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Pretreatment Neutrophil–Lymphocyte Ratio

An Independent Predictor of Survival in Patients With Hepatocellular Carcinoma

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Abstract: The neutrophil-to-lymphocyte ratio (NLR) has been shown to be associated with prognosis in various types of cancer. We evaluated pretreatment NLR as a predictor of poor prognosis in patients with hepatocellular carcinoma (HCC), and we compared the prognostic value of NLR with other prognostic scores.

We retrospectively analyzed 825 patients diagnosed with HCC between October 2008 and May 2012. Baseline data, including the NLR and the Child–Pugh class or Model for End-Stage Liver Disease (MELD) score, were recorded before treatment. The relationships between overall survival (OS) and the study variables were assessed using univariate and multivariate analyses and receiver operating characteristic (ROC) curves. The prognostic value of NLR was assessed using a Kaplan–Meier survival analysis and compared with that of the Barcelona-Clinic Liver Cancer (BCLC) and Tumor, Node, Metastasis (TNM) staging.

The NLR, γ-glutamyltranspeptidase, α-fetoprotein $\geq$400 ng/mL, tumor number $\geq$3, tumor size $\geq$5 cm, lymph node metastasis, portal vein involvement, and Child–Pugh class were significantly associated with OS. The NLR demonstrated the strongest prognostic value (area under ROC curve = 0.811). An NLR $\geq$2.7 was a significant predictor of poor OS ($P < 0.0001$), and the survival period of patients with an NLR $\geq$2.7 decreased with more advanced BCLC and TNM stage.

Pretreatment NLR is a useful prognostic biomarker in HCC patients. The prognostic value of NLR $\geq$2.7 is superior to that of MELD stage or Child–Pugh class, and correlates with that of BCLC and TNM staging scores.

(*Medicine* 94(11):e639)

Abbreviations: AFP = α-fetoprotein, ALB = serum albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, AUC = area under the curve, BCLC = Barcelona-Clinic Liver Cancer, BSC = best supportive care, G-CSF = granulocyte-colony stimulating factor, GGT = γ-glutamyltranspeptidase, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, IL = interleukin, MELD = Model for End-Stage Liver Disease, NLR = neutrophil-to-lymphocyte ratio, OS = overall survival, PT = prothrombin time, ROC = receiver operating characteristic, TBil = total bilirubin, TNM = Tumor, Node, Metastasis.

Editor: Hua Yang.
Received: December 1, 2014; revised: February 6, 2015; accepted: February 15, 2015.
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This study was supported by grants from the National Natural Science Foundation of China (grant nos. 81273743 and 81473641) and the 215 Program from Beijing Municipal Health Bureau (2013-2-11).
The authors have no conflicts of interest to disclose.
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ISSN: 0025-7974
DOI: 10.1097/MD.0000000000000639

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and has the third highest mortality rate among cancers.[1] The incidence of HCC is the highest in countries where the hepatitis B virus (HBV) is endemic.[2] In the Asia-Pacific region, where the incidence of viral hepatitis is relatively high, HCC is a major public health problem, with the incidence of HCC in China alone accounting for 55% of HCC cases worldwide in 2002.[1] In the USA, HCC-related mortality in patients chronically infected with the hepatitis C virus (HCV) has become the fastest-rising cause of cancer-related death.[2] Despite the many recent advances in the treatment of HCC, the 5-year survival rate of HCC patients remains low, compared with other types of cancer.[2,3]

Various methods have been proposed for staging and predicting the prognosis of HCC patients, including the Barcelona-Clinic Liver Cancer (BCLC) staging system, the Tumor, Node, Metastasis (TNM) classification system, the Japanese Integrated Staging score, the Cancer of the Liver Italian Program, the Child–Pugh classification system, and the Model for End-Stage Liver Disease (MELD).[4–6] However, no worldwide consensus has been reached regarding which is the best system for predicting HCC outcomes. In addition, the application of these methods can be cumbersome, and they have been used primarily in clinical trials. The identification of more straightforward prognosticators for HCC, such as serum biomarkers, for the reliable prediction of metastatic recurrence would better facilitate the development of more efficacious therapeutic strategies for HCC.

Mounting evidence suggests that components of the systemic inflammatory response are predictors of outcome in various types of cancer, including HCC.[7–11] Previous studies have shown that a high neutrophil count (NC) is associated with angiogenesis, and that DNA damage and tumor metastasis suppress lymphocyte activity through the upregulation of cytokines that counteract the antitumor immune response.[8–10,12]

Previous studies suggested that pretreatment neutrophil-to-lymphocyte ratios (NLRs) are predictors of tumor recurrence and survival in HCC patients undergoing specific treatments.[9,10] Conversely, it has also been noted that, though NLR was predictive for overall survival (OS) in a univariate

analysis, it was not predictive in a multivariate analysis and was inferior to other inflammation-based prognostic scores.[13] In addition, a Western center study did not support the prognostic value of NLR to guide therapy for HCC, whereas MELD and Child–Pugh score were more predictive.[14] We conducted our current study to determine whether the pretreatment NLR is associated with the clinical outcome of patients with HCC and to compare the prognostic value of the pretreatment NLR with other prognostic methods of predicting survival in Chinese HCC patients. Our findings show that the pretreatment NLR is a useful prognostic biomarker in HCC patients, and that the prognostic value of NLR correlates with that of the BCLC and TNM staging scores.

