Association Between Neutrophil-to-Lymphocyte Ratio with Inflammatory Activity and Fibrosis in Non-alcoholic Fatty Liver Disease

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ABSTRACT

Background: *Inflammation plays an important role in the development and progression of non-alcoholic steatohepatitis (NASH), and NASH is a powerful driving force for the progression of fibrosis. The neutrophil-to-lymphocyte ratio (NLR) is a simple emerging indicator of inflammation. We aimed to assess the potential association between NLR and histological severity of non-alcoholic fatty liver disease (NAFLD).*

Methods: *This retrospective study consisted of 231 patients with biopsy-proven NAFLD in China from August 2017 to September 2019. The steatosis, activity, and fibrosis scoring system were used to evaluate liver biopsy tissue.*

Results: *Of the 231 patients with NAFLD, advanced inflammatory activity was present in 43.3% and significant fibrosis in 25.5% of patients. Multivariate logistic regression analysis showed NLR to be correlated with advanced inflammatory activity (Odds ratio (OR): 0.62, 95% CI: 0.42-0.94, P = .025) and significant fibrosis (OR: 0.57, 95% CI: 0.35-0.94, P = .028). The NLR was inversely associated with the degree of steatosis, lobular inflammation and fibrosis (r = −0.16, P = .014; r = −0.15, P = .019; r = −0.13, P = .046, respectively), but had no association with the severity of ballooning. The multivariate-adjusted models had good predictability for advanced inflammatory activity (area under curves (AUC) 0.790, 95% CI: 0.730-0.850) and for significant fibrosis (AUC 0.798, 95% CI: 0.728-0.868).*

Conclusion: *This study showed negative correlations between elevated NLR levels with advanced inflammatory activity and significant fibrosis in patients with NAFLD. Our results also suggested that NLR could be considered as a simple and noninvasive mark to identify high-risk populations in NAFLD.*

Keywords: *Neutrophil-to-lymphocyte ratio, inflammatory, fibrosis, non-alcoholic fatty liver disease*

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has rapidly become one of the leading causes of chronic liver disease in the world, affecting over 25% of the world's population.[1] In Asia, the prevalence of NAFLD varies from 15 to 45% and is still rising.[2] Noteworthy, the prevalence is up to 60% in the Middle East from a recent study.[3] The histological spectrum of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), to cirrhosis or even hepatocellular carcinoma (HCC).[4] Recently, the new definition of NAFLD has been proposed, and international experts are ongoing to recommend the use of metabolic-associated fatty liver disease (MAFLD) instead of NAFLD as a result of considerable benefits in patients and clinicians.[5] Unlike simple steatosis, a benign lesion, NASH can be regarded as a special type of NAFLD with an increased risk of disease progression and can eventually progress to cirrhosis.[6,7] In addition, fibrosis has been accepted to increase the risk for liver-related complications and cardiovascular disease mortality.[8] Liver biopsy remains the reference standard for diagnosing NAFLD and assessing the stage of fibrosis. However, the exploration and detection of noninvasive methods to determine hepatocyte inflammation and significant fibrosis will still be a major development trend in the field of NAFLD.

It has been suggested that the levels of inflammatory cytokines were higher in patients with NASH.[9] Chronic inflammation may prompt the disease progression to NASH and provide a new direction for seeking noninvasive biological indicators related to NAFLD. Inflammation

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is not only related to NAFLD but also NAFLD-related cardiovascular events. On the one hand, the inflammatory environment has been reported as linked to insulin resistance, subsequent obesity, diabetes, and lipid metabolism disorder, which played an important role in the development of NAFLD.[10,11] On the other hand, the interaction of inflammatory and lipid metabolism may contribute to cardiovascular damage in NAFLD patients.[12] The neutrophil-to-lymphocyte ratio (NLR) is an easily available and low-cost indicator, which can be calculated by conventional white blood cell count. Prior studies demonstrated that NLR plays a prognostic role in patients with hepatocellular carcinoma[13] and cardiovascular disease.[14] A recent study showed that NLR is associated with infectious and inflammatory disease.[15] However, few studies are available on the association between NLR and NAFLD severity, and the results remain controversial. The objective of our study was to evaluate the relationships between NLR and the presence of inflammatory activity and fibrosis in patients with biopsy-proven NAFLD.

