Original Articles

Neutrophil-to-lymphocyte ratio is effective prognostic indicator for post-amputation patients with critical limb ischemia

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ABSTRACT

الأهداف: لتأكيد ما إذا كانت نسبة العدلات إلى الخلايا اللمفاوية (NLR) ونسبة الصفائح الدموية إلى الخلايا اللمفاوية (PLR) هي مؤشرات لتشخيص المرضى بعد البتر مع نقص التروية الحرجة للأطراف (CLI).

الطريقة: شملت هذه الدراسة الرصدية بأثر رجعي ما مجموعه 270 مريضاً في مرحلة ما بعد البتر مع CLI بين يناير 2010 وديسمبر 2014 في مستشفى الأولى من جامعة جيلين تشانغتشون، الصين. سجلت العدلات واللمفاويات التعداد قبل بتر الأطراف. احتُسبت نسبة العدلات إلى الخلايا اللمفاوية وتم تعريف NLR ≥8.08 بأنها مرتفعة. أُجري تحليل الانحدار اللوجستي لاختبار قيمة النذير.

النتائج: وفقاً للتحليل الإحصائي، أشير إلى أن NLR ≥8.08 (OR: 26.228, 95% CI: 5.801-118.583, $p<0.001$), (PLR ≥237.14 (OR: 3.464, 95% CI: 1.289-9.308, $p=0.014$ وأمراض القلب التاجية (OR:2.739,95%CI:1.060-7.082,$p=0.038$) كانت مؤشرات النذير المستقلة للمرضى.

الخاتمة: نسبة NLR و PLR وأمراض القلب التاجية هي مؤشرات النذير المستقلة للمرضى بعد البتر مع CLI.

Objectives: To confirm whether neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are indicators for the prognosis of post-amputation patients with critical limb ischemia (CLI).

Methods: In this retrospective observational study a total 270 post-amputation patients with CLI were included between January 2010 and December 2014 in the First Hospital of Jilin University, Changchun, China. The neutrophil and lymphocyte counts were recorded before amputations. Neutrophil-to-lymphocyte ratio was calculated and NLR ≥8.08 was defined as elevated. Logistic regression analysis was conducted to test the prognostic value.

Results: According to the statistical analysis, it was indicated that NLR ≥8.08 (odds ratio [OR]: 26.228, 95% confidence interval [CI]: 5.801-118.583, $p<0.001$), PLR ≥237.14 (OR: 3.464, 95% CI: 1.289-9.308, $p=0.014$) and coronary heart disease (OR: 2.739, 95% CI: 1.060-7.082, $p=0.038$) were the independent prognostic indicators for the patients.

Conclusion: Neutrophil-to-lymphocyte ratio, PLR, and coronary heart disease are independent prognostic indicators for post-amputation patients with CLI.

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Critical limb ischemia (CLI) is usually caused by a severe blockage, such as arteriosclerotic plaque and thrombus, and so forth in the arteries of the lower extremities that markedly reduces blood flow. The incidence of CLI is 0.35% of the eligible study population in the United States.[1] With a low limb salvage rate and rapid progress, the patients always suffer from a series of ischemic symptoms, such as intermittent claudication, rest pain, and so forth. The pathological terminations are ulcer and gangrene of limb. Once the disease is diagnosed, the systemic treatments, such as bypass surgery and endovascular intervention therapy should be conducted.[2,3] However, there are still 30-50% of the patients with CLI who have to suffer from amputation instead of revascularization due to some reasons.[4] Prognosis for post-amputation patients, especially

with diabetes mellitus, are usually poor.[5,6] According to a domestic study, for post-amputation patients with CLI and diabetes mellitus, 1-year mortality range from 13% to 40% and 5-year mortality range from 39% to 80%, which are higher than some patients with tumor.[7] In recent years, neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been demonstrated to be indicators in systemic inflammatory response (SIR), and SIR has a close connection with prognosis of patients with tumor.[8,9] Similarly, limb ischemia and gangrene can lead to SIR, whose pathological characteristics are inflammatory cell infiltration, metabolite accumulating and tissue necrosis, and so forth. As a trauma stress, amputation can promote SIR as well. However, there is still no related research on the relation between NLR/PLR and the prognosis of post-amputation patients with CLI. We conducted this study to confirm whether NLR and PLR are indicators for the prognosis of post-amputation patients with CLI.

Methods. *Selection criteria.* All of the patients suffered from chronic limb ischemia (>3 weeks) or acute limb ischemia (≤3 weeks) with severe limb ulcer or gangrene (Rutherford category V or VI), and a series of routine examinations, such as blood routine, serum lipid, and protein examination were performed before amputation.

