Translational Oncology
www.transonc.com

Volume 7 Number 2 April 2014 pp. 248–255 248

Preoperative Neutrophil-to-Lymphocyte Ratio as a New Prognostic Marker in Hepatocellular Carcinoma after Curative Resection[1]

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Abstract

BACKGROUND: Preoperative peripheral blood neutrophil-to-lymphocyte ratio (NLR) has been proposed to predict prognosis of hepatocellular carcinoma (HCC). However, the cutoff value of NLR in several studies is not consistent. This study aims to investigate the correlation of preoperative NLR with clinicopathologic features and the prognosis in patients who have undergone resection for HCC. *METHODS*: Clinical data of 256 patients with HCC who underwent radical hepatectomy were retrospectively analyzed. The patients were divided into the low-NLR group (NLR $\leq$ 2.31) and the high-NLR group (NLR > 2.31). A univariate analysis was performed to assess clinicopathologic characteristics that influenced disease-free survival (DFS) and overall survival (OS) in patients. The significant variables were further analyzed by a multivariate analysis using Cox regression. The Kaplan-Meier method was used to assess the DFS and OS rate. *RESULTS*: The value of NLR was associated with tumor size, clinical tumor-node-metastasis (TNM) stage, portal vein tumor thrombus (PVTT), distant metastasis, and aspartate aminotransferase (AST) in HCC. NLR > 2.31, size of tumor > 5 cm, number of multiple tumors, III-IV of TNM stage, PVTT, distant metastasis, and AST > 40 U/l were predictors of poorer DFS and OS. NLR > 2.31, size of tumor >5 cm, III-IV of TNM stage, and AST > 40 U/l were independent predictors of DFS and OS. *CONCLUSION*: Preoperative NLR > 2.31 was an adverse predictor of DFS and OS in HCC after hepatectomy. This study suggested that NLR might be a novel prognostic biomarker in HCC after curative resection.

Translational Oncology (2014) 7, 248–255

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[1] This work was supported in part by the National Natural Science Foundation of China (No. 31370917, No. 30972797, No. 81360309, and No. 81260328), the Natural Science Foundation of Guangxi Province (No. 2013GXNSFCA019012), the Science and Technology Planning Project of Guangxi Province (No. 1140003B-79 and No. 1298003-2-1), the Lijiang Scholarship Foundation and the Science and Technology Planning Project of Guilin City (20100128-5 and 20110119-1-8), the Open Fund of Medical Scientific Research Center of Guangxi Medical University (KFJJ2010-49 and KFJJ2011-06), and the Key Project of Medical Research Foundation, Department of Public Health (Nanning, China) (2011006).

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Received 7 December 2013; Revised 7 January 2014; Accepted 15 February 2014

http://dx.doi.org/10.1016/j.tranon.2014.02.011

Introduction

An estimated 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008. Half of these cases and deaths were estimated to occur in China [1]. There are significant geographical differences in the morbidity and mortality of hepatocellular carcinoma (HCC) all over the world. HCC is one of the common malignant tumors of digestive tract and is the major cancer burden in China [2]. The prognosis of HCC remains poor mainly because of high recurrence and metastasis rates even after surgical resection. Tumor recurrence rates are more than 70% of cases at 5 years [3,4]. Although surgical resection is a potentially curative treatment for HCC and despite improved diagnosis and advances in surgical and nonsurgical therapy, the clinical outcome of HCC remains poor [5]. Therefore, it is of great significance to carry out deep research in diagnosis and prognosis of HCC. Such researches might lead to a breakthrough in the field of HCC diagnosis, treatment, and prevention and furthermore, adoption of effective measures to improve surgical treatment for HCC.

Recently, there is increasing evidence that the presence of systemic inflammation correlates with poor cancer-specific survival. The prognostic value of various markers of systemic inflammatory, including cytokines such as intercellular adhesion molecule 1 and neutrophil-to-lymphocyte ratio (NLR) has been investigated in certain cancer populations [6-14]. Previous studies have demonstrated that an elevated NLR may correlate with a poor prognosis in patients who underwent curative resection of HCC. However, the cutoff value of NLR is not consistent; for instance, it is determined as 2.3 [15], 3.0 [16], and 5.0 [17,18] in different studies. So the cutoff value of NLR in patients who underwent curative resection of HCC should be optimized; otherwise, it is difficult to evaluate the clinical value of NLR and to compare different studies. Our study was designed to determine the optimal value of NLR and to evaluate the correlation of preoperative NLR with clinicopathologic features and prognosis in patients with HCC who underwent curative resection.

