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Platelet indices as a predictor in the differentiation of Behçet's disease from recurrent aphthous stomatitis

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Abstract. – OBJECTIVE: The aim of this retrospective cohort study was to investigate complete blood count parameters in patients with Behçet's disease (BD) who present with oral ulcers and patients with recurrent aphthous stomatitis (RAS) in order to determine whether they could be used as discriminatory biomarkers.

PATIENTS AND METHODS: This study was conducted between January 2019 and January 2023. The study population consisted of three groups: patients with BD who had oral ulcer manifestation (n=85, BD-Group), patients with idiopathic RAS (n=186, RAS-Group) and healthy controls (n=90, HC-Group). All data about participants, on their first application, including sociodemographic and clinical data, comorbidity status, laboratory results were collected retrospectively from the hospital computer records and patients' charts.

RESULTS: The groups were similar in terms of age (p=0.235) and sex distribution (p=0.450). Mean platelet volume (MPV) and plateletcrit values of the BD-Group were significantly lower, while platelet distribution width (PDW) was significantly higher, compared to the other two groups (p<0.001 for all). Low MPV (<9.15) (56.47% sensitivity and 90.86% specificity), high PDW (≥15.75) (75.00% sensitivity and 94.96% specificity) and low plateletcrit (<0.237) (55.29% sensitivity and 79.46% specificity) could significantly distinguish BD patients with oral ulcer onset from patients with RAS.

CONCLUSIONS: PDW, MPV, and plateletcrit may be useful biomarkers in the differential diagnosis of oral ulcers when distinguishing between BD and RAS. However, these results need to be supported by further comprehensive studies.

Key Words:
Oral ulcer, Behçet's disease, Recurrent aphthous stomatitis, Platelet indices, Platelet distribution width, Mean platelet volume, Plateletcrit.

Introduction

Behçet's disease (BD) is a variable vasculitis affecting vessels of any size[1]. Population-based studies[2] have reported that the prevalence of the disease ranges from 20 to 421 per 100,000 individuals in Turkey, one of the countries where BD is most common. It is often defined as a disease that presents with a clinical 'triad', consisting of oral ulcer, genital ulcer and uveitis[3]. However, BD may progress with recurrent inflammatory attacks, adversely affect the central nervous system, gastrointestinal tract and cardiovascular system, and has been reported[2,4,5] to cause significant morbidity. Therefore, long-term regular follow-up of BD patients is important. Mucocutaneous lesions, especially recurrent oral ulcers and genital ulcers, are often the first manifestations of Behçet's disease[1]. Disease onset with oral ulcers is reported[2] in up to 88% of patients. Early detection of oral ulcers caused by BD will contribute to early initiation of treatment, prevention of BD-related major organ involvement, and can reduce adverse outcomes[1]. However, the fact that BD is not the only disease presenting with oral ulcers creates difficulties in the diagnosis, necessitating high awareness and accurate differential diagnosis.

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Recurrent aphthous stomatitis (RAS) is the most common disorder of the oral cavity, presenting with recurrent oral aphthae and accounting for 25% of oral ulcers in adults and 40% in children[6,7]. It should be investigated in the differential diagnosis of BD, inflammatory bowel diseases, celiac disease and connective tissue diseases[8]. Particularly in the context of BD, the distinction of oral ulcer etiology is critical, as it is possible for a BD patient presenting with only an oral ulcer to be misdiagnosed with RAS for many years, leading to major organ involvement due to lack of management for BD[1,8]. However, clinical discrimination of oral ulcers is impossible, especially in the absence of other mucocutaneous involvement in BD, and unfortunately, there are no easily accessible and reliable blood tests allowing differential diagnosis of oral ulcers caused by BD or RAS.

Both BD and RAS have unclarified etiopathogenesis, but there is substantial evidence[1,9] that inflammation plays an important role in the pathophysiology of both. Blood-based inflammation markers have an important role in determining the systemic inflammation burden[9]. Some complete blood count (CBC) parameters associated with inflammation are described in the literature, such as leukocyte, neutrophil, lymphocyte, monocyte, platelet counts, platelet-related markers [mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW)], and some indexes derived from these parameters [neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SII) and pan-immune-inflammation value (PIIV)]. Since they are easily accessible, these parameters have recently found increasing use for differential diagnosis or prognostication in many diseases and cancers[10-16]. However, the role of some of these easily available markers in differentiating mucocutaneous BD from RAS patients has not yet been investigated, while the number of studies on some other parameters is very limited (Table I).

