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Hematological and biochemical alterations in female dogs with mammary cancer and inflammatory carcinoma

[*Alterações hematológicas e bioquímicas em cadelas com neoplasias mamárias e carcinoma inflamatório*]

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ABSTRACT

Hematological and biochemical alterations in animals with neoplasms may result from the direct effects of tumor growth or paraneoplastic syndromes. The objective of this study was to evaluate these hematological and biochemical alterations in female dogs with mammary tumors and with inflammatory carcinoma (IC). Blood samples were collected from 43 female dogs divided into three groups according to clinical staging: Group 1 (G1) - animals in initial stage ($T_{1,2,3}N_0M_0$, N=17), Group 2 (G2) - animals in advanced stage ($T_{1,2,3}N_1M_{0,1}$, N=15) and Group 3 (G3) - animals presenting IC (N=11). Hematological and biochemical parameters obtained were related to patients' clinical staging. Among alterations, the most common were anemia, neutrophilic leukocytosis, monocytosis, increased ALT, AST, and hypoalbuminemia, mainly in dogs in advanced clinical staging and with inflammatory carcinoma.

Keywords: canine, breast cancer, complete blood count, serum biochemistry

RESUMO

Alterações hematológicas e bioquímicas em animais portadores de neoplasias podem resultar dos efeitos diretos do crescimento tumoral ou de síndromes paraneoplásicas. O objetivo deste estudo foi avaliar as alterações hematológicas e bioquímicas em cadelas com neoplasias mamárias e carcinoma inflamatório (IC). Foram coletadas amostras de sangue de 43 cadelas divididas em três grupos de acordo com o estadiamento clínico: grupo 1 (G1) - cadelas em estádio inicial ($T_{1,2,3}N_0M_0$, N=17), grupo 2 (G2) - cadelas em estádio avançado ($T_{1,2,3}N_1M_{0,1}$, N=15) e grupo 3 (G3) - cadelas que apresentaram IC (N=11). Os parâmetros hematológicos e bioquímicos obtidos foram relacionados com o estadiamento clínico das pacientes. Entre as alterações, as mais comuns foram anemia, leucocitose neutrofílica, monocitose, aumento de ALT, AST e hipoalbuminemia, principalmente em cadelas em estadiamento clínico avançado e com carcinoma inflamatório.

Palavras-chave: caninos, câncer de mama, hemograma, bioquímica sérica

INTRODUCTION

Paraneoplastic syndromes (PNS) correspond to a diverse group of clinical changes that occur in distant sites of primary tumors or their metastases, being as prejudicial as its initial cause. In some cases, PNS represent the first clinical sign observed before the diagnosis of a tumor. Although incidence of PNS in veterinary medicine is poorly described, in human medicine, 75% of cancer patients are estimated to have paraneoplastic alterations during the neoplasm evolution (Morrison, 2002).

Direct effects of tumor growth or paraneoplastic syndromes may result in hematological changes in pets with neoplasms (Childress, 2012). Anemia, leukocytosis, thrombocytopenia, and coagulopathies are known to be the most commonly hematological manifestations

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observed (Stockhaus *et al*., 1999; Bailey, 2020). Cancer-related anemia is a frequently complication in human patients with several types of tumors and may have a negative impact on quality of life, prognosis, and treatment response (Blohmer *et al*., 2005). Moreover, several biochemical abnormalities have been described in oncological patients, such as hypercalcemia, hypoglycemia, hypoproteinemia and increase in total alkaline phosphatase and its corresponding isoenzymes (Karayannopoulou *et al.*, 2006; Bailey, 2020).

In human medicine, hematological changes are considered an important determinant prognostic factor in patients with neoplasms. However, in veterinary medicine, these changes and their relation to mammary neoplasms are few explored. Identify paraneoplastic syndrome allows an early diagnosis of tumor, improving the patient's prognosis and quality of life. Studies associating either hematological or biochemical alterations with disease progression in canine mammary neoplasms are scarce (Silva *et al*., 2014; Garrido *et al*., 2015). Furthermore, no study has evaluated the association of these blood alterations with tumor progression in female dogs with inflammatory carcinoma. Therefore, the objective of this study was to evaluate hematological and biochemical alterations in female dogs with malignant mammary neoplasms according to clinical staging and with inflammatory carcinoma.