MATERIALS AND METHODS

Patients and Study Design

The retrospective study included 231 patients with liver biopsy-confirmed NAFLD at the First Affiliated Hospital of Wenzhou Medical University between August 2017 and September 2019. Patients aged 18 years or above were included in the study after excluding the following criteria: liver diseases of other known causes, such as viral hepatitis types B and C, Wilson's disease, autoimmune hepatitis; excessive alcohol consumption (types B and C, WilsonF10 g/day in women); use of medications known to induce steatosis or immunosuppressive; a history of malignancy or hematological diseases before the study; or insufficient clinical and laboratory data. No informed consent was needed because of the retrospective noninterventional study design. The study protocol was approved by the Institutional Clinical Research Ethics Committee, complying with the ethical guidelines of the 1975 Declaration of Helsinki.

Main Points

- *NLR is inversely correlated with steatosis, lobular inflammation, and fibrosis grades.*
- *Elevated NLR is negatively associated with advanced inflammatory activity and significant fibrosis.*
- *NLR can be considered as a potential noninvasive indicator to assess the severity of NAFLD.*

Clinical and Laboratory Data Acquisition

Information on the history of systemic disease, drug use, drinking habits, and demographical variables (sex, age) was acquired by face-to-face interviews. Standing height and weight were measured after taking off shoes and wearing light clothing.

After an overnight fast, blood samples were collected and measured at the hospital Clinical Sample Test Room. The laboratory assessment of the subjects included triglyceride (TG) (mmol/L), total cholesterol (TC) (mmol/L), low-density lipoprotein cholesterol (LDL-c) (mmol/L), high-density lipoprotein cholesterol (HDL-c) (mmol/L), albumin (g/L), aspartate aminotransferase (AST) (U/L), alanine aminotransferase (ALT) (U/L), alkaline phosphatase (ALP) (U/L), gamma-glutamyl transferase (γGT) (U/L), glucose (mmol/L), total bilirubin (TB) (μmol/L), platelet (PLT) ($\times 10^9$), free T3 (pmol/L), free T4 (pmol/L), neutrophils (k/μL), lymphocytes (k/μL) ,which were analyzed with clinical standard laboratory methods. From these parameters, fT3/fT4 ratio (fT3/fT4) and N/L ratio (NLR) were calculated. Body composition was determined using an InBody720 (Biospace, Seoul, Korea) including appendicular skeletal muscle mass (ASM) (kg), visceral fat area (VFA) (cm^2). The ASM, calculated as the sum of the skeletal muscle mass of both bilateral upper and lower limbs, was divided by body weight (kg) and expressed as a percentage (ASM/weight, ASM%).

Liver Biopsy

Liver biopsy specimens were used, hematoxylin and eosin stain and Masson trichrome stains, and were reviewed by 2 experienced pathologists who were unaware of all data. The Bedossa et al[16] classification was used to grade and stage the histological features of NAFLD. The steatosis, activity, and fibrosis (SAF) scoring system consist of 3 components: steatosis (0-3), activity grade (0-4), and fibrosis stage (0-4). The sum of the scores of lobular inflammation (0-2) and ballooning (0-2) was used to determine the inflammation activity grade (A). Patients with at least grade 1 of each of the 3 features (steatosis, ballooning, lobular inflammation) were diagnosed as NASH. According to the SAF score, the mild disease was defined as inflammatory activity stage 0-2 and fibrosis stage 0-1 ($A \leq 2$ and $F < 2$), whereas severe disease was defined as inflammatory activity stage 3-4 and fibrosis stage 2-4 ($A > 2$ and $F \geq 2$).

Statistical Analysis

Continuous variables were expressed as mean (standard deviation) or median (interquartile range) and categorical

variables were expressed as a percentage. The differences between the tertiles of NLR (T1-T3) were assessed by one-way analysis of variance (normal distribution), Kruskal–Wallis H (skewed distribution) test, and Pearson χ^2 test (categorical variables). Spearman rank correlation analysis was used to evaluate the association between NLR and histological features (steatosis, lobular inflammation, ballooning, and fibrosis). The variables with statistically significance ($P < .05$) at the univariate analysis that was entered in the multiple logistic regression models were all checked for the functional form–the log-linearity assumption and multicollinearity. Moreover, we constructed unadjusted and adjusted multiple logistic regression analyses to estimate the correlations between NLR with advanced inflammatory activity and significant fibrosis. For the adjusted logistic regression models, the area under curves (AUC) and Hosmer–Lemeshow goodness-of-fit test were performed. The stratified regression analysis was used to assess any significant interactions in subgroups (age, gender, body mass index, hypertension, and diabetes). A *P*-value of <.05 was considered statistically significant and IBM SPSS software version 24.0 (IBM Corp, Armonk, NY, USA) was used for all analyses.

