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ORIGINAL ARTICLE

Value of hematological indices versus VEGF as biomarkers of activity in Behçet's disease

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Abstract

Objective The aim of this study is to investigate the value of several hematological indices, neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), red blood cell distribution width (RDW), and mean platelet volume (MPV), versus vascular endothelial growth factor (VEGF) as biomarkers of activity in Behçet's disease (BD).

Methods This study included 96 adult BD patients recruited from the Rheumatology Outpatient Clinic, Kasr Al-Ainy Hospital, Cairo University, and 60 healthy subjects as controls. Assessment of BD activity was done using the Behçet's Disease Current Activity Form (BDCAF). The CBC was measured by COULTER LH 750 assay analyzer with special consideration to the NLR, PLR, MPV, and RDW. Serum VEGF level was measured by enzyme-linked immunosorbent assay (ELISA) technique.

Results The NLR, RDW, and PLR were significantly higher in BD patients when compared with healthy controls ($p = 0.011$, < 0.001, < 0.001, respectively), while there was no statistical difference in MPV or VEGF between patients and controls ($p = 0.82$, 0.46). NLR and PLR were significantly higher in BD patients with vascular activity ($p = 0.03$, 0.01). RDW was significantly higher, while MPV was significantly lower in patients with vascular manifestations ($p = 0.04$, 0.004). NLR and PLR were the most valuable predictors of vascular activity ($p = 0.033$, 0.018). PLR was more powerful as a predictor of vascular activity, but it had a lower specificity than NLR.

Conclusion The NLR, PLR, and RDW are significantly higher in BD patients, suggesting their value as promising inflammatory biomarkers in BD. NLR and PLR are the most valuable predictors of vascular activity, while RDW and MPV were not valuable predictors of BD activity, rather implicated in the ongoing vascular inflammatory process in BD. The VEGF was neither a surrogate biomarker of BD activity nor reflecting the ongoing inflammatory process in BD.

Keywords Behçet's disease · MPV · NLR · PLR · RDW · VEGF

Introduction

Behçet's disease (BD) is a chronic, autoimmune, inflammatory disorder characterized by involvement of the mucocutaneous, urogenital, ocular, vascular, neurological, and gastrointestinal systems [1]. BD is a systemic vasculitis with significant neutrophil infiltration, endothelial cell swelling, and fibrinoid necrosis. The diagnosis of BD is only supported by clinical criteria and requires the exclusion of other diagnoses based on clinical presentation. There are no pathognomonic laboratorial findings of BD [2].

Despite the fact that several cytokines and biomarkers have been identified to monitor disease activity in BD, no specific laboratory test was distinguished [3]. These biomarkers included serum endocan [4], serum growth differentiation factor 15 [5], serum alpha 1-acid glycoprotein [6], interleukin-32 [7], interferon gamma [8], interleukin-20 [9], interleukin-6, tumor necrosis factor-α, interleukin-26 [10], and interleukin-2, which was reported to be elevated in BD patients with uveitis [11]. Nitric oxide elevation was also found to be increased during BD activity [9, 12, 13]. On the contrary, direct bilirubin level with its anti-oxidative properties was reported to have an immunomodulatory effect in BD by suppressing inflammation [14]. Moreover, the presence of oxidative stress in BD

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was studied, revealing that there is a relationship between oxidative stress markers and endothelial function in patients with BD [15].

Recently, the neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), red blood cell distribution width (RDW), and mean platelet volume (MPV) have been addressed as novel inflammatory biomarkers that can help in the assessment of disease severity in many autoimmune diseases, such as ankylosing spondylitis [16, 17], rheumatoid arthritis (RA) [18], systemic lupus erythematosus (SLE) [19], and psoriatic arthritis [20].

High NLR and PLR in peripheral blood indicate systemic inflammatory responses [21]. NLR can be increased in response to systemic or local inflammation, e.g., ulcerative colitis and inflammatory arthritis [22]. The significance of NLR in several autoimmune diseases (e.g., familial Mediterranean fever, ulcerative colitis, psoriasis) was recently reported [23–25]. PLR has been suggested as a predictor of cancer and inflammatory conditions [26, 27]. The MPV is another marker that shows platelet count and activity, which has been found to be associated with inflammation [28, 29]. The RDW was reported to be increased in different autoimmune diseases like RA, SLE, ankylosing spondylitis, and ulcerative colitis [30–33]; also, an elevated RDW can be seen in diseases, such as anemia, renal dysfunction, thyroid disease, nutritional deficiencies, cancer, and diabetes mellitus [34].

