CLINICS

CLINICAL SCIENCE

Post-treatment neutrophil-lymphocyte ratio independently predicts amputation in critical limb ischemia without operation

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OBJECTIVES: Limited information is available concerning the post-treatment neutrophil-lymphocyte ratio in critical limb ischemia patients who receive conservative therapy. Accordingly, this study was designed to evaluate the predictive value of the post-treatment neutrophil-lymphocyte ratio in critical limb ischemia patients without surgery.

METHOD: From January 2009 to January 2011, critical limb ischemia patients were admitted to a vascular center. The demographic data, patient histories, comorbidities and risk factors were documented, and the differential cell count was determined at admission and seven days later after conservative therapy. The cutoff value of the post-treatment neutrophil-lymphocyte ratio was determined by an ROC curve. Patients were divided into groups A and B according to the cutoff value. Amputation-free survival was compared between groups. Univariate and multivariate analyses were used to identify independent risk factors.

RESULT: A total of 172 patients were identified with a mean age 71.98 ± 10.09 years; among them, 122 were male. A value of 3.8 was identified as the cutoff value of the post-treatment neutrophil-lymphocyte ratio. Groups A (post-treatment neutrophil-lymphocyte ratio ≥ 3.8) and B (post-treatment neutrophil-lymphocyte ratio < 3.8) showed a significant difference in amputation-free survival ($P < 0.001$). The 1-year, 2-year and 3-year amputation-free survival rates were 79.6%, 55.6% and 46.3%, respectively, in group A; however, in group B, these values were 89.7%, 79.3% and 75.9%, respectively. The post-treatment neutrophil-lymphocyte ratio was identified as an independent predictive factor for amputation in critical limb ischemia patients ($P < 0.001$).

CONCLUSION: The post-treatment neutrophil-lymphocyte ratio is an independent predictive factor for amputation in critical limb ischemia patients. Patients with a post-treatment neutrophil-lymphocyte ratio ≥ 3.8 are likely to suffer from amputation; amputation-free survival usually occurs in patients with a post-treatment neutrophil-lymphocyte ratio < 3.8.

KEYWORDS: Neutrophil-lymphocyte ratio; Critical limb ischemia; Conservative therapy; Amputation; Amputation free survival.

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INTRODUCTION

Critical limb ischemia (CLI) is a widespread disease in China, especially in older people. Although statistics are lacking for the Chinese population, according to epidemiological research of other countries (1), more than 5 million Chinese individuals are estimated to suffer from CLI. Due to long-term chronic ischemia, the end-point for CLI patients is usually amputation. Therefore, a method of identifying CLI patients with a high risk for amputation and performing risk stratification at an early time point is always the focus of clinical practice.

CLI is an atherosclerotic disease that is usually associated with a general inflammatory response. Based on previous studies, C-reactive protein (CRP), platelet aggregation, and the neutrophil to lymphocyte ratio (NLR) have been indicated as effective predictive markers for CLI patients (2). The NLR has universally been accepted as a predictive

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index for stent patency (3,4), amputation-free survival (AFS) (5), and mortality (6-8). However, these studies usually focus on preoperative or postoperative NLR in CLI patients who have undergone an operation or intervention. Little research has been performed on CLI patients who receive conservative treatment. Furthermore, the NLR has been adopted for prognosis evaluation in various medical fields including hearing loss (9), peripheral artery disease (PAD) (10,11), bladder cancer (12), prostatic hyperplasia (13), pancreatic cancer (14,15), and colorectal cancer (16,17).

However, whether the post-treatment NLR (post-NLR) can predict the prognosis in CLI patients is unclear. Therefore, the present study was designed to evaluate the predictive value of the post-NLR in CLI patients who receive conservative therapy.

METHODS

Patients and methods

The objective inclusion criteria for enrolled patients with a diagnosis of CLI consisted of more than two weeks of foot pain associated with an ankle-brachial index of 0.4 or less, an ankle systolic pressure of 50 mm Hg or less, or a toe systolic pressure of 30 mm Hg or less (Rutherford categories 4 and 5 (18). Patients with a) decisive evidence of acute limb ischemia; b) clinical symptoms or signs of sepsis, any other inflammation or a white blood cell (WBC) count $>10*10^9/L$; c) poor data integrity; d) a previous surgery history; or e) any diseases or drug that would affect the lymphocyte count, such as lymphopenia, lymphemia, acute cardinal infraction and corticosteroid use, were excluded from this study.

