

# Prognostic value of inflammation-based indexes for intrahepatic cholangiocarcinoma following curative resection

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Received January 28, 2018; Accepted September 6, 2018

DOI: 10.3892/ol.2018.9618

**Abstract.** It is widely acknowledged that inflammatory indices may serve as effective prognosis indicators for various malignancies. In the present study, the prognostic value of systemic inflammatory biomarkers for patients undergoing curative resection for intrahepatic cholangiocellular carcinoma (ICC) was investigated. Clinical data of ICC patients who underwent curative resection between September 2008 and July 2017 were collected. Inflammatory indicators were analyzed using the Area Under the Receiver Operating Characteristic Curve. Indicators that were significantly associated with the overall survival (OS) were used to establish a systemic inflammation-based score system and tested via nomogram using R software. The neutrophil To lymphocyte ratio (NLR) and lymphocyte to macrophages ratio (LMR) were significantly associated with the OS and disease-free survival of the patients. High NLR and low LMR were associated with worse clinicopathological and survival outcomes. The univariate and multivariate analyses indicated that tumor T stage, incisional margin, NLR and LMR were associated with the OS of the patients. The systemic inflammation-based scoring system based on LMR and NLR demonstrated a stronger discriminatory capacity and may serve as a useful prognostic parameter for patients undergoing curative resection for ICC. Low LMR and high NLR were observed to be associated with poor prognosis and worse clinical outcomes for patients with ICC undergoing curative surgery. A combined inflammation-based scoring system based on LMR and NLR may effectively predict the outcomes and serve as a novel prognostic predictor for these patients.

## Introduction

Primary liver cancer is one of the most common malignant tumors in the world, with particularly high incidence rates in Asia and Africa (1,2). It is the third leading cause of cancer-associated mortality worldwide (3). The major histopathologic types of liver cancer include hepatocellular carcinoma (HCC) and intrahepatic cholangiocellular carcinoma (ICC) (2,4). Although various methods have been implemented to improve the survival of patients with liver cancer, including surgical resection, liver transplantation, transarterial chemoembolization, local ablation, radiotherapy, chemotherapy and molecular targeted drug treatment, the prognosis remains poor, particularly for ICC (5-8).

ICC is the second most common malignancy worldwide and accounts for 15-20% of primary liver cancer (9). Due to its rising incidence and poor prognosis, research into ICC is receiving increasing attention (10,11). Surgical resection remains one of the most effective ways to treat ICC (12,13). However, the clinical outcomes are limited due to the high recurrence and metastasis rates following operation (14). Thus, effective indicators that may predict the prognosis of ICC are essential for the treatment of these patients.

Previous studies have demonstrated that inflammation serves an important role in the development and progression of liver cancer (15,16). It has previously been demonstrated that a number of inflammation-based indicators in the blood are associated with the prognosis of HCC, such as the Glasgow Prognostic Score (GPS), the systemic inflammation score, the neutrophil to lymphocyte ratio (NLR), the lymphocyte to monocyte ratio (LMR), the platelet-to-lymphocyte ratio (PLR), prognostic nutritional index (PNI), prognostic index and red cell distribution width (RDW) (3,17-19). In addition, there are various serum enzyme-associated parameters that have been found to be associated with the clinical outcomes of ICC, including the gamma-glutamyltransferase to platelet ratio (GPR), albumin (ALB) to alkaline phosphatase ratio (APPR),  $\gamma$ -glutamyltransferase to alanine aminotransferase ratio (GAR) and the ALB to  $\gamma$ -glutamyltransferase ratio (AGR) (20,21). However, few studies have considered the prognostic significance of serum inflammatory-based indicators for ICC.

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**Key words:** intrahepatic cholangiocellular carcinoma, prognostic factors, inflammation, neutrophil to lymphocyte ratio, lymphocyte to macrophage ratio

The aims of the present retrospective analysis were to investigate the association between inflammation-based prognostic indicators and the survival of patients undergoing curative surgical resection for ICC.

## Materials and methods

**Patients.** For this retrospective cohort study, 221 patients who were pathologically diagnosed with ICC in the First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) between September 2008 and July 2017 were retrospectively recruited. Patients with active hepatitis, parasitic infection, acute cholangitis or other malignant tumors were excluded. Ultimately, 123 patients following curative resection were enrolled into the study. The institutional ethics committee at the study center approved this study. All participants gave consent after being fully informed of the goal and characteristics of this research.

**Treatment and follow-up.** Blood tests and computed tomography (CT) scans were routinely performed as preoperative tests within 3 days prior to surgery. The clinical staging was based on The American Joint Committee on Cancer (AJCC) 8th Edition Cancer Staging System (22). Each patient was followed-up at least every 2 months following hospital discharge during the first year and every 3 months thereafter. The final follow-up date was September 30th, 2017.

