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The predictive benefits of inflammatory markers in cancers of the liver

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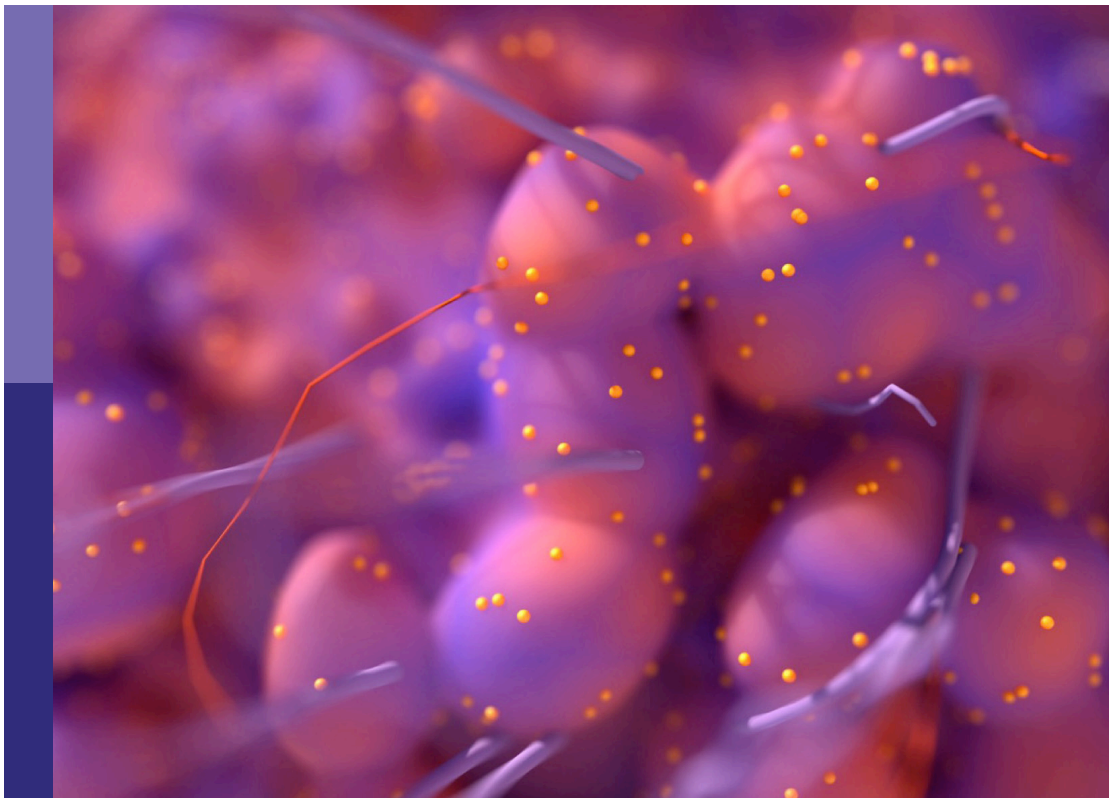

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The predictive benefits of inflammatory markers in cancers of the liver

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Table of contents

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Editorial: The predictive benefits of inflammatory markers in cancers of the liver

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KEYWORDS

liver cancer, inflammatory markers, hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), predictive ability

Editorial on the Research Topic
The predictive benefits of inflammatory markers in cancers of the liver

Primary malignant liver tumours still represent an intricated scenario that challenges clinicians in their strive for achieving prompt and accurate diagnosis, effective therapies and treatments, and the best possible outcomes and quality of life. The frequency and mortality rates of liver tumours mandate a constant effort to improve the tools that can assist physician and surgeons in their clinical endeavours. Biomarkers have shown the potential to be a powerful complimentary tool to optimize patient outcomes by improving diagnosis, prognosis, and treatment response prediction. It is well known that inflammation is a hallmark of cancer, contributing to several aspects of tumour development and progression as well as to the response to therapy. Therefore, inflammatory biomarkers could play a key role in all stages of cancer treatment.

This Research Topic focuses on the various roles and benefits of inflammatory markers in liver cancers.

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is still burdened by high recurrence rates and mortality. Its management is still a matter of debate, and possible therapeutic strategies range from locoregional treatments to immunotherapy and surgery, including liver transplantation. In order to identify the best approach, several factors have to be taken into consideration, including the patient's general conditions, liver disease status, and tumour stage, all included in decision-making algorithms and the recently introduced concepts of therapeutic hierarchy (1, 2). A key factor allowing satisfactory long-term outcomes is accurate pre-intervention prognostication that would assist clinicians in allocating patients to the best possible treatment with a more reliable and personalised approach. In recent years, a strong correlation between systemic inflammation and HCC prognosis has been described, with several systemic and pathological markers associated with survival and recurrence.

Giannone et al. in their review describe the features of the inflammatory microenvironment in all stages of HCC carcinogenesis, with special focuses on serum markers and gene signatures and their ability in predicting HCC recurrence and survival.

The surgical approach represents one of the preferred treatments in early-stage HCC, but in the context of liver cirrhosis, it can be complex and burdened by a high incidence of postoperative complications. Among them, one of the most feared is the post-hepatectomy liver failure (PHLF). Among the inflammatory indexes, AST-to-platelet ratio index (APRI) has the characteristic of reflecting the progression of liver cirrhosis, thanks to the increased AST release consequent to cell damage, and the severity of portal hypertension, represented by a a decrease in circulating platelets values. APRI has been investigated by Fang et al., who retrospectively collected a sample of 488 HCC patients undergoing liver resection, and included it in a nomogram that outperformed the MELD, ALPI and CP scores in predicting PHLF (C-index of 0.845, 95%CI, 0.806-0.884).

In case of single, large (>5 cm) HCC, the recurrence rates are high, even after radical surgery. To optimise outcomes, a two-step approach including Transcatheter Arterial Embolization (TACE) before liver resection has been explored, but heterogeneous results have been observed and no clear oncological benefit demonstrated. Zhang et al. evaluated the presence of circulating tumour cells (CTCs) in patients with large HCC, and found that only in CTC-positive patients, preoperative TACE reduced early recurrence and improved long-term survival, allowing better patient selection and treatment allocation.

The high recurrence rates after HCC resection mandate strict follow-up and frequently, patients need further treatments, including re-do resections, locoregional treatments and salvage liver transplantation. Chen and Wang in their retrospective analysis on 896 HCC-HBV patients identified pre-operative IL-25 levels as predictor of postoperative overall and recurrence-free survival. Patients with IL-25 levels <14.9 μg/ml had significantly better outcomes, representing a valuable diagnostic and prognostic tool, especially in cases of alfa-fetoprotein-negative HCC. In a similar population of patients, Wenpei et al. constructed a combined inflammation and pathology model (CIP) to investigate the predictive value of preoperative neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic inflammation response index (SIRI), and systemic immune inflammation index (SII) for early recurrence in HCC-HBV patients undergoing liver resection. The CIP model showed a good predictive ability, with an AUC of 0.804.

Several international guidelines identify TACE as the one of the principal treatment options for patients unresectable HCC. Although TACE can potentially be repeated in case of incomplete treatment or recurrence, reduced efficacy and refractoriness can be observed. Identifying patients experiencing TACE refractoriness would have great benefits, as the early use of combination therapy confers significant survival advantages. Xia et al. described how high plasma arginase-1 (ARG1) expression was independently associated with a lower incidence of early TACE refractoriness and constructed a nomogram also including tumour size and number and platelet count, predicting refractoriness with an AUC of 0.833 (95%CI 0.791-0.875).

Patients with unresectable HCC often present with a large tumour size, as well as vascular invasion or distant metastases. In these cases, the ability of TACE to achieve complete tumour necrosis is limited and could paradoxically contribute to tumour recurrence and dissemination through increased expression of programmed cell death ligand 1 (PD-L1) and vascular endothelial growth factors (VEGF). Therefore, treatments with immune checkpoint inhibitors (ICIs) and tyrosine kinase inhibitors (TKIs) can be indicated in unresectable HCC in combination with TACE. Guo et al. retrospectively evaluated 98 patients undergoing TACE+ICIs+TKIs and identified low pre-treatment platelet-to-lymphocyte ratio (PLR) values (>98.89) as an independent risk factor for a shorter median overall survival and progression-free survival.

The second most common primary malignant liver tumour is intrahepatic cholangiocarcinoma (ICC), characterised by an aggressive behaviour with only 20-40% of cases amenable of surgery at presentation, and a 5-year survival of only 30-40% after complete resection. In their multicentric analysis on a cohort of 374 patients, Zhang et al. developed a novel classification based on pre-operative inflammatory and immune status (merging together the systemic immune-inflammatory index (SII) and the albumin bilirubin (ALBI) grade) that was able to serve as a reliable prognostic indicator for postoperative overall and recurrence-free survival in patients with ICC.

Several inflammation-based scores have been proposed and evaluated, and He et al. retrospectively analysed 399 ICC patients comparing 8 different scores to determine the one with the best survival outcomes predictive value. The modified Glasgow Prognostic Score (mGPS), a combination of C-reactive protein (CRP) and albumin levels, emerged as the most sensitive, efficient, simple, rapid, and widely applicable preoperative prognostic factor for ICC patients, with elevated mGPS scores indicating a poor prognosis.

Several challenges still exist in the complex field of primary liver tumours, where clinicians face difficulties in obtaining early diagnoses and selecting the optimal treatments to grant patients the best possible outcomes. The dysregulation of the tumour microenvironment, associated with inflammation, is a well-established contributor to carcinogenesis and tumour progression. Therefore, the identification of early, reliable and validated prognostic inflammatory markers is of paramount importance in the context of an increasingly personalized-medicine approach.

Author contributions

GR: Conceptualization, Writing – original draft. DT: Conceptualization, Writing – review & editing.

Conflict of interest

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

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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ORIGINAL RESEARCH
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Evaluation of Preoperative Inflammation-Based Prognostic Scores in Patients With Intrahepatic Cholangiocarcinoma: A Multicenter Cohort Study

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Background: Accumulating evidence has indicated the vital role of inflammation-based score (IBS) in predicting the prognostic outcome of cancer patients. Otherwise, their value in intrahepatic cholangiocarcinoma (iCCA) remains indistinct. The present study aimed to evaluate whether IBSs were related to survival outcomes in iCCA patients.

Method: Clinical characteristics were retrospectively collected in 399 patients diagnosed with iCCA from cohorts of Sun Yat-sen University Cancer Center (SYSUCC) and the First Hospital of Dalian Medical University (FHDMU). The survival curves were constructed with the Kaplan-Meier method and compared with the log-rank test. Univariate and multivariate analyses were conducted to determine the prognostic factors of overall survival (OS) and progression-free survival (PFS). The concordance index and the area under the time-dependent receiver operating characteristic (ROC) curves (AUROCs) were used to compare the predictive value of inflammation-based scores in terms of survival outcomes.

Results: The significant survival differences in OS and DFS were observed when patients were stratified by the modified Glasgow Prognostic Score (mGPS) ($p<0.001$). Multivariate analysis demonstrated that higher mGPS score was independently associated with poor OS and DFS ($p<0.001$). The predictive accuracy of the mGPS was superior to other IBSs (all $p<0.001$) in survival prediction in iCCA patients. The findings were further supported by the external validation cohort.

Conclusion: The mGPS is a sensitive, efficient, simple and widely applicable preoperative prognostic factor for iCCA patients. Thus, more effective therapy and frequent surveillance should be conducted after surgical resection in iCCA patients with higher mGPS scores.

Keywords: intrahepatic cholangiocarcinoma, Modified Glasgow Prognostic Scores, overall survival, progression-free survival, prognosis

BACKGROUND

Intrahepatic cholangiocarcinoma (iCCA) is the second most common malignant tumor ranking after hepatocellular carcinoma (1). Although iCCA patients in different stages can be treated with various modalities, including surgery resection, chemotherapy, and radiation therapy, the overall incidence and mortality have shown a worldwide increase in the past decades (1, 2). Even though surgical resection provided the best chances to obtain prolonged survival, the median progression-free survival (PFS) time was reported to be merely 12 to 36 months in patients with resectable iCCA (3). To optimize risk-benefit assessments and stratify the patients for more individualized treatment, there is an urgent demand to seek an objective, sensitive and reliable prognostic marker for patients with iCCA. Currently, common prognostic markers, such as tumor margins, tumor differentiation, and lymph node metastases, are determined only after surgical resection (2). Therefore, there is continuing momentum in finding a practical pre-operative prognostic marker that could facilitate accurate patient stratification before surgery and improve therapeutic outcomes.

Inflammation, as a new hall marker of cancer (4), plays a vital role in the progression of tumors (5). Tumors produce inflammatory chemokines and cytokines and are locally infiltrated by leucocytes (6). Moreover, the activation of the ongoing systemic chronic inflammatory response will further lead to cachexia (6). According to these pieces of evidence, many inflammation-based scores (IBSs) were proved to be prognostic in various tumors, including Glasgow Prognostic Score (GPS) and modified Glasgow Prognostic Score (mGPS) (7), Prognostic Index (PI) (8), Prognostic Nutritional Index (PNI) (9), systemic immune-inflammation index (SII) (10), neutrophil to lymphocyte ratio (NLR) (11), platelet to lymphocyte ratio (PLR) (12), and lymphocyte to monocyte ratio (LMR) (13). Nonetheless, the research about reliable and valid inflammation-based scores in patients with iCCA after resection remains supplemented. Besides, most previous studies were conducted in a single center with a small number of patients and were mostly concentrated on a certain single IBS (14–17). Thus, for evaluating the validity of the IBSs in iCCA patients, a multicenter study with a large volume of patients would be necessary and imperative. According to these findings, our study aimed to find the best combination of inflammatory factors that could predict survival outcomes for iCCA patients after surgical resection.

METHODS

Study Design and Patient Materials

A total of 399 patients pathologically diagnosed with iCCA from two cohorts were finally enrolled in the present study [292 patients from Sun Yat-sen University Cancer Center (SYSUCC) between January 2000 and December 2018 as the primary cohort and another 107 patients from the first affiliated hospital of Dalian Medical University (FHDMU) between May 2013 and December 2019 as the validation cohort]. The enrolling flowchart of patients was presented in **Figure 1**. Clinical characteristics were retrospectively aggregated from the electronic medical record and were exhibited in **Table 1**. This study obtained the written informed consent from all the patients and was approved by the ethics committees of two participating centers.

Survival Outcomes and Follow-Up

The study's outcome variables, overall survival (OS) and PFS, were calculated from the date of surgery to the date of death and tumor progression, respectively, or the last follow-up. The first post-operative follow-up was conducted at 30 days after surgical resection, then every three months for the first year, and every six months until death or dropout. Follow-up data of two cohorts were retrieved on November 30, 2020.

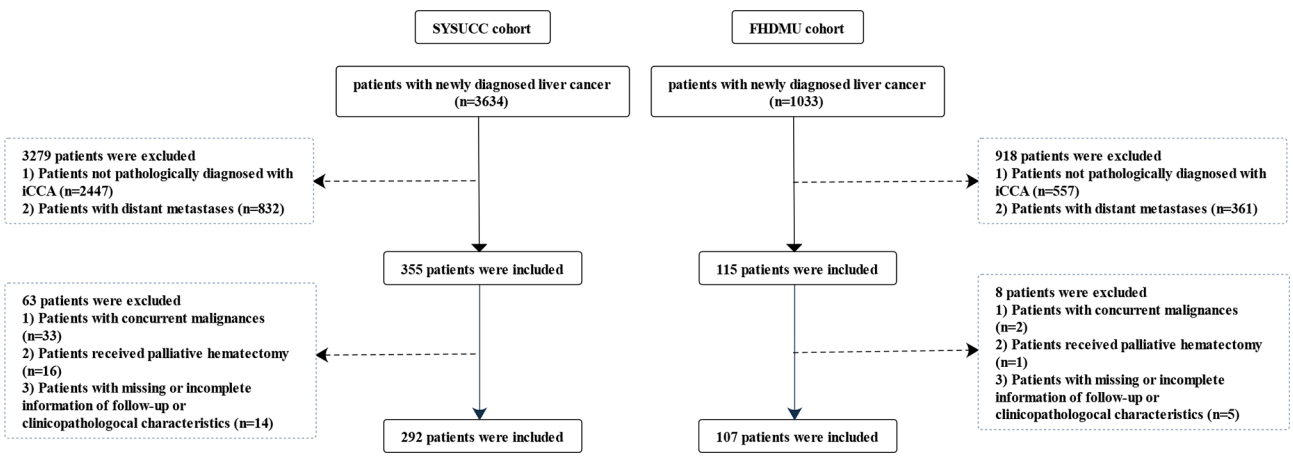

FIGURE 1 | Flow chart of the patient enrolling process.

TABLE 1 | Clinical, radiological, and pathological characteristics of the SYSUCC cohort and FHDMU cohort.

Variables	Primary cohort (n = 292)	Validation cohort (n = 107)	Variables	Primary cohort (n = 292)	Validation cohort (n = 107)
Gender			PI		
Male	181 (62.0%)	62 (57.9%)	0	220 (75.3%)	32 (29.9%)
Female	111 (38.0%)	45 (42.1%)	1	61 (20.9%)	63 (58.9%)
Age (years)			2	11 (3.8%)	12 (11.2%)
≤60	189 (64.7%)	33 (30.8%)	Tumor capsular		
>60	103 (35.3%)	74 (69.2%)	Absence	45 (15.4%)	–
WBC count ($\times10^9$/L)			Uncompleted	37 (12.7%)	–
≤10	259 (88.7%)	92 (86.0%)	Completed	210 (71.9%)	–
>10	33 (11.3%)	15 (14.0%)	Satellite sites		
HGB (g/L)			Absence	201 (68.8%)	106 (99.1%)
≤175	27 (9.20%)	30 (28.0%)	Presence	91 (31.2%)	1 (0.90%)
>175	265 (90.8%)	77 (72.0%)	Thrombus		
PLT ($\times10^9$/L)			Absence	269 (92.1%)	–
≤350	10 (3.40%)	5 (4.70%)	Presence	23 (7.90%)	–
>350	282 (96.6%)	102 (95.3%)	Tumor differentiation		
ALT (U/L)			Low	6 (2.10%)	3 (2.80%)
≤50	236 (80.8%)	55 (51.4%)	Medium	105 (35.9%)	81 (75.7%)
>50	56 (19.2%)	52 (48.6%)	High	181 (62.0%)	23 (21.5%)
AST (U/L)			Microvascular invasion		
≤40	254 (87.0%)	56 (52.3%)	Absence	237 (81.2%)	86 (89.7%)
>40	38 (13.0%)	51 (47.7%)	Presence	55 (18.8%)	11 (10.3%)
GGT (U/L)			Lymph-vessel invasion		
≤60	108 (37.0%)	16 (15.0%)	Absence	273 (93.5%)	–
>60	184 (63.0%)	91 (85.0%)	Presence	19 (6.5%)	–
ALP (U/L)			Macro vascular invasion		
≤125	182 (62.3%)	25 (23.4%)	Absence	274 (93.8%)	95 (88.8%)
>125	110 (37.7%)	82 (76.6%)	Presence	18 (6.20%)	12 (11.2%)
ALB (g/L)			Back membrane invasion		
>40	5 (1.70%)	38 (35.5%)	Absence	114 (39.0%)	90 (84.1%)
≤40	287 (98.3%)	69 (64.5%)	Presence	178 (61.0%)	12 (15.9%)
TBIL (μmol/L)			Imaging tumor size		
≤20.5	265 (90.8%)	54 (50.5%)	≤5 cm	131 (44.9%)	56 (52.3%)
>20.5	27 (9.20%)	53 (49.5%)	≤5 cm	161 (55.1%)	51 (47.7%)
IBIL (μmol/L)			Imaging vascular invasion		
≤15	275 (94.2%)	65 (60.7%)	Absence	271 (92.8%)	97 (90.7%)
>15	17 (5.80%)	42 (39.3%)	Presence	21 (7.20%)	10 (9.30%)
CRP (mg/L)			Imaging LN metastasis		
≤3	172 (58.9%)	35 (32.7%)	Absence	207 (70.9%)	54 (50.5%)
>3	120 (41.1%)	72 (67.3%)	Presence	85 (29.1%)	53 (49.5%)
HBsAg			Imaging LN size		
Absence	162 (55.5%)	105 (98.1%)	Absence	207 (70.9%)	–
Presence	130 (44.5%)	2 (1.9%)	≤1 cm	28 (9.60%)	–
CA19-9 (U/ml)			>1 cm	57 (19.5%)	–
≤35	141 (48.3%)	25 (23.4%)	Tumor size		
>35	151 (51.7%)	82 (76.6%)	≤5 cm	115 (39.4%)	52 (48.6%)
CEA (ng/ml)			≤5 cm	177 (60.6%)	55 (51.4%)
≤5	211 (72.3%)	60 (56.1%)	LN metastasis		
>5	81 (27.7%)	47 (43.9%)	Absence	250 (85.6%)	95 (88.8%)
LCR			Presence	42 (14.4%)	12 (11.2%)
0	21 (7.20%)	–	Nerve tract invasion		
1	271 (92.8%)	–	Absence	96 (89.7%)	96 (89.7%)
mGPS			Presence	11 (10.3%)	11 (10.3%)
0	216 (74.0%)	37 (34.6%)	Peri-origin invasion		
1	67 (22.9%)	43 (40.2%)	Absence	269 (92.1%)	103 (96.3%)
2	9 (3.10%)	27 (25.2%)	Presence	23 (7.90%)	4 (3.70%)
NLR			T stage 8th		
<2.62	194 (66.4%)	36 (33.6%)	1	34 (24.3%)	84 (78.5%)
≥2.62	98 (33.6%)	71 (66.4%)	2	44 (15.1%)	5 (4.7%)
LMR			3	153 (52.4%)	14 (13.1%)
<4.06	125 (42.8%)	–	4	24 (8.20%)	4 (3.7%)
≥4.06	167 (57.2%)	–	N stage 8^{th}		

(Continued)

TABLE 1 | Continued

Variables	Primary cohort (n = 292)	Validation cohort (n = 107)	Variables	Primary cohort (n = 292)	Validation cohort (n = 107)
PLR			Absence	250 (85.6%)	95 (88.8%)
<104.85	172 (58.9%)	24 (22.4%)	Presence	42 (14.4%)	12 (11.2%)
≥104.85	120 (41.1%)	83 (77.6%)	TNM 8th		
SII			I	70 (24.0%)	81 (75.7%)
0	68 (23.3%)	30 (28.0%)	II	37 (12.7%)	2 (1.90%)
1	224 (76.7%)	77 (72.0%)	III	185 (63.4%)	24 (22.4%)
PNI			After operation therapy		
0	277 (94.9%)	48 (44.9%)	Absence	161 (55.1%)	72 (67.3%)
1	15 (5.1%)	59 (55.1%)	Presence	131 (44.9%)	35 (32.7%)

WBC, white blood cell; HGB, hemoglobin; PLT, platelets; ALT, alanine aminotransferase; AST, glutamic-oxalacetic transaminase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; ALB, Albumin; TBIL, total serum bilirubin; IBIL, indirect serum bilirubin; CRP, C-reaction protein; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; LCR, lymphocyte-C-reactive protein ratio; mGPS, modified Glasgow prognostic score; NLR, neutrophil-lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune-inflammation index; PNI, prognostic nutritional index; PI, prognostic Index; LN, lymph node.

Standard Management of iCCA Patients

The indications to resection and contraindications were the same in two centers of this study. The following indications to resection were followed: 1) Clinically diagnosed with iCCA according to the laboratory measurements and the imaging examinations. 2) The tumor was resectable. 3) No distant lymph-node metastasis or distant organ metastasis were observed. The contraindications included inoperable cardiopulmonary dysfunction, large volume of ascites and cachexy. Preoperative blood samples were routinely collected 1 week before surgery or at the preoperative outpatient visit. Routine laboratory measurements of differential leukocyte count and classification, including C-reactive protein (CRP), hemoglobin (HGB), platelet (PLT), tumor biomarkers (alpha-fetoprotein [AFP], carbohydrate antigen 19-9 [CA19-9], carcinoma embryonic antigen [CEA]) and blood biochemistry (serum albumin [ALB], alanine transaminase [ALT], glutamic-oxalacetic transaminase [AST], alkaline phosphatase [ALP], gamma-glutamyl transpeptidase [GGT], indirect bilirubin [IBIL], and total bilirubin [TBIL]) were carried out. The preoperative imaging evaluations for iCCA included abdomen computed tomography (CT), chest CT, pelvis CT and magnetic resonance imaging. Those patients with jaundice or dilated bile ducts routinely underwent biliary drainage. Once the regional LN metastasis was implicated in the preoperative imaging evaluations or suspected during surgery, all resectable regional LNs were dissected. The postoperative pathological stage of iCCA was classified according to the eighth AJCC TNM staging system. Moreover, the adjuvant chemotherapy was routinely implemented in the patients with more advanced or aggressive tumors, particularly those with LN metastasis.

Inflammation-Based Scores

According to our previous study (18, 19) related to the survival predicting performance of IBSs, NLR, PLR, LCR, LMR, PI, GPS, mGPS, PNI, and SII were included and calculated in this multicohort study to identify the IBS with highest accuracy to predict poor OS and PFS in iCCA patients. The details of IBSs were described in **Table 2**.

TABLE 2 | Inflammation-based prognostic scoring systems.

Scoring systems	Score
The modified Glasgow Prognostic Score (mGPS)	
CRP (≤10 mg/L) and albumin (≥35 g/L)	0
CRP (≤10 mg/L) and albumin (<35 g/L)	0
CRP (>10 mg/L) and albumin (≥35 g/L)	1
CRP (>10 mg/L) and albumin (<35 g/L)	2
Lymphocyte-C-reactive Protein ratio (LCR)	
10^4 × lymphocyte count (10^9/L): CRP (mg/L) >6000	0
10^4 × lymphocyte count (10^9/L): CRP (mg/L) ≤6000	1
Neutrophil to lymphocyte ratio (NLR)	
Neutrophil count: lymphocyte count < 5:1	0
Neutrophil count: lymphocyte count ≥ 5:1	1
Lymphocyte to monocyte ratio (LMR)	
Lymphocyte count (×10^9/L): monocyte count (×10^9/L) <3	0
Lymphocyte count (×10^9/L): monocyte count (×10^9/L) ≥3	1
Platelet to lymphocyte ratio (PLR)	
Platelet count: lymphocyte count < 150:1	0
Platelet count: lymphocyte count ≥ 150:1	1
Platelet count: lymphocyte count > 300:1	2
Systemic immune-inflammation index (SII)	
Platelet count (×10^9/L) × neutrophil count (×10^9/L)/ lymphocyte count (×10^9/L) < 305	0
Platelet count (×10^9/L) × neutrophil count (×10^9/L)/ lymphocyte count (×10^9/L) ≥ 305	1
Prognostic Nutritional Index (PNI)	
Albumin (g/L) +5 × total lymphocyte count (10^9/L) ≥45	0
Albumin (g/L) +5 × total lymphocyte count (10^9/L) <45	1
Prognostic index (PI)	
CRP (≤10 mg/L) and white blood cell count (≤11 × 10^9/L)	0
CRP (≤10 mg/L) and white blood cell count (>11 × 10^9/L)	1
CRP (>10 mg/L) and white blood cell count (≤11 × 10^9/L)	1
CRP (>10 mg/L) and white blood cell count (>11 × 10^9/L)	2

*Abbreviations as in **Table 1**.*

Statistical Analysis

Continuous variables were reported with median and interquartile range. Categorical variables were reported with whole numbers and proportions. Proportions were compared using the chi-square test or the Fisher Exact test. Distributions of continuous variables were compared using the Mann-Whitney U test. Maximally selected rank statistic from the R package was employed to identify the optimal cutoff points of NLR, PLR and

LMR. Survival curves were generated using the Kaplan-Meier method and compared with the log-rank test. The Cox regression model was used to perform the multivariate analysis of the predictive factors of OS and PFS. Time-dependent receiver operating characteristic curves (ROC) were analyzed to compare the prognostic ability of these eight inflammation-based scores. The concordance index (C-index) and the area under the ROC curves (AUROCs) were performed using R software version 3.5.0 (The R Foundation for Statistical Computing, Vienna, Austria. http://www.rproject.org). All statistical inferences were based on two-sided p values, with values <0.05 taken to indicate statistical significance.

RESULTS

Patient Characteristics

A total of 399 patients pathological diagnosed with iCCA from two different patient cohorts were enrolled in this study. In the primary cohort, 181 male (62.0%) and 62 female (57.9%) iCCA patients with a median age of 56 years (range, 20–77 years) were enrolled. There were 70 (24.0%) patients diagnosed as TNM stage I, 37 (12.7%) patients as stage II, and 185 (63.4%) as stage III, respectively. Moreover, a majority of patients were assigned into LCR 0 (271, 92.8%), NLR< 2.62 (194, 66.4%), LMR≥ 4.06 (167, 57.2%), PLR< 104.85 (172, 58.9%), SII 1 (224, 76.7%), PNI 0 (277, 94.9%), and PI 0 (220, 75.3%), respectively. Specially, 216 (74.0%) patients had an mGPS of 0, 67 (22.9%) patients had an mGPS of 1, and 9 patients (3.1%) had an mGPS of 2. The validation cohort consisted of 62 males (57.9%) and 45 females (42.1%) with a median age of 64 years (range, 32–88 years). Slightly different from the primary cohort, a majority of patients were assigned into NLR≥2.62 (71, 66.4%), PLR≥ 104.85 (83, 77.6%), SII 1 (77, 72.0%), PNI 1 (59, 55.1%), and PI 1 (63, 58.9%) in the validation cohort, respectively. No significant differences were observed in baseline characteristics between the included patients. Further hematologic, imaging and pathological characteristics are presented in **Table 1**.

Survival Outcomes According to IBSs

The median OS of patients were 39.47 months (95% CI, 31.03–49.87 months) in the primary cohort, and 16.23 months (95% CI, 12.23–24.10 months) in the validation cohort, respectively. The median PFS was 11.23 months (95% CI, 8.87–14.13 months) in the primary cohort, and 12.87 months (95% CI, 10.10–16.97 months) in the validation cohort, respectively. In the primary cohort, mGPS showed an outstanding prediction of both OS (1-year OS rates: 94.4%, 29.2% and 0%; 2-year OS rates: 81.8%, 11.7%, and 0%; 3-year OS rates: 65.8%, 6.23%, 0%) and PFS (1-year PFS rates: 62.9%, 6.1%, and 0%; 2-year PFS rates: 45.8%, 6.1%, and 0%; 3-year PFS rates: 39.4%, 6.1%, 0%). Additionally, poor OS was obtained in patients with higher values of PI and NLR (all P<0.001) and lower values of LMR (P=0.023). Meanwhile, patients with higher values of PI (P<0.001), NLR (P=0.002), and lower values of LMR (P=0.045) showed poor PFS as well. All the details of OS curve and PFS curves in the primary cohort were shown in **Figures 2**, **3**, respectively.

Prognostic Factors for Survival Outcomes

For our primary cohort, the univariate analysis identified 22 hematological, pathological, and radiological elements and IBSs as prognostic factors for OS and PFS (**Table 2**). Additionally, the Cox-regression analysis was conducted to distinguish the independent risk factors of OS and PFS. In the multivariate analysis, only CA19-9 (HR, 1.568; 95% CI, 1.071–2.296; P = 0.021), CEA (HR, 1.677; 95% CI, 1.112–2.528; P = 0.014), mGPS (HR, 37.929; 95% CI, 12.609–113.367; P < 0.001), PI (HR, 0.187; 95% CI, 0.059–0.593; P = 0.004), imaging 9^{th} LN metastasis (HR, 3.179; 95% CI, 1.092–9.256; P = 0.034), and after operation therapy (HR, 1.941; 95% CI, 1.345–2.778; P < 0.001) displayed statistical difference of OS, and the factors independently associated with PFS were: CA19-9 (HR, 1.586; 95% CI, 1.140–2.208; P = 0.006), mGPS (P < 0.001), PI (P < 0.001), imaging LN metastasis (HR, 1.462; 95% CI, 1.168–1.829; P < 0.001), and after operation therapy (HR, 3.571; 95% CI, 1.878–5.150; P < 0.001) (**Table 3**).

The External Validation of Significant Prognostic Factors

According to the statistic results in the primary cohort, an external validation was conducted. The significant prognostic factors, which were defined in the primary cohort, were validated in the FHDMU cohort. The multivariate analysis based on the validation cohort indicated that only mGPS was an independent prognostic factor for both OS and PFS (**Table 4**). In addition, survival was also well separated by mGPS in the external validation cohort (OS, 1-year rates: 79.8%, 52.2%, and 45.4%; 2-year rates: 63.9%, 24.2%, and 15.2%; 3-year rates: 60.1%, 16.1%, 12.6%; P < 0.001; PFS, 1-year rates: 66.3%, 42.4%, and 45.6%; 2-year rates: 51.9%, 23.0%, and 16.7%; 3-year rates: 39.7%, 11.5%, 8.34%; P = 0.003) (**Supplementary Figure 1**).

Comparison of the Predictive Power of IBSs on Survival Outcomes in Two Cohorts

ROC curves and AUROC values were analyzed to contrast the prognostic capacity of eight IBSs in both primary (**Figure 4** and **Table 5**) and validation cohort (**Figure S2** and **Table 5**). The ROC curves were depicted at the 1-, 2-, 3-year follow-ups. C-index was calculated to compare the prognostic power of mGPS to other IBSs. In our primary cohort, the AUROC values of mGPS with OS (1-year 0.897, 2-year 0.813, 3-year 0.743) and PFS (1-year 0.728, 2-year 0.673, 3-year 0.661) were significantly higher than those of any other IBSs (OS: HR, 0.721; 95% CI, 0.705–0.737; all P < 0.001; PFS: HR, 0.645; 95% CI, 0.631–0.659, all P<0.001). The results of the validation cohort presented likewise similarly (OS: HR, 0.651; 95% CI, 0.585–0.717; all P < 0.001; PFS: HR, 0.623; 95% CI, 0.561–0.685; all P<0.005). Thus, the mGPS presented a more powerful prognostic prediction than other IBSs and could divide iCCA patients into subgroups with different survival outcomes more precisely.

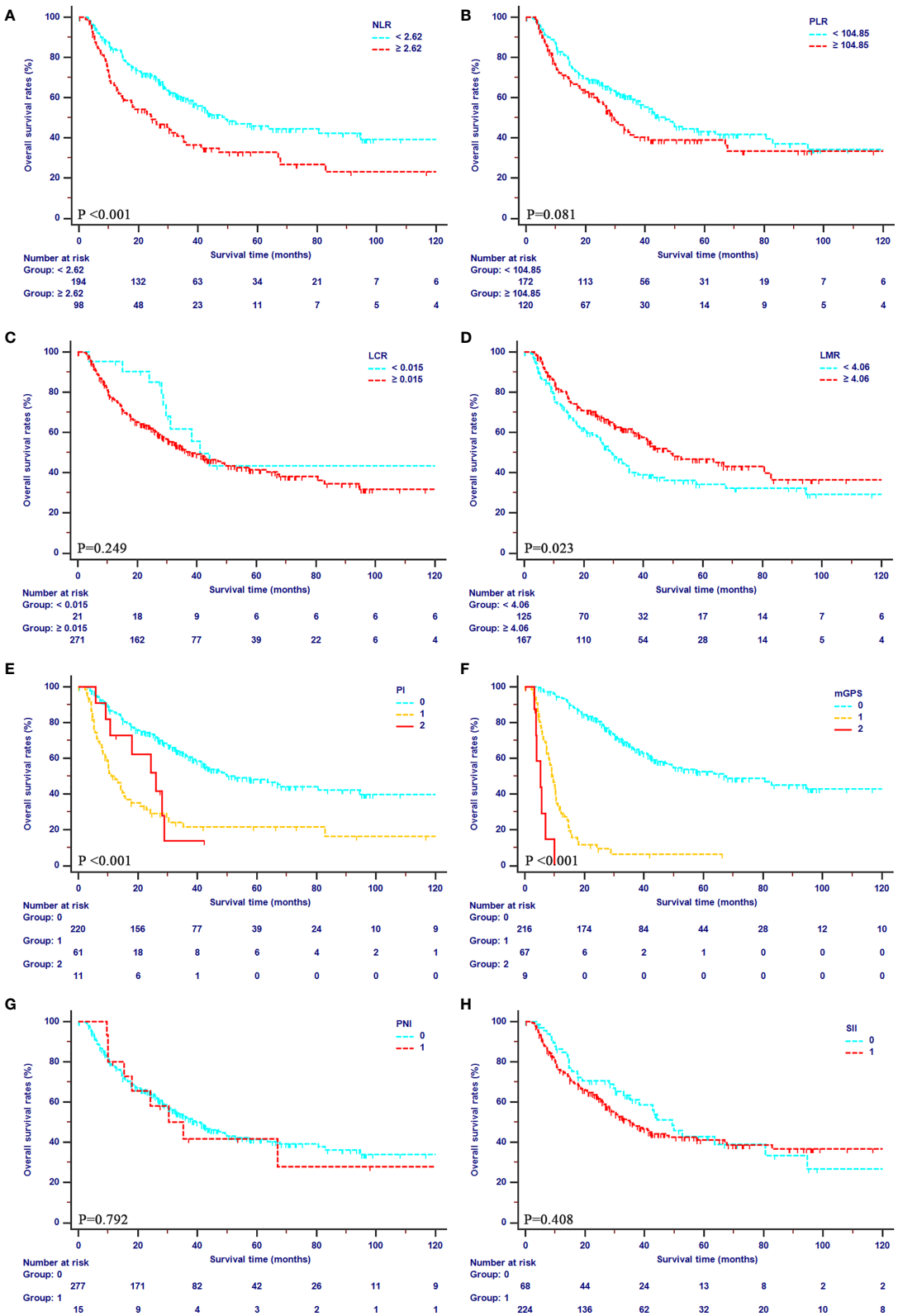

FIGURE 2 | Kaplan-Meier curves for OS in patients with iCCA in the SYSUCC cohort stratified by the inflammation-based score systems. **(A)**, NLR; **(B)**, PLR; **(C)**, LCR; **(D)**, LMR; **(E)**, PI; **(F)**, mGPS; **(G)**, PNI; **(H)**, SII.

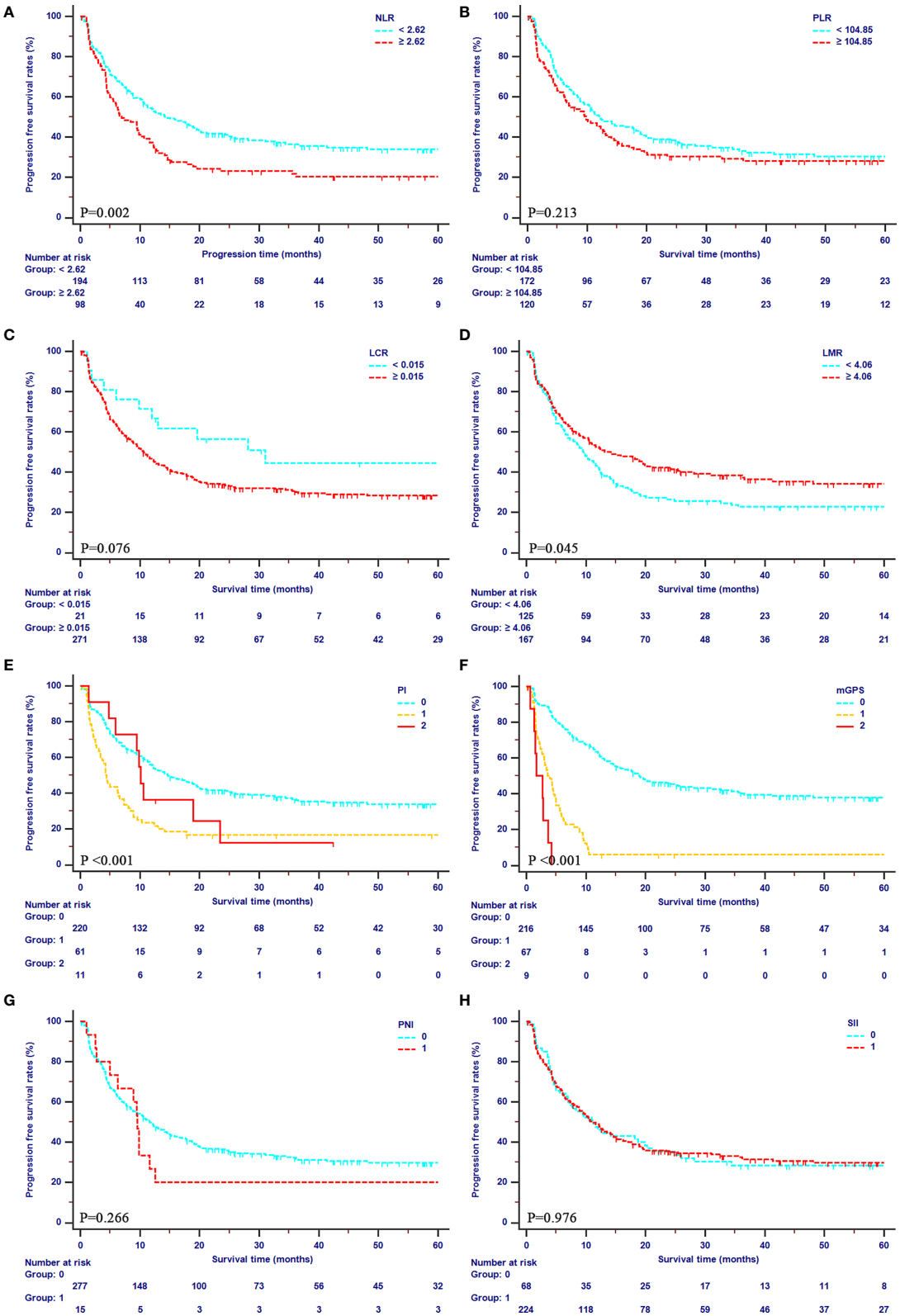

FIGURE 3 | Kaplan-Meier curves for PFS in patients with iCCA in the SYSUCC cohort stratified by the inflammation-based score systems. **(A)**, NLR; **(B)**, PLR; **(C)**, LCR; **(D)**, LMR; **(E)**, PI; **(F)**, mGPS; **(G)**, PNI; **(H)**, SII.

TABLE 3 | Univariate and multivariate analyses of prognostic factors of OS and PFS in the SYSUCC cohort.

