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# The comparison of complete blood count parameters between acute and chronic peripheral arterial disease

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**Abstract**

Recently published data demonstrated that peripheral arterial disease (PAD) is well associated with some hematological parameters. However, data in acute onset PAD is scarce. We aimed to search baseline hematological parameters in angiographically proven lower extremity PAD with chronic and acute thrombotic occlusions. In this retrospective study, angiographically proven 172 PAD patients (121 chronic and 51 acute thrombotic cases) and 80 control subjects (without documented coronary and peripheral arterial disease) were enrolled to this study and divided into three groups as chronic, acute PAD and control groups. Demographic characteristics, laboratory data and angiography findings of the participants were obtained from computerized database and patient files. All groups were statistically similar with regards to baseline clinical and demographic features. WBC and the neutrophils count were found higher whereas the lymphocyte and eosinophil counts were found lower in acute PAD group than chronic PAD and control group. In PAD group (acute and chronic), the median Fontaine stage was significantly positive correlated with N/L ratio and significantly negative correlated with eosinophil count. In multivariable logistic regression analysis, in addition to sex presence of diabetes mellitus and smoking, eosinophil count was found an independent predictor for the development of acute PAD. As well as N/L ratio, eosinophil count is associated with acute development of PAD rather than chronic process and may be used as a diagnostic marker for this purpose in patients undergoing percutaneous intervention.

**Keywords:** Peripheral arterial disease, neutrophil to lymphocyte ratio, eosinophil, thrombotic occlusion

## Introduction

Lower extremity peripheral arterial disease (PAD) is usually associated with atherosclerosis. However, thrombosis is also common especially in patients with recent onset of symptoms and acute limb ischemia [1]. Hemogram parameters, which are easily accessible biomarkers, are routinely used in clinical practice. Recent studies showed that hemogram parameters are associated with intravascular inflammatory activity during the acute vascular events [2,3]. It has been noted that inflammation is an important pathological process underlying atherosclerosis. Inflammation may contribute to initiation and as well as progression of atherosclerosis [4]. In hematological parameters, especially neutrophils may contribute to the development of atherosclerosis and atherothrombotic events [5,6]. Nowadays, hemogram parameters are recognized as prognostic markers of many diseases including atherosclerosis [4,5,7-10]. Although role of hematological parameters has been widely investigated in patients with PAD [7-10], there is no data during acute thrombotic phase.

In current study, we aimed to search baseline hematological parameters in angiographically proven lower extremity PAD with chronic and acute thrombotic occlusions.

## Materials and Methods

Patients who underwent lower extremity angiography due to acute limb ischemia or intermittent claudication between April 2017 and March 2019 at Bolu Abant Izzet Baysal University Hospital were retrospectively enrolled to the study. PAD was defined as presence of >50% luminal narrowing in lower extremity arterial vessels. Patients with sudden onset of symptoms within 14 days and suspicion of acute thrombosis on Doppler ultrasound and/or angiography and the lesions which are easily crossed with wires during percutaneous intervention and/or well responded to catheter directed thrombolysis were categorized as acute (thrombotic) PAD group (n=51) and patients with more than 50% lumen narrowing in

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lower extremity arterial vessels without acute lesion characteristics as chronic PAD group (n=121). Fontaine classification was used for categorization of severity of PAD. Control group (n=80) was selected among patients having normal coronary and having no claudication symptoms with palpable peripheral pulses.

Patients with a history of recent infectious states, surgery, heart failure, acute coronary syndrome, severe valvular disease, previous surgical or endovascular lower limb revascularization, hepatic failure, severe renal insufficiency, presence of malignancies and use of anti-inflammatory and /or immune-modulatory agents were excluded from the study. The institutional board was approved the study. The patients' files were retrospectively reviewed and demographic characteristics, angiographic findings and hemogram parameters of the patients were recorded. By dividing the neutrophil to lymphocyte count the NLR was calculated.