**Demographics and clinical characteristic data.** All clinical data were collected from the patients' medical records in the department of Hepatobiliary Surgery of the First Affiliated Hospital of Xi'an Jiaotong University. Clinical data included age, gender, tumor size, number of nodules and presence or absence of vascular invasion. Furthermore, preoperative biochemical indices were measured, including white blood cell counts (WBC), platelet counts (PLT), neutrophil counts, lymphocyte counts, megakaryocyte counts, RDW,  $\alpha$ -fetoprotein (AFP) levels, alanine transaminase (ALT) levels, aspartate aminotransferase (AST) levels, total bilirubin (TBIL) levels, indirect bilirubin (IBIL) levels, alkaline phosphatase (ALP) levels, ALB levels, hepatitis B surface antigen (HbsAg) levels and hepatitis B virus deoxyribonucleic acid (HBV-DNA) load. NLR was defined as the neutrophil count/lymphocyte count ratio; dNLR was calculated by neutrophil count/(WBC-neutrophil counts) ratio; LMR was defined as the lymphocyte count/megakaryocyte count ratio; PLR was defined as the platelet count/lymphocyte count ratio; PNI was defined as  $ALB + (5 \times \text{lymphocyte count})$ . The primary endpoints of this study were overall survival time (OS) and disease-free survival time (DFS). OS was defined as the time between radical surgery and mortality. DFS was defined as the time between radical surgery and tumor recurrence.

**Statistical methods.** Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Continuous data are presented as the mean  $\pm$  standard deviation and were compared using a unpaired Student's t-test or one-way analysis of variance for normal distribution data with Fisher's LSD post hoc test for the comparison of among different groups, Kruskal-Wallis test was used for multi-group comparison of abnormal distribution. The categorical variables were

compared using a  $\chi^2$  test and a Fisher's exact test. The diagnostic accuracy of all the indicators was determined using receiver operating characteristic (ROC) curve analysis. Indicators that displayed significance were chosen for the next part of the study and the Youden's index was applied to determine the optimal cut-off values (22). Patients were divided into different groups according to these aforementioned cut-off values. Univariate analysis of variables associated with survival was performed using log-rank testing to evaluate clinical factors associated with OS. Multivariate analysis was performed using Cox proportional hazards regression modelling using backward elimination and likelihood ratio testing, and the included variables were those which had significant associations with OS, determined by the univariate analysis. The inflammation-based scoring system was defined as follows: Patients with high NLR and high LMR were assigned a score of 2; patients with high NLR and low LMR or low NLR and high LMR were assigned as score of 1; patients who had low NLR and LMR were assigned a score of 0. Patients were grouped according to this score. The Kaplan-Meier method was used to analyze the long-term effect of the different groups and these were compared using the log-rank test. Finally, nomograms were used to validate the outcomes. Nomograms for possible prognostic factors associated with OS were using R software 3.4.0 (Institute for Statistics and Mathematics, Vienna, Austria). and the model performance for predicting outcome was evaluated by Harrell's concordance index (c-index), as previously described (23).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Patients' characteristics.** A total of 123 patients were recruited to the present study, who had been pathologically diagnosed with ICC and undergone radical resection between September 2008 and July 2017, including 67 males and 56 females. The mean age of the patients was  $56.80 \pm 10.67$  (29-79) years old. The final follow-up date was September 30th, 2017. The median follow-up time was 29.1 months (2-64 months). Following analysis with the Area Under the ROC curve (AUROC), NLR and LMR only were found to be significantly associated with the OS of patients (Fig. 1, data for indicators which were not associated with OS not presented). The optimal cut-off value of OS for LMR and NLR was 3.42 and 2.05, respectively.

According to the cut-off value of NLR and LMR, the cohort was divided into lower and higher groups as presented in Table I. Higher NLR was observed to be associated with a higher ratio of male patients, larger tumor diameter, higher lymph node metastasis rate and increased white blood cell and megakaryocyte counts. Lower LMR was associated with larger tumor diameter, higher lymph node metastasis rate, increased numbers of patients at tumor stage 3-4 (T3-4) and Tumor, Node, Metastasis (TNM) stage 3-4, lower ALB levels and increased megakaryocyte counts.

**Survival outcomes.** The 1-year and 3-year OS for the whole cohort were 37.40 and 5.69%, respectively. The median OS was 9 months. Higher NLR was associated with poor OS and decreased DFS. Patients with higher LMR exhibited higher 3-year OS and 3-year DFS (Fig. 2).

Table I. Associations between clinical characteristics of NLR and LMR.