The vascular endothelial growth factor (VEGF) is a potent cytokine that modulates angiogenesis by acting as an essential mitogen for vascular endothelial cells. VEGF has been reported to be elevated in BD patients especially during disease activity; so, it was proposed as a marker of disease activity [35–37].

The aim of the present study is to investigate the value of several hematological indices (NLR, PLR, MPV, and RDW) versus VEGF as biomarkers of activity in BD.

Patients and methods

Study design

This is a case–control study where subjects were selected based on the presence of BD (cases) or not (controls).

Data source

This study included 96 adult BD patients recruited from the Rheumatology outpatient clinic, Kasr Al-Ainy Hospital, Cairo University, and 60 age- and sex-matched healthy subjects as controls. All patients were diagnosed according to the international study group criteria for the diagnosis of BD [38]. Patients and controls with (1) concomitant autoimmune or autoinflammatory disease, (2) acute or chronic infection, (3) malignancy, (4) systemic disease, such as diabetes mellitus, (5) pregnancy or up to 6 months postpartum, and (6) liver, kidney, and heart failure were excluded from the study. All procedures performed were in accordance with the ethical standards of Kasr Al-Ainy, Cairo University Hospital's research committee and with the 1964 Helsinki declaration ethical standards.

Data collection

BD patients and controls were subjected to full history taking, clinical examination, and laboratory investigations. BD patients were divided into those with active disease and those with inactive disease, using the Behçet's Disease Current Activity Form (BDCAF) [39]. Active disease was defined as a BDCAF value ≥ 2 [40]. The blood samples were obtained in the morning, under fasting conditions. The blood samples were taken before modifying the treatments in patients with activity of the disease. The CBC was measured by COULTER LH 750 assay analyzer with special consideration to the NLR, PLR, MPV (normal 7.5–11.5 fl), and RDW (normal 11–15%). Serum VEGF level was measured by enzyme-linked immunosorbent assay (ELISA) technique. CRP level was measured using an immunonephelometry assay on a Dade Behring Nephelometer BN II device (Siemens Healthcare GmbH, Erlangen, Germany), and ESR was measured using a traditional Westergren method.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences, Version 21.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were analyzed as mean values ± standard deviation or median (range) as appropriate. For categorical variables, differences were analyzed with χ^2 (Chi square) tests. Differences between parametric variables were analyzed by Student's t test. Comparisons between quantitative variables were done using the non-parametric Kruskal–Wallis and Mann–Whitney tests. Pearson's correlation was used. Receiver operator curve (ROC) was done to determine the best cut-off value, sensitivity, and specificity of MPV, RDW, NLR, and VEGF in predicting the disease activity. p value ≤ 0.05 was considered significant.

Results

This study was conducted on 96 BD patients and 60 age- and sex-matched controls. Mean age of BD patients ranged from 18 to 73 years with a mean of 34.9 ± 10.1 years. Disease duration ranged from 1 to 40 years

Table 1 Clinical manifestations of the studied BD patients

N (%)	BD patients (*n* = 96)
Oral ulcers	96 (100%)
Genital ulcers	96 (100%)
Skin manifestations	60 (62.5%)
Articular manifestations	48 (50%)
GIT manifestations	5 (5.2%)
Ocular manifestations	60 (62.5%)
CNS manifestations	7 (7.2%)
Vascular manifestations	50 (52%)
Pulmonary manifestations	7 (7.2%)
Cardiac manifestations	2 (2%)
Genital ulcers	96 (100%)
Skin manifestations	96 (100%)
Articular manifestations	60 (62.5%)
GIT manifestations	48 (50%)

BD, Behçet's disease; CNS, central nervous system; GIT, gastrointestinal

with a mean of 9 ± 7 years. Fifty nine patients had active disease (37.8%), while 37 patients were inactive (23.7%). Eye activity (e.g., anterior uveitis, posterior uveitis, pan uveitis) was present in 17 (17.7%) patients, CNS activity (e.g., stroke, nerve palsy) in 4 (4.1%) patients, and vascular activity (e.g., venous or arterial thrombosis, arterial aneurysm) in 4 (4.1%) patients. The clinical manifestations of the studied BD patients are shown in Table 1, while Table 2 demonstrates demographic characteristics and laboratory investigations of BD patients and controls. The pharmacological treatments used for BD patients are presented in Table 3. There was a significant difference between active BD patients and those in remission regarding the current oral corticosteroid dose (median of 15 mg vs 10 mg, respectively) ($p = 0.035$).

