

## Evaluation of erythrocyte and platelet indices in canine visceral leishmaniasis

Ethem Mutlu TEMİZEL<sup>1</sup>, Hüseyin CİHAN<sup>1</sup>, Zeki YILMAZ<sup>1</sup>, Nilüfer AYTUĞ<sup>1</sup>

<sup>1</sup> Departments of Internal Medicine, Faculty of Veterinary Medicine, Uludag University, 16059, Gorukle /Nilüfer, Bursa.

**Summary:** The purpose of the study was to evaluate erythrocyte indices and platelet indices in dogs with leishmaniasis (CL, *L. infantum*). CL was diagnosed by polymerase chain reaction, and confirmed by western blott in 54 dogs. These dogs were divided into three groups; asymptomatic (n=16), oligosymptomatic (n=16), and symptomatic (n=22). Leishmaniasis free 25 dogs were served as controls. Microcytic or normocytic-hypochromic anemia was observed in dogs with CL. There was not a significant difference on RDW (RBC distribution width) groups. PLT count was slightly lower in dogs with CL in all clinical stages (p<0.05), compared to controls. MPV (mean platelet volume) in dogs with CL was higher (P<0.05) compared with controls, and it was significantly higher in asymptomatic dogs. RBC (red blood cell) and platelet indices may be useful parameters during course of the leishmaniasis. These indices may have diagnostic properties but are not enough to differentiate of the clinical stages of CL. Further comprehensive studies are needed to clinical use of RBC and platelets indices in canine practice.

Key words: dog, erythrocyte, indices, leishmaniasis, platelet

### Canine visceral leishmaniasisli köpeklerde eritrosit ve trombosit indekslerinin değerlendirilmesi

**Özet:** Bu çalışmanın amacı, Canine Leishmaniazis (CL, *L. infantum*) tanısı konan köpeklerde eritrosit ve platelet indekslerindeki değişimleri değerlendirmektir. Bu çalışmanın materyalini, farklı ırk, yaş ve cinsiyetteki 54 köpek oluşturdu. CL (*L. infantum*) tanısı, PCR (polymerase chain reaction) yöntemiyle kondu, Western blott yöntemiyle de teyit edildi. Çalışmayı oluşturan köpekler üç gruba ayrıldı: Bu gruplar, asemptomatik (n:16), oligosemptomatik (n:16) ve semptomatik (n:22) köpeklerin (en az üç karakteristik bulguyu taşıyan köpekler) oluşturduğu gruplar olarak düzenlendi. Ayrıca 25 adet sağlıklı köpek kontrol grubunu oluşturdu. CL'li köpeklerde eritrositlerin morfolojik değerlendirmesinde mikrositik, normositik ve hipokromik oldukları saptandı. Gruplar arasında RDW değeri açısından bir fark saptanmadı. PLT değerinin bütün klinik safhalarda hafif düşük (p<0.05) olduğu belirlendi. MPV değeri CL köpeklerde sağlıklı köpeklere göre (p<0.05) önemli derecede yüksekti. Ayrıca diğer gruplara göre özellikle asemptomatik grupta daha yüksek olduğu saptandı.

Bu çalışmada, eritrosit ve platelet indekslerinin CL'nin seyri esnasında kullanışlı parametreler olarak değerlendirilebileceği, klinik safhaların ayırımında kullanışlı olmamakla birlikte diagnostik uygunluğa sahip olduğu kanısına varıldı. İleride klinik pratikte, eritrosit ve platelet indekslerinin klinik kullanımına ilişkin daha ayrıntılı çalışmalara ihtiyaç duyulmaktadır.

Anahtar sözcükler: Eritrosit, indeks, köpek, leishmaniazis, platelet

### Introduction

Canine Leishmaniasis (CL), caused by *Leishmania infantum*, is among the most important protozoal diseases of dogs worldwide (1, 18). It is estimated that around 50% of dogs infected with CL show no clinical signs of the disease (19). According to Mancianti et al. (11), CL can be categorized into 3 distinct clinical forms, based on the major features observed in infected animals, which can be classified as asymptomatic, oligosymptomatic and symptomatic.

