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Cytological grading of canine mast cell tumors: correlation with histologic grading and survival time[1]

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ABSTRACT.- Modesto T.C., Gundim L.F., Oliveira L.A., Bandarra M.B., Magalhães G.M. & Medeiros-Ronchi A.A. 2023. **Cytological grading of canine mast cell tumors: correlation with histologic grading and survival time.** *Pesquisa Veterinária Brasileira 43:e07099, 2023.* Laboratório de Patologia Animal, Universidade Federal de Uberlândia, Av. Mato Grosso 3289, Uberlândia, MG 38400-900, Brasil. E-mail: talita.cris@hotmail.com.br

Mast cell tumors are one of the most common neoplasia in dogs and cytopathology and/or histopathology examinations are used for diagnosis. Histologic grading is considered the gold standard test to predict the prognosis of this neoplasia. However, studies have been conducted using the cytological grading system to provide similar information in a faster, less invasive, and more accessible way. This study aimed to investigate cytological graduation and correlate it with histological grading and the survival time of dogs diagnosed with cutaneous mast cell tumors at the Veterinary Hospital of "Universidade Federal de Uberlândia" over five years. For that, cytological and histological slides from 72 animals were reviewed. The statistical methods used were the kappa test for agreement between grading systems, the Kaplan–Meier for survival time, Cox regression for comparison of cytological and histological grades and survival time. The cytological grading when compared to the two-tier histologic grading, high and low grades, had a moderate agreement (kappa 0.566). When the correlation between survival time and the cytological grade was evaluated, there was a higher death rate in the group with high-grade mast cell tumors compared to low grade, pointing to a correlation between survival time and cytological grade (p=0.009). In conclusion, the cytological grade is useful to treatment planning and providing prognostic information that precedes tumor removal, showing a good correlation with the two-tier histologic grading and with the survival time of the animals.

INDEX TERMS: Cytopathology, degree, dogs, histopathology, mast cell tumor, neoplasm, prognosis.

RESUMO.- [**Graduação citológica de mastocitomas caninos: correlação com graduação histológica e sobrevida.**] O mastocitoma é uma das neoplasias cutâneas mais comum nos cães e os exames citopatológicos e/ou histopatológicos são utilizados para diagnóstico. A graduação histológica é considerada padrão ouro para prever o prognóstico dessa neoplasia. Contudo, estudos têm sido realizados visando utilizar graduação citológica para fornecer informações semelhantes de maneira rápida, menos invasiva e mais acessível. Esse trabalho objetivou realizar graduação citológica e correlacionar com as graduações histológicas e com a sobrevida de cães diagnosticados com mastocitoma cutâneo no Hospital Veterinário da Universidade Federal de Uberlândia durante 5 anos. Para isso, lâminas de citologia e histologia de mastocitomas de 72 animais foram revisadas. Os métodos estatísticos utilizados foram teste kappa para concordância entre os sistemas de graduação, método Kaplan-Meier para tempo de sobrevida dos animais, e análise pela regressão de Cox para comparação do grau citológico e grau histológico e o tempo de sobrevida global. A graduação citológica quando comparada com a histológica de dois níveis, alto grau e baixo grau, obteve uma concordância moderada (kappa 0,566). Na avaliação da correlação entre sobrevida e grau citológico,

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houve maior taxa de óbito no grupo de cães com mastocitoma de alto grau, comparado aos de baixo grau, sendo observada correlação entre a sobrevida e o grau citológico (p=0,009). Esse estudo concluiu que o grau citológico é útil para o planejamento do tratamento e para fornecer informações prognósticas que antecedem a exérese do tumor, tendo boa correlação com a graduação histológica de dois níveis e com a sobrevida dos animais.

TERMOS DE INDEXAÇÃO: Cão, caninos, citopatologia, graduação, histopatologia, mastocitoma, neoplasia, prognóstico.