Exclusion criteria. Patients with the disease which can lead to the abnormal white blood cell and platelet count, including inflammatory diseases (such as, pulmonary infection and biliary tract infection), leukemia, tumor, severe renal or hepatic dysfunction, abnormal thyroid functions, metabolic syndrome, bone marrow or hematologic disorders, splenectomy, thrombotic thrombocytopenic purpura, idiopathic thrombocytopenic purpura, myeloproliferative disorders, and radiation. Two-hundred and seventy patients complying with the previously mentioned criteria were retrospectively recruited from January 2010-December 2014 in The First Hospital of Jilin University, Changchun, China. These patients included 187 males and 83 females (male/female ratio=2.25:1), aged between 60 and 88 years (mean age: 71±6 years). According to prognosis of the patients, they were divided into 2 groups: poor prognosis group (including myocardial infarction, stroke, and death cases within 30 days) and contrast group. The study protocol, according to principles of Helsinki Declaration, was reviewed and approved by the Ethics Committee of The First Hospital of Jilin University. Patients with acute ischemia (77 cases), usually underwent sudden pallor, pain, and paralysis of limbs. Due to lack of timely and prompt treatments, most of them had suffered from limb gangrene before hospitalization. In spite of revascularization, others also underwent amputation due to irreversible damage or extreme ischemia in this study. Patients with chronic ischemia (193 cases), always underwent long-term intermittent claudication or rest pain, eventually limb ulcer and gangrene. There are 30 cases for minor amputation (toe and foot amputation) and 240 cases for major amputation (above and below knee amputation). Medicine treatments, including vascular dilation and anti-infection, were performed for the patients after amputation. Within 30 days after amputation, there were 9 cases with myocardial infarction or stroke, and 22 cases of death.

All test data of the patients were collected using the Hospital Information System (HISystem V3.0, Zhejiang MediCARE I.T.CO., LTD). We extracted the last routine examination data before amputation. In this study, NLR is the ratio of absolute neutrophil count and PLR is the ratio of absolute platelet count divided by the absolute lymphocyte count.

Statistical analysis. Data were presented as mean±standard deviation (SD) or the absolute number of subjects. The Q-Q plots and the Shapiro-Wilk test were examined to assess data normality. Levene's test was performed for variance homogeneity and Kruskal-Wallis test was applied when analysis of variance was not applicable. Chi-squared or Fisher's exact test was used for comparing the differences between the groups. Receiver operating characteristic (ROC) curves were performed to identify the indicative performances of NLR and PLR in post-amputation patients with critical limb ischemia. The areas under the curves (AUC) were calculated with 95% confidence intervals (CI). The Youden index was performed to determine optimal cut-off values for each indicator. Logistic regression model was conducted to verify prognostic factors. Sensitivity, specificity, positive, and negative predictive values. Positive and negative likelihood ratios were calculated with 95% CIs. The statistical analysis was performed using the Statistical Package for Social Sciences version 19 software (IBM Corp., Armonk, NY, USA). In all analysis, $p<0.05$ was considered statistically significant.

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Results. In this study, the data of blood examination and the history of patients, such as smoking history, hypertension (blood pressure >140/90 mm Hg), diabetes mellitus (fasting blood glucose >7.0 mmol/L or 2 hours postprandial blood glucose >11.1 mmol/L), coronary heart disease, hyperlipidemia (total cholesterol >5.72 mmol/L, or triglyceride >1.70 mmol/L) and cerebral apoplexy were identified according to the electronic medical records (**Table 1**).

As shown in **Table 1**, we observed that the coronary heart disease (p=0.002), NLR (p<0.001), PLR (p<0.001), and albumin (p=0.004) were statistically significant. According to **Figure 1** and **Table 2**, NLR ≥8.08 was primary hazard factor with odds ratio [OR]: 26.228.

Table 1 - General characteristics and history of 270 patients with critical limb ischemia.

