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RESEARCH ARTICLE

The elevated preoperative neutrophil-to-lymphocyte ratio predicts poor prognosis in intrahepatic cholangiocarcinoma patients undergoing hepatectomy

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Abstract A high preoperative peripheral blood neutrophil-to-lymphocyte ratio (NLR) has been reported to be a predictor of poor survival in patients with various cancers. The aim of this study was to evaluate the predictive significance of the NLR in patients undergoing hepatectomy for intrahepatic cholangiocarcinoma (ICC). From 2005 to 2011, 322 patients who underwent hepatectomy for ICC were enrolled in this retrospective study. Clinicopathological parameters, including NLR, were evaluated to identify predictors of overall and recurrence-free survival after hepatectomy. The best cutoff for NLR was 2.49, and 177 of 322 patients (54.9 %) had an NLR≥2.49. The 5-year survival rate after hepatectomy was 51.1 % in patients with NLR<2.49 and 24.8 % in those with NLR≥2.49 (P=0.0001). Univariate analyses revealed that NLR was significantly associated with recurrence-free survival (RFS) and overall survival (OS; both P<0.05). Multivariable analyses revealed that elevated NLR independently predicted poorer OS (P=0.003, hazard ratio [HR]= 1.600). In summary, our results indicate that elevated NLR is a promising independent predictor of poor survival after hepatectomy in patients with ICC.

Keywords Blood neutrophil-to-lymphocyte ratio · Intrahepatic cholangiocarcinoma · Liver resection · Prognosis

Abbreviations
ICC Intrahepatic cholangiocarcinoma
HR Hazard ratio
OS Overall survival

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Introduction

Intrahepatic cholangiocarcinoma (ICC) is a poorly understood biliary malignancy that accounts for an estimated 10 % to 15 % of all primary liver cancers [1]. In the US, the age-adjusted incidence of ICC increased from 0.32 per 100,000 individuals in 1975 to 0.85 per 100,000 individuals in 2000 and is still increasing [2, 3]. Surgery is the only potentially curative treatment option for patients who have resectable ICC. Unfortunately, the clinical outcomes of patients undergoing curative-intent liver resection are disappointing. The 5-year survival rate is only approximately 30 % at the early T1–T2 stages. Patients with an unresectable tumor have a dismal median survival time of approximately 9 months [4, 5]. Furthermore, the roles of adjuvant therapies, including systemic chemotherapy and radiotherapy, remain poorly defined. These

therapies have only modest therapeutic effects [6, 7]. Therefore, identification of novel molecular markers of ICC progression and improved understanding of the molecular mechanisms associated with metastasis and postsurgical recurrence of ICC would be beneficial for the development of effective therapeutic schemes.

Inflammation has emerged as the seventh hallmark of cancer [8]. Systemic inflammatory responses reflect the promotion of angiogenesis and DNA damage and tumor invasion through upregulation of cytokines [9–11]. The neutrophil-to-lymphocyte ratio (NLR) is a simple index of systemic inflammation. An elevated NLR has recently been found to be associated with poorer prognosis in patients with various types of malignant tumors, including colorectal cancer [12, 13], breast cancer [14], pancreatic cancer [15], and hepatocellular carcinoma (HCC) [16]. Furthermore, an elevated NLR significantly correlates with poor outcome in HCC patients undergoing liver transplantation [17], hepatic resection [18], radiofrequency ablation [19], and transarterial chemoembolization [20]. To our knowledge, only one, relatively small-scale, study of 27 patients has found that preoperative NLR may be a prognostic indicator of survival after hepatic resection for ICC [21].

To further clarify the prognostic significance of the NLR for ICC, we evaluated the effect of preoperative NLR on overall survival (OS) and recurrence-free survival (RFS) in a large cohort of ICC patients who underwent hepatic resection.

Materials and methods

Patient recruitment

The 322 ICC patients who underwent curative hepatic resection at the Liver Cancer Institute of Zhongshan Hospital (Fudan University, Shanghai, China) between 2005 and 2011 consisted of 194 males and 128 females. The mean age of the patients was 58 years. Of these patients, 123 (38.2 %) were seropositive for hepatitis B surface antigen (HBs-Ag), and two patients (0.062 %) were seropositive for hepatitis C antibody (HCV-Ab). Their 1-, 3-, and 5-year OS rates were 75.0 %, 47.8 %, and 35.2 %, respectively. Their 1-, 3-, and 5-year RFS rates were 56.6 %, 39.0 %, and 32.3 %, respectively. The study was approved by the Zhongshan Hospital Ethics Committee, and institutional review board protocols were followed when informed consent was obtained from each patient. Patients who underwent preoperative therapies (e.g., transarterial chemoembolization, radiofrequency ablation, or percutaneous ethanol injection) were excluded from the study.