INTRODUCTION

Canine mast cell tumors (MCTs) are one of the most common skin neoplasia in dogs and represent nearly 20% of all cutaneous tumors in dogs (Kiupel 2017). They have a variable biological behavior that ranges from solitary tumors that are treatable with surgical excision, to aggressive neoplasia with high recurrence and metastatic rates (Kiupel et al. 2011, Brocks et al. 2021). Considering these facts, the importance of studies that can contribute to rapid diagnostic tools that provide information about prognostic and predictive factors is clear.

There are numerous factors used to determine the prognosis of canine MCT, like clinical features, histological criteria, immunohistochemical evaluations, and molecular features (Horta et al. 2018). Many available papers concerning cutaneous MCTs suggest schemes to determine the prognosis (Kiupel et al. 2005, Strefezzi et al. 2009, Thompson et al. 2011, Melo et al. 2021). However, currently the therapeutic decisions remain based on the clinical presentation, the histological grade, and the presence of negative prognostic factors (De Nardi et al. 2022). Clinical results based on these parameters show that these are frequently inaccurate, and prognosis may be hard to assess, especially in grade II MCTs (mildly differentiated) (Welle et al. 2008, Horta et al. 2018).

Currently, histological grading of tumors uses the protocol of Patnaik et al. (1984) that grades the tumors in three levels: grade I (well differentiated), grade II (mildly differentiated), and grade III (low differentiated), and also using the system of Kiupel et al. (2011) that grades the tumor as a high or low grade. The limitation of the grading system of Patnaik et al. (1984) is that it attributes high importance to tumor extension and also includes subjective criteria that result in the classification of more than 40% of MCTs as grade II, gathering a broad range of lesions with distinct biological behavior (Gross et al. 2008). Grade II MCTs can account for 80.5% of all MCTs, and they do not correlate with the clinical outcome (Horta et al. 2018). The grading system of Kiupel et al. (2011) decreases the subjectivity of that of Patnaik et al. (1984) and is highly reproducible, which shows a prognostic superiority. Horta et al. (2018) developed a clinical classification system based on the mortality risk of animals with MCT using clinical, histological, immunohistochemical, and molecular criteria, and they concluded that high-grade tumors in the Kiupel system must be included in the intermediary risk group whatever the Patnaik grade. The Kiupel classification should also be used with other prognostic factors.

Some authors propose the evaluation of cell characteristics using cytopathology as a prognostic factor (Strefezzi et al. 2009, Scarpa et al. 2014). In a similar way, Camus et al. (2016), based on the Kiupel classification developed their own algorithm to classify MCTs using cytology, aiming to provide similar information to histological classification. Besides its limits, cytology has the advantage of being a more accessible examination, is less invasive, quicker, and can correctly diagnose around 92-96% of MCTs (Baker-Gabb et al. 2003).

The cytological classification proposed by Camus et al. (2016) showed a good correlation between survival time and histological grade, since dogs with low-grade MCT had an extended survival time. Considering that cytological grade can have the potential for being a helpful tool in therapeutic planning and prognostic determination of dogs with MCT and studies correlating cytological grade and survival time are rare, this study aimed to: realize a retrospective study of MCT cases in dogs attending the Veterinary Hospital of the "Universidade Federal de Uberlândia" (HOVET-UFU); describe breed, sexual, and age features of dogs with MCTs; attribute histological grade to the MCTs using the systems of Patnaik et al. (1984) and Kiupel et al. (2011); attribute cytological grade of MCTs using the algorithm developed by Camus et al. (2016) with Giemsa and Panoptic Fast stains; compare cytological and histological grades of the canine MCTs analyzed; determine the prognostic value of cytological grade, correlating it to survival time of dogs with MCTs.

MATERIALS AND METHODS

Sampling. A retrospective and prospective study was conducted of cases of canine MCT from the archives of the "Laboratório de Patologia Animal" of the Veterinary Hospital of "Universidade Federal de Uberlândia" (HOVET-UFU). Cytological and histological examinations, diagnostic of MCTs that had been analyzed in a five-year period (2016-2021), were used.