The NLR, RDW, and PLR were significantly higher in BD patients when compared with healthy controls ($p = 0.011$, < 0.001, < 0.001, respectively), while there was no statistical difference between BD patients and controls regarding the MPV or VEGF ($p = 0.82$, 0.46, respectively) (Table 4).

The NLR and PLR were significantly higher in BD patients with vascular activity than in those without vascular activity ($p = 0.03$, 0.01, respectively) (Table 5).

The RDW was significantly higher, while MPV was significantly lower in patients with vascular manifestations than in those without ($p = 0.04$, 0.004, respectively) (Table 6).

There was no statistical difference in the NLR or the PLR between patients currently receiving oral corticosteroids and those who are not currently receiving corticosteroids ($p = 0.2$, 0.8, respectively) (Table 7). RDW was significantly increased in both anemic and non-anemic BD patients (16.47 ± 2.32, 15.15 ± 1.67, respectively) in comparison with controls (14.29 ± 1.03) ($p < 0.001$, 0.010, respectively).

Table 2 Demographic characteristics and laboratory investigations of BD patients and controls

Mean ± SD, range *N* (%)	Active BD patients (*n* = 59)	Inactive BD patients (*n* = 37)	Controls (*n* = 60)	*p* value
Age (years)	33.03 ± 9.8 (18–60)	36.2 ± 10.1 (21–73)	36.7 ± 12.6 (24–64)	0.26
Sex (males)	31(83.8%)	48 (81.4%)	51 (85%)	0.86
Smokers (%)	20 (54%)	28 (47.5%)	24 (40%)	0.39
Hb (g/dl)	13.1 ± 1.27 (9.9–15.5)	13.9 ± 1.5 (10.1–16.6)	14.4 ± 1.3 (11.6–17.7)	< 0.001
MCV (fl)	82.42 ± 5.93 (71.6–94.5)	83.64 ± 6.77 (66.6–99.4)	81.9 ± 7.4 (57.9–95.6)	0.48
TLC ($\times 10^3/mm^3$)	9.59 ± 4.66 (4.1–26.4)	8.98 ± 2.99 (3.7–18.7)	7.1 ± 1.7 (4–11.2)	0.001
Neutrophils (%)	59.59 ± 15.97 (34–94)	56.75 ± 14.12 (12–92)	51.2 ± 13.3 (18–76)	0.035
Lymphocytes (%)	30.38 ± 13.59 (5–56)	33.10 ± 12.49 (6–60)	39.3 ± 12 (20–68)	0.003
Platelets ($\times 10^3/mm^3$)	272.5 ± 75.5 (134–421)	277.6 ± 76.3 (168–483)	243.4 ± 50 (150–343)	0.077
ESR (mm/1st hour)	33.6 ± 23.2 (5–80)	19.7 ± 17.9 (2–82)	10.3 ± 9.1(2–50)	< 0.001
CRP (mg/l)	26.71 ± 34.11 (6–169)	11.98 ± 5.15 (6.2–29)	–	0.007
CRP (+ve)	31(52.5%)	14(37.8%)	0 (0%)	< 0.001
Blood urea (mg/dl)	29.88 ± 8.78 (12.8–45)	26.91 ± 8.75 (13–50)	26 ± 6.5 (12.8–40)	0.087
Serum creatinine (mg/dl)	0.82 ± 0.23 (0.30–1.3)	0.73 ± 0.21 (0.30–1.3)	0.79 ± 0.19 (0.4–1.3)	0.132
AST (IU/l)	22.65 ± 9.68 (11–46)	22.08 ± 8.26 (10–45)	24.1 ± 8.4 (12–57)	0.225
ALT (IU/l)	22.86 ± 14.2 (5–60)	20.6 ± 9.97 (7–50)	21.3 ± 9 (8–40)	0.816

BD, Behçet's disease; Hb, hemoglobin; TLC, total leucocytic count; MCV, mean corpuscular volume; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; AST, aspartate transaminase; ALT, alanine transaminase

p values are significant at $p \leq 0.05$

Table 3 Pharmacological treatments of the studied BD patients

Treatment of BD patients ($n = 96$)	N (%)
Oral corticosteroids	79 (82.3%)
Colchicine	74 (77%)
Azathioprine	56 (58.3%)
Cyclophosphamide	42 (43.7%)
Cyclosporine A	34 (35.4%)
Methotrexate	9 (9.3%)
Infliximab	13 (13.5%)
Low-dose aspirin	15 (15.6%)
Warfarin	34 (35.4%)

There was no association between NLR and corticosteroid dose ($p = 0.2$). RDW significantly correlated with hemoglobin level ($r = -0.38$, $p = 0.003$) (Table 8).