Follow-up strategy and postoperative treatment

Patient follow-up and postoperative management were administered following our established guidelines, as previously described [22, 23]. In brief, data were censored at last patient follow-up after discharge. All patients were followed up with monthly screening for recurrence. Tumor markers, such as the CA199 test, liver ultrasonography, and 6-month computerized tomography scanning, magnetic resonance imaging, and bone scans, were selected as needed. If recurrence was suspected, additional investigations, such as hepatic angiography and positron emission tomography-computed tomography (PET-CT) procedures, were performed. When ICC recurrence was confirmed, a second hepatectomy, radiofrequency ablation, percutaneous ethanol injection, transcatheter arterial chemoembolization, or external radiotherapy were performed depending on the number, size, and sites of the recurrent tumor. OS was defined as the interval between the dates of surgery and death or between the dates of surgery and the last observation of surviving patients. Time to recurrence (TTR) was defined as the interval between the dates of surgery and the first recurrence or from the dates of surgery to the dates of last follow-up (for the patients without recurrence).

Statistical analysis

Neutrophil and lymphocyte counts were measured in each patient as part of the routine preoperative work-up. Neutrophil-to-lymphocyte ratios were calculated using the preoperative blood value as the reference. OS and RFS were calculated. Using the Cox proportional hazards model, univariate and multivariate analyses were performed for OS and RFS. The following variables were examined with respect to OS and RFS rate: age, sex, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), carbohydrate antigen 19-9 (CA199), Child–Pugh score, liver cirrhosis, tumor size, number of tumors, lymphonodus metastasis, tumor differentiation, tumor–node–metastasis (TNM) stage (American Joint Committee on Cancer 7th edn. staging for intrahepatic cholangiocarcinoma). Continuous variables were entered into the model.

All data were also expressed as the mean±standard deviation values. Independent χ^2 tests were used to compare categorical variables. Continuous variables were compared using unpaired t tests. Survival curves for OS and RFS were analyzed using the Kaplan–Meier method and compared using the log–rank test. The best cutoff for NLR was determined using a time-dependent receiver operating characteristic curve [24]. P values<0.05 were considered to be statistically significant.

Results

Patient characteristics

We investigated the correlations between the preoperative NLR values, factors, and clinicopathological characteristics.

We found that preoperative NLR was significantly correlated with tumor size (P=0.001), HBsAg (P=0.004), lymphonodus metastasis (P=0.006), TNM (P=0.003), and preoperative serum CA199 level (P=0.045). Other clinical characteristics, including age, sex, HCV, Child–Pugh score, liver cirrhosis, tumor number, and tumor differentiation, were not directly related to the preoperative NLR (Table 1).

NLR as an independent prognostic factor

Clinicopathological parameters, including NLR, were evaluated to identify predictors of ICC patients' OS and RFS. The results for the statistically significant prognostic factors identified using univariate and multivariate analyses are presented in Table 2. High serum CA199 levels were identified as a significant predictor of poor prognosis. Among tumor-related factors, large tumor size, multiple tumors, lymphonodus metastasis, and advanced TNM stage were identified as predictors of poor prognosis (Table 2). A high preoperative NLR was also identified as a predictor of prognosis. The multivariate analyses identified lymphonodus metastasis (P=0.000), multiple tumors (P=0.002), and high CA199 level (P=0.013) as independent predictors of poor prognosis. A high NLR was significantly correlated with OS (P=0.003, hazard ratio=1.600,Table 2).