MATERIAL AND METHODS

This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Minas Gerais (UFMG) under protocol no. 200/2016. Forty-three female dogs with malignant mammary neoplasms, treated at the Veterinary Oncology Service of the Veterinary Hospital (SOV-HV) in UFMG, were included in the present study. The inclusion criteria were female dogs of any breed or age, with malignant mammary neoplasms or inflammatory carcinoma (IC), attended in the period from March 2015 to January 2018. Tumors associated with edema, hyperthermia, erythema, with history of sudden growth in the form of diffuse plaque, were considered as IC due these clinical characteristics. Patients with serological diagnosis of leishmaniasis, hemoparasitosis or that were vaccinated within less than four months were excluded from the study. The parameters of clinical and epidemiological interest such as age, reproductive status, tumor size and histological type were obtained through electronic medical records present in the digital files of HV-UFMG.

The blood samples collection was done once prior to surgical excision as an auxiliary test for surgical risk. For patients with IC, it was performed once at the time of clinical diagnosis. All patients were submitted to vacuum venipuncture of the external jugular, cephalic or lateral saphenous veins, after adequate site antisepsis, with a 5mL syringe with needle. Tubes with dipotassium ethylenediaminetetraacetic anticoagulant (EDTA-K2) were used for complete blood count (CBC) and tubes with coagulant activator were used for biochemical analyzes. Hematological and biochemical analyses were performed by the Clinical Analysis Laboratory (LAC) at HV-UFMG.

CBC was performed by the Veterinary Hematology analyzer Icounter Vet (Diagno, Brazil), through the impedance method, which provides automatic counting of total white blood cells (WBC), basophils, eosinophils, band neutrophils, segmented neutrophils, lymphocytes, monocytes, red blood cells (RBC), hematocrit, platelets, hemoglobin concentration (HGB), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH) and RBC distribution width (RDW). The hematocrit was also evaluated by microhematocrit technique, centrifuged for five minutes at 10,000 rpm, for comparison with automatic result. Classification of anemia intensity was obtained through hematocrit value, in which percentages between 30 and 37% indicated mild anemia, between 20 and 29% moderate anemia, between 13 and 19% severe anemia, and below 13% very severe anemia, as well as anemia type obtained through MCV and MCHC values, in macrocytic/normocytic/ microcytic and hypochromic/normochromic/ hyperchromic. The differential leukocyte concentration and cytomorphological analysis were done with optical microscopy, in 20x, 40x and 100x objectives, using blood films with Diff-Quick stain (Panótico Rápido®, Laborclin, Brazil). Automatic platelet concentration was checked in blood film by counting platelet

average in ten fields of 100x, with subsequent multiplication to 20,000. The hematological reference intervals followed those proposed by Jain (1993).

Biochemistry profile was performed with serum obtained after centrifugation of tubes containing coagulation activator, in a Cobas Mira 5 Biochemical Analyzer (Roche Diagnostics, Switzerland). The analyzes, using commercial Synermed kits (Synermed International Inc, United States), included total protein and fractions by the colorimetric method, creatinine by the kinetic method, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) by the enzymatic method. The biochemical reference intervals followed those proposed by Kaneko *et al*. (1997).

All patients were submitted to 3-view thoracic radiography (ventro-dorsal, latero-lateral right and left) and abdominal ultrasound for clinical staging. This study used TNM system, established by World Health Organization (WHO), which takes parameters such as size of the primary tumor (T_1: 0-3 cm; T_2: 3-5 cm; T_3: >5 cm), involvement of regional lymph nodes (N_0: non-metastatic; N_1: metastatic) and metastases in distant organs (M_0: non-metastatic; M_1: metastatic). The presence of metastases in the axillary or inguinal lymph nodes was confirmed histopatologically after surgical excision, considering the presence of isolated cells, micro or macrometastases as criteria for positivity of nodal involvement.