Materials and Methods

The Source of Specimens and Clinical Data

Two hundred fifty-six cases of patients with HCC underwent hepatic resection at the Affiliated Hospital of Guilin Medical University (Guilin, People's Republic of China) from September 1999 to June 2007, and these patients were recruited for this study. These subjects were confirmed by clinical, serological, ultrasonography (US), computerized tomography, magnetic resonance imaging, and pathologic examination, and HCC diagnoses in this study followed the Primary Liver Cancer Clinical Diagnosis and Staging Criteria (Ministry of Health, Beijing, China). Clinicopathologic characteristics of these patients including NLR, age, gender, hepatitis B surface antigen (HBsAg), α-fetoprotein (AFP), the size and the number of tumors, combined liver cirrhosis, clinical tumor node metastasis (TNM) stage, portal vein tumor thrombus (PVTT), distant metastasis, and aspartate aminotransferase (AST) were collected and detailed in Table 1. All subjects gave written informed consent, and the local ethics committee approved this study. This study was conducted as a retrospective analysis of a prospectively collected computerized database in a single hospital. Among them, 256 patients who met the inclusion criteria were enrolled in this study. Patients were obviated if they 1) were patients with cholangiocarcinoma or were not primary patients with HCC, 2) died in perioperative period, 3) could not provide detailed and needed clinical data, 4) had clinical evidence of infection, immune-system disease, or hematology disease or used hematology-influenced drugs within 1 month, 5) lost contact during the follow-up time, or 6) were HIV positive.

Our research group investigated patients with HCC with long-term follow-up after surgery including using serum AFP test and US examination every 2 months and chest radiography every 6 months during the first two postoperative years and at 3- to 6-month intervals thereafter. Computerized tomography or magnetic resonance imaging scans were performed if recurrence was suspected due to an abnormal AFP test or US examination. The mean postoperative follow-up time was 38.0 months (median, 21.0 months; range, 2.0-161.0 months). Disease-free survival (DFS) was measured from the date of surgery to the date of recurrence, metastasis, death, or last follow-up. Overall survival (OS) was measured from the date of surgery to the date of death or last follow-up.

Selection of Cutoff Score

To avoid predetermined cut point, receiver operating characteristic (ROC) curve analysis was applied to define the cutoff score for preoperative NLR. The score was selected as the cutoff value that was closest to the point with both maximum sensitivity and specificity. Other clinicopathologic parameters used were dichotomized: age (≤55 *vs* >55 years), gender (female *vs* male), HBsAg (negative *vs* positive), AFP level (≤20 *vs* >20 ng/ml), tumor size (≤5 *vs* >5 cm), cirrhosis (yes *vs* no), tumor number (single *vs* multiple), TNM stage (I-II *vs* III-IV), distant metastasis (yes *vs* no), PVTT (yes *vs* no), recurrence (yes *vs* no), and AST (yes *vs* no). Subsequently, the clinicopathologic and prognostic significance of the NLR level in HCC was investigated.

Table 1. Patients with HCC (256 Cases) Categorized by NLR and Their Clinical Pathologic Characteristics.

Clinical Character	Variable	No. of Patients	NLR		χ^2	*P* Value
			≤2.31 n (%)	>2.31 n (%)		
Age (yr)	≤ 55	176	81 (46.0)	95 (54.0)	0.349	.555
	> 55	80	40 (50.0)	40 (50.0)		
Gender	Female	30	15 (50.0)	15 (50.0)	0.102	.750
	Male	226	106 (46.9)	120 (53.1)		
HBsAg	Negative	41	18 (43.9)	23 (56.1)	0.222	.638
	Positive	215	103 (47.9)	112 (52.1)		
AFP (ng/ml)	≤ 20	62	31 (50.0)	31 (50.0)	0.245	.620
	> 20	194	90 (46.4)	104 (53.6)		
Tumor size (cm)	≤ 5	47	36 (76.6)	11 (23.4)	19.869	< .001
	> 5	208	85 (40.7)	124 (59.3)		
Cirrhosis	No	27	14 (51.9)	13 (48.1)	0.255	.614
	Yes	229	107 (46.7)	122 (53.3)		
Tumor no.	Single	163	79 (48.5)	84 (51.5)	0.259	.610
	Multiple	93	42 (45.2)	53 (54.8)		
TNM stage	I-II	109	73 (67.0)	36 (33.0)	29.576	< .001
	III-IV	147	48 (32.7)	99 (67.3)		
PVTT	No	184	98 (53.3)	86 (46.7)	9.434	.002
	Yes	72	23 (31.9)	49 (68.1)		
Distant metastasis	No	218	111 (50.9)	107 (49.1)	7.858	.005
	Yes	38	10 (26.3)	28 (73.7)		
Recurrence	No	164	71 (43.3)	93 (56.7)	2.890	.089
	Yes	92	50 (54.3)	42 (45.7)		
AST (U/l)	≤ 40	117	64 (54.7)	53 (45.3)	4.779	.029
	> 40	139	57 (41.0)	82 (59.0)		

NLR, neutrophil-to-lymphocyte ratio; HBsAg, hepatitis B surface antigen; AFP, α-fetoprotein; TNM, tumor-node-metastasis; PVTT, portal vein tumor thrombus; AST, aspartate aminotransferase.