A ROC analysis for the studied parameters to detect their sensitivity and specificity for prediction of vascular disease activity revealed that NLR and PLR were the most valuable predictors of vascular activity ($p = 0.033$, 0.018, respectively) while PLR was more powerful as a predictor of vascular activity but had a lower specificity than NLR (Table 9) (Fig. 1).

Discussion

BD is a systemic inflammatory disease characterized by recurrent episodes of acute inflammation consisting mainly of neutrophil infiltration around blood vessels in the affected tissues [41]. To date, there is no specific tool or serum marker to identify the activity of BD.

To our knowledge, this is the first study evaluating the value of several hematological indices (NLR, PLR, MPV, and RDW) versus VEGF as biomarkers of activity in BD.

In the present study, our results demonstrated significantly higher NLR in BD patients compared with controls ($p = 0.011$), which is in accordance with Ozturk et al. [42], Rifaioglu et al. [43], and Alan et al. [44], suggesting that innate immune system dysfunction presenting with neutrophil hyperactivity has a fundamental role in the pathogenesis of BD [45, 46]. In addition, Ünlü and his coworkers reported that high NLR may be related to endothelial dysfunction and reflects BD activity [47].

In the current study, NLR was significantly higher in patients with vascular activity ($p = 0.03$), which is in agreement with Okatan and his coworkers [48] who stated that the NLR was significantly higher in active vascular BD patients, suggesting high NLR may be a useful disease activity marker in Behçet's disease. Similarly, Erden and his colleagues reported that high NLR was important for predicting deep venous thrombosis in BD, where NLR of BD patients with deep venous thrombosis was significantly higher than in those without thrombosis ($p < 0.0001$) [49]. Also, Dursun et al. demonstrated that higher NLR was associated with the development of retinal vein occlusion [50]. Furthermore, Ayça and his colleagues found that increased NLR predicted stent thrombosis in myocardial infarction patients [51]. Moreover, according to our results, NLR was found to be a valuable predictor of vascular activity. Considering that vascular complications are amongst the most devastating complications of BD, these data show that NLR could be a promising index for predicting BD vascular activity.

In our study, there was no impact of oral corticosteroids on the results of NLR, where there was no difference in NLR between patients currently receiving corticosteroids and those who are not ($p = 0.2$). Moreover, low dose of corticosteroids has no effect on NLR [52], which was the case in our study where the median dose of corticosteroids received was 15 mg/day in active BD patients and 10 mg/day in BD patients in remission.

In the present study, RDW was significantly higher in BD patients in comparison with controls ($p < 0.001$), which is in agreement with Aksoy et al. [53] and Vayá et al. [54]. This can be attributed to the inflammatory cytokines and the oxidative

Table 4 MPV, RDW, NLR, PLR, and VEGF values in BD patients and controls

	Mean ± SD, range Median, range	Active BD patients ($n = 59$)	Inactive BD patients ($n = 37$)	Controls ($n = 60$)	p value
Blood picture parameters	MPV (fl)	8.95 ± 1.1 (6.8–13.6)	9.05 ± 1.2 (7.2–12.4)	9 ± 0.9 (7.1–10.8)	0.820
	RDW (%)	15.63 ± 2.1 (12.8–24.1)	15.41 ± 1.8 (12.9–20.1)	14.3 ± 1.03 (12.1–16)	< 0.001
	NLR	1.8 (0.6–18.8)	1.7 (0.2–4.4)	1.35 (0.3–3.8)	0.011
	PLR	8.61 (3–60.4)	8.45 (4–21.8)	6.15 (2.78–15)	< 0.001
VEGF (ng/l)		1.74 ± 0.67 (0.78–3.55)	1.73 ± 0.73 (0.76–3.53)	1.7 ± 1.1 (0.2–3.56)	0.461