Parameter	NLR $\leq$ 2.05 (n=25)	NLR $>$ 2.05 (n=98)	P-value	LMR $\leq$ 3.42 (n=57)	LMR $>$ 3.42 (n=66)	P-value
Age, year <sup>b</sup>	56.08 $\pm$ 11.81	56.99 $\pm$ 10.42	0.705	56.58 $\pm$ 11.06	57.00 $\pm$ 10.41	0.828
Sex (male/female)	8/17	59/39	0.011 <sup>a</sup>	32/25	35/31	0.730
BMI, kg/m <sup>2</sup> <sup>b</sup>	23.00 $\pm$ 4.24	23.13 $\pm$ 3.70	0.878	22.50 $\pm$ 3.91	23.63 $\pm$ 3.64	0.101
Tumor diameter, cm <sup>b</sup>	4.81 $\pm$ 2.63	6.46 $\pm$ 3.18	0.018 <sup>a</sup>	6.86 $\pm$ 3.18	5.48 $\pm$ 2.98	0.015 <sup>a</sup>
Differentiated (well/poorly)	17/8	53/45	0.152	28/29	42/24	0.105
Incisal margin (negative/positive)	13/12	63/35	0.259	34/23	42/24	0.650
N (-/+)	22/3	53/45	0.002 <sup>a</sup>	25/32	50/16	0.000 <sup>a</sup>
T (1-2/3-4)	17/8	51/47	0.152	25/32	43/23	0.018 <sup>a</sup>
TNM stage (I-II/III-IV)	11/14	27/71	0.112	10/47	28/38	0.003 <sup>a</sup>
Vascular invasion (absent/present)	18/7	63/35	0.468	34/23	47/19	0.177
WBC count, x10 <sup>3</sup> /ml <sup>b</sup>	5.75 $\pm$ 2.23	7.19 $\pm$ 2.71	0.015 <sup>a</sup>	7.37 $\pm$ 2.86	6.49 $\pm$ 2.47	0.071
Platelet count, x10 <sup>3</sup> /ml <sup>b</sup>	178.96 $\pm$ 83.70	198.01 $\pm$ 93.65	0.356	201.40 $\pm$ 82.81	187.86 $\pm$ 98.96	0.416
Albumin, g/dl <sup>b</sup>	39.05 $\pm$ 5.14	38.07 $\pm$ 5.83	0.455	36.58 $\pm$ 5.21	39.73 $\pm$ 5.72	0.002 <sup>a</sup>
Neutrophil count, x10 <sup>3</sup> /ml <sup>b</sup>	6.51 $\pm$ 14.33	4.82 $\pm$ 2.18	0.562	5.13 $\pm$ 2.46	5.20 $\pm$ 8.86	0.952
Lymphocyte count, x10 <sup>3</sup> /ml <sup>b</sup>	2.38 $\pm$ 5.23	1.47 $\pm$ 0.52	0.391	1.36 $\pm$ 0.45	1.91 $\pm$ 3.23	0.203
Megakaryocyte count, x10 <sup>3</sup> /ml <sup>b</sup>	0.33 $\pm$ 0.17	0.48 $\pm$ 0.21	0.001 <sup>a</sup>	0.54 $\pm$ 0.22	0.37 $\pm$ 0.17	0.000 <sup>a</sup>
TBIL, mmol/l <sup>b</sup>	32.92 $\pm$ 57.42	38.12 $\pm$ 68.62	0.728	37.57 $\pm$ 65.67	36.62 $\pm$ 67.36	0.937
AST, U/l <sup>b</sup>	66.62 $\pm$ 61.00	133.21 $\pm$ 659.63	0.616	163.25 $\pm$ 798.85	69.23 $\pm$ 106.15	0.380
ALT, U/l <sup>b</sup>	68.20 $\pm$ 68.92	131.17 $\pm$ 545.37	0.567	152.14 $\pm$ 652.15	79.28 $\pm$ 148.61	0.411
AFP, ng/ml <sup>b</sup>	12.34 $\pm$ 40.56	31.37 $\pm$ 157.13	0.551	3.16 (1.21-1,440)	3.11(1.22-420)	0.563
CA-199 kU/l (median)	34.04 (0.6-10,000)	185 (7.74-10,000)	0.065	185 (3.55-10,000)	61(0.6-10,000)	0.001
GGT, U/l <sup>b</sup>	162.61 $\pm$ 187.45	241.74 $\pm$ 347.26	0.275	251.52 $\pm$ 396.68	195.71 $\pm$ 204.48	0.320
Child-plug score (A,B/C)	19/6	68/30	0.517	37/20	50/16	0.187

<sup>a</sup>P<0.05, <sup>b</sup>mean $\pm$ standard deviation. NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to macrophage ratio; BMI, body mass index; TNM, Tumor, Node, Metastasis; WBC, white blood cell; TBIL, total bilirubin levels; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP,  $\alpha$ -fetoprotein; CA-199, cancer antigen 199; GGT,  $\gamma$ -glutamyltransferase.

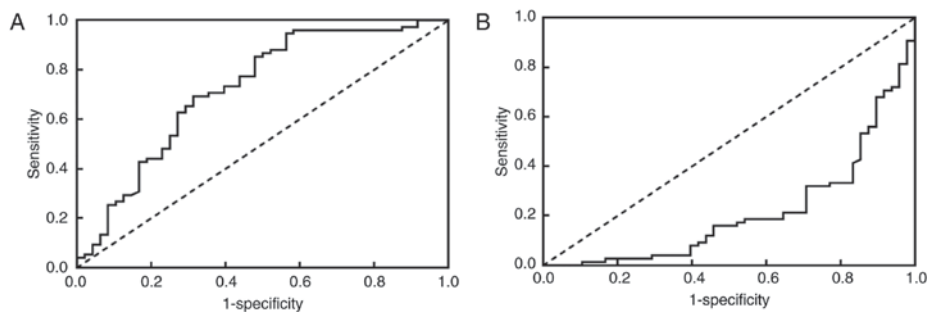


Figure 1. ROC analysis curve for NLR and LMR. (A) The ROC curve of NLR, P<0.05 and AUC=0.645. The cut-off point is 2.05. (B) The ROC curve of LMR, P<0.05 and AUC=0.299. The cut-off point is 3.41. ROC, receiver operating characteristic; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to macrophage ratio; AUC, area under the curve.

