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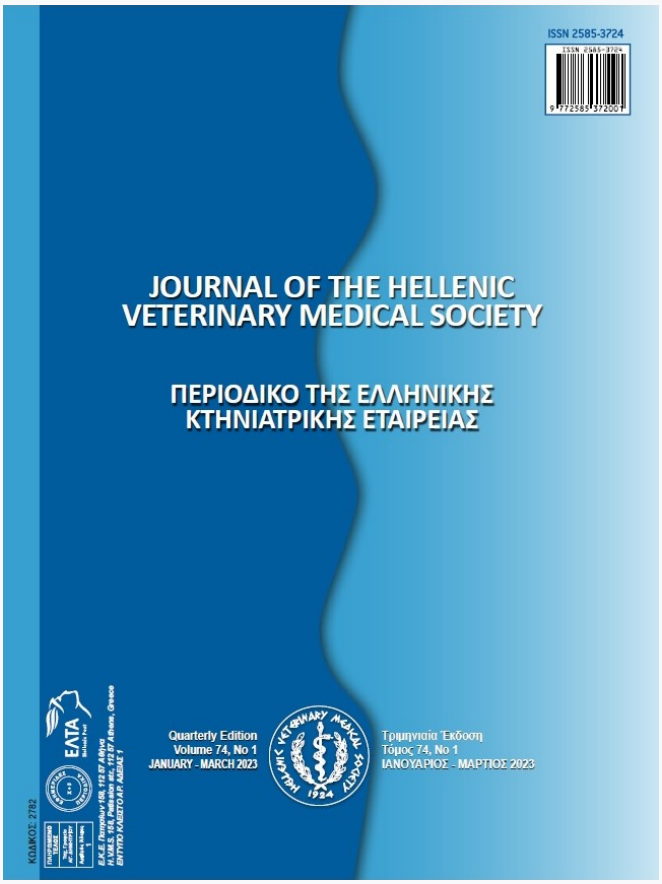

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Research article
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Leukogram patterns significance and prevalence for an accurate diagnosis in dogs

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ABSTRACT: Interpretation of changes in WBC (White Blood Cell) provides valuable information for guiding the veterinarian to establish the diagnosis for a wide range of diseases. Leukocyte changes, both quantitative and qualitative, are always secondary, so the control and therapeutic success are dependent on the identification of the primary condition.The purpose of this study was the association between the magnitude of quantitative changes in leukocytes and the primary conditions in which they occurred, to facilitate a faster orientation of the diagnosis. From dogs with internal affections and based on inclusion and exclusion criteria,the surveyevaluated 447 complete blood counts (CBC).In 272 CBC analyzed, the number of leukocytes was in the physiological range (i.e.6,000-17,000 cells × μL^{-1} blood), but in 131 cases, the leukocytes exceeded the upper limit, and in 44 cases, leukocytes were below the lower limit. In terms of leukocytosis, affections of the digestive system had the highest prevalence, while leucopenia, was more present in the circulatory system pathologies. The cases of leukocytosis depending on the severity were: mild (73 cases), moderate (41), and severe leukocytosis (15) and respectively, two extreme leukocytosis cases, statistically emerging: $p<0.01$ for IBD (inflammatory bowel disease), acute pancreatitis, ehrlichiosis,chronic babesiosis and respectively $p<0.001$ for acute lymphoblastic leukemia.Results revealed that infections source (devoid of parvovirosis), inflammation of the digestive tract was frequently accompanied by moderate leukocytosis, while the parvoviral caused enteritis conducted, in the early stages, to leukopenia. In bronchopneumonia, the leukocytosis was moderate, while inflammation of the anterior airways caused mild leukocytosis. Moderate leukocytosis was found also in the splenic, hepatic, and pulmonary neoplasm, and acute lymphoblastic leukemia developed with severe leukocytosis and chronic leukemia with extreme leukocytosis.

Keywords:canine; clinical context;leukocytosis; leukopenia; WBC.

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INTRODUCTION

Leukocytes or White Blood Cells (WBCs) are major cellular components of the inflammatory and immune response, which protect the body against infections and neoplasm and help to repair damaged tissue. Peripheral blood analysis, by performing the Complete Blood Count (CBC), represents the evaluation of the hematogenous marrow, as regards the production of a sufficient number of cells on the three major hematopoietic lines (myelocytic, erythrocyte, megakaryocyte series) and if the exponents of this series (erythrocytes, leukocytes, thrombocytes) have normal morphology (Weiser et al., 2012; Willard and Tvedten, 2012; Mercedes et al., 2014).