In this study, all of the enrolled patients who received conservative therapy were not candidates for revascularization due to poor general condition, loss of indication, severe local infection or patient unwillingness.

At admission, the demographics (age, gender), history (smoking, drug use), comorbidities and results of laboratory tests (in particular, the neutrophil, lymphocyte, and WBC counts) were recorded in a specific database by doctors and nurses. All enrolled patients received the same standardized conservative therapy plan (antiplatelet, anticoagulation and vasodilator drugs were given intravenously for approximately 7 days). The same blood tests were conducted 7 days after admission.

NLR definition

The pre-treatment WBC and differential counts were evaluated at admission before conservative therapy. The post-treatment counts evaluated taken 7 days later after conservative therapy was adopted. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count (19).

Follow-up after discharge

Discharged patients were followed via outpatient consultation or telephone calls. Each patient received the same unified follow-up form and was followed at the end of the 1st, 3rd, 6th, and 12th month after discharge, then annually thereafter. Telephone follow-up was performed at the same intervals. In this study, the primary end-points were amputation [above-the-knee amputation (AKA), below-the-knee amputation (BKA) or Toe] or 36 months after discharge.

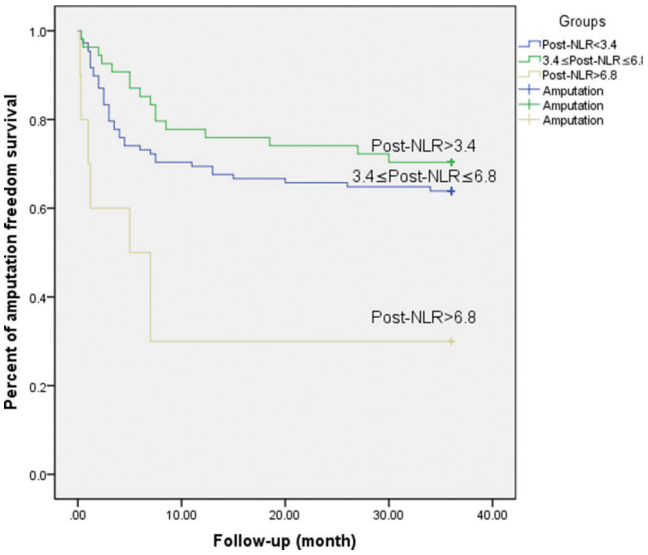

Figure 1 - Association between post-NLR and amputation-free survival (P=0.006).

Analysis method

Data analysis was performed with SPSS version 16 (SPSS Inc, Chicago, IL). Categorical data and continuous variables were compared with the chi-square test or Fisher's exact test and the independent sample t-test, respectively. The incidence of amputation was analyzed by the Kaplan-Meier method, and the difference was compared with a log-rank test. Independent risk factors were identified by multivariate Cox proportional hazards regression analysis. The calculated *P*-values were two-sided, and a *P*-value <0.05 was considered significant.

Ethics

The patients included in this study were recruited from the Vascular Department of West China Hospital, Sichuan University, between January 2009 and January 2011. The study was approved by the Ethics Committee of West China Hospital, Sichuan University.

RESULTS

Based on the exclusion criteria, 12 patients were excluded from the final analysis due to a) determined evidence of acute limb ischemia (n = 1); b) clinical symptoms or signs of sepsis or any other inflammation or WBC counts $>10*10^9/L$ (n = 6); c) poor data integrity (n = 3); or d) a history of previous surgery (n = 2). A total of 172 patients were included in the final outcome analysis.

Cutoff value of post-NLR identification

The analysis of the relationship between post-NLR and AFS was conducted using tertiles of existing data (e.g., Post-NLR <3.4, Post-NLR >6.8 and Post-NLR between 3.4 and 6.8). This grouping method showed a significant discrimination effect (P = 0.006, Figure 1).

