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Mehmet Hamdi Orum, Mahmut Zabit Kara

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Mehmet Hamdi Orum[1], Mahmut Zabit Kara[2]

[1]Kahta State Hospital, Clinic of Psychiatry, Adiyaman - Turkey
[2]Health Sciences University, Antalya Training and Research Hospital, Child and Adolescent Psychiatry, Antalya - Turkey

Sorumlu Yazar:

Mehmet Hamdi Orum, Kahta State Hospital, Clinic of Psychiatry, Adiyaman - Turkey

E-mail: mhorum@hotmail.com

Tel:+90-416-216-10-15/1186

Monocyte to lymphocyte ratio and platelet to lymphocyte ratio in opioid use disorder and marijuana use disorder

Abstract

Objective: There are various methods used in prediction of opioid/marijuana-related situations. However, there is a need for more specific and sensitive markers that are easily applicable and accessible. Complete blood count (CBC) is a convenient method that can be used for this purpose. In this study, we investigated the relationship between CBC values particularly monocyte to lymphocyte ratio (MLR) and platelet to lymphocyte ratio (PLR) and opioid/marijuana use disorder.

Method: We compared CBC values of 56 male patients diagnosed with opioid use disorder (OUD), 56 male patients diagnosed with marijuana use disorder (MUD), and 56 healthy subjects.

Results: Percentage of monocyte (MONO%) was significantly higher in the MUD group compared to the OUD group (p=0.010). The monocyte count (MONO) was significantly different in the MUD group from the other two groups (p=0.018). MLR was significantly higher in the MUD group compared to the OUD group (p=0.049). PLR was significantly different from the other two groups in the OUD group (p=0.038). In the MUD group, the MONO% was declining with age (r=-0.474, p=0.011). The area under the receiver operating characteristic curve of MONO value for MUD was 0.670.

Conclusion: Measurements like lymphocyte and monocyte-related ratios in OUD and MUD can be important in substance monitoring, detection, and differentiation of acute and chronic conditions. In sum, in order to be able to clarify the subject, further studies with fewer limitations are needed.

Keywords: Biomarker, Marijuana, Monocyte to lymphocyte ratio, Opioid, Platelet to lymphocyte ratio

Opioid kullanım bozukluğu ve esrar kullanım bozukluğunda monosit lenfosit oranı ve trombosit lenfosit oranı

Öz

Amaç: Opioid/esrar ile ilgili durumların belirlenmesinde kullanılan çeşitli yöntemler vardır. Bununla birlikte, kolayca uygulanabilir ve erişilebilir olan daha özgül ve duyarlı belirteçlere ihtiyaç vardır. Tam kan sayımı (TKS), bu amaç için kullanılabilecek uygun bir yöntemdir. Bu çalışmada, monosit lenfosit oranı (MLO) ile trombosit lenfosit oranı (TLO) başta olmak üzere TKS değerleri ve opioid/esrar kullanım bozukluğu arasındaki ilişkiyi araştırdık.

Yöntem: Opioid kullanım bozukluğu (OKB) tanılı 56 erkek hasta, esrar kullanım bozukluğu (EKB) tanılı 56 erkek hasta ve 56 sağlıklı birey karşılaştırıldı.

Bulgular: Monosit yüzdesi (MONO%) OKB grubuna göre EKB grubunda anlamlı olarak daha yüksekti (p=0.010). Monosit sayısı (MONO) EKB grubunda diğer iki gruptan anlamlı olarak farklıydı (p=0.018). MLO, EKB grubunda OKB grubuna göre anlamlı olarak yüksekti (p=0.049). TLO, OKB grubunda diğer iki gruptan anlamlı olarak farklıydı (p=0.038). EKB grubunda MONO% yaşla birlikte azalıyordu (r=-0.474, p=0.011). EKB'de MONO değerinin ROC eğrisinin altında kalan alan 0.670 idi.