MPV, mean platelet volume; RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; VEGF, vascular endothelial growth factor

p values are significant at $p \leq 0.05$

Table 5 MPV, RDW, NLR, PLR, and VEGF values in relation to BD activity

Mean ± SD (*p*) Median (*p*)		MPV (fl)		Behçet's disease patients (*n* = 96)							
				RDW (%)		NLR		PLR		VEGF (ng/l)	
Eye activity	Y (*n* = 17)	9.2 ± 1.4	(0.6)	16.1 ± 2.7	(0.34)	3.20	(0.07)	12.00 **(0.14)**		1.7 ± 0.7	(0.8)
	N (*n* = 79)	8.9 ± 1.1		15.4 ± 1.8		1.60		8.15		1.7 ± 0.7	
CNS activity	Y (*n* = 4)	9.6 ± 0.6	(0.09)	18.2 ± 4.6	(0.271)	1.35	(0.4)	8.58 (0.53)		1.8 ± 0.4	(0.529)
	N (*n* = 92)	8.9 ± 1.1		15.4 ± 1.7		1.80		8.53		1.7 ± 0.7	
Vascular activity	Y (*n* = 4)	8.8 ± 0.7	(0.7)	15.5 ± 1.8	(0.867)	5.00	(0.03)	17.98 (0.01)		2.1 ± 0.8	(0.340)
	N (*n* = 92)	9 ± 1.1		15.6 ± 1.9		1.70		8.28		1.7 ± 0.8	

Y, yes; N, no; MPV, mean platelet volume; RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; VEGF, vascular endothelial growth factor; CNS, central nervous system

p values are significantly different at $p \leq 0.05$

stress of the disease, leading to suppression of the maturation of erythrocytes [55].

Although there was a significant difference between BD patients and controls regarding hemoglobin level ($p = 0.001$), which could be due to anemia of chronic disease, RDW was significantly increased in both anemic and non-anemic BD patients in comparison with controls ($p < 0.001$, 0.001). Consequently, the RDW change is attributed to the disease itself rather on the changes in the hemoglobin level.

In this study, there was no difference in the MPV between BD patients and controls, which is in agreement with the findings of Alan et al. [44], Dikker et al. [56], and Ataş et al. [57].

Dikker et al. [56] and Acikgoz et al. [58] demonstrated that there was no difference in the MPV between active and inactive BD patients, which matches our results. Accordingly, MPV was not a valuable predictor of activity in BD.

Regarding the relation of the studied hematological indices with the clinical manifestations of BD, there was a significant increase in the RDW in patients with vascular and CNS manifestations, which is in accordance with several reports linking higher RDW with the occurrence of venous thrombosis, stroke [59], and cardiovascular events (e.g., ischemic heart disease or cerebrovascular accident) [60]. So, in light of our results, RDW was not found to be a valuable predictor of BD activity, rather a novel inflammatory biomarker of BD with an influence in the ongoing vascular disease process.

Furthermore, MPV was significantly lower in patients with vascular manifestations, suggesting that MPV is implicated in the ongoing vascular inflammatory process in BD. Indeed, Lippi et al. [61] stated that there is an inverse association between MPV and the risk of venous thrombosis. Moreover, Gasparyan et al. [62] reported that increased consumption of large platelets at the sites of inflammation

Table 6 MPV, RDW, NLR, PLR, and VEGF values in relation to clinical manifestations of BD

