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Immature granulocytes are a potential biomarker in the early diagnosis of lower extremity subacute arterial thrombosis

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ABSTRACT

Aims: Acute arterial embolism in peripheral arteries is generally diagnosed quickly. In contrast, subacute arterial thrombosis (SAT), which arises as an acute exacerbation of chronic peripheral artery disease (PAD), is often more challenging to diagnose rapidly, primarily due to the development of collateral circulation. This study aims to investigate the role of immature granulocytes (IG) in the early diagnosis of SAT in patients with chronic PAD.

Methods: This retrospective study was conducted at a single center between 2019 and 2021. A total of 99 patients with chronic lower extremity PAD were included in the study. Of these, 27 patients (27.2%) who developed exacerbations in the form of SAT were assigned to the SAT group. The remaining 72 patients (72.8%) with chronic PAD were included in the control group. Blood samples from patients in both groups were collected at their first admission before receiving any treatment. Complete blood count parameters were analyzed using an automatic hematological analyzer and compared between the two groups.

Results: Comparison between the control and SAT groups revealed significant differences in hemogram parameters. These parameters included white blood cell count (WBC, p<0.001), neutrophil count (NEUT#, p<0.001), lymphocyte count (LYMPH#, p=0.004), eosinophil count (EO#, p=0.035), neutrophil percentage (NEUT%, p<0.001), lymphocyte percentage (LYMPH%, p<0.001), monocyte percentage (MONO%, p=0.013), eosinophil percentage (EO%, p=0.008), immature granulocyte count (IG#, p<0.001), immature granulocyte percentage (IG%, p=0.002), neutrophil-to-lymphocyte ratio (NLR, p<0.001). These parameters were statistically significantly different in patients with SAT compared to patients with chronic atherosclerotic lower extremity peripheral artery.

Conclusion: Our study demonstrates that immature granulocytes show 81% sensitivity and %59 specificity for the early diagnosis of SAT. These findings suggest that immature granulocytes may serve as a reliable biomarker for the early detection of SAT.

Keywords: Peripheral arterial disease, diagnosis, immature granulocytes

INTRODUCTION

Acute arterial occlusion (AAO) is generally considered a vascular emergency. If acute arterial occlusion is not diagnosed and treated quickly, it may progress in a short time and lead to loss of limb and even life. There are six known P findings in AAO. These include the "six Ps" of pain, pallor, paralysis, paresthesia, pulselessness, and poikilothermia.[1,2] Patients with acute embolic occlusion tend to have a sudden onset with more severe symptoms, as collateralization in the vascular structure does not occur at this point. Treatment is possible with the ease of early diagnosis in AAO.[3,4]

Arterial occlusions can sometimes be detected in the subacute or chronic phase. SAT develops based on chronic PAD.[5-6] Diagnosis and treatment may be delayed due to collateral circulation. If a delay occurs, it may result in increased morbidity and mortality. The main goal of the treatment is to perform any intervention at the best time and save limb vitality in the best way possible. In such a delay, mortality and morbidity rates increase.

Percentage of immature granulocytes (IG%) is a newly discovered inflammatory serum marker. Usually, there is no IG in the systemic blood of healthy people. Therefore, most physicians do not know these immature granulocytes' number IG (IG#) and % IG. IGs are the precursors of neutrophil cells, and they ınclude myelocytes, promyelocytes, and metamyelocytes. IG% values can accurately reflect inflammation in the body. Some studies have shown that the IG% in the blood increases earlier than parameters such as C-reactive protein (CRP) and leukocytes.[7-9] IG% can be rapidly determined by routine blood serum examination. IG# and IG% can be measured quickly and inexpensively in automatic hematological analyzers in almost every laboratory.[10-12]

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IG% has been measured in numerous ıllness. A study reported the IG count as a prognostic biomarker in patients with severe acute pancreatitis.[9] In this study, we aimed to investigate the role of IG in the early diagnosis of SAT.

METHODS

Before starting this study, approval was obtained from the Kastamonu University Clinical Researches Ethics Committee (Date: 12.01.2022, Decision No: 2020-KAEK-143-138). The Declaration of Helsinki's ethical rules and principles were carried out in all procedures.

This study was conducted retrospectively in our cardiovascular surgery clinic between 2019 and 2021. A total of 99 patients with chronic PAD in the lower extremity were included in the study. The diagnosis of chronic PAD was established based on clinical findings such as claudication, smoking history, previous PAD-related surgeries, and imaging evidence of arterial disease. Patients without hemogram data, those under 18, pregnant women, trauma patients, and those with active infections were excluded from the study.