Variables	OS				PFS			
	Univariate		Multivariate		univariate		multivariate	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Gender (Male: Female)	0.853 (0.610–1.192)	0.351			0.778 (0.581–1.043)	0.093		
Age, years (≤60:>60)	1.137 (0.818–1.579)	0.445			1.007 (0.756–1.340)	0.964		
WBC, $\times10^9$/L (≤10: >10)	2.395 (1.551–3.697)	<0.001	1.850 (0.962–3.557)	0.065	1.541 (1.019–2.329)	0.040	1.864 (0.972–3.574)	0.061
HGB, g/L (≤:175 >175)	1.455 (0.787–2.691)	0.232			1.258 (0.765–2.069)	0.365		
PLT, $\times10^9$/L (≤350: >350)	0.844 (0.373–1.911)	0.684			1.159 (0.545–2.465)	0.701		
ALT, U/L (≤50: >50)	1.443 (0.984–2.117)	0.061			1.225 (0.874–1.717)	0.239		
AST, U/L (≤40: >40)	1.266 (0.805–1.991)	0.307			1.315 (0.896–1.931)	0.162		
GGT, U/L (≤60: >60)	2.003 (1.393–2.879)	<0.001	0.980 (0.618–1.554)	0.931	1.518 (1.126–2.045)	0.006	0.780 (0.530–1.147)	0.206
ALP, U/L (≤125: >125)	2.583 (1.868–3.573)	<0.001	1.194 (0.755–1.890)	0.448	1.864 (1.410–2.463)	<0.001	1.166 (0.792–1.717)	0.435
ALB, g/L (≥35:<35)	1.086 (0.877–1.344)	0.449			0.986 (0.794–1.225)	0.899		
TBIL, μmol/L (≤20.5: >20.5)	1.361 (0.821–2.256)	0.232			1.222 (0.781–1.911)	0.380		
IBIL, μmol/L (≤15: >15)	1.139 (0.599–2.166)	0.691			1.492 (0.873–2.549)	0.143		
CRP, mg/L (≤3: >3)	2.072 (1.499–2.863)	<0.001	0.879 (0.550–1.405)	0.590	2.015 (1.524–2.664)	<0.001	1.229 (0.858–1.760)	0.260
HBsAg (no: yes)	1.012 (0.731–1.402)	0.940			1.180 (0.894–1.557)	0.243		
CA19-9, U/ml (≤35: >35)	1.951 (1.402–2.714)	<0.001	1.568 (1.071–2.296)	0.021	1.939 (1.459–2.575)	<0.001	1.586 (1.140–2.208)	0.006
CEA, ng/ml (≤5: >5)	2.713 (1.940–3.792)	<0.001	1.677 (1.112–2.528)	0.014	1.756 (1.301–2.370)	<0.001	0.898 (0.621–1.299)	0.568
LCR (0: 1)	1.458 (0.766–2.776)	0.249			1.723 (0.937–3.168)	0.076		
mGPS								
0	Ref		Ref		Ref		Ref	
1	9.902 (6.758–14.510)	<0.001	12.609 (7.142–21.251)	<0.001	4.548 (3.278–6.308)	<0.001	4.128 (2.634–6.489)	<0.001
2	41.983 (17.84–98.802)	<0.001	37.929 (12.609–113.367)	<0.001	11.709 (5.482–25.009)	<0.001	5.417 (2.1–13.976)	<0.001
NLR (<2.62: ≥2.62)	1.763 (1.271–2.446)	<0.001	0.890 (0.573–1.382)	0.604	1.562 (1.175–2.076)	0.002	0.959 (0.652–1.410)	0.831
LMR (<4.06: ≥4.06)	0.691 (0.501–0.953)	0.023	0.878 (0.591–1.304)	0.518	0.754 (0.571–0.995)	0.045	0.920 (0.647–1.308)	0.642
PLR (<104.85: ≥104.85)	1.332 (0.963–1.843)	0.081			1.194 (0.903–1.580)	0.213		
SII (0: 1)	1.175 (0.801–1.724)	0.408			1.005 (0.725–1.393)	0.976		
PNI (0: 1)	1.095 (0.558–2.149)	0.792			1.374 (0.782–2.414)	0.266		
PI								
0	Ref		Ref		Ref		Ref	
1	3.092 (2.157–4.433)	<0.001	0.896 (0.510–1.575)	0.703	2.146 (1.549–2.972)	<0.001	0.776 (0.478–1.259)	0.304
2	2.458 (1.189–5.083)	0.015	0.187 (0.059–0.593)	0.004	1.442 (0.734–2.833)	0.289	0.140 (0.048–0.414)	<0.001
Imaging tumor size (≤5 cm: >5 cm)	1.913 (1.368–2.676)	<0.001	1.011 (0.648–1.577)	0.961	1.758 (1.322–2.338)	<0.001	1.170 (0.785–1.744)	0.441
Imaging vascular invasion (no: yes)	1.178 (0.942–1.474)	0.151			1.239 (0.987–1.555)	0.065		
Imaging LN metastasis								
5^{th} LN metastasis	0.049 (0–237.011)	0.486			0.383 (0–49.654)	0.043	0 (0–7.198×10^{144})	0.946
7^{th} LN metastasis	1.031 (0.255–4.171)	0.965			1.476 (0.536–4.062)	0.451		
8^{th} LN metastasis	1.675 (0.976–2.877)	0.061			1.421 (0.881–2.290)	0.150		
9^{th} LN metastasis	3.177 (1.292–7.815)	0.012	3.179 (1.092–9.256)	0.034	2.294 (1.016–5.181)	0.046	1.112 (0.412–2.907)	0.834
12^{th} LN metastasis	2.847 (2.030–3.994)	<0.001	1.272 (0.740–2.185)	0.384	2.714 (1.930–3.817)	<0.001	1.936 (1.146–3.272)	0.014
13^{th} LN metastasis	1.752 (0.773–3.970)	0.179			1.842 (0.906–3.744)	0.092		
14^{th} LN metastasis	0.049 (0–4335.171)	0.667			3.235 (0.450–23.26)	0.243		
16^{th} LN metastasis	2.570 (0.917–8.083)	0.106			2.381 (0.758–7.475)	0.137		
Imaging LN size								
Absence	Ref		Ref		Ref		Ref	
≤1 cm	1.616 (0.987–2.645)	0.056	1.032 (0.422–1.185)	0.521	1.530 (0.964–2.428)	0.071	1.450 (0.724–2.312)	0.141

(Continued)

TABLE 3 | Continued

Variables	OS				PFS			
	Univariate		Multivariate		univariate		multivariate	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
>1 cm	1.948 (1.312–2.893)	0.001	1.872 (0.671–2.881)	0.471	2.188 (1.576–3.038)	<0.001	2.528 (0.672–4.138)	0.341
Tumor capsular (no: yes)	1.003 (0.811–1.240)	0.979			1.018 (0.844–1.226)	0.854		
Satellite sites (no: yes)	1.123 (1.044–1.208)	<0.001	0.946 (0.554–1.615)	0.839	2.147 (1.612–2.860)	<0.001	1.423 (0.923–2.195)	0.110
Thrombus (no: yes)	1.802 (1.087–2.987)	0.022	1.327 (0.736–2.363)	0.336	1.516 (0.955–2.406)	0.078		
Tumor differentiation								
Well	Ref				Ref		Ref	
Moderate	2.172 (0.528–8.930)	0.282			2.810 (0.688–11.484)	0.150	3.668 (0.788–17.080)	0.098
Poor	2.779 (0.682–11.326)	0.154			3.733 (0.922–15.119)	0.065	4.440 (0.957–20.644)	0.057
Microvascular invasion (no: yes)	1.606 (1.078–2.392)	0.020			1.754 (1.249–2.462)	<0.001	1.167 (0.750–1.815)	0.493
Lymph-vessel invasion (no: yes)	1.477 (0.851–2.563)	0.166			1.239 (0.732–2.097)	0.425		
Macrovascular invasion (no: yes)	1.530 (0.828–2.829)	0.175			1.419 (0.838–2.402)	0.193		
Adjacent organ invasion included gallbladder (no: yes)	1.765 (1.111–2.804)	0.016	0.608 (0.205–1.802)	0.369	1.902 (1.280–2.826)	<0.001	1.787 (0.750–4.258)	0.190
LN metastasis (no: yes)	3.304 (2.251–4.850)	<0.001	0.872 (0.427–1.784)	0.709	3.078 (2.158–4.392)	<0.001	0.901 (0.475–1.707)	0.748
Liver capsule invasion (no: yes)	1.240 (0.889–1.730)	0.205	0.418 (0.314–1.306)	0.134	1.391 (1.042–1.858)	0.025	1.093 (0.475–2.516)	0.835
T stage 8th								
1	Ref		Ref		Ref		Ref	
2	2.079 (1.231–3.512)	0.006	1.519 (0.752–3.16)	0.312	1.717 (1.078–2.735)	0.023	1.546 (1.756–4.237)	0.543
3	1.781 (1.153–2.751)	0.009	1.721 (0.101–5.317)	0.724	1.764 (1.226–2.539)	0.002	1.412 (0.428–4.186)	0.142
4	2.482 (1.315–4.686)	0.005	1.892 (0.698–7.972)	0.811	2.207 (1.296–3.758)	0.004	1.394 (0.334–4.257)	0.610
Tumor size (≤5 cm: >5 cm)	2.900 (1.997–4.211)	<0.001	1.301 (0.753–2.248)	0.345	2.164 (1.605–2.916)	<0.001	1.396 (0.903–2.158)	0.134
TNM 8th								
I	Ref		Ref		Ref		Ref	
II	1.602 (0.898–2.857)	0.111	2.571 (0.261–25.445)	0.419	1.362 (0.819–2.267)	0.234	1.519 (0.162–14.205)	0.714
III	2.048 (1.335–3.141)	0.001	2.968 (0.327–26.925)	0.334	1.943 (1.360–2.777)	<0.001	2.540 (0.292–22.100)	0.398
After operation therapy (no: yes)	1.901 (1.372–2.635)	<0.001	1.941 (1.345–2.778)	<0.001	3.176 (2.3678–4.260)	<0.001	3.571 (1.878–5.150)	<0.001

OS, overall survival; PFS, progression-free survival; Ref, reference. Other abbreviations as in ***Table 1****.*

TABLE 4 | External validation of significant prognostic factors in primary cohort.

Variables	OS		DFS	
	HR (95% CI)	P	HR (95% CI)	P
CA19-9, U/ml (≤35: >35)	0.663 (0.354–1.240)	0.198	0.823 (0.484–1.402)	0.474
CEA, ng/ml (≤5: >5)	1.734 (0.983–3.059)	0.057	1.134 (0.763–1.588)	0.631
mGPS				
0	Ref		Ref	
1	6.563 (2.024–21.28)	0.002	6.763 (2.445–18.705)	< 0.001
2	10.598 (2.994–37.514)	< 0.001	9.128 (3.023–27.564)	< 0.001
Imaging LN metastasis (no: yes)	1.141 (0.679–1.917)	0.618	1.317 (0.829–2.092)	0.243
After operation therapy (no: yes)	0.734 (0.430–1.251)	0.256	0.738 (0.456–1.195)	0.217

*Abbreviations as in **Table 3**.*

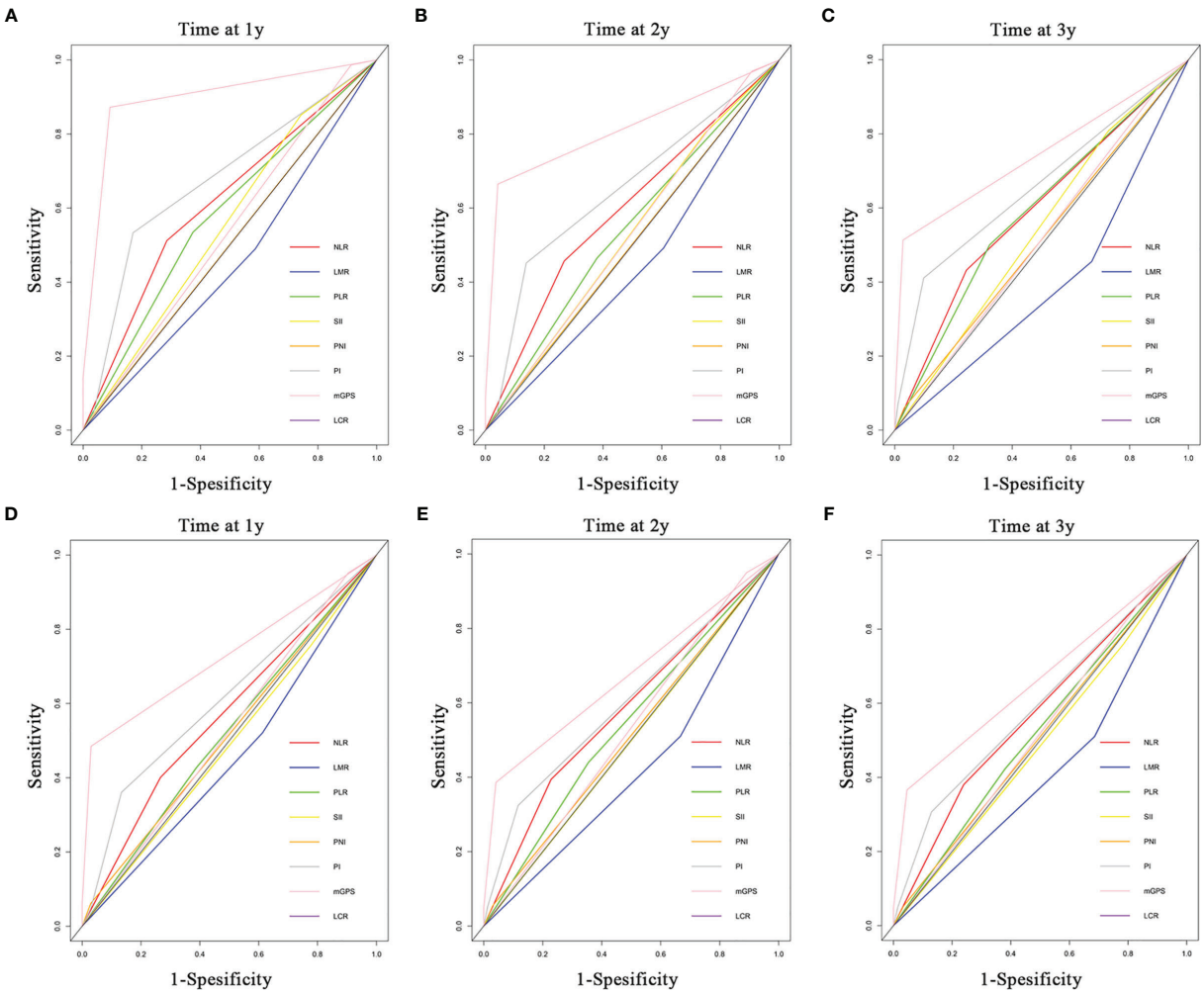

FIGURE 4 | Comparisons of the ROC curves for OS and PFS in the SYSUCC cohort among the inflammation-based score systems. ROC curves of OS at 1 **(A)**, 2 **(B)**, and 3 years **(C)**. ROC curves of PFS at 1 **(D)**, 2 **(E)**, and 3 years **(F)**.

DISCUSSION

Over the past decades, various progresses have been made in prophylaxis and treatment of cholangiocarcinoma (1, 2). Nevertheless, the OS and PFS of iCCA patients remained poor (3). For the prediction of prognosis, the TNM grade system has been applied as the mainstream prognostic assessment system since it was presented. However, the TNM grades can only be

TABLE 5 | Comparisons of the AUROC values and C-index with mGPS and other IBSs.

Cohort	IBS	OS					DFS				
		AUROC			C-index	P	AUROC			C-index	P
		1 year	2 year	3 year			1 year	2 years	3 years		
SYSUCC cohort	mGPS	0.897	0.813	0.743	0.721 (0.705–0.737)	Ref	0.728	0.673	0.661	0.645 (0.631–0.659)	Ref
	NLR	0.613	0.594	0.594	0.603 (0.579–0.627)	<0.001	0.567	0.583	0.570	0.563 (0.542–0.583)	<0.001
	PI	0.676	0.651	0.657	0.618 (0.599–0.636)	<0.001	0.611	0.605	0.590	0.568 (0.553–0.583)	<0.001
	PLR	0.580	0.543	0.589	0.544 (0.523–0.565)	<0.001	0.519	0.542	0.521	0.529 (0.511–0.547)	<0.001
	SII	0.554	0.528	0.540	0.529 (0.512–0.546)	<0.001	0.490	0.499	0.488	0.504 (0.489–0.519)	<0.001
	PNI	0.501	0.503	0.514	0.503 (0.495–0.511)	<0.001	0.516	0.512	0.509	0.504 (0.497–0.511)	<0.001
	LMR	0.451	0.443	0.392	0.554 (0.533–0.575)	<0.001	0.454	0.422	0.412	0.530 (0.512–0.548)	<0.001
	LCR	0.537	0.532	0.514	0.518 (0.509–0.527)	<0.001	0.524	0.529	0.517	0.516 (0.506–0.527)	<0.001
FHDMU cohort	mGPS	0.683	0.693	0.772	0.651 (0.585–0.717)	Ref	0.613	0.648	0.731	0.623 (0.561–0.685)	Ref
	NLR	0.584	0.545	0.593	0.532 (0.468–0.596)	<0.001	0.563	0.522	0.633	0.534 (0.457–0.593)	<0.001
	PI	0.615	0.619	0.703	0.594 (0.526–0.662)	<0.001	0.552	0.570	0.615	0.562 (0.499–0.625)	0.032
	PLR	0.566	0.527	0.460	0.520 (0.469–0.571)	<0.001	0.506	0.471	0.453	0.498 (0.450–0.546)	<0.001
	SII	0.633	0.598	0.585	0.575 (0.522–0.628)	<0.001	0.578	0.550	0.620	0.562 (0.511–0.613)	0.004
	PNI	0.547	0.510	0.453	0.549 (0.485–0.613)	<0.001	0.499	0.466	0.475	0.525 (0.464–0.586)	0.002

AUROC, area under the ROC curves. Other abbreviations as in ***Table 3****.*

calculated according to the postoperative pathological factors, and the systemic inflammation level was not included in the TNM grade system. As a result, the TNM grade system cannot make a preoperative overall assessment to guide the therapeutic strategy. To fill this gap, there has been an urgent demand to explore and validate a pre-operative potential prognostic factor for patients with iCCA. IBS, as a combination inflammation index, can objectively reflect the level of inflammation, and further indicate the prognostic and outcomes of cancer patients.

The present study compared the prognostic efficacy of eight common IBSs in patients with iCCA. The univariate and multivariate analyses were further performed to verify the prognostic factors. The mGPS was identified as a significant prognostic factor for predicting OS and PFS in both SYSUCC and FHDMU cohort. Moreover, it was shown that mGPS was superior to the other IBS indexes for predicting the OS and PFS of iCCA patients. It is worth noting that classical pathological elements and TNM staging system made no significant association with the OS and PFS in this study. The probable reasons for the outcome are as follows. First, the powerful predictive performance of mGPS for OS and PFS might mask the role of pathological elements in the multivariate analysis. As a result, the pathological factors showed no significant difference in multivariate analysis. However, that was not intended to deny the predictive effect of pathological elements. As our univariate analysis presented, the TNM staging system was still a statistically significant predictor of prognosis (P = 0.003 in OS, P<0.001 in PFS). Second, the TNM system is a continuously updating and evolving standard with its graded prognostic effect remains controversial. For instance, invasive liver capsule may not adequately reflect the pathogenesis of iCCA tumor, due to the influence of tumor location and tumor size (20). Additionally, the definition of category T3 could barely indicate the biological extent of iCCA tumor (21). Moreover, the number of lymph nodes determined by preoperative imaging examinations and intraoperative findings cannot objectively indicate lymph node metastasis, which may further lead to misjudgment or underestimation of N stage (20, 21). In addition, a further analysis was conducted to elucidate the relationship between mGPS and some clinical and pathological characteristics (**Supplementary Table 1**). The results demonstrated that there was a significant correlation between mGPS and tumor marker (CA19-9, CEA), satellite sites, microvascular invasion, tumor size, lymph node metastasis and TNM stages. These characteristics were significantly related to the poor survival outcomes. Different from these factors, mGPS could be assessed preoperatively. It was worth noting that the CRP level was the key point between mGPS 0 group and mGPS 1/2 groups. In the present study, mGPS 0 group presented a better survival outcome than mGPS1/2 group did. This could also certify the vital role which inflammation played in tumor progression.

The GPS staging system was firstly established in inoperable non-small-cell lung cancer (22), with two major evaluative dimensions: serum ALB and CRP. Serum ALB may indicate the general status as well as the amount of lean tissue of cancer patients. Furthermore, hypoalbuminemia is also associated with cachexia. ALB has been shown to be a prognostic marker in gastric cancer (23) and pancreatic cancer (24), and the role of albumin as a marker of inflammation has been underscored by recent research in malignancy (25). In addition, iCCA, as a type of liver cancer, can weaken the synthesis function of the liver, further leading to the hypoalbuminemia. On the other hand, CRP is not only a sensitive indicator of the systemic inflammatory response. Accumulating evidence indicated the role of CRP in the tumor development and metastasis (26, 27). Theoretically, high CRP level may be due to the production of cytokines from tumor cells (26). As an acute-phase protein, CRP together with IL-6, TNF, and other cytokines further initiates or sustains the systemic inflammatory response (27). Then, inflammation promotes the tumor proliferation, angiogenesis, invasion, and metastasis as a feedback loop (4). It has been confirmed that high level of CRP was correlated with unfavorable

survival in esophageal carcinoma (28), colorectal carcinoma (29), as well as multiple myeloma (30). Moreover, as a commonly used index, CRP has high sensitivity and cost-effectiveness and is easily obtained in clinical practice.

With the increasing numbers of studies about GPS and survival in patients with cancers, researchers found that hypoalbuminemia regularly occurred with elevated CRP levels (31). Moreover, the survival outcomes of patients with hypoalbuminemia alone were significantly better than patients with elevated CRP levels, indicating that CRP played a more important role in survival prediction. In case of that, GPS was modified into mGPS (32). Since then, the modified GPS has been occupied in colorectal cancer (32), hepatocellular carcinoma, esophageal cancer (33), and ovarian cancer (34) and simultaneously presented robust prognostic prediction. Significantly, this is the very first study which evaluating the prognostic prediction of the common IBSs in iCCA patients. In this large, multicenter cohort study, we compared the survival curves of these eight frequently used IBSs. Surprisingly, mGPS was not only the independent prognostic factor of OS ($P < 0.001$) and PFS ($P < 0.001$) in both of our cohorts, it presented the most powerful performance of prognostic prediction in the common IBSs (all $P < 0.005$). Similarly, mGPS also presented prominent prognostic manifestations in perihilar cholangiocarcinoma (35) and biliary tract cancer (36) in previous studies. Furthermore, by contrast with the pathological prognostic factors, mGPS, as an inflammation-based score, could make the pre-operative prediction of cancer patients to facilitate accurate stratification and further improve the survival outcomes. Besides, assessments of serum albumin and CRP are simple and inexpensive compared to genetic assessments, which are complicated and expensive.

According to the results of the present study, the treatment strategies of patients with higher mGPS should be optimized. Clinical staff should be especially caution about the indications and contraindications of operation and take careful consideration about the overall healthy situation of these patients. The shorter follow-up intervals were conducive to earlier detection of tumor recurrence or progression. And this would further provide an opportunity for early medical intervention in recurrence. Moreover, the inclusion of routine postoperative chemotherapy in the overall treatment strategies may be beneficial.

Certain limitations of the present study merit discussion. First, the retrospective nature is a potential limitation; we enrolled two cohorts from different regions to restrain this limitation. Second, improvements in perioperative management and treatment methods may lead to the heterogeneous antitumor treatments of our patients' cohorts and further interfere with the result of the present study. Third, the underlying mechanism of mGPS and poor survival outcome has not been fully demonstrated. Finally, further extensive trans-regional studies were needed to verify the prognostic power of mGPS in iCCA patients.

In conclusion, the present study, we identified mGPS as a sensitive, efficient, simple, rapid, and widely applicable preoperative prognostic factor for iCCA patients. Elevated mGPS scores indicated poor prognosis for these patients. Thus, more effective therapy and frequent surveillance after treatment should be conducted for the iCCA patients with higher mGPS scores.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Sun Yat-sen University Cancer Center and the first affiliated hospital of Dalian Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Study concept: XL. Study design: CH, CZ, and JL. Drafting of the manuscript: CH, CZ, and JL. Data collecting: CH, CZ, JL, CC, and XH. Data analysis: CH, CZ, JL, and XH. Critical revision of the manuscript: XL. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.672607/full#supplementary-material

Supplementary Figure 1 | Kaplan-Meier curves for OS **(A)** and PFS **(B)** in patients with iCCA in the FHDMU cohort stratified by the mGPS.

Supplementary Figure 2 | Comparisons of the ROC curves for OS and PFS in the FHDMU cohort among the inflammation-based score systems. ROC curves of OS at 1 **(A)**, 2 **(B)**, and 3 years **(C)**. ROC curves of PFS at 1 **(D)**, 2 **(E)**, and 3 years **(F)**.

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ORIGINAL RESEARCH
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A Nomogram Based on Preoperative Inflammatory Indices and ICG-R15 for Prediction of Liver Failure After Hepatectomy in HCC Patients

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Objective: To establish a nomogram based on inflammatory indices and ICG-R15 for predicting post-hepatectomy liver failure (PHLF) among patients with resectable hepatocellular carcinoma (HCC).

Methods: A retrospective cohort of 407 patients with HCC hospitalized at Xiangya Hospital of Central South University between January 2015 and December 2020, and 81 patients with HCC hospitalized at the Second Xiangya Hospital of Central South University between January 2019 and January 2020 were included in the study. Totally 488 HCC patients were divided into the training cohort (n=378) and the validation cohort (n=110) by random sampling. Univariate and multivariate analysis was performed to identify the independent risk factors. Through combining these independent risk factors, a nomogram was established for the prediction of PHLF. The accuracy of the nomogram was evaluated and compared with traditional models, like CP score (Child-Pugh), MELD score (Model of End-Stage Liver Disease), and ALBI score (albumin-bilirubin) by using receiver operating characteristic (ROC) curve, calibration curve, and decision curve analysis (DCA).

Results: Cirrhosis (OR=2.203, 95%CI:1.070-3.824, P=0.030), prothrombin time (PT) (OR=1.886, 95%CI: 1.107-3.211, P=0.020), tumor size (OR=1.107, 95%CI: 1.022-1.200, P=0.013), ICG-R15% (OR=1.141, 95%CI: 1.070-1.216, P<0.001), blood loss (OR=2.415, 95%CI: 1.306-4.468, P=0.005) and AST-to-platelet ratio index (APRI) (OR=4.652, 95%CI: 1.432-15.112, P=0.011) were independent risk factors of PHLF. Nomogram was built with well-fitted calibration curves on the of these 6 factors. Comparing with CP score (C-index=0.582, 95%CI, 0.523-0.640), ALBI score (C-index=0.670, 95%CI, 0.615-0.725) and MELD score (C-ibasedndex=0.661, 95%CI, 0.606-0.716), the nomogram showed a better predictive value, with a C-index of 0.845 (95%CI, 0.806-0.884). The results were consistent in the validation cohort. DCA confirmed the conclusion as well.

Conclusion: A novel nomogram was established to predict PHLF in HCC patients. The nomogram showed a strong predictive efficiency and would be a convenient tool for us to facilitate clinical decisions.

Keywords: ICG-R15, APRI, nomogram, hepatocellular carcinoma, post-hepatectomy liver failure

INTRODUCTION

With the rapid rise in its prevalence, hepatocellular carcinoma (HCC) has become the sixth most aggressive malignant tumor and the second-leading cause of cancer-related deaths worldwide (1). Among all therapeutic strategies, surgical resection remain the mainstay of the curative approach for HCC nowadays (2). Even though surgical technique and perioperative care have significantly improved over the past few years, post-hepatectomy liver failure (PHLF) is still the primary driver of morbidity and mortality after hepatectomy in HCC patients (3, 4). In patients with low liver regeneration capability and reduced function reservation of remnant liver tissue following hepatectomy, PHLF occurs most frequently. Therefore, it is critically important to predict the risk of PHLF, which is essential for surgeons to choose individualized treatment.

To accurately predict PHLF, several articles relating to PHLF have been published (5–8). Although these efforts on the preoperative prediction of PHLF have been made, an effective prediction model is still lacking (9). For many years, the clinical scoring systems, such as CP score (Child-Pugh) (10, 11) and MELD score (Model of End-Stage Liver Disease) (12), are widely used for preoperative assessment of liver function. The Child-Pugh score system has some drawbacks and limitations because of its two subjective clinical variables-ascites and hepatic encephalopathy (11). Similarly, the MELD score is not optimal for the prediction of PHLF. Recent research indicated that a new evidence-based model, called the albumin-bilirubin (ALBI) score (13). has been developed to assess liver function reserve. And it has been proven to be superior in estimating PHLF and survival of HCC patients undergoing liver resection (14, 15). Indocyanine green (ICG) (16), a nontoxic, infrared, and photosensitive dye, can be combined with albumin and beta lipoprotein. As a quantitative test to assess hepatic blood flow and liver function, the ICG clearance test at 15 minutes (ICG-R15) is now commonly used to evaluate reserved liver function in surgical patients. Moreover, ICG-R15 has been proven to be a reliable predictor of PHLF recently (17, 18).

Serum inflammatory indices are a reflection of the systemic inflammatory, which plays a significant role in the pathogenesis and progression of liver cirrhosis (19). Recently, a study has demonstrated that chronic inflammation can increase the operative risk of liver resection (20). However, the exact relationship between Serum inflammatory indices and PHLF is not very clear. And whether the combination of inflammatory indices and ICG-R15 could add more benefit in predicting PHLF is worth exploring.

Therefore, this study aims to investigate the possibility of inflammatory markers in predicting PHLF. Moreover, we develop a nomogram based on ICG-R15 and inflammatory markers, and compare its predictive value with traditional models, such as CP score, MELD score and ALBI score, in HCC patients undergoing hepatectomy.

PATIENTS AND METHODS

Patients

We retrospectively collected the data from 488 HCC patients who underwent partial hepatectomy from the Xiangya Hospital of Central South University and the Second Xiangya Hospital of Central South University in China. By random sampling, 378 patients were selected as training cohorts while another 110 patients were chosen as the validation cohorts. The study was approved by the Ethics Committee of the Xiangya Hospital of Central South University and the Second Xiangya Hospital of Central South University in compliance with the Declaration of Helsinki. Written informed consent was obtained from all patients for use of their data in this study. Barcelona Clinic Liver Cancer (BCLC) criteria were applied to select HCC patients for hepatectomy in the paper.

The inclusion criteria were as follows: 1) diagnosis of HCC confirmed by histology; 2) no anticancer treatments before hepatectomy, including transarterial chemoembolization, radiofrequency ablation, and others; 3) no simultaneous malignancies; 4) no preoperative obstructive jaundice; and 5) no preoperative cardiopulmonary, renal dysfunction or severe encephalopathy.

The exclusion criteria were as follows: 1) Patients with recurrent tumors before hepatectomy; 2) no liver function and coagulation function data on or after postoperative day 5.

Indocyanine Green Test

Prior to surgery, a dose of 50mg ICG (Yichuang Pharmaceutical Co. Ltd., China) dissolved in 10ml of sterile water was injected through a peripheral vein based on the bodyweight of patients (0.5mg/kg). The 15-min retention rate of ICG (ICG-R15) was measured at 15 min after injection using a pulse spectrophotometer (DDG-3300K, Japan). Results were expressed as the percentage of ICG-R15 after injection.

Clinicopathologic Variables

Patients' demographic variables were collected including age, gender, history of diabetes, hypertension, hepatitis B based on discharge diagnosis. The number of tumor nodules, tumor size (major nodule diameter), cirrhosis and ascites were included in patients' imaging data based on contrast-enhanced MRI, contrast-enhanced CT and ultrasound. The following data were recorded based on intraoperative situation: time of

operation, blood loss. Preoperative serum examination included serum α-fetoprotein level (AFP), ICG-R15, creatinine (Cr), albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), alanine transaminase (ALT), aspartate transaminase (AST), prothrombin time (PT), international normalized ratio (INR), neutrophil, lymphocyte, monocyte, platelet, hemoglobin, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), AST-to-platelet ratio index (APRI), lymphocyte-to-monocyte ratio (LMR), and AST-to-neutrophil ratio index (ANRI). ALBI score = 0.66 × lg (TBIL, μmol/L) – 0.085 × (ALB, g/L). The MELD score = 11.2 × ln (INR) + 9.57 × ln (Cr, mg/dL) + 3.78 × ln (TBIL, mg/dL) + 6.43. APRI = [AST level (/ULN)/Platelet counts (109/L)] × 100. NLR was determined by the neutrophils count divided by lymphocytes count. PLR was measured by the platelet count divided by lymphocytes count. LMR was calculated by the lymphocytes count divided by monocytes count. ANRI was calculated by the AST divided by neutrophils count. All preoperative assessments including ICG, blood routine test, liver function test, AFP, and imaging material was arranged on the 1st day after administration (within 1 week before surgery).

Definitions of PHLF

There are various definitions of PHLF that have been used. For example, in the study of Eguschi et al (21), PHLF was diagnosed when three results were present in the patient: (1) hepatic encephalopathy, (2) progressive hyperbilirubinemia, (3) reduced hepaplastin test. The "50-50 criteria" is another definition of PHLF proposed by Balzan (22). But it has some limitations due to its high specificity (97.7%) and low sensitivity (69.6%).

It is in 2010 that a consensus about PHLF was reached. PHLF was defined as a postoperatively acquired deterioration in the ability of the liver to maintain its synthetic, excretory, and detoxifying functions, which are characterized by an increased INR and concomitant hyperbilirubinemia on or after postoperative day 5 by the International Study Group of Liver Surgery (ISGLS) (3). So the diagnosis of PHLF in our study is based on the definition of PHLF proposed by ISGLS.

Statistical Analysis

Continuous variables were expressed as mean ± SD and compared using the Student's *t*-test or Mann–Whitney *U* test. Categorical variables were shown as frequency and compared using either chi-square test or Fisher exact test. Factors whose P values were less than 0.05 in the univariate analysis were subjected to multivariate logistic regression analysis to identify the independent predictors of PHLF. According to the independent PHLF predictors, a nomogram was plotted by using the *rms* package of R (version 4.0.3). ROC curve analysis was used for comparison between our nomogram and other models based on the concordance index (C index). A calibration plot with 1000 bootstrap samples was employed to measure the accuracy of the nomogram. The decision curve analysis (DCA) was conducted to estimate the clinical usefulness of the nomogram through quantifying net benefits at different threshold probabilities. SPSS 26.0 (SPSS Inc, Chicago, IL, USA) and R 4.0.3 software (Institute for Statistics and Mathematics) were performed in our analysis. P< 0.05 was considered statistically significant.

RESULTS

Clinicopathologic Characteristics of Patients

During the study period, 488 patients who met the inclusion criteria were enrolled, including 417 (85.45%) males and 71 (14.55%) females, and divided into the training cohort and validation cohort. The mean age of the 488 patients was 53.08 ± 11.68 (range from 18 to 83) years. The majority of patients (85.24%) were infected with hepatitis B virus (HBV) and cirrhosis was observed in 346 (70.9%) patients. The mean tumor size was (6.53 ± 4.29) cm and 87 (17.83%) patients had multiple tumors. The mean ICG-R15 and APRI were 7.59 ± 7.33 and 0.91 ± 0.74, respectively. PHLF occurred in 42.8% of patients (n =209).

In the training cohort, 378 patients were enrolled and PHLF occurred in 163 patients. For the validation cohort, 110 patients were studied and PHLF occurred in 46 patients. The clinicopathologic characteristics of the patients are listed in **Table 1**. The baseline clinicopathologic data were comparable between the training and validation cohorts.

Univariate and Multivariate Analysis of Factors of PHLF

In the training cohort, the univariate analysis suggested that gender (P=0.023), cirrhosis (P<0.001), lymphocyte (P<0.001), platelet (P<0.001), TBil (P<0.001), DBil (P<0.001), Alb (P<0.001), ALT (P<0.001), AST (P<0.001), PT (P<0.001), INR (P<0.001), tumor size (P=0.002), ICG-15R% (P<0.001), blood loss (P<0.001), operation time (P=0.006), NLR (P=0.025), LMR (P<0.001), ANRI (P<0.001) and APRI (P<0.001) were potential risk factors of PHLF. Then, all these potential risk factors were accepted into the multivariate logistic analysis. Only cirrhosis (P=0.030), PT (P=0.020), tumor size (P=0.013), ICG-R15% (P<0.001), blood loss (P=0.005) and APRI (P=0.011) were independent risk factors of PHLF (**Table 2**).

Nomogram for Post-Hepatectomy Liver Failure

Through multivariate analysis, we found that cirrhosis, PT, tumor size, ICG-R15%, blood loss and APRI were independent risk factors of PHLF. These independent risk factors were further integrated to establish a PHLF estimation nomogram in the training cohort (**Figure 1**). The nomogram showed a better accuracy for PHLF prediction, with a C-index of 0.845 (95%CI, 0.806-0.884) (**Figure 2**). The calibration curves for PHLF prediction revealed sufficient agreement between the nomogram and actual observation (**Figure 3**).

TABLE 1 | Characteristics of patients in training cohort and validation cohort.

Characteristics	Training (n=378)	Validation (n=110)	P value
Age, years	53.29±11.74	52.36±11.50	0.463
Gender			
Male	320	97	0.356
Female	58	13	
Diabetes			
Yes	41	19	0.071
No	337	91	
Hypertension			
Yes	104	27	0.536
No	274	83	
HBsAg			
Positive	321	95	0.707
Negative	57	15	
Cirrhosis			
Yes	261	85	0.095
No	117	25	
Neutrophil, 10^9/L	3.31±1.32	3.01±1.19	0.031
Lymphocyte, 10^9/L	1.42±0.49	1.49±0.63	0.234
Monocyte, 10^9/L	0.46±0.20	0.44±0.18	0.418
Platelet, 10^9/L	158.80±80.65	160.32±77.75	0.861
HB, g/L	139.00±18.38	142.07±17.17	0.118
TBil, μmol/L	13.24±6.04	13.24±5.15	0.994
DBil, μmol/L	6.00±3.20	6.04±2.48	0.926
Alb, g/L	39.90±4.53	39.74±4.35	0.737
ALT, U/L	40.98±37.32	37.94±22.16	0.415
AST, U/L	49.30±38.50	44.56±32.32	0.240
PT, s	13.47±1.39	13.57±1.27	0.485
INR	1.08±0.11	1.09±0.10	0.335
Cre, μmol/L	84.12±19.69	83.98±17.16	0.949
AFP, ng/ml			
≥400	148	39	0.482
<400	230	71	
Tumor size, cm	6.66±4.31	6.06±4.17	0.194
Tumor number			
Solitary	307	94	0.307
Multiple	71	16	
ICG-R15 (%)	7.38±6.40	8.33±9.90	0.234
Blood loss, ml			
≥400	226	63	0.637
<400	152	47	
Operation time,min	206.10±68.30	204.47±61.04	0.822
NLR	2.57±1.38	2.23±1.06	0.006
PLR	119.44±66.87	114.99±57.25	0.527
LMR	3.45±1.37	3.75±1.65	0.055
ANRI	16.66±13.14	17.08±14.25	0.771
APRI	0.92±0.74	0.88±0.75	0.617

Categorical variables are expressed as frequency. Continuous variables are expressed as mean (standard deviation).

ICG-R15, indocyanine green retention rate at 15 min; AFP, α-fetoprotein level; HBsAg, hepatitis be antigen; HB, hemoglobin; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; PT, prothrombin time; INR, international normalized ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; APRI, AST-to-platelet ratio index; ANRI, AST-to-neutrophil ratio index.

Validation of the Nomogram

In the validation cohort, the nomogram also demonstrated a better accuracy for PHLF prediction, with a C-index of 0.854 (95%CI, 0.782-0.926) (**Figure 4**). The calibration curves for PHLF prediction showed good agreement between the nomogram and actual observation (**Figure 5**).

Comparison of Predictive Accuracy for PHLF Between the Nomogram and the Conventional Models in the Training Cohort and Validation Cohort Respectively

In the training cohort, the C-index of the nomogram was significantly higher than CP score (C-index=0.582, 95%CI, 0.523-0.640), ALBI score (C-index=0.670, 95%CI, 0.615-0.725), MELD score (C-index=0.661, 95%CI, 0.606-0.716) (**Figure 2**). DCA has been used to evaluate the clinical value of models that integrates the preferences of patients into the analysis (23, 24). DCA indicated that this nomogram of PHLF prediction added more benefit compared with CP score, ALBI score and MELD score (**Figure 6**). In the validation cohort, we can draw the same conclusion. The C-index of the nomogram was higher than CP score (C-index=0.606, 95%CI, 0.496-0.716), ALBI score (C-index=0.771, 95%CI, 0.678-0.865), MELD score (C-index=0.583, 95%CI, 0.476-0.690) (**Figure 4**). DCA of validation cohort showed that this nomogram was more reliable compared with conventional models too (**Figure 7**).

DISCUSSION

Post-hepatectomy liver failure is one of the most feared complications after hepatectomy in HCC patients. There is a need to prospectively identify HCC patients at risk of PHLF. Therefore, establishing a prediction model of PHLF is necessary to improve clinical decisions.

Many models have been put forward to predict the occurrence of PHLF. But the predictive model is still evolving due to its multifactorial causative factors (25). Based on our clinical data, we performed this study to recognize the risk of PHLF in HCC patients in order to construct a nomogram for predicting PHLF.

In our analysis, we found that tumor size, blood loss(≥400ml), cirrhosis, PT, ICG-R15 and APRI were the independent risk factors for PHLF in HCC patients through the multivariable logistic regression analysis. Based on the risk factors, we developed the nomogram to predict the occurrence of PHLF. As for tumor size, we think the size of tumor influence the scope of resection of liver parenchyma, consequently affecting the volume of the remaining healthy liver. Several reports suggested that patients with a smaller liver remnant have a greater chance of developing PHLF (26, 27). Also, Heng Zou and his team found liver remnant is a good predictor of PHLF (28). As for blood loss, Osamu Aramaki (29) in his article demonstrated that intraoperative blood loss was the most crucial factor related to postoperative complications, including PHLF. Also back in 2007, Marieke T. de Boer found that there is a significant and clinically relevant association between blood loss and postoperative mortality and morbidity (30).

TABLE 2 | Univariable And Multivariable Analyses for preoperative and intraoperative variables of PHLF according to ISGLS criteria in the training cohort.

Variables	Univariable logistic regression		Multivariable logistic regression	
	OR (95%CI)	P value	OR (95%CI)	P value
Age, years	1.018 (1.000-1.036)	0.050		
Gender, (Female vs Male)	0.494 (0.269-0.906)	0.023		
Diabetes, (Yes vs No)	1.158 (0.604-2.219)	0.659		
Hypertension, (Yes vs No)	1.468 (0.933-2.312)	0.097		
HBsAg, (Yes vs No)	1.360 (0.760-2.433)	0.300		
Cirrhosis, (Yes vs No)	2.839 (1.762-4.574)	<0.001	2.203 (1.070-3.824)	0.030
Neutrophil, 10^9/L	0.921 (0.787-1.078)	0.305		
Lymphocyte, 10^9/L	0.291 (0.179-0.474)	<0.001		
Monocyte, 10^9/L	1.596 (0.561-4.537)	0.381		
Platelet, 10^9/L	0.995 (0.992-0.998)	<0.001		
HB, g/L	1.003 (0.992-1.014)	0.629		
TBil, μmol/L	1.075 (1.035-1.117)	<0.001		
DBil, μmol/L	1.192 (1.097-1.296)	<0.001		
Alb, g/L	0.909 (0.867-0.954)	<0.001		
ALT, U/L	1.012 (1.004-1.020)	0.002		
AST, U/L	1.018 (1.010 1.025)	<0.001		
PT, s	1.552 (1.299-1.853)	<0.001	1.886 (1.107-3.211)	0.020
INR	1.761 (1.401-2.213)	<0.001		
Cre, μmol/L	1.004 (0.994-1.014)	0.460		
AFP, (≥400 vs<400 ng/ml)	1.208 (0.796-1.832)	0.374		
Tumor size, cm	1.081 (1.030-1.135)	0.002	1.107 (1.022-1.200)	0.013
Tumor number, (≥2 vs<2)	1.677 (0.998-2.817)	0.051		
ICG-R15 (%)	1.169 (1.115-1.226)	<0.001	1.141 (1.070-1.216)	<0.001
Blood loss, (≥400 vs<400 ml)	2.870 (1.850-4.452)	<0.001	2.415 (1.306-4.468)	0.005
Operation time, min	1.004 (1.001-1.007)	0.006		
NLR	1.194 (1.022-1.395)	0.025		
PLR	0.999 (0.996-1.002)	0.594		
LMR	0.725 (0.614-0.856)	<0.001		
ANRI	1.064 (1.041-1.089)	<0.001		
APRI	7.176 (4.212-12.226)	<0.001	4.652 (1.432-15.112)	0.011

ICG-R15, indocyanine green retention rate at 15 min; AFP, α-fetoprotein level; HBsAg, hepatitis be antigen; HB, hemoglobin; ALB, albumin; TBIL, total bilirubin; DBIL, direct bilirubin; ALT, alanine transaminase; AST, aspartate transaminase; PT, prothrombin time; INR, international normalized ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; APRI, AST-to-platelet ratio index; ANRI, AST-to-neutrophil ratio index.

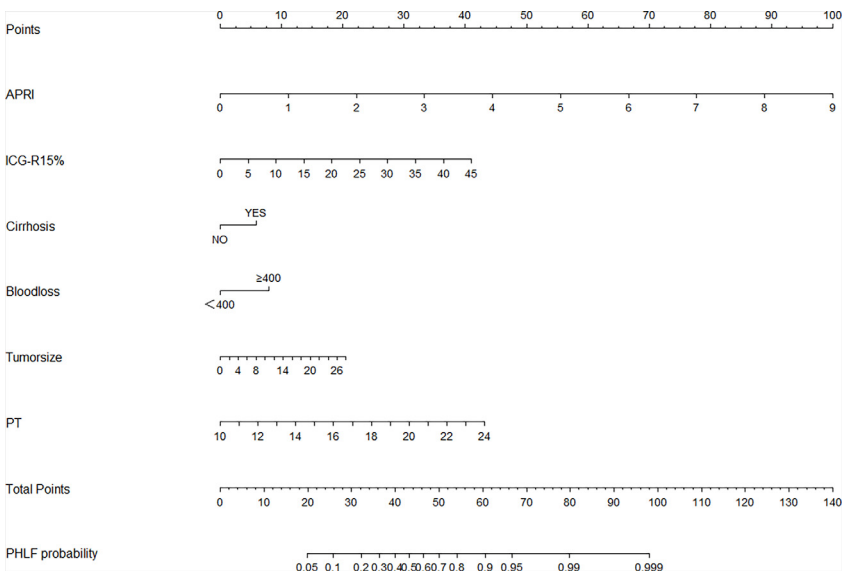

FIGURE 1 | The nomogram was developed in the training cohort and incorporated the AST-to-platelet ratio index (APRI), ICG-R15, tumor size, blood loss, cirrhosis, and prothrombin time (PT). To use the nomogram, an individual patient's value is located on each variable axis, and a line is drawn upward to determine the number of points received for each variable value. The sum of these points is located on the total points axis, and a line is drawn downward to the likelihood of PHLF.

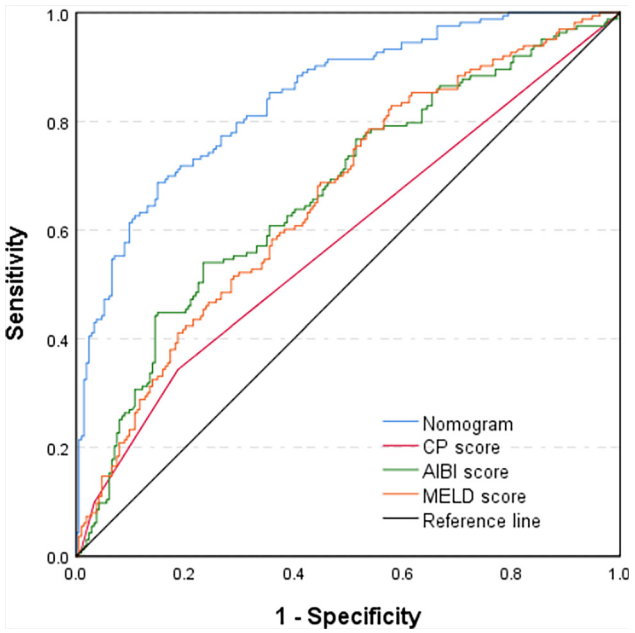

FIGURE 2 | Comparison of predictive accuracy for post-hepatectomy liver failure between the nomogram and the conventional models (CP score, MELD score, and ALBI score) by the training cohort.