Mean ± SD (*p*)		MPV (fl)		BD patients (*n* = 96)							
Median (*p*)				RDW (%)		NLR		PLR		VEGF (ng/l)	
Eye	Y (*n* = 60)	9 ± 1.2	(0.6)	15.6 ± 2	(0.7)	2.10	(0.06)	9.09	(0.35)	1.8 ± 0.7	(0.3)
	N (*n* = 36)	8.9 ± 1		15.4 ± 2		1.45		7.72		1.6 ± 0.7	
Skin	Y (*n* = 61)	8.9 ± 1.2	(0.9)	15.5 ± 1.7	(0.7)	2.10	(0.4)	9.63	(0.2)	1.8 ± 0.7	(0.7)
	N (*n* = 35)	9 ± 0.9		15.7 ± 2.4		1.50		7.90		1.7 ± 0.7	
Articular	Y (*n* = 48)	9 ± 1.1	(0.9)	15.5 ± 1.8	(0.8)	2.10	(0.3)	9.33	(0.41)	1.7 ± 0.7	(0.8)
	N (*n* = 48)	8.9 ± 1.1		15.6 ± 2.1		1.70		8.40		1.8 ± 0.7	
Vascular	Y (*n* = 50)	8.8 ± 0.8	(0.04)	15.9 ± 1.9	(0.04)	2.20	(0.1)	10.21	(0.06)	1.8 ± 0.7	(0.5)
	N (*n* = 46)	9.2 ± 1.4		15.1 ± 1.9		1.55		7.88		1.7 ± 0.7	
CNS	Y (*n* = 17)	9.2 ± 1.1	(0.4)	16.8 ± 2.7	(0.03)	1.60	(0.9)	9.04	(0.81)	1.9 ± 0.7	(0.3)
	N (*n* = 79)	8.9 ± 1.1		15.3 ± 1.7		1.80		8.40		1.7 ± 0.7	
GIT	Y (*n* = 5)	8.5 ± 0.5	(0.3)	15.7 ± 1.9	(0.6)	1.70	(0.9)	7.90	(0.88)	1.7 ± 0.8	(0.9)
	N (*n* = 91)	9 ± 1.1		15.5 ± 1.9		1.80		8.61		1.7 ± 0.7	

Y, yes; N, no; MPV, mean platelet volume; RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; VEGF, vascular endothelial growth factor; CNS, central nervous system; GIT, gastrointestinal tract

p values are significantly different at $p \leq 0.05$

Table 7 MPV, RDW, NLR, PLR, and VEGF values in relation to medications received in BD patients

Mean ± SD (*p*) Median (*p*)		BD patients (*n* = 96)									
		MPV (fl)		RDW (%)		NLR		PLR		VEGF (ng/l)	
Corticosteroids	Y (*n* = 17)	9.02 ± 1.1	(0.45)	15.6 ± 1.9	(0.04)	1.80	(0.21)	8.53	(0.8)	1.76 ± 0.7	(0.28)
	N (*n* = 79)	8.57 ± 0.8		14.1 ± 1.1		1.25		9.04		1.4 ± 0.4	
CsA	Y (*n* = 34)	9.2 ± 1.2	(0.19)	15.5 ± 1.7	(0.9)	2.10	(0.2)	9.24	(0.45)	1.8 ± 0.7	(0.3)
	N (*n* = 62)	8.9 ± 1		15.6 ± 2.1		1.55		8.14		1.7 ± 0.7	
AZA	Y (*n* = 56)	9 ± 1.1	(0.7)	15.6 ± 2.2	(0.6)	1.50	(0.2)	7.90	(0.12)	1.8 ± 0. 7	(0.7)
	N (*n* = 40)	8.9 ± 1.1		15.4 ± 1.6		2.10		10.48		1.7 ± 0.7	
MTX	Y (*n* = 9)	9.1 ± 1	(0.82)	16.5 ± 2	(0.18)	2.30	(0.93)	11.00	(0.2)	1.9 ± 0.8	(0.4)
	N (*n* = 87)	9 ± 1.1		15.5 ± 2		1.70		8.28		1.7 ± 0.7	
IFX	Y (*n* = 13)	8.9 ± 0.8	(0.84)	15.9 ± 2.1	(0.5)	2.80	(0.4)	10.76	(0.3)	1.8 ± 0. 7	(0.7)
	N (*n* = 83)	9 ± 1.2		15.5 ± 2		1.70		8.42		1.7 ± 0.7	

Y, yes; N, no; MPV, mean platelet volume; RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; VEGF, vascular endothelial growth factor; CsA, cyclosporine A; AZA, azathioprine; IFX, infliximab; MTX, methotrexate

p values are significantly different at $p \leq 0.05$

leads to a decrease in the MPV. However, neither NLR nor PLR was associated with vascular or CNS manifestations of BD, which is in accordance with the findings of Alan et al. [44].

In the present study, PLR was significantly increased in BD patients ($p < 0.001$), which is in accordance with Jiang et al. [63] and Alan et al. [44] who reported a significant difference in the PLR between BD patients and controls ($p < 0.001$), concluding that increased PLR was an intrinsic characteristic of BD and can be a new inflammatory marker of BD.

This difference in PLR could not be attributed to corticosteroid intake as there was no statistical difference in the PLR between patients currently receiving corticosteroids and those who are not ($p = 0.8$).

