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Can platelet-related parameters predict the involvement type of brucellosis?

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ABSTRACT:

— ***Objective:*** Brucellosis is a zoonotic disease that affects people all over the globe. Although this disease has been known since ancient times, there is no rapid diagnostic method to predict the complications of this disease. For the early diagnosis of complications in brucellosis patients, it is critical to find novel biomarkers. This study aimed at investigating the association between platelet-related parameters [platelet count, platelet lymphocyte ratio (PLR), and mean platelet volume (MPV)] and focal involvement in brucellosis.

— ***Patients and Methods:*** The patients diagnosed with brucellosis were evaluated retrospectively. The patients were divided into two groups based on whether they had focal involvement. The platelet-related parameters and inflammatory markers were statistically analyzed to predict the different types of brucellosis involvement.

— ***Results:*** A total of 60 patients with brucellosis were included in the study. The mean age of the patients was 54±9.8 years, 68.3% (n=41) were male, and 29 (48.3%) had focal involvement. PLR level was significantly higher in the focal involvement group (p=0.007). However, there was no statistical difference in platelet count or MPV levels (p>0.05). Monocyte, ALT, AST, and total bilirubin levels were lower in the focal involvement group (p=0.033, p=0.006, p=0.004, p=0.038).

— ***Conclusions:*** According to the findings of this study, platelet count or MPV levels could not determine the type of involvement, while PLR could predict the focal involvement.

— ***Keywords:*** *Brucellosis, Diagnosis, Platelet count, Mean platelet volume, Platelet lymphocyte ratio.*

INTRODUCTION

Brucellosis is a widespread zoonosis affecting half a million people yearly and is still endemic in many developing countries[1]. Although brucellosis is found worldwide, it is more prevalent in endemic areas such as Central and South America, the Mediterranean Basin (Portugal, Turkey, Spain, Southern France, Italy, Greece, North Africa), and the Middle East[2]. Since Sir David Bruce discovered *Brucella melitensis* in 1887, brucellosis has been a developing illness. Although *B. abortus* has been eradicated from cattle in many countries, *B. melitensis* and *B. suis* have resurfaced as sources of this infection in cattle, resulting in human infections[3]. Consumption of unpasteurized milk or milk derivatives is the most common method of infection. The symptoms are similar to those of a fever with a wide range of symptoms[1]. Hematogenous and lymphatic pathways typically complicate brucellosis, which can spread to various organs and cause localized symptoms in multiple organs. The osteoarticular, urogenital, and central nervous systems are the most common sites for focal involvement[4].

Acute, subacute, and chronic forms of human brucellosis all show symptoms across various time frames.

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About half of the cases of brucellosis are acute (with symptoms lasting up to three months). In contrast, chronic cases (with symptoms lasting more than six months) necessitate protracted chemotherapeutic treatment, which can be added to the patient's and the healthcare system's costs[5].

Since brucellosis in humans has a diverse and non-specific clinical presentation, laboratory confirmation of the diagnosis is crucial for effective patient care. The diagnosis of brucella infections can be made using nucleic acid amplification assays, serological tests, and culture. Although a more prolonged incubation and the performance of blind subcultures may be required for protracted cases, modern automated blood culture techniques enable the identification of acute cases of brucellosis within the standard 5 to 7 days incubation protocol employed in clinical microbiology laboratories[6]. The diagnosis is mainly based on the patient's history of animal exposure, contaminated products or travels to endemic regions and clinical findings[7,8].

Anemia, leukocytosis or leukopenia, thrombocytopenia, abnormal liver transaminase levels, or elevated inflammatory markers, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) can be detected in any clinical forms of brucellosis. In difficult situations, data are scarce on the values of hematological markers that can help with diagnosis, treatment response, and recurrence. By detecting complex cases early and providing proper therapy, it may be possible to lower relapse rates[9]. Neutrophil and platelet count are parameters affected by the inflammatory response. Platelet plays a role as primitive immune cell against pathogens at the site of inflammation. Bioactive molecules, chemokines, and cytokines are released from the granules of activated platelets[10,11].

Many released inflammatory cytokines affect thrombopoiesis; platelet count and size changes are seen in inflammatory diseases. Mean platelet volume (MPV) correlates with platelet count and is associated with proinflammatory and prothrombotic states. Studies show that MPV is a prognostic marker in the course of the disease and its correlation with inflammatory markers in brucellosis[12-14]. This study aimed to examine the relationship between platelet-related parameters [platelet count, MPV, and platelet/lymphocyte ratio (PLR)] and organ involvement in brucellosis patients.