A CBC is a group of paraclinical blood tests, which determines the number of white and red blood cells circulating in the bloodstream, being a laboratory examination frequently requested by veterinarians and represents a routine evaluation of healthy and diseased animal companions. Microscopic examination and determination of the quantitative number of different blood cells in the blood still form the keystone of clinical veterinary hematology necessary to better assess the health of an animal (Becker et al., 2008; Wood, 2017).

The quantification of hematological parameters associated with the examination of the blood smear brings valuable information for guiding the veterinarian to perform other specific tests to establish the diagnosis for a wide range of diseases. The increase in the total number of WBC is described as leukocytosis, while leukopenia is their decrease. The increase or decrease in each type of WBC has its significance and can help in the diagnostic process (Weiss and Wardrop, 2010; Weiser et al., 2012; Willard and Tvedten, 2012; Wood, 2017; Kostadinović and Lević, 2018).

In general, leukocyte changes are produced by multifactorial pathophysiological mechanisms, which can make it difficult for the clinician to establish the etiological diagnosis, which often requires complementary methods of investigation. Interpretation of changes in WBC provides information about potential conditions that may occur. The quantitative and qualitative assessment of the leukocyte profile associated with the evaluation of the morphological status represents the leukogram. A modified leukogram usually leads to the identification of a pathological process (e.g. inflammation), but not to the establishment of a specific diagnosis. Interpretation of leukocyte changes coupled with clinical symptoms may lead to a diagnosis. Most patterns of leukocyte response are not interpreted in specific diagnoses, except leukemias, whereas responses are interpreted in processes that may occur in an organism. Inflammation is the most important and one of the most common responses of leukocytes. Leukocyte recruitment out of blood vessels and into tissues is essential for the development of an inflammatory response (injury or infection) and in the sequence of events leading to inflammatory diseases (Dhabhar, 2003; Weiser et al., 2012).

The inflammatory response causes an increase in the concentration of immature neutrophils in the blood, and mainly refers to band neutrophils. The left shift occurs if the demand at the tissue level is high, so if the demand at the tissue level far exceeds the ability of the bone marrow to produce neutrophils, their number may fall below reference intervals. A left shift with neutrophilia is usually associated with inflammatory conditions caused by infectious diseases: bacteriosis, virosis, fungal infections, protozooses, or others such as immune-mediated, examples being acute pancreatitis, pyothorax, septic peritonitis, bronchopneumonia, pyometra, prostatitis, acute pyelonephritis, pleurisy, pyoderma, etc. (Weiss and Wardrop, 2010; Weiser et al., 2012; Allison and Little, 2013; Feldman et al., 2015; Nelson and Couto, 2014).

The response to exciting stimuli (stress-inducing factors) by releasing adrenaline is an immediate change and is also known as the *"fight-or-flight"* response. The release of adrenaline leads to cardiovascular changes by increasing blood flow through the microcirculation, especially in the muscles, with the movement of leukocytes from the marginal compartment to the circulating one. The response of leukocytes to stress as a result of corticosteroid release or corticosteroid administration is probably the most common response. Physiological stress is a response of the body mediated by the release of adrenocorticotropic hormone from the pituitary gland and the release of cortisol from the adrenal gland. It occurs in response to major systemic diseases, metabolic disorders, and pain (Dhabhar, 2003; Weiser et al., 2012).

Moreover, it can observe that leukocyte changes, both quantitative and qualitative, are always secondary, so the control and therapeutic success are dependent on the identification of *"the disease behind the disease"*.Thus, the purpose of this study was to assess the association between the magnitude of quantitative changes in WBC and the primary conditions in which they occurred, to facilitate faster orientation of the di-

agnosis to a particular group of diseases.

MATERIALS AND METHODS

This study was performed at the Clinic of Internal Diseases of the Faculty of Veterinary Medicine in Timișoara, in the period between January 2016 and January 2020, all animal owners giving them written consent for this study. To establish the etiological diagnosis, respectively of the primary disease that led to the appearance of leukocyte changes, microscopic examinations of blood smears were performed from each sample, collected to determine the CBC.At the same time, for the elucidation of the diagnosis, in the patients where the ultrasonographic examination was considered useful, ultrasounds were performed with the help of the MyLab 70 XVG device (Esaote, Florence, Italy). Ultrasound helped to establish the diagnosis, especially in the case of tumors from the abdominal cavity, more frequently diagnosed being those located in the spleen.

Also, it was useful in acute and chronic diseases of the digestive tract. In urogenital diseases, the ultrasound examination was of real use in the diagnosis of uterine diseases, especially pyometra, but also in the case of acute or chronic kidney diseases. Due to a large number of diseases with leukocyte changes, firstly, they were grouped by systems, and secondly, a comparison between diseases from a specific system was made.

Animals

The white blood cell count (WBCs) was examined from a total of 447 canine patients who needed hematological tests to confirm a diagnosis, associated with the determination of blood biochemical parameters, or with imaging examinations.