The PLR was significantly higher in BD patients with vascular activity ($p = 0.01$) which is in line with Okatan and his coworkers [48] who stated that the PLR was significantly higher in active vascular BD patients, concluding that high PLR may be a useful disease activity marker in Behçet's disease. Indeed, Erden and his coworkers [49] stated that there was a statistically significant difference in the PLR between BD patients with and without thrombosis ($p < 0.0001$).

The PLR along with the NLR were the most valuable predictors of vascular activity in BD. However, PLR was more

Table 8 Correlation of MPV, RDW, NLR, PLR, and VEGF with different parameters in BD patients

Parameter *r* (*p*)		Behçet's disease patients (*n* = 96)				
		MPV(fl)	RDW (%)	NLR	PLR	VEGF (ng/l)
Age		0.04 (0.7)	0.1 (0.2)	− 0.2 (0.054)	− 0.3 (0.007)	0.2 (0.1)
Disease duration		0.05 (0.6)	0.03 (0.7)	− 0.06 (0.5)	− 0.07 (0.5)	0.08 (0.4)
BDCAF		− 0.13 (0.21)	0.02 (0.9)	0.17 (0.1)	− 0.02 (0.88)	− 0.1 (0.3)
Corticosteroid dose		− 0.09 (0.4)	0.18 (0.08)	0.08 (0.5)	0.03 (0.78)	0.02 (0.8)
Laboratory investigations	ESR	0.01 (0.9)	0.31 **(0.003)**	0.12 (0.3)	0.21 (0.04)	0.06 (0.6)
	Hb	0.03 (0.8)	− 0.38 **(0.003)**	0.01 (0.96)	− 0.18 (0.08)	− 0.12 (0.3)
	MCV	− 0.02 (0.8)	− 0.37 **(< 0.001)**	0.07 (0.5)	− 0.12 (0.26)	− 0.04 (0.7)
	TLC	0.01 (0.9)	0.21 **(0.04)**	0.35 **(0.001)**	0.45 **(< 0.001)**	0.2 **(0.048)**
	Neutrophils	− 0.18 (0.09)	0.05 (0.6)	0.76 **(< 0.001)**	0.8 **(< 0.001)**	0.15 (0.1)
	Lymphocytes	0.14 (0.2)	− 0.04 (0.7)	− 0.73 **(< 0.001)**	− 0.86 (< 0.001)	− 0.18 (0.08)
	Platelets	− 0.35 **(0.001)**	0.21 **(0.04)**	0.04 (0.7)	0.56 (< 0.001)	0.16 (0.1)

MPV, mean platelet volume; RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; VEGF, vascular endothelial growth factor; BDCAF, Behçet's disease current activity form; ESR, erythrocyte sedimentation rate; MCV, mean corpuscular volume; TLC, total leucocytic count; Hb, hemoglobin

Bold values are significant at $p \leq 0.05$

Table 9 ROC analysis for the studied parameters to detect their sensitivity and specificity for prediction of vascular disease activity

	Area under the curve	*p* value	95% Confidence interval		Cut-off	Sensitivity %	Specificity %	PPV	NPV	Accuracy
			Lower bound	Upper bound						
NLR	0.817	0.033	0.585	1.000	4.30	75	91.3	27.27	98.82	90.63
RDW	0.526	0.862	0.214	0.837	–	–	–	–	–	–
MPV	0.444	0.707	0.210	0.678	–	–	–	–	–	–
VEGF	0.647	0.322	0.385	0.908	–	–	–	–	–	–
PLR	0.851	0.018	0.709	0.992	10.56	100	65.2	11.11	100.00	66.67

NLR, neutrophil–lymphocyte ratio; RDW, red cell distribution width; MPV, mean platelet volume; PLR, platelet–lymphocyte ratio; VEGF, vascular endothelial growth factor

p values are significantly different at $p \leq 0.05$

powerful and less specific than NLR as a biomarker of vascular activity. This result is in agreement with Erden et al. [49], where ROC curve analyses for the PLR and NLR in the patients with deep venous thrombosis showed significant value ($p < 0.0001$) with 72.1% sensitivity in NLR and 77% sensitivity in PLR; therefore, NLR and PLR should be evaluated together for a better result of predicting vascular activity in BD patients.