Considering that the liver has abundant blood flow, excessive bleeding inevitably leads to impairment of liver cells, with the liver function decline. Liver cirrhosis has a great effect on liver regeneration after hepatectomy. In other words, Cirrhosis is a negative predictor of liver regeneration and liver function. It is extremely important to evaluate the degree of cirrhosis since it is the dominant risk factor for both PHLF and the prognosis of HCC patients (31). HCC patients with severe cirrhosis have higher morbidity and mortality rates after hepatectomy when compared with non-cirrhotic patients (32). Our study also confirms this point of view that cirrhosis is an independent risk factor of PHLF. Prothrombin time (PT) is an important reflection of coagulation status. It represents an essential parameter in many models that evaluate liver function, such as the Child-Pugh score system. Similarly, PT plays an important role in PHLF according to several studies (33, 34). For decades, ICG-R15 has been applied to test liver function prior to hepatectomy. Especially in Eastern countries, ICG-R15 was the most common approach to select suitable HCC patients for liver resection (35, 36). Admittedly, tumor size, blood loss, cirrhosis, PT and ICG-R15 have been demonstrated to predict PHLF in many studies which are consistent with our conclusion.

In so many serum inflammatory indices, only APRI is a unique independent factor in predicting PHLF in our analysis. APRI was used to predict the degree of liver fibrosis in patients since it is a non-invasive test (37). In 2015, the World Health Organization recommend APRI for non-invasive evaluation for liver cirrhosis in patients with chronic hepatitis B infection. APRI consists of two components, AST and Plt. The progression of liver cirrhosis in HCC patients is inevitably accompanied by sustained damage to liver cells, which results in the release of AST and the increase of its concentration in peripheral blood (38). The platelet count could be decreased because of sequestration and destruction of platelets in the enlarging spleen (portal hypertension) (39). And, Thrombopoietin (TPO) synthesis in the liver is reduced because of liver cirrhosis which could stimulate platelet formation (40). These may explain why APRI, not other inflammatory indices could be used to predict PHLF. In our study, APRI has an OR value of 4.652, which is higher than the other independent risk factors. That means it has a higher correlation with PHLF than others. APRI presented in nomogram confirmed the conclusion as well.

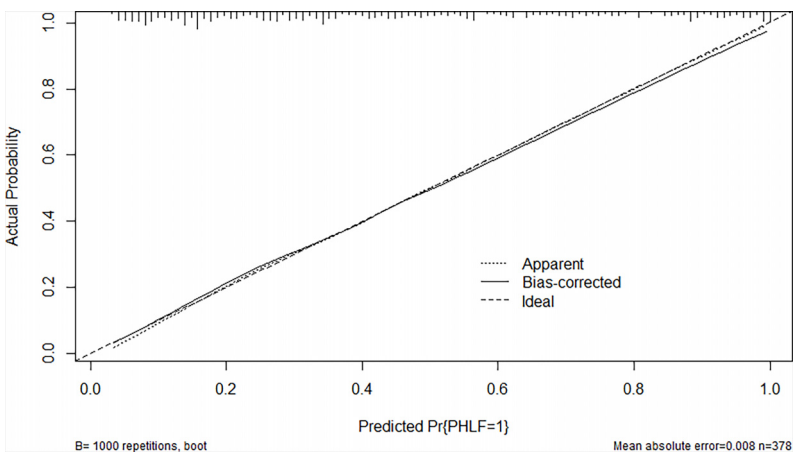

FIGURE 3 | The calibration curve of the nomogram in the training cohort. The x-axis indicates the nomogram predicted probability of PHLF, and the y-axis represents the actual PHLF rate. The dotted line represents a perfect prediction, and the solid line represents the predictive performance of this nomogram. The closer the solid line fit is to the dotted line, the better the prediction of the nomogram will be.

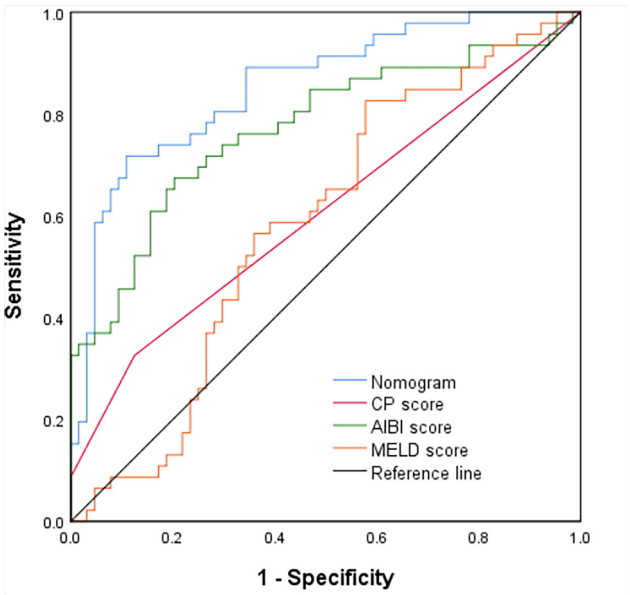

FIGURE 4 | Comparison of predictive accuracy for post-hepatectomy liver failure between the nomogram and the conventional models (CP score, MELD score, and ALBI score) by the validation cohort.

Compared to CP score, ALBI score and MELD score, our nomogram performed well in predicting PHLF and its prediction was supported by the C-index (0.845 and 0.854 for the training cohort and validation cohort, respectively). Traditionally, nomogram is assessed using metrics of diagnostic performances such as specificity, sensitivity and the C-index which fail to determine the clinical value. DCA is a wide-used tool for assessing the benefit of a diagnostic test across a variety of patient preferences for recognizing risks of undertreatment and overtreatment to facilitate decisions about test selection and use. In our study, the DCA indicated that our nomogram brought more benefits than other models in the training cohort and validation cohort. So our nomogram could be used uniformly in clinical practice.

Our nomogram is helpful in predicting PHLF, which can guide therapeutic decisions. By doing this, specific monitoring strategies can be established according to the specific risk categories. For example, if HCC patients are evaluated as a high-risk group of PHLF, we would recommend early use of hepatic protectant, close supervision and intensive care after surgery.

To our knowledge, this is the first nomogram based on inflammatory indices and ICG-R15 to predict PHLF. We emphasized the importance of APRI in the prediction model. However, there are several limitations in the present study.

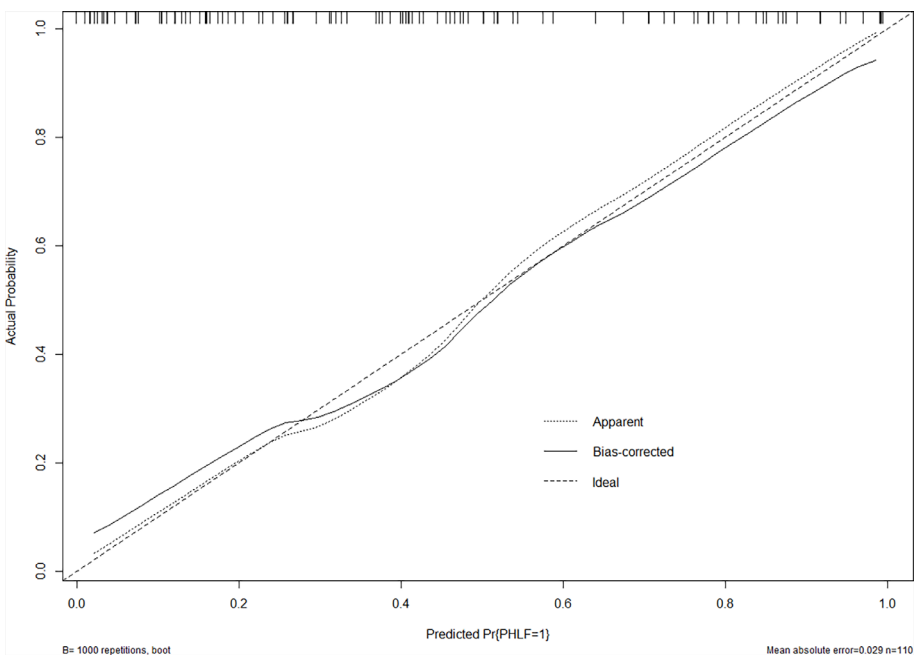

FIGURE 5 | The calibration curve of the nomogram in the validation cohort. The x-axis indicates the nomogram predicted probability of PHLF, and the y-axis represents the actual PHLF rate. The dotted line represents a perfect prediction, and the solid line represents the predictive performance of this nomogram. The closer the solid line fit is to the dotted line, the better the prediction of the nomogram will be.

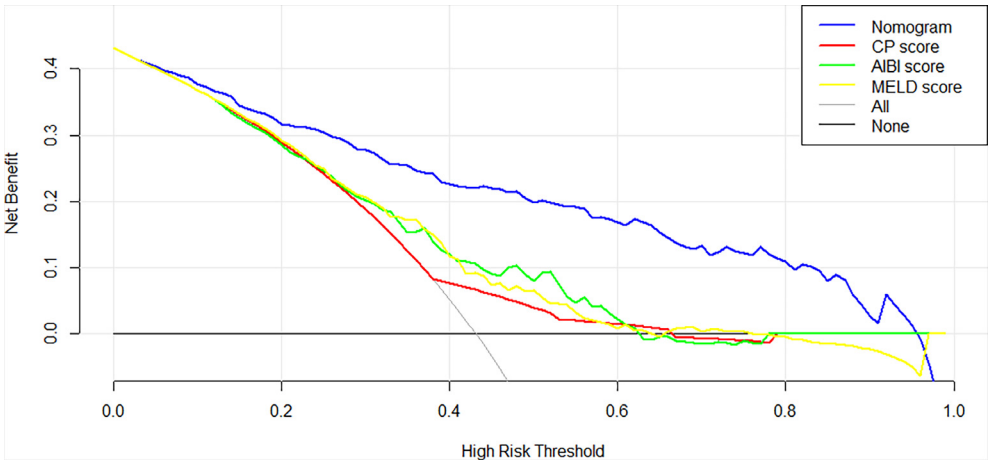

FIGURE 6 | Decision curve analysis of nomogram and the conventional models in the training cohort.

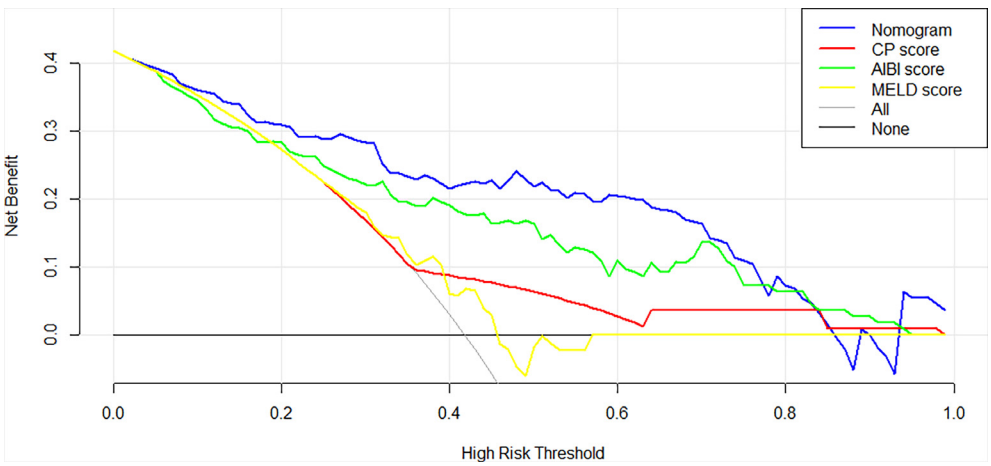

FIGURE 7 | Decision curve analysis of nomogram and the conventional models in the validation cohort.

The main limitation is its retrospective nature. Although our data came from two academic centers, our study has a relatively small sample size. A future multicenter study including a larger number of HCC patients is needed to confirm our findings. Then, as mentioned above, there are many definitions of PHLF, resulting in a wide variation in the incidence of PHLF. We could compare different diagnostic criteria to determine which one is more beneficial to patients. Finally, the main etiology of HCC was chiefly HBV. Even though it didn't play an important role in the prediction of PHLF, it is necessary to include more populations with different etiologies such as alcoholic liver disease or HCV.

In conclusion, we demonstrated that tumor size, blood loss, cirrhosis, PT, ICG-R15 and APRI are the independent risk factors of prediction of PHLF. We present a novel prediction nomogram of PHLF by combining the independent risk factors. The nomogram showed a good predictive performance and would be a convenient tool for us to facilitate clinical decisions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The study was approved by the Ethics Committee of the Xiangya Hospital of Central South University and the Second Xiangya Hospital of Central South University in compliance with the

Declaration of Helsinki. Written informed consent was obtained from all patients for use of their data in this study.

AUTHOR CONTRIBUTIONS

TF and LeZ contributed to conception and design of the sstudy. TF and LeZ organized the database. TF and LeZ performed the statistical analysis. TF wrote the first draft of the manuscript. TF wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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Guiding Value of Circulating Tumor Cells for Preoperative Transcatheter Arterial Embolization in Solitary Large Hepatocellular Carcinoma: A Single-Center Retrospective Clinical Study

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Background: Large hepatocellular carcinoma (LHCC) is highly malignant and prone to recurrence, leading to a poor long-term prognosis for patients. There is an urgent need for measures to intervene in postoperative recurrence. Preoperative Transcatheter Arterial Embolization (TACE) is an effective treatment. However, there is a lack of reliable preoperative indicators to guide the application of preoperative TACE. We, therefore, investigated whether the preoperative status of circulating tumor cells (CTCs) could be used to guide preoperative TACE for HCC treatment.

Methods: This study recruited 361 HCC patients and compared recurrence-free survival (RFS) and overall survival (OS) in patients treated with TACE prior to surgery and those not treated with TACE. Patients were divided into CTC-positive group and CTC-negative group according to CTC status, and the effect of preoperative TACE on RFS and OS was compared in each subgroup.

Results: In CTC-positive patients, preoperative TACE reduces early recurrence and improves long-term survival. However, HCC patients did not benefit from preoperative TACE for the overall population and CTC-negative patients.

Conclusions: Preoperative CTC testing is a reliable indicator of whether HCC patients received TACE preoperatively. CTC positivity was associated with early tumor recurrence, and preoperative TACE could reduce early recurrence and long-term prognosis in CTC-positive patients.

Keywords: preoperative transcatheter arterial embolization, circulating tumor cells, hepatocellular carcinoma, prognosis, TACE

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy globally and the third leading cause of cancer deaths (1). For early-stage HCC, partial hepatectomy prolongs disease-free survival (RFS) and overall survival (OS) in HCC patients (2, 3). However, for HCC patients with large hepatocellular carcinoma (> 5 cm), the tumor is highly malignant and prone to recurrence after surgery, with the vast majority of patients eventually dying due to tumor recurrence (4). This creates an urgent need for appropriate treatment measures to control postoperative recurrence of HCC (5).

Previous studies have reported that preoperative transcatheter arterial embolization (TACE) plays an essential role in improving RFS and OS in patients with HCC (6–15). It has also been suggested that preoperative TACE does not improve RFS and OS in HCC patients (16–25). Therefore, many scholars have speculated that preoperative TACE may only benefit certain particular types of HCC groups, especially those with a higher degree of malignancy (26). However, there is no relevant definition of this special type; there is a lack of relevant and reliable preoperative information to distinguish which HCC patients may benefit from preoperative TACE.

A new study from Zhongshan Hospital reports that positive pre-surgical circulating tumor cells (CTCs) are associated with early postoperative recurrence (27). In addition, it can guide whether postoperative adjuvant TACE should be performed to avoid unnecessary postoperative TACE and achieve precise treatment (28). In the same study, we also noted that in the HCC population with positive preoperative CTC testing, postoperative TACE reduced early postoperative recurrence and facilitated the survival of HCC patients (28). We, therefore, envisaged whether preoperative CTC could guide preoperative adjuvant TACE in HCC patients? Our research team carried out a correlation retrospective study based on this.

MATERIALS AND METHODS

Patient Population

This study recruited HCC patients who underwent preoperative CTC testing at the Department of General Surgery I of Zhongshan People's Hospital from January 2010 to December 2017, with the following inclusion criteria: (1) patients diagnosed with HCC by postoperative pathology, (2) no treatment other than TACE, (3) radical tumor resection (R0) that is, negative macroscopic and microscopic tumor resection margins, (4) complete serological and imaging data, (5) tumor diameter not less than 5 cm, and (6) single tumor. Exclusion criteria were (1) patients younger than 18 years of age, (2) presence of vascular tumor thrombus and distant metastasis, (3) patients with Child-Pugh grade C liver function, (4) no severe vital organ dysfunction, (5) patients who underwent palliative resection, and (6) loss of postoperative follow-up data. The Ethics Committee of Zhongshan People's Hospital has approved the retrospective study, all patients have signed an informed consent form.

Data Collection

All patients underwent abdominal enhanced CT or MRI, chest CT or X-ray scan. Laboratory tests include blood routine, liver and kidney function, coagulation function, hepatitis B surface antigen, hepatitis C antibody, AFP, and other examinations. The basic data of the included study population, such as gender, age, hepatitis B surface antigen, hepatitis C antibody, AFP level, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl aminotransferase (GGT), alkaline phosphatase (ALP), creatinine (Cr), albumin (Alb), total bilirubin (TIBL), direct bilirubin (DIBL), international normalized ratio (INR), platelet count, presence of cirrhosis, Child-Pugh grade, presence of microvascular infiltration (MVI), maximum tumor diameter, pathological classification, extent of liver resection, type of liver resection and other data were collected. Minor liver resection was defined as resection of fewer than three Couinaud liver segments, while major liver resection was defined as resection of three or more liver segments. Non-anatomical liver resection included a limited resection or wedge resection; anatomical resections were defined by the Brisbane 2000 system. The continuous variables are transformed into binary variables, and the cut-off value is the upper and lower lines of the recognized normal value.

Preoperative TACE

Considering that this was a retrospective study, the decision to use TACE prior to surgery was left to the discretion of the treating surgeon and the patient at that time. The patient was placed supine, locally disinfected, draped, and given local anesthetized. The puncture site was chosen to be 2 cm below the inguinal ligament, and the catheter sheath was placed into the femoral artery using the Seldinger technique. Firstly, the DSA technique helps with abdominal trunk and standard hepatic artery angiography to determine the tumor's location, size, and condition of the tumor. Once the tumor is understood, the catheter sheath is continued deeper into the left or right hepatic artery or the vessel that feeds the tumor, 5-fluorouracil (500 mg/m2) or oxaliplatin (100 mg/m2) was injected into the proper hepatic artery, and embolization was performed using different embolization materials. Patients were asked to return to the hospital 4-6 weeks after embolization for follow-up serology, including blood routine, liver and kidney function, coagulation function, AFP, and imaging included abdominal enhanced CT or MRI, chest X-ray scan, etc. All of the above procedures were performed by highly qualified attending physicians who received relevant interventional medicine.

Isolation and Identification of CTC

The Cyttel method is used to detect CTCs, and its main principles include the negative immunomagnetic particle assay and immunofluorescence *in situ* hybridization (im-FISH). Jiangsu Lyle Biomedical Technology Co manufactures the kit. For patients with preoperative TACE, samples were obtained within three days before TACE, while for patients without preoperative TACE, the sample extraction must also be completed within three days before surgery. Generally, we draw 5ml peripheral blood, and process the samples strictly

according to the manufacturer's instructions. Firstly, the samples was treated with negative immunomagnetic powder method to remove leukocytes from the peripheral blood, and isolate rare cells in the blood, and finally obtain CTCs. Then, the im-FISH technique was used to fix and dehydrate the samples, then hybridization with chromosome centromeres 1 and 8, followed by sealing with 4-diamidine-2-phenylindole (DAPI) staining solution, and then observation and counting under a fluorescence microscope (29–32). It defined CTC count ≥1 as CTC-positive (32).

Follow-Up

Each follow-up visit for all patients include AFP, routine blood tests, liver and kidney function tests, coagulation function tests. Enhanced CT or MRI of the abdomen, chest CT, and the bone scan will be performed if tumor residue and signs of tumor recurrence are suspected. The first postoperative follow-up visit would performed one month after the operation. The follow-up frequency was once every 2-3 months within six months after the operation, once every 3-4 months within 6-24 months after the operation, and once every 4-6 months after 24 months after the operation. After a recurrence of HCC, treatment options are chosen according to the recurrence and the patient's general condition. Treatment options include surgical re-resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), TACE, taking targeted drugs, immune drug therapy, and even liver transplantation. OS was defined as the date of surgery until patient death or last follow-up, and RFS was defined as the date of surgery until patient signs of recurrence or last follow-up. Recurrence was classified as early recurrence and late recurrence using a cut-off value of 24 months.

Statistical Analysis

Continuous variables were expressed as median ± square difference (Median ± SD), and categorical variables were expressed as number (n) or percentage (%) of patients. The t-test or Mann-Whitney test was used to compare two groups of continuous variables, and the $\chi 2$ or Fisher's exact test was used to compare two groups of categorical variables. The survival curves of OS and RFS of the patients were plotted using the Kaplan-Meier method, and the OS and RFS of the patients in the preoperative TACE group and the two groups without preoperative TACE were compared using the log-rank. We also used the Landmark analysis method to analyze the results of assessing early recurrence (recurrence 24 months after surgery) and late recurrence. Univariate and multivariate Cox regression models were used to analyze the independent risk factors of each factor on patients' RFS and OS. All statistics and graphs for this study were completed in R (version 3.62). P values < 0.05 were considered statistically significant.

RESULTS

Characteristics of Patients With HCC

Baseline characteristics of the total population of HCC are listed in **Table 1**. The population was divided into positive and negative subgroups based on the preoperative CTCs count. The clinical baseline of each subgroup is shown in **Table 2**. In this study, a total of 361 patients with HCC were enrolled in this study, including 211 patients of CTC-positive (58.4%) and 103 patients of preoperative TACE (28.5%). The median follow-up time of the CTC-positive group was 38.0 months, while the median follow-up time of the CTC-negative group was 44.5 months. The median follow-up time of HCC patients with preoperative TACE was 41.0 months, while patients without TACE were 36.5 months. During follow-up, 134 patients died, and 275 patients developed tumor recurrence. In the CTC-positive and CTC-negative subgroups, the clinicopathological variables were similar, comparable and not statistically significant between patients who underwent preoperative TACE and those who did not ($P > 0.05$; **Table 2**). In the overall population, RFS and OS were similar of patients with and without preoperative TACE; Preoperative TACE did not improve the prognosis of HCC ($P > 0.05$; **Figures 1A, B**).

CTCs Status Affects OS and RFS of HCC Patients

Using survival curves drawn by the Kaplan Meier method, we found that OS (median 39 months vs. 47 months, $P < 0.05$, **Supplementary Figure 1A**) and RFS (median 17.0 months vs. 24 months, $P < 0.05$ **Supplementary Figure 1B**) in CTC-positive group were worse than those in CTC-negative group. We also analyzed the effect of CTCs status on postoperative recurrence patterns using the landmark method. Using a 24-month cut-off, postoperative recurrence was divided into an early recurrence and late recurrence. We found that CTC positive was associated with postoperative early recurrence ($P < 0.05$; **Supplementary Figure 2**) but not with late recurrence ($P > 0.05$; **Supplementary Figure 2**).

The Clinical Efficacy of Preoperative TACE Was Evaluated in Subgroups of CTC-Positive and CTC-Negative Groups

To determine whether CTCs status affects the clinical efficacy of TACE, we stratified patients' CTCs status of and compared the OS and RFS between patients with and without preoperative TACE at different CTCs status. In the CTC-positive group, preoperative TACE prolonged OS and RFS in HCC patients; the difference was statistically significant ($P < 0.05$; **Figures 2A, B**). In CTC-negative group, preoperative TACE could not improve RFS and OS, and the difference was not statistically significant ($P > 0.05$; **Figures 3A, B**).

Univariate and multivariate Cox regression analysis also showed that in CTC-positive group, non-preoperative TACE was an independent risk factor for OS (hazard ratio [HR]= 2.330, 95% confidence interval [*CI*], 1.318-4.120, P <0.05; **Table 3**) and RFS(hazard ratio [HR]= 2.332, 95% confidence interval [CI], 1.584-3.432, P <0.05; **Table 4**) of HCC patients, while in CTC-negative group, preoperative TACE had no effect on OS (hazard ratio [HR]= 0.655, 95% confidence interval [CI], 0.338-1.267, P >0.05; **Table 5**)and RFS (hazard ratio [HR]= 0.805, 95% confidence interval [CI], 0.511-1.269,P >0.05; **Table 6**) of HCC patients.

TABLE 1 | Baseline characteristics of HCC patients for the overall population.

Variable		n = 361	
		n	%
Age (years)	<60	217	60.1
	≥60	144	39.9
Gender	Female	83	23.0
	Male	278	77.0
CTC	Negative	150	41.6
	Positive	211	58.4
HBV	No	16	4.4
	Yes	345	95.6
HCV	No	355	98.3
	Yes	6	1.7
Cirrhosis	No	115	31.9
	Yes	246	68.1
Child-Pugh	A	313	86.7
	B	48	13.3
ALT (U/L)	<50	231	64.0
	≥50	130	36.0
AST (U/L)	<40	126	34.9
	≥40	235	65.1
GGT (U/L)	<45	77	21.3
	≥45	284	78.7
ALP (U/L)	<125	276	76.5
	≥125	85	23.5
Alb (g/L)	<35	55	15.2
	≥35	306	84.8
TIBL (umol/L)	<20.4	300	83.1
	≥20.4	61	16.9
DIBL (umol/L)	<6.8	289	80.1
	≥6.8	72	19.9
CR (umol/L	<84	305	84.5
	≥84	56	15.5
INR	<1.15	248	68.7
	≥1.15	113	31.3
PLT (10^9/L)	<100	100	27.7
	≥100	261	72.3
AFP (ug/mL)	<400	141	39.1
	≥400	220	60.9
Tumor diameter (cm)	<10	207	57.3
	≥10	154	42.7
Edmondson stage	I+II	57	15.8
	III+IV	304	84.2
MVI	No	141	39.1
	Yes	220	60.9
Tumor capsule	Complete	84	23.3
	Absent or Partial	277	76.7
Extent of liver resection	Major liver resection	232	64.3
	Minor liver resection	129	35.7
Type of liver resection	Anatomical	139	38.5
	Non-anatomical	222	61.5
Postoperative TACE	No	181	50.5
	Yes	180	49.5
Site of recurrence	Intrahepatic	217	78.9
	Extrahepatic	28	10.2
	Intrahepatic and extrahepatic	30	10.9
Preoperative TACE	No	258	71.5
	Yes	103	28.5

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; Alb, albumin; TIBL, total bilirubin; DIBL, direct bilirubin; CR, creatinine; INR, international normalized ratio; PLT, blood platelet; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; CTC, circulating tumor cells; MVI, microvascular invasion.

TABLE 2 | Comparison of clinicopathological variables between preoperative TACE and control group in HCC patients with CTC-positive and CTC-negative groups.

Variable		CTC Positive (n=211)			CTC Negative (n=150)		
		Non-TACE (n=148)	TACE (n=63)	*p*	Non-TACE (n=110)	TACE (n=40)	*p*
Age (years)	<60	88 (59.5)	39 (61.9)	0.858	63 (57.3)	27 (67.5)	0.346
	≥60	60 (40.5)	24 (38.1)		47 (42.7)	13 (32.5)	
Gender	Female	41 (27.7)	13 (20.6)	0.366	25 (22.7)	4 (10.0)	0.131
	Male	107 (72.3)	50 (79.4)		85 (77.3)	36 (90.0)	
HBV	No	10 (6.8)	1 (1.6)	0.227	5 (4.5)	0 (0.0)	0.391
	Yes	138 (93.2)	62 (98.4)		105 (95.5)	40 (100.0)	
HCV	No	144 (97.3)	62 (98.4)	1.000	109 (99.1)	40 (100.0)	1.000
	Yes	4 (2.7)	1 (1.6)		1 (0.9)	0 (0.0)	
Cirrhosis	No	49 (33.1)	18 (28.6)	0.627	36 (32.7)	12 (30.0)	0.905
	Yes	99 (66.9)	45 (71.4)		74 (67.3)	28 (70.0)	
Child-Pugh	A	129 (87.2)	52 (82.5)	0.506	98 (89.1)	34 (85.0)	0.691
	B	19 (12.8)	11 (17.5)		12 (10.9)	6 (15.0)	
ALT (U/L)	<50	90 (60.8)	40 (63.5)	0.832	77 (70.0)	24 (60.0)	0.338
	≥50	58 (39.2)	23 (36.5)		33 (30.0)	16 (40.0)	
AST (U/L)	<40	48 (32.4)	22 (34.9)	0.848	42 (38.2)	14 (35.0)	0.869
	≥40	100 (67.6)	41 (65.1)		68 (61.8)	26 (65.0)	
GGT (U/L)	<45	32 (21.6)	16 (25.4)	0.675	22 (20.0)	7 (17.5)	0.913
	≥45	116 (78.4)	47 (74.6)		88 (80.0)	33 (82.5)	
ALP (U/L)	<125	113 (76.4)	48 (76.2)	1.000	86 (78.2)	29 (72.5)	0.611
	≥125	35 (23.6)	15 (23.8)		24 (21.8)	11 (27.5)	
Alb (g/L)	<35	25 (16.9)	13 (20.6)	0.651	12 (10.9)	5 (12.5)	1.000
	≥35	123 (83.1)	50 (79.4)		98 (89.1)	35 (87.5)	
TIBL (umol/L)	<20.4	123 (83.1)	53 (84.1)	1.000	92 (83.6)	32 (80.0)	0.782
	≥20.4	25 (16.9)	10 (15.9)		18 (16.4)	8 (20.0)	
DIBL (umol/L)	<6.8	118 (79.7)	49 (77.8)	0.893	91 (82.7)	31 (77.5)	0.624
	≥6.8	30 (20.3)	14 (22.2)		19 (17.3)	9 (22.5)	
CR (umol/L)	<84	130 (87.8)	51 (81.0)	0.273	92 (83.6)	32 (80.0)	0.782
	≥84	18 (12.2)	12 (19.0)		18 (16.4)	8 (20.0)	
INR	<1.15	99 (66.9)	47 (74.6)	0.343	79 (71.8)	23 (57.5)	0.143
	≥1.15	49 (33.1)	16 (25.4)		31 (28.2)	17 (42.5)	
PLT (10^9/L)	<100	44 (29.7)	16 (25.4)	0.637	25 (22.7)	15 (37.5)	0.109
	≥100	104 (70.3)	47 (74.6)		85 (77.3)	25 (62.5)	
AFP (ug/mL)	<400	54 (36.5)	31 (49.2)	0.116	43 (39.1)	13 (32.5)	0.584
	≥400	94 (63.5)	32 (50.8)		67 (60.9)	27 (67.5)	
Tumor diameter (cm)	<10	69 (46.6)	33 (52.4)	0.538	74 (67.3)	24 (60.0)	0.526
	≥10	79 (53.4)	30 (47.6)		36 (32.7)	16 (40.0)	
Edmondson stage	I+II	20 (13.5)	8 (12.7)	1.000	24 (21.8)	5 (12.5)	0.296
	III+IV	128 (86.5)	55 (87.3)		86 (78.2)	35 (87.5)	
MVI	No	53 (35.8)	19 (30.2)	0.526	48 (43.6)	21 (52.5)	0.437
	Yes	95 (64.2)	44 (69.8)		62 (56.4)	19 (47.5)	
Tumor capsule	Complete	121 (81.8)	45 (71.4)	0.136	31 (28.2)	8 (20.0)	0.424
	Absent or Partial	27 (18.2)	18 (28.6)		79 (71.8)	32 (80.0)	
Extent of liver resection	Major liver resection	94 (63.5)	44 (69.8)	0.468	68 (61.8)	26 (65.0)	0.869
	Minor liver resection	54 (36.5)	19 (30.2)		42 (38.2)	14 (35.0)	
Type of liver resection	Anatomical	57 (38.5)	23 (36.5)	0.905	42 (38.2)	17 (42.5)	0.772
	Non-anatomical	91 (61.5)	40 (63.5)		68 (61.8)	23 (57.5)	
Postoperative TACE	No	72 (48.6)	33 (52.4)	0.730	58 (52.7)	18 (45.0)	0.514
	Yes	76 (51.4)	30 (47.6)		52 (47.3)	22 (55.0)	
Site of recurrence	Intrahepatic	111 (82.2)	26 (72.2)	0.288	62 (78.5)	18 (72.0)	0.599
	Extrahepatic	11 (8.1)	6 (16.7)		7 (8.9)	4 (16.0)	
	Intrahepatic and extrahepatic	13 (9.6)	4 (11.1)		10 (12.7)	3 (12.0)	

TACE, transcatheter arterial chemoembolization; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; AlB, albumin; TIBL, total bilirubin; DIBL, direct bilirubin; CR, creatinine; INR, international normalized ratio; PLT, blood platelet; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; CTC, circulating tumor cells; MVI, microvascular invasion.

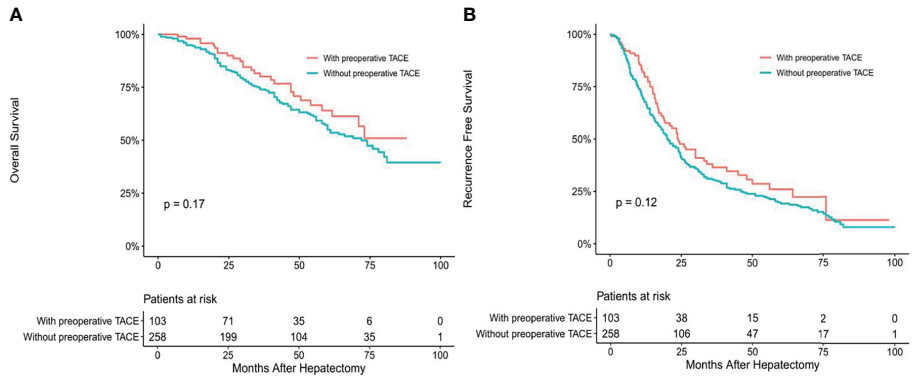

FIGURE 1 | Overall **(A)** and recurrence-free **(B)** survival curves of overall HCC patients with or without preoperative TACE.

Preoperative Adjuvant TACE Can Reduce the Early Recurrence of CTC-Positive Patients

Using landmark analysis and taking 24 months as the cutoff value, we found that preoperative TACE could reduce the early recurrence of patients in CTC-positive group ($P < 0.05$, **Figure 4A**), but could not improve the late recurrence rate of patients ($P > 0.05$, **Figure 4A**). In the CTC-negative group, preoperative TACE could not improve the early and late recurrence ($P > 0.05$, **Figure 4B**).

The Clinicopathological Baseline of CTC-Positive Group and CTC-Negative Group Were Compared

The comparison of clinicopathological variables between the CTC-positive group and CTC-negative group is shown in **Table 7**; the proportion of patients with tumor diameter ≥10cm (48.3.1% vs. 34.7%, $P < 0.05$; **Table 7**) and positive rate of MVI (65.9% vs. 54.0%, $P < 0.05$; **Table 7**) in CTC-positive group was higher than that in CTC-negative group. At the same time, other clinicopathological indicators such as age, sex, HBV, cirrhosis, Child-Pugh, Edmondson stage, and AFP≥400ng/ml were not significantly different($P > 0.05$; **Table 7**).

Comparison of Perioperative Complications Between Patients With Preoperative TACE and Those Without Preoperative TACE

We compared the effects of preoperative TACE on perioperative complications and mortality. We found that preoperative TACE did not increase perioperative mortality, liver failure, bile leakage, ascites, wound infection, and other complications compared to patients without preoperative TACE ($P > 0.05$; **Table 8**).

DISCUSSION

TACE has been one of the most effective and safest local treatment for patients with unresectable HCC (8). Its use of embolic material to occlude the main blood vessels that will

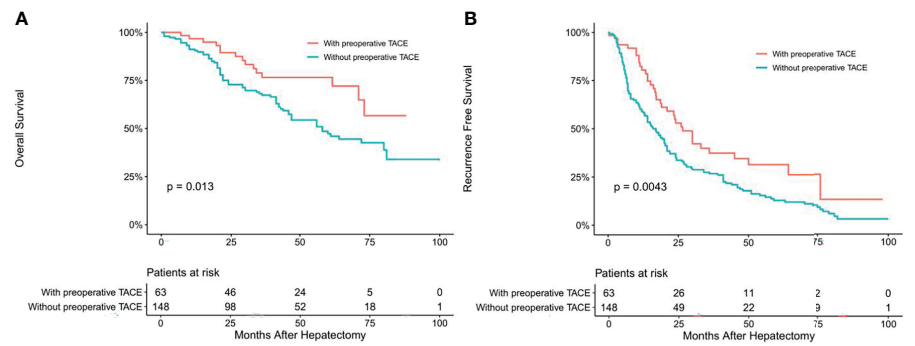

FIGURE 2 | Overall **(A)** and recurrence-free **(B)** survival curves of HCC patients in CTC-positive patients with or without preoperative TACE.

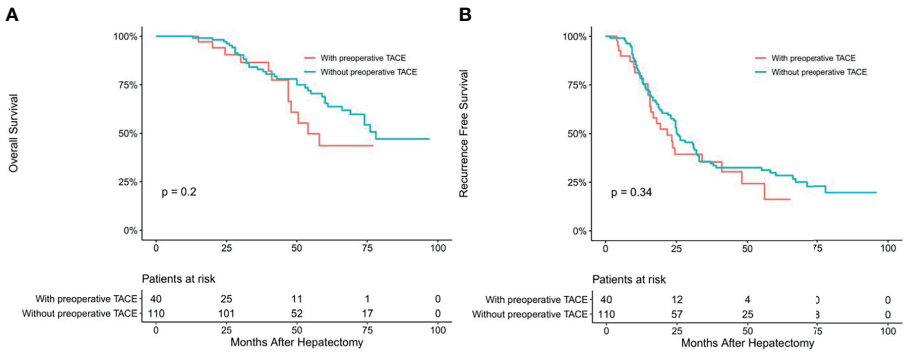

FIGURE 3 | Overall **(A)** and recurrence-free **(B)** survival curves of HCC patients in CTC-negative patients with or without preoperative TACE.

supply the tumor leads to ischaemic necrosis of the tumor and the chemotherapeutic drugs that can effectively kill the tumor tissue or tumor cells. In the last 20 years, many scholars have also applied TACE in the preoperative adjuvant treatment of large HCC (6–25, 33–36). The main objectives of preoperative TACE were: (1) induce tumor volume shrinkage and convert unresectable HCC into resectable HCC; (2) reduce postoperative tumor recurrence and improve long-term patient survival; (3) improve the detection of occult lesions not detected by preoperative imaging beyond TACE (6, 37). However, the effectiveness of preoperative TACE has been controversial (6–25, 33–36), and some scholars believe that preoperative TACE may only be benefit for certain specific groups of HCC, such as patients with large tumor diameters, multiple nodes and invasive HCC (8, 14, 26). However, there is no consensus on which type of HCC patients can benefit from TACE, so there is an urgent need to explore a reliable preoperative indicator to guide preoperative TACE.

CTCs are malignant tumor cells that invade into the peripheral blood *via* epithelial-mesenchymal (EMT) form,

TABLE 3 | Univariate and multivariate Cox regression analyses were used to identify independent risk factors for overall survival in CTC-positive patients.

Variables	HR comparison	UV HR (95% *CI*)	UV p	MV HR (95% *CI*)	MV p*
Preoperative TACE	No vs. yes	2.006 (1.149-3.503)	0.014	2.33 (1.318-4.12)	0.004
Age	≥60 vs <60 years	1.103 (0.716-1.699)	0.656		
Gender	Male vs. female	1.36 (0.808-2.287)	0.247		
HBV	Yes vs. no	2.131 (0.671-6.761)	0.199		
HCV	Yes vs. no	0 (0-Inf)	0.996		
Cirrhosis	Yes vs. no	0.965 (0.605-1.539)	0.881		
Child Pugh	B vs A	1.436 (0.778-2.649)	0.247		
ALT	≥50 vs <50 U/L	1.138 (0.74-1.748)	0.557		
AST	≥40 vs <40 U/L	0.963 (0.615-1.506)	0.867		
GGT	≥45 vs <45 U/L	1.435 (0.819-2.513)	0.206		
ALP	≥40 vs <40 U/L	0.857 (0.514-1.428)	0.553		
Alb	≥35 vs <35 g/L	0.965 (0.552-1.686)	0.900		
TIBL	≥20.4 vs <20.4 umol/L	0.9 (0.489-1.656)	0.734		
DIBL	≥6.8 vs <6.8 umol/L	1.033 (0.607-1.757)	0.904		
CR	≥80.4 vs <80.4 umol/L	0.845 (0.448-1.593)	0.602		
INR	≥1.15 vs <1.15	1.01 (0.637-1.602)	0.966		
PLT	≥ 100 vs <100 × 10^9/L	1.269 (0.769-2.093)	0.352		
AFP	≥400 vs <400ng/mL	3.137 (1.904-5.168)	<0.001	1.925 (1.129-3.28)	0.016
Tumor diameter	<10 vs ≥10cm	0.508 (0.331-0.779)	0.002	0.53 (0.337-0.833)	0.006
Edmondson stage	III+IV vs I+II	2.814 (1.345-5.89)	0.006	2.864 (1.348-6.081)	0.006
MVI	Yes vs. no	3.311 (1.912-5.736)	<0.001	2.159 (1.193-3.907)	0.011
Tumor capsule	Complete vs. incomplete	0.857 (0.514-1.429)	0.553		
Extent of liver resection	Major vs. minor	0.877 (0.562-1.367)	0.562		
Type of liver resection	Anatomical vs. non-anatomical	0.876 (0.572-1.339)	0.469		
Postoperative TACE	Yes vs. no	0.815 (0.533-1.247)	0.346		

TACE, transcatheter arterial chemoembolization; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; Alb, albumin; TIBL, total bilirubin; DIBL, direct bilirubin; CR, creatinine; INR, international normalized ratio; PLT, blood platelet; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, microvascular invasion; CI, confidence interval; HR, hazard ratio; UV, univariable; MV, multivariable.

**Those variables found significant at p < 0.05 in univariable analyses were entered into multivariable Cox-regression analyses.*

TABLE 4 | Univariate and multivariate Cox regression analyses were used to identify independent risk factors for recurrence free survival in CTC positive patients.

Variables	HR comparison	UV HR (95% *CI*)	UV p	MV HR (95% *CI*)	MV p*
Preoperative TACE	No vs. yes	1.699 (1.175-2.455)	0.005	2.332 (1.584-3.432)	≤0.001
Age	≥60 vs <60 years	1.307 (0.953-1.793)	0.097		
Gender	Male vs. female	0.931 (0.662-1.309)	0.680		
HBV	Yes vs. no	0.961 (0.517-1.786)	0.899		
HCV	Yes vs. no	1.533 (0.627-3.746)	0.349		
Cirrhosis	Yes vs. no	0.816 (0.593-1.123)	0.211		
Child Pugh	B vs A	1.426 (0.909-2.237)	0.122		
ALT	≥50 vs <50 U/L	1.171 (0.861-1.594)	0.315		
AST	≥40 vs <40 U/L	1.062 (0.769-1.465)	0.716		
GGT	≥45 vs <45 U/L	1.373 (0.939-2.008)	0.102		
ALP	≥40 vs <40 U/L	1.268 (0.892-1.803)	0.186		
Alb	≥35 vs <35 g/L	0.94 (0.636-1.388)	0.755		
TIBL	≥20.4 vs <20.4 umol/L	0.941 (0.622-1.422)	0.772		
DIBL	≥6.8 vs <6.8 umol/L	1.01 (0.692-1.472)	0.961		
CR	≥80.4 vs <80.4 umol/L	1.185 (0.774-1.814)	0.434		
INR	≥1.15 vs <1.15	1.065 (0.771-1.47)	0.703		
PLT	≥ 100 vs <100 × 10^9/L	1.253 (0.886-1.772)	0.202		
AFP	≥400 vs <400ng/mL	2.617 (1.88-3.643)	<0.001	1.727 (1.214-2.457)	0.002
Tumor diameter	<10 vs ≥10cm	0.468 (0.344-0.636)	<0.001	0.472 (0.34-0.655)	<0.001
Edmondson stage	III+IV vs I+II	1.926 (1.202-3.085)	0.006	1.867 (1.154-3.022)	0.011
MVI	Yes vs. no	2.708 (1.917-3.826)	<0.001	2.03 (1.394-2.958)	<0.001
Tumor capsule	Complete vs. incomplete	0.711 (0.491-1.028)	0.070		
Extent of liver resection	Major vs. minor	0.915 (0.666-1.257)	0.584		
Type of liver resection	Anatomical vs. non-anatomical	1.124 (0.821-1.538)	0.465		
Postoperative TACE	Yes vs. no	1.111 (0.821-1.505)	0.494		

TACE, transcatheter arterial chemoembolization; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; Alb, albumin; TIBL, total bilirubin; DIBL, direct bilirubin; CR, creatinine; INR, international normalized ratio; PLT, blood platelet; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, microvascular invasion; CI, confidence interval; HR, hazard ratio; UV, univariable; MV, multivariable.