PATIENTS AND METHODS

Study Population

We retrospectively reviewed the data of 906 patients who were newly diagnosed with HCC between October 2008 and May 2012 at Beijing Ditan Hospital (Beijing, China), which is affiliated with the Capital Medical University. The study was approved by the ethics committee of Beijing Ditan Hospital (Beijing, China). Patient records and information was anonymized and deidentified prior to analysis. The diagnosis of HCC was based on the detection of a histopathologically confirmed HCC lesion using at least 2 different imaging techniques or the detection of 2 such lesions using 1 imaging technique combined with a serum concentration of α-fetoprotein (AFP) $\geq$400 ng/mL. The imaging techniques used included transabdominal ultrasonography, abdominal computed tomography, magnetic resonance imaging, and hepatic angiography. Patients who were $<$18 years of age, had an active bacterial infection or upper respiratory viral infection, or had received any medications that could affect the NLR, such as granulocyte-colony stimulating factor (G-CSF), interferon, or high-dose steroids, were excluded from our study. Patients for whom data regarding tumor size, tumor number, or the above-mentioned clinical, laboratory, and imaging criteria were unavailable were also excluded. Finally a total of 825 HCC patients were included in our study.

All of the HCC patients were evaluated for potential curative therapies, which included surgical resection and liver transplantation. Patients who were not suitable candidates for curative treatments were treated with locoregional approaches, which included ablation and transarterial embolization. Radiofrequency ablation, microwave ablation, or percutaneous alcohol injection was performed in patients with 2 to 3 tumors that were each $\leq$3 cm in size. Transcatheter arterial chemoembolization and/or lipiodol-transcatheter arterial infusion were performed in patients with $\geq$4 tumors, a tumor $>$3 cm in size, or a Child–Pugh class of A or B. Molecular targeted therapy or systemic chemotherapy, which included sorafenib and FOLFOX (Folinic acid - Fluorouracil - Oxaliplatin) regimens, was performed in HCC patients who had distant metastasis and were not suitable candidates for surgery. The best supportive care (BSC) was provided for patients with distant metastasis or a Child–Pugh class of C. Patients with chronic hepatitis B or C received antiviral therapy, and patients with cirrhosis or liver dysfunction received medical treatment to reduce transaminase activity.[15,16]

Study Variables

The various demographic, medical history, serum biochemical, and clinical characteristics were analyzed at baseline. The patient characteristics analyzed included age, sex, history of alcohol use, history of smoking, and family history of HCC. The following serum biochemical variables were analyzed: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBil), serum albumin (ALB), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (GGT), serum creatinine, prothrombin time (PT), prothrombin activity (PTA), international normalized PT ratio, AFP, white blood cell (WBC) count, absolute NC, absolute lymphocyte count (LC), and absolute platelet count (PLT).

The clinical characteristics included in the analysis were HBV status, HCV status, tumor diameter, tumor number, lymph node metastasis, distant metastasis, portal vein involvement, survival time, staging method (Child–Pugh or MELD), and staging class/score. We determined the BCLC and TNM staging scores for each patient based on the clinical data, as previously described.[4,9] The NLR and platelet to lymphocyte ratio (PLR) were calculated for each patient using the earliest data recorded before treatment. The PLR was calculated by dividing the PLT by the LC. The NLR was calculated by dividing the NC by the LC. Additionally, we recorded the second NLR determination about 1 month after the first course of treatment (surgical resection, locoregional approaches, or BSC), if available, to analyze NLR dynamics over time. Survival was determined based on a 2-year posttreatment follow-up period.

Statistical Analysis

All of the statistical procedures were performed using the SPSS version 19.0 software (IBM, Armonk, NY). The categorical data are reported as the number or percentage of observations. Normally distributed continuous variables are reported as the mean ± standard deviation. Univariate and multivariate analyses of the relationships between OS and the study variables was performed using Cox proportional hazard models. Variables that were shown to be associated with OS in the univariate analysis were evaluated in the multivariate Cox proportional hazard model. The likelihood ratios forward stepwise method was used for the multivariate Cox proportional analysis. A receiver operating characteristic (ROC) curve was constructed for each study variable shown to be significantly associated with OS in the multivariate analysis, and the area under the curve (AUC) was calculated to evaluate the discriminatory capacity of each. The cut off value for the maximum sensitivity and specificity of the NLR was calculated, and the patients were divided into 2 groups based on the NLR cut off value. A Kaplan–Meier survival analysis was performed to compare the OS of the patients in different groups, and the significance of the intergroup difference was evaluated using the log-rank test. A Pearson correlation analysis was performed to determine the relationship between NLR and the BCLC and TNM staging scores. All of the probability values calculated were 2-sided, and the results of comparisons with $P < 0.05$ were considered statistically significant.