We postulated that, although oral ulcers caused by RAS and BD cannot be distinguished morphologically or clinically, possible differences in inflammatory markers (due to differences in the underlying diseases) could be used for discrimination. Therefore, in this study, we aimed to investigate CBC markers in patients with BD presenting with oral ulcers and compare the results to patients with RAS, thereby assessing the performance of these markers in discrimination.

Patients and Methods

Study Design and Ethics

This retrospective cohort study obtained ethical approval from the Ethics Committee of Afyonkarahisar Health Sciences University (Decision date: 03.02.2023, decision No.: 2023/80). The study was conducted in accordance with the principles outlined in the Declaration of Helsinki 1975, as revised in 2013. Due to the retrospective nature of the study, written informed consent was waived.

Study Setting and Data Collection

The study was carried out in the Department of Rheumatology of Afyonkarahisar Health Sciences University from January 2019 to January 2023. Patients who were admitted during this period for RAS or BD were included in the study groups, as well as controls, and sociodemographic and clinical data were collected at admission. Laboratory results were obtained retrospectively from digital hospital records and patient charts.

Participants and Eligibility Criteria

The study population consisted of the following three groups of patients who consecutively applied to the Rheumatology, and Dermatology and Venereology outpatient clinics: BD with the oral ulcer manifestation (n=85, BD-Group), idiopathic RAS (n=186, RAS-Group) and healthy control (n=90, HC-Group).

The diagnosis of BD and pathergy testing was made according to the current International Criteria for Behçet's Disease[17]. Only patients who had a history of recurrent oral ulcers as the initial symptom were included in the BD-Group. Patients with mucocutaneous involvement other than oral ulcers such as erythema nodosum, genital ulcer, severe papulopustular lesion or sweet-like lesion, those with eye, joint, gastrointestinal, cardiovascular or neurological system involvement, and those with concurrent BD activity scores greater than 2 (BDCAF/Behcet's Disease Current Activity Form)[18] at the time of blood sampling at first admission were excluded from the BD-Group.

The RAS group consisted of patients who met the "recurrent oral ulcers diagnostic criteria" by Natah et al[19] in the presence of a negative pathergy test. Patients without pathergy test results, those with a family history of BD, a history of major or herpetiform aphthae, those with known HLA-B51 positivity, and patients with a diagnosis

Table I. Summary of studies that investigated various markers in BD and RAS.

Author (reference)	Study population	Instruments	Findings	Conclusion
Turan et al[8]	Healthy control (n=90) Patients with RAS (n=97) Patients with MBD (n=73)	MPV, RDW, hemoglobin, neutrophils, monocyte, lymphocytes and platelet counts NLR, MLR, PLR, ESR, CRP	ESR, neutrophil count and NLR were significantly. higher in MBD patients. MPV is significantly decreased in BD patients than in healthy control and RAS patients	Decreased MPV (≤10 fL) and increased RDW (≥13.0%) were useful in predicting MBD in patients evaluated with complaints of ROUs
Atalay et al[9]	Healthy control (n=97) Patients with RAS (n=97)	NLR, PLR, SII	SII, NLR and PLR values were significantly higher. in the RAS group compared to the controls .	The results support the role of systemic inflammation in the etiopathogenesis of RAS
Tanacan et al[38]	RAS patients with major aphthae (n=75) RAS patients with minor aphthae (n=123) RAS patients with herpetiform aphthae (n=17)	Hemoglobin, leukocyte, neutrophils, monocyte, lymphocytes and platelet counts, ferritin, NLR, dNLR, PLR, ESR, CRP, SII	USS, neutrophil count, ferritin, SII, NLR, and dNLR were significantly higher in the major and herpetiform aphthae groups compared to the minor aphthae group. Positive, strong, significant correlations were observed between USS, SII, and NLR.	Higher SII, NLR, dNLR and PLR were associated with RAS severity.
Tanacan et al[39]	Patients with active BD (n=103) Patients with inactive BD (n=63)	Hemoglobin, hematocrit, leukocyte, platelet, neutrophil, and lymphocyte count, RDW, ESR, CRP, ferritin, iron, total iron-binding capacity, SII	Higher leukocyte, platelets and neutrophils counts, greater RDW, higher ESR, CRP and ferritin, and higher SII were observed in the active BD patients. SII with a cutoff value of 552 x 10^3/ mm^3 distinguished groups with 81% sensitivity and 82% specificity.	The SII may be used as a marker for the assessment of BD status.
Ekiz et al[12]	Patients with BD (n=61) Patients with RAS (n=60) Healthy control (n=60)	Total cholesterol, LDL-cholesterol, triglyceride, HDL-cholesterol, glucose, urea, creatinine, AST, ALT, leukocyte, MPV, ESR, CRP	The MPV levels and ESR in patients with BD and RAS were significantly higher than the control groups. In the BD and the RAS groups, the disease activity did not affect the MPV levels.	The MPV did not have a predictive value in differentiating the diagnosis of BD and RAS.
Avcı[21]	Patients with RAS (n=51) Healthy control (n=51)	Leukocyte, platelet, neutrophil, and lymphocyte count, RDW, PDW, MPV, NLR, PLR	RDW, PDW, and MPV values were significantly higher in the RAS group compared to the control group.	Increased RDW, PDW, and MPV values could have a diagnostic value in RAS patients.
Sandıkcı and Can[28]	Patients with BD with major organ involvement (n=92) Patients with MBD (n=49) Healthy control (n=96)	CRP, ESR, leukocyte, NLR, PLR, MPV, PDW, RDW	The NLR and RDW levels were higher in the BD group compared to the healthy control group. MPV levels were lower in the BD group than in the control group. The level of complete blood count parameters was similar in active and inactive disease. NLR and PDW values were significantly higher in the BD group with major organ involvement compared to the mucocutaneous BD group.	NLR and PDW both showed good association with major organ involvement in BD. NLR was more suitable to follow up patients with uveitis, MPV for those with erythema nodosum and PDW for neuro Behçet and oral ulcers.