**Those variables found significant at p < 0.05 in univariable analyses were entered into multivariable Cox-regression analyses.*

TABLE 5 | Univariate and multivariate Cox regression analyses were used to identify independent risk factors for overall survival in CTC-negative patients.

Variables	HR comparison	UV HR (95% *CI*)	UV p	MV HR (95% *CI*)	MV p*
Preoperative TACE	No vs. yes	0.655 (0.338-1.267)	0.209		
Age	≥60 vs <60 years	1.215 (0.689-2.142)	0.500		
Gender	Male vs. female	1.398 (0.653-2.99)	0.388		
HBV	Yes vs. no	0.709 (0.22-2.287)	0.565		
HCV	Yes vs. no	4.328 (0.586-31.979)	0.151		
Cirrhosis	Yes vs. no	0.896 (0.486-1.652)	0.724		
Child Pugh	B vs A	1.833 (0.767-4.38)	0.173		
ALT	≥50 vs <50 U/L	0.763 (0.413-1.409)	0.387		
AST	≥40 vs <40 U/L	1.674 (0.884-3.169)	0.114		
GGT	≥45 vs <45 U/L	1.359 (0.61-3.031)	0.453		
ALP	≥40 vs <40 U/L	1.109 (0.565-2.175)	0.763		
Alb	≥35 vs <35 g/L	0.786 (0.352-1.755)	0.557		
TIBL	≥20.4 vs <20.4 umol/L	0.68 (0.305-1.518)	0.346		
DIBL	≥6.8 vs <6.8 umol/L	0.825 (0.398-1.708)	0.604		
CR	≥80.4 vs <80.4 umol/L	0.645 (0.274-1.52)	0.316		
INR	≥1.15 vs <1.15	0.76 (0.387-1.491)	0.424		
PLT	≥ 100 vs <100 × 10^9/L	2.266 (0.962-5.335)	0.061		
AFP	≥400 vs <400ng/mL	2.057 (1.069-3.955)	0.031	2.104 (1.092-4.055)	0.026
Tumor diameter	<10 vs ≥10cm	0.388 (0.219-0.69)	0.001	0.524 (0.293-0.937)	0.029
Edmondson stage	III+IV vs I+II	3.463 (1.461-8.209)	0.005	4.035 (1.662-9.8)	0.002
MVI	Yes vs. no	4.072 (2.101-7.894)	<0.001	4.007 (2.026-7.926)	<0.001
Tumor capsule	Complete vs. incomplete	1.024 (0.56-1.873)	0.939		
Extent of liver resection	Major vs. minor	1.402 (0.792-2.484)	0.246		
Type of liver resection	Anatomical vs. non-anatomical	1.763 (0.933-3.333)	0.081		
Postoperative TACE	Yes vs. no	1.431 (0.808-2.533)	0.219		

TACE, transcatheter arterial chemoembolization; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; Alb, albumin; TIBL, total bilirubin; DIBL, direct bilirubin; CR, creatinine; INR, international normalized ratio; PLT, blood platelet; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, microvascular invasion; CI, confidence interval; HR, hazard ratio; UV, univariable; MV, multivariable.

**Those variables found significant at p < 0.05 in univariable analyses were entered into multivariable Cox-regression analyses.*

TABLE 6 | Univariate and multivariate Cox regression analyses were used to identify independent risk factors for recurrence free survival in CTC negative patients.

Variables	HR comparison	UV HR (95% *CI*)	UV p	MV HR (95% *CI*)	MV p*
Preoperative TACE	No vs. yes	0.805 (0.511-1.269)	0.351		
Age	≥60 vs <60 years	1.092 (0.739-1.614)	0.658		
Gender	Male vs. female	1.236 (0.751-2.033)	0.404		
HBV	Yes vs. no	0.465 (0.188-1.151)	0.098		
HCV	Yes vs. no	3.417 (0.469-24.914)	0.225		
Cirrhosis	Yes vs. no	1.17 (0.765-1.788)	0.470		
Child Pugh	B vs A	1.244 (0.679-2.278)	0.479		
ALT	≥50 vs <50 U/L	0.682 (0.445-1.043)	0.078		
AST	≥40 vs <40 U/L	0.912 (0.616-1.35)	0.646		
GGT	≥45 vs <45 U/L	0.772 (0.481-1.237)	0.281		
ALP	≥40 vs <40 U/L	1.288 (0.821-2.023)	0.271		
Alb	≥35 vs <35 g/L	1.251 (0.651-2.403)	0.502		
TIBL	≥20.4 vs <20.4 umol/L	1.035 (0.622-1.721)	0.894		
DIBL	≥6.8 vs <6.8 umol/L	1.066 (0.659-1.723)	0.795		
CR	≥80.4 vs <80.4 umol/L	0.671 (0.387-1.163)	0.155		
INR	≥1.15 vs <1.15	0.948 (0.622-1.444)	0.803		
PLT	≥ 100 vs <100 × 10^9/L	1.101 (0.706-1.717)	0.671		
AFP	≥400 vs <400ng/mL	4.237 (2.61-6.878)	<0.001	4.291 (2.630-7.000)	<0.001
Tumor diameter	<10 vs ≥10cm	0.545 (0.363-0.82)	0.004	0.536 (0.353-0.814)	0.003
Edmondson stage	III+IV vs I+II	1.798 (1.079-2.996)	0.024	1.82 (1.089-3.042)	0.022
MVI	Yes vs. no	2.605 (1.732-3.919)	<0.001	2.211 (1.465-3.337)	<0.001
Tumor capsule	Complete vs. incomplete	0.823 (0.527-1.284)	0.390		
Extent of liver resection	Major vs. minor	0.875 (0.588-1.303)	0.512		
Type of liver resection	Anatomical vs. non-anatomical	1.335 (0.895-1.992)	0.157		
Postoperative TACE	Yes vs. no	1.254 (0.852-1.844)	0.251		

TACE, transcatheter arterial chemoembolization; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; Alb, albumin; TIBL, total bilirubin; DIBL, direct bilirubin; CR, creatinine; INR, international normalized ratio; PLT, blood platelet; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, microvascular invasion; CI, confidence interval; HR, hazard ratio; UV, univariable; MV, multivariable.

**Those variables found significant at p < 0.05 in univariable analyses were entered into multivariable Cox-regression analyses.*

which reflect the tumor's aggressiveness and is often used for prognostic monitoring in breast, colorectal, and prostate cancers (29, 30, 38, 39). CTCs testing is considered to be a reliable means of early screening for cancer, postoperative recurrence, or metastasis monitoring in HCC patients (40). There are various methods on the market to detect circulating tumor cells, among them the Cyttel method (30–32, 41, 42) and CellSearch™ are the most common (27, 28, 43). The CellSearch™ system assay uses the traditional EpCAM-dependent enrichment method to identify CTCs (41, 44), which has certain limitations. The most important point is that not all peripheral blood CTCs of HCC patients express EpCAM, only 30-40% of HCC cells express EpCAM (45). This results in the low sensitivity of the CellSearch™ system to detect CTCs (44). To overcome this problem, we used a negative immunomagnetic particle method to detect CTCs to improve the assay's sensitivity. Our retrospective study found that patients with positive CTCs had shorter RFS and OS than those with negative CTCs, and the landmark analysis also found that CTCs status was associated with early postoperative recurrence ($P < 0.05$), possibly by causing early recurrence leading to patient death. These findings are consistent with recent studies (46–50).

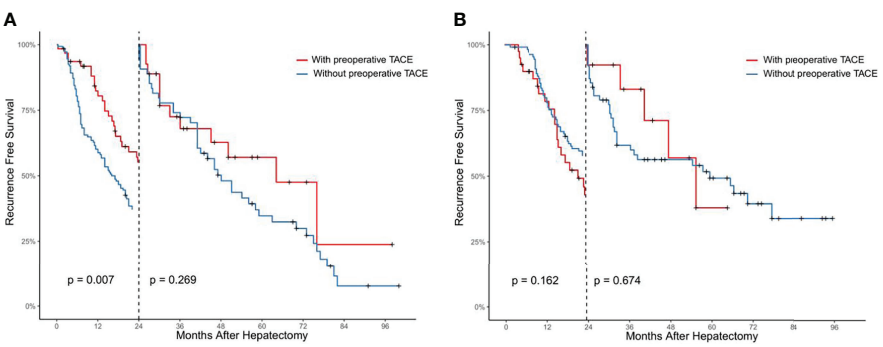

FIGURE 4 | Analysis of the effect of preoperative TACE on early and late postoperative recurrence in CTC positive **(A)** and CTC negative **(B)** HCC patients by landmark method.

TABLE 7 | Relationship between positive and negative CTC and clinicopathological variables.

Variable		Overall (361)	CTC- Negative (n=150)	CTC- Positive (n=211)	*p*
Age (%)	<60years	217 (60.1)	90 (60.0)	127 (60.2)	1.000
	≥60years	144 (39.9)	60 (40.0)	84 (39.8)	
Gender (%)	Female	83 (23.0)	29 (19.3)	54 (25.6)	0.206
	Male	278 (77.0)	121 (80.7)	157 (74.4)	
HBV (%)	No	16 (4.4)	5 (3.3)	11 (5.2)	0.551
	Yes	345 (95.6)	145 (96.7)	200 (94.8)	
HCV (%)	No	355 (98.3)	149 (99.3)	206 (97.6)	0.407
	Yes	6 (1.7)	1 (0.7)	5 (2.4)	
Cirrhosis (%)	No	115 (31.9)	48 (32.0)	67 (31.8)	1.000
	Yes	246 (68.1)	102 (68.0)	144 (68.2)	
Child Pugh (%)	A	313 (86.7)	132 (88.0)	181 (85.8)	0.65
	B	48 (13.3)	18 (12.0)	30 (14.2)	
ALT (%)	<50U/L	231 (64.0)	101 (67.3)	130 (61.6)	0.315
	≥50U/L	130 (36.0)	49 (32.7)	81 (38.4)	
AST (%)	<40U/L	126 (34.9)	56 (37.3)	70 (33.2)	0.481
	≥40U/L	235 (65.1)	94 (62.7)	141 (66.8)	
GGT (%)	<45U/L	77 (21.3)	29 (19.3)	48 (22.7)	0.515
	≥45U/L	284 (78.7)	121 (80.7)	163 (77.3)	
ALP (%)	<125U/L	276 (76.5)	115 (76.7)	161 (76.3)	1.000
	≥125U/L	85 (23.5)	35 (23.3)	50 (23.7)	
Alb (%)	<35g/l	55 (15.2)	17 (11.3)	38 (18.0)	0.112
	≥35g/l	306 (84.8)	133 (88.7)	173 (82.0)	
TIBL (%)	<20.4umol/L	300 (83.1)	124 (82.7)	176 (83.4)	0.965
	≥20.4umol/L	61 (16.9)	26 (17.3)	35 (16.6)	
DIBL (%)	<6.8umol/L	289 (80.1)	122 (81.3)	167 (79.1)	0.705
	≥6.8umol/L	72 (19.9)	28 (18.7)	44 (20.9)	
CR (%)	<84umol/L	305 (84.5)	124 (82.7)	181 (85.8)	0.510
	≥84umol/L	56 (15.5)	26 (17.3)	30 (14.2)	
INR (%)	<1.15	248 (68.7)	102 (68.0)	146 (69.2)	0.900
	≥1.15	113 (31.3)	48 (32.0)	65 (30.8)	
PLT (%)	<100	100 (27.7)	40 (26.7)	60 (28.4)	0.802
	≥100	261 (72.3)	110 (73.3)	151 (71.6)	
AFP (%)	<400ug/mL	141 (39.1)	56 (37.3)	85 (40.3)	0.648
	≥400ug/mL	220 (60.9)	94 (62.7)	126 (59.7)	
Tumor diameter	<10 cm	207 (57.3)	98 (65.3)	109 (51.7)	0.013
	≥10 cm	154 (42.7)	52 (34.7)	102 (48.3)	
Preoperative TACE	TACE	103 (28.5)	40 (26.7)	63 (29.9)	0.587
	Non-TACE	258 (71.5)	110 (73.3)	148 (70.1)	
Edmondson Grade (%)	I+II	57 (15.8)	29 (19.3)	28 (13.3)	0.158
	III+IV	304 (84.2)	121 (80.7)	183 (86.7)	
MVI (%)	No	141 (39.1)	69 (46.0)	72 (34.1)	0.030
	Yes	220 (60.9)	81 (54.0)	139 (65.9)	
Tumor capsule (%)	Complete	84 (23.3)	39 (26.0)	45 (21.3)	0.363
	Absent or Partial	277 (76.7)	111 (74.0)	166 (78.7)	
Extent of liver resection	Major liver resection	232 (64.3)	94 (62.7)	138 (65.4)	0.672
	Minor liver resection	129 (35.7)	56 (37.3)	73 (34.6)	
Type of liver resection	Anatomical	139 (38.5)	59 (39.3)	80 (37.9)	0.870
	Non-anatomical	222 (61.5)	91 (60.7)	131 (62.1)	
Postoperative TACE	No	181 (50.1)	76 (50.7)	105 (49.8)	0.950
	Yes	180 (49.9)	74 (49.3)	106 (50.2)	
Site of recurrence	Intrahepatic	217 (78.9)	80 (76.9)	137 (80.1)	0.780
	Extrahepatic	28 (10.2)	11 (10.6)	17 (9.9)	
	Intrahepatic and extrahepatic	30 (10.9)	13 (12.5)	17 (9.9)	

TACE, transcatheter arterial chemoembolization; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; ALP, alkaline phosphatase; Alb, albumin; TIBL, total bilirubin; DIBL, direct bilirubin; CR, creatinine; INR, international normalized ratio; PLT, blood platelet; AFP, alpha fetoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; MVI, microvascular invasion.

In addition, we divided the overall population into CTC-positive group and negative-group based on CTCs status and explored whether patients in each group would benefit from preoperative TACE. This study suggested that preoperative TACE may prolong survival prognosis by reducing RFS in the CTC-positive population. At the same time, we also showed that non-preoperative TACE was a risk factor for RFS and OS in HCC patients by univariate and multivariate Cox regression analyses. However, in the CTC-negative group, preoperative TACE was not found to reduce

TABLE 8 | Comparison of perioperative complications between patients with preoperative TACE and those without preoperative TACE.

Variable		Overall (361)	Non-TACE (n=258)	TACE (n=103)	*p*
PLF (%)	No	344 (95.3)	243 (94.2)	101 (98.1)	0.196
	Yes	17 (4.7)	15 (5.8)	2 (1.9)	
Abdominal hemorrhage (%)	No	357 (98.9)	254 (98.4)	103 (100.0)	0.475
	Yes	4 (1.1)	4 (1.6)	0 (0.0)	
Bile leakage (%)	No	351 (97.2)	249 (96.5)	102 (99.0)	0.337
	Yes	10 (2.8)	9 (3.5)	1 (1.0)	
Incisional infection (%)	No	330 (91.4)	233 (90.3)	97 (94.2)	0.329
	Yes	31 (8.6)	25 (9.7)	6 (5.8)	
Organ/space infection (%)	No	339 (93.9)	242 (93.8)	97 (94.2)	1
	Yes	22 (6.1)	16 (6.2)	6 (5.8)	
Respiratory infection (%)	No	353 (97.8)	252 (97.7)	101 (98.1)	1
	Yes	8 (2.2)	6 (2.3)	2 (1.9)	
Pleural effusion (%)	No	316 (87.5)	230 (89.1)	86 (83.5)	0.196
	Yes	45 (12.5)	28 (10.9)	17 (16.5)	
Ascites (%)	No	327 (90.6)	236 (91.5)	91 (88.3)	0.473
	Yes	34 (9.4)	22 (8.5)	12 (11.7)	
Other complications (%)	No	351 (97.2)	252 (97.7)	99 (96.1)	0.646
	Yes	10 (2.8)	6 (2.3)	4 (3.9)	
	Absent or Partial	277 (76.7)	111 (74.0)	166 (78.7)	

TACE, transcatheter arterial chemoembolization; PLF, postoperative liver failure.
Comparison of clinicopathological characteristics and perioperative outcomes between patients with and without preoperative TACE in the total population.

postoperative recurrence and improve survival prognosis, and univariate and multivariate Cox regression analyses also showed that preoperative TACE did not improve long-term prognosis by reducing early recurrence in HCC patients but not affecting late recurrence.

Many studies have suggested that early postoperative recurrence of HCC may be associated with occult micrometastases remaining in the liver (26, 50, 51), and many factors influence the patient's early postoperative tumor recurrence, including CTCs status, tumor diameter, tumor number, microvascular invasion, incomplete tumor envelope and satellite nodules (26, 50, 51). In this study, we found that patients with positive CTCs had a relatively larger tumor diameter ($P < 0.05$) and a higher positive rate of MVI ($P < 0.05$), so we hypothesized that the proportion of patients with occult metastases was higher in the CTC-positive group (52). As surgical resection alone does not remove residual occult foci, preoperative TACE can theoretically remove it. This also explains why preoperative TACE reduce early recurrence in CTC-positive patients and prolongs survival prognosis of patient (26, 28, 52). Second, consider that the vast majority of early recurrence are intrahepatic recurrence. According to the "seed" and "soil" theory of HCC recurrence and metastasis after surgery, preoperative TACE causes changes in the tumor microenvironment of hepatocellular carcinoma. Preoperative TACE may act as a herbicide, making it difficult for CTCs (seeds) to grow in the residual liver (soil) (52). Therefore, preoperative CTC testing is relevant to guide preoperative TACE treatment. In the comprehensive management of hepatocellular carcinoma, clinicians need to pay more attention to the clinical value of preoperative CTC testing. For CTC-positive patients, preoperative TACE is necessary to reduce early postoperative recurrence and prolong OS. However, for patients with CTC-negative, preoperative TACE may not be necessary.

In addition to analyzing the impact of preoperative TACE on the prognosis of HCC patients, we also evaluated the impact of perioperative complications of the subsequent surgery with preoperative TACE. The results found that preoperative TACE did not increase the complications such as liver failure, postoperative ascites, and associated postoperative infections ($P > \mathbf{0.05}$). Some papers reported that the effect of preoperative TACE on surgery was rare if the interval between preoperative TACE and surgery was more than four weeks (8). To be precise, the median time from preoperative TACE to surgical resection at our affiliated medical centre is 4.5 weeks (range 3-6 weeks). Secondly, liver resection is only performed by an experienced team of surgeons. The above results may minimise the impact of preoperative TACE in the perioperative period.

Our research has limitations. Firstly, this study is a single-center retrospective study with few cases. Therefore, in the follow-up study, we will conduct a multi-center, large sample prospective study with multiple medical centers to further demonstrate the value of CTC testing as a guide for preoperative TACE. Secondly, most of the population we include were infected with HBV, whereas most HCC patients in western countries are caused by factors such as HCV or alcohol. The result may not be suitable for Western populations.

In conclusion, this study suggests for the first to propose that preoperative CTC testing is a guide to predicting the efficacy of preoperative TACE for HCC. For patients with positive preoperative CTCs, preoperative TACE may be a reliable means to prevent early recurrence and improve patients' postoperative prognosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Zhongshan Hospital Affiliated to Guangdong Medical University, Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology, Huangshi Central Hospital of Edong Healthcare Group, Hubei Polytechnic University, Xiaogan Central Hospital, General Hospital of Central Theater, Qinghai University Affiliated Hospital, and Renmin Hospital of Wuhan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QZ wrote the paper. WH, QZ, and AM provided the data. FX analysed the data. XF, JC, and WZ reviewed and edited the manuscript. All authors read and approved the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.839597/full#supplementary-material

Supplementary Figure 1 | Comparison of overall survival **(A)** and recurrence free survival **(B)** between CTC-positive and CTC-negative patients. Analysis of the effect of CTC status on early and late postoperative recurrence in overall HCC patients by landmark method.

Supplementary Figure 2 | Analysis of the effect of CTC status on early and late postoperative recurrence in overall HCC patients by landmark method.

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A high preoperative serum IL-25 level is a negative prognosis predictor after liver resection for HBV-HCC

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Objective: The aim of this study was to evaluate the association between preoperative IL-25 levels and HBV-HCC patient outcomes following liver surgery.

Methods: This study enrolled consecutive HCC patients that had undergone liver surgery from 2008 to 2015. Baseline patient clinical properties were assessed to establish predictors of postoperative overall survival and recurrence-free survival (OS and RFS, respectively) following liver resection. In addition, serum IL-25 levels were assessed *via* ELISA.

Results: Cox regression analyses revealed IL-25 levels to be independently related to the OS and RFS of 896 HBV-associated HCC patients. An optimal IL-25 cutoff level of 14.9 μg/ml was identified, with 206 patients in this cohort having IL-25 levels above this threshold. Both the OS and RFS of patients with an IL-25 level <14.9 μg/ml were significantly better after liver resection as compared to those of patients with higher preoperative levels of this cytokine ($p < 0.05$). Cox multivariate regression analyses revealed an IL-25 level ≥ 14.9 μg/L to be an independent predictor of poorer RFS and OS. A combination of IL-25 levels and tumor diameter may be an even more reliable predictor of OS.

Conclusions: IL-25 levels are independent predictors of postoperative survival within HCC patients undergoing liver resection.

KEYWORDS

IL-25, hepatitis B virus, hepatocellular carcinoma, prognosis, biomarker

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors of the digestive system, with high morbidity and mortality (1, 2). Its early diagnosis is crucial for timely treatment and improvement of survival rate (3). Although ultrasound, magnetic resonance imaging (MRI), and other imaging techniques have greatly improved the accuracy of HCC diagnosis, their application is limited due to their high cost, strong invasiveness, and insensitivity to small tumors (3). Therefore, convenient, inexpensive, noninvasive, and reproducible serum biomarkers have played an important role in the diagnosis of HCC (4). Alpha-fetoprotein (AFP) is a widely used biomarker for the diagnosis of liver cancer, but its diagnostic accuracy is limited because it has a high false-negative rate in the detection of small tumors and early tumors. In addition, AFP may be elevated in some benign liver diseases, such as chronic hepatitis and cirrhosis without HCC (5). At present, the application of AFP in early screening of liver cancer has been controversial (5).

Therefore, it is very important to find new biomarkers related to liver cancer, achieve multi-indicator combined detection, improve the accuracy of early diagnosis of liver cancer, and reduce the rate of missed diagnosis. Over the years, other tumor markers for HCC have been proposed, such as Golgi protein 73 (GP73), Glypican-3 (GPC3), and cytokeratin 19 (CK-19) (6–8). GP73 is considered a potential marker of liver cancer, but serum GP73 levels may also be elevated in patients with liver parenchymal tumors. Therefore, GP73 detection is not suitable for distinguishing HCC from benign liver disease (6). Liu et al. found that serum GPC3 level was increased in patients with liver cancer; however, GPC3 was not sensitive to distinguish benign diseases from early liver cancer (7). Previous studies have shown that CK-19 expression is related to the invasive behavior of HCC, such as low differentiation, metastasis, and microvascular invasion, which indicates that CK-19 can be used as an indicator of survival and recurrence of HCC patients (8). However, these markers have not been considered effective enough for clinical use as indicators for HCC diagnosis.

Chronic inflammation is often a main driver of oncogenesis, and suppressing such inflammation can thus slow or arrest the physiological progression of cancer (9, 10). Inflammatory factors have been closely linked to many solid tumor types, including HCC (11), with certain cytokines including interleukin-6 (IL-6) serving as key mediators of systemic immune responses (12). There have been several previous reports demonstrating that the serum levels of inflammatory factors can predict the development or prognosis of many forms of cancer, including HCC (13). In addition, the understanding of the relationship between IL-25 and clinicopathological features, as well as the role of IL-25 in assessing the diagnostic role in HCC, has not been fully investigated. These findings may contribute to a more complete understanding of the significance of IL-25 in HCC. Here, to resolve these controversies, we measured serum IL-25 levels to evaluate the individual and combined diagnostic performance of IL-25 and AFP for HCC. The diagnostic ability of IL-25 for AFP-negative HCC was also evaluated. In addition, we analyzed the relationship between serum IL-25 levels and clinicopathological features in patients with HCC, in order to investigate the value of IL-25 in assessing the progression and prognosis of HCC. Herein, we therefore explored the prognostic relevance of preoperative IL-25 levels among hepatitis B virus (HBV)-associated HCC cases that had undergone liver resection.

Patients and methods

Patients

For this study, HBV-infected patients that had undergone liver transplantation conducted by a single surgical team at the 900th Hospital of Logistics Support Force from January 2008 to June 2015 were retrospectively enrolled. All patients had been diagnosed with HCC as per the European Society for the Study of the Liver (EASL) criteria (14), with pathological examination being used to confirm this diagnosis. Selection criteria for cases that participated in this research cohort were as follows: World Health Organization (WHO) preoperative status = 0–1; Child-Pugh Class A; no macrovascular invasion; no distant metastases; and no preoperative chemotherapy, radiosurgery, radiotherapy, or dermal ethanol injections prior to resection of liver. Patients were HBsAg positive and hepatitis C virus (HCV)-Ab negative. The Hospital Institutional Review Committee of the 900th Hospital of Logistics Support Force confirmed the present research, with cases having given the letter of aware satisfaction.

Follow-up

For the first 2 years after surgery, patients experienced follow-up every 3 months, and every 6 months thereafter. Hospital staff blinded to study objectives conducted all follow-up. All patients were regularly monitored for recurrence using approaches including AFP analyses, chest x-rays, and abdominal USG, MRI, or CT scans that were conducted every 3 months. HCC recurrence was diagnosed using the same criteria as were used to diagnose the primary disease before surgery. Approaches to treating recurrent diseases included TACE, PRFA, and PEI, with the exact procedure being selected based on patient- and tumor-specific factors.

Propensity score matching

To diminish the potential for bias inherent in this retrospective analysis, propensity score matching (PSM) was

performed. Specifically, cases with low and high IL-25 were matched *via* a PSM approach as described previously (15). Covariates included in this PSM model are Ishak's inflammation, tumor diameter, AFP, AST, HBeAg, HBV-DNA load, encapsulation of tumor, microvascular invasion, tumor count, and the degree of liver resection. Matching was executed at a 1:1 ratio for cases with low and high IL-25 levels as detailed previously (16).

ELISA

Levels of serum IL-25 were measured in HCC patient samples with the Human IL-25 DuoSet ELISA kit (R&D Systems) based on the provided directions.

Statistical studies

All outcomes are given as median (range) or mean ± standard deviation (SD), and were studied by implementing unpaired Student's *t*-tests or χ^2 assessments as appropriate. The OS and RFS cases were assessed with Kaplan–Meier curves as well as log-rank measurements. Multivariate and univariate methods were used to guide the design of a prognostic nomogram, which was constructed with the "rms" package using R v.3.5.1 (http://www.r-project.org/). This nomogram was assessed based on measurements of the conformity index (C-index), with rcorrp.cens being used to compare the C-index values for this nomogram to those for other nomograms in Hmisc (17). Analyses of the receiver operating characteristic (ROC) curve were implemented to study nomograms and predictors, with $p < 0.05$ as the threshold of significance.

Results

Baseline patient characteristics

Over the defined study period, 933 patients with HBV-associated HCC underwent liver transplantation for curative purposes, and were registered in the present survey. Of these cases, 37 were excluded for reasons including early metastasis or recurrence within 30 days postoperatively (n = 11), surgery (n = 5), liver failure-related mortality within 30 days postoperatively (n = 6), or clinically detected preoperative infection (n = 15), leaving a cohort of 896 patients eligible for these analyses (Figure 1). These patients exhibited a mean age of 52 years (range: 29–75), and were predominantly male (755 male patients and 141 female patients) as shown in Table 1. All patients were positive for HBeAg and the remaining 695 were

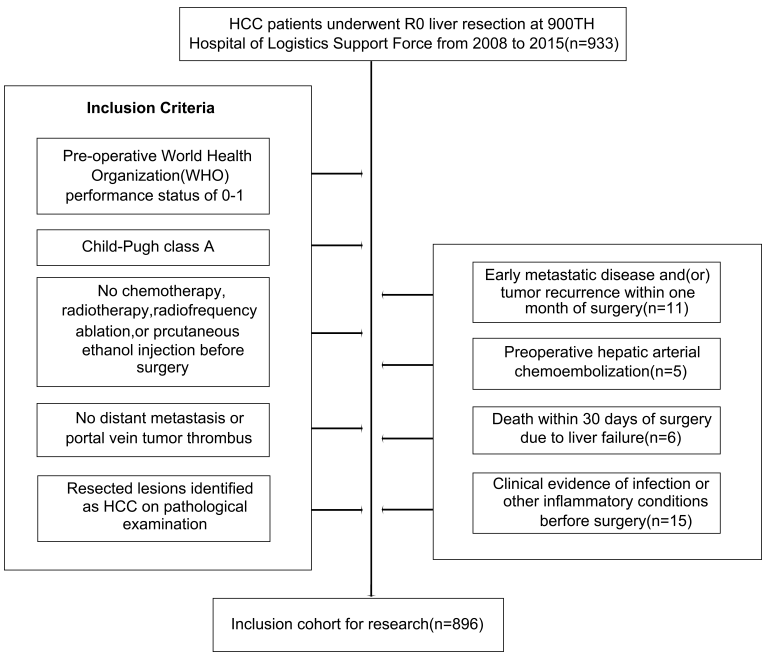

FIGURE 1
Study population selection.

TABLE 1 Patient clinicopathological characteristics.

Characteristics	Total patients (*N* = 896)
Gender	
Male	755
Female	141
Age (years)[a]	52
Liver cirrhosis	
Yes	535
No	361
HBeAg	
Positive	201
Negative	695
AFP (ng/ml)	
≥20	551
<20	345
Alanine aminotransferase (U/L)	
≥40	385
<40	511
Aspartate aminotransferase (U/L)	
≥40	343
<40	553
Total bilirubin (μmol/ml)	
≥17.1	345
<17.1	551
Albumin (g/L)	
≥35	847
<35	49
HBV DNA (IU/ml)	
≥2,000	502
<2,000	394
Ishak inflammation score [a]	6 (2–14)
Ishak fibrosis score [a]	4 (1–6)
Tumor diameter (cm)[a]	4.3 (0.5–17)
Tumor encapsulation	
None	400
Complete	496
Major resection	
Yes	130
No	766
Microvascular invasion	
Yes	325
No	571
Tumor number	
Single	475
Multiple	421
Tumor differentiation	
I/II	145
III/IV	751
Stage of BCLC	
0+A	595
B	301

[a]Age, score of Ishak inflammation, score of Ishak fibrosis, and diameter of tumor are shown as median (range).
HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; AFP, alpha-fetoprotein; BCLC stage, Barcelona Clinic Liver Cancer stage.

negative for HBeAg. All exhibited Child-Pugh A liver function levels, with a median inflammation level of 6 (range: 2–14). A total of 502 cases exhibited HBV DNA levels ≥ 2,000 IU/ml. Primary tumors were a median of 4.3 cm in size (range: 0.5–17 cm), and serum IL-25 levels ranged from 0.25 to 45 μg/L (median: 10.1 μg/L). Under BCLC staging criteria, 595 patients were stage 0 and 301 were stage B. Of these 896 patients, 325 exhibited microvascular, 421 presented with multiple tumors, and 496 exhibited complete tumor encapsulations. In addition, 130 patients underwent major liver resection. Tumors differed markedly in 145 patients (E-S Grades I and II). The median period of follow-up was 41.5 months (range: 9.5–98.5).

IL-25 levels are associated with HCC patient clinicopathological characteristics

Next, ROC curve analyses were used to establish an optimal IL-25 cutoff level capable of differentiating between HCC patient outcomes. The selected cutoff value was 14.9 μg/L, yielding an AUC value of 0.730, a specificity of 0.640, and a sensitivity of 0.757 (Figure 2). In total, 334 and 562 patients were respectively clustered into IL-25-high and -low groups, and there were clear differences in clinical characteristics among these groups (Table 2). Specifically, individuals with high IL-25 levels exhibited higher AFP levels, greater viral loads (≥2,000 IU/ml), and larger tumor sizes (all $p < 0.05$), indicating that higher IL-25 levels are associated with more advanced HCC. After a PSM analysis, 156 patient pairs were generated (Table 3). Following PSM, clinical characteristics did not differ between these cohorts ($p > 0.05$).

IL-25 levels are correlated with HCC patient prognosis

The 3- and 5-year RFS rates of patients in the group of high IL-25 were detected to be considerably decreased in comparison to those of patients in the low IL-25 group (64.1% and 42.2%, respectively, vs. 90.1% and 78.5%, respectively; $p < 0.05$). Higher levels of IL-25 were also associated with decreased 3- and 5-year OS relative to lower IL-25 levels (77.3% and 61.8%, respectively, vs. 97.6% and 95.1%, respectively; $p < 0.05$) (Figures 3A, B).

Following PSM, the 3- and 5-year RFS rates in the IL-25-high group were 61.6% and 42.3%, respectively, whereas they were significantly higher at 89.1% and 76.2%, respectively, in the IL-25-low group ($p < 0.05$). Similarly, following the PSM, the 3- and 5-year OS of cases with high IL-25 levels were 77.6% and 61.1%, respectively, which were significantly decreased as compared to those of cases with low IL-25 levels ($p < 0.05$) (Figures 3C, D)

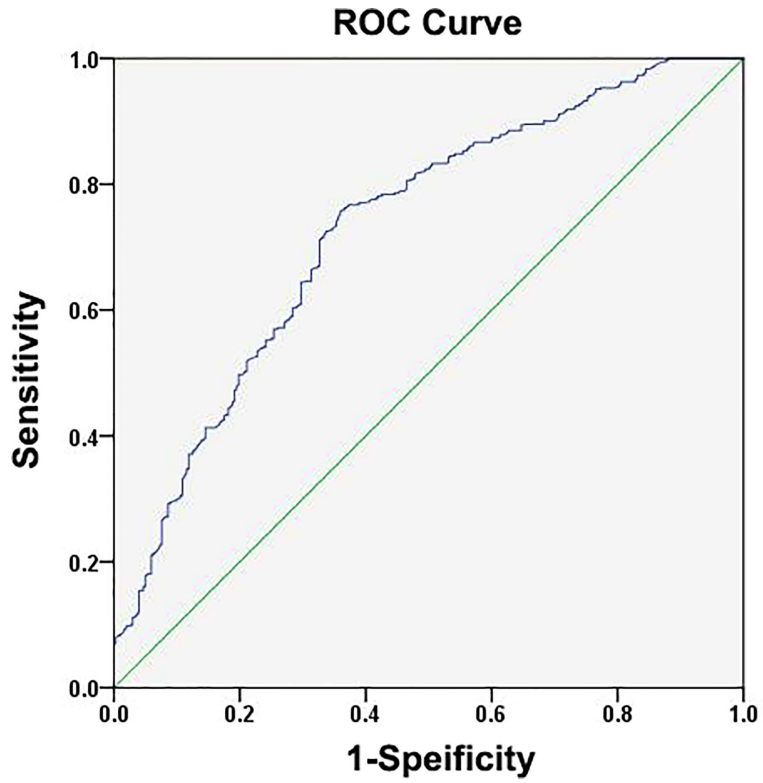

FIGURE 2
Assessments of the receiver operating characteristic (ROC) curve for the specificity and sensitivity of IL-25 in HCC patients.

Identification of factors related to HCC patient prognosis

Cox regression analyses were next applied to detect risk factors independently correlated with HCC patient RFS and OS. In univariate analyses, HBV DNA levels, IL-25 levels, AFP levels, microvascular invasion, tumor encapsulation, tumor number, tumor differences, tumor scale, and cirrhosis were all independently correlated with a decreased RFS (Table 4), whereas HBV DNA levels, IL-25 levels, AFP levels, tumor encapsulation, and invasion were correlated with worse OS. Subsequently, multivariate approach revealed that HBV DNA levels ≥2,000 IU/ml, IL-25 ≥ 14.9 μg/ml, AFP ≥ 20 ng/ml, a lack of complete encapsulation of tumor, multiple tumors, microvascular invasion, and tumor size ≥ 5 cm were independent predictors of worse patient RFS (Table 4), while HBV DNA levels ≥ 2,000 IU/ml, IL-25 ≥ 14.9 μg/L, AFP ≥ 20 ng/ml, a lack of complete tumor encapsulation, and tumor size ≥ 5 cm were independently predictive of worse OS (Table 5). Additional analyses of Cox regression were conducted to detect independent predictors of OS and RFS in the cohort of PSM (Tables 6, 7). In this group, an elevated IL-25 level (≥14.9 μg/ml) remained independently associated with poorer RFS and OS.

Construction and evaluation of nomograms capable of predicting HCC patient survival outcomes

Next, the independent predictors identified in the above multivariate analysis were used to construct nomograms capable of predicting the RFS (Figure 4A) and OS of HCC patients (Figure 4B). The respective values of the C-index for these nomograms of OS and RFS were 0.726 and 0.645. To explore the predictive value of these nomograms, they were next compared with other independent predictors identified above (Table 8). The RFS nomogram C-index value (0.645) was higher than that for HBV DNA (0.542), AFP (0.564), tumor count (0.538), encapsulation of tumor (0.561), IL-25 (0.549), tumor diameter (0.582), and microvascular invasion (0.559) (all $p < 0.001$). The nomogram C-index value (0.726) for OS was higher than that for IL-25 (0.559), HBV-DNA (0.569), AFP (0.584), encapsulation of tumor (0.591), and diameter of tumor (0.635) (all $p < 0.05$). These results thus supported the predictive accuracy of these nomograms, with both the RFS and OS nomograms exhibiting AUC values higher than those for other prognostic risk factors (Table 9).

TABLE 2 HCC patient clinicopathological and demographic characteristics as a function of IL-25 levels.

	Low IL-25 (<14.9 μg/ml), *N* = 562	Elevated IL-25 (≥14.9 μg/ml), *N* = 334	*p*-value
Gender 0.087			
Male	483	272	
Female	79	62	
Age (years)[a]	48.19 ± 10.2	50.23 ± 11.0	0.156
Liver cirrhosis			0.778
Yes	338	197	
No	224	137	
HBeAg			0.869
Positive	125	76	
Negative	437	258	
AFP (ng/ml)			<0.001
≥20	339	212	
<20	223	122	
Alanine aminotransferase (U/L)			0.334
≥40	240	145	
<40	322	189	
Aspartate aminotransferase (U/L)			0.074
≥40	230	113	
<40	332	221	
Total bilirubin (μmol/ml)			
≥17.1	204	141	0.088
<17.1	358	193	
Albumin (g/L)			
≥35	535	312	0.288
<35	27	22	
HBV DNA (IU/ml)			
≥2,000	255	247	0.000
<2,000	307	87	
Ishak inflammation score[a]	5.12 ± 1.73	5.34 ± 2.98	0.102
Ishak fibrosis score[a]	4.73 ± 1.56	5.08 ± 1.36	0.251
Tumor diameter (cm)[a]	5.47 ± 3.67	8.84 ± 4.14	0.002
Tumor encapsulation			0.413
None	249	151	
Complete	313	183	
Major resection			
Yes	92	38	0.049
No	470	296	
Microvascular invasion			
Yes	193	132	0.131
No	369	202	
Tumor number			0.216
Single	289	186	
Multiple	273	148	
Tumor differentiation			
I/II	88	57	0.575
III/IV	474	277	

[a]Age, score of Ishak inflammation, score of Ishak fibrosis, and diameter of tumor are stated as mean ± SD. HbeAg, hepatitis B e antigen; alpha-fetoprotein, AFP.

TABLE 3 HCC patient clinicopathological and demographic characteristics as a function of IL-25 levels after propensity score matching (PSM).

	Low IL-25 (<14.9 μg/ml), *N* = 156	Elevated IL-25 (≥14.9 μg/ml), *N* = 156	*p*-value
Gender			0.732
Male	138	135	
Female	18	21	
Age (years)[a]	50.44 ± 10.59	49.83 ± 10.93	0.522
Liver cirrhosis			0.564
Yes	90	96	
No	66	60	
HBeAg			0.798
Positive	40	43	
Negative	116	113	
AFP (ng/ml)			1.000
≥20	104	104	
<20	52	52	
Alanine aminotransferase (U/L)			1.000
≥40	81	80	
<40	75	76	
Aspartate aminotransferase (U/L)			0.070
≥40	71	88	
<40	85	68	
Total bilirubin (μmol/ml)			0.650
≥17.1	70	75	
<17.1	86	81	
Albumin (g/L)			0.734
≥35	80	77	
<35	76	79	
HBV DNA (IU/ml)			0.650
≥2,000	79	84	
<2,000	77	72	
Ishak inflammation score [a]	4.93 ± 2.62	5.25 ± 2.68	0.304
Ishak fibrosis score [a]	4.61 ± 2.99	4.16 ± 2.72	0.138
Tumor diameter (cm)[a]	8.22 ± 4.48	8.31 ± 4.11	0.508
Tumor encapsulation			0.908
None	95	93	
Complete	61	63	
Major resection	51	46	0.556
Yes			
No	105	109	
Microvascular invasion			0.729
Yes	61	65	
No	95	91	
Tumor number			0.4945
Single	73	66	
Multiple	83	90	
Tumor differentiation			0.376
I/II	15	21	
III/IV	141	135	

[a]Age, Ishak inflammation, and diameter of tumor are expressed as mean ± SD. PSM, propensity score matching.
HBeAg, hepatitis B e antigen; alpha-fetoprotein, AFP.

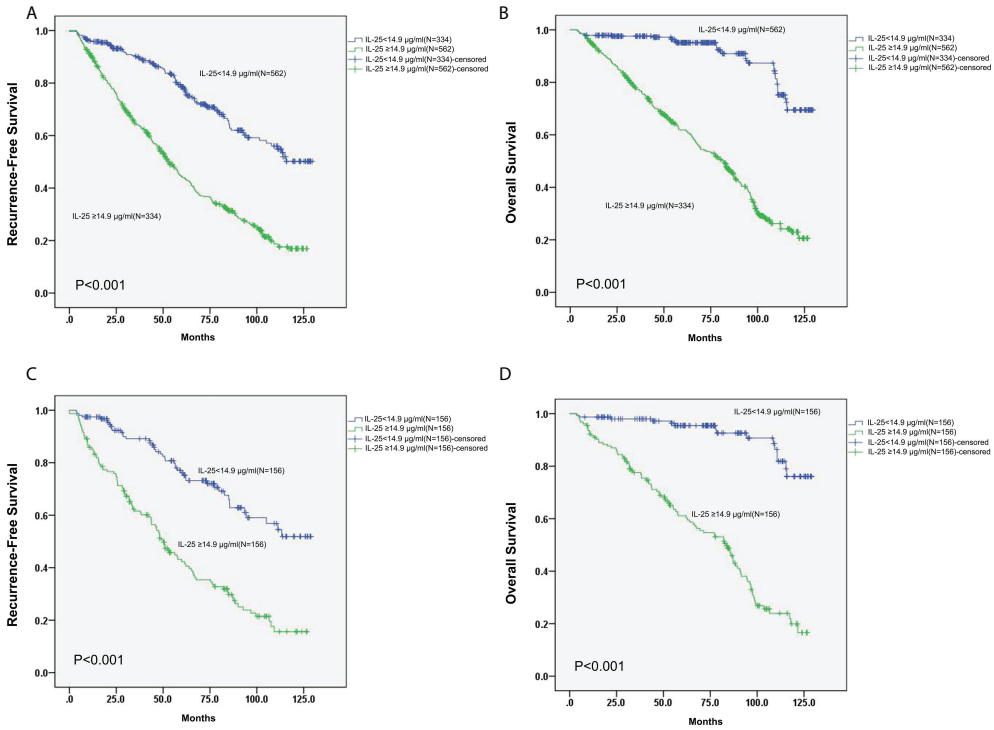

FIGURE 3
(A, B) RFS **(A)** and OS **(B)** curves for all 896 HCC patients with low or high IL-25 levels. **(C)** Curves of RFS for HCC patients in the PSM cohort with low or high IL-25 levels. **(D)** Curves of OS for HCC patients in the PSM cohort with low or high IL-25 levels.

TABLE 4 Multivariate and univariate studies of factors correlated with HCC patient recurrence-free survival.