The onset of new symptoms, including coldness, pallor, and cyanosis, in patients with chronic PAD raised suspicion of subacute arterial thrombosis (SAT). The diagnosis was confirmed through Doppler ultrasonography and contrast-enhanced computed tomography (CT). The detection of thrombosis in combination with collateral circulation on Doppler ultrasonography or contrast-enhanced CT was indicative of subacute arterial thrombosis.

A total of 99 patients with chronic PAD were included in the study. Of these, 27 patients (27.2%) diagnosed with SAT were assigned to the SAT group, while the remaining 72 patients (72.8%) were included in the control group. Hemogram parameters were compared between the SAT and control groups. The diagnostic values of IG count and percentage, white blood cell (WBC) count, neutrophil count and neutrophil-leukocyte ratio (NLR) were determined and the sensitivity and specificity rates of these markers were compared.

The patients' data were retrospectively achieved from the hospital database. Blood sample data from the patient's initial visit were used for analysis before receiving any treatment. Complete blood count (CBC) parameters, calculated using an automated hematology analyzer (XN-1000 Hematology Analyzer, Sysmex Corporation, Japan), were examined.

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 for Windows (SPSS Inc., Chicago, USA). Descriptive statistics for the data were presented as frequencies and percentages for categorical variables and as median (25^{th} percentile, 75^{th} percentile) for continuous variables. The Mann-Whitney U test was used to compare the data between the control and SAT groups, as the data did not follow a normal distribution. The chi-square test was employed to analyze categorical variables. Receiver operating characteristic (ROC) analysis and Youden's index were used to determine the area under the curve (AUC), cut-off values, sensitivity, and specificity. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 99 patients who met the inclusion criteria were included in the study. Of these, 27 patients (27.2%) were assigned to the SAT group. The mean age in the SAT group was 66 years (range: 45-87), with 20 male patients (74%) and seven female patients (26%). In the control group, consisting of 72 patients (72.8%), the mean age was 69 years (range: 50-88), with 53 male patients (73%) and 19 female patients (27%). There were no statistically significant differences between the two groups regarding age (p=0.603) and gender (p=0.962).

Revascularization was performed in patients in the SAT group with total occlusion and ischemia. Patients without total occlusion were managed medically. All surgical interventions were open procedures, and no patients underwent endovascular interventions.

Comparing the SAT and control groups, significant differences were observed in several hemogram parameters (Table 1). These included WBC (p<0.001), NEUT# (p<0.001), LYMPH# (p=0.004), EO# (p=0.035), NEUT% (p<0.001), LYMPH% (p<0.001), MONO% (p=0.013), EO% (p=0.008), IG# (p<0.001), IG% (p=0.002), NLR (p<0.001). These parameters were significantly different in patients with SAT compared to those with chronic atherosclerotic lower extremity PAD.

Table 1. Comparison of hemogram data of the groups

	Control (n=72)	SAT (n=27)	p-value
	Median (IQR)		
WBC	7.46 (5.95; 9.23)	11.71 (9.27; 14.01)	<0.001
NEUT#	4.27 (3.48; 6.48)	8.78 (6.96; 11.22)	<0.001
LYMPH#	1.87 (1.42; 2.54)	1.26 (0.95; 1.87)	0.004
EO#	0.12 (0.07; 0.18)	0.06 (0.01; 0.15)	0.035
NEUT%	60.4 (51.9; 71.1)	79.1 (65.9; 86.7)	<0.001
LYMP%	26.4 (19.2; 34.0)	12.5 (7.2; 23.8)	<0.001
MONO%	8.15 (6.80; 10.05)	7.10 (4.8; 8.4)	0.013
EO%	1.55 (0.80; 2.75)	0.6 (0.10; 1.50)	0.008
IG#	0.03 (0.02; 0.05)	0.08 (0.04; 0.13)	<0.001
IG%	0.4 (0.22; 0.60)	0.7 (0.4; 1.2)	0.002
NLR	2.14 (1.49; 3.74)	6.25 (2.6; 12.1)	<0.001