PATIENTS AND METHODS

Patients

Patients with brucellosis were evaluated retrospectively at Canakkale Onsekiz Mart University Medical Center from January 2018 to January 2021. Only patients diagnosed with brucellosis who were followed up by the Infectious Diseases and Clinical Microbiology Clinic were included. Our local Ethics Committee approved the study (date: 15.12.2021, decision number: 2021-10).

Osteoarticular, hematological, genitourinary, cardiovascular, gastrointestinal, and skin involvements were accepted as complications of brucellosis. The focal involvement was signs of infection in any anatomic region except for hematological involvement. Hematological involvement was defined as hematological abnormalities in laboratory values, except for clinical findings of other possible causes, such as anemia or bleeding, which are not directly associated with brucellosis. Osteoarticular involvement was determined by clinical findings such as back pain, joint pain, swelling and limitation of motion in a joint, as well as findings obtained by direct X-ray, computed tomography, or magnetic resonance imaging. Genitourinary involvement was assumed in patients with testicular pain, redness, and swelling. Gastrointestinal involvement was determined in patients with nausea, vomiting, diarrhea, and abdominal pain. Hepatomegaly and splenomegaly were detected with abdominal USG. Cardiovascular involvement included clinical presentations such as retrosternal pain and cardiac murmur detected by electrocardiography or echocardiography. The patients were first divided into two groups based on whether they had focal involvement.

Patients under 18 years of age, pregnant women, patients with underlying diseases such as rheumatoid arthritis, ankylosing spondylitis, hypercholesterolemia, diabetes, hypertension, myocardial infarction, stroke, long-term drug users, malignancy, other foci of infection, autoimmune diseases, hematological diseases and patients taking antibiotics at the time of admission were not included in the study.

Demographic data, such as the age and gender of patients, clinical findings, and laboratory parameters at admission were retrospectively obtained from hospital records.

Laboratory Analysis

The screening test was the Rose Bengal plate agglutination test. For the patients with possible signs and symptoms of brucellosis, the diagnosis was made using a positive standard agglutination tube test (SAT) and the isolation of *Brucella* species from blood or other cultures. Titers of 1/160 or above on the SAT (Wright or Coombs-Wright agglutination test) were deemed positive for brucellosis. The Coombs-Wright test was used if the Wright titer was negative or slightly positive (1/80). Only the patients who had a fever had their blood cultures taken.

White blood cell (WBC) count, eosinophil count, lymphocyte count, hemoglobin (Hb), platelet count, and MPV were analyzed on the DXH800 hemogram analyzer (Beckman Coulter, Miami, FL). Erythrocyte sedimentation rate (ESR) was measured by the Westergren method on the Vacuplus ESR120 analyzer (Sistat, Ankara, Turkey). C-reactive protein (CRP) was quantified with the nephelometric method on the Image 800 device (Beckman Coulter, Miami, FL). Other biochemistry parameters (albumin, urea, creatinine, aspartate transferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and total bilirubin) were analyzed on the Cobas 6000 device (Roche Diagnostics, Mannheim, Germany).

They were accepted as WBC count <4,000 leukopenia, >11,000 leukocytoses, lymphocyte count < 1,000 lymphopenia, platelet count < 15,0000 thrombocytopenia, Hb <12 g/dL in females and <13 g/dL in males' anemia. The reference interval for MPV was 7.9-10.8 fL in females and 7.4-11.4 in males.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) v17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical examination. The Shapiro-Wilk test was used to examine the data distribution. Continuous data that were normally distributed were expressed as mean ± standard deviation, whereas non-normally distributed data were expressed as median [interquartile range (IQR)]. Categorical data were summarized as numbers (percentages) and compared using Chi-square or Fischer's exact test. Group comparisons for normally distributed data were analyzed with independent sample *t*-test. Levene's test was used to ensure that the variance was homogeneous. The Mann-Whitney U test was assessed for non-normally distributed data. Exact *p*-values were given, and $p<0.05$ was considered statistically significant.

RESULTS

A total of 60 patients was included in the study. The mean age of the patients was 54±9.8 years, and 68.3% of the patients were male. In our study, 48.3% of the patients had focal involvement (Table 1). There were 56 (93.33%) acute brucellosis patients and four (6.67%) subacute brucellosis patients. No chronic brucellosis cases were detected. Of the 19 patients had hepatomegaly, 15 had splenomegaly, and four patients had hepato-splenomegaly. Ten patients had spondylodiscitis, three had arthritis, three had gastrointestinal involvement, two had sacroiliitis, two had endocarditis, and one had a scrotal abscess. No eye or central nervous system involvement were registered.