	Hazard ratio (95% CI)	*p*-value
Univariate studies		
Gender (male vs. female)	0.901 (0.719–1.129)	0.367
Age (years) (≤60 vs. >60)	0.848 (0.695–1.035)	0.105
Alanine aminotransferase (≥40 vs. <40 U/L)	1.212 (1.047–1.302)	0.02
Aspartate aminotransferase (≥40 vs. <40 U/L)	0.504 (0.300–1.741)	0.301
Albumin (<35 vs. ≥35 g/L)	0.806 (0.588–1.105)	0.185
HBV DNA (≥2,000 vs. <2,000 IU/ml)	1.577 (1.564–1.883)	<0.001
Ishak inflammation score (≥3 vs. <3)	1.227 (1.156–1.345)	0.014
Ishak fibrosis score (≥3 vs. <3)	0.825 (0.731–1.267)	0.328
IL-25 (≥14.9 vs. <14.9 μg/L)	1.747 (1.474–2.069)	<0.001
AFP (≥20 vs. <20 ng/ml)	1.649 (1.409–1.929)	<0.001
HBeAg (positive vs. negative)	1.166 (0.995–1.366)	0.058
Tumor encapsulation (yes vs. no)	0.698 (0.603–0.809)	<0.001
Major resection (yes vs. no)	1.168 (0.973–1.403)	0.096
Microvascular invasion (yes vs. no)	1.575 (1.343–1.847)	<0.001
Number of tumor (multiple vs. single)	1.679 (1.377–2.048)	<0.001
Differentiation of tumor (III+IV vs. I+II)	0.560 (0.281–1.899)	0.225
Diameter of tumor (≥5 vs. <5 cm)	1.644 (1.419–1.904)	<0.001
Liver cirrhosis (yes vs. no)	0.056 (0.376–1.442)	0.403

(Continued)

TABLE 4 Continued

	Hazard ratio (95% CI)	*p*-value
Multivariate analysis		
HBV DNA (≥2,000 vs. <2,000 IU/ml)	1.235 (1.133–1.465)	0.013
IL-25 (≥14.9 vs. <14.9 μg/L)	1.494 (1.350–1.786)	<0.001
AFP (≥20 vs. <20 ng/ml)	1.363 (1.254–1.610)	<0.001
Encapsulation of tumor (yes vs. no)	0.785 (0.712–0.879)	0.006
Microvascular invasion (yes vs. no)	1.126 (1.114–1.357)	0.017
Tumor number (multiple vs. single)	1.216 (1.128–1.424)	0.015
Diameter of tumor (≥5 vs. <5 cm)	1.285 (1.188–1.518)	0.003

Assessment of the prognostic value of IL-25 as a predictor of HCC patient survival

For RFS, the C-index value for IL-25 was 0.549, which was considerably greater as compared to that associated with the number of tumors ($p < 0.05$) (Table 8). In a ROC curve analysis for RFS (Table 9), no differences were observed. The AUC value for IL-25 was higher than that for all other predictors with the exception of tumor number ($p < 0.05$), and in an analysis of multivariate for predictors associated with patient RFS, the HR for IL-25 was the greatest. As IL-25 exhibited the greatest specific weight of any factor in a predictive nomogram for HCC patient RFS, this suggested that IL-25 is the most robust predictor of RFS in this patient population (Figure 4A). The C-index value for IL-25 when used to predict HCC patient OS was 0.559, which

TABLE 5 Multivariate and univariate studies of factors correlated with HCC patient overall survival.

	Hazard ratio (95% CI)	*p*-value
Univariate analysis		
Gender (male vs. female)	0.901 (0.719–1.129)	0.367
Age (years) (≤60 vs. >60)	0.848 (0.695–1.035)	0.105
Alanine aminotransferase (≥40 vs. <40 U/L)	1.212 (1.047–1.302)	0.02
Aspartate aminotransferase (≥40 vs. <40 U/L)	1.504 (1.300–1.741)	<0.001
Albumin (<35 vs. ≥35 g/L)	0.806 (0.588–1.105)	0.18
HBV DNA (≥2,000 vs. <2,000 IU/ml)	1.577 (1.564–1.883)	<0.001
Ishak inflammation score (≥3 vs. <3)	1.227 (1.156–1.345)	0.014
Ishak fibrosis score (≥3 vs. <3)	0.825 (0.731–1.267)	0.328
IL-25 (≥14.9 vs. <14.9 μg/L)	1.747 (1.474–2.069)	<0.001
AFP (≥20 vs. <20 ng/ml)	1.649 (1.409–1.929)	<0.001
HBeAg (positive vs. negative)	1.166 (0.995–1.366)	0.058
Tumor encapsulation (yes vs. no)	0.698 (0.603–0.809)	<0.001
Major resection (yes vs. no)	1.168 (0.973–1.403)	0.096
Microvascular invasion (yes vs. no)	0.575 (0.343–1.847)	0.432
Number of tumor (multiple vs. single)	0.679 (0.377–2.048)	0.501
Differentiation of tumor (III+IV vs. I+II)	0.560 (0.281–1.899)	0.356
Diameter of tumor (≥5 vs. <5 cm)	1.644 (1.419–1.904)	<0.001
Liver cirrhosis (yes vs. no)	1.256 (1.176–1.442)	0.003
Multivariate analysis		
HBV DNA (≥2,000 vs. <2,000 IU/ml)	1.235 (1.133–1.465)	0.013
IL-25 (≥14.9 vs. <14.9 μg/L)	1.494 (1.350–1.786)	<0.001
AFP (≥20 vs. <20 ng/ml)	1.363 (1.254–1.610)	<0.001
Tumor encapsulation (yes vs. no)	0.785 (0.712–0.879)	0.006
Diameter of tumor (≥5 vs. <5 cm)	1.285 (1.188–1.518)	0.003

TABLE 6 Multivariate and univariate studies of factors correlated with HCC patient recurrence-free survival in a propensity score matching (PSM) cohort.

	Hazard ratio (95%CI)	*p*-value
Univariate analysis		
Gender (male vs. female)	0.619 (0.476–1.039)	0.536
Age (years) (≤60 vs. >60)	0.654 (0.275–0.982)	0.016
Alanine aminotransferase (≥40 U/L vs. <40 U/L)	0.827 (0.382–1.026)	0.569
Aspartate aminotransferase (≥40 U/L vs. <40 U/L)	1.381 (1.167–1.839)	0.026
Albumin (<35 g/L vs. ≥35 g/L)	0.712 (0.369–1.076)	0.876
HBV DNA (≥2,000 IU/ml vs. <2,000 IU/ml)	1.516 (1.084–1.964)	0.001
Total bilirubin (≥17.1 μmol/ml vs. <17.1 μmol/ml)	0.941 (0.672–1.169)	0.532
Score of Ishak inflammation (≥3 vs. <3)	1.519 (1.287 –1.961)	0.021
Score of Ishak fibrosis (≥3 vs. <3)	0.824 (0.384–1.587)	0.198
IL-25 (≥14.9 vs. <14.9 μg/ml)	1.471 (1.028–1.763)	0.001
AFP (≥20 vs. <20 ng/ml)	1.751 (1.537 –2.571)	<0.001
HBeAg (positive vs. negative)	1.473 (1.021–1.694)	0.029
Encapsulation of tumor (yes vs. no)	0.633 (0.632 –1.469)	<0.001
Major resection (yes vs. no)	0.911 (0.681–1.072)	0.723
Microvascular invasion (yes vs. no)	1.629 (1.169 –2.069)	<0.001
Number of tumor (multiple vs. single)	1.957 (1.037 –2.379)	0.001
Differentiation of tumor (III+IV vs. I+II)	0.996 (0.357 –2.467)	0.267
Tumor diameter (≥5 cm vs. <5 cm)	1.051 (0.863–1.714)	<0.001
Liver cirrhosis (yes vs. no)	1.419 (1.167–1.963)	0.036
Multivariate analysis		
Age (years) (≤60 vs. >60)	0.993 (0.653–1.279)	0.756
Aspartate aminotransferase (≥40 U/L vs. <40 U/L)	0.914 (0.583–1.327)	0.279
HBV DNA (≥2,000 IU/ml vs. <2,000 IU/ml)	1.469 (1.127–1.937)	0.026
Ishak inflammation score (≥3 vs. <3)	1.716 (1.382–1.973)	0.039
IL-25 (≥14.9 vs. <14.9 μg/ml)	1.487 (1.096–1.672)	0.001
AFP (≥20 vs. <20 ng/ml)	0.961 (0.284–1.037)	0.637
Tumor encapsulation (yes vs. no)	0.758 (0.189–0.836)	0.041
Microvascular invasion (yes vs. no)	0.976 (0.376–1.073)	0.583
Number of tumor (multiple vs. single)	0.993 (0.536–1.376)	0.493
Diameter of tumor (≥5 cm vs. <5 cm)	1.072 (0.753–1.539)	0.001
Differentiation of tumor (III+IV vs. I+II)	0.963 (0.365–1.073)	0.367
Liver cirrhosis (yes vs. no)	1.631 (1.256–1.983)	0.034

Values of HRs (95% CI) and p were determined via multivariate and univariate Cox proportional hazard regression studies.
HbeAg, hepatitis B e antigen; AFP, alpha-fetoprotein; PSM, propensity score matching.

was the lowest of all tested predictors (Table 8) in an analysis of the curve of ROC (Table 9). Furthermore, IL-25 exhibited a lower AUC value than any other predictor analyzed in this study, and consistently possessed the least specific weight in a nomogram used to predict patient OS (Figure 4B). As such, we evaluated combinations of IL-25 and other predictors with the goal of defining the most reliable prognostic combination associated with patient OS (Table 9), revealing that a combination of IL-25 and tumor diameter yielded a greater AUC value than any other combination, thus suggesting that these two parameters may represent a more reliable means of predicting HCC patient OS.

Discussion

The onset and progression of HCC are driven in large part by interactions between nascent tumor cells and the surrounding inflammatory milieu (18–22). There is thus clear value in further elucidating the specific roles played by particular inflammatory mediators during the progression of cancer (9, 10). HCC is a common malignant tumor of the digestive system, characterized by aggressive growth and early metastasis, and is the second leading cause of cancer mortality in China (2, 3). Because the early symptoms are not obvious, many patients with liver cancer are diagnosed as advanced stage (23). Systematic screening of

TABLE 7 Multivariate and univariate analyses of factors correlated with HCC patient overall survival in a propensity score matching (PSM) cohort.

	Hazard ratio (95% CI)	*p*-value
Univariate analysis		
Gender (male vs. female)	0.756 (0.417–1.391)	0.426
Age (years) (≤60 vs. >60)	0.851 (0.726–1.109)	0.269
Alanine aminotransferase (≥40 U/L vs. <40 U/L)	0.716 (0.541–1.019)	0.654
Aspartate aminotransferase (≥40 U/L vs. <40 U/L)	1.929 (1.172–2.013)	<0.011
Albumin (<35 g/L vs. ≥35 g/L)	0.651 (0.392–1.103)	0.682
HBV DNA (≥2,000 IU/ml vs. <2,000 IU/ml)	1.419 (1.071–1.939)	0.031
Total bilirubin (≥17.1 μmol/ml vs. <17.1 μmol/ml)	0.817 (0.421–1.253)	0.719
Ishak inflammation score (≥3 vs. <3)	1.461 (1.093–1.865)	0.029
Ishak fibrosis score (≥3 vs. <3)	0.910 (0.612–1.382)	0.349
IL-25 (≥14.9 vs. <14.9 μg/ml)	1.563 (1.192–1.829)	0.016
AFP (≥20 vs. <20 ng/ml)	1.792 (1.461–2.031)	<0.001
HBeAg (positive vs. negative)	1.521 (1.069–2.392)	0.042
Tumor encapsulation (yes vs. no)	0.669 (0.479–0.816)	0.011
Major resection (yes vs. no)	0.719 (0.368–1.310)	0.623
Microvascular invasion (yes vs. no)	1.536 (1.217–1.973)	<0.021
Tumor number (multiple vs. single)	1.604 (1.359–2.679)	<0.041
Tumor differentiation (III+IV vs. I+II)	0.593 (0.431–1.618)	0.161
Tumor diameter (≥5 cm vs. <5 cm)	1.329 (1.195–1.921)	<0.001
Liver cirrhosis (yes vs. no)	0.792 (0.538–1.139)	0.435
Multivariate analysis		
Aspartate aminotransferase (≥40 U/L vs. <40 U/L)	0.651 (0.493–1.079)	0.791
HBV DNA (≥2,000 IU/ml vs. <2,000 IU/ml)	1.3219 (1.167–1.736)	0.031
Ishak inflammation score (≥3 vs. <3)	0.563 (0.352–1.057)	0.259
IL-25 (≥14.9 vs. <14.9 μg/ml)	1.526 (1.056–1.983)	0.049
AFP (≥20 vs. <20 ng/ml)	0.756 (0.328–1.569)	0.129
HBeAg (positive vs. negative)	0.538 (0.393–1.289)	0.563
Tumor encapsulation (yes vs. no)	0.726 (0.357–1.089)	0.369
Microvascular invasion (yes vs. no)	0.574 (0.346–1.147)	0.573
Tumor number (multiple vs. single)	0.692 (0.379–1.639)	0.134
Tumor diameter (≥5 cm vs. <5 cm)	1.328 (1.125–2.537)	<0.011

The values of HRs (95% CI) and p were determined via multivariate and univariate Cox proportional hazard regression analyses.
HBeAg, hepatitis B e antigen; AFP, alpha-fetoprotein; PSM, propensity score matching.

high-risk groups is necessary for early diagnosis. AFP is the most commonly used biomarker for HCC patients, although its sensitivity and specificity are unsatisfactory, especially for early-stage disease (14, 15). Previous studies have shown that the ability of AFP to diagnose liver cancer is relatively poor. Using cutoff values of 17.76 ng/ml and 21.47 ng/ml would result in 60 (35.71%) and 62 (36.90%) of 168 HCC patients being considered negative. Fifteen of 153 healthy controls (9.80%) and 23 of 150 patients with benign liver disease (15.33%) were considered positive, and these inaccuracies supported the inadequacy of AFP as a biomarker (24). Therefore, new and reliable biomarkers are needed to improve the diagnostic level of liver cancer.

IL-25 is an inflammatory IL-17 family cytokine that is best studied as a driver of type 2 immune responses (13, 25–27). In previous reports, IL-25 was shown to perform a central task in the incidence of acute hepatitis (AH), liver fibrosis, and cirrhosis (28–30). As an anti-inflammatory cytokine, IL-25 promotes type 2 cytokine-dependent immunity and limits the production of pro-inflammatory cytokines by inhibiting the expression of type 1 cytokines. Deregulation of IL-25 has been found in many inflammation-related diseases, including helminth parasite infection, inflammatory bowel disease, asthma, severe hepatitis, and NAFLD (31–33). Meanwhile, IL-25 also plays an important role in several human cancers (31–35). However, it is not completely clear whether IL-25 affects the development of HCC. Studies have shown that IL-25 plays a direct role in cancer cells and affects the development of breast cancer (32–34). Previous results showed that IL-25 did not directly affect the growth, apoptosis, or migration of HCC cells. IL-25-induced M2 macrophages attenuated obesity and NAFLD (36). Similarly, Wang et al. reported that IL-25 induces hepatic macrophages to

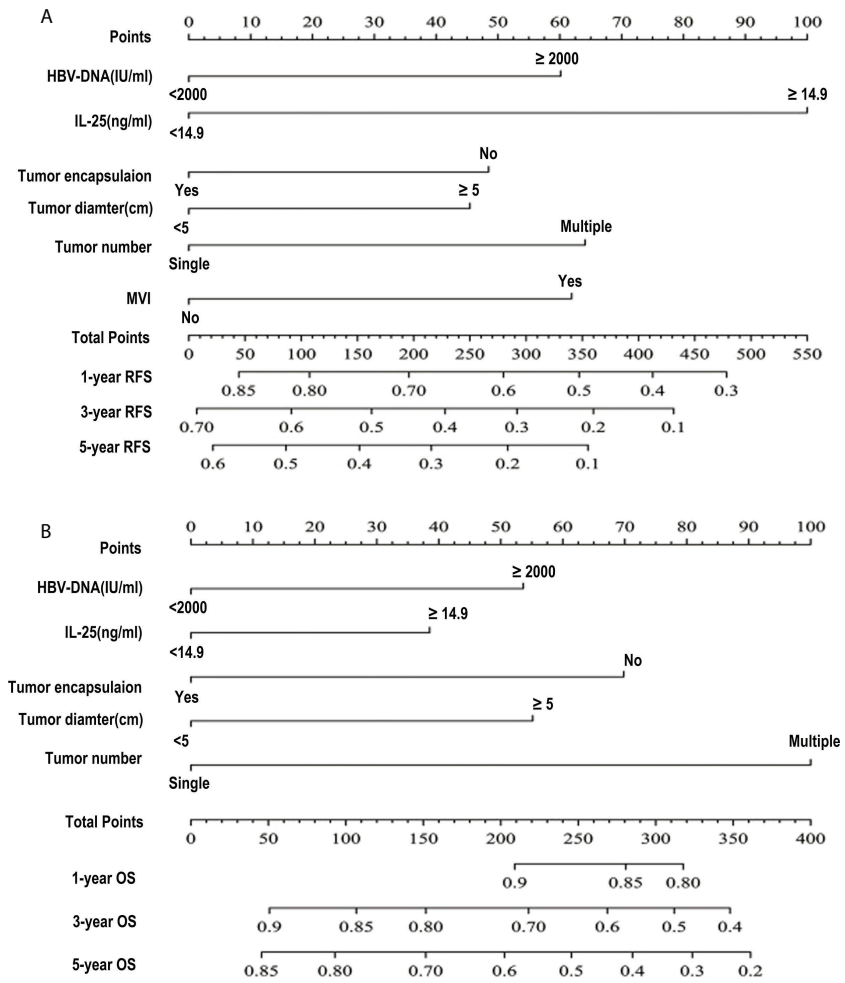

FIGURE 4
HCC patient survival nomogram. **(A, B)** A survival nomogram designed to assess HCC patient RFS **(A)** and OS **(B)**.

TABLE 8 C-index values for predictors of HCC patient survival outcomes.

	RFS				OS			
Variables	**C-index**	**95% CI**	***p*†-value**	***p*‡-value**	**C-index**	**95% CI**	***p*†-value**	***p*‡-value**
Nomogram	0.645	0.605–0.678		<0.001	0.726	0.675–0.756		<0.001
IL-25	0.549	0.534–0.564	<0.001		0.559	0.538–0.579	<0.001	
AFP					0.584	0.558–0.609	<0.001	0.018
Tumor encapsulation	0.561	0.542–0.580	<0.001	0.076	0.591	0.565–0.618	<0.001	<0.001
Tumor diameter	0.582	0.563–0.602	<0.001	0.599	0.635	0.609–0.661	<0.001	<0.001
HBV-DNA	0.542	0.523–0.562	<0.001	0.064	0.569	0.543–0.595	<0.001	0.413
Tumor number	0.538	0.524–0.549	<0.001	0.005				
Microvascular invasion	0.559	0.543–0.575	<0.001	0.323				

AFP, alpha-fetoprotein.
p†-value: nomogram vs. other predictors.
p‡-value: IL-25 vs. other predictors.

TABLE 9 ROC curve results pertaining to analyses of the predictors of recurrence-free and overall HCC patient survival.

	RFS				OS			
Variables	**AUC**	**95% CI**	***p*†-value**	***p*‡-value**	**AUC**	**95% CI**	***p*†-value**	***p*‡-value**
Nomogram	0.615	0.602-0.668		0.006	0.653	0.621–0.723		<0.001
IL-25	0.561	0.539–0.583	0.004		0.559	0.533–0.585	<0.001	
AFP					0.590	0.562–0.618	0.003	0.105
Tumor encapsulation	0.566	0.537–0.595	0.001	0.787	0.599	0.570–0.629	0.001	0.026
Tumor diameter	0.568	0.539–0.597	<0.001	0.649	0.624	0.595–0.653	0.001	<0.001
HBV-DNA	0.567	0.538–0.596	0.032	0.723	0.584	0.555–0.613	<0.001	0.227
Tumor number	0.532	0.513–0.551	<0.001	0.048				
Microvascular invasion	0.571	0.547–0.595	0.019	0.547				
Combination								
IL-25	0.561	0.539–0.583	<0.001		0.559	0.533–0.585	<0.001	
IL-25+AFP					0.618	0.587–0.648	0.067	0.007
IL-25+Tumor encapsulation	0.496	0.466–0.526	<0.001	0.005	0.463	0.432–0.495	<0.001	<0.001
IL-25+Tumor diameter	0.593	0.563–0.622	<0.001	0.006	0.637	0.606–0.668	0.012	<0.001
IL-25+HBV-DNA	0.603	0.574–0.632	0.019	0.001	0.616	0.586–0.646	0.020	0.007
IL-25+Tumor number	0.574	0.548–0.599	0.021	0.128				
IL-25+Microvascular invasion	0.605	0.578–0.633	0.019	0.026				

AFP, alpha-fetoprotein.
p†-value: nomogram vs. other predictors.
p‡-value: IL-25 vs. other predictors.

have an M2 phenotype, negatively regulates the pro-inflammatory immune microenvironment, and improves HDF-induced hepatic steatosis (37). Rizzo et al. reported that IL-25-induced alternatively activated macrophages inhibit colitis (38). In addition, Zhujun Jiang et al. reported that inhibition of IL-25 led to a decrease in the incidence rate of type 2 diabetes T cells and macrophages in the primary tumor microenvironment, as well as enhanced breast tumor invasion and subsequent lung metastasis (31). These findings suggest that macrophages are the key targets of IL-25, and the activation of M2 phenotype may be the main pathway by which IL-25 promotes the development of HCC. Herein, we found that elevated preoperative IL-25 levels were correlated with features of more advanced HCC and with poorer clinical outcomes (RFS and OS) within HBV-associated HCC cases following the resection of the liver. Tumor recurrence differed significantly between cases with low and high levels of serum IL-25 determined *via* a multivariate analysis approach, with elevated preoperative IL-25 levels being independent predictors of decreased OS and RFS in these cases. Importantly, high IL-25 levels functioned as an accurate predictor of long-lasting survival in cases with early-stage disease. While IL-25 levels were the best-identified predictor of RFS in this study, a combination of IL-25 levels and tumor diameter was better able to predict HBV-associated HCC patient OS. The mechanisms behind these effects are not fully understood, but some researchers believe that IL-25-induced dysregulation of intestinal microbiota promotes hepatocellular carcinoma through alternate activation of macrophages in the tumor microenvironment. Together, these results provide clear evidence that preoperative serum IL-25 levels can predict HCC patient prognosis.

The primary limitation of this research is that it was a single-center retrospective analysis, and it is thus susceptible to potential bias with respect to patient selection. Future large-scale multi-center studies validating and expanding upon our results will thus be essential to affirm the clinical relevance of serum IL-25 as a prognostic biomarker in HBV-HCC patients.

Conclusion

This study suggests that serum IL-25 levels may be an independent and useful tumor marker for the diagnosis of liver cancer. IL-25 is still valuable in the diagnosis of AFP-negative HCC and can be used as a supplement to AFP in the diagnosis of HCC. The combined diagnosis of the two markers greatly improves the early diagnostic accuracy of HCC. In addition, IL-25 values are associated with several pathological features that represent tumor aggressiveness and/or poor prognosis. Finally, IL-25 could help in the customized management of cases with risk factors for HCC recurrence after liver resection.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of 900TH Hospital of Logistics Support Force (LLH-20150801). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception and design: S-hC and XW; Administrative support: S-hC and XW; Provision of study materials or patients: S-hC and XW; Collection and assembly of data: All authors; Data analysis and interpretation: All authors; Manuscript writing: All authors; Final approval of manuscript: All authors.

Conflict of interest

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

Publisher's note

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Plasma arginase-1 as a predictive marker for early transarterial chemoembolization refractoriness in unresectable hepatocellular carcinoma

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Objective: To explore the relationship between plasma arginase-1 (ARG1) and early transarterial chemoembolization (TACE) refractoriness in patients with hepatocellular carcinoma (HCC) and develop nomograms for predicting early TACE refractoriness.

Methods: A total of 200 patients with HCC, treated with TACE, were included in the study, including 120 in the training set and 80 in the validation set. Pre-treatment enzyme-linked immunosorbent assay was used to detected the plasma ARG1 levels of the patient, and independent predictors of early TACE refractoriness were determined using a multivariate logistic regression model, based on which a predictive model was developed using a nomogram.

Results: Risk of early TACE refractoriness was negatively correlated with plasma ARG1 levels, and multivariate logistic analysis showed tumour size (OR = 1.138, 95% CI = 1.006-1.288, P = 0.041), multiple tumors (OR=4.374, 95% CI = 1.189-16.089, P = 0.026), platelet count (OR = 0.990, 95% CI = 0.980-0.999, P = 0.036), and plasma ARG1 levels (OR = 0.209, 95% CI = 0.079-0.551, P = 0.002) to be independent prognostic factors for early TACE refractoriness.The AUC value for the nomogram of the training cohort was 0.786 (95% CI = 0.702−0.870), and the validation set AUC value was 0.833 (95% CI = 0.791-0.875).The decision curve analysis suggested that the nomogram had good clinical utility.

Conclusion: High plasma ARG1 expression was associated with a lower incidence of early TACE refractoriness. The nomogram constructed based on four independent prognostic factors could facilitate an individualised prediction of the incidence of early TACE refractoriness.

KEYWORDS

hepatocellular carcinoma, arg1, TACE refractoriness, prognostic factor, predictive models

Introduction

Hepatocellular carcinoma (HCC) is estimated to be one of the most common malignant tumors in the world and one of the most common causes of cancer-related death (1, 2). Intrahepatic metastasis and multi-center origin are the biological characteristics of HCC, which has an insidious onset and a high degree of malignancy (3). According to the Barcelona Clinic Liver Cancer (BCLC) and European Society for Medical Oncology guidelines, transarterial chemoembolization (TACE) is the most important treatment option for patients with unresectable HCC in the early and middle stages (4–6); however, many years of clinical practice has shown proven that repeated TACE can weaken the therapeutic effect and cause TACE refractoriness due to its heterogeneity and the limitations of TACE surgery (7). Studies have found that in cases experiencing TACE refractoriness, the early use of combination therapy confers significant survival benefits and protection of liver function (8). The time required for identifying TACE refractoriness and the adjustment of the corresponding treatment strategy can have various effects on overall survival (OS) in patients with unresectable HCC. Accordingly, early detection of TACE refractoriness and transfer to systemic therapy can have important clinical effects (9).

Arginase-1 (ARG1), an enzyme that converts arginine to urea in the urea cycle, is mainly found in hepatocytes around the hilar of the liver. It can hydrolyze L-arginine, generate urea and L-ornithine, and detoxify ammonia. ARG1 is a more sensitive and specific marker of hepatocyte differentiation compared with other hepatocyte markers. Previous studies have shown that the expression level of ARG1 in liver tissues and paracancerous tissues is significantly higher than that in HCC tissues, and the disease-free survival (DFS) and overall survival (OS) of patients with HCC are related to the expression of ARG1, indicating that ARG1 may play a regulatory role in the occurrence and development of HCC (10, 11).

Previous studies had primarily focused on the relationship between TACE refractoriness and common imaging and laboratory indicators. In this study, by investigating the association between pre-treatment plasma ARG1 levels and early TACE refractoriness in patients with HCC, we aimed to identify patients who may show TACE refractoriness by developing a novel plasma ARG1-based nomogram.

Materials and methods

Patients

The study cohort comprised patients receiving TACE treatment in the Affiliated Cancer Hospital of Zhengzhou University from September 2017 to December 2020. The study was conducted in line with the guidelines of the Declaration of Helsinki and was approved by the Ethics Review Committee of the Affiliated Cancer Hospital of Zhengzhou University & Henan Cancer Hospital (approval number: 2016ct004). Due to the retrospective nature of the study, the ethics committee waived the requirement for informed consent. The data was analyzed anonymously. HCC was defined by pathological evidence or diagnosed using the non-invasive criteria of the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases.

The training and validation cohorts were subjected to the same inclusion and exclusion criteria. The inclusion criteria were as follows: (1) Patients should be over 18 years old with at least one measurable target lesion on the liver; (2) Patients should show indications and no contraindication for TACE; (3) TACE performed as monotherapy; (4) Before the first TACE treatment, the status score of Eastern Cooperative Oncology Group performance should be 0; (5) Patients at BCLC stage A or B, on whom radical surgical resection or ablation could not be performed; (6) Patients with good liver function with Child-Pugh A or B grade; and (7) Patients with complete clinical data. The exclusion criteria were as follows: (1) Patients who have received systemic therapy such as radiotherapy, chemotherapy, targeted therapy, and immunotherapy, (2) Patients who have received surgery or radiofrequency ablation within 6 months after the first TACE; (3) Those with severe heart disease, or those with irrecoverable blood clotting disorder or renal dysfunction.

TACE procedure

As reported previously (12), all TACE procedures were performed by at least two experienced interventional radiologists, and TACE was performed through the traditional femoral artery approach under local anesthesia. 5F RH catheters (Terumo, Tokyo, Japan) were first used for routine angiography, and then microcatheters (Terumo, Tokyo, Japan) were used for super selective arterial catheterization to enter the blood supply branch of the tumor. A mixed solution containing lipiodol (Laboratories Guerbet, Paris, France) and doxorubicin (Haizheng Pharmaceutical, Taizhou, China) was administered into the tumor-feeding vessels, followed by the injection of gelatin sponge particles (500 mm-700 mm; ALICON Dr. SCI&TEC Co., Ltd., Hangzhou, China) to supplement embolization until blood flow nearly ceased. The dose of doxorubicin was 50-70 mg and that of lipiodol was 5-20 ml; the specific dose should be adjusted according to the patient's tumor number and size, liver function, blood vessel distribution, and body surface area.

Definition of TACE refractoriness

As previously reported by the Japan Society of Hepatology (JSH), TACE refractoriness was mainly defined in terms of an insufficient response of the treated tumor or a progressive tumor response, indicated through the following observations: 1) viable lesions more than 50% after two or more TACE; 2) two or more intrahepatic lesions after TACE; 3) an increase in the extent of intrahepatic vascular invasion; 4) occurrence of extrahepatic metastasis; and 5) a continuous increase in the levels of tumor markers, following a temporary decrease in the levels after TACE (13). As had been reported previously, if the patient developed TACE refractoriness within 12 months after the initial TACE, we defined it as early TACE refractoriness (14).

If viable tumors were detected and liver function was adequate, TACE was repeated every 6–8 weeks. Follow-up included check-up for general health condition, liver function tests, routine blood investigations, and contrast-enhanced CT or MRI examination of the liver

ARG1 measurements

Blood samples of the patients were collected before the first TACE; the plasma was then separated by centrifugation at 1000 × g for 10min at 4°C and stored at -80°C until ARG1 analyses. Human plasma ARG1 levels were quantified using an ELISA kit. Measurements were performed according to the manufacturer's (Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) instructions; the lowest and highest detectable levels of the kit were 0.9375 ng/mL and 30 ng/mL, respectively, and all samples were diluted 3-fold with the sample diluent prior to the experiment. Furthermore, 50 mL of both sample and standard were added to the corresponding wells, followed by the addition of 100 mL of horseradish peroxidase-labeled detection antibody to each well, and incubation at 37°C for 60 min. Thereafter, the liquid was discarded, and each well was washed five times with 350 μL of washing solution; 50 μL each of substrate A and B was added next and the mixture was incubated at 37°C for 15 min in the dark. Next, 50 mL of stop solution was added to each well, and using a plate reader (Thermos Fisher Scientific, China), absorbance was measured at 450 nm, after 15 min.

Statistical analyses

SPSS (version 13.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and a binary logistic regression model was used to incorporate statistically significant variables into the multivariate analysis to identify predictors associated with the development of early TACE refractoriness. Nomograms were developed using R programming (Fundamentals of Statistical Computing version 4.1.2, Vienna, Austria), and the performance of each was evaluated using the consistency index (c-index). The c-index value ranges from 0.5 to 1, where a higher c-index indicates better predictive ability of the model. The value of the c-index ranged from 0.5 to 1. Decision curve analysis (DCA) was used to evaluate the accuracy of the model and net clinical benefits. Statistical significance was set at $P < 0.05$.

Results

ARG1 expression and cutoff value

A total of 228 patients were enrolled in the study. Till December 2020, 200 (200/228, 87.71%) patients developed TACE refractoriness during follow-up and were included in the analysis, whereas 28 were excluded. The patients were divided into a training set and a validation set according at a ratio of 3:2. The ARG1 levels in the training and validation sets were 45.38 ± 28.07 ng/mL and 45.38 ± 27.68 ng/mL, respectively. There was no significant difference in the expression of ARG1 between the two groups (P=1.000). In the training set, the area under the ROC curve of ARG1 was 0.687 (0.592-0.781), the best cut-off value was 59.49 ng/mL, the sensitivity was 48.3%, and the specificity was 85.0% (Figure 1). The ARG1 expression levels were further used to classify the patients as belonging to the high expression group (>59.49 ng/mL) and the low expression group (<59.49 ng/mL).

Baseline characteristics of the patients

There was no statistically significant difference in the baseline data of the training and validation sets of the patients (Table 1). There were 120 patients in the training set, of which 60

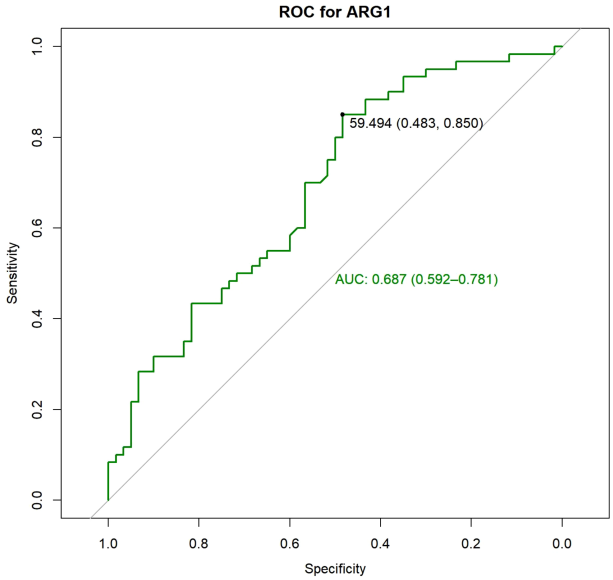

FIGURE 1
ROC analysis was performed and the best cut-off value was determined to be 59.49 ng/ml, at which both sensitivity and specificity were high.

patients showed TACE refractoriness within 12 months of follow-up (early TACE refractoriness), indicating an incidence rate of 50%. The incidence of early TACE refractoriness in the validation set was 48.75%, and there was no statistically significant difference between the two groups (P=0.888). All baseline characteristics of patients in training set and validation set were not significantly different (P>0.05).

In the training set, 95% of the patients were male with an average age of 55.48 ± 11.15 years. Viral hepatitis was the main cause of HCC (98.3%), including infection with hepatitis B or C virus. Patients with single tumors accounted for approximately 46.7% of all patients and those with multiple tumors accounted for 53.3%; among the patients with multiple tumors, those with 2–3 tumors accounted for approximately 28.1%, and those with ≥ 4 tumors accounted for approximately 25.2%. The average size of the largest tumor in all patients was 7.12 ± 3.66 cm. According to the BCLC staging criteria, the proportion of patients with stages A and B was 49.2% and 50.8%, respectively. TACE refractoriness was mainly characterized by new intrahepatic lesions (71.7%), vascular invasion (20.0%) and extrahepatic lesions (8.3%).

Prognostic factors affecting patients with early TACE refractoriness

Univariate analysis of the predictors of early TACE refractoriness showed that BCLC stage (OR=2.110, 95% CI=1.014-4.350, P=0.046), tumor size (OR=1.202, 95% CI=1.074-1.346, P=0.001), occurrence of multiple tumors (OR=3.471, 95% CI=1.635-7.370, P=0.001), platelet count (OR =0.987, 95%CI=0.979-0.995,P=0.003), alanine aminotransferase levels (OR=1.017, 95%CI= 1.001-1.033, P=0.035), and high ARG1 levels (OR=0.189, 95% CI=0.079-0.451, P<0.001) were predictors of early TACE refractoriness. Among them, high platelet count and high ARG1 levels were protective factors against early TACE refractoriness. Multivariate analysis showed that tumor size (OR =1.138, 95% CI=1.006–1.288, P=0.041), occurrence of multiple tumors (OR=4.374, 95% CI = 1.189–16.089, P=0.026), platelet count (OR =0.99, 95%CI=0.980–0.999, P=0.036), and high ARG1 levels (OR=0.209, 95%CI=0.079–0.551, P=0.002) were independent predictors of early TACE refractoriness (Table 2).

Establishment of a predictive model for early TACE refractoriness based on plasma ARG1 levels

We established a nomogram based on the significant predictors identified by binary logistic regression analysis of the univariate and multivariate analysis data (Figure 2), including those on tumor size, tumor number, platelet count, and ARG1 expression. The AUC value obtained for the nomogram of the training cohort was 0.786 (95% CI=0.702–0.870), and the validation set AUC value was 0.833 (95% CI=0.791–0.875), indicating a good diagnostic value and some significance for prediction of early TACE refractoriness in individuals.

The DCA result for the nomogram is presented in Figure 3. In the training set, the decision curve demonstrated that if the threshold probability of a patient or physician was between 9% and 75%, using the developed nomogram to predict the incidence of early TACE refractoriness was more beneficial, than when using the treat-all-scheme or treat-none schemes. Therefore, the developed nomogram was more beneficial than the treat-all scheme or the treat-none scheme for decision-making regarding treatment administration. Further, the validation set showed better results.

Presentation of a patient with early TACE refractoriness

The clinical data of a 56-year-old female patient with hepatocellular carcinoma has been presented here. The platelet count, tumor size, number of lesions, and plasma ARG1 levels of the patient were 67.7×109/L, approximately 7.42 cm, 2, and 22.6 ng/mL, respectively. The above data were consistent with the nomogram-total score of 220, which corresponds to a probability of early TACE resistance of approximately 82%

TABLE 1 Baseline characteristics of patients with TACE refractoriness in training set and validation set.

Variables	Training set N = 120	Validation set N = 80	*P*-value
Sex n (%)			0.944
Male	114 (95.0)	77 (96.2)	
Female	6 (5.0)	3 (3.8)	
Age(years)	55.48 ± 11.15	54.05 ± 10.28	0.362
Hepatitis n(%)			0.368
None	2 (1.7)	4 (5.0)	
HBV	90 (75.0)	56 (70.0)	
HCV	28 (23.3)	20 (25.0)	
BCLC n(%)			0.751
A	59 (49.2)	42 (52.5)	
B	61 (50.8)	38 (47.5)	
Child-Pugh n(%)			0.539
5	49 (40.8)	39 (48.8)	
6	60 (50.0)	35 (43.8)	
7	11 (9.2)	6 (7.5)	
Tumor number n(%)			0.885
single	56 (46.7)	39 (48.8)	
multiple	64 (53.3)	41 (51.2)	
Tumor size, cm	7.12 ± 3.66	7.51 ± 3.27	0.448
Tumor position			0.544
Single	58 (48.3)	43 (53.8)	
Double	62 (51.7)	37 (46.2)	
ARG1, n(%)			0.736
<59.49 ng/mL	82 (68.3)	52 (65.0)	
>59.49 ng/mL	38 (31.7)	28 (35.0)	
AFP, n(%)			0.748
<400 ng/mL	68 (56.7)	48 (60.0)	
≥ 400 ng/mL	52 (43.3)	32 (40.0)	
Ascites n(%)			0.974
None	90 (75.0)	59 (73.8)	
Have	30 (25.0)	21 (26.2)	
RBC, $\times10^{12}$/L	4.66 (1.18)	4.45 (1.47)	0.258
PLT,$\times10^{9}$/L	144.16 (45.69)	140.24 (39.86)	0.533
ALT, U/L	43.96 (24.20)	47.76 (26.93)	0.299
AST, U/L	33.43 (15.27)	33.04 (14.06)	0.853
TBIL, mmol/L	21.71 (11.68)	21.03 (10.88)	0.681
ALB, g/L	38.66 (5.14)	39.62 (5.41)	0.206

BCLC, Barcelona clinic liver cancer; AFP, alpha-fetoprotein; RBC, red blood cell; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin.

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TABLE 2 Univariate and multivariate analyses for predictive factors of early TACE refractoriness.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Gender	1.000	0.194-5.165	1.000			
Age	1.018	0.985-1.052	0.284			
Hepatitis-B	0.957	0.058-15.768	0.975			
Hepatitis-C	1.154	0.065-20.342	0.922			
BCLC	2.110	1.014-4.350	0.046	0.378	0.103–1.381	0.141
Child-Pugh score = 6	1.312	0.615-2.797	0.482			
Child-Pugh score = 7	2.148	0.556-8.296	0.268			
Multiple tumors	3.471	1.635-7.370	0.001	4.374	1.189–16.089	0.026
Tumor size, cm	1.202	1.074-1.346	0.001	1.138	1.006–1.288	0.041
Tumor position	1.307	0.637-2.678	0.465			
ARG1>59.49 ng/mL	0.189	0.079-0.451	<0.001	0.209	0.079–0.551	0.002
AFP > 400 ng/mL	0.762	0.369-1.571	0.462			
Ascites	1.429	0.622-3.285	0.400			
RBC, $\times10^{12}$/L	1.206	0.885-1.644	0.235			
PLT,$\times10^{9}$/L	0.987	0.979-0.995	0.003	0.990	0.980–0.999	0.036
ALT, U/L	1.017	1.001-1.033	0.035	1.015	0.996-1.034	0.115
AST, U/L	0.994	0.971-1.018	0.614			
TBIL, mmol/L	1.002	0.972-1.034	0.888			
ALB, g/L	1.031	0.961-1.106	0.395			

BCLC, Barcelona clinic liver cancer; AFP, alpha-fetoprotein; RBC, red blood cell; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; ALB, albumin.

(Figure 2). The CT image results from March 2020 are shown in Figure 4A; two TACE treatments were performed in March and May 2020. In August 2020, the patient developed a portal vein tumor thrombus (Figure 4B), which indicated disease progression. Furthermore, disease progression occurred 5 months after the patient's first TACE treatment, suggesting that the patient developed early TACE refractoriness. Predicted results of the nomogram were consistent with the observed condition of the patient.

Discussion

To our knowledge, this was the first study to report the correlation between plasma ARG1 levels and the efficacy of TACE in unresectable HCC patients. This study found that plasma ARG1 expression was an independent predictor of early TACE refractoriness. We developed a nomogram based on ARG1 expression levels, combined with different tumor parameters, to predict the risk of early TACE refractoriness in unresectable HCC patients treated with TACE. Incorporation of ARG1 expression and the associated clinical prognostic factors into an easy-to-use nomogram could facilitate an individualized assessment of the risk of developing early TACE refractoriness preoperatively.

Recent studies have confirmed that ARG1 can be induced in alternately activated (M2) macrophages and is involved in the occurrence and development of tumors, mainly due to the anti-inflammatory response, tumor immunity, tumor proliferation, metastasis, and immunosuppression (15). Therefore, altered expression of ARG1 may lead to changes in the metabolism of

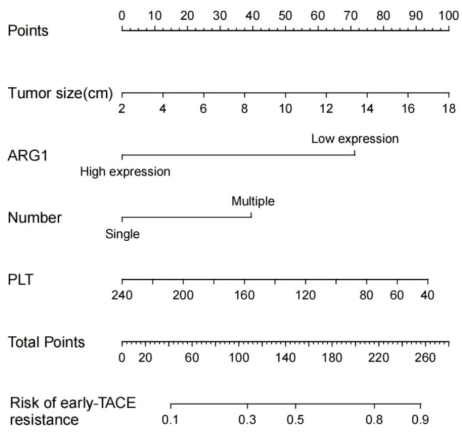

FIGURE 2
Prognostic nomogram for early TACE refractoriness. Each value of the patient is indicated on the axis with the variable. A line is drawn upward to determine the number of points received by each variable. Then, the total number of points are calculated and a line is drawn downward to determine the prediction probability of early TACE refractoriness.

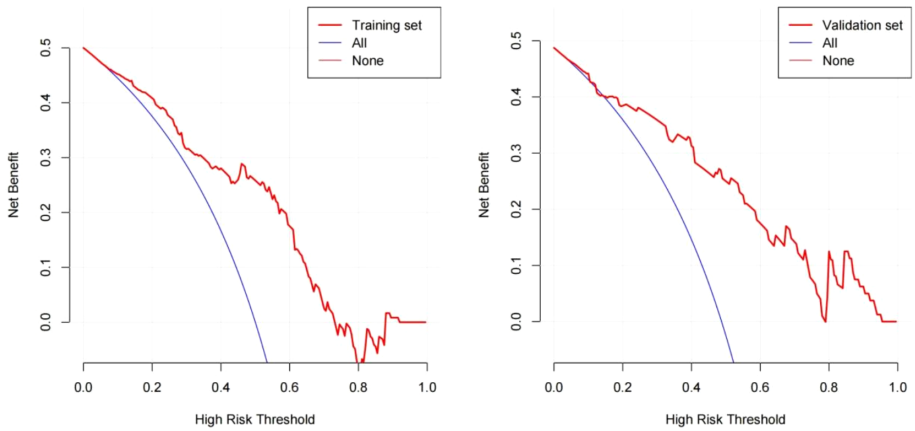

FIGURE 3
Decision curve analysis for the nomogram based on clinical characteristics. The red polyline represents the nomogram. The horizontal line with an ordinate of 0 represents all negative samples. For these participants, the treat-none scheme was applied. The blue fine line represents all positive samples, and for these participants, the treat-all scheme was applied.

the liver tissue and have marked effects on the metabolic and growth statuses of tumor cells. Previous studies have shown that expression of ARG1 was closely related to HCC differentiation, histological type, Edmondson grade, and other indicators that indicate the degree of tumor differentiation. With a decrease in ARG1 expression, the differentiation degree of HCC worsened, suggesting that ARG1 may be a molecular marker for determining the degree of HCC differentiation (10, 16).