The statistically significant factors for RFS that was identified using univariate and multivariate analyses are presented in Table 3. Among tumor-related factors, large tumor size, multiple tumors, lymphonodus metastasis, and advanced TNM stage were identified as predictors of poor prognosis for RFS. Preoperative NLR was identified as a predictor of tumor recurrence. Multivariate analyses identified multiple

Table 1 Correlation between the factors and clinicopathological characteristics in ICC (n=322)

Clinicopathological indexes		NLR		P values
		<2.49 (n=145)	≥2.49 (n=177)	
Age (year)	≤50	44	41	0.146
	>50	101	136	
Sex	Female	54	74	0.405
	Male	91	103	
HBsAg	Negative	77	122	0.004
	Positive	68	55	
HCV	Negative	144	176	1*
	Positive	1	1	
CA199 (U/ml)	≤37	81	79	0.045
	>37	64	98	
Child–Pugh	A	142	169	0.356*
	B or C	3	8	
Liver cirrhosis	No	102	134	0.279
	Yes	43	43	
Tumor size (cm)	≤5	80	65	0.001
	>5	65	112	
Tumor number	Single	110	134	0.974
	Multiple	35	43	
Lymphonodus metastasis	Yes	16	40	0.006
	No	129	137	
Tumor differentiation[a]	Poor	24	44	0.133
	Moderated	93	108	
	Well	28	25	
TNM[b]	I+II	123	125	0.003
	III+IVa	22	52	

NLR neutrophil-to-lymphocyte ratio, *HBsAg* hepatitis B surface antigen, *HCV* hepatitis C virus, *CA19-9* carbohydrate antigen 19-9, *TNM* tumor–node–metastasis

* Fisher's exact tests; chi-square tests for all other analyses

[a] Tumor differentiation was determined according to the British Society of Gastroenterology guidelines on the management of cholangiocarcinoma"

[b] TNM stage: American Joint Committee on Cancer 7th edn. staging for intrahepatic cholangiocarcinoma

Table 2 Univariate and multivariate analyses of factors in relation to overall survival using the Cox proportional hazards model (n=322)

Variables	OS	
	HR (95 % CI)	P values
Univariate analysis†		
Age (≤50 vs. >50)	1.079 (0.780–1.493)	0.647
Sex (female vs. male)	1.143 (0.850–1.538)	0.376
HBsAg (negative vs. positive)	1.090 (0.505–2.352)	0.826
HCV (negative vs. positive)	1.333 (0.330–5.380)	0.686
CA199 (U/ml, >37 vs. ≤37)	1.601 (1.198–2.138)	0.001
Child–Pugh (A vs. B)	1.090 (0.505–2.352)	0.826
Liver cirrhosis (no vs. yes)	1.187 (0.863–1.633)	0.292
Tumor size (cm, >5 vs. ≤5)	1.486 (1.107–1.994)	0.008
Tumor number (multiple vs. single)	1.636 (1.191–2.248)	0.002
Lymphonodus metastasis (yes vs. no)	2.895 (2.066–4.058)	0.000
Tumor differentiation[a] (P vs. M,W)	1.126 (0.839–1.511)	0.428
TNM[b] (I+II vs. III+IVa)	2.459 (1.798–3.364)	0.000
NLR (high vs. low)	1.782 (1.322–2.402)	0.000
Multivariate analysis†		
CA199(U/ml, >37 vs. ≤37)	1.451 (1.083–1.945)	0.013
Tumor size(cm, >5 vs. ≤5)	1.263 (0.922–1.729)	0.146
Tumor number (multiple vs. single)	1.644 (1.192–2.268)	0.002
Lymphonodus metastasis (yes vs. no)	2.359 (1.667–3.340)	0.000
NLR (high vs. low)	1.600 (1.178–2.174)	0.003

NLR neutrophil-to-lymphocyte ratio, *HBsAg* hepatitis B surface antigen; HCV,hepatitis C virus; CA19-9, carbohydrate antigen 19-9; TNM, tumor–node–metastasis; CI, confidential interval; HR, hazard ratio; P, poor differentiation; M, moderated differentiation; W, well differentiation

a Tumor differentiation was determined according to the British Society of Gastroenterology guidelines on the management of cholangiocarcinoma

b TNM stage: American Joint Committee on Cancer 7th edn. staging for intrahepatic cholangiocarcinoma

† Cox proportional hazards regression

tumors (P=0.000) and lymphonodus metastasis (P=0.000) as independent predictors of tumor recurrence (Table 3).

Selection of the best cutoff point for the NLR

Using a time-dependent receiver operating characteristic curve, the best cutoff for NLR (NLR=2.49) was determined for postoperative prognosis. The patients were divided into two groups: a low (<2.49) NLR group (n=145) and a high (≥2.49) NLR group (n=177).

