The Platelet-Lymphocyte Ratio Predict the Risk of Amputation in Critical Limb Ischemia

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Abstract
Background: The aim of this study was to evaluate the platelet-lymphocyte ratio (PLR) in patients with critical limb ischemia (CLI) and determine its association with amputation risk.

Materials and methods: One hundred and forty-one patients with CLI were included in this study. The PLR was calculated using the platelet and lymphocyte counts, and the patients were divided into two groups based on the PLR value. The primary endpoint was amputation, and the secondary endpoints were mortality and major amputation.

Results: The PLR was significantly higher in the group with amputation compared to the group without amputation. The area under the receiver operating curve (AUC) for the PLR was 0.76, indicating its potential as a prognostic indicator.

Conclusion: The platelet-lymphocyte ratio has a significant association with the risk of amputation in patients with CLI, and it can be used as a prognostic marker.

Keywords: Platelet; Lymphocyte; Amputation

Introduction
Critical Limb Ischemia (CLI) is a serious condition characterized by chronic limb ischemia that is not relieved by standard medical or non-invasive interventions. It is associated with a high risk of amputation. While treatment options have improved in recent years, the risk of major amputation remains high.

Patients with CLI comprise a subset of high-risk patients with generalized atherosclerotic disease of the arterial tree. The presence of systemic atherosclerosis is associated with a low-grade systemic inflammatory response. Previous studies have suggested that elevated preoperative C-Reactive Protein (CRP) levels, neutrophil-lymphocyte ratio (NLR), thrombocytosis and platelet aggregation may also represent potential prognostic markers in peripheral arterial disease.

The aim of the study was to investigate the effect of the PLR value on amputation risk in patients with CLI. The PLR might represent significant prognostic indices in patients with CLI and could provide superior prognostic information.

Material and Methods
One hundred and forty-one patients with CLI were included in the study. These patients were assessed by a vascular team consisting of cardiologists, interventional radiologists and cardiovascular surgeons. The PLR was calculated using the platelet and lymphocyte counts, and the patients were divided into two groups based on the PLR value. The primary endpoint was amputation, and the secondary endpoints were mortality and major amputation.

Results: The PLR was significantly higher in the group with amputation compared to the group without amputation. The area under the receiver operating curve (AUC) for the PLR was 0.76, indicating its potential as a prognostic indicator.

Conclusion: The platelet-lymphocyte ratio predicts the risk of amputation in patients with CLI. Stratification of CLI patients according to admission PLR should be considered in the limb survival analyses of future adjuvant and neoadjuvant trials to validate these findings.
The admission complete blood count was recorded. Levels of hemoglobin (Hb), white blood cells (WBC), platelet, neutrophil, and lymphocyte were measured using a Siemens Advia 2120 analyzer.

After the decision was made for patients as inoperable CLI, they were called in for follow-ups at the first, third, and ninth months and then with six-month intervals. Patients were contacted during routine check-ups at our centre on a periodic, but not uniform, basis. During the follow-up, the following events were registered: 1. Mortality for all causes, 2. Major or minor amputation. Latest statuses of the patients were ascertained via both phone and Social Security Death Index at the end of the study.
Statistical Analyses

Continuous data were described using median, interquartile range (IQR) and 95% confidence intervals (CI). Categorical variables were summarized as percentages. The effect of PLR on outcome was studied by constructing a receiver operating characteristic curve with amputation as the primary variable.

Estimations of risk were performed using Cox regression. Potential prognostic factors were entered into univariate regression models of midterm limb survival. Significant univariate correlates were then entered into a reverse stepwise multivariate regression model to test for independence. Analyses were performed using SPSS software. A p value <0.05 was considered statistically significant.

Results

Out of the 133 patients in the study, 83.5% were male and 16.5% were female. The median age was 69 years and median follow-up was 39 months. 76 (57%) patients had non-reconstructable (percutaneous or surgical revascularisation) arterial outflow vessels. 21(16%) patients were inoperable due to the comorbidities. 12(9%) patients didn’t have suitable vein grafts. 5(3%) patients didn’t accept the revascularisation(radiological or surgical revascularisation). All of them had Rutherford classification 4 or 5 with multilevel disease.

Overall, there were a total of 28 (26.9%) deaths and 46 (34%) amputation over a mean follow-up of 46 months. The amputations were above ankle in 34 (74%), below ankle in 12 (26%).

The results of univariate limb survival analysis for each of the demographic and hematologic data are shown in Table 1. All patients were grouped in two, those undergone amputation and those without amputation. Diabetes mellitus, hemoglobin and platelet levels, mean PLR, PLR ≥ 160 (Table 1) were strong univariable predictor of amputation.