The inclusion criteria were: (a) only dogs with MCT that had samples collected from the same nodule for cytological analysis using fine needle aspiration (FNA) and for histological analysis after surgical removal and sent for excisional biopsy were included; (b) a maximum of an eight-week interval between the cytological diagnostic and excisional biopsy for sample collection for histopathology examination; and (c) samples that had at least 100 viable mastocytes for microscopy. Dogs with subcutaneous mast cell tumors or who were submitted to adjuvant or neoadjuvant therapy were excluded. All dogs went through surgery as a curative purpose.

From the medical records of the animals, we accessed the data for age, sex, breed, and tumor location to certify that the collected samples for histopathology were from the same nodule aspirated for the cytological examination. Regarding anatomical localization of the tumors, they were shared between the head, trunk, limbs, and genital area.

Romanowsky staining methods (Giemsa and/or Panoptic Fast) were used to stain the cytological slides. The samples from the excisional biopsy of the MCTs were fixed in 10% formaldehyde, soaked in paraffin, cut at 4 μm, and stained in hematoxylin and eosin (HE), and in addition, they were also stained in Toluidine blue.

Sample grading. Three pathologists analyzed the cytological samples. The cytological grade was determined based on the algorithm developed by Camus et al. (2016), which uses the following criteria: low granularity of mast cells, cellular pleomorphism, binucleation or multinucleation, mitotic figures, and anisokaryosis (Fig.1 and 2). The tumor was classified as high grade if it presented few granules or at least two of the other criteria (Fig.2).

At least two pathologists analyzed all histopathological samples, and both were blinded from the previous cytological findings, and

they were graded according to Patnaik et al. (1984) and Kiupel et al. (2011) (Fig.3 and 4). In the case of disagreement, a third pathologist was involved.

Patient follow-up. To determine the survival time, dogs with MCTs were followed from the excisional biopsy until the day of death, or for at least a period of six months. The evaluation of global survival (GS) time was made, and was defined by the time after biopsy until the day of death due to any cause. The bitches that were still alive were crossed out of the GS time.

Statistical analysis. All statistical analyses were made using commercial software (IBM SPSS Statistics v. 19, IBM, Somers, NY, USA and Prism v. 5.0, GraphPad, San Diego/CA, USA). We calculated the level of agreement (kappa), the specificity and sensibility in the comparison of cytological and histological grades of Patnaik et al.