Sonuç: EKB ve OKB'de lenfosit ile ilişkili oranlar, maddenin takibi, saptanması ve akut/kronik durumların ayırımında önemli olabilir. Konuyu açıklığa kavuşturmak için, daha az kısıtlılığa sahip daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Biyobelirteç, Esrar, Monosit lenfosit oranı, Opioid, Trombosit lenfosit oranı

INTRODUCTION

Substance use disorder (SUD) can result in judicial and social problems (1), neuropsychiatric disorders (2,3), infectious diseases, gastrointestinal diseases, intentional injuries, unintentional injuries,

cardiovascular diseases, and a number hematologic changes including thrombocytopenia, thrombocytopenic purpura, toxic granulation of neutrophils, anemia, lymphocytopenia, eosinophilia, and leukocytosis (1, 4, 5).

Lymphocyte-related ratios are the values obtained by the combined calculation of two of the complete blood count (CBC) parameters (6, 7). Platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) are current parameters added to inflammation markers (8-10). Ozkan et al. (11) showed that patients with heroin dependence trended towards statistically significantly higher NLR compared to healthy controls. According to the Cicek et al. (12)'s study, the mean NLR and PLR levels of patients with heroin dependence were significantly higher than the control subjects. Oseni et al. (13) stated that the NLR did not differ between the marijuana use disorder (MUD) and control subjects. Guzel et al. (14) reported that mean platelet volume (MPV) and percentage of lymphocyte were significantly lower in synthetic cannabinoid use disorder compared to healthy controls. Guzel et al. (14) stated that although there was statistically significant difference between groups in terms of NLR, there was no significant difference for PLR values. Orum et al. (5) reported that PLR, monocyte to lymphocyte ratio (MLR), and percentage of monocyte (MONO%) were significantly lower in opioid use disorder (OUD) group compared to healthy controls. According to our best knowledge, there is no study comparing the lymphocyte-related ratios of OUD and MUD. In this study, we aimed to examine the MLR, PLR, basophil to lymphocyte ratio (BLR), and NLR together with other CBC values in the SUD and investigate the diagnostic value of the lymphocyte-related ratios, especially MLR and PLR, in the SUD.

METHOD

Study Population

In this retrospective cohort study, CBC especially in terms of white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW_CV), platelet count (PLT), platelet distribution width (PDW), plateletcrit (PCT) and MPV, neutrophil (NEU), lymphocyte (LYM), NLR, PLR, BLR, and MLR of 56 male patients diagnosed with OUD and 56 male patients diagnosed with MUD without any psychiatric treatment history who admitted to the Alcohol-Drug Addiction Research Treatment and Training Centre (AMATEM) outpatient clinic of Kahta State Hospital and diagnosed with OUD (with the method of snorting) and MUD were compared with the data of 56 male healthy subjects who were similarly distributed in regard to age. Local ethics committee approval was obtained, and all study participants provided written informed consent (2019/7-7).

The patients consisted of individuals with no substance detected in the urine, without any withdrawal or intoxication symptoms. Depending on the type of substance that these patients frequently preferred (substances that were positive in their urine toxic scans up to 1 month ago and confirmed verbally), they were classified as MUD and OUD. The control group consisted of healthy male volunteers without a self and family history of a SUD who were recruited from the hospital staff.

Inclusion Criteria

Patients with SUD who were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders-5th Edition (DSM-5) (15) criteria were included. Patients who were admitted to the AMATEM outpatient clinic every 2 weeks regularly and whose urine toxic screening test was negative for the last 4 weeks were included in the study. According to the toxicological analysis findings, only MUD and OUD were included in the patient group. All of them were within normal limits in terms of their intelligence level.

Exclusion Criteria

According to the toxicological analysis findings, those who used substances other than marijuana and opioids were excluded from the patient group. Ten patients with benzodiazepine, 14 patients with stimulant, 7 patients with phencyclidine, 22 patients with sedative-acting antipsychotics, 2 patients with biperiden, 25 patients with ethyl glucuronide and 18 patients with multiple drug use were excluded from the study. Individuals who had incomplete information according to the patient registry system were not included in the study. In this way, 11 patients were excluded from the study. Patients who had comorbid additional psychiatric diagnosis, hypertension, diabetes mellitus, severe neurological, immunological or systemic diseases according to the patient registry system were excluded. In the control group, 9 people who had substance use history, 12 people who used alcohol use, 6 people who have incomplete information were excluded from the study. The group of healthy controls did not have any psychiatric diagnosis, hypertension, diabetes mellitus, severe neurological, immunological or systemic diseases which may affect the results. Thirteen patients with dull intelligence, 7 patients with borderline intelligence, and 4 patients with mild mental retardation were excluded from the study.