Inclusion criteria were: WBCs count from dogs examined for a first opinion, regardless of breed, age, sex, or diagnosed condition; WBCs count of dogs brought to the consultation for a second opinion if they have not received any medication for at least 14 days before.

Exclusion criteria were: WBCs count from individuals under treatment; WBCs count from patients who consumed food previous to blood collection.

Blood samples

Blood samples were collected from the antebrachial cephalic vein in vacutainers with anticoagulant (EDTA K3, Vacutest Kima, Arzegrande, Italy) for WBCs count, and with gel and clot activator tubes (Vacutest Kima, Arzegrande, Italy) for the determination of blood biochemical parameters. The WBCs count was performed within 2-4 hours after the blood sampling. At the same time, blood smears were performed from the collected blood samples, which were stained using Diff Quick method (RAL Diff-Quick, RAL Diagnostics, Martillac, France). The WBCs count was accomplished with two automatic devices: ProCyte DXTM (IDEXX, Maine, USA) and Advia 2120i (Siemens, Erlangen, Germany).

In this study, leukocytosiswas considered when WBC >17.000 × μL^{-1} and leukopenia, when WBC <6000 × μL^{-1}.

Statistical analysis

The individual values of the WBCs were processed in average amount, depending on the primary disease that generated the WBC modification, using the statistical program SPSS 20.0 (IBM, USA). The comparison of the number of leukocytes in the groups of dogs constituted according to the primary disease was performed by one-way ANOVA, expressed as mean ±SEM (Standard Error Mean).

The statistical values were considered as follows: *$0.01 \leq p<0.05$, significant; **$0.001 \leq p<0.01$, highly significant; ***$p<0.001$, very high significant.

RESULTS

From the total number of 447 WBC count, which was analyzed, in 272 of them, the number of leukocytes was in the physiological range (between 6,000-17,000 cells × µL $blood^{-1}$), in 131 WBC count the number of leukocytes exceeded the upper limit, and 44 in leukograms, the leukocytes were below the lower limit. Regarding the 175 modified WBC count registered; it was found that 74.85% of cases evolved with leukocytosis, and 25.15% with leukopenia.

The leukocytosis and leukopenia cases and their prevalence are presented in Table 1.

In Table 2 the descriptive statistics on leukocyte changes depending on the primary condition are presented.

In this study, 87% of the cases with leukocytosis could be classified as mild or moderate magnitude. In 11.4% of the cases, the increase was severe and only 1.6% was considered extreme leukocytosis, according to Table 3.

Table 1. The prevalence of leukocytosis and leukopenia depending on the affected system and types of diseases

No.	Affected systems/types of diseases	Prevalence	
		Leukocytosis no. of cases/%	Leukopenia no. of cases/%
1.	Digestive	40/30.8%	12/27.3%
2.	Cardiovascular	22/16.8%	22/50.0%
3.	Genitourinary	12/9.1%	4/9.1%
4.	Respiratory	12/9.1%	3/6.8%
5.	Neuromuscular	7/5.3%	1/2.3%
6.	Endocrine	17/12.9%	-
7.	Neoplastic	21/16.0%	2/4.5%
	Total	**131 cases**	**44 cases**

Table 2. Descriptive statistics on leukocyte changes depending on the primary condition

Primary condition	Mean	Standard Deviation	Standard error	Minimum	Maximum
Nonspecific gastroenteritis	20,215.00	2,857.03	903.47	17,600.00	27,410.00
Inflammatory bowel disease (IBD)	28,150.00	10,895.43	3,852.11	17,650.00	48,090.00
Acute pancreatitis	27,469.09	12,846.88	3,873.48	640.00	43,400.00
Chronic hepatitis	22,543.33	5,620.33	3,244.90	17,290.00	28,470.00
Parvovirosis	3,420.00	1,074.05	438.48	2,490.00	5,100.00
Acute lymphoblastic leukemia	73,620.00	--	--	40,460.00	110,400.00
Splenic neoplasia	24,685.00	4,373.54	1,546.28	19,320.00	33,410.00
Ehrlichiosis	31,375.00	9,834.79	4,917.39	20,370.00	42,300.00
Acute babesiosis	4,281.05	1,078.11	247.33	2,270.00	5,990.00
Chronic babesiosis	27,160.00	9,166.60	3,240.88	18,820.00	42,070.00
Dirofilariosis	17,840.00	--	--	16,980.00	18,700.00
Chronic bronchopneumonia	31,302.50	17,808.31	8.904,15	20,070.00	57,510.00
Pyometra	22,700.00	18,075.28	9.037,64	5,450.00	48,170.00
Cushing syndrome (hyperadrenocorticism)	18,372.50	883.34	441,67	17,530.00	19,490.00

Table 3. Classification of leukocytosis according to the severity

Leukocytes (thousand/mm³)	Number of cases	Percentage
Mild leukocytosis (17,000-25,000)	73	55.70%
Moderate leukocytosisn (25,000-40,000)	41	31.30%
Severe leukocytosis (40,000-100,000)	15	11.40%
Extreme leukocytosis (>100,000)	2	1.60%

Digestive diseases

The diseases of the digestive system followed by leukocytosis were represented in order by: nonspecific (non-parvo) gastroenteritis, Inflammatory Bowel Disease (IBD), acute pancreatitis, chronic hepatitis, parvoviral enteritis, and pharyngeal angina.

