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RESEARCH ARTICLE

The Tumor Architecture and Mitotic Index of Canine Cutaneous Mast Cell Tumors as a Prognostic Indicator

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A B S T R A C T

The aim of the study was: to evaluate the histomorphological alterations in canine mast cell tumors and the modification between these alterations; to assess the influence of histomorphological alterations in the clinical evolution of dogs with mast cell tumors; to evaluate the epidemiological and clinical characteristics of the tumor and verify if they are related to the death of the animal. Histopathological slides of mast cell tumors were analyzed for tumor extension, architecture, necrosis, eosinophilic infiltrate, margins, binucleated cells, mitotic index. These data were compared to the survival of the animals. Tumor architecture correlated with histological grade (HG) according to Kiupel (p=0,000002), mitoses was relationship with HG according to Patnaik grading system (p=0.003). Grade II tumors were more frequently observed in mixed-breed dogs (p=0.045), and animal death was related to presence of tumor ulceration (p=0.006) and age (p=0.001). In the correlation matrix, as association was observed between the number of eosinophils and tumor extension (0.221) and architecture (0.338); number of mitoses and architecture (0.339); Patnaik grading system and bleeding (0.190). In the log-rank test, architecture (p=0.006), number of mitoses (p=0.000), HG according Patnaik (p=0.045), and HG according Kiupel (p=0.000) influenced the survival of the animals. It was concluded that some histomorphological alterations, such as architecture, histological grade, number of mitoses and ulceration, can be used as prognostic factors in MCT in dogs.

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INTRODUCTION

Mast cell tumor (MCT) is one of the most common neoplasms in dogs, representing 16.78 and 21.8% of skin neoplasms (Costa *et al*., 2017; Suepaul *et al*., 2019). The most stricken breeds are Boxer, Labrador Retriever, and Golden Retriever and there is no sexual predisposition (Pierini *et al*., 2019).

The canine mast cell tumors clinical behavior is variable, some show less aggressive behavior, while others grow aggressively and metastasize (Kiupel, 2017). Several factors have been used to predict the behavior of MCT, such as: histopathological grade (Sledge *et al*., 2016), clinical stage (WHO) (Welle *et al*., 2008), location (Śmiech *et al*., 2018; Reynolds *et al*., 2019), mitotic index (Romansik *et al*., 2007), breed (Pierini *et al*., 2019), number of tumor nodules (single or multiple) (Fulani *et al*., 2008), depth of subcutaneous tissue invasion (Thompson *et al*., 2011) and cell proliferation markers, including proliferating nuclear cell antigen and Ki67 (Berlato *et al*., 2015).

The histopathological grade is superior to all these factors in predicting survival time. Willmann *et al.* (2021) reported that dogs with grade 3 tumors are more likely to have recurrence and metastasis, as well as animals with high-grade mast cell tumors. However, this grade depends on the subjective assessment of the histological characteristics of neoplastic cells and has reproducibility problems that lead to up to 50–60% disagreement among experienced pathologists, especially in the assessment of borderline cases (grade II) (Strefezzi *et al*., 2003).

Consequently, the study of other histomorphological variables that can help in determining the prognosis is necessary (Horta *et al*., 2018), mainly because they can be obtained from slides stained with hematoxylin-eosin routinely used for histopathological diagnosis, avoiding other high-cost and complex techniques.

In this perspective, the present study aimed to: evaluate the histomorphological alterations in canine mast cell tumors and the correlation between them; assess the influence of histomorphological alterations on the clinical

evolution of dogs with mast cell tumors; evaluate the epidemiological and clinical characteristics of the tumor and verify if they are related to the animal's death.

MATERIALS AND METHODS

A retrospective study of canine MCT was carried out from the archive of the Animal Pathology Laboratory of the Veterinary Hospital of the Federal University of Uberlândia, Brazil (HOVET-UFU), from January 2016 to June 2019. The inclusion criteria were: dogs with MCT diagnosed from histopathological evaluation, which had undergone excisional biopsy.