RESULTS

Patient Characteristics and Outcomes

A total of 825 HCC patients were included in our study. The baseline characteristics of the study population are shown in Table 1. The patients ranged in age from 25 to 75 years, and the mean age was 54.5 ± 9.8 years. The HCC cohort consisted of 690 men (83.6%) and 135 women (16.4%), among whom 116 (14.1%) of the patients had a family history of HCC. Hepatitis B, hepatitis C, and alcoholic liver disease was diagnosed in 739, 56, and 117 patients, respectively.

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TABLE 1. Univariate and Multivariate Analyses of OS in Patients With HCC (N = 825)

Variable	Value[†]	Univariate HR (95% CI)	Multivariate HR (95% CI)
Age, y	54.5 ± 9.8	0.985 (0.976–0.995)*	
Men/women	690/135	0.649 (0.485–0.868)*	
Family history of HCC	116	1.137 (0.858–1.507)	
History of smoking	321	1.207 (0.993–1.468)	
History of alcohol use	325	1.324 (1.210–1.448)*	
Number of tumors ≥3	292	2.892 (2.380–3.516)*	1.412 (1.140–1.749)*
Tumor size ≥5 cm	268	3.928 (3.224–4.785)*	1.895 (1.514–2.371)*
Lymph node metastasis	77	3.905 (3.024–5.041)*	1.879 (1.432–2.465)*
Portal vein involvement	207	9.072 (7.325–11.236)*	4.284 (3.281–5.595)*
ALT, IU/L	57.2 ± 73.8	1.001 (1.000–1.002)	
AST, IU/L	70.5 ± 86.3	1.003 (1.002–1.003)*	
TBil, μmol/L	27.2 ± 26.2	1.009 (1.007–1.011)	
ALB, g/L	36.4 ± 6.5	0.963 (0.949–0.977)*	
ALP, IU/L	122.7 ± 81.0	1.005 (1.004–1.006)*	
GGT, IU/L	104.2 ± 113.6	1.004 (1.003–1.004)*	1.001 (1.000–1.002)*
Cr, μmoI/L	69.6 ± 29.7	1.005 (1.002–1.007)*	
PTA, %	76.1 ± 18.7	0.983 (0.978–0.988)*	
WBC, 10^9/L	4.6 ± 1.9	1.182 (1.126–1.241)*	
ANC, 10^9/L	2.8 ± 1.5	1.395 (1.319–1.475)*	
ALC, 10^9/L	1.2 ± 0.6	0.517 (0.431–0.619)*	
PLT, 10^9/L	112.0 ± 67.8	1.003 (1.001–1.004)*	
NLR	2.8 ± 1.9	1.343 (1.298–1.390)*	1.171 (1.121–1.222)*
PLR	99.9 ± 58.2	1.008 (1.007–1.010)*	
AFP ≥400 ng/mL	204	3.015 (2.467–3.686)*	1.363 (1.084–1.711)*
MELD score	5.9 ± 5.0	1.057 (1.042–1.072)*	
Child–Pugh class			
A	513	Reference	
B	253	2.037 (1.652–2.511)*	1.598 (1.275–2.004)*
C	59	4.959 (3.638–6.760)*	4.943 (4.484–7.012)*

AFP = α-fetoprotein, ALB = serum albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CI = confidence interval, Cr = serum creatinine, GGT = γ-glutamyltranspeptidase, HCC = hepatocellular carcinoma, HR = hazard ratio, ALC = absolute lymphocyte count, MELD = Model for End-Stage Liver Disease, ANC = absolute neutrophil count, NLR = neutrophil–lymphocyte ratio, OS = overall survival, PLR = platelet–lymphocyte ratio, PLT = platelet count, PTA = prothrombin activity, TBil = total bilirubin, WBC = white blood cell.

[†] Data are presented as the number of observations or the mean ± standard deviation.

* $P < 0.05$.

At the time of diagnosis, 513 (62.2%), 253 (30.7%), and 59 (7.1%) of the patients were classified as Child–Pugh class A, B, or C, respectively. A total of 268 (32.5%) of the patients had a tumor ≥5 cm in size, and 292 (35.4%) of the patients had ≥3 tumors. Seventy-seven (9.3%) of the patients had lymph node metastasis, and 208 (25.2%) of the patients had portal vein involvement. The mean levels of ALT and TBil were 57.2 IU/L and 27.2 μmol/L, respectively, and the mean NLR was 2.8.