Statistical Analysis

SPSS13.0 (SPSS Inc, Chicago, IL) and MedCalc statistical software version 11.3.0.0 (MedCalc Software, Broekstraat 52 Mariakerke, Belgium) were used in analyzing the data. The Pearson χ^2 test was used to compare qualitative variables. Univariate analysis was performed to determine the significance of variables using the logistic regression model for the response rate and the Cox regression model for DFS and OS. Survival curve was estimated by Kaplan-Meier analysis, and the log-rank test was used to examine the difference of survival distributions between groups. Subsequently, the variables with $P < .05$ were subjected to multivariate analysis. Cox proportional hazards regression model was used to determine the independent prognostic factors. A value of $P < .05$ was considered significant.

Results

An Optimal Cutoff Value for Elevated NLR

According to the ROC curve, the optimal cutoff value of preoperative NLR that had a relatively high specificity was 2.31. The area under the ROC curves was 0.723 with a 95% confidence interval (95% CI) for the area between 0.664 and 0.777. A cutoff value of 2.31 presented a sensitivity of 59.1% and a specificity of 79.4% (Figure 1).

The Preoperative NLR in Patients with HCC and Its Relationship with Clinical Pathologic Characteristics

As shown in Table 1, the relationship between preoperative peripheral blood NLR and clinical pathologic characteristics was investigated. One hundred thirty-five patients (52.73%) identified as high-NLR group had an elevated NLR (>2.31), and 121 patients (47.27%) were identified as low-NLR (≤2.31) group. Preoperative NLR level was closely correlated with the tumor size (range, >5cm) (χ^2 = 19.869; $P < .001$), clinical TNM stage (χ^2 = 29.576; $P < .001$), PVTT (χ^2 = 9.434; P = .002), distant metastasis (χ^2 = 7.858; P = .005), and AST (χ^2 = 4.779, P = .029). No obvious correlations with age, gender, HBsAg, AFP (>20 ng/ml), and combination of liver cirrhosis and the number of tumors were observed ($P > .05$).

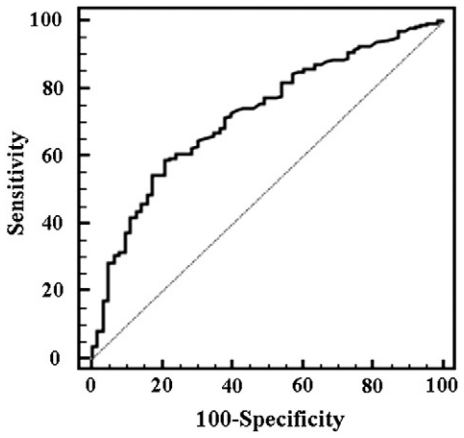

Figure 1. ROC curve to assess the predictive value of the NLR in patients with HCC who underwent curative resection is shown.

Association of NLR or Clinical Pathologic Index between Postoperative DFS and OS

Kaplan-Meier survival analysis showed that NLR > 2.31 was associated with a shorter DFS (Figure 2*A*) and OS (Figure 2*B*). Univariate analysis revealed that obvious association existed between clinical parameters and both DFS and OS (Table 2). Mean DFS in patients with NLR ≤ 2.31 was 69.47 months (95% CI, 56.93-82.01) compared with 30.23 months (95% CI, 21.99-38.48) in patients with NLR >2.31 ($P < .001$). Mean OS in NLR ≤ 2.31 group and NLR > 2.31 group was 76.15 months (63.35-88.96) and 37.96 months (28.52-47.40), respectively ($P < .001$). In addition to high-NLR group (NLR > 2.31), size of tumor >5cm, multiple tumor number, III-IV of TNM stage, and combination of PVTT, distant metastasis, and AST > 40 U/l were also associated with a shorter DFS and OS, and recurrence was associated with a shorter OS (Table 2). As mentioned above, the cutoff value of NLR was selected as 3.0 [16] or 5.0 [17,18] in previous reports, so we also evaluated the patients with HCC in this study using these cutoff values. Kaplan-Meier survival analysis showed that NLR >3.0 (Figure 2, *C* and *D*) and 5.0 (Figure 2, *E* and *F*) were associated with a shorter DFS and OS, but there are 81 (31.64%) cases with NLR >3.0 in 256 patients with HCC (Figure 2, *C* and *D*) and only 29 (11.33%) cases with NLR >5.0 in 256 patients with HCC (Figure 2, *E* and *F*).