SAT: Subacute arterial thrombosis, IQR: Interquartile range, WBC: White blood cell, NEUT#: Neutrophil count, LYMPH#: Lymphocyte count, EO#: Eosinophil count, NEUT%: Neutrophil percent, LYMPH%: Lymphocyte percent, MONO%: Monocyte percent, EO%: Eosinophil percent, IG#: Immature granulocyte count, IG%: Immature granulocyte percent, NLR: Neutrophil leucocyte ratio; The data has been presented as median, interquartile range. p<0.05 is considered significant

In the ROC analysis (Table 2 and Figure 1), moderate to high predictive values were observed for the following tests: WBC (cut-off: 9.25, AUC: 0.783), NEUT# (cut-off: 6.74, AUC: 0.820), NEUT% (cut-off: 73.9, AUC: 0.801), IG# (cut-off: 0.35, AUC: 0.759), IG% (cut-off: 0.65, AUC: 0.699), NLR (cut-off: 4.76, AUC: 0.793). When immature granulocyte parameters (IG#, IG%, were added to the routine hemogram parameters in SAT patients, immature granulocytes showed 81% sensitivity and 59% specificity for the early diagnosis of SAT (Table 2).

Table 2. ROC analysis values of hemogram data in SAT group

	Cut-off	AUC	95% CI	p-value	Sensitivity %	Specificity %
WBC	9.25	0.783	0.68-0.89	0.000	77	76
NEUT#	6.74	0.820	0.72-0.91	0.000	77	77
NEUT%	73.9	0.801	0.69-0.90	0.000	70	84
IG#	0.35	0.759	0.65-0.86	0.000	81	59
IG%	0.65	0.699	0.58-0.81	0.002	55	80
NLR	4.76	0.793	0.68-0.89	0.000	70	86

SAT: Subacute arterial thrombosis, AUC: Area under the curve, CI: Confidence interval, WBC: White blood cell, NEUT#: Neutrophil count, NEUT%: Neutrophil percent, IG#: Immature granulocyte count, IG%: Immature granulocyte percent, NLR: Neutrophil leucocyte ratio, p<0.05 is considered significant

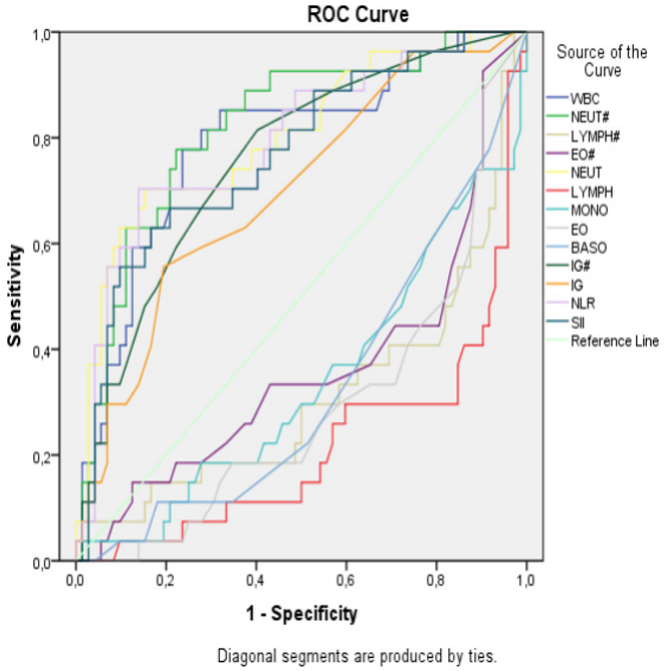

Figure. In the ROC analysis

Moderate-high predictive properties were detected in the following tests; WBC (cut off: 9.25, AUC: 0.783), NEUT# (cut off: 6.74, AUC: 0.820), NEUT% (cut off: 73.9, AUC: 0.801), IG# (cut off: 0.35, AUC: 0.759), IG% (cut off: 0.65, AUC: 0.699), NLR (cut off: 4.76, AUC: 0.793)

ROC: Receiver operating characteristic, WBC: White blood cell, AUC: Area under the curve, NEUT#: Neutrophil count, NEUT%: Neutrophil percent, IG#: Immature granulocyte count, NLR: Neutrophil-leukocyte ratio

DISCUSSION

AAO is a well-recognized vascular emergency that, if diagnosed and treated promptly, can minimize morbidity and mortality. However, diagnosing SAT can present significant challenges. SAT often develops based on chronic PAD, and its diagnosis may be delayed due to the presence of collateral circulation, which can mask the typical symptoms of acute ischemia. This delay in diagnosis and treatment leads to a higher risk of limb loss and increased mortality, underscoring the need for timely identification of SAT. Early diagnosis can improve outcomes and reduce postoperative complications and associated medical costs.[5]