The study was conducted according to the Helsinki Declaration and approved by the our university clinical researches ethics committee (2019/385). A written and verbal informed consent was obtained from each subject.

**Statistical analysis**

SPSS (version 16; SPSS Inc. Chicago, Illinois) software was used to perform statistical analysis. Data were presented as mean ± standard deviation and nonparametric data were expressed as number and percentages of the total. For homogeneous distributed data, one-way ANOVA test was used, and Tukey's HSD was performed for post hoc analyses. Heterogeneously distributed parameters were compared with Kruskal-Wallis test. Data between the groups were compared with Chi-square test for qualitative variables and student-t test or Mann-Whitney U test for quantitative variables. The correlations of hematological parameters with other study parameters were evaluated either via Pearson or Spearman correlation tests. A logistic regression analysis was used to determine independent factors for acute PAD. A p value below 0.05 were considered significant.

**Results**

There were 121 patients in chronic PAD group and 51 patients in acute thrombotic group. Eighty subjects were enrolled in the control group. Demographic and clinical characteristics of control and PAD groups were similar (Table 1).

The WBC (9.08(5.29-23.70) vs. 8.17(2.15-22.70), 7.16(3.83-11.80) respectively; p<0.001) and the neutrophils count (6.11(2.90-21.70) vs. 5.31(1.68-21.50), 4.18(1.70-11.80) respectively; p<0.001) were found higher whereas the lymphocyte (1.16(0.58-4.07) vs. 1.65(0.51-5.40), 2.13(0.50-5.99) respectively; p=0.02) and eosinophil counts (0.087(0.003-0.350) vs. 0.151(0.002-1.050), 0.119(0.002-0.620) respectively; p=0.04) were found lower in acute PAD group than chronic PAD and control group. Compared to control group median neutrophil and WBC counts were significantly higher in PAD groups with a significantly higher level in acute thrombotic group (p<0.001) (Table 2). Mean lymphocyte count was significantly lower in acute PAD group than chronic PAD and control group (p=0.02). As expected, the NLR was higher in acute PAD group than chronic PAD and control group (3.50(0.98-37.16) vs. 3.09(0.88-32.39), 1.92(0.60-11.81) respectively; p<0.001) (Table 2).

Frequencies of severity of critical limb ischemia in chronic and acute PAD groups was as follow: Fontaine stage IIa (non-disabling claudication): 14 (11.6%), versus 0, Fontaine stage IIb (disabling claudication): 43 (35.5%), versus 4 (7.8%), Fontaine stage III (ischemic rest pain): 25 (20.7%), versus 41 (80.4%), Fontaine 4 (ulceration or gangrene): 39 (32.2), versus 6 (11.8%). Correlation analysis revealed that there was a significantly weak positive correlation between Fontaine stage and NLR, and a significantly very weak negative correlation and eosinophil count in PAD groups (acute and chronic) (r=0.179, P=0.019 and r= -0.225, P<0.01) (Table 3).

In multivariable logistic regression analysis, eosinophil count: OR=2.14, 95% CI(1.97,2.29) P<0.01, presence of diabetes mellitus: OR=3.19, 95% CI(2.88,3.42) P<0.01, gender: OR=3.01, 95% CI(2.60,3.50) P<0.01 and smoking: OR=2.36, 95% CI(2.01,2.63) P<0.01 were independent predictors of acute PAD (Table 4).