Surgical resection was performed in 90 (10.9%) of the patients, and locoregional approaches were administered in 698 (89.3%) of the patients. The remaining 39 patients (4.7%) received BSC. At the end of the 2-year follow-up period, 414 (50.2%) of the patients had survived, whereas 411 (49.8%) of the patients had died. The median OS period was 24 months. The 6-, 12-, and 24-month OS rates were 82.9%, 66.2%, and 50.2%, respectively.

Prognostic Value of Pretreatment NLR in HCC

To identify predictors of HCC survival, the prognostic value of 26 variables were evaluated. The univariate analysis showed that age, sex, history of alcohol use, tumor number ≥3, tumor size ≥5 cm, lymph node metastasis, portal vein involvement, AST, TBil, ALB, ALP, GGT, Cr, PTA, WBC, ANC, ALC, PLT, NLR, PLR, AFP ≥400 ng/mL, MELD score, and Child–Pugh class were associated with OS in HCC patients ($P < 0.05$, Table 1). In the multivariate Cox regression analysis, only NLR, tumor number ≥3, tumor size ≥5 cm, lymph node metastasis, portal vein involvement, Child–Pugh class, GGT, and AFP ≥400 ng/mL were significantly associated with OS ($P < 0.05$, Table 1).

To compare the predictive value of NLR, GGT, AFP ≥400 ng/mL, tumor number ≥3, tumor size ≥5 cm, lymph node metastasis, portal vein involvement, and Child–Pugh class for the prognosis of HCC, we compared the ROC curves of these parameters. As shown in Figure 1, the ROC curve for NLR (0.811) had the highest AUC, compared with that for tumor number ≥3 (0.666), tumor size ≥5 cm (0.702), lymph node metastasis (0.592), portal vein involvement (0.748), Child–Pugh class (0.640), GGT (0.745), and AFP ≥400 ng/mL (0.634) at 24 months posttreatment. The AUC value for NLR was also higher than that of the other study variables at 6 months (0.789)

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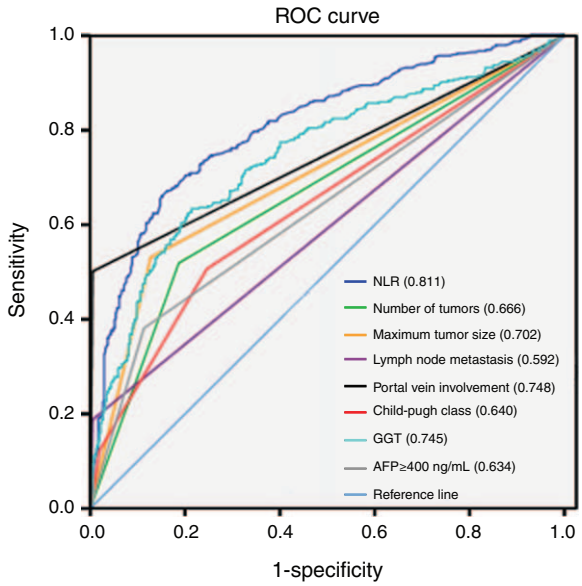

FIGURE 1. ROC curves for the study variables shown to be associated with OS in the multivariate analysis. The AUC was calculated to evaluate the discriminatory capacity of each variable for predicting survival in patients with HCC. AFP = α-fetoprotein, AUC = area under the curve, GGT = γ-glutamyltranspeptidase, HCC = hepatocellular carcinoma, NLR = neutrophil-to-lymphocyte ratio, OS = overall survival, ROC = receiver operating characteristic.

and 12 months (0.817) after treatment. These results indicated that the pretreatment NLR in HCC patients is of significant prognostic value.

We calculated the cut off value, sensitivity, specificity, positive predictive value, and negative predictive value of the pretreatment NLR. Using a sensitivity of 66.2% and a specificity of 84.8% as optimal conditions, the cut off value for NLR was determined to be 2.7. The Kaplan–Meier analysis showed that the OS of patients with an NLR $\geq$2.7 was significantly poorer at 6, 12, and 24 months after treatment than that of patients with an NLR <2.7. As shown in Figure 2A, the 6-, 12-, and 24-month OS rates of the patients with an NLR $\geq$2.7 (66.3%, 39.2%, and 19.0%, respectively) were significantly lower ($P < 0.0001$) than those of patients with an NLR <2.7 (94.1%, 84.4%, and 71.2%, respectively).

Impact of NLR Dynamic on OS in HCC

We tested the reproducibility of our findings by using another NLR determination at a second timepoint (Figure 2B). The second NLR determination was available in 83.2% of patients (686 of the 825 patients) and was performed 37 days (median, 95% CI 30–49) after the first course of treatment. Elevated NLR levels remained associated with poor OS. As shown in Figure 2B, the 6-, 12-, and 24-month OS rates of the patients with posttreatment NLR $\geq$2.7 (65.2%, 50.9%, and 30.8%, respectively) were significantly lower ($P < 0.0001$) than those of patients with posttreatment NLR <2.7 (94.9%, 84.0%, and 68.5%, respectively, Figure 2B).