The post-NLR appeared to have a significant influence on the outcome in a continuous manner. The post-NLR appeared to be negatively correlated with the outcome. An ROC curve was used to identify the cutoff value, which is shown in Figure 2. In the ROC analysis setting, amputation was set as the state variable. The C-statistic (area under the curve) was 0.740. A

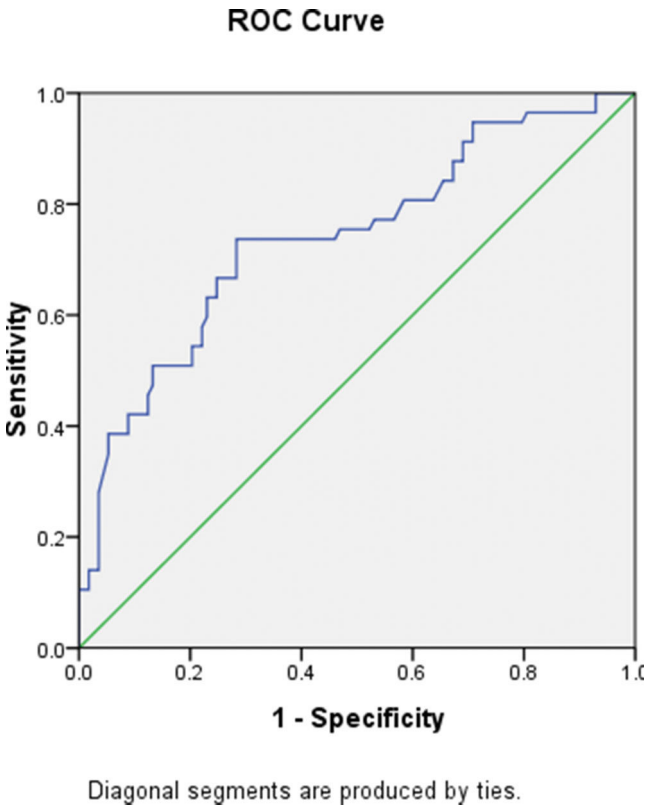

Figure 2 - *Power of diagnostic value* (C-statistic is 0.740).

post-NLR of 3.8 was selected as the cutoff value because it had the maximum discriminative power (sensitivity 70.2%, specificity 71.7%). Seventy-four patients (43%) had a post-NLR ≥3.8, and 98 patients had a post-NLR <3.8.

Baseline characteristics

A total of 172 patients with mean age of 71.98 ± 10.09 years were included, and 70.9% (n = 122) of these patients were male. The mean follow-up duration was 34.7 months (range 3.25 to 62.5 months). The 74 patients with a post-NLR ≥3.8 were collectively called group A, and the 98 patients with a post-NLR <3.8 were called group B. The mean ages in groups A and B were 71.83 ± 11.00 and 72.00 ± 9.52, respectively. The baseline characteristic comparison between group A and group B is shown in Table 1. The incidence of diabetes mellitus, the pre-NLR, the post-NLR, the monocyte count, and the cholesterol level were significantly different between the two groups.

Impact of the post-NLR on amputation

Overall, the AFS rates in group A (post-NLR ≥3.8) and group B (post-NLR <3.8) were 43.2% and 82.7%, respectively. Furthermore, the 1-year, 2-year and 3-year incidence rates of AFS in group A were 83.8%, 67.6% and 43.2%, respectively. By contrast, the respective 1-year, 2-year and 3-year AFS rates were 89.8%, 82.7% and 82.7% in group B. Thus, 42 patients suffered from amputation during follow-up in group A, whereas only 17 patients received amputations in group B (P < 0.001). A detailed comparison of amputation instances is shown in Table 2. Moreover, the survival outcome showed a significant difference between groups A and B (P < 0.001), which is shown in Figure 3.

Table 1 - Baseline characteristics of 172 CLI patients compared according to post-NLR.