Variable	Total (n=270)	Poor prognosis (n=31)	Contrast group (n=239)	Value	*P*-value
Gender, n (%)					
Male	**187 (69.3)**	17 (54.8)	170 (71.1)	3.421	0.096*
Female	**83 (30.7)**	14 (45.2)	69 (28.9)		
Age (yrs)	**70.711 ± 6.361**	70.484 ± 6.212	70.741 ± 6.393	0.034	0.854†
Progress classification, n (%)					
Acute	**77 (28.5)**	10 (32.3)	67 (28.0)	0.240	0.673*
Chronic	**193 (71.5)**	21 (67.7)	172 (72.0)		
Smoking history, n (%)	**192 (71.1)**	25 (80.6)	167 (69.9)	1.550	0.292*
Hypertension, n (%)	**152 (56.3)**	19 (61.3)	133 (55.6)	0.355	0.571*
Diabetes mellitus, n (%)	**113 (41.9)**	16 (51.6)	97 (40.6)	1.371	0.252*
Coronary heart disease, n (%)	**119 (44.1)**	22 (71.0)	97 (40.6)	10.276	0.002*
Hyperlipidemia, n (%)	**52 (19.3)**	7 (22.6)	45 (18.8)	0.248	0.630*
Cerebral apoplexy, n (%)	**67 (24.8)**	7 (22.6)	60 (25.1)	0.094	1.000*
NLR	**7.883 ± 8.006**	20.117 ± 16.287	6.297 ± 4.122	51.941	<0.001†
PLR	**232.766 ± 142.468**	382.827 ± 253.428	213.302 ± 107.386	16.959	<0.001†
MCV (fL)	**87.831 ± 8.767**	87.342 ± 6.103	87.894 ± 9.064	0.689	0.406†
RDW (%)	**14.148 ± 1.842**	14.161 ± 1.704	14.146 ± 1.863	0.447	0.504†
	31.130 ± 6.679	27.381 ± 7.807	31.616 ± 6.377	8.322	0.004†

Data are presented as mean±standard deviation (SD) or the absolute number. *Chi-squared or Fisher's exact test, †Kruskal-Wallis test. NLR - neutrophil to lymphocyte ratio, PLR - neutrophil to lymphocyte ratio, MCV - mean corpuscular volume, RDW - red cell distribution width

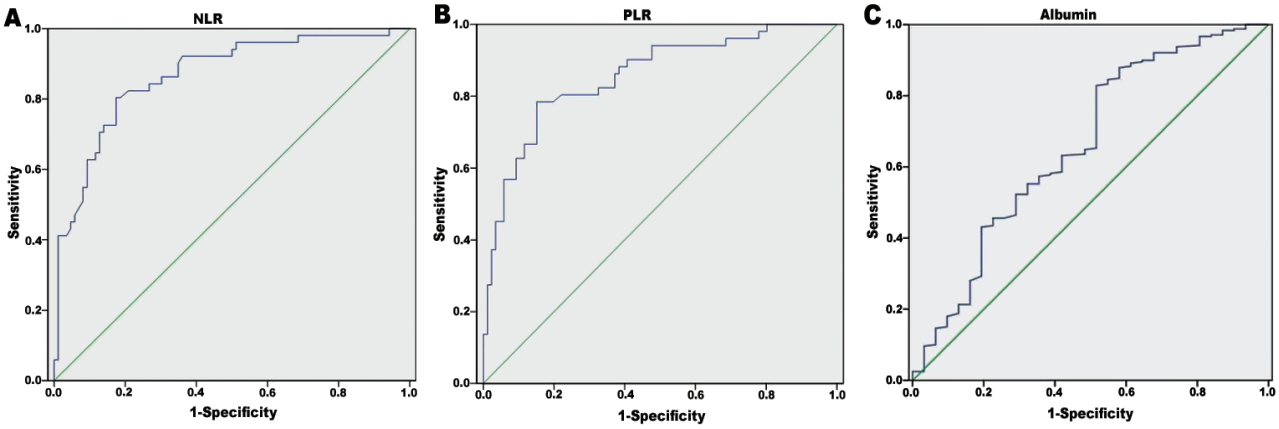

Figure 1 - The receiver operating characteristic (ROC) curves for **A**) neutrophil to lymphocyte ratio (NLR) (the area under the curve (AUC) is 0.898 and the cut-off value is 8.08, with a sensitivity of 0.935 and a specificity of 0.757 for NLR), **B**) platelet to lymphocyte ratio (PLR) (the AUC is 0.727 and the cut-off value is 237.14, with a sensitivity of 0.774 and a specificity of 0.644 for PLR), and **C**) albumin in amputated patients with critical limb ischemia (the AUC is 0.659 and the cut-off value is 25.35, with a sensitivity of 0.828 and a specificity of 0.484 for albumin).

Table 2 - Multi-variate Logistic regression of 270 patients with critical limb ischemia.