RESULTS

Patient Characteristics

This retrospective study included 231 NAFLD patients who underwent liver biopsy, with a mean age of 42.1 ± 12.2 years, among which 70% were obese (BMI ≥ 25 kg/m^2). Most (74.5%) patients were male and 99 (42.9%) patients had diabetes or hypertension. Advanced inflammatory activity ($A > 2$) was identified in 100 (43.3%) patients, whereas 59 (25.5%) patients had significant fibrosis ($F \geq 2$). However, only one patient had cirrhosis (F4). A total of 211 of the 231 patients had ballooning, and lobular inflammation was found in 212 patients. Data were divided according to the NLR levels into tertiles: T1, T2, and T3. The clinical and laboratory data of the patients according to NLR tertiles were shown in Table 1.

Correlation Between Neutrophil-to-Lymphocyte Ratio with Advanced Inflammatory Activity and Significant Fibrosis

As shown in univariate analysis (Table 2), BMI, TC, TG, LDL-c, ALT, AST, ALP, fT3/fT4, and VFA were positively associated with advanced inflammatory activity. A similar observation was found in patients with significant fibrosis. BMI, TC, LDL-c, ALT, AST, ALP, fT3/fT4, and VFA were also positively associated with significant fibrosis. ASM (%) was negatively correlated with advanced inflammatory activity and significant fibrosis. In Spearman correlation analysis, NLR was inversely associated with steatosis, lobular inflammation and fibrosis grades ($r = -0.16$, $P = .014$; $r = -0.15$, $P = .019$; $r = -0.13$, $P = .046$, respectively) (Figure 1).

Table 3 displayed the unadjusted and adjusted correlations between the NLR levels and tertiles of NLR with advanced inflammatory activity and significant fibrosis, respectively. In the unadjusted model (crude model), NLR was associated with advanced inflammatory activity (OR: 0.68, 95% CI: 0.48-0.96, $P = .030$). In the adjusted model 1, after adjustment for ASM (%) and VFA, the result remained statistically significant (OR: 0.64, 95% CI: 0.44-0.93, $P = .021$). After further adjustment for LDL-c, AST, ALP, fT3/fT4, ASM (%) and VFA (model 2), the correlation between NLR and advanced inflammatory activity were still persisted (OR: 0.62, 95% CI: 0.42-0.94, $P = .025$). For SF, AST did not meet the linear assumption, so it did not enter the multiple regression models. It is worth noting that NLR was shown to be no significantly associated with significant fibrosis in the unadjusted model (OR: 0.67, 95% CI: 0.43-1.02, $P = .060$). However, after adjustment for ASM (%), and VFA (model 1), and further adjustment for LDL-c, ALT, ALP, fT3/fT4, ASM (%), and VFA (model 2), NLR had a significant association with significant fibrosis (OR: 0.59, 95% CI: 0.37-0.94, $P = .028$; OR: 0.57, 95% CI: 0.35-0.94, $P = .028$, respectively). Similar results were also noted when NLR was regarded as a categorical variable (tertiles, T1-T3). Compared with T1, the association between NLR with advanced inflammatory activity and significant fibrosis were statistically significant for the second tertiles after adjusting for model 1 (OR: 0.36, 95% CI: 0.18-0.72, $P = .004$; OR: 0.38, 95% CI: 0.18-0.84, $P = .016$, respectively) and model 2 (OR: 0.33, 95% CI: 0.15-0.76, $P = .009$; OR: 0.29, 95% CI: 0.11-0.73, $P = .009$, respectively), and the significant trend also maintained (all *P* for trend <.05 for model 1 and model 2).

In the adjusted logistic regression model (model 2), the area under the receiver operating characteristic (ROC) curve (AUC) for the detection of advanced inflammatory activity was 0.790 (95% CI: 0.730-0.850) (Fig 2) with good calibration (Hosmer–Lemeshow goodness-of-fit test, $P = .138$), whereas the AUC of 0.798 (95% CI: 0.728-0.868) (Fig 3) for predicting patients with significant fibrosis had good calibration (Hosmer–Lemeshow goodness-of-fit test, $P = .539$).