Prognostic comparisons of the low and high NLR groups

The mean OS time was 55.8±3.9 months in the low NLR group and 39.6±3.1 months in the high NLR group. The results for the overall survival rates for patients in the low and high NLR groups are presented in Fig. 1. We found that for the low NLR group, the 1-, 3-, and 5-year OS rates were 84.7 %, 57.1 %, and 51.1 %, respectively. These results were significantly higher compared with the high NLR group who had rates of 67.2 %, 40.1 %, and 24.8 % for 1-, 3-, and 5-year OS, respectively (P=0.0001).

The results for RFS rates for patients in the low and high NLR groups are presented in Fig. 2. We also found that a NLR≥2.49 was significantly correlated with ICC recurrence after hepatic resection. The 1-, 3-, and 5-year RFS rates were significantly lower in the high (51.8 %, 33.0 %, and 26.8 %, respectively) compared with the low NLR group (62.3 %, 46.0 %, and 38.4 %, respectively; P=0.0145).

Table 3 Univariate and multivariate analyses of factors in relation to recurrence-free survival using the Cox proportional hazards model (n=322)

Variables	RFS	
	HR (95 % CI)	P values
Univariate analysis[†]		
Age (≤50 vs. >50)	1.160 (0.841–1.598)	0.366
Sex (female vs. male)	1.063 (0.794–1.422)	0.683
HBsAg (negative vs. positive)	0.660 (0.271–1.605)	0.360
HCV (negative vs. positive)	0.645 (0.206–2.018)	0.451
CA199 (U/ml, >37 vs. ≤37)	1.289 (0.969–1.714)	0.081
Child–Pugh (A vs. B)	0.660 (0.271–1.605)	0.360
Liver cirrhosis (no vs. yes)	1.254 (0.915–1.719)	0.159
Tumor size (cm, >5 vs. ≤5)	1.349 (1.012–1.798)	0.042
Tumor number (multiple vs. single)	1.839 (1.345–2.515)	0.000
Lymphonodus metastasis (yes vs. no)	2.532 (1.792–3.577)	0.000
Tumor differentiation[a] (P vs. M,W)	1.304 (0.976–1.740)	0.072
TNM[b] (I+II vs. III+IVa)	2.042 (1.483–2.813)	0.000
NLR (high vs. low)	1.426 (1.069–1.902)	0.016
Multivariate analysis[†]		
Tumor size (cm, >5 vs. ≤5)	1.212 (0.888–1.654)	0.225
Tumor number (multiple vs. single)	1.787 (1.304–2.449)	0.000
Lymphonodus metastasis (yes vs. no)	2.388 (1.686–3.383)	0.000
NLR (high vs. low)	1.253 (0.924–1.698)	0.146

NLR neutrophil-to-lymphocyte ratio, *HBsAg* hepatitis B surface antigen, *HCV* hepatitis C virus, *CA19-9* carbohydrate antigen 19-9, *TNM* tumor–node–metastasis, *CI* confidential interval, *HR* hazard ratio, *P* poor differentiation, *M* moderated differentiation, *W* well differentiation

[†] Cox proportional hazards regression

[a] Tumor differentiation was determined according to the British Society of Gastroenterology guidelines on the management of cholangiocarcinoma

[b] TNM stage: American Joint Committee on Cancer 7th edn. staging for intrahepatic cholangiocarcinoma

Discussion

Inflammatory markers have long been linked with malignancy, and mounting evidence suggests that inflammatory factors and cells are closely related to tumor progression. Early in the 18th century, Virchow first observed leukocytes in neoplastic tissues [11], which led to the hypothesis that inflammation has an important role in the development of malignant disease. Tumor-induced systemic proinflammatory effects have recently been widely investigated. The tumor-generated inflammatory response is thought to cause upregulation of cytokines, inflammatory mediators, and inflammatory corpuscles, which result in an increased propensity for tumor recurrence and metastasis. This response is carried out by inhibition of apoptosis, promotion of angiogenesis, and damage of DNA [9–11, 25].