Except for patients with IC, all female dogs underwent surgical excision as initial therapy for mammary neoplasms. Mastectomies were performed according to the number, size and location of the tumors, respecting the principles of oncological surgery and lymphatic drainage of the mammary chain (Horta *et al*., 2014). The surgical specimens' samples were immediately immersed in 10% buffered formalin aqueous solution and sent for histopathological analysis. Histological sections of 4 µm were obtained from mammary fragments of surgical excision or from necropsy material of female dogs who died due to IC. All samples were processed using the routine paraffin embedding technique and stained by Hematoxylin-Eosin technique. The identification of the histological type followed the classification proposed by Cassali *et al*. (2020). Survival time of patients were obtained through a maximum period of 2 years or until obit.

Comparisons between groups and hematological and biochemical alterations were performed using the Kruskal-Wallis global test. All variables were tested on the specific survival curve in univariate and multivariate analysis. Values were considered statistically significant when $P<0.05$. The reference intervals for CBC and biochemistry were used as control according to Jain (1993) and Kaneko *et al.* (1997), respectively. In addition, hematological and biochemical alterations were correlated with clinical staging and presence of IC. Therefore, three distinct groups were included: Group 1 (G1) - animals in early clinical stage ($T_{1,2,3}N_0M_0$, N=17), Group 2 (G2) - animals in advanced clinical stage ($T_{1,2,3}N_1M_{0,1}$, N=15) and Group 3 (G3) - animals presenting IC (N=11). Data are presented through tables and graphics.

RESULTS

The mean age at diagnosis was 10.82±2.86 years (minimum at 6 years and maximum at 15.5 years old). The majority (70.73%) were not castrated at the time of diagnosis and in the other patients, castration occurred late, after the third heat, according to Schneider (1969). The most frequent histological type was carcinoma in mixed tumor, followed by micropapillary and papillary carcinomas, according to Table 1.

Hematological and biochemical parameters according to the groups and reference intervals are described in Table 2 and 3, respectively. Anemia was the most frequent hematological alteration, observed in female dogs of groups G2 and G3, corresponding to 53.8% of the cases (14/26). Related to type, 71.43% (10/14) of anemias were classified as normocytic normochromic and regarding the grade, 57.14% (8/14) of female dogs presented mild anemia and 35.71% (5/14), moderate anemia. In groups G2 and G3, neutrophilic leukocytosis was frequently observed, indicating the presence of an inflammatory process. Serum biochemical alterations were observed in the enzymes ALT between groups G1 and G2, in AST between groups G1 and G2 and between groups G1 and G3.

Table 1. Clinic-pathological characteristics of female dogs in early and advanced clinical staging of mammary neoplasms and IC

Variables	G1	G2	G3	TOTAL
N	17(39,53%)	15(34,88%)	11(25,58%)	43(100%)
Age				
mean ± SD	8.76±3.34	11.94±1.94	11.76±3.30	10.82±2.86
Reproductive status				
Castrated	0(0%)	6(46.15%)	6(54.55%)	12(29.27%)
Non castrated	17(100%)	7(53.85%)	5(45.45%)	29(70.73%)
Histological type				
Carcinoma in mixed tumor	13(76.47%)	3(20%)	0(%)	16(37.21%)
Micropapillary carcinoma	0(0%)	2(13.33%)	6(54.55%)	8(18.60%)
Papillary carcinoma	3(17.65%)	1(6.67%)	2(18.18%)	6(13.95%)
Pleomorphic lobular carcinoma	0(0%)	2(13.33%)	2(18.18%)	4(9.30%)
Solid carcinoma	0(0%)	3(20%)	0(0%)	3(6.98%)
Carcinosarcoma	0(0%)	2(13.33%)	0(%)	2(4.65%)
Malignant adenomyoepithelioma	1(5.88%)	1(6.66%)	0(%)	2(4.65%)
Cribriform carcinoma	0(0%)	0(%)	1(9.09%)	1(2.32%)
Osteosarcoma	0(%)	1(6.67%)	0(%)	1(2.32%)