Table 1. Clinical and Laboratory Characteristics of the Subjects According to NLR Tertiles

	Tertiles of NLR			
Variables	T1	T2	T3	*P*
N	76	78	77	
Sex (male)	61 (80.3%)	59 (75.6%)	52 (68.4%)	.188
Diabetes (yes)	24 (31.6%)	20 (25.6%)	22 (28.6%)	.717
Hypertension (yes)	16 (21.1%)	18 (23.1%)	24 (31.2%)	.310
Age (years)	42.3 ± 11.7	39.7 ± 11.4	44.5 ± 13.3	.050
BMI (kg/m^2)	26.9 (24.5-28.8)	27.4 (25.0-30.2)	26.8 (23.9-27.8)	.207
TG (mmol/L)	2.4 (1.3-3.0)	2.1 (1.3-2.7)	2.0 (1.3-2.2)	.180
TC (mmol/L)	5.4 ± 1.2	4.9 ± 1.1	5.3 ± 1.0	.018
LDL-c (mmol/L)	3.2 ± 1.0	2.9 ± 0.9	3.1 ± 0.9	.121
HDL-c (mmol/L)	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	.062
Albumin (g/L)	46.4 (44.7-48.5)	46.5 (44.2-49.1)	45.6 (43.5-48.1)	.415
ALT (U/L)	76.8 (35.3-108)	73.1 (30.0-94.0)	68.6 (24.5-87.0)	.107
AST (U/L)	46.6 (26.3-64.3)	45.6 (24.8-54.5)	44.3 (23.5-48.5)	.111
ALP (U/L)	82.3 (67.5-91.8)	84.5 (68.0-100.3)	84.6 (64.0-99.0)	.931
γGT (U/L)	71.4 (36.5-86.5)	71.2 (30.5-98.3)	63.2 (27.5-79.5)	.077
Glucose (mmol/L)	5.9 (4.9-6.7)	5.9 (4.9-6.3)	5.7 (4.9-6.2)	.860
PLT (×10^9)	240.5 ± 57.4	234.5 ± 57.2	258.1 ± 63.8	.039
TB (μmol/L)	15.4 (11.0-17.8)	14.3 (10.0-16.3)	15.2 (10.0-17.0)	.505
fT3/fT4	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	.674
ASM (%)	30.3 ± 3.1	29.6 ± 3.0	29.4 ± 3.2	.126
VFA (cm^2)	106.6 (86.9-123.8)	108.6 (94.3-125.3)	108.7 (90.8-120.8)	.710

BMI, body mass index; TG, triglyceride; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; γGT, gamma-glutamyltransferase; PLT, platelet; TB, total bilirubin; fT3/fT4, free T3/free T4 ratio; ASM, appendicular skeletal muscle mass; VFA, visceral fat area; NLR, neutrophil-to-lymphocyte ratio.

Subgroup Analyses

To determine the impact of potential confounding factors, we further analyzed the correlation between NLR with advanced inflammatory activity and significant fibrosis in subgroups (Table 4). However, all the selected effect modifications for age, gender, BMI, hypertension, or diabetes on the correlation between NLR with advanced inflammatory activity and significant fibrosis were not statistically significant (all *P* for interaction >.05).

DISCUSSION

In this cross-sectional study, higher NLR levels were negatively associated with advanced inflammatory activity and significant fibrosis in liver biopsy-confirmed NAFLD patients estimated using the SAF score. Moreover, in the histological features, the correlations between NLR and steatosis, lobular inflammation, and fibrosis grades were found.

The NLR is the ratio of the absolute number of neutrophils to lymphocytes, which has been widely used to evaluate inflammatory diseases. As mentioned earlier, NLR has played an important role in infections, cancer, and cardiovascular diseases.[13-15] Recently, NLR has been used to predict prognosis in cirrhotic patients. Especially for patients with hepatitis B virus-related decompensated cirrhosis, NLR was shown to be a highly accurate indicator for predicting early poor outcomes.[17]

Conflicting and inconclusive data have been reported on the association between NLR levels and the histological severity of NAFLD. The results of our study were in concordance with a recent study by Khoury et al,[18] who reported that NLR was significantly associated with significant fibrosis grade and advanced inflammatory activity in both univariate and multivariate analysis. In 101 patients with biopsy-proven NAFLD in another study, those