When these 4 variables were included in a multivariate regression modeling, diabetes mellitus (OR,2.9; 95%CI, 1.3-6.5 P=0.01), hemoglobin levels (OR, 0.9; 95%CI, 0.75-1.15, P=0.5), platelet levels (OR, 1; 95%CI, 1-1.02, P=0.6), PLR ≥ 160 (OR, 3.1; 95%CI, 1.2-7.4, P=0.02) remained as independent factors associated with midterm amputation (Table 2).

Discussion

The findings of the present study demonstrate that PLR obtained from a universally available low-cost test (CBC with differential) provides relevant information regarding the risk of amputation in patients who are admitted with non-reconstructable CLI.

Chronic inflammation is both an etiological factor and physiological consequence of peripheral arterial disease [3,8]. Experimental models provide compelling evidence for the role of inflammation in the initiation, progression, and complication of atherosclerosis, confirmed in the clinical setting [9]. High levels of hs-CRP are correlated with angiographic coronary artery disease progression [10,11]. The peripheral vasculature, both hs-CRP [7,10,11], fibrinogen and serum amyloid A [9] are related to the progression of atherosclerosis in peripheral arterial disease. Akinci et al. [12] demonstrated that circulating levels of acute phase reactants were associated with amputation risk in diabetic foot. Similarly, Violi et al. [13] reported that elevated levels of CRP were associated with poor long-term prognosis in patients with peripheral artery disease. Previous studies have shown that progression of atherosclerosis is associated with increased vascular events [3,14]. Progression of atherosclerosis and additional vascular events increase limb ischemia. Based on the findings of the present study, we also think that amputation may increase in patients with peripheral vascular disease with high inflammatory markers.

Thrombocytosis is commonly associated with a peripheral arterial disease and has been widely reported as an adverse prognostic marker [7]. The proinflammatory phase associated with systemic inflammation results in release of various immunological mediators such as interleukin (IL)-1, IL-3, and IL-6, which increase circulating platelet counts as a result of megakaryocyte proliferation [15,16]. It is well documented that subjects with pathologically increased platelet counts, that is, thrombocytosis, have an enhanced risk of thrombotic complications [17,18]. Experimental animal studies indicate that blood platelets both initiate atherogenesis and trigger its complications [19]. It is shown that, in healthy individuals with platelet count lower than normal, platelet aggregation increases when number of platelets increase(>250×10⁹/L, >150×10⁹/L [17,18]. Platelets play an important role in the progression of atherosclerosis. According to current research platelets interact with endothelial cells and leukocytes [20] and release inflammatory substances leading to adhesion and transmigration of monocytes [21]. These monocytes support inflammatory processes in the vessel wall promoting atherosclerotic lesions [22]. An elevated platelet count leading to an elevated PLR might therefore lead to an increase in vascular endpoints. Robless et al. found that whole blood platelet aggregation was significantly enhanced in PAD (Peripheric Arterial Disease) patients [7]. Also, previous studies have indicated that lymphopenia is associated with the progression of atherosclerosis. Lymphopenia may be caused by lymphocyte apoptosis in atherosclerotic lesions, which gradually increases with atherosclerotic burden. Likewise, a reduced lymphocyte count has also been associated with poorer prognosis [23-25].

Thrombocytosis and lymphocytopenia both correlate with the degree of host systemic inflammation, and the PLR reflects a novel marker incorporating both hematologic indices. Several studies has shown a relationship between the PLR and prognosis in colorectal and pancreatic cancer [26-30].

PLR value in vascular surgery is one of the subjects that have been recently studied, Gary and colleagues reported that PLR value of greater than 150 is a significant marker in PAD, similar to our study, PLR ≥ 160 is associated with a high proportion of amputation in patients with PAD.

The limitations of our study include the possibility of selection bias, the relatively small number of patients with a highly prevalent disease, and the possibility of missing some outcomes using the Social Security Death Index. Only 1 measurement of admission full blood count and calculation of PLR was included in the analysis, and it was not possible to determine whether an acute and brief inflammation was responsible for the correlation observed. Additionally, inflammatory markers, such as C-reactive protein, myeloperoxidase, and interleukin-6, were not analyzed and compared to PLR.

|                          | Multivariate OR | S.E. | P    |
|--------------------------|-----------------|------|------|
| Diabetes mellitus        | 2.88 (1.28-6.48)| 0.41 | 0.01 |
| Hemoglobin (mg/dL)       | 0.93 (0.75-1.15)| 0.11 | 0.51 |
| Platelet (10³/mm³)       | 1.01 (0.99-1.02)| 0.01 | 0.64 |
| PLR ≥ 160                | 3.1 (1.22-7.4)  | 0.46 | 0.02 |

Table 2: Multivariate (Cox Proportional Hazards) Limb Survival Analysis (SE Standard error of regression coefficient, OR Odds ratio).
The results of the present study suggest that significant prognostic information can be obtained from routine blood results in patients with CLI. Stratification of CLI patients according to admission PLR should be considered in the limb survival analyses of future adjuvant and neoadjuvant trials to validate these findings.

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