Hematological analysis

Venous blood samples were obtained from antecubital vein of both patient and control group between 8 and 10 a.m. after at least 8 h of starving. The samples were centrifuged within 30 minutes and in the same day, centrifugation was followed in the "CELL-DYN 3700 SL analyzer (Abbott Diagnostics, Chicago, U.S.A.)" device at the Kahta State Hospital biochemistry laboratory. Reference intervals

were determined as PLT: 142-424 (10^3/uL), PDW: 0-1000 (fL), PCT: 0-1000 (fL), MPV: 6.8-10.8 (fL), RBC: 4.04-6.13 (10^6/uL), MCV: 80-97 (fL), MCH: 25-33.5 (pg), RDW_CV: 11.6-15.8 (%).

Statistical analysis

SPSS for Windows statistical package version 22 (SPSS Inc., Chicago, IL, United States) was used for all statistical analyses. The numerical data were expressed as means and standard deviations, and the categorical data were expressed as frequencies and percentages. Normal distribution suitability was assessed using visual and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk test). Student's t test was used for normal distributions, and one-way ANOVA was used for three independent groups. Mann–Whitney U test and Kruskal Wallis test were used for those with no normal distribution. A post-hoc Tukey Honestly Significant Difference test was used when a significant difference was found between the three independent groups. The relationship between the variables was assessed by Spearman correlation test. Receiver operating characteristic (ROC) curve analysis was used to measure the diagnostic value of monocyte count (MONO). A value of less than 0.05 was considered statistically significant.

RESULTS

The mean age was 23.64 ± 5.30 years in the OUD group, 23.75 ± 5.55 years in the MUD group, and 26.21 ± 4.80 years in the control group. The ratio of males 100.00% in both patients and control groups, and there was no significant difference in terms of age between the two groups (p=0.121). The mean duration of OUD was 4.98 ± 4.42 years. The mean duration of MUD 6.42 ± 5.16 years.

According to the comparison of CBC values with student's t test, MONO% was significantly higher in the MUD group compared to the OUD group (p=0.010). Again in terms of MONO%, MUD group was lower than control group, OUD group was higher than control group, but this was not significant. The MONO was significantly different in the MUD group from the other two groups (p=0.018). MLR was significantly higher in the MUD group compared to the OUD group (p=0.049). There were no significant differences between the control group and the other two groups for MLR. PLR was significantly different from the other two groups in the OUD group (p=0.038). There was no significant difference between groups in terms of NLR and BLR (p=0.970 and p=0.473). RDW_CV showed a significant difference between all groups (p=0.000). Other variables showed no significant differences. A comparison of sociodemographic variables and CBC values of the OUD, MUD, and control groups are given in table 1.

Spearmen correlation analysis findings between age and CBC values were also given in table 2 separately. In the OUD group, the percentage of neutrophil (NEU%) was increasingly correlated

with age (r=0.507, p=0.010), and percentage of lymphocyte (LYM%) and MONO% were declining with age (r=-0.472, p=0.017; r=-0.458, p=0.021). In the MUD group, the MONO% was declining with age (r=-0.474, p=0.011). There was no correlation with age in the control group (Table 2).

ROC curve analysis performed to assess the diagnostic value of MONO is shown in figure 1. The area under the ROC curve of MONO value for MUD was 0.670. The optimal cut-off value for MONO was 0.55, and its sensitivity and specificity for diagnosis of MUD were 64.3% and 48.1%, respectively.