The univariate analysis of OS-associated indicators are presented in Table II. Tumor diameter, degree of tumor differentiation, lymph node metastasis, resection margin,

T stages, TNM stage, vascular invasion, NLR, LMR and macrophage counts were found to be associated with the OS for the cohort. In order to exclude the colinear regression

Table II. Univariate and Multivariate analysis of the clinical characteristic factors associated with OS.

Parameter	Univariate analysis			Multivariate analysis		
	P-value	HR	95% CI	P-value	HR	95% CI
Age (years)						
<60; ≥60	0.588	1.145	0.701-1.872	-	-	-
Sex						
Male; female	0.459	0.829	0.504-1.362	-	-	-
BMI (kg/m <sup>2</sup> )						
<24; ≥24	0.615	0.879	0.532-1.452	-	-	-
Tumor diameter						
<5 cm; ≥5 cm	0.031 <sup>a</sup>	1.773	1.053-2.984	0.788	1.087	0.592-1.996
Differentiated						
Well; poorly	0.036 <sup>a</sup>	0.589	0.359-0.967	0.147	0.675	0.397-1.148
Incisal margin						
Negative; positive	0.010 <sup>a</sup>	2.036	1.184-3.502	0.190 <sup>a</sup>	2.132	1.132-4.016
N						
N-; N+	0.007 <sup>a</sup>	1.990	1.204-3.288	0.091	1.642	0.924-2.918
T						
T1-2; T3-4	0.006 <sup>a</sup>	2.027	1.228-3.347	0.014 <sup>a</sup>	2.015	1.155-3.516
TNM stage						
1; 2; 3; 4	0.003 <sup>a</sup>	1.605	1.179-2.185	NA	NA	NA <sup>b</sup>
Vascular invasion						
Yes; No	0.017 <sup>a</sup>	1.863	1.117-3.105	0.362	1.316	0.729-2.374
NLR						
≤2.05; >2.05	0.031 <sup>a</sup>	1.029	1.003-1.055	0.046 <sup>a</sup>	1.033	1.001-1.067
LMR						
≤3.42; >3.42	0.000 <sup>a</sup>	0.686	0.547-0.819	0.023 <sup>a</sup>	0.789	0.643-0.968
WBC count (x10 <sup>3</sup> /ml)	0.056	1.093	0.998-1.197	-	-	-
Platelet count (x10 <sup>3</sup> /ml)						
<100; ≥100	0.253	1.494	0.751-2.973	-	-	-
Albumin (g/dl)						
<35; ≥35	0.363	0.778	0.453-1.337	-	-	-
Neutrophil count (x10 <sup>3</sup> /ml)	0.685	1.011	0.964-1.060	-	-	-
Lymphocyte count (x10 <sup>3</sup> /ml)	0.769	1.020	0.896-1.161	-	-	-
Macrophages count (x10 <sup>3</sup> /ml)	0.009 <sup>a</sup>	4.173	1.435-12.133	0.279	2.064	0.556-7.665
TBIL (mmol/l)						
<20.5; ≥20.5	0.441	1.223	0.733-2.038	-	-	-
AST (U/l)						
<45; ≥45	0.580	0.866	0.520-1.433	-	-	-
ALT (U/l)						
<45; ≥45	0.296	0.756	0.447-1.278	-	-	-
AFP (ng/ml)						
<400; ≥400	0.403	1.297	0.705-2.386	-	-	-
CA-199 (kU/l)						
<35; ≥35	0.789	0.928	0.537-1.603	-	-	-
Child-plug score						
A; B; C	0.422	1.207	0.763-1.911	-	-	-
ALP (U/l)						
<100; ≥100	0.249	1.357	0.808-2.278	-	-	-
GGT (U/l)						
<50; ≥50	0.277	1.370	0.777-2.414	-	-	-

<sup>a</sup>P<0.05. <sup>b</sup>TNM stages were excluded to avoid the influence of colinear regression. OS, overall survival; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to macrophage ratio; BMI, body mass index; TNM, Tumor, Node, Metastasis; WBC, white blood cell; TBIL, total bilirubin levels; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP,  $\alpha$ -fetoprotein; CA-199, cancer antigen 199; GGT,  $\gamma$ -glutamyltransferase; CI, confidence interval; HR, hazard ratio.