Independent Predictors of DFS and OS in the Stepwise Multivariate Cox Proportional Hazards Model

The Cox proportional hazards model was used to examine the association between clinicopathologic factors and DFS/OS after surgical resection of HCC (Table 3). After adjusting other confounding factors, except recurrence factor for OS, seven associated factors (high NLR, size of tumor >5 cm, multiple tumor number, III-IV of TNM stage, and combination of PVTT, distant metastasis, and AST > 40 U/l) were analyzed for DFS and OS using the stepwise multivariate Cox proportional hazards model. Four factors were significant in the Cox proportional hazards model. The hazard ratio (HR), 95% CI, and P values of the four independent predictors are listed in Table 3. A stepwise multivariate Cox proportional hazards model revealed that high NLR (HR, 1.690; 95% CI, 1.247-2.291; P = .001), size of tumor > 5 cm (HR, 1.974; 95% CI, 1.200-3.247; P = .007), III-IV of TNM stage (HR, 1.727; 95% CI, 1.183-2.520; P = .005) and AST > 40 U/l (HR, 1.888; 95% CI, 1.391-2.563; $P < .001$) were independent predictors for DFS (Table 3). High NLR (HR, 1.639; 95% CI, 1.212-2.218; P = .001), size of tumor >5 cm (HR, 1.922; 95% CI, 1.168-3.162; P = .010), III-IV of TNM stage (HR, 1.806; 95% CI, 1.236-2.638; P = .002), and AST > 40 U/l (HR, 1.916; 95% CI, 1.415-2.595; $P < .001$) were independent predictors for OS (Table 3).

Kaplan-Meier analysis of DFS and OS in 256 patients with HCC Based on Statistically Significant Clinical Parameters

We established a preoperative prognostic score model by calculating the number of independent predictors (NLR, size of tumor, TNM stage, and AST) for each patient. Each factor was allotted a score of 1, and then patients were divided into five categories by their risk scores (RSs) (0, 1, 2, 3, and 4). For example, "RS = 0" means patients without any of the above factors; this group occupied 8.59% (22 of 256). "RS = 4" means patients with all four factors; it occupied 26.56% (68 of 256) of patients carrying all four