Table 2. The Results of Univariate Analysis

Variables	OR (95% CI) for A > 2	*P*	OR (95% CI) for SF	*P*
Sex (Male)	0.73 (0.40-1.32)	.293	0.71 (0.37-1.37)	.312
Diabetes (yes)	1.13 (0.64-2.01)	.675	0.81 (0.41-1.58)	.536
Hypertension (yes)	0.99 (0.54-1.80)	.974	0.70 (0.34-1.43)	.329
Age (years)	1.00 (0.97-1.02)	.656	0.99 (0.97-1.02)	.492
BMI (kg/m^2)	1.15 (1.07-1.25)	<.001	1.15 (1.05-1.25)	.002
TG (mmol/L)	1.27 (1.01-1.60)	.041	1.19 (0.93-1.52)	.166
TC (mmol/L)	1.35 (1.07-1.71)	.012	1.39 (1.07-1.80)	.014
LDL-c (mmol/L)	1.47 (1.10-1.96)	.009	1.50 (1.09-2.06)	.013
HDL-c (mmol/L)	0.60 (0.15-2.35)	.466	1.58 (0.34-7.28)	.560
Albumin (g/L)	0.99 (0.93-1.06)	.802	0.97 (0.90-1.05)	.440
ALT (U/L)	1.01 (1.01-1.02)	<.001	1.01 (1.01-1.02)	<.001
AST (U/L)	1.03 (1.02-1.04)	<.001	1.02 (1.01-1.03)	<.001
ALP (U/L)	1.01 (1.00-1.02)	.027	1.02 (1.00-1.03)	.013
γGT (U/L)	1.00 (1.00-1.01)	.183	1.00 (1.00-1.01)	.162
Glucose (mmol/L)	1.05 (0.87-1.27)	.619	1.16 (0.94-1.42)	.163
PLT (×10^9)	1.00 (1.00-1.01)	.937	1.00 (1.00-1.01)	.190
TB (μmol/L)	1.03 (0.99-1.06)	.153	1.00 (0.96-1.04)	.943
fT3/fT4	1.66 (1.19-2.31)	.003	1.82 (1.25-2.64)	.002
ASM (%)	0.88 (0.80-0.96)	.003	0.82 (0.74-0.91)	<.001
VFA (cm^2)	1.02 (1.01-1.04)	<.001	1.02 (1.01-1.03)	.001

BMI, Body mass index; TG, triglyceride; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP: alkaline phosphatase; γGT: gamma-glutamyltransferase; PLT: platelet; TB, total bilirubin; fT3/fT4, free T3/free T4 ratio; ASM, Appendicular skeletal muscle mass; VFA, Visceral fat area; NLR, neutrophil-to-lymphocyte ratio; OR, odds ratio; CI, confidence interval; A>2, advanced inflammatory activity; SF, significant fibrosis.

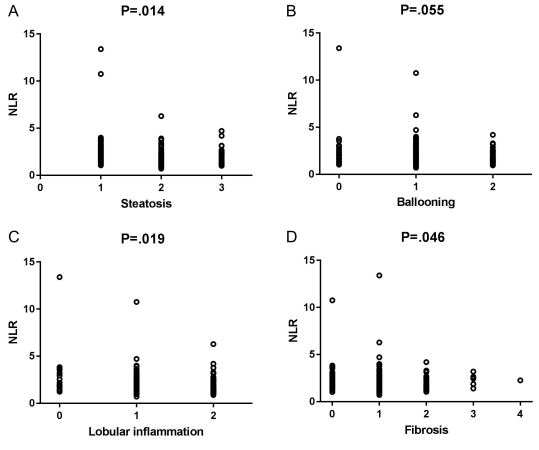

Figure 1. NLR in correlation with histological features of NAFLD. The NLR was negatively correlated with steatosis, lobular inflammation and fibrosis grade (all P < .05), but no correlation with ballooning.

with NASH patients had elevated NLR levels compared with non-NASH subjects and the NLR was significantly related to the histological features of NAFLD (steatosis, inflammation, ballooning, and fibrosis).[19] A prospective study with 873 NAFLD patients and 150 healthy controls demonstrated that NLR correlated positively with NASH and the presence of fibrosis.[20] In contrast, a large cohort study revealed that NLR is not related to NAFLD severity, especially inflammation and fibrosis.[21] This conclusion denies that NLR as a noninvasive indicator for inflammation and fibrosis in NAFLD patients. In the present study, we provide evidence for the first time on the association between NLR levels and inflammation/fibrosis among the Chinese adult population.