**Table 1**. General characteristics of the study groups

| | Control (n=80) | Chronic PAD (n=121) | Acute PAD (n=51) | P value |
|---|---|---|---|---|
| **Age (years)*** | 66±7 | 68±10 | 68±13 | 0.31 |
| **Body mass index, kg/m2*** | 26±3 | 27±5 | 26±4 | 0.23 |
| **Systolic BP, mmHg*** | 122±15 | 127±16 | 125±10 | 0.87 |
| **Diastolic BP, mmHg*** | 74±9 | 71±9 | 72±10 | 0.72 |
| **Creatinine, mg/dl+** | 0.82(0.66-2.14) | 1.08(0.58-1.32) | 0.97(0.68-1.12) | 0.69 |
| **Fasting glucose, mg/dl+** | 102(76-413) | 118(42-497) | 115(56-337) | 0.10 |
| **Male/Female, n** | 56/24 | 69/51 | 33/18 | 0.22 |
| **Hypertension, n** | 50(63%) | 70(58%) | 28(55%) | 0.09 |
| **Smoking, n** | 18(23%) | 29(24%) | 10(20%) | 0.32 |
| **Diabetes mellitus, n** | 31(38%) | 44(36%) | 20(39%) | 0.25 |
| **Hyperlipidemia, n** | 23(28%) | 29(24%) | 13(25%) | 0.21 |

* Mean ±standard deviation, + Median (minimum-maximum), PAD: Peripheral artery disease, BP: Blood pressure

**Table 2**. Hemogram parameters of the study cohort

| | Control (n=80) | Chronic PAD (n=121) | Acute PAD (n=51) | P value |
|---|---|---|---|---|
| **Hemoglobin, g/L*** | 13.4±1.5 | 13.0±1.9 | 13.0±2.0 | 0.28[a] |
| **MPV, fl *** | 8.1±1.5 | 7.8±1.5 | 7.7±1.4 | 0.24[a] |
| **Platelet counts, k/mm3+** | 240(130-391) | 242(63-501) | 225(152-391) | 0.5[4a] |
| **WBC count, ×109/L+** | 7.16(3.83-11.80) | 8.17(2.15-22.70) | 9.08(5.29-23.70) | <0.001[a]<br><0.02[b] |
| **Eosinophil count, ×109/L+** | 0.119(0.002-0.620) | 0.151(0.002-1.050) | 0.087(0.003-0.350) | 0.11[a]<br>0.04[b] |
| **Lymphocyte count, ×109/L+** | 2.13(0.50-5.99) | 1.65(0.51-5.40) | 1.16(0.58-4.07) | 0.02[a]<br>0.009[b] |
| **Neutrophil count, ×109/L+** | 4.18(1.70-11.80) | 5.31(1.68-21.50) | 6.11(2.90-21.70) | <0.001[a]<br>0.019[b] |
| **NLR+** | 1.92(0.60-11.81) | 3.09(0.88-32.39) | 3.50(0.98-37.16) | <0.001[a]<br>0.019[b] |

* Mean ±standard deviation, + Median (minimum-maximum), a difference between all groups, bdifference between chronic and acute thrombotic PAD groups
PAD: Peripheral artery disease, MPV: Mean platelet volume, WBC: White blood cell, NLR: Neutrophil to lymphocyte ratio.

**Table 3**. Correlation analysis results between study parameters with Fontaine stage

| | r | p |
|---|---|---|
| **Age** | -0.12 | 0.33 |
| **Fasting glucose** | 0.12 | 0.31 |
| **Systolic BP, mmHg** | 0.27 | 0.02 |
| **Eosinophil count** | -0.32 | 0.01 |
| **Lymphocyte count** | 0.34 | <0.001 |
| **Neutrophil count** | -0.17 | 0.25 |
| **NLR** | 0.36 | 0.001 |

BP: Blood pressure, NLR: Neutrophil to lymphocyte ratio.