Most cases that developed with leukocytosis were those diagnosed with nonspecific gastroenteritis in 13 cases (32.5%). In terms of prevalence, acute pancreatitis, with 10 cases (25.0%), was very close to the nonspecific gastroenteritis, followed by IBD with 9 cases (22.5%). Comparatively, chronic hepatitis, parvoviral enteritis, and pharyngeal angina were diseases with the lowest prevalence of leukocytosis (Table 4).

Regarding the absolute number of WBCs, moderate leukocytosis was ascertained in chronic hepatitis, IBD, and acute pancreatitis, while in nonspecific gastroenteritis, the increase in leukocyte count was considered mild.

Table 4. Prevalence of leukocytosis and leukopenia by groups of diseases

No.	Affected systems/ types of diseases	Groups of diseases	Prevalence (cases / %)	
			Leukocytosis	Leukopenia
1.	Digestive	1.Nonspecific gastroenteritis	13/32.5%	2/16.6%
		2. Acute pancreatitis	10/25.0%	1/8.3%
		3. Inflammatory bowel disease (IBD)	9/22.5%	–
		4. Chronic hepatitis	4/10.0%	–
		5. Parvoviral enteritis	3/7.50%	9/75.0%
		6. Pharyngeal angina	1/2.5%	-
2.	Cardiovascular	1. Babesiosis	10/45.6%	19/86.4%
		2. Dirofilariosis	4/18.2%	-
		3. Ehrlichiosis	4/18.2%	2/9.1%
		4. Heart failure	2/9.0%	1/4.5%
		5. Juvenile polyarteritis syndrome	1/4.5%	–
		6. Anaplasmosis	1/4.5%	-
3.	Genitourinary	1. Acute kidney injury	7/58.4%	–
		2. Glomerulonephritis	2/16.6%	–
		3. Pyometra	3/25.0%	2/50.0%
		4. Prostatitis	-	1/25.0%
		5. Urolithiasis	-	1/25.0%
4.	Respiratory	1.Acute bronchopneumonia	4/33.4%	-
		2.Chronic bronchopneumonia	3/25.0%	-
		3. Tracheobronchitis	2/16.7%	-
		4.Eosinophilic bronchopneumonia	1/ 8.3%	-
		5. Laryngotracheitis	1/8.3%	-
		6. Tracheal collapse	1/8.3%	-
		7. Tracheal stenosis	-	3/-
5.	Neuromuscular	1. Meningoencephalitis	4/57.1%	-
		2. Epilepsy	2/28.6%	1/-
		3. Puerperal eclampsia	1/14.3%	-
6.	Endocrine	1. Cushing Syndrome	10/58.8%	-
		2. Diabetes mellitus	5/29.8%	-
		3. Diabetes insipidus	2/11.7%	-
7.	Neoplastic	1. Splenic neoplasms	9/42.8 %	-
		2. Liver neoplasms	3/14.3%	-
		3. Acute lymphoblastic leukemia	3/14.3%	-
		4. Chronic leukemia	2/9.5%	-
		5.Cutaneous lymphoma	1/4.8%	2/-
		6.Pulmonary neoplasms	3/14.3%	-

Among the diseases of the digestive system that evolved with leukopenia, the highest prevalence had parvoviral enteritis in 9 cases (75.0%), followed by nonspecific gastroenteritis in two cases (16.6%), and acute pancreatitis, with one case (8.3%), respectively (Table 4).

In absolute terms, the magnitude of leukopenia was appreciated as mild in the case of nonspecific gastroenteritis, moderate in parvoviral enteritis, and severe in the evolution of a case of acute pancreatitis where the number of leukocytes decreased below $1000 \times \mu L^{-1}$ blood.

Cardiovascular diseases

Diseases of the cardiovascular system were analyzed in44 WBC counts, equally divided between leukocytosis and leukopenia cases. Leukocytosis was found in 10 cases babesiosis (representing 45.6% from leukocytosis cases); 4 cases (18.2%), with heartworm disease and ehrlichiosis, followed by heart failure in two cases (9.0%), and juvenile polyarteritis respectively anaplasmosis with one case (4.5%) (Table 4).