Table 2. Mean hematological parameters of female dogs in early and advanced clinical staging of mammary neoplasms and IC

Parameter	G1	G2	G3	Reference Interval (Jain *et al.*, 1993)
Hematocrit	43.6±6.7	36.4±8.7	32.5±6.3	37-55 (%)
HGB	15.2±2.3	13.0±3.6	10.7±2.6	12-18 (g/dL)
RBC	6.6±0.9	5.5±1.4	4.8±1.0	5.5-8.5 (10^6/µL)
MCV	66.6±5.3	66.8±4.4	68.4±4.6	60-77 (fL)
MCHC	34.4±3.1	35.4±2.7	32.8±2.4	31-36 (g/dL)
MCH	23.7±3.1	23.6±2.4	22.4±1.7	19-24.5 (pg)
RDW	13.5±1.2	12.8±1.0	14.6±2.3	12-15 (%)
WBC	9,1±3.2	18.9±9.6	25.8±22.7	6-17 (x10^3/µL)
Neutrophils	6761±2490	15825±8677	21546±7958	3-11.5 (x10^3/µL)
Band Neutrophils	27.1±56.2	117.4±259.0	668.9±1710.9	0-300 (/µL)
Lymphocytes	1423±895	1486±1006	1269±993	1000-4800 (/µL)
Monocytes	452±238	915±902	1895±2284	150-1350 (µL)
Eosinophils	443±346	318±476	356±506	100-1250 (/µL)
Platelets	324±132	385±343	256±265	200-500 (x10^3/µL)

Table 3. Mean biochemical parameters of female dogs in early and advanced clinical staging of mammary neoplasms and IC

Parameter	G1	G2	G3	Reference Interval (Kaneko *et al.*, 1997)
ALT	55.8±32.1	175.5±229.6	74.2±47.4	0-110(U/L)
AST	30.6±10.3	779.9±205.7	1348.6±436.6	0-100(U/L)
ALP	61.7±63.2	39.2±26.2	115.0±156.9	20-156(U/L)
Urea	32.8±8.8	42.4±33.3	47.9±52.5	20-56(mg/dL)
Creatinine	0.9±0.2	0.83±0.27	1.21±0.55	0.5-1.5(mg/dL)
Total Protein	6.7±1.4	6.3±0.8	5.9±1.0	5.4-7.5(g/dL)
Albumine	3.0±0.5	2.3±0.5	2.6±0.6	2.3-3.1(g/dL)
Globulin	3.8±1.6	4.1±1.0	3.3±1.1	2.7-4.4(g/dL)

The CBC analysis showed a significant difference in the values of RBC (P<0.001) and HGB (P<0.01) between groups G1 and G3, respectively. There was also a significant difference when hematocrit values were compared between groups G1 and G2, as well as between groups G1 and G3, respectively, characterizing anemia (Fig. 1).