Epidemiological (age, sex, breed) and clinicopathological data (location, tumor size, and presence of ulceration) were collected from medical records. Additionally, three age groups were distinguished: young (0-1 year), adults (2-8 years) and elderly (>8 years). Eight tumor locations were distinguished: head, limbs, genitals, tail, abdomen, chest, flank, inguinal region (Costa *et al*., 2017). According to the tumor size, three groups were distinguished: <2cm, 2-5cm and >5cm (Murphy *et al*., 2006).

The paraffin blocks corresponding to each medical record were routinely sectioned and stained with hematoxylin and eosin for routine histopathology examination. For each MCT, histological grading was assessed according to both the Patnaik *et al*. (1984) and Kiupel *et al*. (2011) classifications. The association of the two grading systems was also used, according to Berlato *et al*. (2021). Tumor grade was independently assessed by three pathologists and when there was disagreement regarding grade, the diagnosis assigned by two pathologists was considered.

MCT were also evaluated to characterize tumor extent (infiltration of tumor cells into the dermis, subcutaneous tissue, and muscle tissue), architecture (nests (Fig. 1A) or cords (Fig. 1B)), intratumoral necrosis, hemorrhage, collagen hyalinization, desmoplasia, and tumor-associated tissue eosinophilia in a semiquantitative analysis (absent, discrete, moderate, and intense). Other assessments were the number of binucleated cells and mitotic index according to Elston *et al*. (2009).

The follow-up time of the dogs was at least 12 months. Information was collected regarding the cause of death of the animal, survival, appearance of new tumors and recurrence, overall survival (OS) was defined as time from surgery to death or euthanasia due to MCT-related cause, or end of the follow-up.

Chi-square test was used to assess the relationship between the following variables: histological grading by Patnaik *et al*. (1984) and Kiupel *et al*. (2011) and histological changes (number of binucleated cells, mitotic index, tumor extent, architecture, eosinophilic infiltrate, intratumoral necrosis, hemorrhage, collagen hyalinization and desmoplasia); epidemiological characteristics (age, breed, tumor size, ulceration of the neoplasm) and histological grading; epidemiological characteristics and death.

When the variables showed statistical association, a logistic regression test was performed. The calculation of the correlation between the histological variables was performed using the Spearman correlation matrix, with r>7 considered a strong correlation, r between 0.3 and 0.7 a moderate correlation and r<0.3 a weak correlation.

The OS of patients was estimated using the Kaplan–Meier test followed by the log-rank, Breslow, and Tarone–Ware tests to compare survival curves according to the histological variables studied (histological grade (HG) according to Patnaik *et al.* (1984) and Kiupel *et al.* (2011) and the association of both in animals with grade II tumors, and histological alterations). The concomitant influence of prognostic variables on OS was investigated using multivariate Cox regression analysis. The tests were performed using the Statistics Base program for Windows V.20 (IBM® SPSS®) software, with a significance level of 5% ($p<0.05$).

RESULTS

A total of 123 animals were included in this study, most of them females (65.29%), crossbreed (48.82%) ($p=2.2^{-6}$) and with a mean age of 8.95 years (Table 1). One hundred and two animals had a single nodule (82.93%) and the others (17.07%) had multiple nodules, totaling 141 tumors. Among these, most were in the abdominal region (22.61%) and limbs (20.87%) and most were not ulcerated (62.22%). The size of the neoplasms ranged from 0.2 to 25cm, with a mean of 4.49cm, and most tumors (45.95%) were between 2-5cm (Table 1). In the evaluation of the HG of MCT, the majority presented grade II (60.99%) and were classified as low grade (64.54%) (Table 1).

When the histological alterations were compared with the HG, an association was observed between the cellular arrangement of the neoplasms in cords and low grade according Kiupel grade system (p=2.907-6) (Table 2), with the odds ratio being 0.272. It was also observed that grade III neoplasms had a greater number of mitoses (p=0.03) (Table 2).

In the analysis of Spearman's correlation matrix, the histological variables that showed correlation were: TATE, tumor extension, and architecture; architecture and binucleated cells, number of mitoses, Patnaik grade system, and Kiupel grade system; bleeding and grade according to Patnaik grade system.

Survival assessment: The mean follow-up period was 560 days (range 1–1400). In the follow-up period, 73 dogs were lost to follow-up and data on postsurgical follow-up were available for 50 dogs. Only three dogs (6%) had recurrence, one animal developed metastasis (2%) and 31 dogs died (62%).