Leukopenia was found in 19 cases of babesiosis (representing 86.4% ofleukopenia cases), followed by ehrlichiosis with 9.1% (two cases) and heart failure with 4.5% (one patient) (Table 4).

The total number of WBCs in diseases of the cardiovascular system that evolved with leukocytosis varied within large limits, with increases of mild magnitude in heartworm disease and moderate magnitude in ehrlichiosis, chronic endocarditis, and chronic babesiosis.

Leukopenia, had an increased prevalence in acute babesiosis, in which the reduction in the number of leukocytes was generally of small magnitude and a lower prevalence of ehrlichiosis, but which evolved with leukopenia of moderate magnitude.

Urogenital diseases

Leukocytosis had the highest prevalence in acute kidney disease with 7 cases (58.4% from all urogenital) that led to acute renal failure, followed by 3 cases (25.0%) pyometra and glomerulonephritis 2 cases (16.6%), the last oneevolving to chronic renal failure (Table 4). Leukopenia revealed in pyometra,in two cases, followed by prostatitis and urolithiasis, one case each, the increase of leukocytes being of moderate magnitude.

Respiratory diseases

Leukocytosis was found in the acute bronchopneumonia, 4 cases (33.4%), chronic bronchopneumonia, and tracheobronchitis having a lesser incidence, with 3 cases(25.0%) and 2 cases (16.7%) respectively. Eosinophilic bronchopneumonia, laryngotracheitis, and tracheal collapse are other respiratory diseases that evolved with leukocytosis, but with a prevalence of 8.3% (one case each) (Table 4).

Leukopenia is the examined dogs was only in the tracheal stenosis (3 cases).

The magnitude of leukocytosis in respiratory diseases was different depending on the type of disease. Thus, severe leukocytosis was found in chronic bronchopneumonia, while in acute bronchopneumonia, the magnitude of leukocytosis was moderate. Finally, in the case of tracheobronchitis, the magnitude of leukocytosis was mild.

Neuromuscular diseases

Neuromuscular diseases were the fewest diseases that evolved with leukocytosis (Table 4), so meningoencephalitis had the highest prevalence:4 cases (57.1%), followed by epileptiform syndrome in two cases (28.6%) and one case (14.3%) of puerperal eclampsia.Leukocytosis in neuromuscular diseases ranged in wide limits, so mild increases were observed in puerperal eclampsia and epileptiform syndrome, and severe leukocytosis in meningoencephalitis. Only one case with epileptiform syndrome evolved with leukopenia.

Endocrine diseases

Table 4 shows the prevalence of endocrine disorders that developed with leukocytosis. Thus, it can be seen that hyperadrenocorticism has the highest percentage, with 10 cases(58.8%), among the dogs studied. Also, diabetes mellitus estimated 29.5% (5 cases) of all cases of leukocytosis, and diabetes insipidus 11.7% (2 cases).

The magnitude of leukocytosis, in absolute value, was small in the case of diabetes insipidus and hyperadrenocorticism, and in the case of diabetes mellitus was observed a moderate leukocytosis.

Neoplastic diseases

Neoplastic diseases that evolved with leukocytosis were numerous, and by far, the splenic neoplasm was the most frequent(42.8%) (Table 4). Cutaneous lymphoma with one case (4.8%) and chronic leukemia(two cases representing 9.5%) were the neoplasms with the lowest percentage. With medium prevalence (14.3%) regarding leukocytosis were the acute lymphoblastic leukemia, liver neoplasm, and lung neoplasm, with 3 cases each. The only neoplastic disease that evolved with leukopenia,in the dogs studied, was lymphoma.

Regarding the absolute number of WBCs, moderate leukocytosis was found in the splenic, hepatic, and pulmonary neoplasm. Acute lymphoblastic leukemia evolved with severe leukocytosis, chronic leukemia with extreme leukocytosis, while in cutaneous lymphoma, the increase in leukocytes was of mild magnitude.

The increase in the number of leukocytes, in acute and chronic lymphoblastic leukemia, occurred due to the absolute number and the percentage of lymphocytes (Figure1).

Statistical analysis

The statistical comparison of the number of leukocytes in the dog groups according to the primary disease performed by one-way ANOVA test is presented in Figure2.