Finally, patients were categorized into 4 groups according to the changes in NLR: pretreatment and posttreatment NLR <2.7 (n = 347, cohort 1), pretreatment NLR <2.7 and posttreatment NLR $\geq$2.7 (n = 84, cohort 2), pretreatment NLR $\geq$2.7 and posttreatment NLR <2.7 (n = 66, cohort 3), and pretreatment and posttreatment NLR $\geq$2.7 (n = 189, cohort 4). As shown in Figure 2C, patients with lower posttreatment NLR had an improved OS when compared with patients with higher posttreatment NLR (cohort 1 vs 2, $P < 0.0001$; cohort 1 vs 4, $P < 0.0001$; cohort 3 vs 2, $P = 0.004$; cohort 3 vs 4, $P < 0.0001$). Persistence of NLR levels (cohort 1, 4) retained prognostic significance, whereas a new elevation of NLR (cohort 2) was associated with a dismal prognosis.

Correlation of Pretreatment NLR Cut Off Value With BCLC and TNM Staging of HCC

The relationship between the pretreatment NLR and the BCLC and TNM stages for the prognosis of HCC was evaluated. Positive linear correlations were observed between the NLR and the BCLC ($r = 0.462$, $P < 0.001$) and TNM ($r = 0.363$, $P < 0.001$) stages. The NLR increased with increasing BCLC stage (Figure 3A), with NLR values of 2.08 ± 1.18 in stage 0/A; 2.70 ± 1.71 in stage B; 3.80 ± 1.93 in stage C; and 4.34 ± 2.41 in stage D (stage 0/A vs B, $P < 0.0001$; stage B vs C, $P < 0.0001$; stage C vs D, $P = 0.1251$). Likewise, the NLR increased with increasing TNM stage (Figure 3B), with NLR values of 1.70 ± 0.69 in stage I; 2.23 ± 1.38 in stage II; 3.82 ± 2.12 in stage III; and 3.75 ± 1.66 in stage IV (stage I vs II, $P = 0.0972$; stage II vs III, $P < 0.0001$; stage III vs IV, $P = 0.5735$).

We also performed Kaplan–Meier survival analyses to evaluate the relationships between the NLR cut off value and the BCLC and TNM stages for the prognosis of HCC. The OS of patients with an NLR $\geq$2.7 was significantly poorer than that of patients with an NLR <2.7 for all BCLC stages ($P < 0.0001$ to $P = 0.0006$), and the OS period of patients with an NLR $\geq$2.7 decreased with more advanced BCLC stage throughout the 2-year follow-up (Figure 4A–D). The OS of patients with an NLR $\geq$2.7 was significantly poorer than that of patients with an NLR <2.7 for TNM stages II, III, and IV ($P < 0.0001$ to $P = 0.0365$, Figure 4F–H), whereas the OS of the small number of TNM stage I patients (n = 2) with an NLR $\geq$2.7 was not significantly different than that of the TNM stage I patients with an NLR <2.7 ($P = 0.7518$, Figure 4E). The OS period of patients with an NLR $\geq$2.7 also decreased with more advanced TNM stage throughout the 2-year follow-up.

DISCUSSION

During the last decade, multiple studies have shown that the pretreatment NLR is a predictor of clinical outcome in various malignancies, including HCC.[7,11,13] In our current study, the pretreatment NLR was an independent prognostic factor for reduced OS in HCC patients, and the prognostic value of NLR $\geq$2.7 is superior to that of other common serum biochemical variables, tumor characteristics, MELD stage or Child–Pugh class. The findings were reproducible at a second independent time point with another NLR determination. By subgroup analyses, we also found that elevated NLR correlated with BCLC and TNM stage ($r = 0.462$, $P < 0.001$; $r = 0.363$, $P < 0.001$, respectively), with patients with a higher NLR tending to have greater liver involvement and metastasis. These findings suggest that high NLR is predictive of an aggressive HCC phenotype that contributes to poor OS.

Although measured easily, the clinical relevance of NLR is complex because it represents a combination of factors related to both inflammation and host immunity. Chronic HBV or HCV infection are associated with HCC, and both types of viral