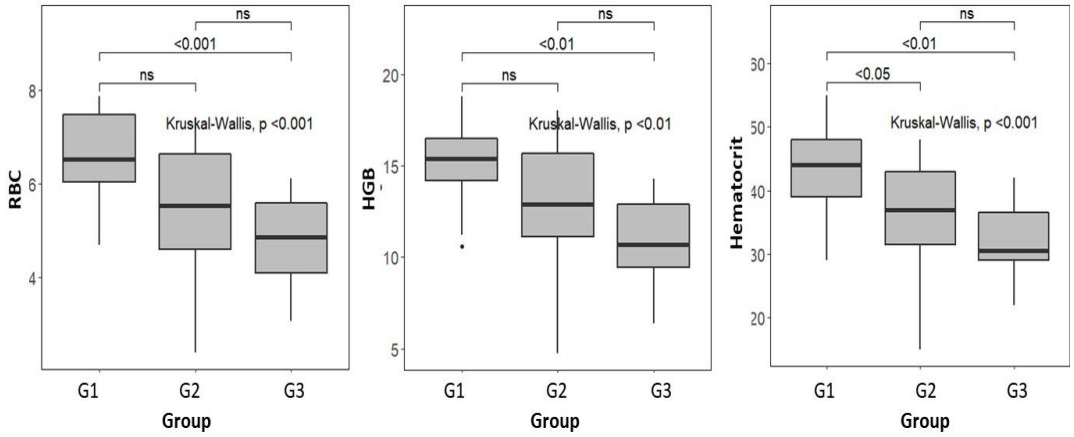

Figure 1. RBC, HGB and hematocrit boxplot according to groups of female dogs in early and advanced clinical staging of mammary neoplasms and IC.

The total WBC analysis showed a significant difference between groups G1 and G2 (P<0.001) and between groups G1 and G3 (P<0.05). There was also significant difference in segmented neutrophils between groups G1 and G2 (P<0.001) and groups G1 and G3 (P<0,05), characterizing neutrophilic leukocytosis (Fig. 2).

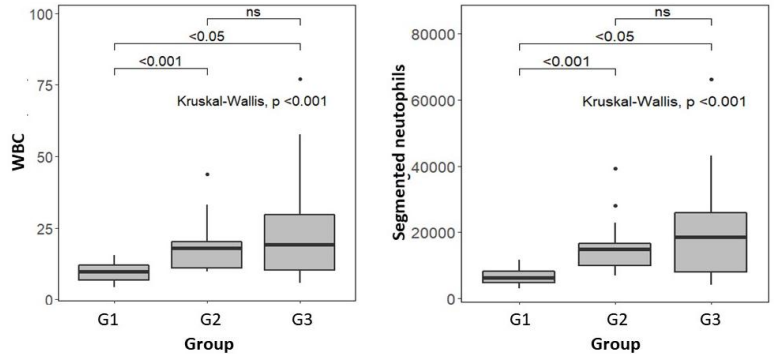

Figure 2. WBC and segmented neutrophils boxplot according to groups of female dogs in early and advanced clinical staging of mammary neoplasms and IC.

The biochemical profile analysis showed a significant difference in ALT values between groups G1 and G2 (P<0.05), in AST between groups G1 and G2 (P<0.0001) and between groups G1 and G3 (P<0.01), as well as in albumin concentration between groups G1 and G2 (P<0.01) (Fig. 3).

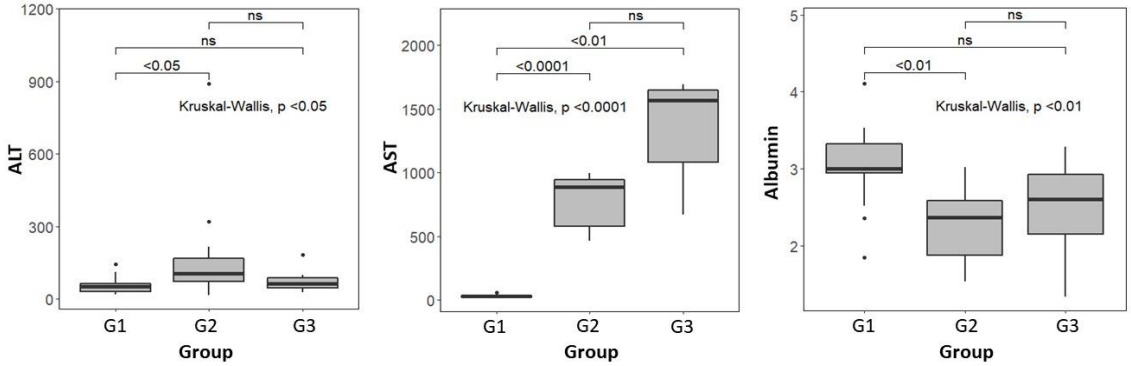

Figure 3. ALT, AST and albumin boxplot according to groups of female dogs in early and advanced clinical staging of mammary neoplasms and IC.

Survival time was statistically significant according to groups, although the other hematological and biochemicals variables were tested but did not show significant correlations (Fig. 4). Group G1 did not reach the median, while G2 and G3 obtained median survival time of 74 and 19 days, respectively (P<0,0001).