Regarding the analysis of the influence of epidemiological and tumor characteristics on clinical evolution (Chi-square test), there was a correlation between death and tumor ulceration (p=0.006) and age >8 years (p=0.001), with the risk of death in elderly dogs 40.5% higher than in adult animals. Clinical outcome was not related to tumor location, breed, or size (p>0.05) (Table 3).

The mean OS was 698 days (95% confidence interval [CI], 543–853), and the median was 604 days (95% CI, 386–822). Of the prognostic variables considered, only number of mitoses, degree, and architecture showed a significant correlation with OS (Table 3).

Animals with MCT arranged in nests had a median of 90 days and died earlier than animals with neoplasms arranged in cords (p=0.006). Animals with high-grade MCT did not reach the median (604 days) and died earlier

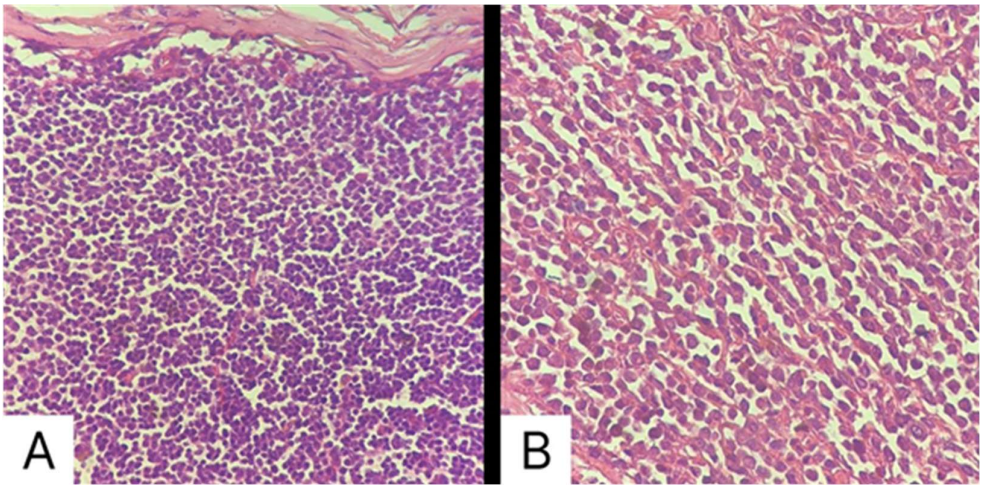

Fig. 1: A) Dog, mast cell tumor, 40x magnification. Observe neoplastic cells arranged in nests. B) Mast cells arranged in cords.