The most frequent clinical signs of CL include lymphadenomegaly, weight loss, dermatological changes and onychogryphosis (1). Anaemia and thrombocytopenia are the most common laboratory abnormalities that encountered in CL (13, 17). Diagnosis of CL is generally

based on clinical signs and confirmed by serological, direct examination of smears, PCR and Western Blotting (1, 11, 18).

Despite practical application of red blood cell (RBC) indices in dogs [RBC indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and RBC distribution width (RDW)], platelet indices are not currently used in daily clinical practice. These measurements, known as erythrocyte or RBC indices, provide important information about various types of anemias. Platelet indices provide clinical information about the underlying conditions of thrombocytopenia. Platelet volume indices; such as plateletcrit (PCT), the percentage of blood volume occupied by platelets, mean

platelet volume (MPV), representing the average platelet size, and platelet distribution width (PDW), representing the heterogeneity of platelet size, are now routinely available with modern haematology analysers and characterise changes in platelet size. In CL, some studies have focused attention on haemostatic parameters possibly associated with clinical manifestations (13, 14). Despite many of studies, especially the immunology of CL, few investigations have examined thrombocytopenic manifestations in naturally infected animals. Despite limited works have been published on routine utility of RBC indices (7), there is no information available on the diagnostic value of platelet indices in CL.

Thus, the aim of this study was to compare the changes in the RBC and platelet indices in dogs with CL and healthy dogs, and to evaluate whether RBC and platelet indices could be used to differentiate asymptomatic CL from oligosymptomatic and symptomatic CL in canine practice.

### Materials and Methods

The study was conducted on 25 healthy dogs and 54 dogs which were detected as naturally infected with *L. infantum* by Western Blot and PCR test. Dogs of different ages (2-12 years), breeds, both sexes (24 males, 30 females) and an average weight of 6.4±2.2 kg were used (study group). Clinical examinations were performed in each dog.

Separated serum and anticoagulated (EDTA) blood samples were stored at -20°C until forwarded to the

Department of Animal Production, Epidemiology and Ecology, University of Turin (TO, Italy), to perform Western Blot and PCR tests. White blood cell count (WBC) and RBC and platelet indices were determined using an automatic analyzer with optical scatter and impedance methods (CELL-DYN 3500; Abbott, Wiesbaden, Germany).

The positive animals were divided into 3 groups based on the clinical signs of CL including asymptomatic (with no clinical signs of infection, n=16), oligosymptomatic (with up to three clinical signs of infection; in general lymphoid adenopathy, slight decrease of weight and/or opaque hair, n=16), symptomatic (with more than three severe signs of infection, i.e., cutaneous alterations such as depilation, furfuraceous eczema, and ulcers; onychogryphosis n=22), as described previously (12).

Data were analyzed using variance analysis (ANOVA), and expressed as Mean ± SE. Tukey tests were used to test the differences among groups. P value less than 0.05 was considered significant (SPSS version 13.0, Illinois, USA).

### Results

Symptomatic dogs showed various clinical signs including dermatitis (20/22, 90.9%), weight loss (19/22, 86.3%), apathy (19/22, 86.3%), lymphadenopathy (15/22, 68.1%), epistaxis (6/22, 27.2%), fever (6/22, 27.2%), hepatosplenomegaly (6/22, 27.2%), conjunctivitis (3/22, 13.6%), and locomotion problems (2/22, 9.0%).