DISCUSSION

Our study suggests that there is a relationship between hematological parameters and SUD, especially the immune cells. There was no change in parameters associated with red blood cells except RDW_CV. The duration of SUD was greater in the MUD group. According to the literature, the age of starting marijuana use is lower than the age of starting to use opioids. This result is compatible with the literature. There was a significant relationship between PLR and OUD. Our results are similar to some statements in the literature, but incompatible with some. Rasheed and Iqtidar (16) investigated the hematologic parameters of 100 heroin dependents and suggested a statistically significant decrease in HGB, HTC, RBC, PLT in heroin addicts as compared to control subjects. Verde Méndez (17) demonstrated that for both sexes, the levels of HTC and HGB were similar in the control groups and opiate addicts. Savov et al. (18) established a heroin macrocytosis in chronic heroin abusers. Again, there was no change in parameters associated with platelets. Guzel et al. (14) designed a study and found that WBC, MCH, RDW_CV, MCV, MPV, MONO, and NEU parameters were significantly higher in synthetic cannabinoid group to control group.

On the other hand, our results suggest that the use of opioids and marijuana causes a change in the immune response and these results are consistent with literature. Opioids have been shown to inhibit immune parameters such as natural killer (NK) activity, lymphocyte proliferation, and nitric oxide production. Neural modulation of immunity by opioids is a well-documented phenomenon that involves both neuroendocrine and autonomic efferent pathways (19). Banerjee and Sarkar's study (20) indicate that the monocyte count was significantly lower in heroin smokers than in control persons. Pérez-Castrillón et al. (21) suggest that opioids play an important role in the depression of monocyte chemotaxis observed in intravenous drug abusers. Novick et al. (22) reported that NK activity was reduced significantly in parenteral heroin abusers compared with methadone maintenance patients and apparently healthy individuals. Δ(9)-tetrahydrocannabinol (THC), the major product of marijuana, exhibits immunosuppressive activity. Another product of marijuana, cannabidiol (CBD), while not psychotropic, also modulates immune function. THC inhibits the migratory capability of macrophage-

like cells resident in the central nervous system, such as microglia, toward nodes of microbial invasion. THC and CBD have been reported to exert developmental and long-term effects on the immune system suggesting that exposure to these substances during an early stage in life has the potential to alter the fundamental neuroimmune response to select microbial agents in the adult (23).

Endocannabinoid receptors, in particular the cannabinoid receptor type 2, are involved in the development and modulation of immune and hematologic cells. Information derived from studies performed on chronic marijuana consumers have shown that this substance can reduce the number of T and B lymphocytes and increase the number of eosinophils (13, 24). According to our study, the use of marijuana affects MONO% more than opioid use. Again, the MONO is associated with marijuana use but not with opioid use. MONO% correlated with age in the MUD group. These results and ROC curve analysis suggest that the use of marijuana and monocyte-related parameters are related. In our study, monocyte-associated biomarkers were found to be more relevant to marijuana use than opioid use. The mononuclear phagocyte system includes the promonocytes and their precursors in the bone marrow, the monocytes in the peripheral blood, and the macrophages in the tissues. Macrophages play major roles in both innate and acquired immunity to infections. For innate immunity, macrophages produce acute-phase cytokines, phagocytose and kill microbes; for acquired immunity, macrophages present antigen and release cytokines. Studies of lung alveolar macrophages showed that marijuana smoking had little effect on phagocytic capacity but did cause some metabolic and morphological changes in the cells (23, 24).

Self-report with such as Addiction Severity Index (ASI), Addiction Profile Index (API), and Drug Abuse Screening Test (DAST) is inexpensive and relatively accurate, but has limitations particularly due to participant's denial or recall bias (25, 26). For this reason, laboratory evidence of OUD and MUD is needed. Measures related to OUD and MUD are mostly in the field of forensic medicine and emergency medicine. Measurements in OUD and MUD are important in substance monitoring and detection. It is also important to monitor the treatment response and determine the level of compliance (27). Biomarkers are markers of a biological process or state, which are useful for clinicians and patients if they provide information about the current status or future risk of disease. It is an easy and inexpensive method to utilize hematologic parameters in cases where we cannot get clear information from patients. For instance, there is an important research area associated with hematologic markers of alcoholism. For alcohol users, the markers known as state markers are divided into two groups: relapse markers and screening markers. Relapse markers are also sensitive to acute alcohol consumption. Screening markers may be useful for early identification before alcohol use disorder emerges. Carbohydrate-deficient transferrin (CDT), RBC, and MCV are the hematological state markers used for this purpose (28, 29). The increased biomarker work related to heroin, as in alcohol, will facilitate the planning of follow-up and treatment of these patients, increase their utility

in judicial cases, and can be used to differentiate between chronic and acute conditions. In our study, there were changes about MONO, MONO%, MLR, and PLR. CBC values and calculations made using both of these values are intended to be used as OUD and MUD biomarkers.