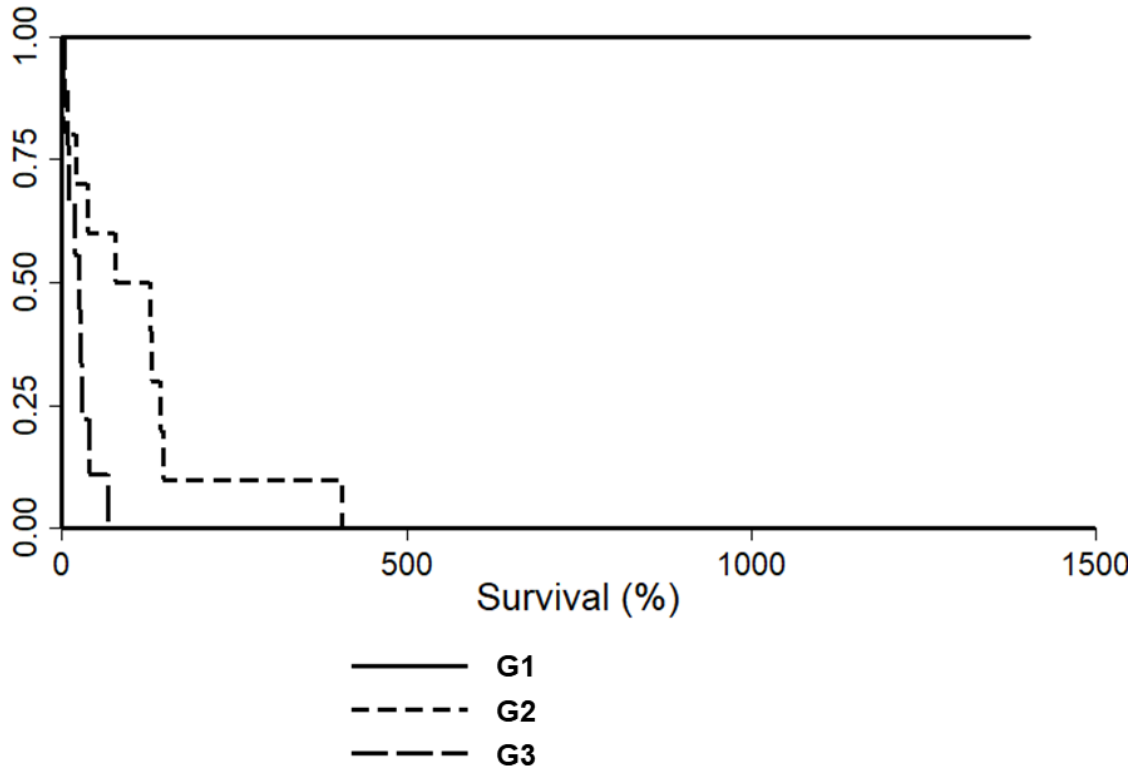

Figure 4. Survival time according to groups of female dogs in early and advanced clinical staging of mammary neoplasms and IC.

DISCUSSION

Due common hematological alterations observed in animals with mammary gland tumors, the normocytic normochromic anemia presented in female dogs with advanced disease (G2) and with IC (G3) corroborates with previous studies that associated it with tumor progression (Silva *et al.*, 2014). Normocytic normochromic anemia is characteristic of chronic disease or inflammation anemia and its mechanism is associated with tumor secretion of cytokines responsible for promoting iron sequestration, reduction of red blood cells half-life and erythropoietin secretion (Cançado and Chiattone, 2002; Mangieri, 2016; Silva *et al*., 2014).