A cellular imbalance with the dominance of neutrophils over lymphocytes that seems vital in the inflammatory response is the NLR.[22] Non-alcoholic fatty liver can be considered a chronic low-grade inflammation state, which is

Table 3. Association with Advanced Inflammatory Activity and Significant Fibrosis in Different Models

	Crude Model		Model 1		Model 2	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
NLR as a continuous variable						
A > 2	0.68 (0.48-0.96)	.030	0.64 (0.44-0.93)	.021	0.62 (0.41-0.94)	.025
SF	0.67 (0.43-1.02)	.060	0.59 (0.37-0.94)	.028	0.57 (0.35-0.94)	.028
NLR as a categorical variable (tertile)						
A > 2						
T1	Reference		Reference		Reference	
T2	0.43 (0.22-0.82)	.011	0.36 (0.18-0.72)	.004	0.33 (0.15-0.76)	.009
T3	0.55 (0.29-1.04)	.064	0.44 (0.22-0.90)	.024	0.48 (0.23-1.02)	.057
Trend test		.030		.009		.021
SF						
T1	Reference		Reference		Reference	
T2	0.50 (0.24-1.03)	.058	0.38 (0.17-0.84)	.016	0.29 (0.11-0.73)	.009
T3	0.55 (0.27-1.12)	.097	0.42 (0.19-0.93)	.032	0.43 (0.18-1.02)	.054
Trend test		.108		.027		.029

OR, odds ratio; A>2, advanced inflammatory activity; SF, significant fibrosis; NLR, neutrophil-to-lymphocyte ratio.
Crude model did not adjust for other covariates; Model 1: adjusted for ASM and VFA; Model 2: further adjusted for LDL-c, AST, ALP, fT3/fT4, ASM and VFA to *A* > 2; LDL-c, ALT, ALP, fT3/fT4, ASM and VFA to SF, respectively.

related to liver inflammation and fibrosis.[23] A study in rats reported that inhibition of neutrophil myeloperoxidase could induce oxidative stress, suggesting a critical role of low levels of neutrophils in oxidative stress.[24] Oxidative stress can contribute to liver inflammation and fibrosis by impairing mitochondrial function, depleting energy, and damaging DNA, lipids, and proteins.[25] Although the infiltration of neutrophils into the liver promotes the progression of NASH, they also create new vascular regeneration channels to repair an advanced liver injury.[26] Therefore, whether neutrophils have a promoting effect in early inflammation but play a repairing role in the later stage

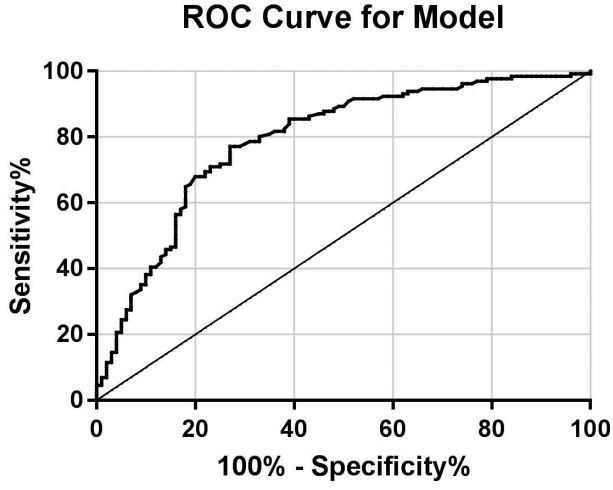

Figure 2. The area under the curve of the multivariate-adjusted models for advanced inflammatory activity.

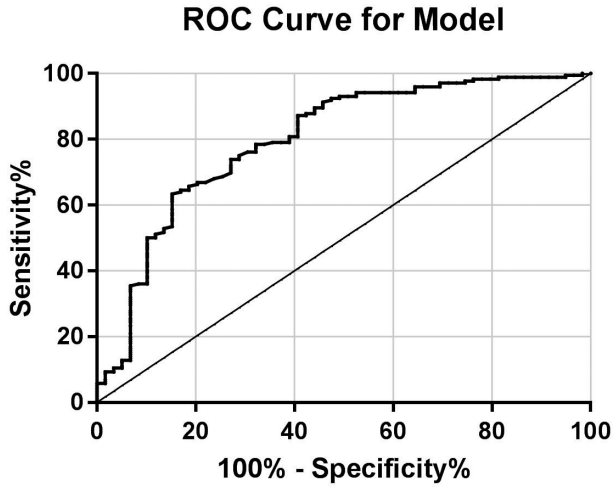

Figure 3. The area under the curve of the multivariate-adjusted models for significant fibrosis.

