

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

Murat Songur C\*, Erdal Simsek, Omer Faruk Cicek, Kemal Kavasoglu, Sameh Alagha, Mehmet Karahan, Tugba Avcı and Irfan Tasoglu

Department of Cardiovascular Surgery, Turkiye Yuksek Ihtisas Training and Research Hospital Ankara, Turkey

### Abstract

**Background:** The aim of this study was to PLR (platelet-lymphocyte ratio) might represent significant prognostic indices in patients with CLI (critical limb ischemia) and might provide superior prognostic information.

**Materials and methods:** One hundred and forty one patients presented with CLI to our hospital between February 2007 and June 2012 who could not have radiological or surgical revascularization and had medical treatment are included in our study (n:133). Primary end point was determined as amputation (limb survival) and all-cause death and separate analyses were performed.

**Results:** Out of the 133 patients in the study. 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 24 (77%), below ankle in 7 (23%). All patients were grouped in two, those undergone amputation and those without amputation. Diabetes mellitus, hemoglobin and platelet levels, mean PLR,  $PLR \geq 160$  were strong univariable predictor of amputation.

**Conclusion:** 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.

**Keywords:** Platelet; Lymphocyte; Amputation

### Introduction

Critical Limb Ischemia (CLI) is an entity with high mortality and high risk of limb amputation. Although treatment options, especially endovascular treatment possibilities, improved in the last decades, mortality and amputation rates are still high [1,2].

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 [3,4]. Previous studies have suggested that elevated preoperative C-Reactive Protein (CRP) levels [5,6], neutrophil-lymphocyte ratio (NLR), thrombocytosis and platelet aggregation [3,7,8] may also represent potential prognostic markers in peripheral arterial disease.

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

### Material and Methods

One hundred and forty one patients presented with CLI to our hospital between February 2007 and June 2012 who could not have radiological or surgical revascularisation and had medical treatment are included in our study. These patients were assessed by the vascular team consisting of cardiologists, interventional radiologists and cardiovascular surgeons and accepted as ineligible for endovascular intervention or conventional surgery; thus getting medical therapy instead. All patients with CLI defined by the TASC II (Trans-Atlantic Inter-Society Consensus) for the management of peripheral arterial disease. The arterial anatomy of the lower extremities was assessed by digital subtraction angiography with use of either stepping techniques with a single contrast material bolus or multiple contrast material injections. Inclusion criteria were persistent rest pain for more than 2 weeks or ischemic skin lesions, ankle pressure below 50 mmHg or,

in patients with diabetes and incompressible vessels, absent palpable ankle pulses or toe pressure below 30 mmHg. Standard treatment included cilostazol, statins, analgesics, antithrombotic drugs (aspirin), cardiovascular risk factor control, hemorrheologic agents (such as pentoxifylline), local wound care and antibiotics, if indicated. The local ethics committee approved the study protocol.

Patients with infected ischemic wounds (n=4), active cancer (n=1), unstable coronary disease (n=2) and steroid use (n=1) were excluded from outcome analysis. Eight patients were excluded according to these criteria. Thus 133 patients were included the study.

Demographic characteristics, medical histories, risk factors, laboratory studies (Table 1) were collected by the research team using a standardized database. Prior surgical or percutaneous revascularization was documented (Table 1). Any subsequent surgical intervention was also documented as follows: major amputation (above the level of the ankle) or minor amputation (below the level of the ankle).

Primary end point was determined as amputation (limb survival) and all-cause death were performed. The effect of PLR on outcome was studied by constructing a receiver operating characteristic curve. Using a PLR cutoff of  $\geq 160$ , the area under the receiver operating characteristic curve was 0.65 (95% confidence interval [CI] 0.55-0.75) (Figure 1).

\*Corresponding author: Murat Songur C, Department of Cardiovascular Surgery, Turkiye Yuksek Ihtisas Training and Research Hospital Ankara, Turkey, Tel: +90 505 376 21 00; E-mail: [drmuratsongur@hotmail.com](mailto:drmuratsongur@hotmail.com)