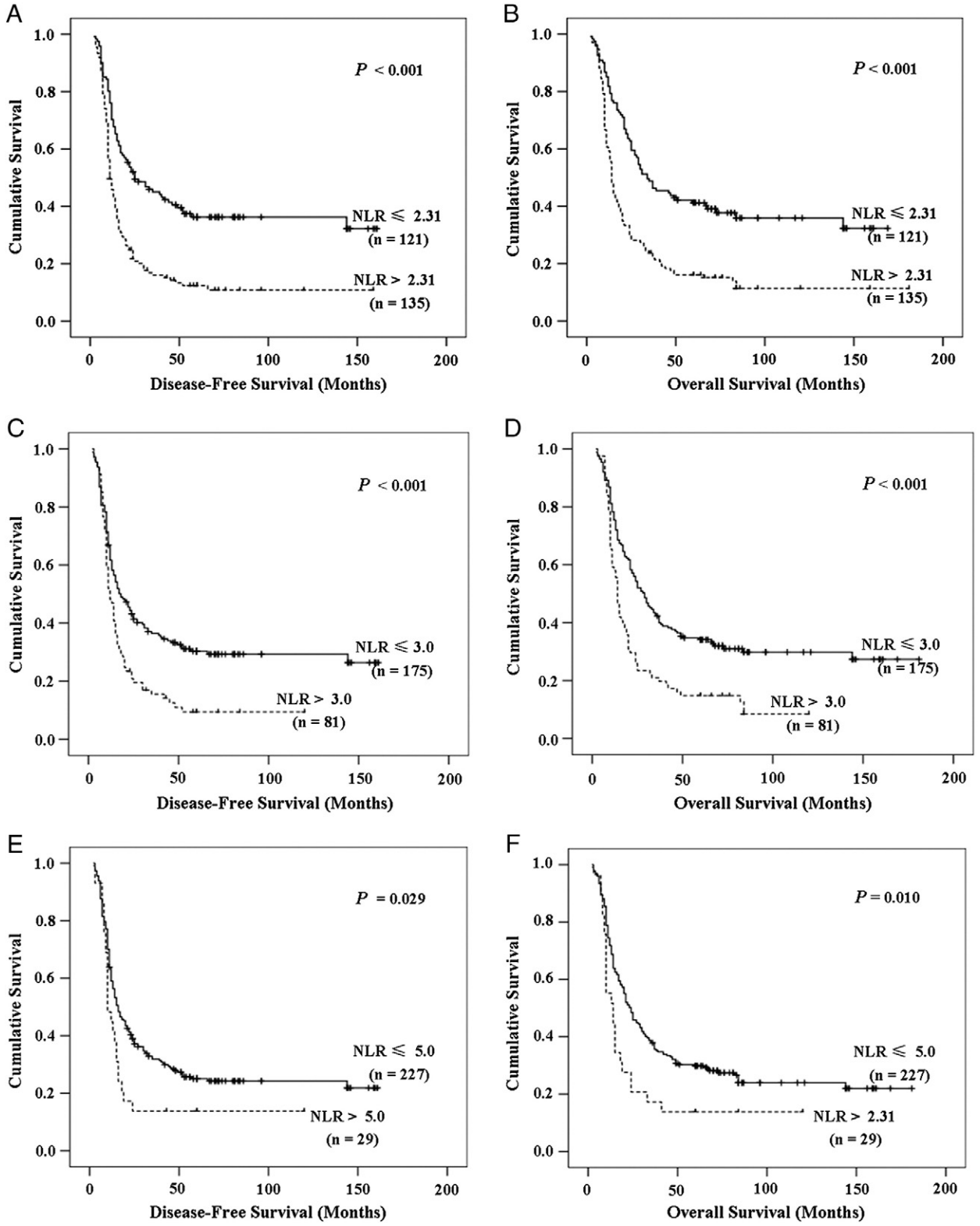

Figure 2. (A and B) Kaplan-Meier survival analysis of patients with NLR > 2.31 having a shorter DFS and OS. The solid line represents the NLR ≤ 2.31, whereas the dashed line represents the NLR > 2.31. (C and D) Kaplan-Meier survival analysis of patients with NLR > 3.0 having a shorter DFS and OS. The solid line represents the NLR ≤ 3.0, whereas the dashed line represents the NLR > 3.0. (E and F) Kaplan-Meier survival analysis of patients with NLR > 5.0 having a shorter DFS and OS. The solid line represents the NLR ≤ 5.0, whereas the dashed line represents the NLR > 5.0.

factors (Figure 3). Because no significant difference were observed in DFS and OS between patients whose RS equals 0 or 1 (Figure 3, *A* and *C*; $P = .132$ and $P = .145$, respectively), these patients were merged as score ≤ 1 group. By combining four independent predictors, patients with different RSs showed distinguishable DFS (RS ≤ 1 *vs* RS = 2, $P < .001$; RS = 2 *vs* RS = 3, $P = .037$; and RS = 3 *vs*

Table 2. Association between NLR, Clinical Parameters, and DFS/OS.

Clinical Character	Category	No. of Patients	DFS (Mo)			OS (Mo)		
			Mean	95% CI	*P* Value	Mean	95% CI	*P* Value
NLR	≤ 2.31	121	69.47	56.93-82.01	< .001	76.15	63.35-88.96	< .001
	> 2.31	135	30.23	21.99-38.48		37.96	28.52-47.40	
Age (yr)	≤ 55	176	48.07	38.75-57.40	.526	56.48	45.99-66.98	.292
	> 55	80	50.74	36.96-64.51		55.96	42.70-69.22	
Gender	Female	30	54.99	33.09-76.89	.167	63.04	42.58-83.51	.095
	Male	226	47.49	39.35-55.63		55.50	46.42-64.58	
HBsAg	Negative	41	48.52	28.94-68.10	.834	53.32	34.40-72.24	.968
	Positive	215	48.60	40.26-56.95		57.00	47.56-66.45	
AFP (ng/ml)	≤ 20	62	45.72	30.28-61.17	.867	54.07	39.10-69.03	.764
	> 20	194	49.74	40.80-58.69		57.48	47.52-67.43	
Tumor size (cm)	≤ 5	47	98.96	79.86-118.07	< .001	111.32	89.55-133.08	< .001
	> 5	208	37.63	30.00-45.26		42.50	34.96-50.04	
Cirrhosis	No	27	44.74	21.99-67.50	.334	48.33	26.29-70.38	.518
	Yes	229	49.22	40.98-57.45		58.08	48.89-67.27	
Tumor no.	Single	163	58.20	47.84-69.56	.001	66.93	55.42-78.44	.003
	Multiple	93	28.03	20.41-35.65		33.78	26.12-41.44	
TNM stage	I-II	109	79.01	65.64-92.37	< .001	92.80	78.33-107.27	< .001
	III-IV	147	25.45	18.90-32.00		28.78	22.26-35.29	
PVTT	No	184	58.24	48.50-67.98	< .001	69.04	58.32-79.75	< .001
	Yes	72	22.21	14.72-29.69		23.63	16.22-31.03	
Distant metastasis	No	218	54.26	45.51-63.02	< .001	63.65	53.91-73.38	< .001
	Yes	38	15.97	10.03-21.92		19.61	13.06-26.15	
Recurrence	No	164				48.70	39.15-58.25	.002
	Yes	92				66.52	52.39-80.64	
AST (U/l)	≤ 40	117	71.03	58.08-83.98	< .001	82.74	68.40-97.08	< .001
	> 40	139	30.93	22.84-39.03		34.67	26.70-42.64	