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BD: Behcet's disease, CRP: C-reactive protein, dNLR: Derived neutrophil to lymphocyte ratio, ESR: Erythrocyte sedimentation rate, HC: Healthy control, HDL: High density lipoprotein, LDL: Low density lipoprotein, MBD: Mucocutaneous Behcet's disease, MCV: Mean corpuscular volume, MLR: Monocyte to lymphocyte ratio, MPV: Mean platelet volume, NLR: Neutrophil to lymphocyte ratio, PCT: Plateletcrit, PDW: Platelet distribution width, PIIV: Pan-immune-inflammation value, PLR: Platelet to lymphocyte ratio, RAS: Recurrent aphthous stomatitis, RDW: Red cells distribution width, ROU: Recurrent oral ulcer, SII: Systemic immune-inflammation index, USS: Ulcer severity score.

of incomplete BD or were likely to progress to BD were not included in the RAS-Group.

The HC-Group consisted of individuals with complaints of hair loss and negative pull test and without any known systemic disease. Pull test was done as follows: about 40 strands of hair from different areas of the scalp were grasped and gently pulled. Obtaining six or fewer hairs was interpreted as a negative pull test (indicating normal shedding), while obtaining more than six hairs was interpreted as a positive pull test (active hair loss)[8]. Individuals who had used any oral supplements in the last 3 months, those diagnosed with alopecia or anagen effluvium, and patients with a history of oral ulcer were excluded from the HC-Group.

The common exclusion criteria of the groups were: being <18 and >45 years old, having a body mass index of >30 kg/m^2, being diagnosed with and active infection, using any anti-inflammatory (colchicine, non-steroidal anti-inflammatory drug, steroid), immunosuppressive, immunomodulatory, anticoagulant-antiplatelet drugs, and iron and vitamin supplement in the last 3 months, pregnancy or breastfeeding, presence of malignancy, hematological diseases [including anemia (hemoglobin <11.9 g/dL in females, <13.6 g/dL in males) and neutropenia (absolute neutrophil count <1,500 mm^3)]. Also, any subjects with rheumatological (except Behçet's disease), autoimmune, pulmonary, cardiac, and psychiatric diseases, as well as diabetes, hypertension, gluten enteropathy and/or inflammatory bowel disease or immunodeficiency were excluded.

Laboratory Analysis and Marker Calculation

Blood samples were drawn from the antecubital vein at the first admission under sterile conditions and samples were analyzed by the certified local laboratory (the Biochemistry Laboratory of Afyonkarahisar Health Sciences University). Blood tests included CBC parameters (hemoglobin, mean corpuscular volume, red cells distribution width, MPV, PDW, PCT and absolute neutrophil, lymphocyte, monocyte and platelet count), C-reactive protein and erythrocyte sedimentation rate (ESR). All measurements were made via the use of calibrated standard measuring devices and according to the manufacturer's recommendations.

Using the results of CBC parameters, NLR, MLR, PLR, SII and PIIV were calculated.