A total of 3 patients, 2 of whom with focal involvement, had thrombocytopenia. 8 of 12 patients with MPV values below the lower reference limit showed focal involvement. None of the two patients with leukopenia had focal involvement. 5 of 9 patients with leukocytosis, 2 of 3 with lymphopenia, and 10 of 23 with anemia had focal involvement.

Rose-bengal test and SAT ≥1/160 were positive in all the patients. There was no difference in platelet or MPV levels ($p>0.05$), while PLR was statistically higher in the focal involvement group ($p=0.007$). The monocyte, ALT, AST, and total bilirubin levels were higher in the non-focal involvement group ($p=0.033$, $p=0.006$, $p=0.004$, $p=0.038$) (Table 2). The difference in blood tests, according to the subtypes of focal presentations, could not be analyzed due to the insufficient number of patients included in the study for each subgroup.

DISCUSSION

In this study, we examined the predictive value of platelet-related parameters for focal involvement in brucellosis. For this platelet count, MPV and PLR, which are easily accessible, fast, simple and cost-effective parameters, were evaluated. As a result of our study, the PLR level was significantly higher in the focal involvement group, while no significant difference was observed in platelet and MPV levels.

Several hematologic abnormalities are reported in brucellosis since its primary involvement is in the reticuloendothelial system. Thrombocytopenia is one of these abnormalities. Thrombocytopenia caused by *Brucella* has been documented in the 2.4-33%[13]. Although anemia, thrombocytopenia, and leukopenia are expected hematological consequences of acute brucellosis, severe

Table 1. Demographic, clinical, and microbial characteristics of brucellosis patients according to involvement type.

	Focal Involvement (+) (n=29)	Focal Involvement (-) (n=31)	*p*-value[1]
Age[2], years (mean±SD)	56.6±13.9	50.9±14.0	0.126
Gender[3]			0.783
Female, n (%)	10 (34.5%)	9 (29.1%)	
Male, n (%)	19 (65.5%)	22 (70.9%)	
Fever[3], n (%)	20 (68.9%)	26 (83.8%)	0.227
Brucellosis[3]			0.613
Acute brucellosis, n (%)	28 (96.6%)	28 (90.3%)	
Subacute brucellosis, n (%)	1 (3.4%)	3 (9.7%)	
Hepatomegaly[3], n (%)	9 (31.0%)	10 (32.3%)	0.919
Splenomegaly[3], n (%)	8 (27.6%)	7 (22.6%)	0.769
Hepato-splenomegaly[3], n (%)	0 (0%)	4 (12.9%)	0.045
Blood culture positivity[3], n (%)	1 (3.4%)	4 (12.9%)	0.355

[1]$p<0.05$ was considered significant. [2]Data were expressed as mean±SD (standard deviation). [3]Data were expressed as numbers and percentages [n (%)].

Table 2. Summary of laboratory findings of the brucellosis patients according to involvement type.

	Focal involvement (+)	Focal involvement (-)	*p*-value[1]
WBC2, 10^3/µL	7.5 (6.45-8.28)	7.70 (4.98-10.01)	0.739
Lymphocyte[3], 10^3/µL	2.12±0.81	2.48±0.92	0.213
Monocyte[2], 10^3/µL	0.55 (0.47-0.86)	1.19 (0.75-1.60)	**0.033**
Eosinophil[2], 10^3/µL	0.3 (0.20-0.48)	0.50 (0.27-0.61)	0.086
Hemoglobin[2], g/dL	13 (12.05-13.48)	12.05 (12-14)	0.830
Platelet count[3], 10^3/µL	253.17±79.1	222.42±68.46	0.112
MPV[3], fL	8.33±1.03	8.35±0.68	0.923
PLR[3]	126.17±51.51	94.65±33.93	**0.007**
CRP[2], mg/dL	0.864 (0.622-1.245)	1.050 (0.755-1.345)	0.265
Albumin[2], g/dL	3.90 (3.15-4.22)	3.40 (3.08-3.88)	0.477
ESR[2], mm/h	46 (24.75-80.25)	80.5 (65.75-85)	0.100
Urea[3], mg/dL	42.36±17.30	40.23±14.82	0.522
Creatinine[3], mg/dL	1.02±0.37	0.87±0.25	0.272
AST[2], U/L	25.6 (16.4-38.5)	40 (34.5-48.75)	**0.004**
ALT[2], U/L	27.25 (15-38.73)	42.5 (40-50.25)	**0.006**
ALP[2], U/L	78 (59.25-109.5)	84 (78-98.75)	0.892
GGT[2], U/L	64 (28-78.75)	66 (43.25-98.25)	0.368
Total bilirubin[2], mg/dL	0.84 (0.31-1.10)	1.03 (0.88-1.28)	**0.038**