As a conclusion, the known effect of opioid and marijuana on lymphocytes, neutrophils, platelets led us to investigate the effect of OUD on PLR, MLR, BLR, and NLR. There is relationship between the OUD and MUD and hematological parameters including MONO%, RDW_CV. Retrospectively our results of CBC showed that OUD and MUD was related to PLR and MLR; was not related to BLR and NLR. This study provides the opportunity to reach NLR, BLR, PLR and MLR values of OUD and MUD patients in one study.

Our study has several limitations. The retrospective nature of the study is the most important limitation and prospective studies are needed. By increasing the number of samples, it was thought that it would be important to evaluate the data related to individual genders and ages. Studies with larger sample sizes will reveal more clearly the relationship between opioids, marijuana and MLR and PLR. Long-term studies are needed to see what happens after detoxification and remission periods. Substance use frequency and length of substance use are some other important points that may affect blood parameters. Studies are needed to clarify these points. The possible effect of smoking on the results is unknown. The life style and nutritional characteristics of the individual are also other confounding factors. Further studies are needed to confirm these results and explain the underlying mechanisms.

Conflicts of Interest

The authors declared that there were no conflicts of interest regarding the publication of this paper.

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Table 1. Comparison of Socio-Demographic Variables and Complete Blood Count Values of OUD (n=56), MUD (n=56) and Control (n=56) Groups

	OUD (n=56)	**MUD (n=56)**	**Control (n=56)**	**η^2**	**p**
	Mean±SD	**Mean±SD**	**Mean±SD**		
Age (years)	23.64± 5.30	23.75 ± 5.55	26.21 ± 4.80	0.051	0.121
Male **Gender**	56 (100.00%)	56 (100.00%)	56 (100.00%)		
WBC (10^3/uL)	9.51 ± 2.68	9.31 ± 3.20	8.05 ± 1.74	0.059	0.084
RBC (10^6/uL)	5.26 ± 0.51	5.35 ± 0.43	5.29 ± 0.39	0.006	0.779
HGB (g/dL)	15.49±1.23	15.80 ± 1.59	15.82 ± 0.86	0.015	0.541
HTC (%)	46.32± 3.66	47.34 ± 4.12	46.97 ± 2.68	0.015	0.551
MCV (fL)	88.32± 4.63	88.49 ± 4.82	88.89 ± 4.23	0.003	0.891
MCH (pg)	29.49± 1.97	29.52 ± 1.95	29.98 ± 2.04	0.013	0.582
MCHC (g/dL)	33.38± 1.19	33.35 ± 1.30	33.72 ± 1.37	0.017	0.504
RDW_CV (%)	11.69± 0.63	12.17 ± 0.92	11.19 ± 0.98	0.183	0.000
PLT (10^3/uL)	233.63± 55.52	249.68 ± 68.54	245.42 ± 49.96	0.014	0.571
PDW (fL)	19.28± 1.38	19.34 ± 1.65	18.54 ± 1.53	0.055	0.099