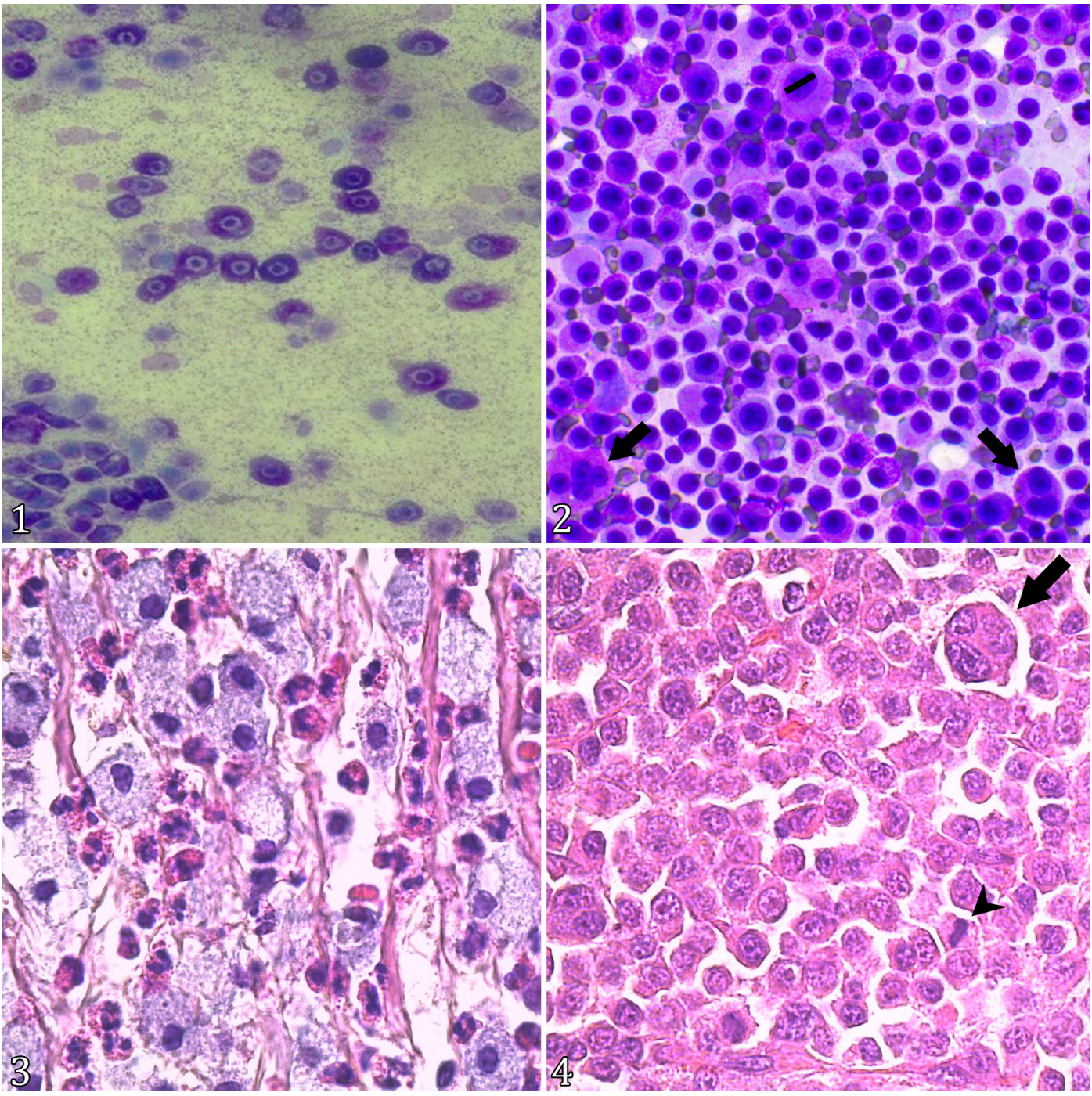

Fig.1-4. Photomicrography of mast cell tumors, skin, dog. (**1**) Low-grade mast cell tumor showing round cells with cytoplasmatic granules. Cytology. Fast Panoptic, obj.40x. (**2**) High-grade mast cell tumor with the presence of binucleated and multinucleated cells (arrows) and anisokaryosis (bar). Cytology. Fast Panoptic, obj.40x. (**3**) Grade I mast cell tumor according to Patnaik et al. (1984) and low grade according to Kiupel et al. (2011) showing well-differentiated mast cell proliferation with apparent granules and organized in a line. Histology. HE, obj.100x. (**4**) Grade III mast cell tumor according to Patnaik et al. (1984) and high grade according to Kiupel et al. (2011) showing a proliferation of mast cells, multinucleated cells (arrow), and mitotic figure (arrowhead). Histology. HE, obj.100x.

(1984) and Kiupel et al. (2011), with the last one being the gold standard method. The agreement level (kappa value [K]) considered K<0 as disagreement, 0-0.20 as poor agreement, 0.21-040 as reasonable agreement, 0.41-0.60 as mild agreement, 0.61-0.80 as substantial agreement, and 0.81-1.00 as almost perfect agreement, all according to Landis & Koch (1977).

The analysis of the GS time used the Kaplan-Meier method with the appliance of the Log-Rank to compare the survival time curves according to cytological and histological grades. Cox regression analysis compared the predictable variables of cytological and histological grades for canine MCT and GS time. The level of significance was $p \leq 0.05$ in almost all of the tests.