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Risc Factors	All Patients N=133	Non amputation N=87	Amputation N=46	Univariate OR	P
Age (years)	66.8 (33-91)	67.8 (42-91)	65.1 (33-89)	0.98 (0.95-1.01)	0.24
Age > 75 years	44 (33.1%)	30 (34.5%)	14 (30.4)	1.2 (0.56-2.59)	0.64
Male Gender	111 (83.5%)	72 (82.8%)	39 (84.8%)	1.16 (0.44-3.1)	0.76
Diabetes	55 (41.4%)	27 (31%)	28 (60.9%)	0.29 (0.14-0.61)	<b>0.001</b>
Hypertension	61 (45.9%)	36 (41.4%)	25 (54.3%)	0.59 (0.29-1.2)	0.15
Hyperlipidemia	62 (46.6%)	42 (48.3%)	20 (43.5%)	1.2 (0.6-2.5)	0.6
Current Smoker	90 (67.7%)	63 (72.4%)	27 (58.7%)	1.85 (0.87-3.9)	0.11
Chronic Obstructive Pulmonary Disease	56 (42.1%)	34 (39.1%)	22 (47.8%)	0.6 (0.3-1.1)	0.14
Previous Myocardial Infarction	33 (24.8%)	22 (25.3%)	11 (23.9%)	1.08 (0.47-2.47)	0.86
Stroke	17 (12.8%)	9 (10.3%)	8 (17.4%)	1.1 (0.3-4.2)	0.8
Previus Vascular Surgery	34 (25.6%)	18 (20.7%)	16 (34.8%)	0.46 (0.2-1.03)	0.06
Previus Stent	8 (6%)	5 (5.7%)	3 (6.5%)	0.95 (0.2-4.2)	0.95
<b>Medical Treatment</b>					
Statin Use	23 (17.3%)	13 (14.9%)	10 (21.7%)	0.78 (0.28-2.1)	0.63
ASA Use	45 (33.8%)	27 (31%)	18 (39.1%)	0.62 (0.2-1.79)	0.37
Cilastazol Use	92 (69.2%)	65 (74.7%)	27 (58.7)	0.8 (0.4-2.8)	0.5
<b>Laboratory Data</b>					
Urea (mg/dL).	44.2 (19-128)	44.2 (23-128)	44.3 (19-119)	1 (0.98-1.02)	0.97
Creatinine (mg/dL)	1 (0.5-3.8)	1.1 (0.5-3.8)	0.9 (0.6-1.5)	0.38 (0.12-1.26)	0.11
Hemoglobin (mg/dL)	13.6 (7.9-19.2)	13.9 (7.9-17.3)	13 (9-19.2)	0.78 (0.65-0.95)	<b>0.01</b>
Platelet (103/mm <sup>3</sup> )	325 (73-959)	298 (73-865)	374 (108-959)	1.02 (1.01-1.05)	<b>0.02</b>
White blood cells (×109/L)	9.6 (3.9-24)	9.2 (3.9-24)	10.6 (4.2-19.9)	1.12 (1.01-1.25)	<b>0.03</b>
Neutrophil (×109/L)	7.1 (2.3-44.6)	6.8 (2.3-44.6)	7.6 (2.3-17.4)	1.03 (0.96-1.1)	0.41
Lymphocyte (×109/L)	2.2 (0.5-20.5)	2.4 (0.5-20.5)	1.9 (0.8-3.6)	0.7 (0.45-1.14)	0.16
PLR	171.4 (15.9-667)	151.3 (15.9-502.9)	208.9 (44.4-667)	1.05 (1.01-1.08)	<b>0.01</b>
PLR ≥ 160	58 (43.6%)	28 (32.2%)	30 (65.2%)	0.25 (0.12-0.54)	<b>&lt;0.001</b>

Table 1: Baseline characteristics of patients. divided according to amputation.

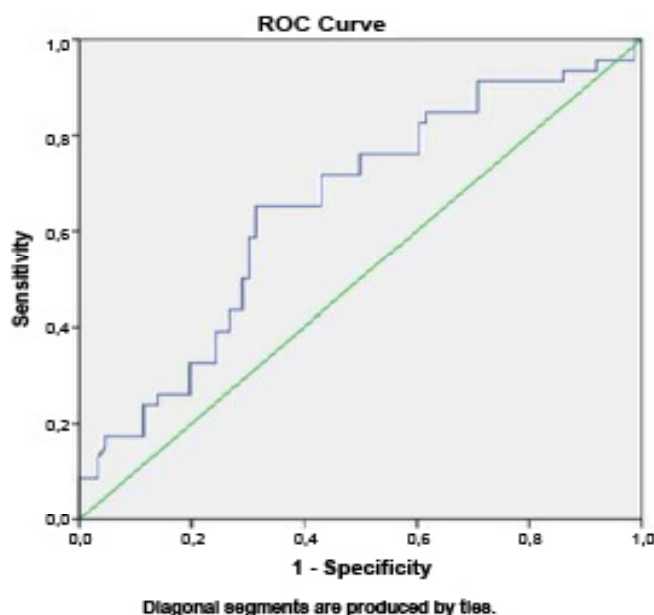


Figure 1: Using a PLR cutoff of ≥160, the area under the receiver operating characteristic curve was 0.65 (95% confidence interval [CI] 0.55-0.75).

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, hemoglobine and platelet levels, mean PLR,  $PLR \geq 160$  (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),  $PLR \geq 160$  (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]. In 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 ( $>250 \times 10^9/L$ ,  $>150 \times 10^9/L$  [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 (Peripheral 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,  $PLR \geq 160$  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 ( $10^9/mm^3$ )	1.01 (0.99-1.02)	0.01	0.64
$PLR \geq 160$	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 Ods 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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