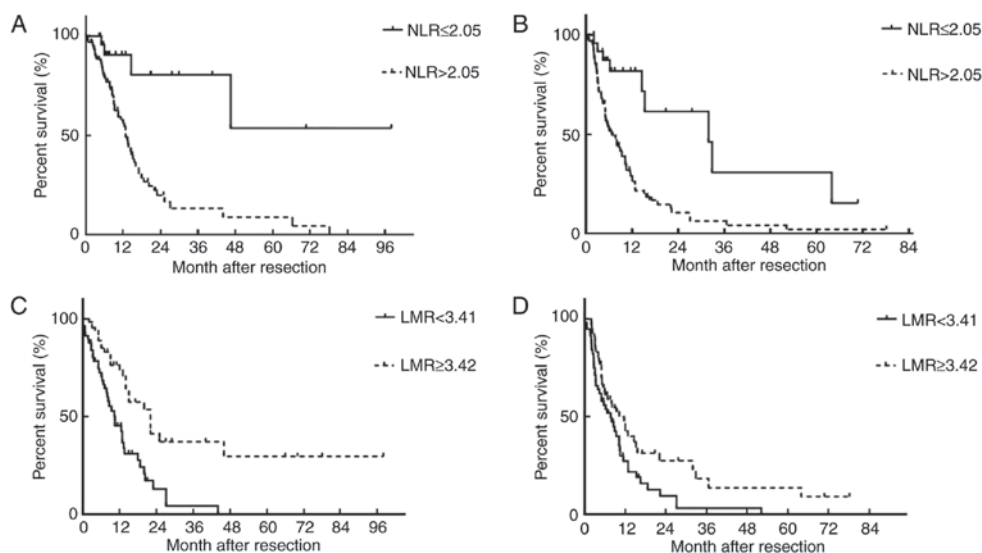


Figure 2. OS and DFS of patients with different NLR and LMR scores. (A) The OS of patients with low NLR compared with high NLR;  $P < 0.001$ . (B) The DFS of patients with low NLR compared with high NLR;  $P < 0.001$ . (C) The OS of patients with low LMR compared with high LMR;  $P < 0.001$ . (D) The DFS of patients with low LMR compared with high LMR;  $P < 0.001$ . OS, overall survival rate; DFS, disease-free survival rate; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to macrophage ratio.

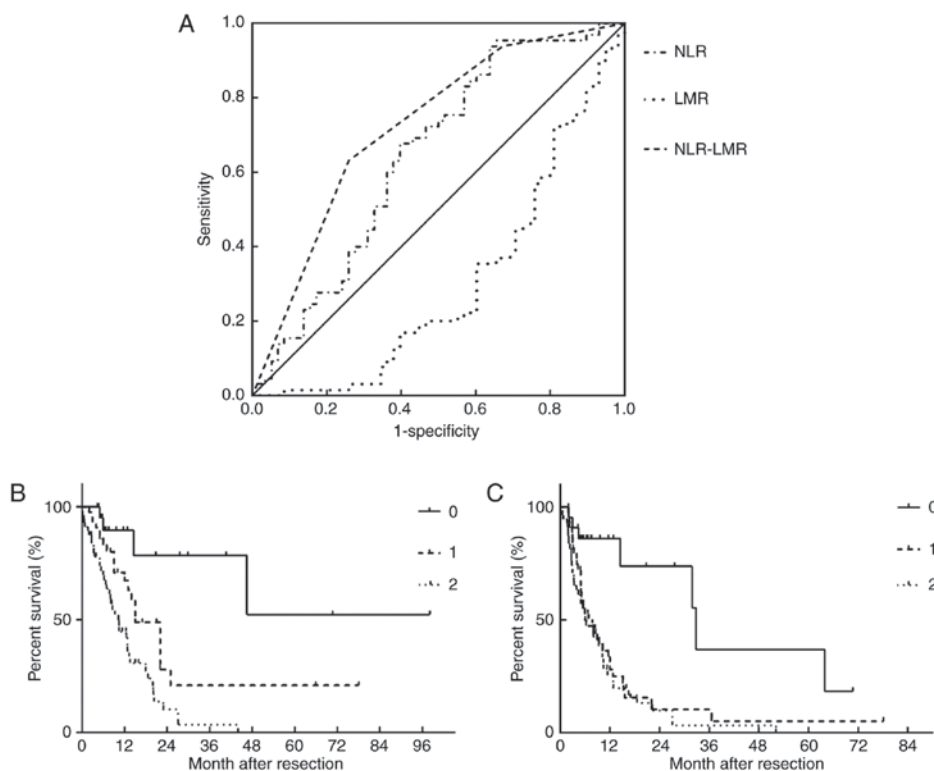


Figure 3. Comparison of the AUROC values and long-term effect on the OS of ICC patients based on inflammatory scores system. (A) The ROC curve for NLR, LMR and inflammation-based scoring system; AUROC values were 0.645, 0.299 and 0.724, respectively. (B) A comparison of the OS between the different groups based on the inflammatory-based system;  $P < 0.001$ . (C) A comparison of the DFS between the different groups based on the inflammatory-based system;  $P < 0.001$ . AUROC, Area Under the Receiver Operating Characteristic Curve; OS, overall survival rate; ICC, intrahepatic cholangiocellular carcinoma; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to macrophage ratio.

amongst the related factors, TNM stages were not included in the multivariate analysis. The results showed that the T stage, resection margin, NLR and LMR were significantly associated with the OS of patients with ICC.

To comprehensively evaluate the association between the inflammation-based score and the OS, patients were divided

into three groups: Score 0 group ( $n=23$ ); score 1 group ( $n=44$ ); and score 2 group ( $n=56$ ). The ROC analysis was used to determine the discriminatory capacity of the NLR, LMR and inflammation-based score system, as presented in Fig. 3A. The AUROC of NLR, LMR and the inflammation-based scoring system was 0.645, 0.299 and 0.724, respectively.

Table III. Association between clinical characteristics and the inflammation-based scoring system.