Our study found that the average ARG1 level of 60 patients with early TACE refractoriness was 36.55 ng/mL and that of patients without early TACE was 54.22 ng/mL; the former value was significantly lower than the latter, and the difference was statistically significant ($P < 0.05$). In unresectable HCC patients treated with TACE, the risk of early TACE refractoriness decreased with an increase in ARG1 expression, possibly because the increased ARG1 expression

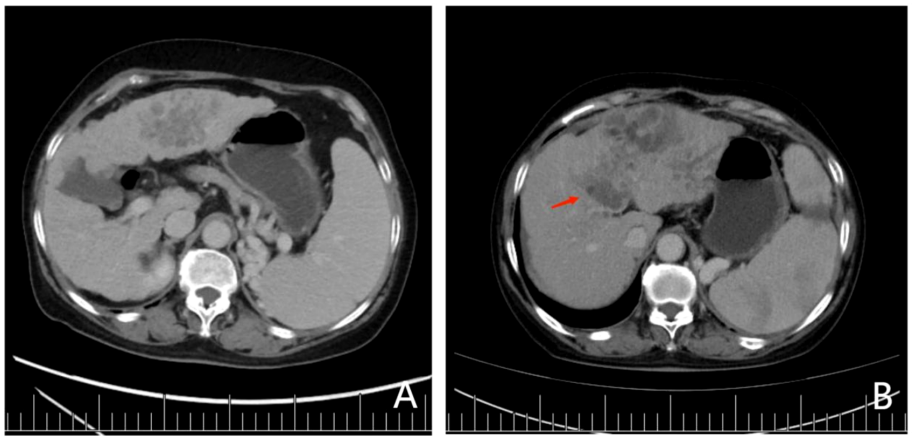

FIGURE 4
(A) Preoperative contrast-enhanced CT showed two lesions in the patient's liver with cirrhosis. The largest lesion was 7.42 cm in diameter, located in the left lobe of the liver. In contrast-enhanced CT, lesions in the arterial phase were significantly enhanced, although they were weakened in the delayed phase, which was in accordance with the diagnostic criteria for hepatocellular carcinoma. Portal vein blood flow was unobstructed, and no portal vein tumor thrombus was found. **(B)** After 2 cycles of TACE treatment, lesions in the left hepatic lobe were enlarged, and tumor thrombus was seen in the portal vein (red arrow).

increased the catalysis and consumption of arginine and reduced the amount of arginine in the microenvironment (17). Hepatoma cells are malignant tumor cells with arginine deficiency. Arginine is an essential amino acid. The growth of cancer cells is inhibited in the absence of arginine. A reduction in the levels of amino acids in HCC tissues inhibits the growth and reproduction of tumor cells, improves the efficacy of TACE, and reduces the risk of tumor progression and TACE refractoriness after repeated TACE. If exogenous arginine is administered to the tumor microenvironment to deplete arginine, tumor growth can be inhibited. Currently, the antitumor drug PEGylated recombinant human arginase is being tested in clinical trial including patients with unresectable HCC (18).

However, the effect of ARG1 on patient survival remains controversial. Obiorah et al. reported that patients with high ARG1 expression have a shorter median time to recurrence (19), whereas Mao et al. found that patients with low ARG1 expression in HCC had shorter DFS and OS (11). Our study showed that the expression of plasma ARG1 in unresectable HCC patients is negatively correlated with the probability of early TACE refractoriness, that is, the higher the expression of plasma ARG1, the lower the risk of early TACE refractoriness and the better the prognosis. Our results were similar to Mao's findings, suggesting that the assessment of plasma ARG1 levels would be helpful in assessing the risk of early TACE refractoriness in patients with HCC treated with TACE.

There is no consensus yet regarding the risk factors affecting TACE refractoriness, and the results of various studies differ widely. By studying the molecular indicators and clinical biochemical indicators, some researchers found that microRNA, TP53 mutation, the M2 isoform of pyruvate kinase expression level, AFP level, and interleukin-8 expression level can affect the outcome of TACE (14, 20–22). Shehta et al. found thrombocytopenia to possibly be an important predictor of tumor recurrence after hepatectomy in HCC patients with cirrhosis, which is similar to our findings (23). However, the pathophysiological mechanism remains to be explored further. The results of our study showed that BCLC stage, tumor size, the presence of multiple tumors, platelet count, and plasma ARG1 level as shown by our univariate analyses may be predictive factors for early TACE refractoriness. However, in multivariate analyses we found that only tumor number, tumor size, and platelet and plasma ARG1 levels were independent predictive factors for early TACE refractoriness. Moreover, our univariate analyses indicated that BCLC and ALT were predictors of early TACE refractoriness. However, our multivariate analyses of these factors instead yielded statistically insignificant associations. One possible explanation for this seeming contradiction is that since tumor size and number were both strongly correlated with BCLC stage, they therefore may also be related to ALT. In other words, we managed to obtain a complete result from analyzing only a single factor. And by eliminating the influences of other factors *via* our multivariate analyses, we revealed that neither BCLC stage nor ALT independently influence the prediction of early TACE refractoriness.

In recent years, many studies have attempted to establish a predictive model for the prognosis and response to TACE treatment. However, relatively few studies have used TACE refractoriness as an endpoint (24, 25). Current research mainly focuses on predicting postoperative response to TACE, and there are very few studies on the early prediction of TACE refractoriness, most of them being retrospective, focusing on the relationship between a certain index and TACE refractoriness. Furthermore, only a few predictive models integrate multiple clinical indicators. Existing models are mostly based on local samples and lack clinical and external validations (26). In this study, we developed a nomogram based on ARG1 expression level, combined with different tumor parameters, to predict the risk of early TACE refractoriness in patients with unresectable HCC. The logistic regression model included ARG1, tumor size and number, and platelet counts, and the results showed the model c-index to be higher (0.833), indicating that models incorporating ARG1 expression level had higher prediction accuracy. The DCA suggested that the nomogram had good clinical utility. Many previous studies have thoroughly discussed the relationships between tumor size, number, and hepatic physiology-related indicators and TACE efficacy. Increases in either tumor size or number lead to increased |tumor burdens that markedly influence liver cancer treatment outcomes (20, 27–29). Hu et al. retrospectively analyzed the association between TACE refractoriness and various biomarkers; they proposed that the main risk factors for refractory TACE include AFP, some liver function indicators and tumor imaging findings (30). Taken together, these previous results are consistent with our findings.

Our study had some limitations. First, we used the definition of TACE refractoriness provided by the JSH, which is applicable in clinical practice; however, since TACE treatment itself is highly heterogeneous, the concept of "refractoriness" has certain limitations and presently, there is no international consensus regarding its definition. Second, this is a single-center retrospective study with a small sample size, which may lead to potential data selection bias. Therefore, the results of this study may need to be further verified by prospective, multicenter, large sample, randomized controlled trials. Third, this predictive model is temporarily not applicable to patients with advanced HCC or those receiving TACE combined with other treatments. The correlation between ARG1 and TACE refractoriness in these specific patient populations will be the topic of our continued research in this direction.

In conclusion, to our knowledge, we showed for the first time that the expression of plasma ARG1 in unresectable HCC patients is negatively correlated with the probability of early TACE refractoriness. The expression of plasma ARG1 before TACE in patients with unresectable HCC can be used as one of the candidate biomarkers for predicting early TACE refractoriness. A nomogram based on tumor size, tumor number, platelet, and plasma ARG1 levels could help predict the possibility of early TACE refractoriness before TACE treatment to achieve an individualized prediction of early TACE refractoriness in different patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The ethics of the research program has been approved by the Ethics Committee the Affiliated Cancer Hospital of Zhengzhou University and Henan Cancer Hospital review board. The ethics committee waived the requirement of written informed consent for participation.

Author contributions

Conceptualization and design the study: H-LL and H-TH; Provision of study materials or patients: W-LX, LZ, D-YZ, Q-JY, and C-YG; Collection and assembly of data: W-LX, YG, X-HZ, and S-JX; Data analysis and interpretation: X-HZ, YG, YZ,LZ, and D-YZ; Manuscript writing: W-LX; Manuscript reviewing: H-LL, H-TH, and W-JF; Final approval of manuscript: All authors.

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Conflict of interest

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

Publisher's note

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Predictive value of preoperative inflammatory indexes for postoperative early recurrence of hepatitis B-related hepatocellular carcinoma

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Objective: To investigate the predictive value of preoperative neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic inflammation response index (SIRI), and systemic immune inflammation index (SII) for early recurrence after liver resection in patients with hepatitis B-related hepatocellular carcinoma.

Methods: A retrospective study was conducted on 162 patients who underwent hepatitis B-related hepatocellular carcinoma (HCC) resection between January 2013 and April 2016. The Youden index was utilized to calculate the optimal cut-off value. The Pearson Chi-square test was applied to analyze the relationship between inflammatory indexes and common clinical and pathological features. The Kaplan-Meier method and Log-Rank test were implemented to compare the recurrence-free survival rate within 2 years of the population. The Cox regression analysis was used to identify the risk factors for early postoperative recurrence.

Results: The best cut-off values of SIRI, PLR, NLR and SII were 0.785, 86.421, 2.231 and 353.64, respectively. Tumor diameter, degree of tumor differentiation, vascular invasion, SIRI>0.785, PLR>86.421, NLR>2.231 and SII>353.64 were risk factors for early recurrence. Combining the above seven risk factors to construct a joint index, the AUC of the joint prediction model was 0.804. The areas under the ROC curves of SIRI, PLR, NLR, and SII were 0.659, 0.725, 0.680, and 0.723, respectively. There was no significant difference in the predictive ability between the single inflammatory index models, but the predictive performance of the joint prediction model was significantly higher than that of the single

inflammatory index models. The patients with lower SIRI, PLR, NLR, SII and joint index value had longer recurrence-free survival within 2 years.

Conclusion: The joint index CIP, constructed by combining preoperative SIRI, PLR, NLP and SII with pathological features, can better predict the early recurrence of HBV-related HCC patients after surgery, which is beneficial in identifying high-risk patients and assisting clinicians to make better clinical choices.

KEYWORDS

inflammatory indexes, hepatocellular carcinoma, recurrence, predictive models, hepatitis B virus

1 Introduction

Primary liver cancer is one of the most malignant and influential cancers in the world. Its incidence is increasing year by year, but the treatment methods are extremely limited (1). Hepatocellular carcinoma (HCC) is the most common pathological type of primary liver cancer, accounting for about 75%-90% of all cases. The causes of HCC include hepatitis B virus (HBV) infection, hepatitis C virus infection, aflatoxin infection, alcohol consumption, and non-alcoholic fatty liver disease which has attracted much attention in recent years (2, 3). In China, HBV-related HCC accounts for the largest proportion. Although the infection rate of HBV is decreasing with the application of antiviral drugs, there is still a considerable base of HBV-related HCC in China (4).

The treatment methods for early and middle stage HCC include radiofrequency ablation, liver transplantation, and hepatectomy, among which hepatectomy is the most widely used. Tumor recurrence is a major complication after hepatectomy and a leading cause of cancer-related death. It is usually divided into early recurrence and late recurrence by 2 years (5, 6). Previous studies have shown that the early recurrence rate of HCC is as high as 30-50%, accounting for more than 70% of the total tumor recurrence (6–8). Timely identification of high-risk patients with early recurrence after surgery is very important for prolonging the survival time of patients and improving the quality of life after surgery. At present, there are many predictive models established in different medical centers based on risk factors related to early recurrence (such as male, large Tumor diameter, high serum AFP, vascular invasion, low tumor differentiation, etc.) and imaging features (9, 10). However, there is no consensus on the best tool for risk stratification.

The construction of clinical prediction models based on inflammation-related indicators is a research hotspot in recent years. Since the 20th century, the theory of cancer-related inflammation has been enriched and developed, and the role of inflammation in tumorigenesis, proliferation, invasion, and metastasis has been gradually elucidated (11, 12). Inflammatory indexes constructed based on peripheral blood neutrophil, lymphocyte, monocyte and platelet counts have been developed for cancer research due to their non-invasive, clinically readily available and low-cost nature. In a variety of malignant tumors, these inflammatory indexes have a good effect in predicting prognosis (13–15). In HCC, these inflammatory indicators such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic inflammatory response index (SIRI) and systemic immune inflammation index (SII) have also shown high application value in predicting HCC prognosis (16–18). However, previous studies only included 1-2 inflammatory markers. There is still a paucity of literature that combines these 4 important inflammatory markers to study early recurrence of HBV-related HCC after surgery. In this study, we compared the effects of four inflammatory markers models on the early recurrence of HBV-related HCC after surgery in the same population and constructed a common prediction model based on inflammatory markers and pathological characteristics. By comparing the predictive efficacy of each model, we found a more robust and accurate model that could predict the early recurrence of HBV-related HCC after surgery.

2 Materials and methods

2.1 Patient and clinical sample collection

We investigated 162 patients with HBV-related HCC who underwent hepatectomy at Shanxi Provincial Cancer Hospital from January 2013 to April 2016. Clinical and demographic data and detailed treatment information for all enrolled patients were extracted from the electronic medical record. The inclusion criteria were as follows: (1) HCC was diagnosed by postoperative pathology; (2) negative surgical margins; (3) liver reserve function Child-Pugh grade A or B; (4) patients over 18 years old; (5) complete clinical and pathological data were available. Exclusion criteria: (1) acute infection or high fever before surgery; (2) other malignant tumors, immune or hematological diseases; (3) preoperative anti-tumor therapy; (4) loss of critical data. This retrospective chart review

study involving human participants complied with institutional and national research Council ethical standards and the 1964 Declaration of Helsinki.

The baseline clinical data of the patients were collected and analyzed, including gender, age, body mass index (BMI), diabetes mellitus, drinking history, family history of cancer, hepatitis B surface antigen (HBsAg), AJCC stage, tumor number, tumor diameter, tumor differentiation, vascular invasion, liver cirrhosis, postoperative treatment (including postoperative ablation or TACE), serum alpha-fetoprotein (AFP) and preoperative blood routine test. Routine blood samples were collected within 7 days before surgery. AJCC staging was performed according to the 8th edition of the classification of the Union for International Cancer Control and the American Joint Committee on Cancer (AJCC) (19).

Peripheral blood inflammation-related indicators refer to relevant indicators that can reflect systemic inflammation based on immune cells of the human circulatory system (13–18). The formula for the calculation of various inflammation-related indicators based on blood routine test was as follows: SIRI = neutrophil×monocyte/lymphocyte (13); PLR = platelet/lymphocyte (18); NLR= neutrophil/lymphocyte (18), SII = platelet ×neutrophil/lymphocyte (17).

2.2 Follow-up

Follow-up began after surgery and was performed every 3 months in the outpatient clinic for the first 2 years until recurrence or loss of follow-up. During follow-up, examinations included physical examination, AFP testing, and abdominal imaging scans. If there was no evidence of recurrence, chest X-ray and abdominal ultrasound were preferred. If abdominal ultrasound suggested recurrence, abdominal enhanced CT scan or magnetic resonance scan should be performed to confirm the diagnosis. In addition, chest CT enhancement should be used to rule out lung metastases, and positron emission tomography should be used to rule out metastases in other sites. Outpatient and inpatient medical record systems were combined with telephone follow-up. Recurrence-free survival (RFS) was defined as the time from the date of surgery to the first recurrence or loss to follow-up.

2.3 Statistical analysis

Taking the recurrence within 2 years as the outcome, ROC analysis was performed on the original values of inflammatory markers as variables, and the best cut-off value was found by the Youden index. The population was divided into high and low groups by cut-off value. The Pearson Chi-square test was used to evaluate the association between SIRI, PLR, NLR, SII and clinical and pathological data. The Kaplan-Meier method and Log-Rank test were used to analyze the differences between groups and recurrence-free survival rate within 2 years. The Cox regression analysis was used to identify the risk factors for early postoperative recurrence. According to the results, the regression coefficients of the included variables were obtained by binary logistic regression equation, and the joint predictors were obtained by the regression coefficients. The SPSS 24.0 and GraphPad Prism 9 were used for statistical analysis and drawing. The MedCalc(20.218) was used to conduct DeLong's test. P<0.05 considered that the difference was statistically significant.

3 Results

3.1 Baseline data

A total of 162 HCC patients who underwent partial hepatectomy were enrolled. 58 patients had recurrence within 2 years, including 37 cases of intrahepatic recurrence, 7 cases of extrahepatic recurrence and 14 cases of both intrahepatic and extrahepatic recurrence, with a recurrence rate of 35.8%. ROC analysis showed that the optimal cut-off points of SIRI, PLR, NLR and SII were 0.785, 86.421, 2.231 and 353.64, respectively. HCC patients were divided into low SIRI group (≤0.785) and high SIRI group (>0.785), low PLR group (≤86.421), high PLR group (>86.421), low NLR group (≤2.231), high NLR group (>2.231), low SII group (≤353.64) and high SII group (>353.64). Table 1 detailed the association of different groups with the clinical and pathological features. Of the 162 patients, 125 (77.2%) were male and 37 (22.8%) were female. 107 cases (66.0%) were under 60 years, and 55 cases (34.0%) were over 60 years. There were 93 patients (57.4%) in the low SIRI group and 69 patients (42.6%) in the high SIRI group. There were 74 patients (45.7%) in the low PLR group and 88 patients (54.3%) in the high PLR group. There were 98 patients (60.5%) in the low NLR group and 69 patients (39.5%) in the high NLR group. There were 93 patients (57.4%) in the low SII group and 64 patients (42.6%) in the high SII group. The results showed that SIRI, PLR, NLR and SII were significantly correlated with tumor diameter ($P< 0.05$). PLR was significantly different in age and liver cirrhosis ($P<0.05$). NLR was associated with the type of tumor differentiation and the use of TACE/ablation after surgery ($P< 0.05$). SII had a statistically significant difference in HBsAg (+) ($P< 0.05$).

3.2 Differences in RFS between groups

The difference between SIRI ≤ 0.785 group and SIRI>0.785 groups was tested by Log-Rank test, and P<0.001 (Figure 1A), indicating that there was a significant difference between groups in terms of recurrence-free survival rate. The 2-year recurrence-free survival rate of the SIRI ≤ 0.785 group was 76.3%, the other was 47.8%.

The difference between PLR ≤ 86.421 group and PLR>86.421 group was tested by Log-Rank test, and P<0.001 (Figure 1B), indicating that there was a significant difference between groups in terms of recurrence-free survival rate. The 2-year recurrence-free survival rate of the PLR ≤ 86.421 group was 82.7%, the other was 48.2%.

The difference between NLR ≤ 2.231 group and NLR>2.231 group was tested by Log-Rank test, and P<0.001 (Figure 1C), indicating that there was a significant difference between groups in terms of recurrence-free survival rate. The 2-year recurrence-free survival rate of the NLR ≤ 2.231 group was 77.8%, the other was 42.9%.

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TABLE 1 Baseline Characteristics of 162 Patients with HCC.

Variables	No.(%)	SIRI		χ^2	P value	PLR		χ^2	P value	NLR		χ^2	P value	SII		χ^2	P value
		⩽ 0.785	> 0.785			⩽ 86.421	> 86.421			⩽2.231	>2.231			⩽353.64	>353.64		
All patients	162 (100.0)	93	69			74	88			98	64			93	69		
Gender																	
Male	125 (77.2)	71	54	0.083	0.774	61	64	2.148	0.143	78	47	0.832	0.362	73	52	0.221	0.639
Female	37 (22.8)	22	15			13	24			20	17			20	17		
Age																	
≤60	107 (66.0)	64	43	0.746	0.388	55	52	4.160	0.041	65	42	0.008	0.927	63	44	0.279	0.597
>60	55 (34.0)	29	26			19	36			33	22			30	25		
BMI																	
⩽28.0	150 (92.5)	80	63	1.068	0.301	67	76	0.677	0.410	86	57	0.064	0.800	84	59	0.887	0.346
>28.0	12(7.5)	13	6			7	12			12	7			9	10		
Diabetes																	
No	151 (93.2)	86	65	0.187	0.665	70	81	0.413	0.521	91	60	0.049	0.825	87	64	0.040	0.842
Yes	11(6.8)	7	4			4	7			7	4			6	5		
Alcohol																	
No	130 (80.2)	74	56	0.063	0.802	60	70	0.060	0.807	79	51	0.021	0.885	76	54	0.299	0.584
Yes	32 (19.8)	19	13			14	18			19	13			17	15		
Family cancer history																	
No	150 (92.5)	86	66	0.691	0.406	59	83	0.080	0.777	92	60	0.001	0.974	89	63	1.321	0.250
Yes	12(7.5)	7	3			5	5			6	4			4	6		
HBsAg (+)																	
No	27 (16.7)	15	12	0.045	0.831	8	19	3.363	0.067	15	12	0.331	0.565	9	18	7.680	0.006
Yes	135 (83.3)	78	57			66	69			83	52			83	51		
Tumor number																	
Multiple	24 (14.8)	15	9	0.299	0.585	11	13	0.000	0.987	16	8	0.449	0.503	16	8	0.988	0.320
Solitary	138 (85.2)	78	60			63	75			82	56			77	61		
Tumor diameter																	
⩽5	101 (62.3)	69	32	13.056	0.000	55	46	8.326	0.004	69	32	6.869	0.009	70	31	15.533	0.000
>5	61 (37.7)	24	37			19	42			29	32			23	38		
Differentiation																	
poor	60 (37.0)	30	30	2.138	0.144	23	37	2.072	0.150	27	33	9.572	0.002	30	30	2.138	0.144
Moderate and well	102 (63.0)	63	39			51	51			71	31			63	39		

(Continued)

TABLE 1 Continued

Variables	No.(%)	SIRI		χ^2	P value	PLR		χ^2	P value	NLR		χ^2	P value	SII		χ^2	P value
		⩽ 0.785	> 0.785			⩽ 86.421	> 86.421			⩽2.231	>2.231			⩽353.64	>353.64		
Vascular invasion																	
No	147 (90.7)	87	60	2.049	0.152	70	77	2.408	0.121	91	56	1.322	0.250	86	61	0.780	0.377
Yes	15(9.3)	6	9			4	11			7	8			7	8		
Liver cirrhosis																	
No	26 (16.0)	14	12	0.161	0.689	6	20	6.376	0.012	16	10	0.014	0.905	13	13	0.695	0.404
Yes	136 (84.0)	79	57			68	68			82	54			80	56		
N																	
No	157 (97.0)	90	67	0.014	0.905	73	84	1.371	0.242	95	62	0.001	0.982	91	66	0.639	0.424
Yes	5(3.0)	3	2			1	4			3	2			2	3		
AJCC stage																	
I-II	154 (95.0)	88	66	0.089	0.765	72	82	1.450	0.228	92	62	0.741	0.389	88	66	0.089	0.765
III-IV	8(5.0)	5	3			2	6			6	2			5	3		
Postoperative treatment (Ablation or TACE)																	
No	135 (83.3)	78	57	0.045	0.831	64	71	0.975	0.323	88	47	7.459	0.006	81	54	2.227	0.136
Yes	27 (16.7)	15	12			10	17			10	17			12	15		
AFP>400																	
No	112 (69.1)	65	47	0.059	0.809	70	42	0.611	0.434	65	47	0.059	0.809	73	39	0.152	0.697
Yes	50 (30.9)	28	22			28	22			28	22			31	19		
Child-Pugh grading																	
A	154 (95.0)	89	65	0.189	0.664	72	82	1.450	0.228	94	60	0.388	0.533	90	64	1.364	0.240
B	8(5.0)	4	4			2	6			4	4			3	5		

SIRI, Systemic Inflammation Response Index; PLR, Platelet-to-lymphocyte ratio; NLR, Neutrophil-to-Lymphocyte Ratio; SII, Systemic Immune-Inflammation Index; BMI, Body mass index; N, Regional lymph node metastasis; AFP, alpha fetoprotein; TACE, transcatheter arterial chemoembolization.

The difference between SII ≤ 353.64 group and SII>353.64 group was tested by Log-Rank test, and P<0.001 (Figure 1D), indicating that there was a significant difference between groups in terms of recurrence-free survival rate. The 2-year recurrence-free survival rate of the SII ≤ 353.64 group was 76.3%, the other was 47.8%.

3.3 Univariate and multivariate COX regression analysis

Cox univariate analysis of tumor diameter (>5cm vs ≤5cm: *HR*=1.700, 95%*CI*: 1.015-2.845, *P*=0.044), degree of differentiation (poorly differentiated vs moderately or well differentiated: *HR*=2.438, 95%*CI*: 1.449-4.103, *P*<0.001), vascular invasion (invasion vs no invasion: *HR*=3.053, 95%*CI*: 1.519-6.136, *P*=0.002), SIRI (>0.785 vs ≤0.785: *HR*=2.738, 95%*CI*:1.609-4.660, *P*<0.001), PLR(>86.421 vs ≤86.421, *HR*=3.060, 95%*CI*: 1.649-5.676, *P*< 0.001), NLR(>2.231 vs ≤2.231, *HR*=2.613, 95%*CI*: 1.536-4.443, *P*< 0.001), SII(>353.64 vs ≤353.64, *HR*=2.547, 95%*CI*: 1.497-4.334, *P*<0.001) (Table 2). Multivariate analysis showed that only tumor differentiation (poor differentiation vs moderate-high differentiation: *HR*=2.043, 95%*CI*: 1.152-3.623, *P*=0.014) was an independent risk factor for early recurrence within 2 years (Table 2).

3.4 Joint index construction

According to the results of univariate and multivariate analysis, based on the available clinical and pathological parameters, the 4

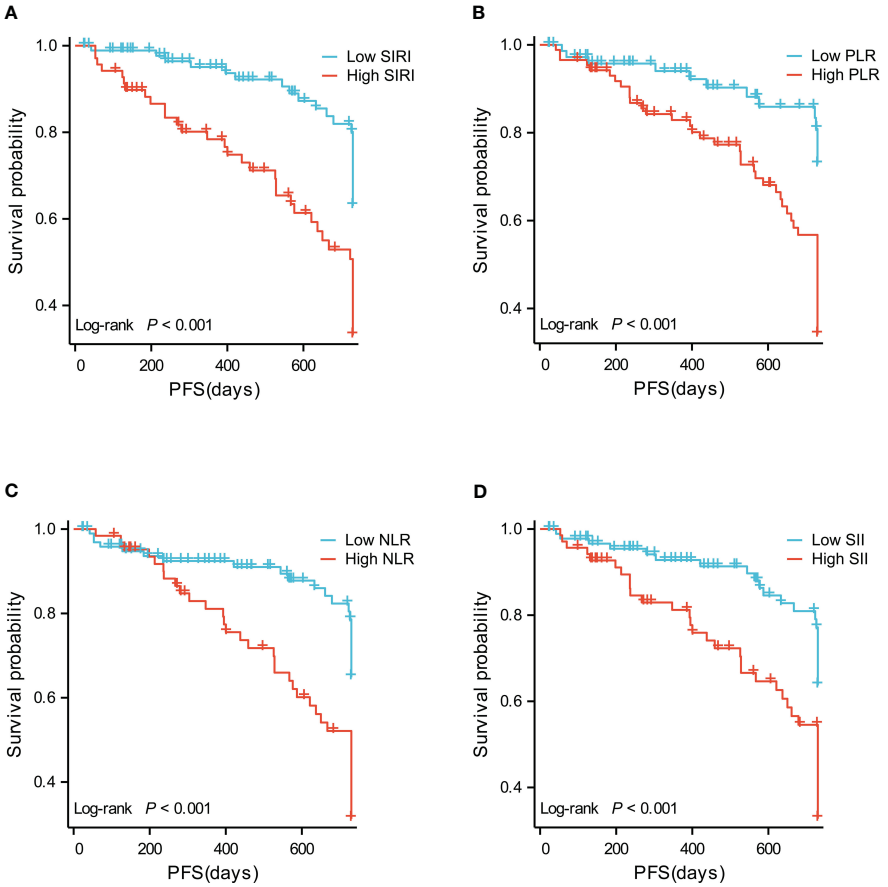

FIGURE 1
(A) Recurrence-free survival curve of patients with high SIRI and low SIRI. **(B)** Recurrence-free survival curve of patients with high PLR and low PLR. **(C)** Recurrence-free survival curve of patients with high NLR and low NLR. **(D)** Recurrence-free survival curves of patients with high SII and low SII.

TABLE 2 Univariate and multivariate analysis of RFS within 2 years.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Gender	162				
Female	37	Reference			
Male	125	1.154 (0.611-2.179)	0.658		
Age	162				
≤60	107	Reference			
>60	55	1.104 (0.642-1.898)	0.720		
BMI>28	162				
≤28	143	Reference			
>28	19	1.848 (0.873-3.916)	0.109		
Diabetes	162				
No	151	Reference			
Yes	11	0.485 (0.151-1.552)	0.222		
Drinking history	162				

(Continued)

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TABLE 2 Continued

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
No	130	Reference			
Yes	32	1.432 (0.795-2.578)	0.232		
HBSAg(+)	162				
No	27	Reference			
Yes	135	0.748 (0.387-1.443)	0.386		
Tumor number	162				
Solitary	138	Reference			
Multiple	24	0.887 (0.420-1.872)	0.754		
Tumor diameter (5cm)	162				
≤5	101	Reference			
>5	61	1.700 (1.015-2.845)	0.044	1.338 (0.763-2.343)	0.309
Differentiation	162				
Moderate and well	102	Reference			
poor	60	2.438 (1.449-4.103)	<0.001	2.043 (1.152-3.623)	0.014
Vascular invasion	162				
No	147	Reference			
Yes	15	3.053 (1.519-6.136)	0.002	1.959 (0.936-4.097)	0.074
Cirrhosis	162				
No	26	Reference			
Yes	136	0.574 (0.304-1.085)	0.087		
AJCC Staging	162				
I-II	154	Reference			
III-IV	8	1.609 (0.581-4.456)	0.360		
Postoperative treatment (Ablation or TACE)	162				
No	135	Reference			
Yes	27	1.274 (0.687-2.364)	0.442		
SIRI					
≤0.785	93	Reference			
>0.785	69	2.738 (1.609-4.660)	<0.001	1.690 (0.848-3.368)	0.136
PLR	162				
≤86.421	74	Reference			
>86.421	88	3.060 (1.649-5.676)	<0.001	1.758 (0.859-3.600)	0.123
NLR	162				
≤2.231	98	Reference			
>2.231	64	2.613 (1.536-4.443)	<0.001	1.113 (0.554-2.238)	0.763
SII	162				
≤353.64	93	Reference			
>353.64	69	2.547 (1.497-4.334)	<0.001	1.319 (0.674-2.581)	0.419

(Continued)

TABLE 2 Continued

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
AFP (ng/ml)	162				
≤400	112	Reference			
>400	50	1.124 (0.649-1.946)	0.676		

SIRI, Systemic Inflammation Response Index; PLR, Platelet-to-Lymphocyte Ratio; NLR, Neutrophil-to-Lymphocyte Ratio; SII, Systemic Immune-Inflammation Index; BMI, Body mass index; N, Regional lymph node metastasis; AFP, alpha fetoprotein.

inflammatory indicators combined with tumor diameter, degree of differentiation and vascular invasion were used to construct a combined inflammation and pathology model, referred to as CIP. Scoring criteria: tumor diameter (>5cm=1,≤5cm=0), degree of differentiation (undifferentiated and poorly differentiated=1, moderately and well differentiated=0), vascular invasion (with invasion=1, no invasion=0), SIRI (>0.785 = 1,≤0.785 = 0), PLR (>86.421 = 1,≤86.421 = 0), NLR(>2.231 = 1,≤2.231 = 0), SII (>353.64 = 1,≤353.64 = 0). According to the regression coefficient of binary logistic equation, the joint index calculation formula was as follows: CIP=0.331×Tumor diameter +1.141×degree of differentiation +0.970×vascular invasion +0.469×SIRI+1.152×PLR+0.630×NLR+0.031×SII. The area under the ROC curve of joint index CIP was 0.804 (the best cut-off value was 1.48), which was higher than that of other single indexes (SIRI=0.659, PLR=0.725, NLR=0.680, SII=0.723) (Figure 2A). DeLong's test was performed using MedCalc (20.218), and it was found that there was no significant difference between the single inflammation indexes, and the joint index CIP was significantly different from the single inflammation models (P<0.05) (Table 3). The KM curve showed that the group with a lower joint index had a longer RFS (P<0.001) (Figure 2B). To avoid the interaction between variables, univariate and multivariate cox analysis were performed again after removing the factors included in the combined index. The joint index was an independent risk factor for early recurrence and had good predictive value (Table 4).

4 Discussion

Cancer-associated inflammation can be divided into two categories: local inflammation and systemic inflammation. Local inflammation is mainly related to the immune response in the tumor microenvironment, which usually occurs before the appearance of tumors. Systemic inflammation is a continuous response to malignant tumors mediated by cytokines, inflammatory proteins, and immune cells (20). HBV-related HCC is an inflammation-driven tumor, which mostly occurs based on chronic hepatitis, and its development, proliferation and metastasis are seriously affected by the inflammatory environment (21). Currently, there is no consensus on the time point of early recurrence, which ranges from 6 months to 2 years in most studies (22–24). It is generally accepted that recurrences within 2 years represent "true recurrences," whereas after this period, "recurrences" are thought to be largely caused by "*de novo*" tumors (6). Our study used 2 years as the cutoff point. Whether 2 years is the best cut-off point to determine early recurrence needs further study.

There have been many studies on the effects of inflammatory indexes on RFS and overall survival of HCC after surgery. However, there are few studies on the effect of inflammatory indexes on early

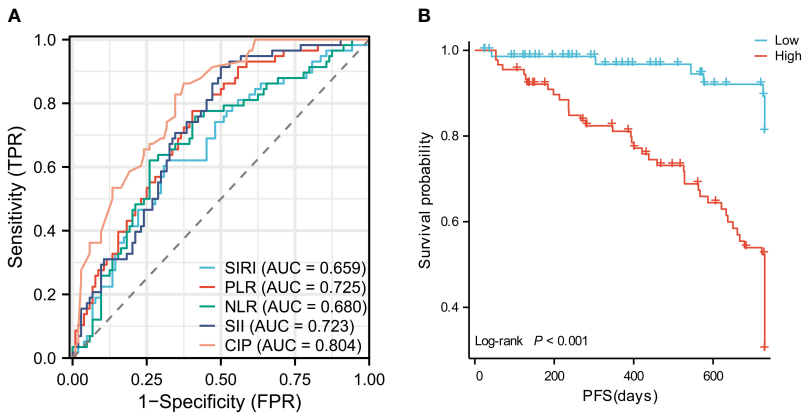

FIGURE 2
(A) Receiver operating characteristic analysis of SIRI, PLR, NLR, SII,CIP. **(B)** Recurrence-free survival curves of patients with high CIP and low CIP.

TABLE 3 DeLong's test between models.

Model 1	Model 2	P value
SIRI	PLR	0.191
SIRI	NLR	0.5528
SIRI	SII	0.062
SIRI	CIP	0.0006
PLR	NLR	0.3621
PLR	SII	0.9536
PLR	CIP	0.0115
NLR	SII	0.2426
NLR	CIP	0.0012
SII	CIP	0.0091

SIRI, Systemic Inflammation Response Index; PLR, Platelet-to-lymphocyte ratio; NLR, Neutrophil-to-Lymphocyte Ratio; SII, Systemic Immune-Inflammation Index; CIP, combined inflammation and pathology index.

recurrence (within 2 years) of liver cancer after surgery. In 2021, Wu et al. proposed that inflammatory indexes could be combined with clinical risk factors to construct a more effective prediction model for early recurrence (25). Our study focused on patients with HBV-related HCC, explored the predictive value of SIRI, PLR, NLR, and SII for early recurrence, and constructed a joint index CIP combining inflammatory indicators and pathological features. Compared with the model constructed by single inflammatory indexes, the CIP model had better predictive ability (AUC=0.804). It is worthy of further research and promotion.

Studies showed that all 4 inflammatory indexes were associated with tumor diameter and high SIRI, PLR, NLR and SII were associated with shorter RFS within 2 years. This may be due to the role of immune cells that constitute the inflammatory markers. Elevated levels of circulating neutrophils have been linked to the stimulation of tumor-derived cytokines, such as granulocyte colony-stimulating factor, platelet-derived growth factor, and Interleukin-8, which mobilize bone marrow-derived cells and splenocytes and lead to their circulation and migration to organs

TABLE 4 Univariate and multivariate analysis of RFS within 2 years.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Gender	162				
Female	37	Reference			
Male	125	1.154 (0.611-2.179)	0.658		
Age	162				
≤60	107	Reference			
>60	55	1.104 (0.642-1.898)	0.720		
BMI>28	162				
≤28	143	Reference			
>28	19	1.848 (0.873-3.916)	0.109		
Diabetes	162				
No	151	Reference			
Yes	11	0.485 (0.151-1.552)	0.222		
Drinking history	162				
No	130	Reference			
Yes	32	1.432 (0.795-2.578)	0.232		
HBSAg (+)	162				
No	27	Reference			
Yes	135	0.748 (0.387-1.443)	0.386		
Tumor number	162				
Solitary	138	Reference			
Multiple	24	0.887 (0.420-1.872)	0.754		
Cirrhosis	162				

(Continued)

TABLE 4 Continued

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
No	26	Reference			
Yes	136	0.574 (0.304-1.085)	0.087		
AJCC Staging	162				
I-II	154	Reference			
III-IV	8	1.609 (0.581-4.456)	0.360		
Postoperative treatment (Ablation or TACE)	162				
No	135	Reference			
Yes	27	1.274 (0.687-2.364)	0.442		
AFP (ng/ml)	162				
≤400	112	Reference			
>400	50	1.124 (0.649-1.946)	0.676		
CIP					
≤1.48	73	Reference			
>1.48	89	5.851 (2.770 - 12.360)	< 0.001	5.851 (2.770 - 12.360)	< 0.001

SIRI, Systemic Inflammation Response Index; PLR, Platelet-to-Lymphocyte Ratio; NLR, Neutrophil-to-Lymphocyte Ratio; SII, Systemic Immune-Inflammation Index; BMI, Body mass index; N, Regional lymph node metastasis; AFP, alpha fetoprotein.

(26). Neutrophils are thought to drive tumor progression through immunosuppression, direct enhancement of tumor cell survival, invasiveness, and metastatic ability, extracellular matrix remodeling, and angiogenesis (27). Lymphocytes control tumor growth by inducing cytotoxic cell death and secreting cytokines, and decreased levels of lymphocytes can impair host immune function and accelerate tumor progression (28). Monocytes can infiltrate tumors and further differentiate into Tumor-associated macrophages, which can induce apoptosis of CD8+ T cells with anticancer activity and promote tumor growth, invasion, and migration (29). It has been suggested that macrophage populations, as indicated by peripheral blood mononuclear cell counts, are correlated with tumor burden. In this study, it was found that platelet-related parameters such as PLR and SII had better predictive performance. It has been proposed that platelets can promote tumor growth and metastasis through the release of mediators such as vascular endothelial growth factor and platelet-derived growth factor. Additionally, platelets may also protect tumor cells from natural killer cells and promote epithelial-mesenchymal transition. It is suggested that high platelet counts may be associated with a poor prognosis in HCC.

Tumor markers such as alpha-fetoprotein and abnormal prothrombin are effective indicators to judge the prognosis of HCC, but many patients have normal tumor markers when they are diagnosed with HCC. Therefore, it is essential to find more prognostic biomarkers for clinical decision-making. Peripheral blood inflammatory markers are highly generalizable due to their non-invasive and easy availability. Future prospective multicenter studies with larger sample sizes and studies targeting other HCC etiologies are necessary to verify the results of this study.

5 Conclusion

The joint index CIP constructed by combining preoperative SIRI, PLR, NLP and SII with pathological features, can better predict the early recurrence of HBV-related HCC patients after surgery, which is helpful to identify high-risk patients and assist clinicians to make better clinical decisions.

Data availability statement

The datasets presented in this article are not readily available because hepatocellular carcinoma. Requests to access the datasets should be directed to 953168503@qq.com

Ethics statement

The studies involving human participants were reviewed and approved by Shanxi Provincial Cancer Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

GW is the main writer. LY, MJ and WB collected the data. LY also organized the data and participated in writing the paper. LLB analyzed the data. NZ and NY reviewed and revised the manuscript. LLX designed the overall topic and guiding the paper. All the

authors contributed to the original draft preparation and reviewing and editing of the study.

Conflict of interest

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

Publisher's note

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Construction and validation of a novel prognostic model for intrahepatic cholangiocarcinoma based on a combined scoring system of systemic immune-inflammation index and albumin-bilirubin: a multicenter study

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Background: The degree of inflammation and immune status is widely recognized to be associated with intrahepatic cholangiocarcinoma (ICC) and is closely linked to poor postoperative survival. The purpose of this study was to evaluate whether the systemic immune-inflammatory index (SII) and the albumin bilirubin (ALBI) grade together exhibit better predictive strength compared to SII and ALBI separately in patients with ICC undergoing curative surgical resection.

Methods: A retrospective analysis was performed on a cohort of 374 patients with histologically confirmed ICC who underwent curative surgical resection from January 2016 to January 2020 at three medical centers. The cohort was divided into a training set comprising 258 patients and a validation set consisting of 116 patients. Subsequently, the prognostic predictive abilities of three indicators, namely SII, ALBI, and SII+ALBI grade, were evaluated. Independent risk factors were identified through univariate and multivariate analyses. The identified independent risk factors were then utilized to construct a nomogram prediction model, and the predictive strength of the nomogram prediction model was assessed through Receiver Operating Characteristic (ROC) survival curves and calibration curves.

Results: Univariate analysis of the training set, consisting of 258 eligible patients with ICC, revealed that SII, ALBI, and SII+ALBI grade were significant prognostic factors for overall survival (OS) and recurrence-free survival (RFS) ($p < 0.05$). Multivariate analysis revealed the independent significance of SII+ALBI grade as a risk factor for postoperative OS and RFS ($p < 0.05$). Furthermore, we conducted an analysis of the correlation between SII, ALBI, SII+ALBI grade, and clinical features, indicating that SII+ALBI grade exhibited stronger associations with

clinical and pathological characteristics compared to SII and ALBI. We constructed a predictive model for postoperative survival in ICC based on SII +ALBI grade, as determined by the results of multivariate analysis. Evaluation of the model's predictive strength was performed through ROC survival curves and calibration curves in the training set and validation set, revealing favorable predictive performance.

Conclusion: The SII+ALBI grade, a novel classification based on inflammatory and immune status, serves as a reliable prognostic indicator for postoperative OS and RFS in patients with ICC.

KEYWORDS

intrahepatic cholangiocarcinoma, SII, ALBI, SII+ALBI grade, nomogram, prognosis

1 Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most prevalent primary liver cancer distinguished by its aggressive nature, accounting for approximately 15–20% of all biliary malignancies (1, 2). The worldwide incidence of ICC has been consistently rising at a yearly rate of 15% over the past few decades (1). Curative surgical resection currently stands as the gold-standard treatment for ICC. However, only about 20%–40% of patients who get curative surgical resection survive 5 years or more (3, 4). Therefore, the identification of novel prognostic indicators for distinguishing ICC patients who would benefit from curative surgical resection is crucial for developing personalized treatment strategies.

Increasing evidence suggests that in addition to common factors such as lymph node metastasis, tumor size, and vascular invasion, nutritional status and inflammatory levels play a significant predictive role in the prognosis of curative surgical resection for tumors (5, 6). Among them, the Systemic Immune-Inflammation Index (SII) is a fresh quantitative indicator used to assess individual immune status and inflammation levels (7, 8). It is calculated based on parameters such as platelet, neutrophil, and lymphocyte counts. SII is frequently used to assess patients' preoperative nutritional status and precisely evaluate their individual surgical risks (8). Additionally, the Albumin–bilirubin (ALBI) grade is a composite indicator that comprehensively evaluates patients' liver function and reserves. Its introduction was first compared to Child-Pugh classification in hepatocellular carcinoma (HCC) patients in 2015, demonstrating superior predictive capability for survival following liver resection and postoperative liver failure (9). A growing body of literature indicates a close association between SII, ALBI, and the prediction of prognosis and survival in patients with HCC, ICC, and other malignancies (9–13). However, whether the combined application of SII and ALBI can improve the prognostic prediction in patients with ICC remains inconclusive. This research seeks to identify the combined application of SII and ALBI in predicting postoperative survival after curative resection for ICC and attempt to construct a survival prognostic model based on SII and ALBI.

2 Materials and methods

2.1 Patient selection

This study included all patients who received curative surgical resection for ICC between January 2016 and January 2020 at People's Hospital of Zhengzhou University, Cancer Hospital of Zhengzhou University, and The First Affiliated Hospital of Zhengzhou University. Following were the inclusion criteria: 1) Patients whose pathological confirmation with ICC followed a curative surgical resection; 2) Patients aged 18 years or older; 3) No prior anticancer treatment before surgery; 4) No concurrent occurrence of other malignant tumors. Following were the exclusion criteria: 1) Perioperative mortality; 2) Patients with hematological disorders and autoimmune diseases; 3) Incomplete clinical or laboratory data; 4) Patients requiring a second surgery for tumor recurrence; 5) Incomplete follow-up information. 258 patients from People's Hospital of Zhengzhou University and Cancer Hospital of Zhengzhou University were chosen as the training set, while a total of 116 patients from The First Affiliated Hospital of Zhengzhou University were chosen as the validation set. The 8th edition of the American Joint Committee on Cancer (AJCC) staging method was used to evaluate all patients who were included, and all patients were monitored until January 2023.

This study received ethical approval from the Institutional Review Boards of Zhengzhou University People's Hospital (Ref No. 2023-012), Zhengzhou University Cancer Hospital (Ref No. 2023-203), and Zhengzhou University First Affiliated Hospital (2021-KY-1137-002). Written informed consent was obtained from all patients prior to their participation in the study.