Variable	OR	95% CI lower	95% CI upper	*P*-value
Coronary heart disease	2.739	1.060	7.082	0.038
NLR ≥8.08	26.228	5.801	118.583	<0.001
PLR ≥237.14	3.464	1.289	9.308	0.014
Albumin ≤25.35	1.845	0.722	4.713	0.201

NLR - neutrophil to lymphocyte ratio, PLR - neutrophil to lymphocyte ratio, OR - odds ratio, CI - confidence interval

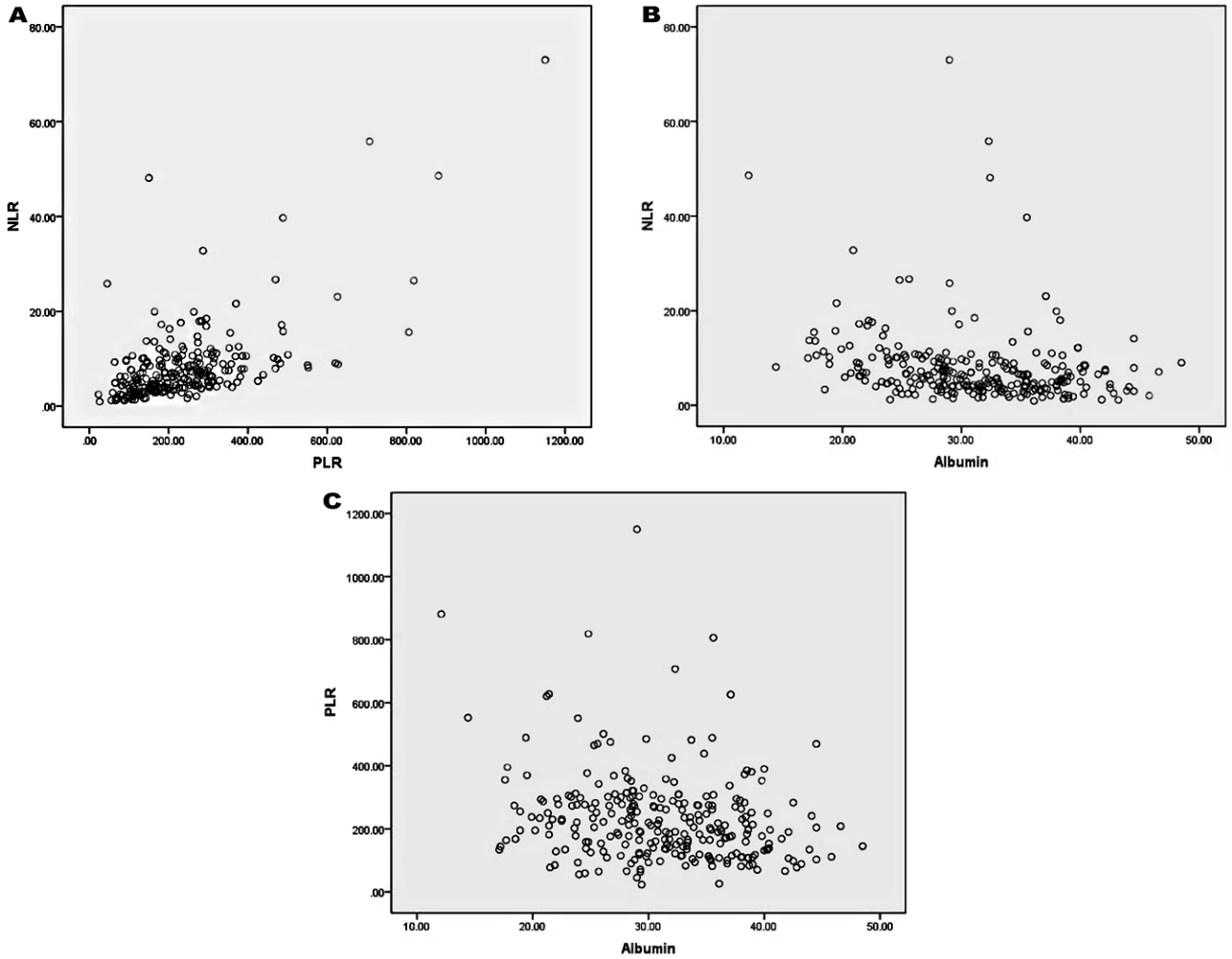

Figure 2 - Correlation among neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and albumin for patients in 2 groups. **A**) There was a positive correlation between NLR and PLR, R=0.618, $p<0.001$. **B**) There was a negative correlation between NLR and albumin, R=-0.233, $p<0.001$. **C**) There was a negative correlation between PLR and albumin, R=-0.238, $p<0.001$.

Sensitivity and specificity of NLR were greater than the others. In **Figure 2**, it was shown that NLR, PLR, and albumin were linearly associated with each other.