Regarding the serum VEGF level, no significant difference was found between BD patients and controls ($p = 0.8$); also, there was no difference in the serum VEGF level between active and inactive BD patients. In accordance, Shaker et al. reported that serum VEGF level did not correlate with BD activity [64]; thus, according to our results, VEGF was not a surrogate biomarker of BD activity and does not reflect the ongoing inflammatory process in BD.

In light of our results, there is an evidence of increased NLR, PLR, and RDW in BD patients, suggesting their value as promising inflammatory biomarkers in BD. The PLR and NLR were the most valuable predictors of vascular activity, while RDW and MPV were not valuable predictors of BD activity, rather implicated in the ongoing vascular inflammatory process. The VEGF was neither a surrogate biomarker of BD activity nor reflecting the ongoing inflammatory process in BD.

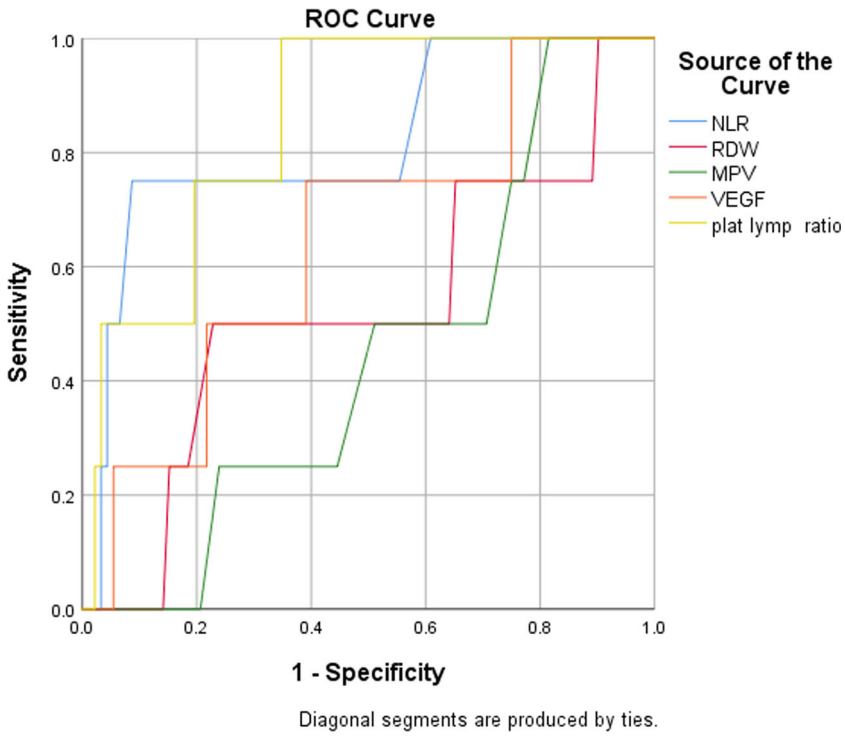

Fig. 1 ROC analysis showing NLR and PLR as most valuable predictors of vascular activity, with PLR as the more powerful but had lower specificity than NLR

Conclusion

The NLR, PLR, and RDW are significantly increased in BD patients. The PLR and NLR were the most valuable predictors of vascular activity, while RDW, MPV, and serum VEGF are not surrogate biomarkers of BD activity.

Recommendations

In light of our results, we suggest the use of NLR and PLR as inexpensive, readily available inflammatory biomarkers as predictors of vascular activity in BD.

Compliance with ethical standards

Disclosures None.

Abbreviations *BD*, Behçet's disease; *ESR*, erythrocyte sedimentation rate; *NLR*, neutrophil–lymphocyte ratio; *MPV*, mean platelet volume; *RDW*, red blood cell distribution width; *PLR*, platelet–lymphocyte ratio; *VEGF*, vascular endothelial growth factor; *RA*, rheumatoid arthritis; *SLE*, systemic lupus erythematosus; *BDCAF*, Behçet's Disease Current Activity Form; *CBC*, complete blood count; *ELISA*, enzyme-linked immunosorbent assay; *CNS*, central nervous system; *GIT*, gastrointestinal; *CsA*, cyclosporine A; *AZA*, azathioprine; *IFX*, infliximab; *MTX*, methotrexate; *MCV*, mean corpuscular volume; *TLC*, total leucocytic count; *Hb*, hemoglobin; *ALT*, alanine transaminase; *AST*, aspartate transaminase; *BUN*, blood urea nitrogen

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