2.2 Clinical variables

Patient clinical and pathological data included age, gender, HBV infection, obstructive jaundice, tumor differentiation, tumor number, tumor size, perineural invasion, microvascular invasion, and the AJCC 8th TNM Stage. Laboratory test results were collected

from one week before surgery, including carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), alanine transaminase (ALT), aspartate transaminase (AST), albumin, bilirubin, white blood cell count (WBC), lymphocyte count (LY), neutrophil count (NEUT), platelet count (PLT), hemoglobin (HGB), prothrombin time (PT), international normalized ratio (INR) and activated partial thromboplastin time (APTT). Additionally, the calculation methods for the two immune-inflammatory markers, ALBI and SII, were as follows: ALBI = $\log_{10}$bilirubin (mol/L) * 0.66 - albumin (g/L) * 0.085, SII = platelet count * neutrophil count/lymphocyte count. Subsequently, Subsequently, the X-tile software (Yale University, New Haven, CT, USA) was employed to compute the optimal cutoff values for overall survival (OS) and recurrence-free survival (RFS) with respect to SII and ALBI. Based on the results, ALBI ≥ -2.50 was defined as the high ALBI group, and ALBI < -2.50 as the low ALBI group. Similarly, SII ≥ 470 was defined as the high SII group, and SII < 470 as the low SII group. In the subsequent analysis, the combination of low SII and low ALBI was defined as SII+ALBI Grade A, the combination of high SII and high ALBI was defined as SII+ALBI Grade C, and the remaining combinations were defined as SII+ALBI Grade B.

2.3 Statistical analysis

The Kolmogorov-Smirnov test was used in research to determine if continuous variables were normally distributed. Mean and standard deviation (SD) were used to represent normally distributed data, whereas interquartile range (IQR) was used to represent non-normally distributed variables. For group comparisons, the Mann-Whitney rank sum test and the student t-test were used. The baseline features of categorical variables were compared using the chi-square test and Fisher's exact test. Cox proportional hazards regression analysis was used for the univariate analysis. Cox backward stepwise regression models were employed for the multivariate analysis. GraphPad Prism (version 8.0) was used to create Kaplan-Meier survival curves for OS and RFS based on the grouping of ALBI, SII, and ALBI+SII. Additionally, ROC survival curves were drawn, and the three groups' areas under the curve (AUC) were contrasted. Statistical significance was defined as $p<0.05$.

2.4 Follow-up

For the included patients, follow-up began after the surgical procedure. Within the first year postoperatively, monthly follow-up visits were conducted, followed by follow-up visits every three months for the next two years. The last follow-up was performed on January 2023. Overall survival was determined as the interval between the date of curative surgical resection and the last examination or the date of death from any cause. Recurrence-free survival was determined as the interval between the date of curative surgical resection and the most recent follow-up, the occurrence of tumor recurrence or advancement in any way, or the patient's death for any reason.

2.5 Development and assessment of nomogram

Based on the results of the Cox backward stepwise regression model, predictive models for OS and RFS were constructed using nomogram models. The accuracy of the models was assessed by plotting ROC survival curves and calibration curves for the training and validation sets based on the models. The construction and evaluation of the models were performed using R software (version 4.2.1).

3 Result

A total of 374 patients (172 male and 202 female) who underwent curative surgical resection for pathologically confirmed ICC from January 2016 to January 2020 were included in this study. The median age of the patients was 59 years, ranging from 28 to 80 years. The median follow-up time was 12 months (1-91 months). The 1-year, 2-year, and 3-year OS rates were 52.1%, 23.3%, and 10.9%, respectively. The 1-year, 2-year, and 3-year RFS rates were 29.2%, 15.5%, and 5.3%, respectively. As can be seen in Table 1, the baseline data and clinicopathological traits of the training set (n=258) and validation set (n=116) were examined for their association. The two cohorts' distributions were balanced ($p>0.05$).

3.1 Survival analysis for OS and RFS

Through univariate survival analysis of the included variables, we found that SII [OS: hazard ratio (HR) = 1.574, 95% CI = 1.126-2.201, p = 0.008; RFS: HR = 1.590, 95% CI = 1.181-2.140, p = 0.002], ALBI [OS: HR = 1.692, 95% CI = 1.220-2.346, p = 0.002; RFS: HR = 1.980, 95% CI = 1.291-3.038, p = 0.002], and SII+ALBI grade [OS: HR = 2.717, 95% CI = 1.701-4.341, p < 0.001; RFS: HR = 3.078, 95% CI = 1.822-5.198, p < 0.001] were prognostic factors for OS and RFS in patients with ICC after surgical resection (Figures 1, 2). Additionally, the results of the multivariate survival analysis also indicated that SII+ALBI grade [OS: HR = 2.230, 95% CI = 1.371-3.628, p = 0.001; RFS: HR = 2.355, 95% CI = 1.359-4.082, p = 0.001] was an independent risk factor for OS and RFS in postoperative ICC patients (Figures 1, 2). The detailed results of the univariate and multivariate analyses are presented in Table 2.

In addition, we plotted the ROC survival curves for SII, ALBI, SII+ALBI grade, Child-pugh Grade and AJCC 8th TNM stage. By comparing the area under the ROC curves, we found that SII+ALBI grade demonstrated a superior survival predictive effect (Figure 3).

3.2 Correlation analysis of SII, ALBI and SII+ALBI with clinical and pathological features

Through chi-square tests, we found that compared to SII and ALBI, SII+ALBI grade exhibited better correlations with age,

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TABLE 1 Comparison of clinicopathological characteristics in training and validation sets.

Variables	All patients (n=374)	Training set (n=258)	Validation set (n=116)	p value
Sex				0.884
Male	172(46.0%)	118(45.7%)	54(46.6%)	
Female	202(54.0%)	140(54.3%)	62(53.4%)	
Age (years)				0.076
≤65	250(66.8%)	165(64.0%)	85(73.3%)	
>65	124(33.2%)	93(36.0%)	31(26.7%)	
Obstructive jaundice				0.965
No	310(82.9%)	214(82.9%)	96(82.8%)	
Yes	64(17.1%))	44(17.1%)	20(17.2%)	
HBV infection				0.467
No	239(63.9%)	168(65.1%)	71(61.2%)	
Yes	135(36.1%)	90(34.9%)	45(38.8%)	
AFP (ng/ml)				0.085
<20	309(82.6%)	219(84.9%)	90(77.6%)	
≥20	65(17.4%)	39(15.1%)	26(22.4%)	
CEA (ng/ml)				0.491
<5	242(64.7%)	164(63.6%)	78(67.2%)	
≥5	132(35.3%)	94(36.4%)	38(32.8%)	
CA19-9 (U/ml)				0.387
<37	149(39.8%)	99(38.4%)	50(43.1%)	
≥37	225(60.2%)	159(61.6%)	66(56.9%)	
Child–Pugh Grade				0.639
Grade A	334(89.3%)	233(90.3%)	101(87.1%)	
Grade B	40(10.7%)	25(9.7%)	15(12.9%)	
Tumor number				0.995
= 1	303(81.0%)	209(81.0%)	94(81.0%)	
>1	71(19.0%)	49(19.0%)	22(19.0%)	
Tumor size				0.366
<5.0cm	158(42.2%)	105(40.7%)	53(45.7%)	
≥5.0cm	216(57.8%)	153(59.3%)	63(54.3%)	
Tumor differentiation				0.627
Well	36(9.6%)	27(10.5%)	9(7.8%)	
Moderate	280(74.9%)	193(74.8%)	87(75.0%)	
Poor	58(15.4%)	38(14.7%)	20(17.2%)	
Perineural invasion				0.109
No	194(51.9%)	141(54.7%)	53(45.7%)	
Yes	180(48.1%)	117(45.3%)	63(54.3%)	
Microvascular invasion				0.727

(Continued)

TABLE 1 Continued

Variables	All patients (n=374)	Training set (n=258)	Validation set (n=116)	p value
No	205(54.8%)	143(55.4%)	62(53.4%)	
Yes	169(45.2%)	115(44.6%)	54(46.6%)	
AJCC 8th edition T stage				0.053
T_{1a}/T_{1b}	171(45.7%)	117(45.3%)	54(46.6%)	
T_2	147(39.3%)	95(36.8%)	52(44.8%)	
T_3/T_4	56(15.0%)	46(17.8%)	10(8.6%)	
AJCC 8th edition N stage				0.252
N_0	279(74.6%)	188(72.9%)	91(78.4%)	
N_1	95(25.4%)	70(27.1%)	25(21.6%)	
AJCC 8th edition M stage				0.311
M_0	368(98.4%)	255(98.8%)	113(97.4%)	
M_1	6(1.6%)	3(1.2%)	3(2.6%)	
ALT (ng/ml)	53(45-61)	50(44-59)	60(44-75)	0.259
AST (ng/ml)	48(42-53)	45(38-52)	53(41-65)	0.222
Albumin (ng/ml)	40.54(39.90-41.18)	40.80(40.05-41.54)	39.98(38.75-41.20)	0.245
Bilirubin (ng/ml)	28.16(22.35-33.97)	26.88(20.08-33.68)	31.00(19.79-42.20)	0.520
PT (s)	12.30(12.17-12.44)	12.27(12.11-12.44)	12.37(12.13-12.62)	0.509
INR	1.21(1.00-1.41)	1.17(0.95-1.39)	1.28(0.83-1.74)	0.611
APTT (s)	32.00(31.36-32.65)	31.92(31.10-32.74)	32.18(31.16-33.20)	0.718
WBC (10^9/L)	6.88(6.59-7.16)	6.76(6.44-7.09)	7.13(6.55-7.70)	0.248
HGB (g/L)	131(129-133)	130(128-133)	131(128-135)	0.710
NEUT (10^9/L)	5.19(4.65-5.72)	4.94(4.38-5.51)	5.73(4.54-6.92)	0.183
LY (10^9/L)	1.66(1.43-1.88)	1.71(1.39-2.03)	1.53(1.41-1.65)	0.470
PLT (10^9/L)	215(207-223)	215(205-225)	215(200-231)	0.946
SII	706(741-946)	796(685-907)	950(728-1172)	0.172
ALBI	-2.67(-2.74 - -2.60)	-2.70(-2.77 – -2.62)	-2.60(-2.73 – -2.48)	0.207

obstructive jaundice, HBV infection, CA19-9, CEA, Child–Pugh Grade, tumor size, tumor differentiation, perineural invasion ($p<0.05$, Table 3).

3.3 Development and assessment of nomogram

Based on the results of Cox multivariate survival analysis, we established a nomogram prediction model using R software for postoperative OS and RFS in patients with ICC, incorporating various variables including SII+ALBI grade (Figure 4). In addition, we plotted the ROC survival curves for the training and validation sets based on the predictive model. The AUC values for 1–3-year OS in the training set were 0.804, 0.820, and 0.763, respectively, while for the validation set, they were 0.731, 0.793, and 0.781. The AUC values for 1–3-year RFS in the training set were 0.751, 0.742, and 0.822, respectively, and for the validation set they were 0.768, 0.738, and 0.745 (Figure 4). We also plotted the calibration curves of the training and validation sets for 1–3-year survival using both models, and the results consistently demonstrated the excellent predictive ability of the model for postoperative survival in ICC patients (Figures 5, 6).

4 Discussion

Curative surgical resection represents the gold standard for the treatment of ICC (14). The decision to proceed with surgical resection is often based on the patient's imaging data and the

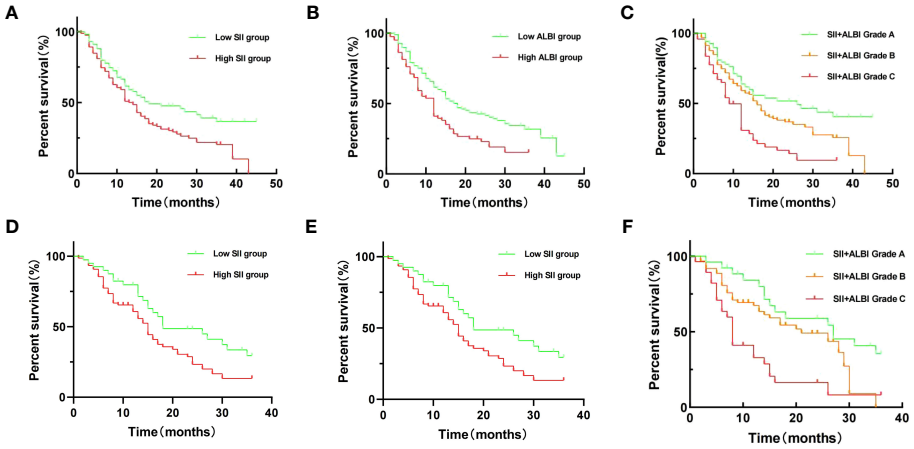

FIGURE 1
Kaplan–Meier overall survival (OS) curves of patients with intrahepatic cholangiocarcinoma (ICC) after radical resection according to different prognostic factors in the training set and validation set. **(A–C)** Kaplan–Meier OS curves according to the Systemic Immune-Inflammation Index (SII) **(A)**, albumin–bilirubin (ALBI) **(B)**, and SII+ALBI grade **(C)** of training set. **(D–F)** Kaplan–Meier OS curves according to SII **(A)**, ALBI **(B)**, and SII+ALBI grade **(C)** of validation set.

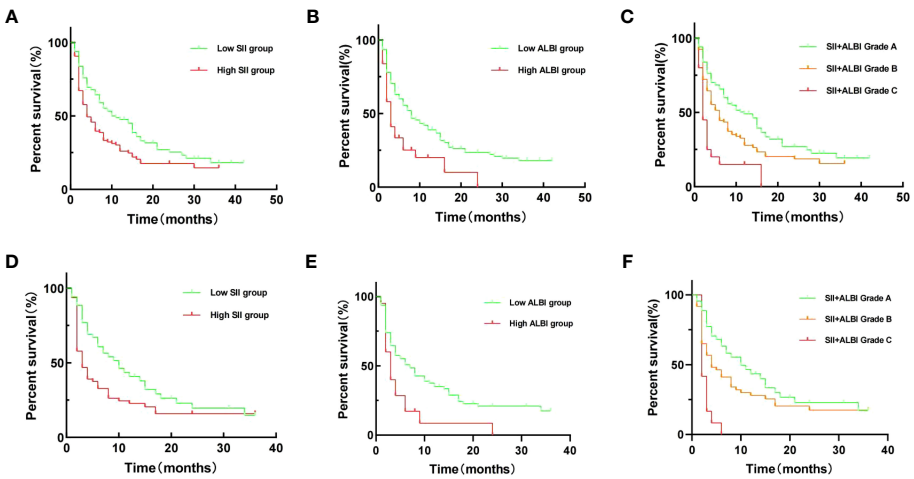

FIGURE 2
Kaplan–Meier recurrence-free survival (RFS) curves of patients with intrahepatic cholangiocarcinoma (ICC) after radical resection according to different prognostic factors in the training set and validation set. **(A–C)** Kaplan–Meier OS curves according to the Systemic Immune-Inflammation Index (SII) **(A)**, albumin–bilirubin (ALBI) **(B)**, and SII+ALBI grade **(C)** of training set. **(D–F)** Kaplan–Meier OS curves according to SII **(A)**, ALBI **(B)**, and SII +ALBI grade **(C)** of validation set.

TABLE 2 Univariate and multivariate analyses of the prognosis for intrahepatic cholangiocarcinoma (ICC) after radical resection in the training set.

Variables	OS				RFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value
Sex Female vs. Male	0.918(0.672-1.256)	0.594			1.040(0.773-1.400)	0.794		
Age (years) >65 vs. ≤65	0.947(0.683-1.314)	0.744			0.806(0.590-1.101)	0.176		

(Continued)

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TABLE 2 Continued

Variables	OS				RFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value
Obstructive jaundice Yes vs. no	0.923(0.533-1.600)	0.776			0.928(0.575-1.499)	0.761		
HBV infection Yes vs. no	0.722(0.515-1.012)	0.059			0.861(0.629-1.179)	0.351		
AFP (ng/ml) ≥20 vs. <20	0.856(0.549-1.333)	0.491			1.051(0.702-1.575)	0.808		
CEA (ng/ml) ≥5 vs. <5	1.958(1.427-2.685)	<0.001			1.417(1.044-1.923)	0.025		
CA19-9 (U/ml) ≥37 vs. <37	2.067(1.464-2.920)	<0.001	1.520(1.037-2.228)	0.032	1.779(1.297-2.440)	<0.001	1.764(1.273-2.445)	0.001
ALT (ng/ml)	1.001(0.999-1.003)	0.292			1.001(0.999-1.002)	0.404		
AST (ng/ml)	1.003(1.000-1.005)	0.032			1.002(0.999-1.004)	0.127		
Albumin(ng/ml)	0.959(0.933-0.986)	0.003			0.966(0.941-0.992)	0.010		
Bilirubin(ng/ml)	1.003(1.001-1.005)	0.055			1.004(1.001-1.006)	0.002		
PT(s)	1.057(0.948-1.180)	0.318			1.028(0.927-1.139)	0.606		
INR	0.938(0.821-1.071)	0.342			0.959(0.873-1.054)	0.385		
APTT(s)	0.997(0.973-1.021)	0.782			0.991(0.970-1.013)	0.442		
Child–Pugh Grade Grade A vs. Grade B	2.146(1.216-3.789)	0.008						
SII High group vs. low group	1.574(1.126-2.201)	0.008			1.590(1.181-2.140)	0.002		
ALBI High group vs. low group	1.692(1.220-2.346)	0.002			1.980(1.291-3.038)	0.002		
SII+ALBI Grade Grade B vs.Grade A Grade C vs.Grade A	 1.519(1.013-2.278) 2.717(1.701-4.341)	 0.037 <0.001	 1.347(1.013-2.053) 2.230((1.371-3.628)	 0.037 0.001	 1.493(1.091-2.042) 3.078(1.822-5.198)	 0.012 <0.001	 1.225(1.004-1.696) 2.355(1.359-4.082)	 0.032 0.002
Tumor number 1 vs. >1	1.426(0.978-2.079)	0.065			1.853(1.306-2.628)	0.001	1.614(1.114-2.339)	0.011
Tumor size (cm) >5.0 vs. ≤5.0	1.402(1.013-1.939)	0.041			1.293(0.954-1.751)	0.097		
Tumor differentiation Moderate vs. well Poor vs. well	 2.193(1.142-4.209) 3.258(1.578-6.729)	 0.018 0.001	 1.685(1.105-3.314) 2.654(1.244-5.662)	 0.028 0.012	 1.646(1.063-2.812) 2.333(1.251-4.350)	 0.029 0.008	 1.334(1.073-2.302) 2.068(1.089-3.925)	 0.035 0.026
Perineural invasion Yes vs. no	1.691(1.232-2.322)	0.001			1.246(0.927-1.676)	0.145		
Microvascular invasion Yes vs. no	1.993(1.451-2.737)	<0.001	1.548(1.112-2.156)	0.010	1.473(1.092-1.987)	0.011	1.364(1.004-1.853)	0.047
AJCC 8th edition T stage T_2 vs. T_{1a}/T_{1b} T_3/T_4 vs. T_{1a}/T_{1b}	 1.342(0.947-1.903) 1.514(0.982-2.332)	 0.098 0.060			 1.062(0.689-1.637) 1.527(0.985-2.367)	 0.784 0.058		

(Continued)

TABLE 2 Continued

Variables	OS				RFS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value
AJCC 8th edition N stage N_1 vs. N_0	1.840(1.307-2.592)	<0.001	1.452(1.011-2.085)	0.043	1.421(1.027-1.965)	0.034		
AJCC 8th edition M stage M_1 vs. M_0	1.620(0.400-6.556)	0.499			0.988(0.244-3.991)	0.986		

presence of accompanying symptoms. However, even among patients with similar disease stages and grades, there exists significant heterogeneity in the prognosis and clinical response to curative surgical resection (15). Therefore, the identification of a robust intraoperative and postoperative risk prediction tool holds paramount importance.

As a composite index of platelet, lymphocyte, and monocyte counts, SII provides a direct reflection of the body's inflammatory status. Increasing evidence suggests that platelets and monocytes can interact with tumor cells through various mechanisms, promoting tumor cell survival and metastasis, enhancing cancer cell invasion, proliferation, and immune evasion, thereby modulating the interplay between the host and tumor (16–19). On the other hand, lymphocytes play a crucial role in cell-mediated immune destruction of cancer cells by activated T cells and other lymphocytes, while tumors can also release cytokines such as IFN-γ and TNF-α to regulate various immune functions in the body (20, 21). Furthermore, numerous studies have confirmed that SII is an independent prognostic factor for postoperative survival in various digestive system malignancies, including HCC, ICC, and gallbladder cancer (8, 22–25). Similarly, in our study, a lower SII was significantly associated with improved postoperative survival and reduced recurrence rates, further validating this observation.

Albumin-bilirubin, calculated based on serum albumin and bilirubin levels, provides an intuitive reflection of a patient's immune status and liver function; ALBI was initially proposed by Johnson et al. in 2014 as an alternative to the Child-Pugh classification for assessing liver function in HCC patients, overcoming its limitations (26). Increasing evidence suggests that ALBI is a reliable indicator of liver functional reserve. A multicenter cohort study demonstrated that the predictive performance of the Barcelona Clinic Liver Cancer (BCLC) staging system based on ALBI score is comparable to or even superior to that based on the Child-Pugh classification (27). Subsequently, the predictive ability of ALBI for the prognosis of HCC and ICC patients has been

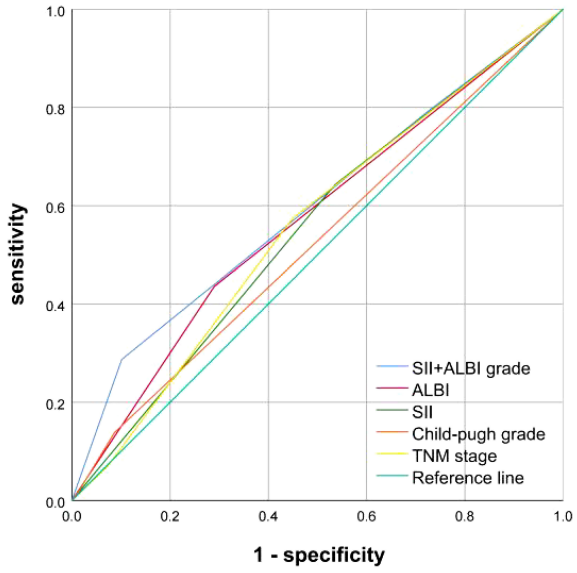

FIGURE 3
Comparison of SII, ALBI, SII+ALBI grade, Child-pugh Grade and TNM stage in predicting OS.

TABLE 3 Relationship of SII, ALBI and SII+ALBI grade with clinicopathological characteristics of intrahepatic cholangiocarcinoma (ICC) after radical resection in the training set.

	SII		X^2	p-value	ALBI		X^2	p-value	SII+ALBI Grade			X^2	p value
	Low group	High group			Low group	High group			Grade A	Grade B	Grade C		
Sex			1.768	0.184			0.012	0.912				3.294	0.193
Male	60	80			97	43			44	69	27		
Female	41	77			81	37			26	70	22		
Age			0.911	0.340			0.002	0.964				7.010	0.030
≤65	61	104			114	51			38	99	28		
>65	40	53			64	29			32	40	21		
Obstructive jaundice			0.017	0.897			18.259	<0.001				10.157	0.006
No	83	130			159	54			62	118	33		
Yes	18	27			19	26			8	21	16		
HBV infection			6.834	0.009			3.805	0.051				11.015	0.004
No	56	112			109	59			35	95	38		
Yes	45	45			69	21			35	44	11		
AFP (ng/ml)			0.947	0.331			1.351	0.245				2.280	0.320
<20	83	136			148	71			56	119	44		
≥20	18	21			30	9			14	20	5		
CEA (ng/ml)			9.779	0.002			0.269	0.604				7.978	0.019
<5	76	88			115	49			54	83	27		
≥5	25	69			63	31			16	56	22		
CA19-9 (U/ml)			5.880	0.015			7.205	0.007				16.720	<0.001
<37	48	51			78	21			41	44	14		
≥37	53	106			100	59			29	95	35		
SII							0.008	0.930				156.891	<0.001
Low group					70	31			70	31	0		
High group					108	49			0	108	49		
ALBI			0.008	0.930								145.410	<0.001

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TABLE 3 Continued

	SII		X^2	p-value	ALBI		X^2	p-value	SII+ALBI Grade			X^2	p value
	Low group	High group			Low group	High group			Grade A	Grade B	Grade C		
Low group	70	108							70	108	0		
High group	31	49							0	31	49		
SII+ALBI grade			156.891	<0.001			145.410	<0.001					
Grade A	70	0			70	0							
Grade B	31	108			108	31							
Grade C	0	49			0	49							
Child–Pugh grade			0.171	0.679			54.684	<0.001				31.612	<0.001
Grade A	91	142			177	56			69	130	34		
Grade B	10	15			1	24			1	9	15		
Tumor Number			2.840	0.092			0.004	0.947				2.383	0.304
=1	87	122			144	65			61	109	39		
>1	14	35			34	15			9	30	10		
Tumor Size(cm)			19.244	<0.001			2.223	0136				7.382	0.025
≤5	58	47			67	38			38	49	18		
>5	43	110			111	42			32	90	31		
Tumor differentiation			1.062	0.588			5.498	0.064				6.736	0.151
Well	13	14			23	4			10	17	11		
Moderate	73	120			133	60			49	108	36		
Poor	15	23			22	16			11	14	2		
Perineural invasion			9.145	0.002			10.043	0.002				19.220	<0.001
No	67	74			109	32			52	72	17		
Yes	34	83			69	48			18	67	32		
Microvascular invasion			0.500	0.480			1.296	0.255				1.679	0.432
No	58	84			102	40			42	76	24		
Yes	42	73			75	40			27	63	25		

(Continued)

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TABLE 3 Continued

	SII		X^2	p-value	ALBI		X^2	p-value	SII+ALBI Grade			X^2	p value
	Low group	High group			Low group	High group			Grade A	Grade B	Grade C		
AJCC 8th edition T stage			7.729	0.021			3.851	0.146				3.737	0.443
T1a/T1b	55	62			80	37			38	59	20		
T2	35	60			61	34			22	52	21		
T3/T4	11	35			37	9			10	28	8		
AJCC 8th edition N stage			2.403	0.121			0.671	0.413				0.398	0.819
N_0	79	109			127	61			53	100	35		
N_1	22	48			51	19			17	39	14		
AJCC 8th edition M stage			0.043	0.836			1.804	0.179				1.244	0.537
M_0	100	155			177	78			70	137	48		
M_1	1	2			1	2			0	2	1		

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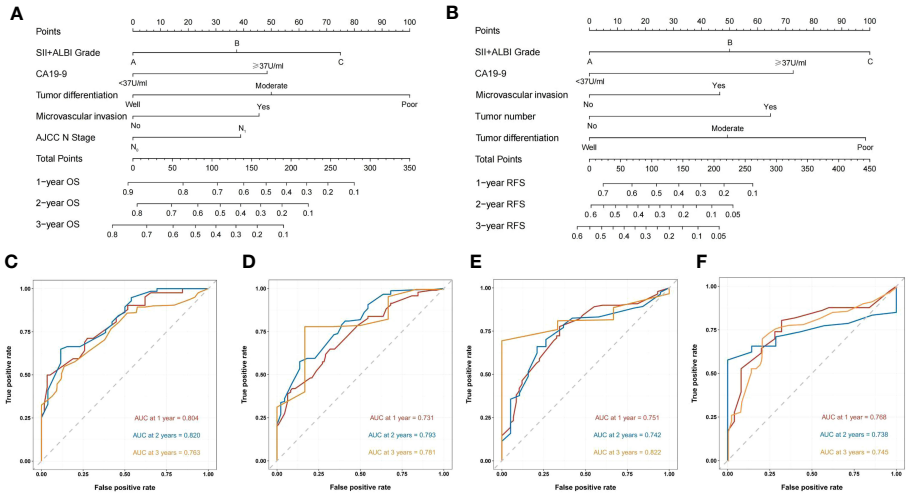

FIGURE 4
Construction and validation of the nomograms. Nomograms incorporating SII + ALBI Grade and other clinicopathological parameters for OS **(A)** and RFS **(B)** prediction in the training cohort. ROC survival curves of the training set for OS **(C)** and RFS **(D)** based on the model. ROC survival curves of the validation set for OS **(E)** and RFS **(F)** based on the model.

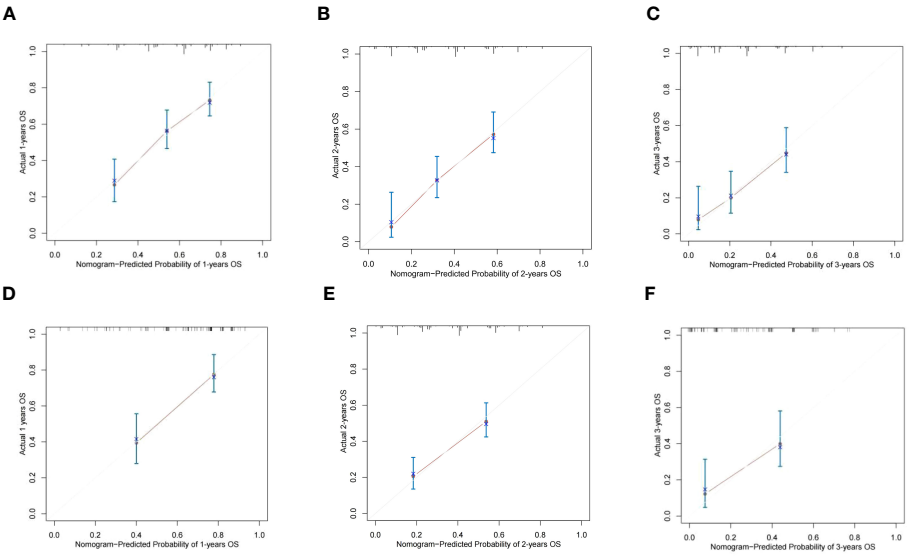

FIGURE 5
The calibration curves of the nomograms between predicted and observed 1-, 2-, and 3-year OS of patients in the training set **(A–C)** and the validation set **(D–F)**. The dashed line of 45° represents the perfect prediction of the nomogram.

validated in multiple independent cohorts, including those from Japan, China, and other countries (28–30). Consistent with the findings of these studies, in our research, the low ALBI group exhibited significantly higher OS and RFS rates compared to the high ALBI group.

In our study, we took into consideration the patients' inflammatory status, immune capacity, and liver function, by combining SII and ALBI, which were categorized into three grades: A, B, and C. Through the construction of Kaplan-Meier survival curves and ROC survival curves, we found that the SII+ALBI grade had better predictive ability and discrimination when compared separately to SII and ALBI. Therefore, we included the SII+ALBI grade as an independent grade index in our model and confirmed that the nomogram predictive model incorporating SII+ALBI grade for OS and RFS demonstrated good predictive performance. Additionally, we analyzed the correlation between SII+ALBI grade and clinical and pathological characteristics. Surprisingly, for indicators such as microvascular invasion and 8th edition AJCC N stage, which showed no significant correlation with individual SII or ALBI, the SII+ALBI classification still exhibited a correlation. Therefore, we believe that the SII+ALBI classification can

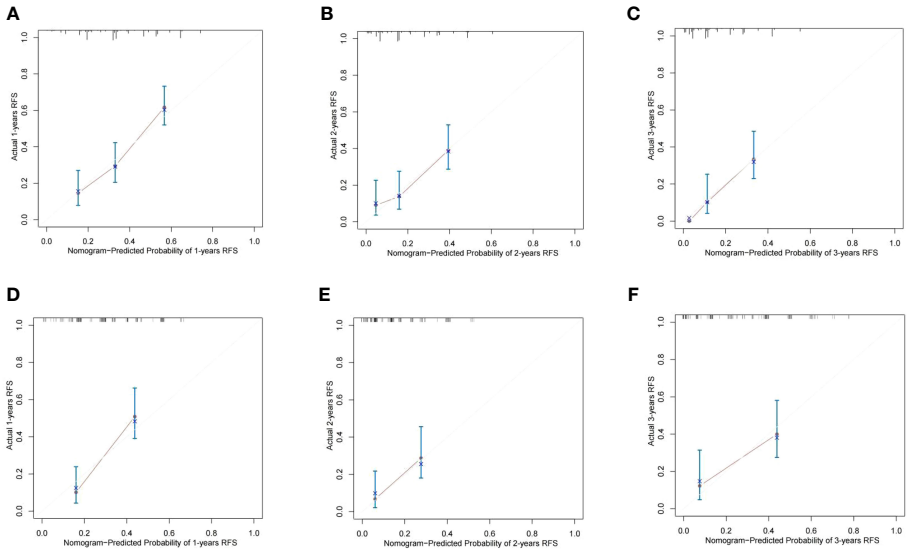

FIGURE 6
The calibration curves of the nomograms between predicted and observed 1-, 2-, and 3-year RFS in the training set **(A–C)** and the validation set **(D–F)**. The dashed line of 45° represents the perfect prediction of the nomogram.

better reflect the patients' clinical and pathological characteristics to a certain extent.

After reviewing relevant research, we found that our study is the first to combine SII and ALBI and construct a prognostic survival model based on SII+ALBI grade. In our model, SII+ALBI grade carries a significant weight, which is closely related to representing the immune-inflammatory status and liver function. Additionally, we plotted ROC survival curves and calibration curves for the training and validation sets based on the predictive model. The results demonstrated excellent predictive ability of the model for postoperative survival in patients with ICC.

In addition, our study has the following limitations. Firstly, although it is a multicenter retrospective study, the sample size involved in the study is relatively small, with a total of 374 cases. Secondly, due to the retrospective nature of this study, selection bias is unavoidable, and we only included patients who underwent surgical resection without receiving other treatments prior to surgery. Thirdly, despite our efforts to minimize the impact of confounding factors on the study results, individual differences in various laboratory parameters cannot be completely eliminated. Therefore, further large-scale prospective multicenter studies are still needed to validate our findings.

5 Conclusion

In conclusion, this multicenter study included a sample of 374 patients with ICC who underwent surgical resection in three tertiary hospitals. Based on univariate, multivariate, and clinical significance analyses, multiple relevant indicators incorporating the SII+ALBI grade were incorporated to construct a nomogram predictive model for OS and RFS. The model demonstrated excellent accuracy in survival prediction. To our knowledge, this is the first clinical prediction model for ICC that includes the SII +ALBI grade. We believe that this model can provide better guidance for the management of ICC and has the potential for broad application.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study received ethical approval from the Institutional Review Boards of Zhengzhou University People's Hospital (Ref No. 2023-012), Zhengzhou University Cancer Hospital (Ref No. 2023-203), and Zhengzhou University First Affiliated Hospital (2021-KY-1137-002). Written informed consent was obtained from all patients prior to their participation in the study. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

HZ is the first author. HZ, QL and HY conceived and designed the study. HZ, GH, ZY, KC and BM collected and offered the data. HZ, GH and ZY performed follow-up and statistical analysis. HZ wrote the manuscript. HY critically revised the manuscript for

important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Inflammation-related prognostic markers in resected hepatocellular carcinoma

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Hepatocellular carcinoma is usually detected late and therapeutic options are unsatisfactory. Despite marked progress in patient care, HCC remains among the deadliest cancers world-wide. While surgical resection remains a key option for early-stage HCC, the 5-year survival rates after surgical resection are limited. One reason for limited outcomes is the lack of reliable prognostic biomarkers to predict HCC recurrence. HCC prognosis has been shown to correlate with different systemic and pathological markers which are associated with patient survival and HCC recurrence. Liver inflammatory processes offer a large variety of systemic and pathological markers which may be exploited to improve the reliability of prognosis and decision making of liver surgeons and hepatologists. The following review aims to dissect the potential tools, targets and prognostic meaning of inflammatory markers in patients with resectable HCC. We analyze changes in circulant cellular populations and assess inflammatory biomarkers as a surrogate of impaired outcomes and provide an overview on predictive gene expression signatures including inflammatory transcriptional patterns, which are representative of poor survival in these patients.

KEYWORDS

HCC, biomarkers, genetic signatures, inflammation, patient outcome

1 Introduction

Hepatocellular Carcinoma (HCC) is the most common primary liver cancer accounting for about 80% of all cases and it ranks as the third leading cause of cancer deaths worldwide (1). Like cholangiocarcinoma, HCC shows a dismal prognosis with a relative 5-year survival rate of approximately 20% (2). Despite the constant and progressive evolution of the therapeutic algorithms on which decision strategy is based, in clinical

practice several issues remain to be addressed. First, a reliable prognostic clinical marker to predict HCC outcome is still missing. Among the prognostic indicators, the most common is plasmatic alpha-protein (AFP), which correlates with tumor behavior and risk of recurrence and survival (3–5). However, in 15–30% of HCC, AFP levels remain in a normal range and the heterogeneity of studies prevents from formulating clear recommendations (6, 7). Secondly, the complex treatment allocation process does not always reflect in a complete therapeutic arsenal. Effective and validated peri-operative therapies are still lacking and the inability to accurately detect more aggressive tumors could lead surgeons to validate complex and high morbidity resections on patients with an elevated risk of recurrence (8). In the last years several authors reported a strong correlation between systemic inflammation and HCC prognosis with different systemic and pathological markers associated with survival and recurrence. For example, high values of platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR) and other similar scores seem to predict poor long-term outcomes after treatment (9–11). This relationship is also evident on a molecular level as gene expression alterations are at the basis of these inflammatory cell shifts on which cancer develops and progresses (12). In this review, we provide a comprehensive overview and update on the prognostic meaning of inflammatory modifications in patients with resectable HCC. We analyze changes in circulant cellular populations and assess inflammatory biomarkers as a surrogate of impaired outcomes and provide an overview on predictive gene expression signatures including inflammatory transcriptional patterns, which are representative of poor survival in these patients.

2 Inflammatory microenvironment in HCC carcinogenesis and prognosis

A large body of knowledge has demonstrated that a dysregulation in tumor microenvironment (TME) contributes to carcinogenesis and tumor progression (13). Chronic inflammation is considered as an excessive, abnormal, and prolonged form of cellular immune responses interacting with other factors in the development of the neoplastic process (14). A large panel of innate immune cells in the tumor microenvironment (macrophages, neutrophils, dendritic cells, innate lymphoid cells, myeloid-derived suppressor cells, and natural killer cells) as well as adaptive immune cells (T cells and B cells) are linked to tumor progression and outcome (15). Tumors control their microenvironment by a large number of tumor-associated factors promoting its establishment, growth, survival, and spread by shaping a pro-tumoral local cytokine milieu (15). This cause-effect relationship is well described in HCC patients and several mechanisms have been shown to be related to tumor development, progression, and overall survival. The majority of HCCs occur in injured liver after stimulation with different inflammation-triggering agents, as viruses, alcohol, drugs, toxins, or obesity (16). Alterations in inflammatory cell populations and a dysregulation of genes and protein expression pattern have been correlated with long-term outcomes in HCC patients. Among many others, these involve an upregulation of several metalloproteinases (MMP) and downregulation of C-type Lectin-like Receptor 2 (CLEC2) which were found to be associated with impaired survival (17). Similarly, hyperexpression of PD-1 and PD-L1 in neoplastic hepatocytes and lymphocytes infiltrating the tumor is a marker of poor survival, while in slowly growing HCC these markers are barely expressed (17). Other authors demonstrated that TNF, IL6 and CCL2 mutations are those most significantly associated with outcomes and considerably longer survival was seen in patients with higher levels of both TNF and IL6 (18, 19). To our knowledge, out of the mentioned markers, targeted therapies have been developed for PD-1 and PD-L1, while the clinical trials targeting the other mentioned markers have so far been unsuccessful, at least in the context of HCC (20–27). The above-mentioned markers have been summarized in Table 1. In regard to cell populations (Figure 1), Kuang and co-workers found that peritumoral stroma of HCC tissues was enriched with neutrophils and their levels could serve as a powerful predictor for poor survival in HCC patients (32). Accordingly, high inflammatory cytokine levels in the tumor can promote local and systemic neutrophilia (33). Lymphocytes are at the same time involved in tumor progression, and an enhanced infiltration of specific subtypes within the tumor samples, as CD8+ and CD3+ T cells, CD20+ B cells and CD56+ NK cells, was found to be present in patients with longer survival (18, 34). A recent study (28) identified a structure formed by specific cell populations and its role in immunotherapy resistance. It was found that a subpopulation of macrophages with high expression of osteopontin (SPP1), in combination with CAFs (cancer-associated fibroblasts) mediates resistance to immune checkpoint inhibitors. Blocking SPP1, a phosphoprotein with a previously identified regulatory role in the TME (35), rendered the

TABLE 1 Markers of the inflammatory microenvironment of HCC patients.

Type of marker	Study	Expression change	Prognostic meaning
MMP1, MMP10, MMP12	Critelli et al., 2017 (17);	Upregulation	Decreased Survival
CLEC2	Critelli et al., 2017 (17);	Downregulation	Decreased Survival
PD1	Critelli et al., 2017 (17);	Upregulation	Decreased Survival
PDL1	Critelli et al., 2017 (17);	Upregulation	Decreased Survival
TNF	Chew et al., 2010 (19); Chew et al., 2012 (18)	Upregulation	Increased Survival
IL6	Chew et al., 2010 (19); Chew et al., 2012 (18)	Upregulation	Increased Survival
CCL2	Chew et al., 2010 (19); Chew et al., 2012 (18)	Upregulation	Increased Survival
SPP1	Liu et al., 2023 (28)	Upregulation	Decreased Survival

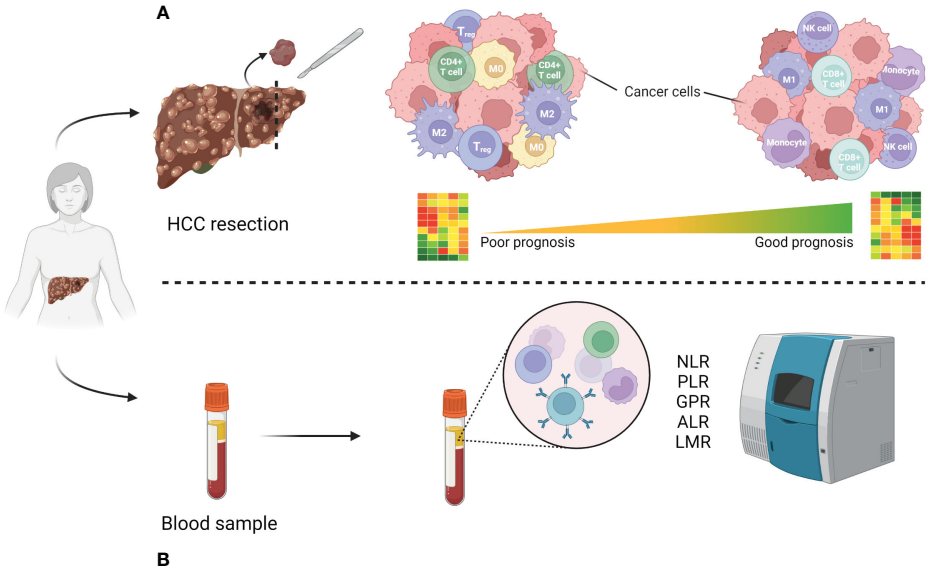

FIGURE 1
Immune cell population difference analysis in poor vs good prognosis patients. **(A)** High-risk resected patient tissue with poor prognosis tends to be enriched with regulatory immune cells (Treg, CD4+ T cell), type 2 macrophages (M2) as well as non-activated macrophages (M0), as opposed to natural killer (NK) cells, CD8+ cytotoxic T cells, type 1 macrophages (M1) and monocytes in good prognosis patients (29–31). **(B)** Most used inflammatory markers analyzable from patient blood samples. Created using BioRender.

tumors more responsive to immunotherapy in an animal model. It was therefore marked as a target for further clinical studies in the context of HCC, but to our knowledge, no such trials are currently in progress. It is also worth noting that this study focused on a restricted number of cases and did not explore the potential of SPP1 as a serum inflammatory marker.

3 Serum inflammatory markers

Based on the strong association between tumor microenvironment and natural history of tumors, modifications in circulating inflammatory markers highlight more aggressive diseases and therefore predict poor outcomes. These patterns have been implemented in clinical practice as scores, which have the advantage of being easy to approach, calculated with routine laboratory tests, thus with limited costs, and available before surgical treatment. The most diffused and described serum inflammatory marker in resected HCC is undoubtedly the NLR (10, 11, 36–40). An increased NLR, despite the different cut-offs used by the authors, seems associated with reduced overall survival and disease-free survival rates after curative resection. Neutrophil count, rather than reduced lymphocytes, could probably explain these results, knowing that elevated neutrophils associated independently with poorer survival and impaired performance status in HCC (41). Although other publications did not support the prognostic value of NLR at univariate or multivariate analysis (11, 36, 40, 42), two meta-analyses confirmed the significant correlation with impaired prognosis in resected patients (43, 44). Another well-established immunity-related score found to be predictive of long-term outcomes in resected HCC is the PLR. Several studies confirmed a strong association between oncologic outcomes and an elevation of this index and, unlike NLR, this biomarker has almost always confirmed its prognostic role at multivariate analysis (10, 11, 38–40, 44, 45). Other less explored scores are the gamma-glutamyl transpeptidase-to-lymphocyte ratio (GLR) (11, 36), the aspartate aminotransferase-to-lymphocyte ratio (ALR) (11, 46) or the lymphocyte-to-monocyte ratio (39, 40), all more or less related to long-term outcomes. A summary of these inflammatory biomarkers as well as studies assessing their prognostic role is shown in Table 2. In order to increase the accuracy of these biomarkers, some authors developed new scores by combining these aforementioned values together or by adding other non-inflammatory variables in the formula. The first group includes indexes as the A-G-P score, a predictive model to accurately predict survival by analyzing at the same time the ALR, the GLR and the PLR (11). This equation demonstrated to be an excellent independent predictor of OS in resected patients and, at the same time, being able to stratify patients with HCC according to the resulting score well (11). On the other hand, other formulas have been developed starting from these inflammatory markers and other serum values, as nutritional indexes. This is the case of the Glasgow prognostic score (GPS) and the modified GPS, calculated from the CRP and the albumin level (47, 48), the prognostic nutritional index (PNI) combining lymphocyte count and serum albumin (29, 49) or the inflammation-immunity-nutrition score (IINS), a combination of CRP, lymphocyte count and serum albumin level (30). All these equations, although not systematically integrated in clinical practice, have been widely described as factors of impaired survival in literature.