Factor	Group A (post-NLR ⩾3.8)	Group B (post-NLR <3.8)	*P*-value
Gender (male/female)	50/24	72/26	NS
Smoking	40	60	NS
Hypertension	36	56	NS
Diabetes mellitus	8	22	0.046
Heart disease	18	26	NS
CAD	10	14	NS
Statin use	21	27	NS
Age	71.83 ± 11.00	72.00 ± 9.52	NS
Glucose	7.12 ± 3.25	6.52 ± 2.45	NS
Neutrophil count	5.42 ± 2.52	5.29 ± 2.19	NS
Lymphocyte count	1.48 ± 0.49	1.40 ± 0.60	NS
Pre-NLR	6.27 ± 5.05	4.37 ± 2.49	0.004
Post-NLR	7.57 ± 4.46	2.20 ± 0.99	<0.001
Monocyte	0.59 ± 0.23	0.48 ± 0.18	0.001
WBC	6.85 ± 1.36	6.47 ± 1.26	NS
Albumin	36.72 ± 4.34	37.99 ± 3.96	0.049
Potassium	3.88 ± 0.49	3.90 ± 0.49	NS
Creatinine	84.33 ± 31.11	83.70 ± 28.60	NS
HDL	1.11 ± 0.38	1.17 ± 0.39	NS
LDL	2.56 ± 0.82	2.35 ± 0.71	NS
TG	1.82 ± 1.40	1.45 ± 0.96	NS
Cholesterol	4.41 ± 0.97	4.00 ± 0.97	0.009
Social status			NS
Bachelor or greater education	12	8	
Middle school education	76	43	
Primary school education	18	15	
Living condition			NS
Nursing home	21	14	
Private home	85	52	
Fontaine grade			NS
II	2	4	
III	48	66	
IV	24	28	

CAD: coronary artery disease; Pre-NLR: pre-treatment neutrophil to lymphocyte ratio; Post-NLR: post-treatment neutrophil to lymphocyte ratio; WBC: white blood cell; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglyceride. *P < 0.05 was considered a significant difference; NS indicated no significance.

Table 2 - Amputation comparison between Groups A and B

	Group A (post-NLR ⩾3.8)	Group B (post-NLR <3.8)	*P*-value
Amputation/Total	42	17	<0.001
AKA	1	1	NS
BKA	12	5	0.016
Toe	29	11	<0.001

*AKA: above-knee amputation; BKA: below-knee amputation.
**P* < 0.05 was considered a significant different; NS indicated no significance.

Independent risk factor identification

Risk factors for CLI patients, including diabetes mellitus, pre-NLR, post-NLR, monocyte albumin and cholesterol level, were included in the multivariate analysis (Cox proportional hazards model). The analysis results showed that the post-NLR and albumin were independent risk factors of amputation (hazard ratios [95% confidence interval]: 1.140 [1.086, 1.197] and 0.914 [0.856, 0.976], respectively). These data are shown in Table 3.

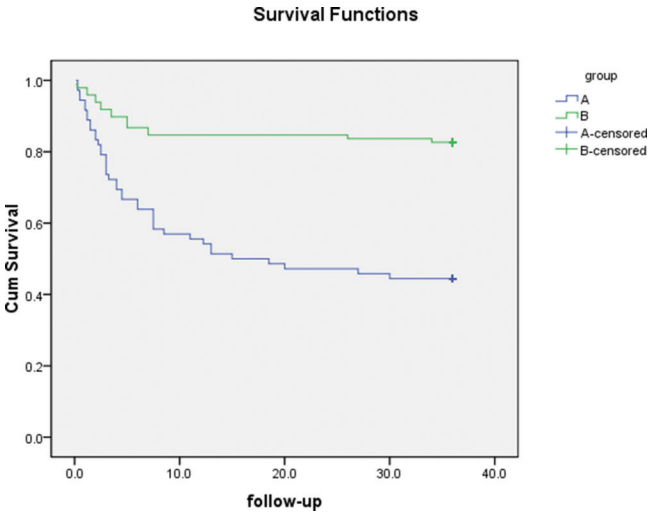

Figure 3 - Survival outcome comparison between groups A and B ($P < 0.001$).