[1]$p<0.05$ was considered significant. [2]Data were expressed as median (interquartile range). [3]Data were expressed as mean±SD (standard deviation). WBC, white blood cell; MPV, Mean platelet volume; PLR, platelet/lymphocyte ratio; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase.

thrombocytopenia (platelet count <20,000) is uncommon. In this study, we observed thrombocytopenia in 5% of the patients; none had severe thrombocytopenia. Thrombocytopenia can be caused by hypersplenism, reactive hemophagocytosis, or immunosuppression[15].

Since MPV is a marker of platelet activation and generation, combining MPV values with platelet counts in brucellosis patients could be beneficial[13,16-18]. There are various studies on MPV levels in brucellosis. Kader et al[12] compared Brucellosis patients grouped according to SAT titer with the control group. The patient group found the MPV level statistically significantly lower than the control group. According to Oztürk et al[19], MPV values were lower in patients with brucellosis than in control groups. Some researchers reported that it might be useful to investigate MPV values together with platelet count in patients with brucellosis since MPV is an indicator of platelet activation and production[14,20,21]. Togan et al[22] reported that MPV was not a useful marker in acute brucellosis, but they did not investigate the MPV for clinical subtypes. Sen et al[11] reported that MPV was significantly lower in complicated brucellosis when specific organ involvement was present than in uncomplicated brucellosis. As a result, they suggested[11] that MPV may also be a useful inflammatory marker in determining the involvement of particular organs in brucellosis. Aydin et al[23] reported that MPV was significantly lower in *Brucella epididymal-orchitis* than in non-*Brucella epididymal-orchitis*.

Limited studies[9,16-18] show the predictive value of MPV in the involvement type of brucellosis. In a study[9], the authors hypothesized that leukocyte, neutrophil, platelet, MPV, and ESR were significantly different in individuals with focal involvement compared to those without, and that these parameters could be used to diagnose focal involvement. Conversely, in our study, in which we evaluated the platelet-related parameters according to the involvement in brucellosis, we found no difference in MPV levels between the focal involvement group and those without the focal involvement group. Differences may be observed in MPV values obtained from different hematology analyzers using different methodologies and techniques[16,17]. Significant differences (2-50%) have been reported in MPV measurements of various complete blood count systems, depending on the measurement time after puncture[17,18]. Another factor influencing the MPV results is the age gap between the two groups. In our study, all blood samples were studied with the same analyzer. In addition, the patients who had underlying diseases were excluded. No selection was made according to the age groups of the patients.

PLR has been associated with many inflammatory diseases. Olt et al[24] did not find PLR significant in their study comparing the brucellosis and healthy control groups. In another study[25], PLR levels were similar in the brucellosis patient group and the healthy control group, while a significant difference was found between subacute and chronic brucellosis. In Sen et al[11] study, it was reported that PLR level predicted complications and focal involvement. Copur et al[26] found the PLR level to be similar in the group with and without focal involvement in their study (p=0.970). However, we found that PLR level significantly predicted focal involvement in our study (p=0.007).

Limitations

Our study had some limitations. This study was conducted retrospectively and in a single center. Therefore, the number of patients was low. In addition, the absence of a control group was among the limitations of the study.

CONCLUSIONS

Several studies in literature have been published on using platelet-related parameters in brucellosis, but the available studies still need clarifications. In addition, the need for a control group in published retrospective studies is a limitation of these studies. Prospective observational cohorts in which patient homogenization is ensured and preanalytical and analytical errors are minimized can be used to evaluate the clinical use of these parameters. In conclusion, it is thought that PLR level may be helpful in predicting focal involvement in brucellosis.

Ethics Approval:
This study was approved by Canakkale Onsekiz Mart University Medical School Clinical Research Ethi-cal Committee (date: 15.12.2021, decision number: 2021-10).

Informed Consent:
Since it was a retrospective study, informed consent was not required.

Conflict of interest:
There is no conflict of interest.

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None.

Availability of Data and Materials:
The data generated and analyzed in the presented study are available from the corresponding author on re-quest.

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