SII was calculated with the following formula: SII ($\times10^3$)=Absolute neutrophil count ($\times10^3$) × Absolute platelet count ($\times10^3$)/Absolute lymphocyte count ($\times10^3$)[9].

PIIV was calculated with the following formula: PIIV ($\times10^6$)=Absolute neutrophil count ($\times10^3$) × Absolute monocyte count ($\times10^3$) × Absolute platelet count ($\times10^3$)/Absolute lymphocyte count ($\times10^3$)[20].

Statistical Analysis

Statistical significance was defined as $p<0.05$. All analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Data were presented as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables, depending on the normality of distribution. Categorical variables were reported as frequency (percentage). To assess the normal distribution of variables, histogram and Q-Q plots were utilized. Normally distributed variables were analyzed using the one-way analysis of variances (ANOVA), while non-normally distributed variables were analyzed using the Kruskal-Wallis test. The Chi-square test, Fisher's exact test, or Fisher-Freeman-Halton test were employed for categorical variables. Bonferroni correction was applied to adjust for pairwise comparisons. Discrimination performance was evaluated through Receiver Operating Characteristic (ROC) curve analysis. Optimal cut-off points were determined using the Youden index. Logistic regression analysis was conducted to calculate odds ratios.

Results

The mean age of the HC-Group was 29.30±5.80, that of the RAS-Group was 30.67±7.84, and that of the BD-Group was 30.45±6.97. Fifty-two patients (57.78%) in the HC-Group, 122 (65.59%) in the RAS-Group, and 54 (63.53%) in the BD-Group were females. There was no significant difference between the groups in terms of age ($p=0.235$) and sex distribution ($p=0.450$). The MPV ($p<0.001$) and PCT ($p<0.001$) values of the BD-Group were significantly lower, while the PDW ($p<0.001$) was significantly higher compared to the RAS and control groups. Absolute neutrophil count ($p=0.036$) and NLR ($p=0.028$) values of the BD-Group were significantly lower than that of the HC-Group. The PDW of the RAS-Group was significantly lower than that of the HC-Group ($p<0.001$) (Table II).

With a cut-off value of <9.15, MPV could significantly distinguish patients with BD from

Table II. Summary of demographics and laboratory measurements with regard to groups.

	HC-Group (n=90)	RAS-Group (n=186)	BD-Group (n=85)	*p*
Age, years	29.30±5.80	30.67±7.84	30.45±6.97	0.235
Sex				
Female	52 (57.78%)	122 (65.59%)	54 (63.53%)	0.450
Male	38 (42.22%)	64 (34.41%)	31 (36.47%)	
Body mass index, kg/m^2	24.37±2.94	23.91±3.20	23.73±2.71	0.347
Pathergy test (n=271)				
Negative	-	186 (100.00%)	49 (57.64%)	<0.001
Positive	-	0 (0.0%)	36 (42.35%)	
Smoking status (n=101)				
Smoker	-	7 (7.87%)	2 (16.67%)	0.438
Ex-smoker	-	7 (7.87%)	0 (0.00%)	
Non-smoker	-	75 (84.27%)	10 (83.33%)	
Alcohol use (n=101)				
User	-	4 (4.49%)	0 (0.00%)	1.000
Ex-user	-	0 (0.00%)	0 (0.00%)	
Non-user	-	85 (95.51%)	12 (100.00%)	
Duration of symptoms, months	-	60 (36 - 120)	90 (51 - 162)	0.240
Hemoglobin, g/dL	14.63±1.44	14.31±1.63	14.47±1.50	0.266
MCV, fL	85.4 (83.2 - 87.9)	85.85 (82.5 - 88.7)	85.9 (83.0 - 89.0)	0.633
RDW, %	13.0 (12.4 - 13.4)	12.8 (12.4 - 13.5)	13.1 (12.3 - 13.85)	0.785
MPV, fL	10.20±1.12	10.23±0.96	8.96±1.54*#	<0.001
PDW, fL	12.6 (11.7 - 16.1)	11.6 (10.7 - 13.3)*	16.4 (14.7 - 17.1)*#	<0.001
PCT, %	0.28±0.06	0.28±0.06	0.24±0.07*#	<0.001
Neutrophil (x10^3)	3.88 (3.30 - 4.64)	4.01 (3.10 - 5.06)	4.50 (3.50 - 5.40)*	0.036
Lymphocyte (x10^3)	2.19 (1.88 - 2.70)	2.17 (1.80 - 2.59)	2.19 (1.80 - 2.60)	0.580
Monocyte (x10^3)	0.54±0.15	0.55±0.18	0.53±0.18	0.547
Platelet (x10^3)	275.16±58.69	276.98±62.65	276.58±69.94	0.975
NLR	1.71 (1.39 - 2.28)	1.94 (1.38 - 2.38)	2.05 (1.62 - 2.48)*	0.028
MLR	0.23 (0.19 - 0.30)	0.24 (0.20 - 0.32)	0.24 (0.19 - 0.30)	0.519
PLR	125.47 (93.82 - 147.83)	125.72 (101.04 - 154.66)	123.75 (105.20 - 156.32)	0.690
SII (x10^3)	480.78 (370.57 - 608.05)	517.04 (360.20 - 687.25)	504.69 (410.28 - 756.22)	0.086
PIIV (x10^6)	249.17 (163.88 - 325.02)	274.18 (162.08 - 418.81)	268.97 (191.87 - 420.90)	0.367
CRP (mg/L)	3.00 (0.50 - 3.13)	1.30 (0.40 - 4.13)	2.78 (1.10 - 3.70)	0.442
ESR (mm/h)	7.5 (4 - 11)	8 (5 - 17)	10 (5 - 17)	0.113