TABLE 2 Prognostic meaning of different serum inflammatory markers in resected hepatocellular carcinoma in aforementioned studies.

Type of marker	Study	Cut-off assessed	Number of patients	Prognostic meaning	Role at multivariate analysis
Neutrophil to lymphocyte ratio (NLR)	Sullivan et al., 2014 (42)	–	75	Not predictive of OS	–
	Lu et al., 2016 (37)	2.81	963	Shorter OS and RFS	Independent risk factor for OS and RFS
	Zheng et al., 2017 (39)	–	370	Shorter OS and RFS	Lost
	Wang et al., 2019 (10)	2.92	239	Shorter OS and RFS	Independent risk factor for OS and RFS
	Dai et al., 2020 (36)	2.5	302	Shorter OS and DFS	Lost
	Wu et al., 2021 (11)	2.33	347	Shorter OS, no differences in DFS	Lost
	Silva et al., 2022 (38)	1.715 for OS 2.475 for DFS	161	Shorter OS and DFS	Lost
	Zhou et al., 2022 (40)	4.191 for OS 2.271 for RFS	91	Shorter OS, no differences in RFS	Lost
Platelets to lymphocyte ratio (PLR)	Zheng et al., 2017 (39)	275 for RFS a 298 for OS	370	Shorter OS and RFS	Independent risk factor for OS and RFS
	Wang et al., 2019 (10)	128.1	239	Shorter OS and RFS	Independent risk factor for OS and RFS
	Wu et al., 2021 (11)	117.09	347	Shorter OS, no differences in DFS	Independent risk factor for OS
	Kim et al., 2022 (45)	132	159	Shorter OS and RFS	Independent risk factor for OS
	Silva et al., 2022 (38)	115.05 for OS 100.25 for DFS	161	Shorter DFS	Independent risk factor for DFS
	Zhou et al., 2022 (40)	302.104 for OS 228.644 for RFS	91	Shorter OS and RFS	Independent risk factor for OS and RFS
Gamma-glutamyl transpeptidase to platelet ratio (GPR)	Dai et al., 2020 (36)	0.35	302	Shorter OS and DFS	Independent risk factor for OS and DFS
	Wu et al., 2021 (11)	0.48	347	Shorter OS and DFS	Independent risk factor for OS and DFS
Aspartate aminotransferase to lymphocyte ratio (ALR)	Chen et al., 2021 (46)	26.6 for OS 27.9 for RFS	983	Shorter OS and RFS	Independent risk factor for OS and RFS
	Wu et al., 2021 (11)	31	347	Shorter OS and DFS	Independent risk factor for OS and DFS
Lymphocyte-to-monocyte ratio (LMR)	Zheng et al., 2017 (39)	–	370	Shorter OS and RFS	Lost
	Zhou et al., 2022 (40)	3.785 for OS 4.633 for RFS	91	No differences	–

OS, Overall Survival; RFS, Recurrence-Free Survival; DFS, Disease-Free Survival.

4 Gene signatures

An emerging toolset potentially complementing the classical predictive markers in the clinics are transcriptional gene signatures (GS). They refer to expression values of a group of genes, and are mostly representative of a condition, healthy, diseased or both. The expression pattern of genes is often correlated with the activity of their products and can therefore infer on the cell processes these genes are a part of. Recent technological advancements enable the collection and analysis of large quantities of biological data, as in cases of gene expression values across the genomes of multiple cells. This kicked off the development of gene signatures in several diseases and cancer. Majority of GS have been assessed as predictive tools and are derived from data obtained using techniques such as quantitative PCR (qRT-PCR), hybridization arrays (oligonucleotide, cDNA), RNA sequencing etc., that all

have the analysis of levels of RNA production in common. Most signatures focus on messenger RNA transcription, while some of them are based on microRNA (miRNA) (31), long non-coding RNA (lncRNA) (50) or protein expression (51).

Contrary to most classical prognostic pathological or clinical features, the analysis of gene signatures allows a profound molecular profiling of the tumour environment. As cancer is a multicellular disease often involving several systems within the body, analysing gene expression patterns from multiple cell types facilitates identification of dysregulated pathways and their comprehension. Gene signatures provide a list of differentially expressed genes (DEG), upregulated or downregulated between the compared groups, usually diseased and non-diseased or healthy conditions. Tissues that are presumably not affected but surrounding the cancer area are usually considered as non-diseased, while healthy tissue is obtained from regions distant from the affected area. Out of the selected genes, some are linked to a poor prognosis or high risk while others are marked as good-prognosis or low risk genes. Therefore, the combination of both poor and good prognosis gene expression pattern allows a classification of patients into high and low-risk groups. The predictive capacity of a signature is mostly measured using machine learning-derived ROC (Receiver Operating Characteristics) and AUC (Area Under the ROC Curve) values, while some authors also use confidence intervals. The closer the AUC value is to one, the more accurate the predictive signature is (52). Recent analyses have studied the drawbacks of gene signatures, notably their redundancy and possibilities of improving them (53). Even with drawbacks, these signatures can be efficient for a statistically important number of patients and therefore their use in clinical practice should not be ignored.

4.1 Gene signatures predicting HCC recurrence and survival in resected patients

To date, most signature-based studies focus on predicting recurrence as well as survival in HCC patients. A study from 2020, found that 66% of patients experienced HCC recurrence over a period of 8 years emphasizing the drastic recurrence rates of HCC (54). Although still debated, the classification of tumor recurrence into early and late recurrence is strongly linked to the tumour origin. Secondary tumours originating from leftover cancer cells of the resected tumour within two years after surgery are defined as early recurrence, whereas tumours originating from novel cancer cells of the same organ (*de novo* tumour) more than two years after surgery are considered as events of late recurrence (55).

As early as 2008, the first collection of 186 genes was published in the pioneering work from Hoshida et al., highlighting 73 poor and 113 good prognosis genes being predictive for survival in liver disease (56–59). The authors established a robust signature of DEGs from tissues surrounding HCC of 106 resected patients which was then validated in another cohort of 234 patients. They managed to overcome the technical difficulty to analyse more commonly available formalin- and paraffin-treated (FFPE) tissues instead of depending on snap frozen tissues. This signature has since been further studied and validated in additional cohorts. A 5-gene signature from frozen liver tissues was reported (TAF9, RAMP3, HN1, KRT19, and RAN) predicting survival from HCC in 314 HCC patients (60). Depending on the differential expression of these five genes, patients were stratified into poor and good prognosis groups, and the signature was validated in external cohorts of patients. As reported by Nault and co-workers (60), the comparison of the two signatures described above validated the findings from both articles, also as the signatures provide similar output, i.e., a comparable stratification of patients in their corresponding poor and good survival groups. More recently, a signature specific for early recurrence in HCC has been described, which was not based on coding genes but on 25 lncRNAs, another type of RNA relevant in HCC development (50). This signature had better predictive performance than multiple other factors, including serum AFP. Interestingly, the high and low-risk groups correlated with the immune characterization of the tissue of these patients; for example, the low-risk group showed higher levels of tumour-infiltrating lymphocytes. Another 9-gene survival signature with links to immune microenvironment was derived from the analysis of 274 resected HCC patient tissues by another group (61). Of the four upregulated (C2HC1A, MARCKSL1, PTGS1, CDKN2B) and five (CLEC10A, PRDX3, PRKCH, MPEG1, LMO2) downregulated genes in poor prognosis patients, several signature genes have direct or indirect roles in cancer immune environment (CLECL10A, PTGS1, C2HC1A). Even though they focused on data from HCC patients of viral aetiology, their established signature is seemingly outperforming the previously established ones (61). Finally, a more recurrence-specific gene signature had been identified by comparing recurrence and non-recurrence HCC tissues from 85 patients (62). Within the selected genes, two (HMGA1 and RACGAP1) were found to be particularly relevant for recurrence in HCC patients. Interestingly, both genes have recently been studied for their role in cancer immunity (63, 64). However, while some of the signature genes are known to have roles in HCC, they are generally parts of unrelated pathways and do not necessarily interact with each other.

4.2 Inflammatory gene signatures

As single-cell resolution in transcriptomic analysis boosted our understanding of the HCC microenvironment (65, 66), signatures derived from immune cell populations or linked to immunity in HCC have been increasingly explored in the recent years. However, most of these studies tend to use a variety of patient tissues as source, including results from not only resected patients, but also biopsies of advanced HCC or data found in online databases, mainly from The Cancer Genome Atlas (https://www.cancer.gov/tcga). To our knowledge, resection-specific immune gene signatures have yet to be established. A study from 2021 established a robust immune-related gene signature containing seven genes from TCGA-derived data of 372 patients with a variety of backgrounds (histological grade, clinical stage, survival rate etc.) (67). Six out of seven genes (S100A8, BIRC5, CACYBP, NR0B1, RAET1E, SPP1)

were associated with high-risk of survival, while SPINK5 was identified as a low-risk factor. On the cellular level they found that immunosuppressive cell groups such as CD4+, Treg cells, M0 and M2 macrophages, as well as neutrophiles were more abundant in the high-risk groups compared to the low-risk ones (Figure 1). This signature, however, needs further testing before it can be confidently applied in patients. Another recent study used a similar but more focused approach, as they report developing an eight gene signature based on M2-like tumour associated macrophages from both patient biopsies and resections (68). Similar findings were reported by two independent studies, whose 6 and 8 immune-related gene signatures had an AUC of 0.71 and 0.68, respectively (69, 70). Finally, Shi and co-workers reported a non-invasive immune signature for early-stage HCC based on the analysis of cells from patient blood samples using single cell cytometry (65). In this dynamic immune atlas, they identify mainly lymphocyte (sub) types characterizing advanced stages of HCC using only patient blood samples. In general, most recent immune signatures tend to have less than 10 genes and their AUC values vary from 0.65 to 0.75. These have been summarized in Table 3. Of note, all the listed studies report the tendency of presence of contrasting immune cell types within the high-risk compared to low-risk group: the high-risk patient group tends to be enriched with macrophages and Tregs while B, NK, cytotoxic T cells and mast cells are less represented.

5 Challenges & future directions

Inflammation is a key player in the natural history of HCC and thus the relationship between some inflammatory-based tools and patients' prognosis are closely linked by the disease biology of hepatocarcinogensis (71–74). This observation offers an opportunity to predict long-term outcomes as precise as possible if compared to current markers. Although some of the biological markers above cited clearly show a direct and independent connection with recurrence and survival after liver resection for HCC, they are far from being extensively implemented in clinical practice. Limitations of the currently available serum biomarkers are the difficulty in standardizing reliable cut-offs as well as the universal validation of their prognostic role, regardless of underlying patient pathologies or cancer aetiology. When assessing the above-mentioned ratio (NLR, PLR, etc.), cut-off values are determined by the AUC and therefore always different among all the studies. As a result, we found that authors use various values to define cases with impaired outcomes and, sometimes, these values are significantly different if considering the type of outcome assessed, as recurrence or survival (38, 40). A recent meta-analysis assessing the role of NLR, found that among 13 included studies the cut-off values ranged between 1.505 and 5.0, and only a few studies used the same ratio (43).

Another issue to solve is the large-scale applicability of these markers in clinical practise. This review focusses on resected patients which represent a large minority of all diagnosed HCC. This type of lesion often develops on an immunity-altered host which can distort the results and thus the direct correlation between serum markers and prognosis. Furthermore, authors usually analyze specific subgroups of HCC patients in order to create a homogeneous cohort, as tumors in well-compensated cirrhosis (40). In 2016, Lu et al. assessed the utility of the NLR and used subgroup analysis to examine this potential relationship separately in patients in BCLC stages 0/A, B, or C (37). The authors found that this marker may be a good predictor of survival in early/intermediate stage, whereas it was

TABLE 3 A summary of immune-related predictive signatures in HCC: their predictive power, data origin and the defined genes.

Signature	Study	AUC	Good/Poor prognosis genes	Data origin	Patients
An Inflammatory Response-Related Gene Signature Can Impact the Immune Status and Predict the Prognosis of HCC	Zhuo et al., 2021 (69)	0.685, 0.626, 0.605 at 1, 2, and at 3 years	SERPINE1 ADORA2B, MEP1A, P2RX4, ITGA5, NOD2, RIPK2, SLC7A	TCGA LIHC&ICGC	>400
Survival prediction and response to immune checkpoint inhibitors: A prognostic immune signature for HCC	Ying et al., 2021 (70)	0.71 at 5-year survival	FYN, IGF1, MASP1, NR3C2, TGFBR3 BIRC5	TCGA&GEO	>400
Identification of a prognostic and therapeutic immune signature associated with HCC	Peng et al., 2021 (67)	0.77, 0.73, and 0.74 in predicting 1-, 3-, 5-year overall	SPINK5 BIRC5, CACYBP, NR0B1, RAET1E, S100A8, SPP1	TCGA, GEO & ICGC	>400
M2-like tumor-associated macrophage-related biomarkers to construct a novel prognostic signature, reveal the immune landscape, and screen drugs in HCC	Qu et al., 2022 (68)	1, 3, and 5 years was 0.728, 0.689, and 0.663,	KLF2 LIM3, PAM, PDLIM7, FSCN1, DPYSL2, ARID5B, LGALS3	TCGA, GEO & ICGC	>400
Single-cell immune signature for detecting early-stage HCC and early assessing anti-PD-1 immunotherapy efficacy	Shi et al., 2022 (65)	–	Cells, no genes specified	PBMC at resection	~50

TCGA, The Cancer Genome Atlas; LIHC, Liver Hepatocellular Carcinoma; ICGC, International Cancer Genome Consortium; GEO, Gene Expression Omnibus; PBMC, peripheral blood mononuclear cells.

not associated with risk of overall survival (OS) or tumor recurrence in patients with stage C disease. Similar limitations are found when comparing the potential of transcriptional signatures. Despite the very promising results from a decade of development, no predictive transcriptomic signature is used in a clinical setting. Like the serum biomarkers, the AUC values used to quantify the power of GS vary significantly, and do not have confirmed utility until the signatures are confirmed by other teams or in clinical settings. Also, as we mentioned earlier and for the purpose of this review, resection-specific immune/inflammatory gene signatures have been scarce. However, a promising immune signature has been recently identified using artificial intelligence on transcriptomics of resected patients (75). The authors argue their approach would allow for the bypass of technical bias and restriction induced with a more "classical" gene signature approach. Moreover, patient samples used are often restricted to small numbers, a single country, patient population or aetiology, potentially affecting the applicability of these signatures without validation in other cohorts (61, 62, 68). An additional important limitation of the transcriptional signatures is their dependency on patient liver tissues. Non-invasive methods, such as described by Shi and co-workers (65), should thus be prioritized in the future. Initiatives to translate transcriptional signatures into minimal-invasive blood surrogates have already been taken with a recently published eight-protein signature termed PLSec (76). It is based on the 186-gene PLS (56, 58) and is predictive for survival, as well as recurrence of HCC in advance fibrosis patients. The very encouraging data are based on the analysis of 400 patients in total and pave the wave for further consolidation in larger cohorts. Out of the eight, 6 proteins were marked as high-risk, including vascular cell adhesion molecule 1 (VCAM-1), insulin-like growth factor-binding protein 7 (IGFBP-7), gp130, matrilysin, IL-6, and C-C motif chemokine ligand 21 (CCL-21), and 2 were defined as low-risk-associated serum proteins, angiogenin and protein S. Collectively, new combinations of classical and novel blood-based biomarker signatures will likely have the biggest impact in transforming patient care.

Finally, beyond the pure prognostic meaning, another non-negligible potential of these biomarkers lies undoubtedly in the possibility of guiding therapeutic approaches in advanced disease. Finding a biomarker which could accurately predict tumor progression or response to specific treatments would mean opening the door to precision medicine in HCC, as already established in other cancers (77). Although immunotherapy is the first-line option in these patients, with drugs targeting different checkpoints of the immune system, no correlation between tissue and serum inflammatory markers and chemotherapy sensibility have been demonstrated in literature to date. Other non-inflammatory biomarkers have been tested with usually poor or not significant results (78). Currently, there is no established role or indication for molecular or genetic testing in HCC due to the absence of specific benefit. Only a few mutations can influence the therapeutic algorithm in HCC but exclusively in case of progression after first-line administration, and in certain circumstances (79). Similarly, ramucirumab, another second-line option, has shown better outcomes in advanced HCC with AFP > 400 ng/ml previously treated with sorafenib, leading international drug agencies to approve this anti-VEGF drug in this setting (80). However, the restriction of ramucirumab to patients with AFP > 400 ng/ml does not mean that this should be the agent of choice for that population (81). Further trials are therefore urgently needed to identify new biomarkers for precision medicine in HCC.

Author contributions

FG: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. NS: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. PP: Validation, Writing – review & editing. CS: Supervision, Writing – review & editing. TB: Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing – review & editing. JL: Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

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

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Prognostic value of platelet-to-lymphocyte ratio in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization and tyrosine kinase inhibitors plus immune checkpoints inhibitors

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Purpose: To investigate the prognostic value of platelet-to-lymphocyte ratio (PLR) in patients with unresectable hepatocellular carcinoma (uHCC) treated with transarterial chemoembolization (TACE) and tailored tyrosine kinase inhibitors (TKIs) plus immune checkpoints inhibitors (ICIs).

Materials and methods: Ninety-eight patients from May 2018 to January 2022 in our hospital were enrolled in this study. The receiver operating characteristic (ROC) curve analysis was performed and the corresponding Youden index was used to determine the optimal PLR cut-off. Overall survival (OS), progression-free survival (PFS), and adverse events (AEs) of patients were evaluated based on the PLR cut-off. The factors affecting survival were assessed using univariate and multivariate Cox proportional hazards regression analyses.

Results: The PLR cut-off was 98.89. There were 49 patients in the low pretreatment PLR group (PLR ≤ 98.89) and 49 patients in the high PLR group (PLR > 98.89). Patients with low pretreatment PLR had significantly longer median OS (25.7 months vs 16.1 months; $P < 0.001$) and PFS (14.9 months vs 10.2 months; $P < 0.001$) than those with high pretreatment PLR. The multivariate analysis revealed that ALT, tumor size, and PLR are risk factors affecting OS. The three independent factors affecting PFS are tumor size, AFP, and PLR. The AEs were tolerable and manageable.

Conclusion: The low pretreatment PLR (PLR ≤ 98.89) was an independent protective factor for the survival outcomes of patients in this study. PLR was helpful for clinicians to predict the prognosis and identify the patients with uHCC who were most likely to benefit from TACE + TKIs + ICIs.

KEYWORDS

platelet-to-lymphocyte ratio, hepatocellular carcinoma, transarterial chemoembolization, tailored tyrosine kinase inhibitors, immune checkpoint inhibitors

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death in the world (1). Patients who are diagnosed with early-stage HCC have the opportunity to undergo curative treatments (2, 3). Since the onset of HCC is insidious, a majority of patients with HCC are diagnosed at intermediate or advanced stage and are not suitable for curative resection (4).

According to the guidelines (5, 6), transarterial chemoembolization (TACE) has been recommended as a standard treatment for intermediate and advanced HCC. Since the efficacy of TACE is associated with tumor size, vascular invasion and distant metastasis (7), it is challenging to achieve complete tumor necrosis using TACE alone. In addition, TACE could increase the expression of programmed cell death ligand 1 (PD-L1) and vascular endothelial growth factors (VEGF) as a result of the hypoxic microenvironment after embolization, contributing to the tumor recurrence and metastasis (8, 9).

It is known that immune checkpoints, including programed cell death protein 1 (PD-1), programed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), can suppress the T-cell-mediated immune responses, which permits cancer cells to escape from the immune destruction (10). Immune checkpoint inhibitors (ICIs) such as camrelizumab and atezolizumab act to block the interaction of immune checkpoints and the corresponding ligands. As a result, tumor-reactive T cells are able to overcome the negatively regulatory mechanisms caused by immune checkpoints and facilitate an effective anti-tumor response (11).

Angiogenic factors such as VEGF can bind to VEGF receptors (VEGFRs) to suppress immune responses by inducing vascular abnormalities, inhibiting antigen presentation, or enhancing the activity of regulatory T cells to suppress the immune system (12, 13). Tyrosine kinase inhibitors (TKIs) can exactly block the intracellular domain of VEGFR to impede the immunosuppression effects of VEGF (14).

Thus, systemic therapy, including ICIs and TKIs, has been recommended as the first-line treatment for patients with advanced HCC (15). Based on the guidelines for primary liver cancer (16), it is recommended to combine TACE with systemic therapy to enhance the efficacy of TACE. And many studies have investigated the efficacy of TACE and TKIs plus ICIs, demonstrating significantly higher tumor response and survival benefits (17–19).

Some studies have shown that inflammatory and immune environments play an important role in the formation and progression of HCC (20, 21). And many studies have evaluated the effects of various inflammatory and immune biomarkers in predicting the outcomes of patients with malignant tumors (22–24). High platelet counts can stimulate angiogenesis and tumor proliferation by enhancing the secretion of growth factors, such as VEGF and platelet-derived growth factors (25). Decreased lymphocyte counts are related to an insufficient immunologic reaction to the tumor, which consequently enable tumor progression and metastasis (26). Increased platelet counts along with decreased lymphocyte counts lead to an elevated PLR, which is associated with unfavorable clinical outcome in HCC patients receiving TACE alone or TACE plus TKIs (27–29). However, the prognostic value of PLR for uHCC patients treated with TACE + TKIs + ICIs has not been evaluated.

This study aimed to investigate the effectiveness of pretreatment PLR in predicting the survival outcomes of uHCC patients treated with TACE + TKIs + ICIs.

Materials and methods

Patients

The Institutional Review Board in our hospital approved this retrospective study, and the informed consent was waived. This study was conducted in accordance with the Declaration of Helsinki.

Patients with uHCC received TACE + TKIs + ICIs between May 2018 and January 2022 in our hospital were enrolled in this study. HCC was diagnosed by pathological examination or noninvasive criteria based on the European Association for the Study of the Liver (EASL) guidelines (6). A multidisciplinary team determined the patients' treatment decisions.

The inclusion criteria for this study were as follows: 1) age ≥ 18 years; 2) confirmed diagnosis with uHCC; 3) Eastern Cooperative

Oncology Group (ECOG) scores ≤ 1; 4) Child-Pugh A or B; 5) adequate cardiac, renal and coagulation function; 6) treated with TACE + TKIs + ICIs.

The exclusion criteria were as follows: 1) previous HCC-related treatments, including hepatic resection, liver transplantation, systemic therapy, local ablation or TACE; 2) Child-Pugh C; 3) presence of other malignancies in addition to HCC; 4) incomplete data.

Treatment protocol

TACE was performed under local anesthesia via right femoral artery. The Seldinger technique and angiography were performed to identify the tumor-feeding arteries and assess the tumor burden. According to the tumor burden, 5-15mL of emulsion containing 10-20 mg of doxorubicin hydrochloride (Hisun Pharmaceutical Co.LTD, Zhejiang, China) was mixed with 5-10 mL of lipiodol (Lipiodol Ultrafluido, Guerbet, France) and injected into the tumor-feeding arteries through a 3-F microcatheter. Finally, an appropriate amount of gelatin sponge particles (350-560 μm; Cook) was injected into the tumor-feeding arteries to induce embolization.

TKIs including sorafenib (800 mg), lenvatinib (8 or 12 mg), and apatinib (500 mg) were administered orally daily. ICI immunotherapy with intravenous fixed-dose camrelizumab (200 mg) was performed every 3 weeks until disease progression or unexpected toxicity was observed. The dose and interval of TKIs were adjusted according to the toxicity and disease conditions. The administration of TKIs and ICIs should be stopped when unacceptable toxicity occurred or no clinical benefits were observed. What's more, TKIs and ICIs were discontinued for 3 days before and after TACE.

Outcomes and follow-up

All laboratory indicators and radiological data were collected within 7 days of initial treatment. PLR was calculated as absolute platelet count divided by absolute lymphocyte count prior to the initial treatment. All patients were followed up every 4-8 weeks. The laboratory and imaging information of patients were recorded at each appointment. Two radiologists with more than 10 years experience in abdominal radiology evaluated the imaging examinations. Both of them were blinded to the patients' clinical information. TACE was recommended if the patient had a residual tumor or disease progression during the follow-up. Adverse events (AEs) in this study were monitored and recorded by experienced nurses according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (30).

Overall Survival (OS) and Progression-Free Survival (PFS) were outcomes of this study. OS was defined as the interval from the initial treatment to death or the last follow-up. PFS was defined as the time between the initial treatment and disease progression according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) (31), death or the last follow-up.

Statistical analysis

SPSS version 24.0 (IBM, Chicago, Illinois, USA) was used for the statistical analyses. Continuous variables and categorical variables were presented as median (interquartile range) and frequencies (percentages), respectively. The time-dependent ROC curve analysis was performed and the corresponding Youden index was used to determine the optimal PLR cut-off for patients with uHCC. Continuous variables at baseline for the high and low PLR groups were compared using Student's t-test or Mann-Whitney U test. Chi-square test or Fisher's exact test was used to compare categorical variables. OS and PFS curves were drawn by the Kaplan-Meier method and were compared using log-rank tests. Risk factors related to OS and PFS were identified by univariate and multivariate Cox proportional hazards regression analysis. Factors with $P < 0.05$ at univariate analysis were included in the multivariate analysis. $P < 0.05$ was considered statistically significant.

Results

Baseline statistics

During the follow-up, a total of 128 uHCC patients treated with TACE + TKIs + ICIs were enrolled in this study. However, thirty patients were excluded according to the exclusion criteria (Figure 1). The ROC curve analysis was performed and the Youden index suggested that the optimal PLR cut-off was 98.89. The area under the ROC (AUC) curve was 0.77 (Figure 2). According to the cut-off, forty-nine (50.0%) patients with PLR > 98.89 were divided into the high PLR group and the rest 49 (50.0%) patients with PLR ≤ 98.89 were divided into the low PLR group. The baseline characteristics of these patients were presented in Table 1, with no statistical difference between the two groups.

OS and PFS

The Kaplan–Meier analysis showed that the median OS (mOS) of patients in the low PLR group was higher than that of those in the high PLR group (25.7 months vs 16.1 months; $P < 0.001$) (Figure 3A). Similarly, the median PFS of patients in the low PLR group was also higher than the high PLR group (14.9 months vs 10.2 months; $P < 0.001$) (Figure 3B).

Risk factors affecting OS and PFS

Univariate Cox proportional hazards regression analysis revealed that alanine transaminase (ALT) (hazard ratio [HR]: 1.035; 95% confidence interval [CI]: 1.002-1.069; $P = 0.039$), tumor size (HR: 1.186; 95% CI: 1.059-1.328; $P = 0.003$), and PLR (HR: 8.547; 95% CI: 2.902-25.170; $P = 0.000$) were risk factors affecting OS (Table 2). The factors related to PFS (Table 3) included tumor size (HR: 1.135; 95% CI: 1.031-1.249; $P = 0.010$), alpha-feto

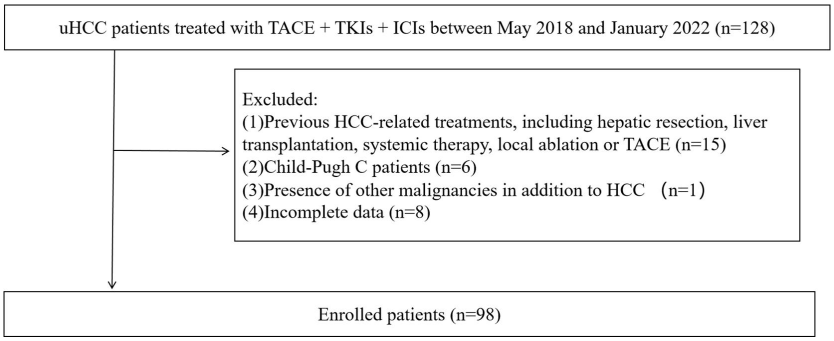

FIGURE 1
Flow chart.

protein (AFP) (HR: 2.516; 95% CI: 1.281-4.940; P = 0.007), and PLR (HR: 4.882; 95% CI: 2.336-10.205; P = 0.000). Multivariate Cox proportional hazards regression analysis identified three risk factors affecting OS: ALT (HR: 1.022; 95% CI: 1.006-1.038; P = 0.006), tumor size (HR: 1.121; 95% CI: 1.045-1.202; P = 0.001), and PLR (HR: 6.680; 95% CI: 3.055-14.606; P = 0.000). Three independent factors affected PFS: tumor size (HR: 1.110; 95% CI: 1.037-1.188; P = 0.003), AFP (HR: 1.940; 95% CI: 1.095-3.437; P = 0.023) and PLR (HR: 3.540; 95% CI: 2.004-6.254; P = 0.000).

Safety

All AEs were presented in Table 4. There was no treatment-related death observed in this study. The most common TACE-related AEs were postembolization syndrome that included nausea (57.2%), vomiting (34.7%), abdominal pain (59.2%), and fever (83.7%). And the most common drug-related AEs were hypertension (24.5%), fatigue (49.0%), headache (14.3%), skin capillary hyperplasia (18.4%), hypothyroidism (22.4%), and pneumonia (2.0%). For grade 3 or 4 AEs, only nausea, fever, hypertension and fatigue had incidences of >5%. (Table 4).

There was no statistical difference in the incidence of most AEs between the two groups. However, the incidence of some immunotherapy-related adverse events (irAEs) of any grade in the low PLR group was significantly higher than that in the high PLR group, with no statistical difference in grade 3 or 4 AEs (Table 4).

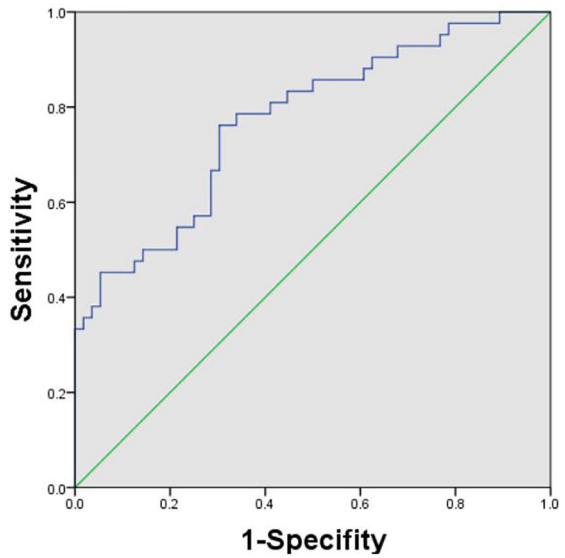

FIGURE 2
Receiver operating characteristic curve analysis was performed to determine the optimal cut-off for PLR. The cut-off was 98.89. PLR, platelet-to-lymphocyte ratio.

Discussion

It is known that the prognosis of patients with uHCC is poor due to drug resistance, frequent recurrence, and metastasis [32]. With the advent of immunomodulatory antibodies and molecular-targeted drugs, a new combination strategy combining TACE + TKIs + ICIs has shown favorable results for uHCC patients (17, 19). However, given that the biological heterogeneity of uHCC and the tumor microenvironment might impair treatment effectiveness, not all patients can benefit from this treatment and the high medical cost is also a worrisome issue. Therefore, it is warranted to identify the patients who are most likely to benefit from this triple therapy.

Accumulating evidence indicates that the inflammatory tumor microenvironment contributes to tumor occurrence and progression and may affect the prognosis of patients with malignancies (33, 34). PLR, as a biomarker that correlates systemic inflammation and immune function, has been shown to be a prognostic factor in various tumors (Chen et al., 2020; 35; 36). In the present study, we evaluated the prognostic value of pretreatment PLR for uHCC patients treated with TACE + TKIs + ICIs.

Our results suggested that patients with low pretreatment PLR had better prognosis than those with high pretreatment PLR. The mOS increased from 16.1 to 25.7 months (P < 0.001), and the corresponding median PFS increased from 10.2 to 14.9 months (P < 0.001). This indicated that pretreatment PLR grading could predict the survival outcomes of uHCC patients treated with TACE + TKIs + ICIs.

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TABLE 1 The baseline characteristics of patients.

Characteristics	PLR ≤ 98.89	PLR >98.89	P value
Age(years)	57.2 ± 6.54	56.8 ± 5.10	0.693
ALT	33.1 ± 16.6	30.2 ± 16.7	0.384
AST	38.9 ± 17.3	43.2 ± 15.1	0.189
TB (μmol/L)	16.9 ± 5.77	16.6 ± 5.2	0.808
Albumin(g/dl)	36.2 ± 5.0	35.4 ± 5.8	0.469
PLT	136.8 ± 59.2	140.34 ± 63.7	0.382
Tumor size (cm)	7.1 ± 3.7	8.0 ± 4.5	0.251
Sex			0.493
male	44	42	
female	5	7	
Treatment protocols			0.670
TACE + sorafenib + camrelizumab	22	24	
TACE + lenvatinib + camrelizumab	17	13	
TACE + apatinib + camrelizumab	10	12	
Number of tumors			0.686
1	27	24	
≥2	22	25	
BCLC stage			0.211
B	22	15	
C	27	34	
Cirrhosis			1.000
Yes	47	48	
No	2	1	
ascites			0.289
Yes	14	20	
No	35	29	
Portal vein invasion			0.225
Yes	20	27	
No	29	22	
Extrahepatic metastases			0.200
Yes	24	32	0.153
No	25	17	
AFP (ng/ml)			0.538
<400	18	22	
≥400	31	27	
Child-Pugh			0.815
A	36	38	
B	13	11	
ECOG performance			0.076
0	39	30	
1	10	19	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; PLT, platelet; BCLC, Barcelona Clinic Liver Cancer; AFP, a-fetoprotein; ECOG, Eastern Cooperative Oncology Group.

Univariate and multivariate Cox proportional hazards regression analysis showed that ALT, tumor size, and PLR were independent risk factors for OS and that tumor size, AFP (≥400 ug/L) and PLR were predictors for PFS. The results suggested that patients with larger tumors had a higher risk of all-cause mortality and tumor progression than those with smaller ones. It may be accounted for that the larger HCC generally has significant necrosis and inflammation pathophysiologically, which contribute to carcinogenesis and tumor progression (33, 37). What's more, the larger HCCs have poorer response to TACE than smaller ones (38).

Immunotherapy related hepatotoxicity often presents as an increase in ALT or AST (39). Our results suggested that elevated ALT levels before TACE + TKIs + ICIs could predict the OS of uHCC patients, which was consistent with previous research results (40). Therefore, it is challenging for clinicians to manage the patients' liver function. AFP is one of the biomarkers of HCC, and we found that elevated AFP levels were correlated with tumor progression. It indicated that AFP could be used as a potential biomarker to predict the tumor progression in uHCC patients treated with TACE + TKIs + ICIs. In addition, our results showed that patients with low pretreatment PLR had lower risks for tumor progression and all-cause mortality than those with high PLR, indicating that PLR is a promising biomarker to predict the survival outcomes of uHCC patients treated with TACE + TKIs + ICIs.

In reference to AEs, this study suggested that TACE + TKIs + ICIs was well-tolerated and its side effects were manageable. The incidences of some irAEs including skin capillary hyperplasia and hypothyroidism were significantly higher in the low PLR group than those in the high PLR group (P = 0.033, P = 0.002, respectively). It might be accounted for the stronger antitumor immune response in the low PLR group. And this result indicated that a low pretreatment PLR might be a predictor of the occurrence of irAEs. As the triple therapy may elicit strong immune responses, these irAEs should be carefully supervised in clinical practice.

While our study showed that the combination therapy of TACE + TKIs + ICIs was a promising approach to treat uHCC, it was also significant to delve into the potential role of second-line immunotherapy in uHCC if patients' responses to the combination therapy were inadequate or the disease progressed. Some studies (41, 42) had investigated the efficacy of second-line

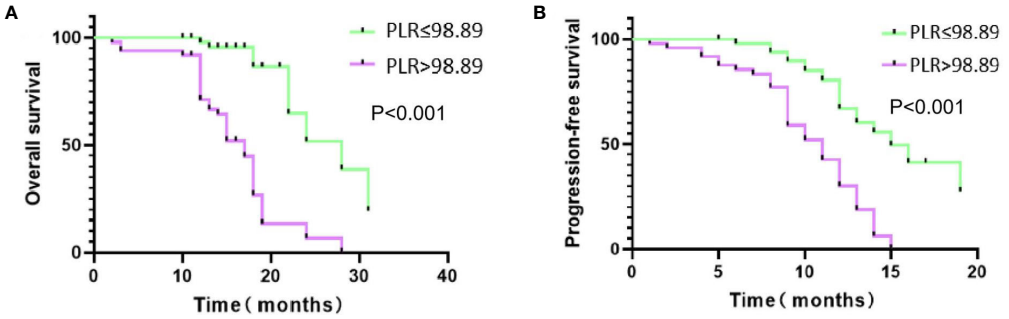

FIGURE 3
Kaplan-Meire (K-M) curves of two groups. **(A)** K-M curve for overall survival (OS); **(B)** K-M curve for progression-free survival (PFS).

TABLE 2 Univariate and multivariate Cox proportional hazards regression analysis of risk factors for OS.

Varaiable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age (years)	0.970 (0.899-1.046)	0.429		
ALT	1.035 (1.002-1.069)	**0.039**	1.022 (1.006-1.038)	**0.006**
AST	0.985 (0.954-1.017)	0.356		
TB	1.004 (0.913-1.105)	0.929		
Albumin	0.919 (0.842-1.003)	0.058		
PLT	0.997 (0.987-1.007)	0.548		
Tumor size (cm)	1.186 (1.059-1.328)	**0.003**	1.121 (1.045-1.202)	**0.001**
Sex		0.535		
Male	1			
Female	0.667 (0.186-2.399)			
Number of tumors		0.609		
1	1			
≥2	0.777 (0.296-2.041)			
BCLC stage		0.501		
B	1			
C	1.864 (0.303-11.464)			
cirrhosis		0.323		
Yes	1			
No	0.294 (0.026-3.330)			
ascites		0.456		
Yes	1			
No	0.6148 (0.193-2.122)			
Portal vein invasion		0.187		
Yes	1			
No	3.206 (0.843-10.200)			

(Continued)

TABLE 2 Continued

Varaiable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Extrahepatic metastasis		0.310		
Yes	1			
No	2.103 (0.501-8.823)			
AFP		0.560		
<400	1			
≥400	1.295 (0.543-3.087)			
Child-Pugh		0.198		
A	1			
B	3.355 (0.801-12.055)			
ECOG performance		0.692		
0	1			
1	1.177 (0.526-2.632)			
PLR		**0.000**		**0.000**
PLR ≤98.89	1		1	
PLR >98.89	8.547 (2.902-25.170)		6.680 (3.055-14.606)	

OS, overall survival; HR, hazard ratio; CI, confidence interval; ALT, alanine transaminase; AST, aspartate aminotransferase; TB, total bilirubin; PLT, platelet; BCLC, Barcelona Clinic Liver Cancer; AFP, a-fetoprotein; ECOG, Eastern Cooperative Oncology Group; PLR, platelet-to-lymphocyte ratio.
The bold values means $P < 0.05$, which is considered statistically significant.

TABLE 3 Univariate and multivariate Cox proportional hazards regression analysis of risk factors for PFS.

Varaiable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age (years)	1.007 (0.944-1.075)	0.828		
ALT	0.995 (0.967-1.022)	0.697		
AST	1.010 (0.984-1.036)	0.446		
TB	0.968 (0.886-1.058)	0.476		
Albumin	1.010 (0.916-1.115)	0.835		
PLT	0.996 (0.989-1.004)	0.370		
Tumor size (cm)	1.135 (1.031-1.249)	**0.010**	1.110 (1.037-1.188)	**0.003**
Sex		0.230		
Male	1			
Female	0.574 (0.232-1.420)			
Number of tumors		0.423		
1	1			
≥2	1.333 (0.6600-2.690)			
BCLC stage		0.567		
B	1			

(Continued)

TABLE 3 Continued

Varaiable	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
C	1.461 (0.399-5.344)			
cirrhosis		0.078		
Yes	1			
No	0.268 (0.061-1.167)			
ascites		0.172		
Yes	1			
No	0.489 (0.175-1.366)			
Portal vein invasion		0.997		
Yes	1			
No	1.002 (0.342-2.937)			
Extrahepatic metastasis		0.662		
Yes	1			
No	0.662 (0.250-1.755)			
AFP		**0.007**		**0.023**
<400	1		1	
≥400	2.516 (1.281-4.940)		1.940 (1.095-3.437)	
Child-Pugh		0.963		
A	1			
B	0.969 (0.253-3.704)			
ECOG performance		0.864		
0	1			
1	1.063 (0.526-2.151)			
PLR		**0.000**		**0.000**
PLR ≤98.89	1		1	
PLR >98.89	4.882 (2.336-10.205)		3.540 (2.004-6.254)	

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; ALT, alanine transaminase; AST, aspartate aminotransferase; TB, total bilirubin; PLT, platelet; BCLC, Barcelona Clinic Liver Cancer; AFP, a-fetoprotein; ECOG, Eastern Cooperative Oncology Group; PLR, platelet-to-lymphocyte ratio.
The bold values means $P < 0.05$, which is considered statistically significant.

TABLE 4 Adverse events of two groups.

Adverse events	Any grade			Grade III or IV		
	PLR ≤ 98.89 (N,%)	PLR>98.89 (N,%)	P value	PLR ≤ 98.89 (N,%)	PLR>98.89 (N,%)	P value
Nausea	28(57.2%)	27 (55.1%)	0.839	4 (8.2%)	3 (6.1%)	0.696
Vomiting	17 (34.7%)	19 (38.8%)	0.675	1 (2.0%)	1 (2.0%)	1.000
Abdominal pain	29 (59.2%)	30 (61.2%)	0.836	1 (2.0%)	2 (4.1%)	0.560
Fever	41 (83.7%)	39 (79.6%)	0.602	7 (14.3%)	5 (10.2%)	0.513
Hypertension	12 (24.5%)	13 (26.5%)	0.817	3 (6.1%)	2 (4.1%)	0.648
Fatigue	24 (49.0%)	25 (51.0%)	0.840	3 (6.1%)	4 (8.2%)	0.696

(Continued)

TABLE 4 Continued

Adverse events	Any grade			Grade III or IV		
	PLR ≤ 98.89 (N,%)	PLR>98.89 (N,%)	P value	PLR ≤ 98.89 (N,%)	PLR>98.89 (N,%)	P value
Headache	7 (14.3%)	6 (12.2%)	0.766	0 (0)	0 (0)	1.000
Skin capillary hyperplasia	9 (18.4%)	1 (2.0%)	**0.033**	2 (4.1%)	0 (0)	0.155
Hypothyroidism	11 (22.4%)	1 (2.0%)	**0.002**	0 (0)	0 (0)	1.000
Pneumonia	1 (2.0%)	0 (0.0%)	0.317	0 (0)	0 (0)	1.000

PLR, platelet-to-lymphocyte ratio.
The bold values means P < 0.05, which is considered statistically significant.

immunotherapy, such as pembrolizumab and nivolumab, in advanced HCC and showed favorable results. However, given the variability in treatment response observed in immunotherapy, it is crucial to understand the responses to second-line treatments to optimize treatment selection and sequencing (43). In addition, it is also warranted to identify predictive biomarkers to aid in stratifying patients who are most likely to benefit from second-line immunotherapy.

Our study had some limitations. First, it was a retrospective and single-center study, which might cause selection bias. Second, the number of patients enrolled in this study was limited. Third, the cut-off value of PLR in our study was determined by the ROC curve analysis, which might not be representative. Therefore, further randomized case-controlled trials with a larger sample size are demanded to validate our findings.

Conclusion

Our study suggested that the low pretreatment PLR (PLR ≤ 98.89) was an independent protective factor for the prognosis with uHCC patients treated with TACE + TKIs + ICIs. What's more, the lower pretreatment PLR might also be an indicator of the occurrence of irAEs. Considering that PLR is an easily accessible indicator in clinical practice, it was helpful for clinicians to predict the prognosis and identify the patients with uHCC who were most likely to benefit from TACE + TKIs + ICIs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The Institutional Review Board of Wuhan Union Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this study was a retrospective study.

Author contributions

YG: Investigation, Writing – original draft. WW: Investigation, Writing – review & editing. BS: Investigation, Writing – review & editing. TG: Funding acquisition, Writing – review & editing. KS: Visualization, Writing – review & editing. CZ: Conceptualization, Supervision, Writing – review & editing. XL: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

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

Publisher's note

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