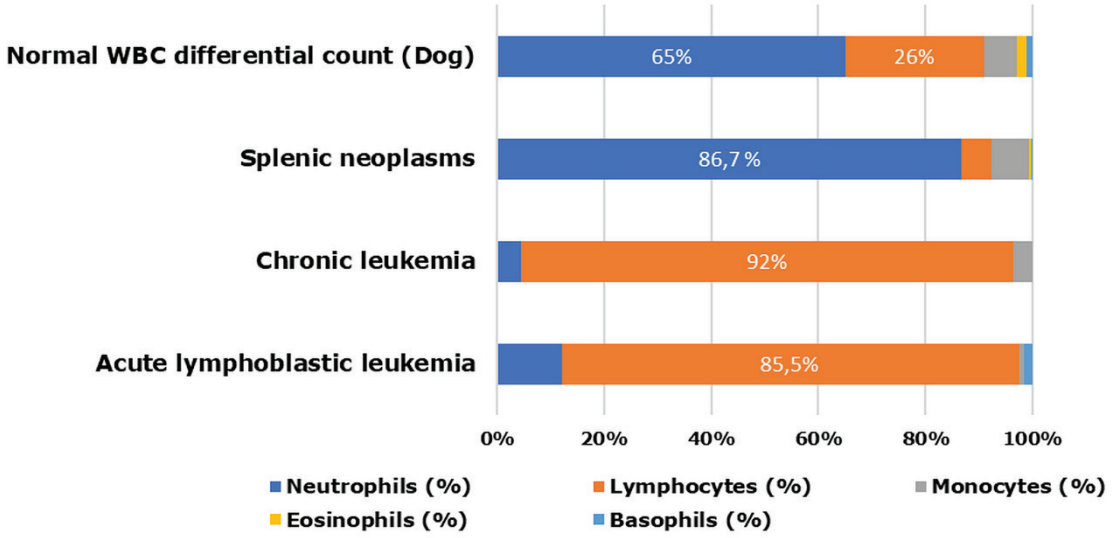

Figure1. The average values of WBC differential count in neoplastic diseases that have evolved with leukocytosis

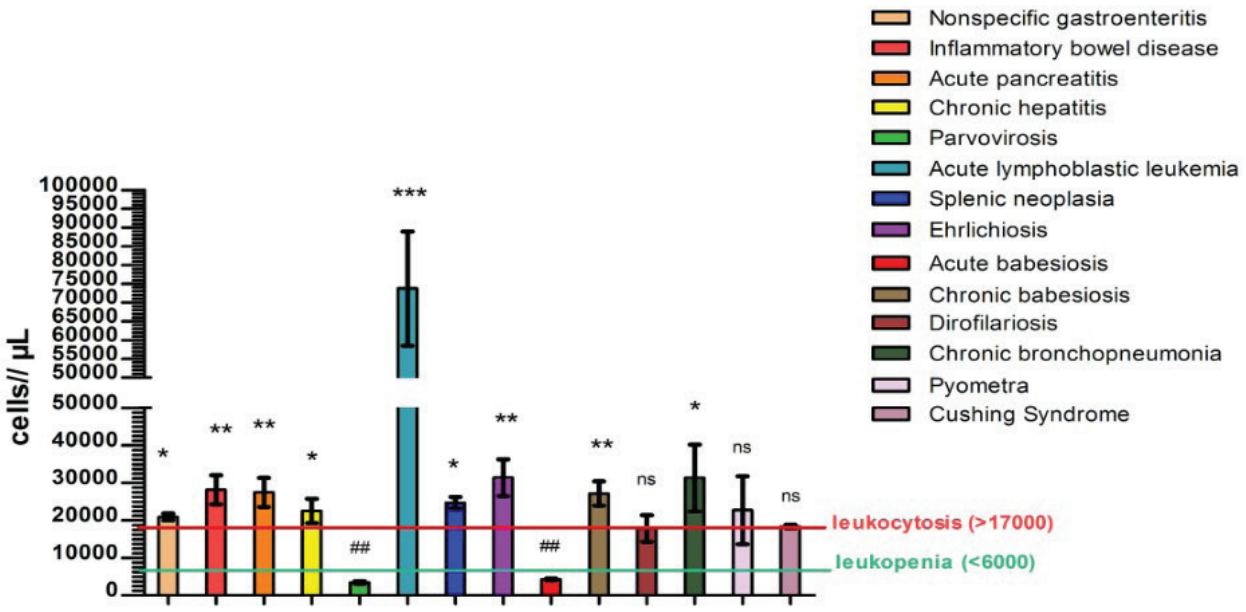

Figure2. The statistical comparison of leukocytes in the dog groups according to the primary disease
Legend: *0.01 ≤ p<0.05, significant; **0.001 ≤ p<0.01, highly significant; ***p<0.001, very high significant; ns - no significant.

DISCUSSION

In this study, leukocytosis was considered when WBC >17.000 × µL^{-1}, and leukopenia when WBC <6000 × µL^{-1}. According to the severity, the number of cases which evolved with leukocytosis was: 73 with mild leukocytosis (17,000-25,000thousand/mm³), 41 with moderate leukocytosis (25,000-40,000 thousand/mm³), 15 with severe leukocytosis (40,000-100,000 thousand/mm³) and 2 with extreme leukocytosis (>100,000 thousand/mm³).