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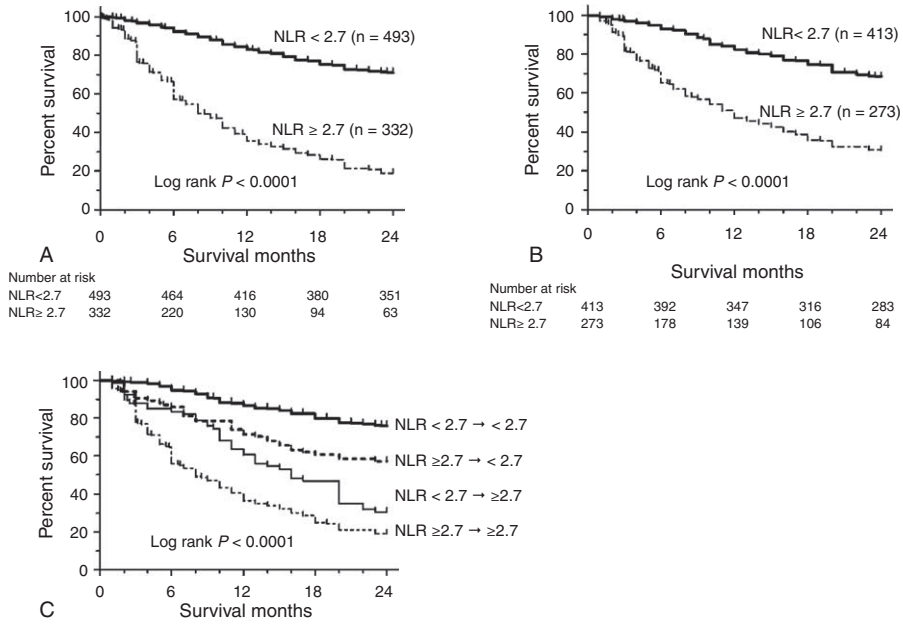

FIGURE 2. (A) Kaplan–Meier survival curves for HCC patients with an NLR ≥2.7 and those with an NLR <2.7 for the 2-year follow-up period. (B) Confirmation of the prognostic significance of NLR using the cut off <2.7 and ≥2.7 at a second independent timepoint using a second NLR determination. (C) Impact of NLR dynamic between the first and second NLR determination on OS. HCC = hepatocellular carcinoma, NLR = neutrophil-to-lymphocyte ratio, OS = overall survival.

infection cause persistent inflammation. The systemic and local inflammatory responses to viral infection may provide a microenvironment that is favorable for tumor growth and metastasis.[8] A high NLR has been shown to be associated with more numerous tumor-associated macrophages[17] and an increased level of interleukin (IL)-17,[18] which may promote systemic neutrophilia through the secretion of IL-6, IL-8, and CC chemokine ligand 2. In addition, the expression of G-CSF and macrophage colony-stimulating factor at high levels in tumor and peritumoral tissues, respectively, is associated with an elevated NC.[19–21] Neutrophils are a primary source of circulating vascular endothelial growth factor, which plays a key role in angiogenesis,[22] and neutrophils can contribute to metastasis by promoting the motility of tumor cells and the adhesion of metastatic tumor cells in liver sinusoids.[23,24]

Lymphocytopenia can indicate the suppression of innate cellular immunity, which may attenuate lymphocyte-mediated antitumor immunity.[25] Neutrophils have been shown to suppress the cytotoxic activity of lymphocytes, natural killer cells, and activated T cells through the production of arginase, nitric oxide, and reactive oxygen species.[26] Therefore, patients with elevated NLR may have mild lymphocytopenia and neutrophilic leukocytosis, which creates a microenvironment that favors metastasis and suppresses cellular immunity.[8] Thus, NLR may

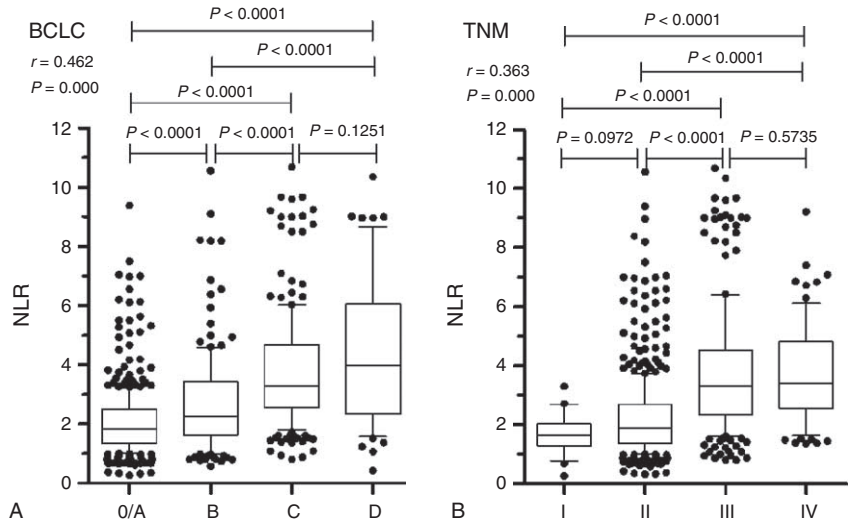

FIGURE 3. Box plots of the NLR and the BCLC and TNM stages of the patients with HCC. BCLC = Barcelona-Clinic Liver Cancer, HCC = hepatocellular carcinoma, NLR = neutrophil-to-lymphocyte ratio, TNM = Tumor, Node, Metastasis.