NLR, neutrophil-to-lymphocyte ratio; HBsAg, hepatitis B surface antigen; AFP, α-fetoprotein; TNM, tumor-node-metastasis; PVTT, portal vein tumor thrombus; AST, aspartate aminotransferase.

RS = 4, $P < .001$) (Figure 3*B*) and OS (RS ≤ 1 *vs* RS = 2, $P < .001$; RS = 2 *vs* RS =3, $P = .015$; and RS = 3 *vs* RS = 4, $P < .001$) (Figure 3*D*). Surprisingly, the proportion of patients with HCC with RS = 4 was very high, occupying 26.56% (68 of 256) of total patients (Figure 3*A*). The DFS and OS in 68 patients with a score of 4 decreased sharply, and all these patients showed much shorter DFS and OS.

Discussion

Experimental and clinical data indicate that chronic inflammation significantly contributes to cancer development. The presence of systemic inflammation is associated with poor survival in certain tumors [15]. Inflammation can promote all stages of tumor development through multiple mechanisms, which include predisposing tumor cell to proliferation and resistance to apoptosis, induction of DNA mutations, and promotion of angiogenesis, invasion, and metastasis [19]. The prognostic value of some systemic inflammatory markers such as C-reactive protein [15] and NLR have been investigated in tumor patients.

Inflammatory environments can accelerate the progression of metastasis by neutrophi- mediated mechanisms [20]. NLR reflects an inflammatory status; a preoperatively high ratio is most likely to reflect more aggressive disease and hence represents poorer outcome. Patients with tumor and elevated NLR have a relative lymphocytopenia and neutrophilic leukocytosis, which denote that the balance is tipped in favor of protumor inflammatory response leading to poor oncologic outcome. The measurement of NLR would be of substantial value in evaluating prognosis of some malignancies like colon cancer, gastric cancer [6,7], lung cancer [8], renal cell carcinoma [9,10], breast cancer [11], colorectal cancer [12], pancreatic cancer [13], and soft-tissue sarcoma [14].

NLR as a prognostic marker in patients with HCC attracted more and more researchers' attention [15-18]. As we know, the NLR is a marker of systemic inflammation that is easily measured, easily calculated from routinely available data, highly repeatable, and inexpensive [21]. In this study, we authenticated that the optimal cutoff value of NLR was 2.31 according to the ROC curve (Figure 1). NLR appeared to be associated with tumor size, clinical TNM stage, PVTT, distant metastasis, and AST in HCC (Table 1). The NLR > 2.31 was identified as a factor for lower survival in patients with HCC. Patients with elevated NLR (>2.31) had a significantly shorter DFS and OS than those with low NLR (≤ 2.31) (Figure 2, Table 2). Consistent with previous findings [16-18], NLR >3.0 (Figure 2, *C* and *D*) and 5.0 (Figure 2, *E* and *F*) were also associated with a shorter DFS and OS, but there were 81 (31.64%) cases with NLR >3.0 in 256 patients with HCC (Figure 2, *C* and *D*) and only 29 (11.33%) cases with NLR >5.0 in 256 patients with

Table 3. Cox Multivariate Proportional Hazards Model of Independent Predictors on DFS and OS.

Variable	HR (95% CI)	*P* Value
DFS		
NLR (≤ 2.31 *vs* > 2.31)	1.690 (1.247-2.291)	.001
Tumor size, cm (≤ 5 *vs* > 5)	1.974 (1.200-3.247)	.007
Tumor no. (single *vs* multiple)	1.167 (0.864-1.576)	.313
TNM stage (I-II *vs* III-IV)	1.727 (1.183-2.520)	.005
PVTT (no *vs* yes)	1.192 (0.850-1.672)	.309
Metastasis (no *vs* yes)	1.463 (0.996-2.147)	.052
AST, U/l (≤ 40 *vs* > 40)	1.888 (1.391-2.563)	< .001
OS		
NLR (≤ 2.31 *vs* > 2.31)	1.639 (1.212-2.218)	.001
Tumor size, cm (≤ 5 *vs* > 5)	1.922 (1.168-3.162)	.010
Tumor no. (single *vs* multiple)	1.045 (0.771-1.416)	.776
TNM stage (I-II *vs* III-IV)	1.806 (1.236-2.638)	.002
PVTT (no *vs* yes)	1.400 (0.995-1.970)	.054
Distant metastasis (no *vs* yes)	1.377 (0.934-2.030)	.106
AST, U/l (≤ 40 *vs* > 40)	1.916 (1.415-2.595)	< .001

CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; TNM, tumor-node-metastasis; PVTT, portal vein tumor thrombus; AST, aspartate aminotransferase.