Data are given as mean ± standard deviation or median (1st quartile - 3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. *: Significantly different from HC-Group, #: Significantly different from idiopathic RAS-Group. Abbreviations: BD: Behcet's disease, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, HC: Healthy control, MCV: Mean corpuscular volume, MLR: Monocyte to lymphocyte ratio, MPV: Mean platelet volume, NLR: Neutrophil to lymphocyte ratio, PCT: Plateletcrit, PDW: Platelet distribution width, PIIV: Pan-immune-inflammation value, PLR: Platelet to lymphocyte ratio, RAS: Recurrent aphthous stomatitis, RDW: Red cells distribution width, SII: Systemic immune-inflammation index

patients with RAS, as demonstrated by 56.47% sensitivity and 90.86% specificity [(AUC=0.768, 95% CI: 0.700 - 0.836; p<0.001 and OR=12.897, 95% CI: 6.681 - 24.895; p<0.001)]. With a cut-off value of ≥15.75, PDW could distinguish patients with BD from patients with RAS, as demonstrated by 75.00% sensitivity and 94.96% specificity (AUC=0.860, 95% CI: 0.790 - 0.929; p<0.001 and OR=56.500, 95% CI: 20.099 - 158.824; p<0.001). With a cut-off value of <0.237, PCT could distinguish BD from RAS with 55.29% sensitivity and 79.46% specificity [AUC=0.684, 95% CI: 0.612 - 0.756; p<0.001 and OR=4.785, 95% CI: 2.742 - 8.350; p<0.001)] (Table III, Figure 1).

Discussion

The present study demonstrated that BD patients had significantly lower MPV and PCT values and significantly higher PDW and NLR values and neutrophil counts compared to controls. Similar results were found for MPV, PCT and PDW values when patients wi-

Table III. Performance of MPV, PDW and PCT to discriminate patients with mucocutaneous BD and idiopathic RAS.

	MPV (fL)	PDW (fL)	PCT (%)
Cut-off	<9.15	≥15.75	<0.237
Sensitivity	56.47%	75.00%	55.29%
Specificity	90.86%	94.96%	79.46%
Accuracy	80.07%	88.89%	71.85%
PPV	73.85%	86.67%	55.29%
NPV	82.04%	89.68%	79.46%
AUC (95.0% CI)	0.768 (0.700 - 0.836)	0.860 (0.790 - 0.929)	0.684 (0.612 - 0.756)
p for AUC	<0.001	<0.001	<0.001
OR (95% CI)	12.897 (6.681 - 24.895)	56.500 (20.099 - 158.824)	4.785 (2.742 - 8.350)
p for OR	<0.001	<0.001	<0.001

AUC: Area under ROC curve, BD: Behcet's disease, CI: Confidence intervals, MPV: Mean platelet volume, OD: Odds ratio, PCT: Plateletcrit, PDW: Platelet distribution width, RAS: Recurrent aphthous stomatitis, PPV: Positive predictive value, NPV: Negative predictive value.