RESULTS

At total 83 samples from 72 dogs were present in this study. From these, 62 dogs had samples of only one cutaneous nodule, nine had samples of two cutaneous nodules in different anatomical locations, and one dog had three samples of cutaneous nodules also from different locations. Regarding the anatomical locations, 52 animals had tumors on the trunk (63%), followed by 15 animals on the limbs (18%), 10 on the genital region (12%), and six on the head (7%).

From the 72 dogs, 56 were females (77.8%) and 16 were male (22.2%). The average age of the animals was 9.38 years, and ranged from two to 20 years old. Mixed breed dogs were in the majority, represented by 38 animals (53%), followed by Labrador Retriever with 11 animals (15%), five Pit bulls (7%), four Pinschers (6%), three Boxers and three Basset Hounds (4% each), and two Maltese (3%). The breeds that accounted for less than two animals were entered in the "Others" group, and amounted to six animals (8%).

The MCT grading based on Patnaik showed 15 (18%) samples with grade I, 55 (66.3%) grade II, and 13 (15.7%) grade III. The Kiupel system showed 66 (79.5%) low-grade MCTs and 17 (20.5%) high grade. When it came to the cytological grade based on the algorithm of Camus et al. (2016), there were 61 (73.5%) low-grade MCTs (Fig.1) and 22 (26.5%) high grade (Fig.2).

The correlation between the histological and cytological grades, which had the Kiupel et al. (2011) grading system as the gold standard method, showed a mild agreement, with a kappa of 0.566, sensibility of 76.5%, and specificity of 86.4% (Table 1). The cytological grade correctly predicted the histological grade in 84.3% of cases. However, there was disagreement in 13/83 cases, and nine (40.9%) of these were a false positive, that means cases classified as high grade in cytology were classified as low grade in histology. Furthermore, four of these were false negative and were confirmed as high grade in histology while they were considered low grade in cytology (Table 1). The correlation between the histological grading using the system of Patnaik et al. (1984) and the cytological samples showed a poor correlation, with a kappa value of 0.154.

It was possible to follow-up the clinical evolution of 26 dogs (26/72, 36.11%) during an average period of 455 days (ranging from 1–1400) and 11 animals died (11/26, 42.3%). The average GS time of the dogs with MCTs was of 835 days (confidence interval – CI of 95%, 592-1,077), and the median was 1,000 days (CI of 95%, 220-1,780).

Regarding cytological grade, the high-grade MCT group had a higher death rate (4/6, 67%) than the low-grade MCT group (7/20, 35%). Dogs with high-grade MCTs showed a lower survival time, with a correlation between survival time and cytological grade (p=0.009). The average survival time of the dogs with high-grade MCTs was 138 days with a median of seven days. The dogs with low-grade MCTs showed an average survival time of 957 days with a median of 1,000 days (Table 2) (Fig.5). At Cox regression analysis, the dogs with high-grade MCTs in cytology showed 5.026 more chance of dying compared to those with low-grade cytology (p=0.018).

With the histological grade based on Kiupel et al. (2011), the death rate in the group of dogs with high-grade MCTs (3/6, 50%) was higher than the rate of the group with low-grade MCTs (8/20, 40%), but there was no statistical correlation between survival time and histological grades based on the system of Kiupel et al. (2011) (p=0.566). The average survival time of dogs with high-grade MCTs was 595 days with a median of 70 days. The dogs with low-grade MCTs showed an average survival time of 864 days with a median of 1,000 days (Table 2) (Fig.6). Cox regression could not be used for the risk analysis because the risk rate of the two groups (high and low grades) were not proportional over time.

At the evaluation of the correlation between survival time and histological grade according to Patnaik et al. (1984), the higher death rate belonged to the grade III MCT group (3/4,

Table 1. Correlation of the histological and cytological grades of dogs with mast cell tumors

Cytology grade	Histology grade (Kiupel et al. 2011)*		
	High grade	Low grade	Total
High grade	13	9	22
Low grade	4	57	61
TOTAL	17	66	83
Kappa value		0.566	
Sensibility		76.5%	
Specificity		86.4%	
For positive test results, probability of being:			
False positive		40.9%	
Truly positive[a]		59.09%	
For negative test results, probability of being:			
False negative		6.6%	
Truly negative[b]		93.44%	

* The gold standard pattern is the histological grading of Kiupel et al. (2011); [a] Positive predictable value, [b] negative predictable value.