Parameter	Inflammation score			P-value
	0 N=23	1 N=44	2 N=56	
Ages, years <sup>f</sup>	55.65±12.21	58.05±9.25	56.30±11.15	0.615
Sex (male/female)	8/15	26/18	33/23	0.110
BMI, kg/m <sup>2</sup> <sup>f</sup>	23.12±4.41	23.87±3.16	22.51±3.94	0.208
Tumor diameter, cm <sup>f</sup>	4.83±2.70 <sup>d</sup>	5.83±3.05	6.88±3.21 <sup>d</sup>	0.023 <sup>a</sup>
Differentiated (well/poorly)	15/8	27/17	28/28	0.351
Incisal margin (negative/positive)	20/3	29/15	27/29	0.004 <sup>a</sup>
N (-/+)	19/4	31/13	25/31	0.002 <sup>a</sup>
T (1-2/3-4)	15/8	29/15	24/32	0.040 <sup>a</sup>
TNM stage (I-II/III-IV)	11/12	19/25	8/48	0.001 <sup>a</sup>
Vascular invasion (absent/present)	17/6	30/14	34/22	0.490
WBC count, x10 <sup>3</sup> /ml <sup>f</sup>	5.57±2.22 <sup>c,d</sup>	6.99±2.44 <sup>c</sup>	7.38±2.89 <sup>d</sup>	0.022 <sup>a</sup>
Platelet count, x10 <sup>3</sup> /ml <sup>f</sup>	178.17±87.22	191.00±103.41	203.16±83.99	0.528
Albumin, g/dl <sup>f</sup>	39.87±4.02	39.06±6.83	36.99±5.07	0.064
Neutrophil count, x10 <sup>3</sup> /ml <sup>f</sup>	2.45±5.46 <sup>d</sup>	4.40±1.57	5.17±2.49 <sup>d</sup>	0.007 <sup>a</sup>
Lymphocyte count, x10 <sup>3</sup> /ml <sup>f</sup>	2.85±6.35 <sup>c,d</sup>	1.64±0.55 <sup>c</sup>	1.34±0.44 <sup>d</sup>	0.012 <sup>a</sup>
Megakaryocyte, x10 <sup>3</sup> /ml <sup>f</sup>	0.31±0.17 <sup>d</sup>	0.40±0.16 <sup>e</sup>	0.54±0.22 <sup>d,e</sup>	0.000 <sup>a</sup>
TBIL, mmol/l <sup>f</sup>	25.57±43.09	45.99±85.16	34.77±61.13	0.463
AST, U/l <sup>f</sup>	59.97±57.28	80.69±123.95	67.69±106.11	0.716
ALT, U/l <sup>f</sup>	64.86±69.91	196.06±796.56	79.31±149.86	0.420
AFP, ng/ml <sup>f</sup>	3.06 (1.41-206.00)	3.12(1.22-420.66)	3.16(1.21-1,440)	0.555 <sup>b</sup>
CA-199, kU/l (median)	44.08 (0.6-10,000)	95.11(0.8-10,000)	185(3.55-10,000)	0.697 <sup>b</sup>
GGT, U/l <sup>f</sup>	154.49±182.39	290.97±458.74	203.54±215.42	0.204
Child-plug score (A, B/C)	20/3	30/14	37/19	0.161

<sup>a</sup>P<0.05. <sup>b</sup>Kruskal-Wallis test. BMI, body mass index; TNM, Tumor, Node, Metastasis; WBC, white blood cell; TBIL, total bilirubin levels; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP,  $\alpha$ -fetoprotein; CA-199, cancer antigen 199; GGT,  $\gamma$ -glutamyltransferase. <sup>c</sup>Represent group Inflammation score 0 has significance with group Inflammation score 1; <sup>d</sup>represent group Inflammation score 0 has significance with group Inflammation score 3; <sup>e</sup>represent group Inflammation score 1 has significance with group Inflammation score 2. <sup>f</sup>Mean±standard deviation.

When comparing the OS and DFS rates of different groups based on the inflammation-based score system, patients in the higher score group had worse prognosis, as presented in Fig. 3B and C.

The clinical characteristics of different inflammatory-based score groups were further compared. The three groups exhibited differences in the tumor diameter, incisal margin, lymph node metastasis, T stage, TNM stage, serum ALB level, white blood cell count, and lymphocyte and megakaryocyte counts (Table III).

To verify the results, a nomogram was established, using the indicators that were significantly associated with the OS. The results were identified to be the same as those for the Cox regression analysis (Fig. 4A). The Harrell's c-indexes of the nomograms for prediction of the OS of patients with ICC were 0.74 (95% CI: 0.677-0.803). Calibration curves for 1-year, 2-year and 3-year nomograms (Fig 4B-D) revealed no deviations from the reference line and no need for recalibration. Thus, the nomogram verified that the NLR and LMR may serve as effective indicators for the prognosis of ICC.