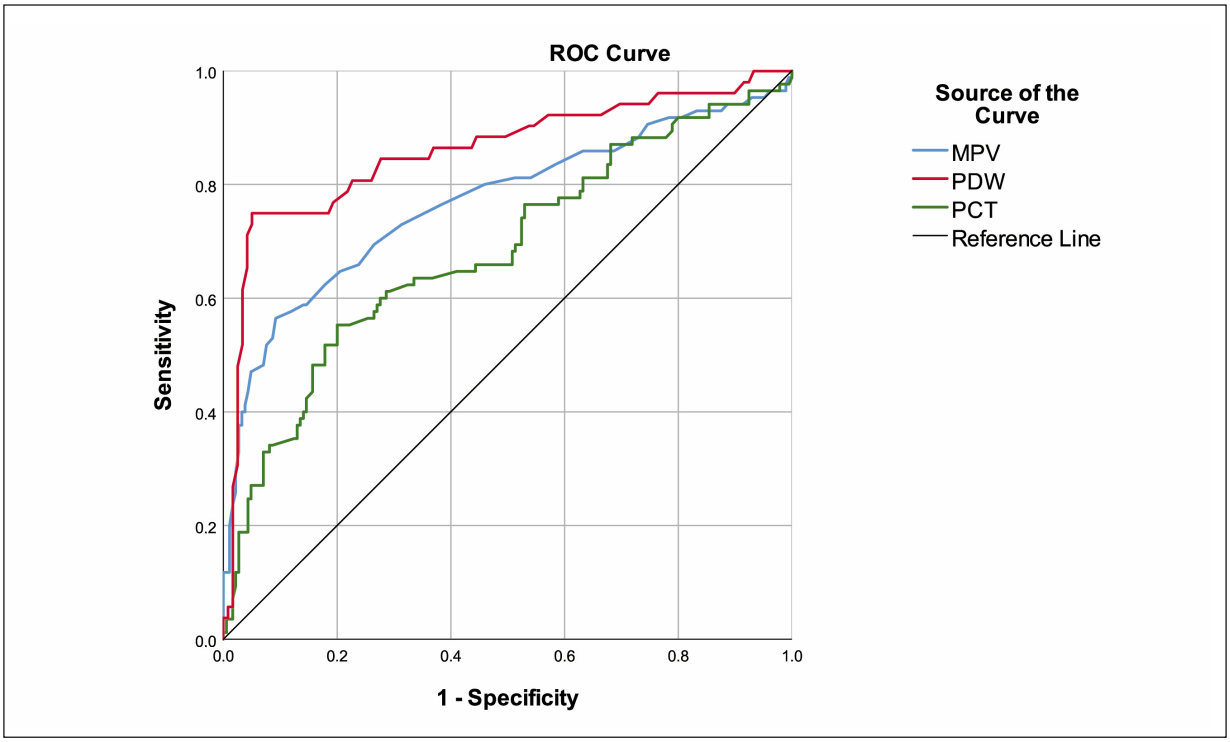

Figure 1. ROC curves of MPV, PDW and PCT to discriminate patients with mucocutaneous BD and idiopathic RAS (BD: Behcet's disease, MPV: Mean platelet volume, PCT: Plateletcrit, PDW: Platelet distribution width, RAS: Recurrent aphthous stomatitis, ROC curve: Receiver Operating Characteristic).

th BD were compared to patients with RAS. As a result, we found that low MPV, high PDW, and low PCT were able to significantly distinguish BD patients presenting with oral ulcers from patients with RAS.

The diagnosis of BD is almost entirely based on clinical criteria, as there is no pathognomonic blood test or histopathological finding[12]. Although the sensitivity of the skin pathergy test has been reported to be 40-80% in some regions, in some other countries, accuracy drops to 20%, reducing the diagnostic value of the test[1]. It is well known that patients presumed to have RAS are diagnosed with BD many years later, and this period was reported to be approximately 4 years in a study[2] from Turkey. Therefore, instruments are needed to predict which patients with oral ulcers not yet meeting the criteria for BD might be at risk for BD[8]. It is well known[21] that platelets play a key role in the pathogenesis of local and systemic

inflammation. Changes in platelet parameters can affect the hemostatic and thrombotic status and have been associated with many pathologies[22]. The relevance of some platelet-related parameters in the context of vascular complications has been investigated in prior studies[22,23] on BD. However, the relationship of these parameters to BD with oral ulceration has been somewhat irrelevant.