Table 2. Correlation between survival time of dogs with mast cell tumor and cytology and histology grades

Medium survival time		No. of animals	No. and % of death	p-value
Cytology grade				
High grade	138	6	4 (67%)	0.009
Low grade	957	20	7 (35%)	
Histology grade (Kiupel et al. 2011)				
High grade	595	6	3 (50%)	0.556
Low grade	863	20	8 (40%)	
Histology grade (Patnaik et al. 1984)				
Grade I	411	2	1 (50%)	0.071
Grade II	943	20	7 (35%)	
Grade III	120	4	3 (75%)	

75%) when compared to the grade II (7/20, 35%) and grade I (1/2, 50%) groups, and it did not reveal any statistical correlation between survival time and histological grade based on Patnaik et al. (1984) (p=0.071). The average survival time of dogs with grade III MCT was 120 days with a median of 30 days. The dogs with grade II MCT showed an average survival time of 411 days with a median of 212 days (Table 2) (Fig.7). Similar to the analysis of the system of Kiupel et al (2011), the Cox regression did not apply to analyze the risk rate as the risk rates of the groups (grade I, grade II, and grade III) were not proportional over time.

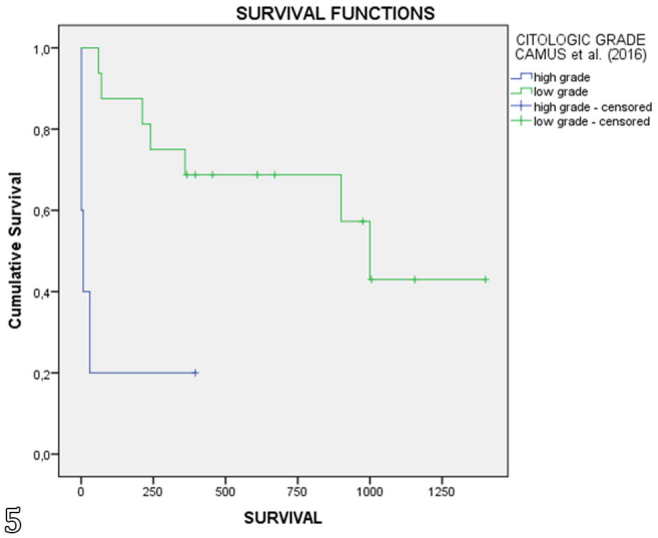

Fig.5. Survival time curve for animals with mast cell tumors (MCTs). Cytology grade: average global survival (GS) time of dogs with high-grade MCTs of 138 days and low-grade MCTs of 957 days (p=0.009).

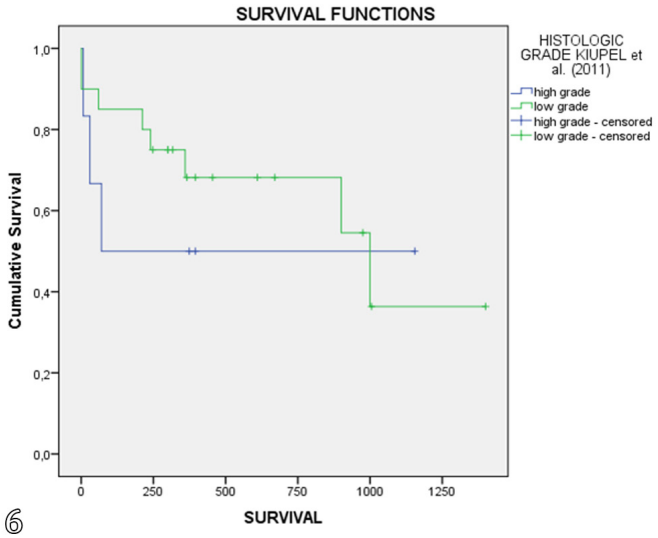

Fig.6. Survival time curve for animals with mast cell tumors (MCTs). Graded according to Kiupel et al. (2011): average global survival (GS) time of dogs with a high-grade MCT was 595 days and with a low-grade MCT was 864 days (p=0.566).