PCT (%)	0.17 ± 0.04	0.18 ± 0.04	0.17 ± 0.03	0.017	0.498
MPV (fL)	7.81 ± 1.83	7.91 ± 1.91	7.52 ± 1.14	0.010	0.665
NEU (10^6/uL)	5.85 ± 2.71	5.79 ± 2.76	4.71 ± 1.70	0.012	0.154
LYM (10^3/uL)	2.77 ± 0.62	2.48 ± 0.62	2.50 ± 0.78	0.037	0.214
MONO (10^3/uL)	0.55 ± 0.16	0.72 ± 0.33	0.55 ± 0.17	0.099	0.018
BASO (10^3/uL)	0.08 ± 0.03	0.08 ± 0.03	0.08 ±0.03	0.001	0.967
EOS (10^3/uL)	0.22 ± 0.14	0.21 ± 0.11	0.18 ± 0.17	0.009	0.707
NEU%	58.33± 9.85	60.14 ± 8.89	57.82 ± 9.42	0.012	0.629
LYM%	31.86± 8.53	28.32 ± 7.52	33.92 ± 8.77	0.042	0.191
MONO%	6.27 ± 1.73	7.96 ± 2.41	6.87 ± 1.79	0.112	0.010
BASO%	0.97 ± 0.31	1.00 ± 0.31	1.05 ± 0.33	0.009	0.697
EOS%	2.53 ± 1.59	2.53 ± 1.34	2.34 ± 2.03	0.003	0.897
NLR	2.30 ± 1.61	2.38 ± 1.09	2.43 ± 2.74	0.001	0.970
PLR	87.50± 25.46	103.49± 30.78	107.90± 39.80	0.088	0.038
MLR	0.20 ± 0.07	0.29 ± 0.12	0.25 ± 0.18	0.073	0.049
BLR	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.05	0.023	0.403

One-Way ANOVA and Post Hoc Tukey were used. Degrees of freedom=167

Notes: □2: Effect Size; OUD: Opioid use disorder, MUD: marijuana use disorder, WBC: White Blood Cell; RBC: Red Blood Cell; HGB: Hemoglobin; HTC: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW_CV: Red Blood Cell Distribution Width_Coefficent of Variation; PLT: Platelet; PDW: Platelet Distribution Width; PCT: Platelecrit; MPV: Mean Platelet Volume; NEU: Neutrophil; LYM: Lymphocyte; MONO: Monocyte; BASO: Basophil; EOS: Eosinophil; NEU%: Percentage of Neutrophil; LYM% Percentage of Lymphocyte; MONO%: Percentage of Monocyte; BASO%: Percentage of Basophil; EOS%: Percentage of Eosinophil; NLR: Neutrophil to Lymphocyte Ratio; PLR: Platelet to Lymphocyte Ratio; MLR: Monocyte to Lymphocyte Ratio; BLR: Basophil to Lymphocyte Ratio

Table 2. The Correlation Between Age and Complete Blood Count Parameters of Three Groups

Parameters	**OUD**		**Parameters**	**MUD**		**Parameters**	**Control**	
	r	**p**		**r**	**p**		**r**	**p**
NEU%	0.507	0.010	NEU%	0.111	0.575	NEU%	-0.246	0.217
LYM%	-0.472	0.017	LYM%	-0.023	0.909	LYM%	0.215	0.281

MONO%	-0.458	0.021		MONO%	-0.474	0.011		MONO%	-0.013	0.949
BASO%	-0.248	0.233		BASO%	0.198	0.313		BASO%	0.091	0.653
EOS%	-0.069	0.742		EOS%	0.200	0.307		EOS%	0.182	0.364
MONO	0.047	0.824		MONO	-0.349	0.069		MONO	-0.023	0.910
BASO	0.206	0.324		BASO	0.102	0.604		BASO	-0.039	0.845
EOS	0.202	0.333		EOS	0.261	0.179		EOS	0.172	0.391
BLR	0.210	0.313		BLR	0.141	0.473		BLR	-0.084	0.677
MLR	0.101	0.631		MLR	-0.331	0.086		MLR	-0.155	0.440

$p<0.05$, Spearmen Correlation Analysis was used.