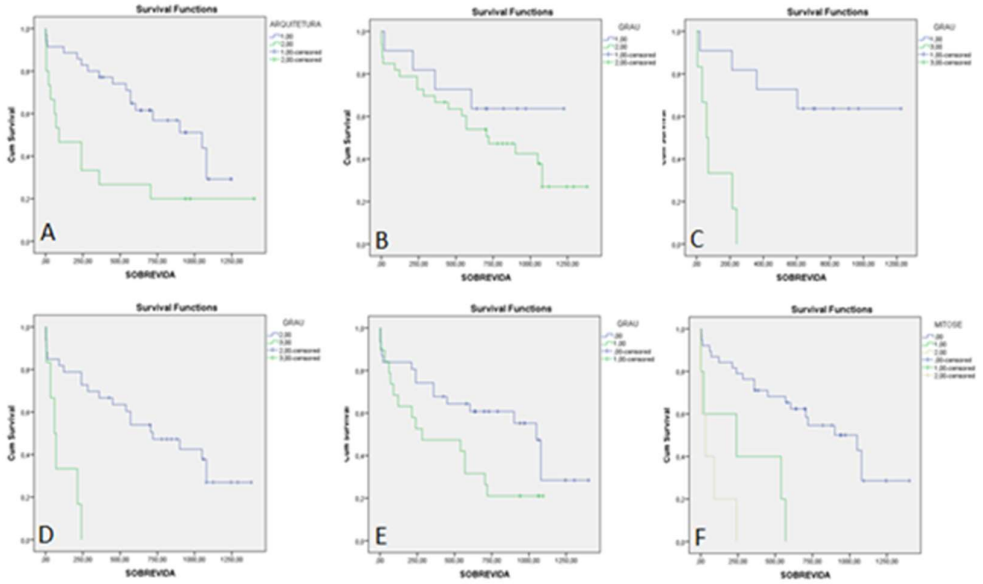

Fig. 2: Survival curves for dogs with MCT. A: Architecture (1: Cords; 2: Nests): median 705 days and MCT with architecture in nests had a median of 90 days (p = 0.006); B: Comparison between HG I and II second Patnaik *et al.* (1984) (p = 0.29); C: Comparison between HG I and III (p = 0.001); D: Comparison between HG II and III (p = 0.000); E: HG according to Kiupel *et al.* (2011): median 604 days and animals with high grade had a median of 283 days (p = 0.045). F: Mitoses (1: < 5; 2: 5–7; 3: > 7).

Table 1: Epidemiological data and histological grade of canine cutaneous MCT in dogs treated at the Veterinary Hospital of the Federal University of Uberlândia. Uberlândia-MG, January 2016 to June 2019.

	n	%		n	%		n	%
Sex (n=121)			Location (n=115)			Patnaik (n=141)		
Female	79	64.23	Abdomen	26	22.61	1	28	18.86
Male	42	34.15	Limbs	24	20.87	2	86	60.99
Breed (n=114)			Genital	20	17.39	3	27	19.15
Crossbreed	55	48.24	Head	18	15.62	Kiupel (n=141)		
Boxer	13	11.4	Thorax	11	9.57	Low grade	91	64.54
Labrador	12	10.53	Inguinal	7	6.09	High grade	50	35.46
Poodle	7	6.14	Flank	5	4.35			
Pit Bull	6	5.26	Others	4	3.48	Kiupel/Patnaik(n=33)		
Cocker	4	3.51	Ulceration (n=90)			Grade II/low grade	20	60.61
Rottweiler	2	1.75	Yes	34	37.78	Grade II/high grade	13	39.39
Basset	2	1.75	No	56	62.22			
Brazilian Terrier	2	1.75	Age (n=110)					
Teckel	2	1.75	Adult	57	51.82			
Yorkshire	2	1.75	Elderly	53	48.18			
Pug	2	1.75	Size (n=11)					
Beagle	2	1.75	<2	28	25.23			
Golden Retriever	1	0.88	2–5 cm	51	45.94			
Lhasa Apso	1	0.88	> 5 cm	31	28.83			
Pinscher	1	0.88						

than those classified as low grade (p=0.045). Dogs with grade III MCTs according to Patnaik dying earlier (p=0.000) and the median was 604 days. In the evaluation of the number of mitoses, the median was 604 days and dogs with MCT with 5–7 and > 7 mitoses per high-power field did not reach the median (median of 240 and 33 days, respectively) and died earlier than those dogs with MCT with < 5 mitoses per high-power field (p=0.000) (Fig. 2).

Table 2: Correlation between histological parameters and grade and death of dogs with MCT. Uberlândia-MG, January 2016 to June 2019.

Histological features	Chi-square			Log-rank
	Patnaik grade	Kiupel grade	Death	
Architecture	0.895	2.907-6*	0112	0.006*
Necrosis	0.235	0.551	0.598	0.690
Hemorrhage	0.491	0.811	0.933	0.980
Hyalinization	0.886	0.617	0.838	0.805
Desmoplasia	0.668	0.642	0.382	0.274
Eosinophils	0.416	0.631	0.262	0.175
Binucleated cells	0.895	0.278	0.257	0.078
Mitosis	0.003*	0.063	0.300	0.000*
Patnaik grade	-	-		0.045*
Kiupel grade	-	-	-	0.000*

* p<0.05

Table 3: Correlation between epidemiological findings and grade and death of dogs with MCT. Uberlândia-MG, January 2016 to June 2019.

