLR |   |   |   |   |
| ≤ 79.59 | Ref | 0.91(0.63-1.32) | 0.6268 | 0.6268 |
| 79.59~155.32 | 0.91(0.63-1.32) | 0.6268 | 0.6268 |
| >155.32 | 0.97(0.63-1.49) | 0.8763 | 0.8763 |
| ALBI | ≤-2.9 | Ref |
|------|-------|-----|
|      | -2.9~2.39 | 1.17(0.79-1.73) | 0.4376 |
|      | >2.39 | 1.37(0.89-2.12) | 0.1524 |
| APRI | ≤0.42 | Ref |
|      | 0.42~1.02 | 1.23(0.82-1.84) | 0.3174 |
|      | >1.02 | 2.12(1.37-3.28) | 0.0007 |
| SII  | ≤274.86 | Ref |
|      | 274.86~810.49 | 1.15(0.78-1.71) | 0.4764 |
|      | >810.49 | 1.35(0.87-2.11) | 0.1819 |
| ANRI | ≤6.7 | Ref |
|      | 6.7~17.69 | 0.99(0.66-1.48) | 0.9663 |
|      | >17.69 | 1.37(0.87-2.17) | 0.1717 |
| ALRI | ≤18.59 | Ref |
|      | 18.59~44.38 | 1.05(0.71-1.56) | 0.8071 |
|      | >44.38 | 1.93(1.26-2.95) | 0.0026 |
| PNI  | ≤45.17 | Ref |
|      | 45.17~51.2 | 0.98(0.68-1.41) | 0.8991 |
|      | >51.2 | 0.55(0.35-0.87) | 0.0104 |
| PT (s) | ≤11.9 | Ref |
|      | 11.9~13.9 | 1.04(0.71-1.51) | 0.8488 |
|      | >13.9 | 1.16(0.75-1.81) | 0.4972 |

**Albumin (g/L)**
|                |                |                |
|----------------|----------------|----------------|
| **≤ 36.9**     | **Ref**        | **0.4076**     |
| **36.9~42.5**  | **0.86(0.6-1.23)** | **0.0326**    |
| **>42.5**      | **0.61(0.39-0.96)** | **0.0326**    |
| **ALT (U/L)**  |                |                |
| **≤ 28**       | **Ref**        | **0.5611**     |
| **28~59**      | **1.12(0.76-1.67)** | **0.5611**    |
| **>59**        | **1.14(0.73-1.78)** | **0.5694**    |
| **DBIL (umol/L)** |            |                |
| **≤ 3.1**      | **Ref**        | **0.3228**     |
| **3.1~6.27**   | **1.22(0.82-1.82)** | **0.3228**    |
| **>6.27**      | **1.25(0.81-1.93)** | **0.3119**    |
| **TBIL (umol/L)** |            |                |
| **≤ 9.5**      | **Ref**        | **0.6878**     |
| **9.5~17**     | **0.92(0.62-1.37)** | **0.6878**    |
| **>17**        | **1.14(0.74-1.76)** | **0.5525**    |
| **AFP (ng/ml)** |                |                |
| **≤ 400**      | **Ref**        | **0.0329**     |
| **>400**       | **1.48(1.03-2.13)** | **0.0329**    |

**DFS Prognostic Nomogram**

Following the identification of independent prognostic factors for DFS, a nomogram was constructed for DFS prediction at one and three years (Figure 1A). This was followed by an internal verification in the validation cohort. The training set C-index was 0.691 (95% CI: 0.644-0.738). The predicted and actual values demonstrated an agreement for both DFS time points by calibration curves in both cohorts (Figure 2).

**OS Prognostic Nomogram**

The OS at three and five years was pitched employing the nonogram based on the independent prognostic factors (Figure 1B). The internal validation was assessed in the validation cohort. The training set C-index was 0.713 (95% CI: 0.670-0.756). The calibration curves of the OS at both time frames
demonstrated conspicuous agreement for the predicted nonogram figures and actual observation in both cohorts (Figure 3).

**Assessment of the value of the nomograms in clinical application**

Employing their nomogram scores, the samples were stratified into high- (≥Q3), medium- (Q1-Q3), and low-risk subgroups (≤Q1) using the first quartile and third quartile as the cutoff value. While the high-risk, medium-risk, and low-risk sub-groups were 73, 145, and 72 in the training cohort patients, these numbers for the validation set were 31, 62, and 31 patients, respectively. There DFS demonstrated an evident variation among the three risk subgroups in both cohorts with the high-risk group displaying a lower DFS rate vs. that of the other groups (p <0.001). This trend was on similar lines for the OS with a shorter value for the high-risk subgroup vs. the other groups (p <0.001) (Figure 4).

**Discussion**

Solitary HCCs are a subtype of HCCs. While the BCLC system is the most extensively adopted HCC staging approach, there are challenges in the staging of solitary HCCs with a diameter of more than 5 cm [9]. Shen et al established the first nomogram for postoperative solitary HCCs in Asia that demonstrated superiority over the traditional staging system [10]. Although this nomogram demonstrated good calibration and discrimination, it only included three clinicopathological factors (tumor size, microvascular invasion, and macrovascular invasion). In this study, the successful development of two nomograms for predicting 1- and 3-year disease-free survival (DFS) and 3- and 5-year overall survival (OS) in solitary HCC patients post-curative liver resection is shown integrating clinicopathological factors with hematologic markers and was further validated in the validation set. The models performed comparably in both cohorts, as predicted.