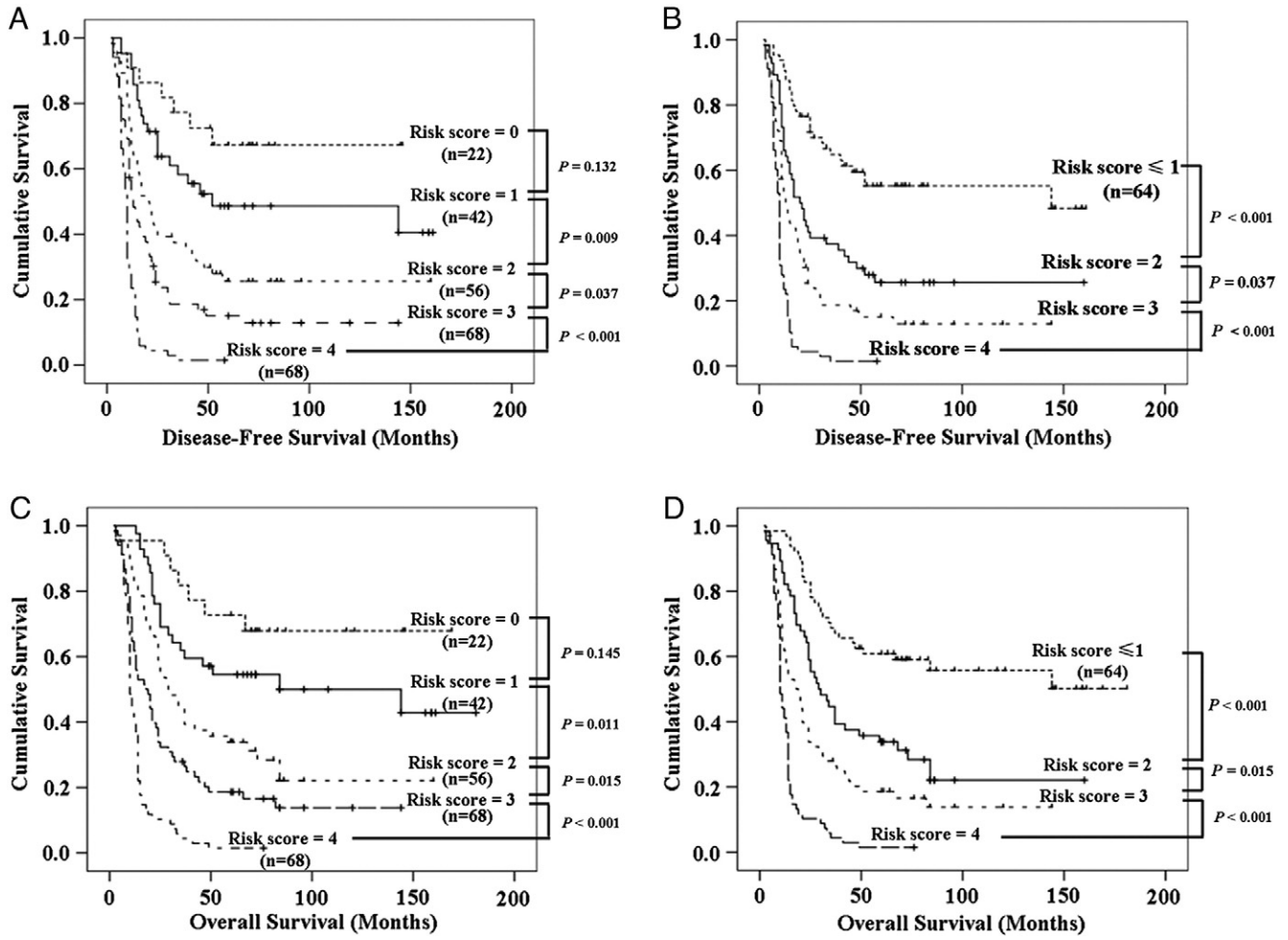

Figure 3. Kaplan-Meier analysis of DFS and OS in 256 of patients with HCC who underwent curative resection by four independent predictors. According to RS calculated from the four independent predictors (NLR >2.31, size of tumor >5 cm, III-IV of TNM stage, and AST >40 U/l), all patients were divided into five groups (0, 1, 2, 3, and 4) (A and C) or four groups (≤ 1, 2, 3, and 4) (B and D); and DFS (A and B) or OS (C and D) is compared.