	Patnaik grade	Kiupel grade	Death
Breed	0.045*	0.336	0.23
Location	0.216	0.216	0.475
Ulceration	0.623	0.385	0.006*
Age	0.289	0.77	0.001*
Tumor size	0.893	0.876	0.472

* chi-square test p<0.05

Table 4: Multivariate Cox regression analysis of overall survival of dogs with MCT compared to predictor variables. Uberlândia-MG, January 2016 to June 2019.

Variables	Standard error	p-value	Hazard rate	CI (95%)
Patnaik	0.570	0.244	1.941	0.636–5.931
Kiupel	0.552	0.835	1.122	0.380–3.312
Extension	0.343	0.551	1.227	0.626–2.404
Architecture	0.479	0.352	0.641	0.251–1.637
Necrosis	0.665	0.532	0.660	0.179–2.430
Hemorrhage	0.667	0.509	1.553	0.420 – 5.743
Hyalinization	0.477	0.789	0.880	0.346–2.242
Eosinophils	0.256	0.834	1.055	0.639–1.744
Binucleation	0.176	0.163	0.782	0.554–1.105
Mitosis	0.290	1.602	2.719	0.907–2.830

In the multivariate analysis between OS and the predictor variables studied for canine MCT, there was no correlation between the variables analyzed (Table 4).

DISCUSSION

Mongrel dogs were the most affected by MCT, as described by Horta *et al*. (2018) and Kluthcovsky *et al*. (2020) in studies also carried out in Brazil. These data reflect the population stratum of the HOVET-UFU, since the animals treated were mostly crossbreed. Warland and Dobson (2013) in a study carried out in the United Kingdom, Śmiech *et al*. (2018) in Poland and Moore *et al*. (2020) in Australia, described that the most affected breeds were Boxers and Labradors, as described in this study.

The mean age of the animals (8.95 years) was similar to that described by Kluthcovsky *et al*. (2020) and Moore *et al*. (2020). In this study elderly dogs died more frequently than adults, and Shoop *et al*. (2015) described that elderly dogs are more likely to develop MCT than young dogs. Likewise, Śmiech *et al*. (2018) reported that elderly dogs are at greater risk of having high-grade tumors.

Although elderly dogs are more likely to develop MCT than young dogs (Shoop *et al*., 2015; Reynolds *et al*., 2019) did not observe a significant association between age and HG. The present study confirmed this observation.

There is no consensus in the literature regarding the most frequent location of MCT. In this study, the abdominal region was most affected (22.61%), while in the study carried out by Reynolds *et al*. (2019), the tumors occurred more frequently on the nose and face. On the other hand, Horta *et al*. (2018) described limbs and trunk as the most common locations, while Pierini *et al*. (2019) and Kluthcovsky *et al*. (2020) also observed more neoplasms in the trunk region. Śmiech *et al*. (2018) described that the risk of MCT in the scrotum region is higher.

Although no correlation was observed between tumor location and histological grading in this trial (p=0.893), other studies indicate a greater chance of high-grade MCT being in the inguinal region, on the head (Reynolds *et al*., 2019), and on the scrotum (Śmiech *et al*., 2018). Tumor ulceration was related to the death of the dogs in this study. Pierini *et al*. (2019) described that ulceration of tumors showed a strong correlation with tumor size and lymph node metastasis. Kiupel (2017) explains that ulcerated, large, and invasive tumors are usually malignant, however, the possibility of malignancy in tumors that do not demonstrate these characteristics should not be ruled out.

The mean size of the neoplasms in the present study (4.49cm) was larger than that observed by Pierini *et al*. (2019), who observed tumors ranging from 0.5 to 10cm, with an average of 2cm, a fact that may be associated with a delay in seeking treatment. As reported by Reynolds *et al*. (2019), there was no correlation between tumor size and tumor grade. On the other hand, Natividade *et al*. (2014) described that larger tumors were more often of high HG. The influence of tumor size on the prognosis of MCT is still questionable and this variable is not considered in determining the staging of the disease.

HG proved to be an excellent prognostic factor capable of predicting the behavior of MCT. Dogs with high-grade MCT showed lower survival than dogs with low-grade tumors, and dogs classified as grade III died earlier than those classified as grades I and II.