In this present study, the clinicopathological features of solitary HCCs, inclusive of tumor diameter, tumor capsule, and macrovascular invasion emerged as significant predictors for both DFS and OS. These features are well-known underlying risk factors and are evidently also associated with HCC recurrence and poor prognosis [10, 21, 23]. Macrovascular invasion refers to thrombosis bordering the tumor in the portal vein with a blurred boundary, with at least one imaging modality confirming this finding in all patients [24]. In our nomograms, macrovascular invasion emerged as the most robust predictor for the recurrence and long-term survival of solitary HCCs post-surgery. Studies have demonstrated an improved staging of solitary HCCs staging by considering tumor size with a cutoff at 5 cm [25], which is on the same lines as our results.

The incidence of microvascular invasion or MVI increases with the increase of tumor size with the MVI incidence at 31 %, 41%, and 58% in solitary HCC tumors measuring ≤5 cm, 5.1 to 6.5 cm, and ≥ 5 cm 6.5 cm, respectively [26]. Currently, MVI is defined as tumor cells within an endothelium-lined vascular
lumen only seen in microscopy and is predominantly diagnosed by post-operative histopathological examination [27]. A previous study demonstrated that MVI over the Milan criteria was a better predictor of recurrence and OS in HCC patients subjected to surgical resection [28]. Shen et al documented that MVI was linked to the outcome post- hepectomy in solitary HCC patients [10]. Our study too displayed that the 1 and 3-year DFS of HCC patients without MVI were longer compared with those of patients with MVI. Another crucial predictor of HCC survival is age with our work also documenting a worse prognosis for patients who were over 60 years in age.

Our work also demonstrated that the blood-based biomarkers AFP and AST are independent risk factors for DFS and OS of solitary HCCs, respectively. Alpha-fetoprotein (AFP) is a glycoprotein synthesized by the fetal yolk sac and liver during development. Since its discovery in HCC patient sera by Tatarinov in the mid-1960s, AFP has found extensive use in diagnosing liver cancer clinically [29]. Recently, a study has corroborated the oncogenic role of AFP in HCC progression by inhibiting the HuR-mediated Fas/FADD apoptotic signaling pathway [30]. This is suggestive of a promising therapeutic approach via AFP blocking and monitoring in HCC patients displaying AFP overexpression. Research has documented the association of high AFP serum levels and HCC recurrence post-hepatic resection [21], which is consistent with our results. Several biochemical tests are employed for diagnosing and managing liver diseases given the slew of biochemical, synthetic, and excretory functions of the liver. These tests as a whole are referred to as liver function tests. One of the most frequently prescribed and handy tests in clinical practice—the Aspartate aminotransferase (AST) test—is indicative of hepatocellular damage. ASTs are present in hepatocytes at high levels catalyzing the conversion of aspartate and α-ketoglutarate to glutamate and oxaloacetate. Upon cell death or plasma membrane damage, AST leaks into the blood resulting in its elevated levels [31]. An association has been unveiled between high AST levels and increasing HCC aggression (tumor size, multifocality, portal vein invasion, and AFP levels) [32]. Also, previous work revealed that the transaminases in HCC patients are associated with neoplastic hepatocyte proliferation [33]. Studies have also revealed an increased preoperative serum AST level was an adverse and independent prognostic factor for HCC OS challenging hepatitis B–related cirrhosis [34]. Our results are also suggestive of the correlation between poor prognosis and high preoperative AST levels. Thus, routine AST tests can emerge as valuable prognostic aspects in HCC management.

There are few limitations in this work. First, as ours is a retrospective analysis that made the exclusion of selection biases not possible. Second, the patient sample set was from China, which limits the representation of the global population. Third, robust conclusions are compromised due to the limited sample size. As our next step, we would increase the sample size and combine this global data to further hone the predictive accuracy of our nomogram to other populations.

To summarize, the establishment and validation of two novel nomograms combining clinicopathological features and hematological biomarkers to predict DFS and OS in solitary HCC patients post- curative liver resection are presented. Their potential usage in supplying reference data for guiding personalized treatment plans clinically and follow-up management in these patients is a plausible and promising research avenue ahead.
Abbreviations

APRI, AST to platelet ratio index; SII, systemic immune-inflammation index; ANRI, AST to neutrophil ratio index; ALRI, AST to lymphocyte ratio index; PNI, prognostic nutritional index; PT, Prothrombin time; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DBIL, Direct Bilirubin; TBIL, total bilirubin; AFP, α-fetoprotein

Declarations

Ethics approval and consent to participate (Not applicable)

Consent for publication (Not applicable)

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

XXC acquired and analyzed the clinical data and was a major contributor in writing the manuscript. WXQ and XKX acquired and analyzed the clinical data. ZFC and ZWH acquired the clinical data. PYL performed the data analysis. CC designed the study and approved the final version of manuscript. All authors read and approved the final manuscript.

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Figures
Figure 1

Nomogram predicting the 1-year and 3-year DFS (A) and 3-year and 5-year OS (B). The overall points of corresponding independent factors were computed per patient to then estimate these values.
Figure 2

The calibration curves of the 1- and 3-year DFS prediction in the training (A, B) and validation (C, D) groups.
Figure 3

The calibration curves of the 3- and 5-year OS prediction in the training (A, B) and validation (C, D) groups.
Figure 4

Nonogram-based values of DFS and OS in the training (A, C), and validation cohorts (B, D). The high-risk, medium-risk, and low-risk groups are illustrated in blue, green, and red lines, respectively.