HCC (Figure 2, *E* and *F*). That means that more patients with HCC are excluded using NLR >3.0 or 5.0; therefore, the cutoff value 2.31 of preoperative NLR had a higher sensitivity in patients with HCC than 3.0 or 5.0. It is noteworthy that 2.31 of preoperative NLR as an optimal cutoff value in patients with HCC is confirmed not only by this retrospective study but also by some prospective clinical trials [15,22].

The association between elevated NLR and poor prognosis is complex and remains to be elucidated. NLR is derived from the value of neutrophils and lymphocytes, both of which are major parts of white blood cells. Neutrophils mediate inflammatory response by release of arachidonic acid metabolites and platelet-activating factors, whereas a relative lymphopenia reflects the cortisol-induced stress response [23]. On the one hand, relatively increased number of circulating neutrophils may increase the levels of circulating angiogenesis-regulating chemokines, growth factors, and proteases (for instance, CXCL8, also known as IL-8 [24], vascular endothelial growth factor, matrix metallopeptidase 9 [25], and intercellular adhesion molecule 1 [26], all of which contribute to cancer development and progression by regulating cell growth, angiogenesis, or inflammation [27] and could serve as a predictor for poor survival in patients with HCC [28]). However, the host's immune response to tumor is lymphocyte dependent. Patients with elevated NLR usually have relative lymphocytopenia, and this may result in poorer lymphocyte-mediated immune response to tumor, leading to a worse prognosis and a greater chance of tumor recurrence and metastases. As we know, lymphocytes play key roles in cytotoxic cell death and cytokine production that inhibits tumor cells' proliferation and metastatic competence [29]; therefore, patients with HCC with weaker lymphocytic infiltration in tumor would have worse prognosis [30].

Up to now, there have been some different models that have limited prognostic value in HCC [31,32]. On the basis of multivariate analysis, we have established a simple preoperative prognostic multiple-factor score model; we found that high NLR, size of tumor > 5 cm, III-IV of TNM stage, and AST > 40 U/l were identified as independent prognostic factors for DFS (Figure 3, *A* and *B*, and Table 3) and OS (Figure 3, *C* and *D*, and Table 3). This is consistent with several previous reports that tumor size >5 cm was a significant risk factor of recurrence after liver resection [33-35] and AST is an independent predictor for DFS in patients with HCC [36-38]. Patients with HCC with small tumors (<5 cm) have a better prognosis [39,40]; larger tumors (>5 cm) are reported to be associated with greater likelihood of vascular invasion and higher recurrence risk [33,34].

The follow-up data by univariate analysis revealed that tumor size > 5 cm, multiple tumor number, III-IV of TNM stage, PVTT, distant metastasis, and AST > 40 U/l were associated with a shorter DFS and OS, and recurrence was associated with a shorter OS (Table 2). Although univariate analysis in this study showed that multiple tumor number, PVTT, and distant metastasis were preoperative prognostic predictors of poor DFS and OS, none of these factors were identified as independent predictors by multivariate analysis (Table 3). However, this result did not mean that these factors are not associated with recurrence and metastasis and are not potential prognostic factors for HCC after resection. For example, tumor number indicating a unifocal or multifocal tumor origin is an important determinant of prognosis in patients with HCC undergoing several kinds of treatments, and individuals with solitary HCC have relatively better survival rate and prognosis than those with multinodular tumors [41]. Previous study has also shown that PVTT is an independent predictor of microvascular invasion [42]. The main cause of metastatic and recurrence in HCC is that tumor cells tend to invade portal veins leading to PVTT, which is a unique manner of HCC dissemination and is associated with poor prognosis of HCC [43,44]. PVTT, arising from the invasion of HCC cells into the portal vein, is well acknowledged as a special type of metastasis in HCC [45] that is characterized by vascular invasion and a more aggressive phenotype.

Taken together, our results showed that high NLR (> 2.31) was an independent predictor for DFS and OS; elevated preoperative NLR reflecting tumor burden, invasion, and metastasis indirectly suggested that NLR might be a novel biomarker for HCC prognosis. We established a multiple-factor scoring system in which NLR is a major component to predict each patient's prognosis. According to the four independent predictors (high NLR, size of tumor > 5 cm, III-IV of TNM stage, and AST > 40 U/l) of the score model, the patients with postoperative HCC were separated into four distinct RS groups with significantly different prognoses. Of note, limited by the retrospective nature of this study and the small single-center sample size, further multicenter, larger prospective studies are required to validate this finding.

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