Accordingly to the literature, most cases of leukocytosis in dogs develop below 40,000 leukocytes × µL^{-1} blood. In a study on 182 dogs with leukocytosis, 151 dogs (83%) had between 17,500-39,990 leukocytes × µL^{-1} blood, which is considered to be mild to moderate leukocytosis, and only 5% had extreme leukocytosis (between 61,050-127,500 leukocytes × µL^{-1} blood) (Willard and Tvedten, 2012).

The quantitative and qualitative changes in leukocytes found in this study are consistent with the infor-

mation and clinical studies from the literature. Thus, in the case of nonspecific gastroenteritis, such as acute gastritis, the body responds to inflammation by leukocytosis with neutrophilia and a left shift of the *Arneth* index, either as a result of increased migration of neutrophils to the site of inflammation or as a result of increased production and release of neutrophils from the bone marrow, which exceeds the consumption of the inflammatory injury (Meyer and Harvey, 2004; Weiser et al., 2012).

In the case of acute pancreatitis, leukocytosis is the consequence of inflammation and necrosis of peripancreatic tissues, caused by the activation and release in the interstitial area of pancreatic enzymes (Meyer and Harvey, 2004; Weiss and Wardrop, 2010).

Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders of the intestine. In the IBD, the physio-pathological mechanism may be considered to be the accumulation of large numbers of polymorphonuclear leukocytes in the mucosa and epithelial crypts of the intestine, which represents a hallmark of the active phase of IBD. In dogs with IBD, hematological changes such as neutrophilia and monocytosis have been frequently described as reflecting chronic, active inflammation (Brazil et al., 2013; Mercedes et al., 2014).

Regarding chronic liver diseases, a study by Elhiblu et al.(2015) on dogs with liver cirrhosis revealed a left shift of neutrophilic leukocytosis, associated with decreased lymphocyte and platelet counts.

In the case of parvoviral enteritis, leukopenia with neutropenia occurs either because the virus is cytotoxic to hematopoietic stem cells or as a result of endotoxemia resulting from gastrointestinal necrosis leading to a depletion of bone marrow leukocyte precursors (Weiss and Wardrop, 2010).

El-Zahar et al. (2019)observed that the erythrocytes and the leucocytes count, neutrophils, and lymphocytes were significantly decreased in dogs with parvoviruses compared to clinically healthy dogs. The platelets, monocytes, eosinophils, and basophils did not vary significantly.

In acute pancreatitis that evolved with a total number of 640 leukocytes, some explanations could be the installation of a consuming leukopenia or as a consequence of depletion of leukocyte precursors in the maturation and storage compartment of the bone marrow, which occurs as a result of endotoxemia.

In chronic babesiosis the leukocytosis was moderate and in acute babesiosis, the reduction in the number of leukocytes was generally of small magnitude, comparatively with those obtained by Eichenberger et al.(2016), in a clinical study performed on 15 dogs with babesiosis, where was observed moderate leukopenia with mild to moderate neutropenia, along with severe thrombocytopenia before the identification of *Babesia canis* on blood smears.

In another study,it was observed a statistically significant association between leukocytosis and monocytosis, respectively between thrombocytopenia and lymphopenia in dogs with babesiosis and leukocytosis with neutrophilia in the acute phase of the disease, especially in the first 10-11 days (Geta and Mălăncuș, 2013). Also, in other clinical trials, the hematological changes found in *Babesia canis* infection were anemia, thrombocytopenia, and inconsistent leukocyte changes, such as leukocytosis, leukopenia, neutrophilia, neutropenia, and eosinophilia (Furlanelloet al., 2005;Lobetti, 2011;Zygneret al., 2012).

In the case of ehrlichiosis, it was observed equally the presence of both leukopenia and leukocytosis. In general, chronic forms of ehrlichiosis are characterized by pancytopenia due to suppression and destruction of the bone marrow, with hypoplasia of all precursors in the bone marrow(Aguirre et al., 2004; Moraret al., 2015;Wanerand Harrus, 2013).

A study by Harrus et al. (1997) on 100 cases of monocytic ehrlichiosis in dogs, confirmed by the presence of anti-*E.canis* antibodies by indirect immunofluorescence, it was found that the main hematological changes were leukopenia, lymphopenia, anemia, and thrombocytopenia.

In the present study, it was observed that the pyometra evolved with both,leukocytosis and leukopenia, a situation that can be explained by the evolutionary moment at which the disease was diagnosed. Thus, in the early stages is an increased influx of leukocytes into the uterus that exceeds the production of the bone marrow, and with the chronicity of the disease, the rate of consumption becomes lower, which leads to exceeding the production-consumption balance, tilting the balance to production, and consequently, to the increase in the number of leukocytes in the blood, a fact confirmed also by other authors(Weiser et al., 2012).