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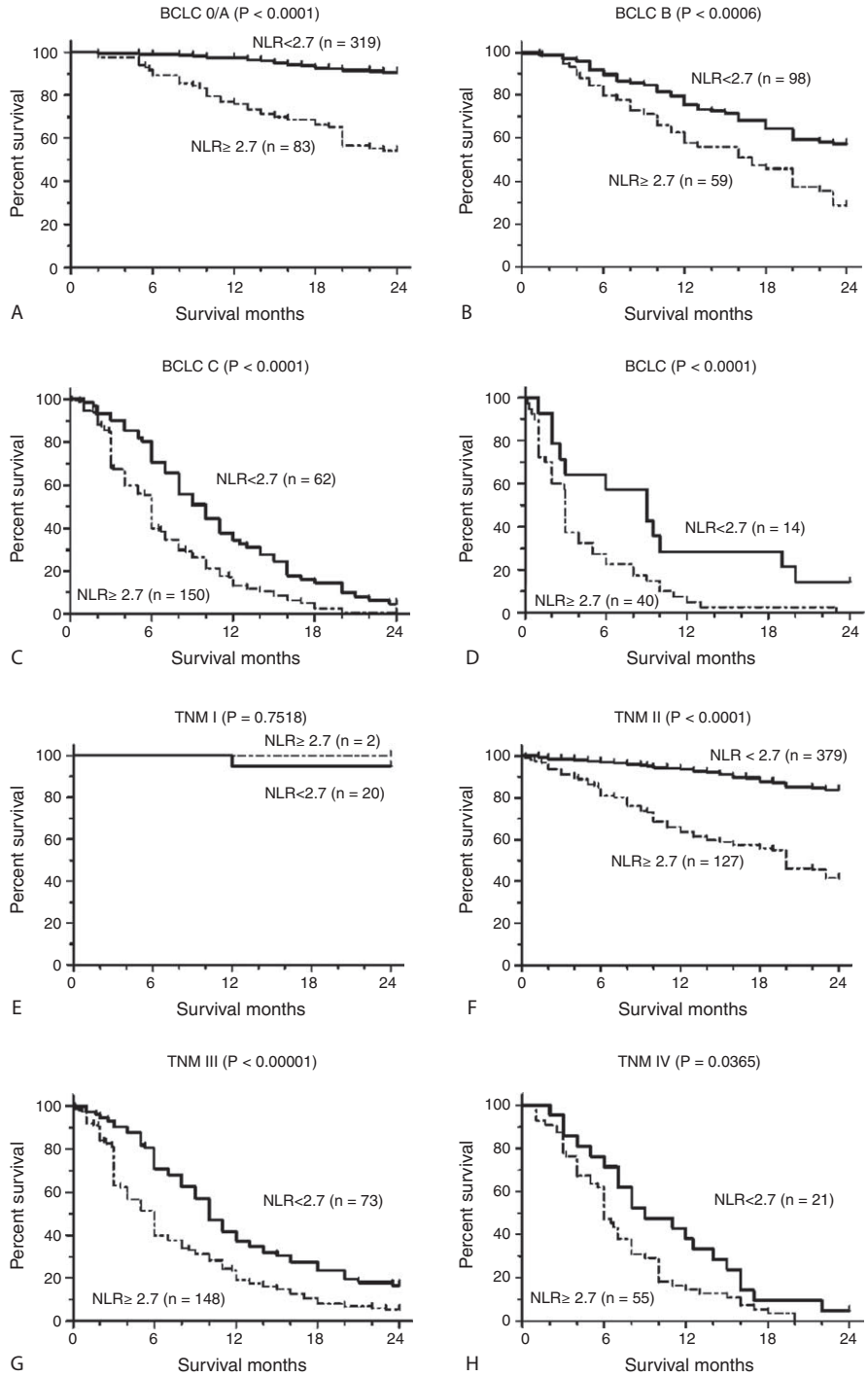

FIGURE 4. BCLC- and TNM-stage-specific Kaplan–Meier survival curves for HCC patients with an NLR ≥2.7 and those with an NLR <2.7. BCLC = Barcelona-Clinic Liver Cancer, HCC = hepatocellular carcinoma, NLR = neutrophil-to-lymphocyte ratio, TNM = Tumor, Node, Metastasis.

reflect the balance between tumorigenic inflammatory processes and antitumor cellular immunity.

In our current study, the prognostic value of the pretreatment NLR was superior to that of GGT, AFP, tumor number, tumor size, lymph node metastasis, portal vein involvement, and Child–Pugh class. We determined that an NLR of 2.7 was the best cut off value for distinguishing between patients with a poor prognosis and those with a better prognosis. This finding is similar to that of Mano et al,[10] who also found that elevated NLR was an independent predictor of survival in HCC patients. However, such findings are inconsistent with those of certain previous studies.[14,27] The retrospective, single-center design of

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our study may have influenced our results, thus contributing to differences between our findings and those of previous investigations. However, our stringent exclusion of patients with fever or a concurrent bacterial or nonhepatitis viral infection was likely sufficient to minimize the influence of confounding comorbidities with inflammatory components.