**Table4**. Multivariable logistic regression analyses results for predicting acute PAD

| | Adjusted OR (95%CI) | P | Unadjusted OR (95%CI) | p |
|---|---|---|---|---|
| **Age, year** | 1.12(1.06-1.8) | 0.47 | 1.37(1.26-1.88) | 0.23 |
| **Sex, female** | 2.03(1.97-2.1) | 0.06 | 3.01(2.60-3.50) | <0.01 |
| **Smoking** | 2.99(2.77-3.11) | <0.01 | 2.36(2.01-2.63) | <0.01 |
| **Presence of DM** | 1.99(1.78-2.27) | 0.02 | 3.19(2.88-3.42) | <0.01 |
| **MPV** | 0.54(0.32-1.24) | 0.54 | 0.81(0.62-1.94) | 0.45 |
| **Platelet counts** | 2.31(2.10-5.10) | 0.82 | 1.03(0.45-2.40) | 0.90 |
| **WBC count** | 1.72(1.70-2.07) | 0.11 | 1.61(0.77-2.79) | 0.62 |
| **Eosinophil count** | 1.94(1.78-2.14) | 0.03 | 2.14(1.97-2.29) | <0.01 |
| **NLR** | 1.04(0.41-3.07) | 0.61 | 2.17(1.93-4.12) | 0.2 |

PAD: Peripheral artery disease, MPV: Mean platelet volume, WBC: White blood cell, NLR: Neutrophil to lymphocyte ratio, DM: Diabetes mellitus, OR: Odds ratio, CI: Confidence interval

## Discussion

To the best of our knowledge, this is the first study showed that eosinophil may have a role in acute thrombotic occlusion of patients having PAD. In this study, we found significant differences between the acute and chronic PAD groups for the level of neutrophil and eosinophil count and the results of our study indicated that initial eosinophil count may play an important role in the detection of acute thrombotic PAD development.

Shah et al. in their observational study researched eosinophil and lymphocyte counts in general healthy population for unheralded incidence of cardiovascular (CVS) events. They included 775 231 individuals ≥30 years old without CVS disease. Fifty-five thousand and four subjects presented with CVS disease over a median follow-up of 3.8 years. Reduced lymphocyte and eosinophil levels were found to be strongly related with increasing short-term incidence of heart failure and coronary caused death.

Additionally, low eosinophil levels were found negatively correlated with PAD (hazard ratio (HR) 0.63, 95% confidence interval (CI) 0.44 to 0.89) [11]. High eosinophil levels were found to be slightly correlated with increased risk of PAD (HR 1.03, 95% CI 1.01 to 1.04, P=0.003). In addition, no correlation was found between high eosinophil levels and other CVS diseases (angina pectoris, ischemic stroke, and myocardial infarction (MI) etc.). It is uncertain how strongly eosinophil counts can be associated with the several CVS diseases. Although we found lower lymphocyte counts in PAD patients in accordance with Shah et al. we found no significant difference between eosinophil counts of control subjects and angiographically proven severe PAD. However, eosinophil counts were significantly lower in acute thrombotic peripheral artery occlusions. Shah et al. evaluated data of previously healthy subjects electronically and thrombotic nature of the presentations could not be defined in their study. Also they did not define the hemogram parameters at the time of CVS events.

Immediately after acute coronary events the eosinophil levels increase, and large number of eosinophils are detected in the content of coronary thrombus [6]. Additionally, lower eosinophil levels were found to be associated with worse outcome in patients undergoing percutaneous coronary intervention [12]. It was reported that, compared to unstable angina eosinophil counts were lower in MI which has a broader burden of thrombi [13]. Therefore, there may be a probable negative correlation between eosinophil count and thrombus burden. The role of eosinophils on the pathogenesis of thrombus is unclear. The content of eosinophilic granules may activate the inflammatory cells. It has been reported that eosinophilic granule proteins like major basic protein and peroxidase directly activate platelets and leads thrombus formation by inhibiting thrombomodulin [14,15]. In addition, release of potent peroxidase products by activated eosinophils may induce prothrombotic and proinflammatory state in endothelial cells [16]. Eosinophils are the resource of interleukin 17, a circulating pro-inflammatory cytokine, which is associated with coronary instability [17]. A major eosinophilic infiltration was shown in larger red thrombi rather the atherosclerotic plaques [10]. Another suggested mechanism is that the tissue factors released from eosinophils may adhere to factor VII and trigger the coagulation cascade [18]. Combining our finding of lower eosinophil counts in acute thrombotic PAD with mentioned reports, it can be hypothesized that eosinophils may accumulate in thrombotic lesions with a resultant decrease in systemic circulation.