Table 1. White blood cell and erythrocytes indices (Mean ± SEM) in asymptomatic, oligosymptomatic, symptomatic dogs naturally infected by *Leishmania infantum*

Tablo 1. Doğal olarak *Leishmania infantum* ile enfekte asemptomatik, oligosemptomatik ve semptomatik köpeklerde total lökosit düzeyi ve eritrosit belirteçleri

Dogs	n	WBC x10 <sup>3</sup> /μl	RBC x10 <sup>6</sup> /μL	Hgb mg/dl	HCT %	MCV fL	MCH pg	MCHC g/dL	RDW %
Healthy	25	10.3±0.6	7.4±0.1 <sup>a</sup>	17.2±0.6 <sup>a</sup>	42.5±0.6 <sup>a</sup>	69.7±0.7 <sup>a</sup>	22.5±0.3	33.2±0.5 <sup>a</sup>	17.0±0.3
Asymptomatic	16	12.4±0.9	6.2±0.3 <sup>ab</sup>	11.4±0.7 <sup>ab</sup>	34.5±0.6 <sup>ab</sup>	60.0±0.7 <sup>b</sup>	23.3±0.4	29.5±0.3 <sup>b</sup>	17.3±0.4
Oligosymptomatic	16	11.3±0.4	6.1±0.2 <sup>ab</sup>	10.8±0.9 <sup>ab</sup>	32.4±1.2 <sup>ab</sup>	60.7±0.6 <sup>b</sup>	22.7±0.6	29.2±0.4 <sup>b</sup>	16.9±0.5
Symptomatic	22	19.8±0.7	4.0±0.7 <sup>b</sup>	8.8±0.4 <sup>b</sup>	28.6±0.9 <sup>b</sup>	55.7±0.5 <sup>b</sup>	24.7±0.2	26.3±0.2 <sup>b</sup>	16.6±0.2

p<0.05, indicates a significant difference among healthy, asymptomatic, oligosymptomatic and symptomatic groups.

<sup>a,b,c</sup> differences between the values involving different letters on the same column is found to be statistically significant

Table 2. Platelet indices (Mean ± SEM) in asymptomatic, oligosymptomatic, and symptomatic dogs naturally infected by *Leishmania infantum*

Tablo 2. Doğal olarak *Leishmania infantum* ile enfekte asemptomatik, oligosemptomatik ve semptomatik köpeklerde trombosit belirteçleri

Dogs	n	PLT x 10 <sup>3</sup> /μL	MPV fL	PCT %	PDW 10 GSD
Healthy	25	399 ± 33 <sup>a</sup>	12.8 ± 0.6 <sup>a</sup>	0.54 ± 0.04 <sup>a</sup>	19.4 ± 0.4 <sup>a</sup>
Asymptomatic	16	149 ± 20 <sup>b</sup>	22.0 ± 2.7 <sup>b</sup>	0.43 ± 0.19 <sup>b</sup>	23.2 ± 2.8 <sup>b</sup>
Oligosymptomatic	16	164 ± 13 <sup>b</sup>	16.3 ± 2.2 <sup>c</sup>	0.34 ± 0.12 <sup>b</sup>	20.4 ± 0.6 <sup>ab</sup>
Symptomatic	22	187 ± 35 <sup>b</sup>	15.6 ± 2.4 <sup>c</sup>	0.31 ± 0.02 <sup>b</sup>	22.1 ± 0.8 <sup>b</sup>

p<0.05 indicates a significant difference among healthy, asymptomatic, oligosymptomatic and symptomatic groups.

<sup>a,b,c</sup> differences between the values involving different letters on the same column is found to be statistically significant

The RBC and platelet indices are shown in Table 1 and 2. RBC counts, Hct and Hgb were significantly lower ( $p<0.05$ ) in symptomatic dogs compared with healthy controls. MCV and MCHC values were significantly lower ( $p<0.05$ ) in all groups of CL than that of the healthy dogs. Platelet count and PCT ( $P<0.05$ ) values in dogs with leishmaniosis were significantly lower than those of healthy dogs. Dogs with leishmaniosis had a higher PDW ( $p<0.05$ ) values, as compared to the healthy dogs. MPV values in 3 groups relating with leishmaniosis were higher at statistically varies importance ( $p<0.05$ ) compared with the healthy dogs. Also asymptomatic dogs had a higher MPV value compared to the symptomatic ( $p<0.05$ ) and oligosymptomatic ( $p<0.05$ ) dogs.