Halazun et al. [26] is the first to report that NLR is associated with hepatic malignancy. And many studies have revealed that elevated NLR is correlated with adverse survival outcome in patients with various solid tumors [13–15, 18, 21, 27]. Furthermore, preoperative NLR has shown a significant correlation with poor outcome in HCC patients undergoing

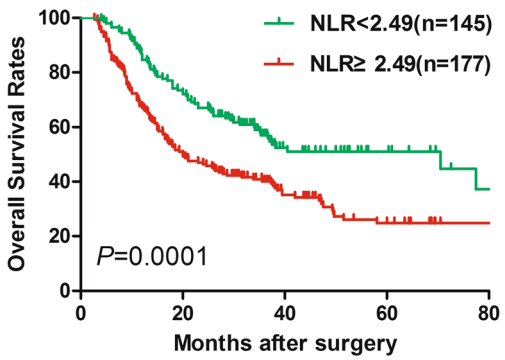

Fig. 1 Comparison of overall survival rates in the low (<2.49) and high (≥2.49) NLR groups. The 1-, 3-, and 5-year overall survival rates were 84.7 %, 57.1 %, and 51.1 %, respectively, in low (<2.49) NLR group, which were significantly higher compared with the high (≥2.49) NLR group (67.2 %, 40.1 %, and 24.8 %, respectively; P=0.0001)

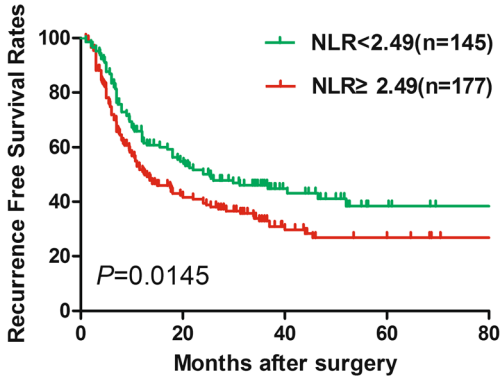

Fig. 2 Comparison of recurrence-free survival rates in the low (<2.49) and high (≥2.49) NLR groups. The recurrence-free survival rate was significantly higher in the low NLR group than in the high NLR group (P=0.0145)

liver transplantation [17, 28]. In addition, increased NLR has also been significantly linked to impaired aortic elastic properties [29] and in-hospital mortality in patients with ST elevation myocardial infarction undergoing percutaneous coronary intervention [30] or pulmonary embolism [31]. To investigate these findings in more detail, we assessed whether preoperative NLR was correlated with ICC recurrence and survival after hepatectomy. We found that the pretreatment NLR may be a significant prognostic factor for ICC patients and has potential for use as a predictor of survival after surgical treatment. High NLR (≥2.49) showed notable correlation with early recurrence and poor overall survival of ICC patients.

Similarly, recent studies have shown that the derived neutrophil-to-lymphocyte ratio (dNLR, ratio of neutrophil count to leukocyte count — neutrophil count), which employs the leukocyte–neutrophil count instead of the lymphocyte count as the denominator, also has prognostic value in cancer [32–34]. Therefore, we further evaluated the prognostic significance of dNLR in ICC. We found that preoperative dNLR was significantly correlated with preoperative NLR, and the Spearman's rank correlation coefficient between them was 0.915 (P<0.001). The best cutoff value for dNLR was 1.71, and 186 of 322 patients (57.8 %) had a high dNLR (dNLR≥ 1.71). The univariate analysis revealed that the OS and RFS were significantly different between patients with high dNLR and those with low dNLR (HR=1.48, P=0.010; HR=1.42, P=0.018, respectively). The 5-year OS rate was significantly lower in the high dNLR group compared with the low group (30.9 % vs. 41.6 %), so was the mean OS time (41.3±3.1 vs. 53.9±3.8 months, P=0.0093; Fig. S1). We also found that the high dNLR group had a notably lower 5-year RFS rate (26.6 % vs. 39.9 %, P=0.0163; Fig. S2). However, the multivariate analysis demonstrated that dNLR was not an independent prognostic factor for ICC patients (P=0.147). Employing the leukocyte–neutrophil value instead of the lymphocyte value leads to the inclusion of the lymphocytes together with the monocytes, which are known to increase in patients with cancer. And the elevated monocyte count has been notably correlative with poor prognosis in cancer victims [35, 36]. The decreased count of lymphocyte and the increased count of monocyte have inverse effects on the dNLR outcome. Hence, NLR could be considered as a better predictor for the prognosis in cancer than dNLR. The studies of Proctor et al. [33] and Dirican et al. [37] also reported the superiority of NLR in predicting prognosis in cancer. Except for NLR and dNLR, various predictors derived from the combination of peripheral blood cellular components of systemic inflammatory response and coagulation have been demonstrated, such as lymphocyte-to-monocyte ratio (LMR) [38, 39] and platelets-to-lymphocyte ratio (PLR) [40, 41], to evaluate clinical outcome in a wide variety of cancers. For instance, studies have shown that decreased LMR predicts poor prognosis in patients with soft tissue sarcoma [38] or pancreatic cancer [42]. And it might be partly owing to lymphocytes' defending host against tumor cells and monocytes' contribution to tumor progression [38]. However, the intrinsic mechanisms of these predictors are left not well-answered by previous investigations.