Discussion. Critical limb ischemia is a severe arteriosclerotic plaque or thrombus blockage in the arteries of the lower extremities that markedly reduces blood flow, whose major symptoms are intermittent claudication, rest pain, limb ulcer, and gangrene. Amputation is inevitable once a limb gangrene appear. The randomized, controlled trials, which served as basis for the New England Society for Vascular Surgery (SVS) safety and efficacy objective performance goals (OPGs) showed that mortality and major amputation rates at

30 days were 2.8% and 2% compared with 2.7% and 1.9% for the patients with CLI.[10-12] A close connection between mortality and infectious complications (such as, sepsis, pneumonia and urinary tract infection, and so forth) has been confirmed by Curran et al's study.[13] For patients with CLI, limb gangrene always comes with tissue necrosis, systemic infection, and inflammation, which can increase mortality. Even worse, harmful metabolites and pain stress lead to poor prognosis as well. As a result, some of them might be very weak to undergo amputation, which causes traumatic stress to the body. At the same time, NLR had been confirmed as the indicator for inflammation in circulatory, respiratory, digestive, urinary disease, and solid tumor.[14-17] Also, PLR had been demonstrated to have a close connection with prognosis of patients with tumor.[18,19] Similarly, NLR and PLR could indicate the inflammation, which is induced by limb gangrene and demonstrate the risk level for the patient with CLI to suffer from amputation. Therefore, NLR and PLR might be the prognostic indicators for post-amputation patient with CLI.

According to the ROC curves, the values of AUC NLR was 0.898 and PLR was 0.727. This means that, especially NLR have highly clinical significance. As shown in **Table 2**, the OR values of NLR ≥8.08 and PLR ≥237.14 were 26.228 and 3.464 (p<0.005). This meant that the patients with NLR ≥8.08 or PLR ≥237.14, were controlling the other factors, are more likely (26.228 times and 3.464 times than the other patients) to undergo the worse prognosis. Moreover, the combination of NLR and PLR had been verified more indicative in prognosis by the Logistic regression analysis.

In recent years, some studies[20-23] on the relationship between NLR and prognosis of patients with non-small cell lung cancer concluded the cut-off values between 2.093 to 5.0. In Wu's study,[24] the cut-off value of NLR for patients with non-small cell lung cancer was 2.68, which was much less than the value in this study. Acute inflammation is almost simultaneous with a sharper increasing count of white blood cell and neutrophil cell than chronic inflammation. Critical limb ischemia often combines with limb gangrene, which can lead to limb infection and acute inflammation. Based on the oncothlipsis effect of tumor and other reasons, patients with non-small cell lung cancer always suffer from obstruction or compression of airway and intrapulmonary infection, which always lead to chronic inflammation. Therefore, the cut-off value of NLR in this study is greater than in other studies, and it is more indicative for systemic acute inflammation. Moreover, the connection between NLR and amputation has been confirmed by other studies as well.[25,26] Luo's study[27] showed that patients with a post-treatment NLR ≥3.8 are likely to suffer from amputation. It is still less than the value in the present study. With NLR ≥3.8, the patient takes higher risk to suffer from amputation. With NLR ≥8.08, the post-amputation patient is likely to receive a worse prognosis. Actually, the results are not contrary to the studies. Besides, coronary heart disease was another prognostic factor (OR: 2.739, 95%CI: 1.060-7.082, p=0.038). Arteriosclerosis is a systemic disease. Coronary arteriosclerosis merely is a local manifestation of systemic arteriosclerosis. Patients with coronary heart disease always undergo arteriosclerosis of limb arteries, mesenteric arteries, renal arteries, and carotid arteries, which could restrain the tolerance of relevant organs.[28] It might be one reason of the worse prognosis. In our study, we pointed out that NLR and PLR are the indicators for the prognosis of the post-amputation patients with CLI for the first time. The results are significant for the clinical treatment and evaluation of the patients with CLI. However, as a single-center and retrospective study, there are some limitations. Additional larger sample, multi-center, and prospective studies are necessary in future studies.

In conclusion, NLR, PLR, and coronary heart disease are independent prognostic indicators for post-amputation patients with CLI. With NLR ≥8.08, PLR ≥237.14 and coronary heart disease, the patients are more likely to suffer from poor prognosis (including myocardial infarction, stroke, and death cases within 30 days) than patients without those risk factors. Especially NLR ≥8.08, which is a primary risk factor for the patients (OR: 26.228, 95%CI: 5.801-118.583, p<0.001).

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