In the present study we found that neutrophil count was increased in PAD with higher levels in acute thrombotic occlusions. NLR is increased in both PAD groups compared to control. However NLR was found similar between chronic cases and acute presentation. NLR has been associated various conditions including coronary artery disease and PAD [5,19]. An elevated NLR has been reported to predict mortality in chronic critical limb ischemia [20]. Erturk et al. had investigated NLR in 508 symptomatic PAD patients retrospectively. They categorized patients to high (>3.0) and low (≤3.0) NLR groups. The patients in the higher NLR group were older and the frequency of critical limb ischemia was found to be high in this group (P=0.019). The frequency of renal failure and intermittent claudication was found to be higher in the low NLR group (P=0.012 and P=0.019). In high NLR group, the cardiovascular mortality was found significantly higher compared to low NLR group (23.6%versus 9.8%; P<0.001) [21]. We have found a weak but significant correlation between severity of PAD, assessed by Fontaine stage, and NLR. However, we did not have a follow up data to assess its value for future prognosis.

There are some suggestions for the association of NLR with CVS events. Arbel et al. reported that a high NLR may be useful to predict all-cause mortality in patients with ST-elevation MI independent of a serum C-reactive protein [22]. They suggested that reduced lymphocyte level was induced by increased concentration of cortisol, and activated neutrophils induced the production of various molecules related to impairment of tissues, such as myeloperoxidase. Furthermore some authors suggested that increased cortisol levels in patients with MI may be responsible for reduced eosinophil count in MI compared to unstable angina [8]. Our similar finding of higher NLR in PAD and lower eosinophil counts in acute thrombotic presentation may be because of cortisol and other mediators.

Recently Velioglu et al. [19] reported mean platelet volume (MPV) as an independent predictor of PAD. However, Ozluk et al. [23] did not find significant changes in MPV levels in angiographically documented PAD patients. We also did not find a significant change in mean MPV levels of PAD patients.

## Limitations

The major limitation of our study is that it was retrospective and single centered. Another limitation is the lack of measurement of other factors like CRP, major basic protein, eosinophilic cationic protein, peroxidase, and other cytokine levels that may interfere with inflammation and thrombosis. Lack of clinical follow-up is another limitation. The control groups were selected only according to their physical examination. It should be better to select the control patients with doppler USG.

## Conclusion

In the present study a negative correlation was found between the blood eosinophil count and thrombotic state in PAD and a positive correlation of NLR with presence and severity of PAD. Therefore, presence of low eosinophil and high neutrophil counts and a high NLR in a recent onset symptomatic PAD may lead physician to a higher probability of acute thrombotic state rather than chronic occlusion.

**Conflict of interests**
*The authors declare that they have no competing interests.*

**Financial Disclosure**
*All authors declare no financial support.*

**Ethical approval**
*The study was conducted according to the Helsinki Declaration and approved by the our university clinical researches ethics committee (2019/385).*

## References

1. W1. Shammas N, Weissman N, Coiner D, et al. Treatment of subacute and chronic thrombotic occlusions of lower extremity peripheral arteries with the excimer laser: a feasibility study. Cardiovasc Revasc Med. 2012;13:211–4.

2. Taşoğlu I, Çiçek OF, Lafcı G, et al. Usefulness of neutrophil/lymphocyte ratio as a predictor of amputation after embolectomy for acute limb ischemia. Ann Vasc Surg. 2014;28:606-13.

3. Saskin H, Ozcan KS, Duzyol C, et al. Are inflammatory parameters predictors of amputation in acute arterial occlusions? Vascular. 2017;25:170-7.

4. Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32:2045-51.