### Discussion and Conclusion

While most symptomatic dogs exhibit dermal abnormalities, some may present other signs in conjunction with or in the absence of skin lesions. Clinical signs of infections are various and involve many organs (1, 4). In the present study, dermal problems were predominant clinical signs in symptomatic and oligosymptomatic dogs, in agreement with the previously published studies.

The possible causes of the anaemia in CL include blood loss due to epistaxis, haematuria and haemorrhagic diarrhea, haemolysis or others. It usually manifests as normocytic, normochromic, poorly regenerative anemia with medullary hypoplasia (18). In contrary, in a study reported in humans with visceral leishmaniasis by Kager and Rees (7), RBCs were microcytic (low MCV) and hypochromic (low MCHC), which increased during follow up but microcytosis persisted up to a year after cure (4, 17). Reis et al. (17) reported that symptomatic dogs had more severe anemia. In the present study, reticulocyte count and bone marrow analysis could not be performed to evaluate whether dogs with leishmaniasis has a regenerative or non-regenerative anemia. Regardless of reticulocyte count and cytological examination of bone marrow, regeneration of anemia can be evaluated by use of RBC and platelet indices. Increased MCV and RDW in dogs and human with anemia are thought to be associated with regenerative anemia. When RBC count decreases in peripheral circulation, young RBCs which cannot be matured yet in bone marrow release to compensate body requirements into circulation, resulting in anisocytosis (increased RDW) and megaloblastic anemia with high regeneration capability. In this study, hypochromic and microcytic anemia were detected in symptomatic group. The anemia can be attributed to infection of the bone marrow by *L. infantum*, inducing infiltration by lymphocytes, plasma cells and macrophages that could contribute to a decrease in erythrocyte production (4). Observed microcytic RBC

may be related with the non-regenerative anemia in this study population.

Haemostatic abnormalities have been reported in canine and humans leishmaniasis (9, 13, 14). In the present study, observed decrease in platelet count in dogs with CL may be resulted from sequestration, utilization, destruction, and decreased or ineffective production of platelets in CL (6). The high MPV indicated that accelerated platelet turnover. An elevated MPV may be a risk marker for platelet activation (16). Moreover, it is well-known that immune thrombocytopenia is correlated with an increased MPV (2, 8). Dominguez and Torano (5) have demonstrated that in the first phases of Leishmania infection is able to interact directly with the platelets by a specific mechanism termed “immune adherence”, with the formation of large aggregates. Observed increase in MPV in dogs with leishmaniosis may be explained with immune mechanism (10) or increased turnover in CL. Corona et al. (3) have demonstrated that platelet count of the marked symptomatic group is lower than other groups in naturally infected dogs with *L. infantum*. In the present study, all groups were thrombocytopenic and it is a remarkable fact that MPV values ( $p<0.001$ ) in asymptomatic dogs was higher than the other groups. In the present study, PDW and MPV responses in all groups of CL were higher than the healthy group. This observation is consistent with the findings in a study of dogs with inflammatory disease, in which increases in MPV and PDW were interpreted as the release of large platelets (megathrombocytes) from bone marrow in response to greater demand for platelets (15).

In conclusion, RBC and platelet indices provide a lot of clinical information about the underlying conditions of anemia and thrombocytopenia. Their use can be of great help in clinical practice since they are routinely generated by automated cell counters. Our results showed that RBC and platelets indices have diagnostic properties to evaluate haemostatic responses, but could not give an opportunity to distinguish asymptomatic dogs from oligosymptomatic or symptomatic dogs suffering from leishmaniasis. Further comprehensive studies are needed to establish the role of RBC and platelets indices in CL.

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**Address for correspondence:**

Dr.Ethem Mutlu Temizel  
 Uludağ Üniversitesi Veteriner Fakültesi  
 İç Hastalıkları Anabilim Dalı  
 Gorukle Kampüsü  
 Gorukle/Nilüfer/Bursa, Turkey. 16059  
 Tlf: 02242940812 e-mail:ethem@uludag.edu.tr