Immature granulocytes (IGs) are generally absent in the peripheral blood of healthy individuals. However, under conditions such as severe inflammation, infection, or acute tissue injury, IGs can be detected in the blood as an early marker for these pathologies. The presence of IGs has been well-documented in the literature as a prognostic marker in a variety of diseases, including sepsis, acute pancreatitis, and myocardial infarction.[7-9] In inflammatory conditions, IGs are typically detected earlier than conventional markers, such as CRP or white blood cell count, making them valuable for early diagnosis.[10-12] Neutrophils, the most abundant type of granulocytes, play a central role in the body's immune response. Neutrophils are produced from hematopoietic stem cells and undergo various stages of maturation, with immature forms (promyelocytes, myelocytes, and metamyelocytes) typically confined to the bone marrow. However, immature forms are released into the peripheral blood during conditions such as infection or acute inflammation. These IGs contribute significantly to the immune response, and studies have shown that they are involved in the pathophysiology of vascular diseases, including atherosclerosis and thrombosis.[13,14] Their role in inflammation and tissue repair has been linked to both the onset and progression of thrombotic events, making them essential candidates for early diagnostic markers in arterial thrombosis.[15,16]

In our study, we found that IGs, specifically IG count (IG#) and percentage (IG%), along with routine hemogram parameters such as WBC, neutrophils, and the NLR, were significantly elevated in patients with SAT compared to those with chronic PAD alone. The ROC analysis showed that IGs and these inflammatory markers had moderate to high predictive values for diagnosing SAT. Specifically, WBC, NEUT#, NEUT%, IG#, and NLR demonstrated good diagnostic performance with AUC values ranging from 0.699 to 0.820. These findings indicate that immature granulocytes show 81% sensitivity and 59% specificity for the early diagnosis of SAT. This suggests that immature granulocytes could serve as a valuable biomarker for the early detection of SAT, thereby improving diagnostic precision and treatment outcomes. Studies in other cardiovascular contexts further support the importance of IGs in vascular diseases. For instance, Korkut et al.[17] demonstrated that IG levels could predict mortality in patients with ST-elevation myocardial infarction (STEMI), with sensitivity and specificity rates of 72% and 77%, respectively. Similarly, studies on critical COVID-19 patients revealed that IGs were positively correlated with thrombotic complications, reinforcing their role in thrombotic processes.[18] Additionally, Karahan et al.[19] found IGs to be valuable in assessing the severity of vaso-occlusive crises in sickle cell anemia. These findings align with our results, suggesting that IGs could be an essential biomarker for arterial thrombosis, including in PAD-related complications such as SAT.

Acute arterial embolism (AAE) can usually be diagnosed quickly, but SAT, particularly in patients with chronic PAD, may have a more insidious onset. This delay in diagnosis is often associated with poorer outcomes, including increased amputation rates and prolonged hospital stays.[20] Given that both SAT and AAE may present with similar clinical symptoms, a reliable early biomarker such as IGs could significantly enhance the ability to differentiate between these conditions, ensuring timely and appropriate intervention. In clinical practice, angiography remains the gold standard for

diagnosing arterial occlusions, but this invasive procedure is not always readily available in all clinical settings. Therefore, a non-invasive biomarker, like IGs, that can help predict SAT and guide decision-making could be highly beneficial.

Limitations

While our study highlights the potential role of IGs as an early diagnostic tool for SAT, it is essential to note that the study's retrospective nature is a limitation. Future prospective studies with larger sample sizes and multicenter designs are needed to validate these findings further and clarify the pathophysiology of IGs in vascular thrombosis. Additionally, while IGs show promise as a diagnostic marker, their role in guiding treatment decisions and improving long-term outcomes remains to be fully explored.

CONCLUSION

Our study suggests that immature granulocytes, exceptionally IG# and IG%, may be valuable biomarkers with high sensitivity (81%) and specificity (59%) for the early diagnosis of SAT. These tests are both simple and cost-effective. Future prospective studies are required to further elucidate the role of immature granulocytes as biomarkers in vascular thrombosis.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of the Kastamonu University Clinical Researches Ethics Committee (Date: 12.01.2022, Decision No: 2020-KAEK-143-138).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available in the "ZENODO" repository, https://doi.org/10.5281/zenodo.7805604.

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