Table 3 - Multivariate analysis of factors affecting overall amputation in patients with critical limb ischemia

Factor	Hazard ratio(95% confidence interval)	*P*-value
Post-NLR	1.140 (1.086, 1.197)	<0.001
Albumin	0.914 (0.856, 0.976)	0.007
Diabetes mellitus, pre-NLR, Monocyte and cholesterol	NS	

*pre-NLR: pre-treatment neutrophil-lymphocyte ratio; post-NLR: post-treatment neutrophil-lymphocyte ratio.
*P < 0.05 indicated a significant difference. NS indicated no significance.

DISCUSSION

The post-NLR was identified as an independent predictive factor of amputation in CLI patients who did not undergo surgery. CLI patients usually suffer from malnutrition (20) due to sleeplessness and lack of appetite, leading to chronic ischemic pain. Moreover, variable albumin is a crucial index for evaluating a patient's nutritional condition, and hypoproteinemia is a risk factor for amputation in PAD (21,22). In this study, albumin was identified as an independent protective factor, which is in accordance with previous studies.

With respect to previous studies, major lower limb amputations (AKA or BKA) at 1 year occur in approximately 20% of untreated CLI patients (23,24). In this study, the 1-year amputation rate was 16.2% in group A (post-NLR $\geq$3.8) and 10.2% in group B (post-NLR $<$3.8). Therefore, no significant difference in the final outcome was observed for untreated CLI patients and patients who received conservative therapy. This result needs to be confirmed by a systematic review or randomized controlled trial in the future. However, this study suggests that CLI patients with a higher post-NLR have a worse prognosis.

A risk-stratification model is an important clinical tool that uses existing clinical data to predict a patient's prognosis and provides a rational discrimination between risky and non-risky patients. The NLR contains crucial information regarding the patient's inflammatory condition. Moreover, the NLR is different from other inflammation markers. It is an inexpensive and readily available marker that is directly calculated from the neutrophil and lymphocyte counts and can easily be obtained from a complete blood cell test on admission.

Furthermore, CLI is a form of chronic atherosclerosis, and fibrosis progression is associated with the general immune-inflammatory response. Repeated local inflammatory responses involving the neutrophils and lymphocytes lead to fibrosis, which aggravates ischemia. The NLR is an effective systematic inflammatory marker that represents the inflammatory condition. When severe ischemia occurs and infection and immune disease are simultaneously excluded, a systematic increase in the post-NLR could represent an irreversible ischemic condition that may lead to an endpoint event (e.g., amputation). The NLR predicts endpoint events in other medical fields, including myocardial ischemia (25) and severe sepsis (26).

In this study, all of the enrolled patients underwent conservative therapy; however, compared with all CLI patients over the same period of time, the proportion of patients receiving conservative therapy was still low. Previous studies have shown that revascularization procedures (e.g., surgery and angioplasty/stenting) can produce a better prognosis than conservative therapy (27-29). In terms of patients who are suitable candidates for revascularization, surgery, intervention or a hybrid procedure is still the optimal choice.

Although this study is prospective with respect to patient collection and discharge follow-up, selection bias cannot be denied. Nearly all of the enrolled patients used antiplatelet therapy, but only 48 subjects (27.9%) received statins. In addition, the post-NLR could not be compared with other inflammatory markers, such as C-reactive protein or the erythrocyte sedimentation rate, because they are not routine tests performed at admission. Moreover, isolated patients with anemia and a hemoglobin level of less than 70 g/L received transfusion therapy. This confounding factor was not included in the analysis in this study.

The post-NLR is an effective marker for amputation in CLI patients who do not undergo an operation. When the post-NLR $\geq$3.8, CLI patients are more likely to suffer from amputation compared with patients with a post-NLR $<$3.8. To avoid amputation and increase AFS, revascularization procedures are still the optimal choice.

AUTHOR CONTRIBUTIONS

Luo H,* Yuan D,* Zhao J and MA Y conceived and designed the study. Luo H, Yuan D, and Yang Y participated in the analysis interpretation. Huang B, Yang H, Zeng G, Chen X, Wang T and Luo H collected the data. Luo H, Yang H, and Xiong H wrote the article. Zhao J and Yuan D revised the article. Huang B, Yang H and Wu Z performed the statistical analysis. Zhao J coordinated the study.
*These authors contributed equally to this manuscript.

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