In conclusion, our results demonstrated that a pretreatment NLR $\geq$2.7 is a strong predictor of poor survival in patients with HCC, and the prognostic value of an NLR $\geq$2.7 correlates with BCLC and TNM stage scores. Therefore, a pretreatment NLR $\geq$2.7 is a useful prognostic biomarker for predicting clinical outcomes in HCC patients. Future prospective, randomized studies with larger samples are warranted to confirm our results. Future investigations of possible links between immunological factors that influence the NLR and processes that promote tumor metastasis are also warranted to clarify the clinical relevance of NLR levels in cancer patients.

REFERENCES

1. Feng K, Yan J, Li X, et al. A randomized controlled trial of radiofrequency ablation and surgical resection in the treatment of small hepatocellular carcinoma. *J Hepatol.* 2012;57:794–802.
2. El-Serag HB. Hepatocellular carcinoma. *N Engl J Med.* 2011;365:1118–1127.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin.* 2013;63:11–30.
4. Camma C, Cabibbo G. Prognostic scores for hepatocellular carcinoma: none is the winner. *Liver Int.* 2009;29:478–480.
5. Edge SB, Byrd D, Compton CC. *AJCC Cancer Staging Manual. From the AJCC Cancer Staging Manual.* 7th ed. New York: Springer; 2010.
6. Zhang JF, Shu ZJ, Xie CY, et al. Prognosis of unresectable hepatocellular carcinoma: comparison of seven staging systems (TNM, Okuda, BCLC, CLIP, CUPI, JIS, CIS) in a Chinese cohort. *PloS One.* 2014;9:e88182.
7. Bhatti I, Peacock O, Lloyd G, et al. Preoperative hematologic markers as independent predictors of prognosis in resected pancreatic ductal adenocarcinoma: neutrophil–lymphocyte versus platelet–lymphocyte ratio. *Am J Surg.* 2010;200:197–203.
8. Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002;420:860–867.
9. Jaiswal M, LaRusso NF, Burgart LJ, et al. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res.* 2000;60:184–190.
10. Mano Y, Shirabe K, Yamashita Y, et al. Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival after hepatectomy for hepatocellular carcinoma: a retrospective analysis. *Ann Surg.* 2013;258:301–305.
11. Sharaiha RZ, Halazun KJ, Mirza F, et al. Elevated preoperative neutrophil:lymphocyte ratio as a predictor of postoperative disease recurrence in esophageal cancer. *Ann Surg Oncol.* 2011;18:3362–3369.
12. Dan J, Zhang Y, Peng Z, et al. Postoperative neutrophil-to-lymphocyte ratio change predicts survival of patients with small hepatocellular carcinoma undergoing radiofrequency ablation. *PloS One.* 2013;8:e58184.
13. Kinoshita A, Onoda H, Imai N, et al. Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. *Br J Cancer.* 2012;107:988–993.
14. Sullivan KM, Groeschl RT, Turaga KK, et al. Neutrophil-to-lymphocyte ratio as a predictor of outcomes for patients with hepatocellular carcinoma: a Western perspective. *J Surg Oncol.* 2014;109:95–97.
15. Bruix J, Sherman M. American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology.* 2011;53:1020–1022.
16. Forner A, Reig ME, de Lope CR, et al. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis.* 2010;30:61–74.
17. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer.* 2004;4:71–78.
18. Kono H, Fujii H, Ogiku M, et al. Role of IL-17A in neutrophil recruitment and hepatic injury after warm ischemia-reperfusion mice. *J Immunol.* 2011;187:4818–4825.
19. Poon RT, Fan ST, Lo CM, et al. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg.* 2002;235:373–382.
20. Poon RT, Fan ST, Lo CM, et al. Improving survival results after resection of hepatocellular carcinoma: a prospective study of 377 patients over 10 years. *Ann Surg.* 2001;234:63–70.
21. Ziparo V, Balducci G, Lucandri G, et al. Indications and results of resection for hepatocellular carcinoma. *Eur J Surg Oncol.* 2002;28:723–728.
22. Kusumanto YH, Dam WA, Hospers GA, et al. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis.* 2003;6:283–287.
23. McDonald B, Spicer J, Giannais B, et al. Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms. *Int J Cancer.* 2009;125:1298–1305.
24. Wu Y, Zhao Q, Peng C, et al. Neutrophils promote motility of cancer cells via a hyaluronan-mediated TLR4/PI3K activation loop. *J Pathol.* 2011;225:438–447.
25. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science.* 2011;331:1565–1570.
26. Muller I, Munder M, Kropf P, et al. Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms? *Trends Immunol.* 2009;30:522–530.
27. Rashid F, Waraich N, Bhatti I, et al. A pre-operative elevated neutrophil:lymphocyte ratio does not predict survival from oesophageal cancer resection. *World J Surg Oncol.* 2010;8:1.

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