Notes: OUD: Opioid Use Disorder; MUD: Marijuana Use Disorder; NEU%: Percentage of Neutrophil; LYM%: Percentage of Lymphocyte; MONO%: Percentage of Monocyte; BASO%: Percentage of Basophil; EOS%: Percentage of Eosinophil; MONO: Monocyte Count; BASO: Basophil Count; EOS: Eosinophil Count; BLR: Basophil to Lymphocyte Ratio; MLR: Monocyte to Lymphocyte Ratio

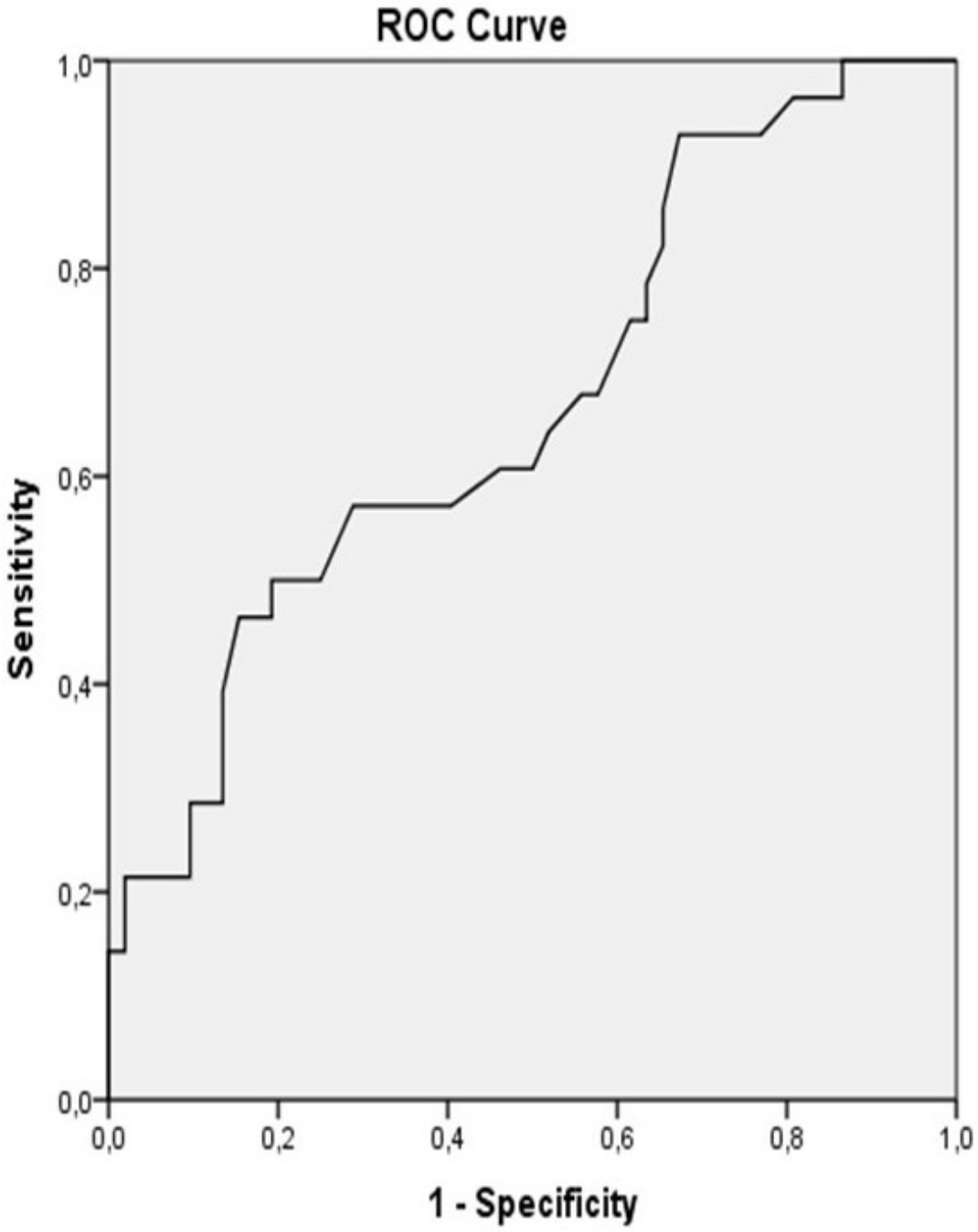

Figure 1. Receiver Operating Characteristic (ROC) Curve Analysis Performed to Assess the Diagnostic Value of Monocyte Count