## Discussion

Inflammation has long been reported to be associated with the development and progression of liver cancer (24). Inflammation may contribute to the cancer microenvironment and promote the proliferation of cancer cells (25,26). The cell-mediated component of the immune system serves an important role in the immune response to a tumor. Levels of peripheral blood cells, such as WBCs, neutrophils granulocytes and lymphocytes, may reflect the inflammatory status of patients (27). It has previously been reported that high numbers of tumor-infiltrating lymphocytes correlate with better prognosis in patients with breast cancer (28). Neutrophils are capable of producing cytokines and chemokines, including vascular endothelial growth factor (VEGF), which may promote tumor angiogenesis and cancer cell proliferation, whilst acting to suppress lymphocyte-mediated cytotoxicity (29,30). Furthermore, megakaryocyte and platelet numbers have been reported to be associated with a cancer-promoting environment. Increasing evidence has demonstrated that serum inflammatory indicators,

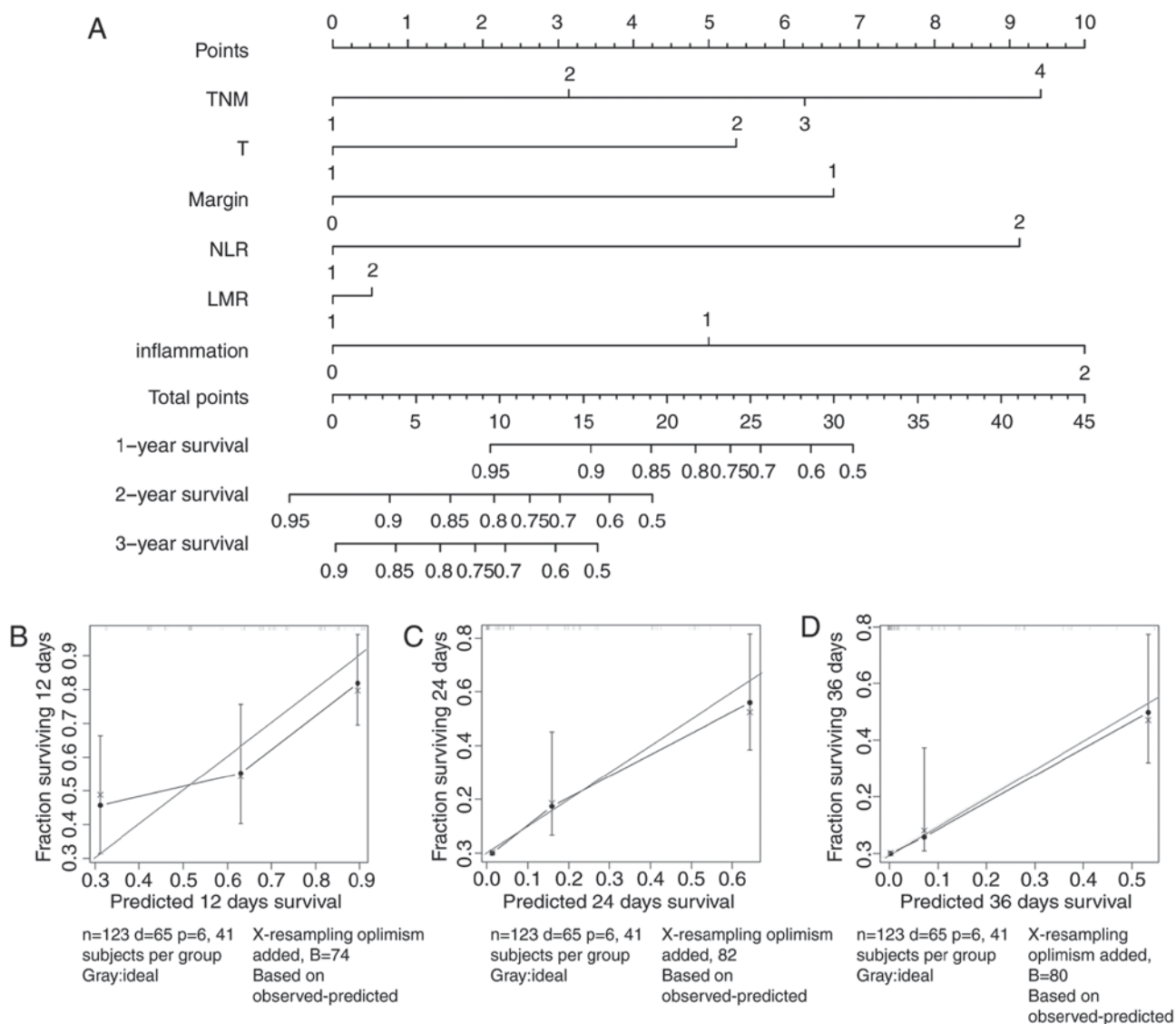


Figure 4. Nomograms of the parameters for the OS of patients undergoing curative resection for ICC. (A) Nomograms established a prognostic model using clinicopathological characteristics and pretreatment inflammatory biomarkers to predict OS for ICC. Nomograms may be interpreted by summing up the points assigned to each variable, which is indicated at the top of scale. The total points may be converted to predict the 3-year probability of mortality and recurrence or metastasis for a patient in the lowest scale. The Harrell's c-indexes for OS prediction were 0.74 (95% CI: 0.677-0.803). Calibration curves for (B) 1-year OS, (C) 2-year OS and (D) 3-year OS using nomograms with clinicopathological characteristics and pretreatment inflammatory biomarkers are presented. The x-axis is the nomogram-predicted probability of survival and y-axis is actual survival. The reference line is 98 and indicates perfect calibration. OS, overall survival rate; ICC, intrahepatic cholangiocellular carcinoma; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to macrophage ratio; TNM, Tumor, Node, Metastasis.

such as NLR, PLR, LMR, RDW and PNI, are associated with the prognosis of various cancer types (3,19,31,32).