Table 4. Association Between NLR and Advanced Inflammatory Activity or Significant Fibrosis in Selected Subgroups

Variables	N	OR (95% CI) for *A* > 2	*P* for Interaction	OR (95% CI) for SF	*P* for Interaction
Gender			.82		.20
Male	172	0.68 (0.45-1.03)		0.77 (0.49-1.21)	
Female	59	0.62 (0.32-1.21)		0.41 (0.18-0.96)	
Age (years)			.13		.57
>50	66	0.99 (0.53-1.85)		0.81 (0.38-1.74)	
≤50	165	0.54 (0.33-0.88)		0.62 (0.36-1.05)	
Diabetes			.28		.53
Yes	66	0.47 (0.22-1.00)		0.51 (0.20-1.31)	
No	165	0.75 (0.52-1.08)		0.71 (0.45-1.13)	
Hypertension			.92		.12
Yes	58	0.70 (0.35-1.39)		0.95 (0.61-1.49)	
No	173	0.67 (0.45-1.01)		0.55 (0.33-0.93)	
BMI (kg/m^2)			.63		.33
≤25	70	0.59 (0.28-1.22)		0.45 (0.18-1.13)	
>25	161	0.72 (0.49-1.04)		0.75 (0.48-1.17)	

OR, odds ratio; *A* > 2, advanced inflammatory activity; SF, significant fibrosis; BMI, body mass index; NLR, neutrophil-to-lymphocyte ratio.

(advanced inflammation and fibrosis) remains to be further studied.

The inflammatory environment is a risk factor for cardiovascular disease, which plays a possible prevalent role in the pathogenesis of arterial stiffness and endothelial dysfunction.[12,27-28] A recent study showed that assessing the potential association between the vascular status (including arterial stiffness, endothelial function, and cognitive performance) and inflammatory markers in NAFLD patients is expected to seek therapeutic targets for the NAFLD-related cardiovascular events.[12] Further studies are required to evaluate the connections between inflammatory markers (e.g., NLR) and indexes of vascular disease in patients with NAFLD.

Although our study is a retrospective, cross-sectional study, and did not clarify the in-depth mechanism between NLR and the severity of NAFLD. However, this research has a relatively large sample size and is similar to most previous studies. In recent years, simple, noninvasive scores (i.e., FIB-4 and NAFLD fibrosis score (NFS)) showed a high degree of accuracy in the exclusion of advanced fibrosis in NAFLD patients.[29,30] Unfortunately, as for our study, only 7 (3.0%) patients with NAFLD had advanced fibrosis ($F \geq 3$). Future research with larger sample sizes of NAFLD patients with advanced fibrosis is needed to explore the utility of NLR compared with noninvasive scores in excluding advanced fibrosis in patients with NAFLD. Nonetheless, it is plausible that NLR, as a potential surrogate indicator for predicting NAFLD patients with liver inflammation and fibrosis, may be helpful for the early identification of high-risk populations in NAFLD.

The major strength of our study is the assessment of liver disease with biopsy-proven NAFLD, which is still considered the gold standard for evaluating liver disease severity. However, several limitations of our work should be considered. First, the cross-sectional design of this study could not infer the causal relationship between changes in NLR and the severity of NAFLD. Second, the patients in the present study were all Chinese who were conducted in a single hospital; thus, further studies are needed to estimate the utility of these associations in different races or populations. Finally, our study did not evaluate the effects of systemic inflammatory indicators (e.g., CRP) and proinflammatory cytokines in the relationship between the NLR with liver inflammation and fibrosis because of lacking relevant data.

In conclusion, NLR levels were negatively associated with advanced inflammatory activity and significant fibrosis in patients with NAFLD. Accumulating evidence supports that NLR, as a simple and easily available marker in predicting the disease severity of NAFLD. The NLR

is expected to be combined with other indicators into future diagnostic models.

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Informed Consent: No informed consent was needed because of the retrospective non-interventional study design.

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