Neutrophilic leukocytosis is a common finding in animals with IC and may be justified due infection and inflammatory process that is characterized by swelling, secretion, and redness or by tumor-associated necrosis (Childress, 2012; Ribeiro *et al*., 2013). Neutrophilic leukocytosis, accompanied by normal levels of mononuclear cells, is considered the most important hematological finding in dogs affected by mammary carcinomas and it is related to a poor prognosis, since animals that presented larger tumors, metastases and death progression showed higher neutrophils concentrations (Estrela-lima *et al*., 2012). This white blood cells are the most common cell in blood and increase in cases of microorganism presence or not, as in the Systeminc Inflammatory Response Syndrome (SIRS) (Childress, 2012). SIRS may be defined as a clinical manifestation of the systemic inflammatory response and results from the imbalance between anti-inflammatory and inflammatory cytokines, without any site of infection (Silverstein and Sanotoro-beer, 2013). This condition is associated with cancer cells and tumor microenvironment complex interactions, that induce tissue damage and start an acute phase response, stimulating cytokines and chemokines to act in whole organism (Balkwill and Mantovani, 2001). In 2016, the first case of SIRS was described in a woman with IC, contributing to the negative disease outcome (Boshier *et al.*, 2016). Canine mammary IC is frequently associated to ulcers as an infection focus and neutrophilic leukocytosis can be pronounced either by cancer inflammation promotion or infectious response, requiring additional tests to differentiate the cause (Boshier *et al.*, 2016; Souza *et al*., 2009). This present study can infer that animals with advanced stage of mammary neoplasms and IC associated with neutrophilic leukocytosis has a worse prognosis, since the increase of condition in advanced stages and IC. More studies are necessary to elucidate the behavior of neutrophilic leukocytosis as a prognostic factor in female dogs with mammary neoplasms.

Increased ALT and AST are mainly associated to liver injury and skeletal muscle damage (Allison, 2012). When associated to high levels of ALT, increased AST supports the suspect of hepatic injuries, such as metastasis in the cancer context (Hall and German, 2016; Mogrovejo *et al.*, 2014; Stockham and Scott, 2008). On the other side, high serum concentrations of AST associated to high serum of creatine kinase and normal ALT, may suggest that the damage is from muscle origin (Hall and German, 2016). Alterations in these enzymes were observed in the present study and may be related to muscle injuries induced by neoplasia in the abdominal muscles and intense catabolism, as well as associated with presence of metastasis. In addition, unlike the increased ALT between groups, high levels of AST were observed according to tumor progression. Although not completely elucidated the mechanism, an unexplained high AST was identified in a human patient with advanced breast cancer before its completely resection and chemotherapy, returning to normal levels before treatment (Birtle *et al.*, 2003). Further studies need to be performed to better understand increased AST implications in the context of breast cancer.

Regarding the albumin analysis, there was a difference between groups G1 and G2, characterized by a relative hypoalbumineamia. In addition, because it is a protein of acute phase, albumin tends to reduce its serum levels under inflammatory stimuli (Cerón *et al*., 2005).

Anemia, neutrophilic leukocytosis, monocytosis, increased serum levels of ALT, AST and hypoalbuminemia were the most frequent hematological and biochemical alterations found in female dogs with mammary neoplasms in initial, advanced and IC clinical staging. It was noticed that these alterations tend to be more pronounced with disease progression, being as potential prognostic markers. These changes can

be associated with paraneoplastic syndromes and directly tumor effects on animal systems, and because of it, their analysis should be considered to stablish parameters for prognosis and treatment of patients. It is also evidenced that those abovementioned hematological and biochemical alterations are more pronounced in female dogs with IC, which may contribute to the worse prognosis and lead systemic changes in the patients.

Advanced clinical staging, histological type and progression to IC are known as relevant prognostic factors (Cassali *et al.*, 2020). In this study, clinical staging and IC diagnosis are important factors that interfere in survival time, showing that even with hematological alterations in the time of diagnosis, these parameters defined patients' survival.

CONCLUSION

Normocytic normochromic anemia, neutrophilic leukocytosis, monocytosis, increased serum levels of ALT, AST and hypoalbuminemia were the most frequent hematological and biochemical alterations found in female dogs due to disease progression. Advanced clinical staging and IC diagnosis associated to hematological parameters provide prognostic information for canine patients with mammary neoplasms.

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