PDW is a blood parameter that can be easily obtained from CBC and evaluates platelet size variability, changes in platelet activation, and heterogeneity in platelet morphology[21] and is an important marker for coagulation activation[21,24]. The PDW value does not change in the case of simple platelet swelling, so PDW is considered more valuable than MPV as a platelet activation marker[24]. It has been claimed that PDW is a useful biomarker in the diagnosis or prognosis of some diseases such as acute gangrenous appendicitis[25], liver steatosis[26], infectious mononucleosis[27] and Crohn's disease[28] as well as BD[22]. However, the number of studies of PDW on BD and RAS is very limited, and as far as we know, there is no study investigating the PDW difference between BD and RAS. In the present study, the median PDW of the BD-Group was significantly higher than that of both the controls and the RAS-Group, while the median PDW of the RAS-Group was significantly lower than that of the controls. High PDW (≥15.75) was able to distinguish BD patients presenting with oral ulcer from RAS patients with 75.00% sensitivity and 94.96% specificity. In a retrospective study[21], RAS patients' mean PDW values were significantly higher than those of the healthy control group. In another study[28], PDW levels of BD patients with major organ involvement were significantly higher than mucocutaneous BD patients. PDW was related to oral ulcers and was higher in the patients with neuro-BD than in those without. Citirik et al[22] stated that PDW values were significantly higher in patients with ocular BD than in healthy individuals. PDW appears to be related to the clinical diversity of BD, but the results regarding its usability in RAS patients are conflicting. Our study also showed that increased PDW in a patient with an oral ulcer favors BD rather than RAS.

Mean platelet volume is another easily available blood parameter that is automatically measured during routine CBC studies and reflects platelet function, activity, production and stimulation and has a good correlation with platelet function and activation[21,29]. MPV has been shown[29] to be a sign of inflammation intensity in various diseases. However, studies[8,12,29] investigating the prognostic value of MPV in some inflammatory diseases, including BD and RAS, endocrine diseases, cancers, and cardiovascular diseases, offer conflicting results. We investigated the usability of MPV to differentiate between RAS and BD. As a result, we found that the mean MPV values of BD-Group were significantly lower than both RAS-Group and controls. Low MPV (<9.15) was the means of distinguishing patients with BD from patients with RAS (sensitivity: 56.47%, specificity: 90.86%). Turan et al8 conducted a similar study and as a result, they reported that decreased MPV may be a predictor of BD in selected patients presenting only with oral ulcers and that more attention should be paid to BD in individuals with recurrent oral aphthae, especially in individuals with MPV ≤10.0 fL. In another similar study by Ekiz et al[12], while MPV levels of the patients in the BP and RAS groups were significantly higher than the control group, there was no significant difference between the BP and RAS groups. Moreover, MPV levels were not associated with RAS and BD disease activity and vascular involvement. However, Ekiz et al[12] included both active and inactive BD patients when making this comparison. In a retrospective study[21], the mean MPV value of RAS patients was found to be significantly higher than the healthy subjects. Our results support the results of Turan et al[8]. They stated that the reasons why MPV levels of BD patients presenting only with oral ulcers are lower than those of RAS patients and healthy individuals may be: (i) Inclusion of only patients with inactive BD in the study may have resulted in low MPV levels, as MPV levels may differ at various stages of inflammation. (ii) Patients with active BP have higher oxidative stress production compared to inactive ones, and the relationship of oxidative stress with the inflammation burden is well known. Therefore, less oxidative stress load in inactive patients may have resulted in lower MPV levels[8]. We largely agree with their comments, but we think that the following should also be considered. Although MPV has an important advantage as a prognostic marker because it is an easily obtainable parameter, it can be affected by many factors such as age, sex, race and ethnicity, lifestyle and genetic factors[29]. This may be one of the reasons for the conflicting results obtained in the literature. Therefore, we think that more comprehensive studies are needed to confirm the utility of MPV in the distinction between RAS and BD.

Plateletcrit is an important instrument used to detect quantitative abnormalities of platelets30. It is a measure derived from MPV and platelet count, which has been assessed in various diseases such as inflammatory bowel diseases[11], coronary artery disease[31], diabetes mellitus[13], pulmonary tuberculosis[32], and psoriasis vulgaris[33], among others. However, as with the previous two variables (MPV and PDW), the association of PCT with BD and RAS has not been adequately studied. To the best of our knowledge, this is the second study investigating PCT values in patients with RAS and BD, and the first study demonstrating its utility in differentiating these two diseases. Our results showed that the mean PCT value of the BD-Group was significantly lower than both the controls and the RAS-Group. Low PCT (<0.237) significantly differentiated BD patients with oral ulcers from RAS patients with 55.29% sensitivity and 79.46% specificity. Turan et al8 reported no difference in PCT value in RAS and BD patients. In a case-control study[18], PCT value of BD patients was significantly higher than that of the healthy controls and PCT was stated as one of the most valuable predictors for BD. In another study[34], it was shown that PCT was significantly higher in BD patients compared to healthy subjects. Citirik et al[22] stated that PCT levels were significantly higher in patients with ocular BD than healthy subjects. As can be seen, PCT appears to be a potentially useful marker for distinguishing whether a patient with oral ulcers has BD or RAS, although the desired predictive performance from a biomarker is low. We hope that our results will be supported by further studies.