Although the association between inflammatory-based indicators and HCC has been extensively studied, little is known about the usefulness of these indicators in ICC. In the present study, it was revealed that NLR, LMR and the inflammation-based score based on these may serve as useful indicators in the prognosis of patients with ICC. Patients with lower LMR, higher NLR or higher inflammation scores may have worse pathological and clinical outcomes.

NLR as a prognostic factor for liver cancer has been widely reported (33-35). It was demonstrated to be associated with worse clinicopathological characteristics and it is also been reported to be an independent predictor of long-term survival for various malignant tumors (32,33,35,36). In the present study,

the optimal cut-off value of NLR was 2.05. In the multivariate analysis, the hazard ratio was 1.033 (95% CI, 1.001-1.067; P<0.05), which is concordant with previous studies (17,37). Patients with higher NLR exhibited tumors of larger diameters and at more advanced stages, which is consistent with previous studies (3,38,39).

LMR is a favorable prognostic factor for clinical outcomes in patients with HCC (24). In the present study it has been identified that LMR is also associated with the prognosis of ICC. Lymphocytes and monocytes are vital for the development and prognosis of various cancer types and are involved in the development of tumors through the release of various soluble factors, which may be essential for tumor angiogenesis, invasion and metastasis (40,41). In the present study, lower LMR was demonstrated to be associated with worse prognostic and

clinical outcomes. Although studies have previously reported LMR to be an independent factor for HCC, this is, to the best of our knowledge, the first evidence to suggest that lower LMR correlates with worse prognosis, therefore may be a potential clinical indicator for patients with ICC (24,42).

The current study identified NLR and LMR to be better predictors compared with other inflammatory indicators for patients with ICC. By combining the two indicators together, it was discovered that the prognostic significance of the inflammation-based system was improved compared with the simple use of a single index. With AUROC analysis, this inflammatory model had a stronger predictive ability compared with NLR or LMR alone. On the basis of this inflammatory score model, it was also discovered that the higher scoring groups had worse prognostic and clinicopathological outcomes.

Many studies have reported  $\gamma$ -glutamyl transferase (GGT)-associated enzymes, including AGR, GPR and GAR, along with other indicators such as PNI, to be associated with the prognosis of ICC (20,43,44). However, the present study demonstrated that these parameters were not significantly associated with the prognosis of ICC. This may be due to the fact that the tumors of the enrolled patients were at a later stage compared with previous studies. The patients in this cohort displaced high serum GGT levels and low serum PNI levels. High GGT levels may reflect disorders of the bile tract whilst low PNI may reflect the nutrition state of the patient, however these indicators may not be suitable as OS predictors for patients in the later tumor stage.

Currently, a lot of studies have established various nomograms using the risk factors for the survival and recurrence of the ICC patients (9). In 2013, Wang *et al* (45) established a nomogram using preoperative carcinoembryonic antigen levels and carbohydrate antigen19-9 levels, tumor size, presence or absence of vascular invasion, nodal status and direct invasion or local metastasis in a cohort of 367 ICC Asian patients. Furthermore, in 2014, Hyder *et al* (46) built a nomogram from a cohort of 514 patients from 13 Western and Eastern centers, in which the patients' age, tumor size, number of lesions, nodal status, vascular invasion status and presence or absence of underlying cirrhotic liver parenchyma were included. These two studies may predict the prognosis of patients with ICC and Doussot *et al* (47) verified this in 2015. This present study focused chiefly on the inflammation indicators for the prognosis of ICC and nomograms were used to verify the results. However, internal validation using the calibration curves and c-index demonstrated that the nomogram established in the present study was comparable with previous studies.

The present study poses a number of limitations. Firstly, the results were based on a single center retrospective study, which may generate biases in the data analysis. Secondly, two systems were established: The nomograms and the systemic inflammation-based system. Whilst these may effectively predict the prognosis of patients with ICC, the present study was unable to establish which one was superior. Thirdly, owing to the limited number of patients, further indicators, including GPS were not measured; hence, a full assessment of inflammatory indicators for ICC was not able to be made.

In the present study, it was demonstrated that low LMR and high NLR are associated with poor prognosis and worse clinical outcomes for patients with ICC undergoing curative surgery. A combined inflammation-based score system based on LMR and NLR may effectively predict the outcomes and serve as a novel prognostic predictor for these patients.

### Acknowledgements

Not applicable.

### Funding

This study was supported by the Project of Development and Innovation Team of Ministry of Education (grant no. IRT\_16R57).

### Availability of data and materials

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

### Authors' contributions

YW, YL and LH conceived and designed the experiments. YW, FR and ZX acquired data and performed statistical analysis. FR, YL, YC and CS interpreted the data. YW, LH, XZ and CS wrote the manuscript. XZ and YL revised the manuscript. XZ was involved in the acquisition of data. All authors approved the final version of the manuscript.

### Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The institutional ethics committee at the study center approved this study. All participants gave consent after being fully informed of the goal and characteristics of this research.

### Patient consent for publication

Written informed consent was obtained from all participants prior to publication.

### Competing interests

The authors declare that they have no competing interests.

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