The exact reason for the observation of NLR rising among patients with malignancy that have poorer RFS and OS remains largely unclear. It has been shown that a high NLR, which is associated with the presence of systemic inflammation, indicates the relative depletion of lymphocytes, which impairs the host immune reactions against malignancy [21, 28, 43, 44]. In addition, increased neutrophils are regarded as the reservoirs of vascular endothelial growth factor (VEGF) [45]. Patients with elevated NLR have a relative neutrophilia and may have higher level of circulating VEGF due to increased numbers of circulating neutrophils which secret VEGF. Increased vascularity, vascular invasion, and increased tendency for HCC metastasis and recurrence can result from these changes [28]. In a previous study, we found that ICC cells that express higher level of CXCL5 are likely to recruit more neutrophils to the tumor foci. This process establishes a tumor-promoting microenvironment, amplifies the inflammatory response, and facilitates ICC metastasis and recurrence [46]. Patients with a high NLR have a relative depletion of lymphocytes, which may lead to an impaired defence against cancers. Overall, patients with a high NLR have a relative neutrophilia and lymphocytopenia. This condition results in an imbalance in the inflammatory cascade and in the host immune modulatory response to cancers, which may potentially create an almost perfect microenvironment for further continuous tumor proliferation and metastasis [28]. We also found that elevated NLR was positively correlated with larger tumor size, greater lymph node involvement, and advanced TNM stage. Tumor size and lymphonodus metastasis are two relatively putative clinicopathological markers of ICC

invasiveness and metastasis [6, 47], and both were found to be independently prognostic indicators for ICC. These results support the hypothesis that a high NLR could be an indicator of immunosuppressive status during the development of malignancies.

IL-17 is a proinflammatory cytokine that promotes HCC growth [48]. IL-17 also initiates neutrophil recruitment by CXC chemokines (e.g., CCL2 released from IL-17-producing T cells) [48–50]. Motomura et al. [17] reported that there is a correlation between elevated NLR in peripheral blood and upregulation of IL-17 in peritumoral regions in hepatoma. Furthermore, our previous study indicated that IL-17 positive cells and neutrophil infiltration into the ICC are related to the aggressiveness of the tumor [23]. Therefore, IL-17 may be a key molecule involved in the relationship between NLR and ICC metastasis.

Using preoperative NLR for prediction of recurrence and outcome has potentially valuable implications with targeting preoperative and postoperative therapies to improve survival outcomes of cancer patients, which is supported by lots of emerging evidence that the use of several anti-inflammatory and anti-angiogenic agents improves survival and decreases recurrence rates in hepatic carcinoma and other malignancies. For instance, selective cyclooxygenase-2 inhibitors can limit growth and prevent proliferation in HCC cell lines [51], possibly by suppressing VEGF activity [52]. These therapies may also have a significant potential role in ICC patients, especially with an elevated NLR, because they may have much higher level of circulating VEGF than others. By suppressing VEGF, and hence angiogenesis, these therapies may have a profound effect on outcomes of patients with ICC. Mano et al. [18] reported that a high infiltration of tumor-associated macrophages, which express many cytokines (e.g., IL-6 and IL-8) in the lesions, is associated with a high NLR. And these cytokines can promote systemic neutrophilia [53, 54]. Therefore, anti-inflammatory treatment may be beneficial to the ICC patients with an elevated NLR. However, thorough investigations and independent validations to further verify the hypothesis are needed.

In conclusion, our results demonstrate that elevated NLR significantly increases the risk of tumor recurrence and death and indicate that NLR is a novel independent predictor for prognosis after hepatic resection in patients with ICC. Preoperative NLR measurement may provide a simple method to identify patients with the poorest prognosis.

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Conflicts of interest None

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