However, there was no statistical difference between grades I and II survival in the Patnaik grading system, as noted by Sabattini *et al*. (2015) and Horta *et al*. (2018). In this sense, it is recommended to use the grading system proposed by Kiupel *et al*. (2011), which, in addition to generating less disagreement between observers (Sabattini *et al*., 2015), better predicts tumor behavior. Although Berlato *et al*. (2021) reported a statistical difference between the survival of grade II-low grade and grade II-high grade, this finding was not confirmed in the present study.

The classification proposed by Kiupel *et al*. (2011) does not include the arrangement of cells as a prognostic factor. Nevertheless, this study confirmed a correlation between architecture and HG and between architecture and mitotic index, in addition to lower survival in animals with MCT with cells arranged in nests. Horta *et al*. (2018) observed similar results and suggested that tumor architecture be included as a criterion for malignancy regardless of histological grading.

Grade III and high-grade tumors had a higher mitotic index (r=0.351 and r=0.301, respectively) (Fig. 1), which validates the HG of this study, since the number of mitoses was used as a grading criterion in both classifications. Likewise, Thompson *et al*. (2011) highlighted the mitotic index as a good prognostic factor in subcutaneous MCT. However, Romansik *et al*. (2007) stated that animals with

a high mitotic index have lower survival rates, regardless of HG. A factor that can interfere in the evaluation of the number of mitoses and its correlation with grade is the choice of fields for evaluation and, in this sense, Bertram *et al*. (2019) recommend better standardization of field selection for mitotic evaluation, since this choice is made subjectively.

In addition to cell differentiation, Patnaik *et al*. (1984) described the presence of necrosis and hemorrhage as criteria for malignancy. In the present study, only a weak association was observed between hemorrhage and Patnaik grade system (r=0.190) and these factors were not associated with animal death. Similarly, Horta *et al*. (2018) did not observe a correlation between these findings, however they described a significant relationship between necrosis and mitotic index, which is a prognostic factor.

The tumor-associated tissue eosinophilia (TATE) has been used in different types of cancers. Necroctic tumor cells, Th2, lymphocytes, and mast cells produce and release cytokines and chemokines, that attract the eosinophils to the tumor site. Activated eosinophils are capable of synthesizing and releasing a wide variety of biologically active mediators that can have both positive and negative effects on target cells (Galietta *et al*., 2023). TATE was used as a malignancy factor in Patnaik grading system, but was not used as a parameter of malignancy in Kiupel grading system.

Natividade *et al*. (2014) described that the amount of eosinophils is not a prognostic factor since it does not influence the survival of the animals this finding was confirmed in the present study. In addition to the absence of interference in survival, the amount of eosinophils showed a weak correlation with the architecture (r=0.338), tumor extension (r=0.221), and number of mitoses (r=0.190), which are good prognostic factors. Despite the lack of correlation between the parameters evaluated in the multivariate analysis, Preziosi *et al*. (2007) described tumor invasiveness and number of mitoses as independent prognostic indicators and Sledge *et al*. (2016) recommended a joint approach between clinical, histopathological, and molecular evaluation to better define the treatment and prognosis of the animal.

Despite conflicting data in the literature, this study highlights the usefulness of tumor architecture and mitotic index as a histomorphological prognostic indicator in canine cutaneous MCT. In summary, our research confirmed that canine MCT with nest architecture have a highest TATE, greater tumor extension, and greater amount of mitoses; grade III tumors hemorrhage more often; animals with tumors arranged in nests are more often classified as high grade and have lower survival rates; survival is lower in dogs with neoplasms with a high mitotic index and HG III and high grade. Animals with ulcerated tumors and older die more often.

One of the limitations of this study is that the reading of the slides may present some degree of subjectivity. For future studies, the use of computational systems is recommended to correct this fact and confirm the prognostic value of the alterations studied. In addition, further studies are needed to verify whether these variables are independent prognostic factors. Therefore, we suggest that tumor architecture and mitotic index be evaluated together with HG for a better prognostic evaluation.

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Authors contribution: LF performed the experiment, analyzed the tissue samples and wrote the article. EC analyzed the data. IP analyzed the tissue samples, TC monitored the survival of animals by telephone and analyzed the data. AAM idealized the project and coordinated the revision. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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