5. Balta S, Celik T, Mikhailidis DP, et al. The Relation Between Atherosclerosis and the Neutrophil–Lymphocyte Ratio. Clin Appl Thromb Hemost. 2016;22:405-11.

6. Kotani K. Neutrophil/Lymphocyte Ratio and the Oxidative Stress Burden. Letters to the Editor. Can J Cardiol. 2015;31:365.e9.

7. Tuncay A, Elcik D. The effect of neutrophil/lymphocyte ratio and tomography measurements at the time of diagnosis in predicting aneurysm rupture in patients with abdominal aortic aneurysms. Turk J Vasc Surg. 2021;30:1-6

8. Jiang P, Wang DZ, Ren YL, et al. Significance of eosinophil accumulation in the thrombus and decrease in peripheral blood in patients with acute coronary syndrome. Coron Artery Dis. 2015;26:101-6.

9. Wang J, Li Q, Li SJ, et al. Relationship of coronary collateral circulation with eosinophils in patients with unstable angina pectoris. Clin Interv Aging. 2016;11:105-10.

10. Sakai T, Inoue S, Matsuyama TA, et al. Eosinophils may be involved in thrombus growth in acute coronary syndrome. Int Heart J. 2009;50:267-77.

11. Shah AD, Denaxas S, Nicholas O, et al. Low eosinophil and low lymphocyte counts and the incidence of 12 cardiovascular diseases: a CALIBER cohort study. Open Heart. 2016;3:e000477.

12. Toor IS, Jaumdally R, Lip GYH, et al. Eosinophil count predicts mortality following percutaneous coronary intervention. Thromb Res. 2012;130:607-11.

13. Sincer I, Gunes Y, Mansiroglu AK, et al. Differential value of eosinophil count in acute coronary syndrome among elderly patients. Aging Male. 2019:1-4.

14. Mukai HY, Ninomiya H, Ohtani K, et al. Major basic protein binding to thrombomodulin potentially contributes to the thrombosis in patients with eosinophilia. Br J Haematol. 1995;90:892-9.

15. Rohrbach MS, Wheatley CL, Slifman NR, et al. Activation of platelets by eosinophil granule proteins. J Exp Med. 1990;172:1271-4.

16. Wang JG, Mahmud SA, Thompson JA, et al. The principal eosinophil peroxidase product, HOSCN, is a uniquely potent phagocyte oxidant inducer of endothelial cell tissue factor activity: a potential mechanism for thrombosis in eosinophilic inflammatory states. Blood. 2006;107:558-65.

17. Liuzzo G, Trotta F, Pedicino D. Interleukin-17 in atherosclerosis and cardiovascular disease: the good, the bad, and the unknown. Eur Heart J. 2013;34:556-9.

18. Moosbauer C, Morgenstern E, Cuvelier SL, et al. Eosinophils are a major intravascular location for tissue factor storage and exposure. Blood. 2007;109:995-1002.

19. Velioglu Y, Yuksel A. Complete blood count parameters in peripheral arterial disease. Aging Male. 2019;22:187-91.

20. Spark JI, Sarveswaran J, Blest N, et al. An elevated neutrophil–lymphocyte ratio independently predicts mortality in chronic critical limb ischemia. J Vasc Surg. 2010;52:632-6.

21. Erturk M, Cakmak HA, Surgit O, et al. Predictive value of elevated neutrophil to lymphocyte ratio for long-term cardiovascular mortality in peripheral arterial occlusive disease. J Cardiol. 2014;64:371-6.

22. Arbel Y, Shacham Y, Ziv-Baran T, et al. Higher neutrophil/lymphocyte ratio is related to lower ejection fraction and higher long-term all-cause mortality in ST-elevation myocardial infarction patients. Can J Cardiol. 2014;30:1177-82.

23. Ozluk OA, Ber I, Peker T, et al. Mean platelet volume levels in the presence of angiographically documented peripheral artery disease. Int J Clin Exp Med. 2015;8:2899-904.