The SII and PIIV are new inflammation markers that have been increasingly investigated for various diseases as prognostic and predictive biomarkers in recent years[14,15,20,35-37]. We should also emphasize that these two markers whose usability has not been investigated before in the differentiation of RAS and BD were also investigated in this study, but no significant results were obtained about them. Although median SII and PIIV values of both the RAS and BD groups were higher than those of the controls, this difference was not significant. Also, there was no significant difference between the SII and PIIV values of the RAS and BD groups. Tanacan et al[38] showed that higher SII was associated with RAS severity. In another study[39], higher SII was observed in active BD patients compared to inactive ones. Atalay et al[9] stated that the SII values of RAS patients were higher than controls. In these studies, the patient population was not selected only from patients with oral ulcers. Therefore, this is likely to explain the lack of differences observed in our study. Although there are studies[38,39] that found increased SII to be associated with RAS[38] and BD[39] severity, the necessary data for the use of SII and PIIV in the differential diagnosis of BD and RAS in patients with recurrent oral aphthae are not available, and our study does not yield such a favorable result.

As a general comment, mucocutaneous lesions are not only the most common initial symptom of BD but may actually be an important cornerstone for disease control. This is because increased mucocutaneous activity has been associated with major organ involvement in young BD patients[40,41]. Therefore, if the specific changes in blood biochemistry of an oral ulcer, which is insufficient to make a diagnosis of BD with our current information but is actually a precursor of future BD disease, can be discovered, major organ involvement and thus morbidity and mortality in most of the BD patients can be prevented with early treatments and precautions. Our study showed that PDW, MPV, and PCT are biomarkers with the potential to be used for this purpose. If our results are supported by further trials, morbidities caused by late BD complications can be reduced. SII and PIIV were not found to be useful markers in the differentiation of BD and RAS patients with oral ulcers without organ involvement.

Limitations

Although this study provides important information regarding previously unresearched or insufficiently researched markers in RAS and BD patients, some of its limitations should be taken into account when interpreting the results. It is a single-center study with retrospective data collection. These limit both the reliability and generalizability of the results. Also, being a retrospective study, it was impossible to assess additional data (such as the time between presentation for oral ulcer and diagnosis of BD). Data covering all patients could not be obtained for some of the variables included in the study. Patients who were followed up with RAS may be diagnosed with BD years later. Finally, although patients with recurrent oral aphthae complaints less than 3 years were excluded, it may still be possible that patients with RAS before this period could have affected the results.

Conclusions

The present study showed that baseline MPV and PCT values of BD patients with oral ulcer manifestation were significantly lower and PDW values were significantly higher compared to patients with RAS patients and healthy subjects. Low MPV (<9.15), low PCT (<0.237) and high PDW (≥15.75) values appear to have some value in discriminating BD patients with oral ulcer onset from patients with RAS. This study is the first to investigate the utility of PDW in patients with RAS and BD, and to demonstrate the role of PDW and PCT in differentiating these two diseases. Therefore, its results need to be supported by further comprehensive studies.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interests.

Ethics Approval

The study was approved by the Ethics Committee of Afyonkarahisar Health Sciences University (date: 03.02.2023, approval number: 2023/80, Ethics Committee code: 2011-KAEK-2).

Informed Consent

Due to the retrospective nature of the study, written informed consent was waived.

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Authors' Contributions

Conceptualization E.A. and T.Ç.; methodology E.A., S.A., D.K.E, D.M. and T.Ç.; software E.A. and T.Ç.; investigation E.A., S.A., D.K.E, D.M. and T.Ç.; resources E.A., S.A., D.K.E, D.M. and T.Ç.; data curation E.A., P.S.B., Y.E.,G.H.S., B.A., İ.B. and T.Ç.; writing–original draft preparation E.A., S.A., D.K.E, D.M., P.S.B., Y.E., G.H.S., B.A., İ.B. and T.Ç.; writing–review and editing E.A., S.A., D.K.E, D.M., P.S.B., Y.E., G.H.S., B.A., İ.B. and T.Ç.; supervision: E.A. and T.Ç.; Project Administration E.A. and S.A. All authors have read and agreed to the published version of the manuscript.

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