In animals with moderate to severe inflammation,

infections, and neoplasm of the respiratory tract, leukocytosis is observed with a left shift of the Arneth index, while leukopenia is common in animals with sepsis. Tracheal collapse progresses to leukocytosis due to stress-induced by dyspnea(Nelson and Couto, 2014).

Leukocytosis found in epileptiform syndrome is due to stress and pain, to which the body responds by releasing adrenocorticotropic hormone (ACTH) by the pituitary gland. Consequently, cortisol is released from the adrenal glands with the appearance of leukocytosis with neutrophilia because steroids cause a decrease in the adhesive capacity of neutrophils, resulting in higher retention in circulation (Meyer and Harvey, 2004; Weiser et al., 2012).

Endogenous release of corticosteroids (hyperadrenocorticism) causes leukocytosis with neutrophilia, a phenomenon due to decreased tissue consumption by loss of adhesion of neutrophils, and increased release of them from the bone marrow storage compartment (Feldman et al., 2015).

Leukocytosis observed in dogs with diabetes mellitus could be explained by the fact that in dogs with diabetes type 1, innate immunity is altered, is characterized by increased production of pro-inflammatory cytokines without a concomitant change in the production of anti-inflammatory cytokines. This may be an explanation for the common infectious and inflammatory complications associated with diabetes in dogs (DeClueet al., 2012).

Moreover, in a study conducted by Aleksandrovskii(1992),on dogs with diabetes, it was found that in the case of hyperglycemia, the functional status of neutrophils is altered, so neutrophilia occurs as a result of leukocyte activation.

The magnitude of lymphocytosis is directly proportional to the absolute number of leukocytes, while the percentage of neutrophils is inversely proportional to the magnitude of leukocytosis. In the case of splenic neoplasm, leukocytosis occurred due to neutrophilia, but a slight monocytosis is also observed. In the literature, the number of leukocytes in dogs with metastases is significantly higher than in those without metastases, the increase in leukocytes can be explained by a neutrophilic leukocytosis induced by infections, inflammation, and tissue necrosis associated with neoplasm metastases (Mercedes et al., 2014).

In some clinical studies performed in patients with acute lymphoblastic leukemia, the main hematological change was the alteration of the number of leukocytes, which can have values from very low (<4,000 × μL^{-1}) to very high (>100,000 × μL^{-1}). Leukocytosis in patients with acute lymphoblastic leukemia occurs by the circulating neoplastic lymphocytes, and in extreme cases, can exceed 500,000 × μL^{-1}(Couto, 1985).

In another clinical study by Vernau and Moore(1999) on a total of 73 cases of chronic lymphocytic leukemia (CLL), blood lymphocyte counts ranged from 15,000 to 1,600,000 × μL^{-1} with a mean of approximately 166,000 × μL^{-1}, with a percentage of 73% cases of CLL involved a proliferation of T lymphocytes. In a total of 38 cases of acute leukemia, the leukocyte counts varied from 3300 ±450,000 × μL^{-1} with a mean of approximately 133,000 × μL^{-1}. Approximately 54% of cases had counts <100,000 × μL-1 and 23% of cases had counts <50,000 × μL-1.

CONCLUSION

We observed that leukocytosis was mainly due to increased neutrophil counts and was more frequently associated with infections, inflammation, and endogenous release of corticosteroids due to stress, except in cases of leukemia where the increase was due to lymphocytosis.

Leukopenia found in the early stages of severe infections and inflammation was due to a reduction in the absolute number of neutrophils being more common in babesiosis, parvoviral enteritis, pyometra, and acute pancreatitis.

Both leukocytosis and leukopenia have been found in conditions such as babesiosis and pyometra, depending on the character ofthe evolutionary stage. In the case of pyometra, the presence of both leukocytosis and leukopenia can be explained by the evolutionary moment at which the disease was diagnosed.

Infections and nonspecific inflammation of the digestive tract are more frequently accompanied by moderate leukocytosis, while specific enteritis leads, at least in the early stages, to leukopenia. In bronchopneumonia, the leukocytosis is moderate, while inflammation of the anterior airways causes mild leukocytosis. In neuromuscular diseases, leukocytosis ranged in wide limits, and mild increases were observed in puerperal eclampsia and epileptiform syndrome, while severe leukocytosis was present in meningoencephalitis.

The severity of leukocytosis in dogs with diabetes mellitus was moderate, also moderate leukocytosis was found in the splenic, hepatic, and pulmonary neoplasm. Acute lymphoblastic leukemia evolved with severe leukocytosis and chronic leukemia with extreme leukocytosis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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