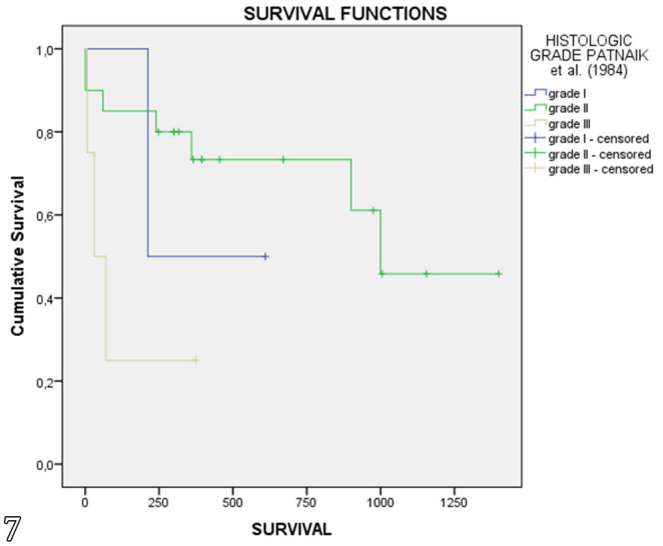

Fig.7. Survival time curve for animals with mast cell tumors (MCTs). Graded according to Patnaik et al. (1984): average GS of dogs with grade I MCT of 411 days, grade II MCT of 943 days, and grade III MCT of 120 days (p=0.071).

DISCUSSION

This study evaluated 72 dogs with MCTs. Regarding the characteristics of the studied population, 77.8% (56/72) were female and 22.2% (16/72) were male. Souza et al. (2018) also verified a higher frequency in females in its study, which was different from Pierini et al. (2019) and Costa et al. (2017) who did not note any sexual predisposition to MCT occurrence. Regarding age, Shoop et al. (2015) reported a higher risk of developing MCTs in dogs older than 10 years and the average age in this paper (9.38 years) was similar to that reported by Kluthcovsky et al. (2020). Mixed breed dogs represented 53% of all animals with MCTs. This result reflects the population stratum of the HOVET-UFU that mainly attends mixed breed dogs. Moreover, other studies from Brazil also describe mixed breeds as the most affected with MCT (Horta et al. 2018, Kluthcovsky et al. 2020). The Labrador breed, observed in 15% of the animals of this paper, was also among the most frequent breed quoted in the studies conducted by Warland & Dobson (2013) and Śmiech et al. (2018). There is no congruence in the literature regarding the most frequent locations of MCT. In this paper, the trunk showed the higher involvement (63%), also described by Pierini et al. (2019) and Kluthcovsky et al. (2020).

Cytological evaluation is indicated as a screening examination for all patients that show cutaneous nodules, and it is a quick, low invasive, and cheap way to diagnose cutaneous MCT in dogs (Marcos & Santos 2011), and the cytological diagnosis of MCT is successfully reached in all cases attending the HOVET-UFU. However, it may be hard to differentiate the aggressive and less aggressive forms in cytology, aiming to help in clinical conduct. With that in mind, some studies have been conducted to verify the usage of the cytological grading method for MCT, which is precise and reproducible, to use it as a prognostic factor for MCT. Even with the proposes of cytological grading of Scarpa et al. (2014), Hergt et al. (2016) and Camus et al. (2016), the histopathology grading remains

the gold standard method (Patnaik et al. 1984, Kiupel et al. 2011, Willmann et al. 2021).

Camus et al. (2016), in their study, had as one of their goals to generate a high agreement between the cytology and histology grades in order to predict tumor behavior before surgery. This paper shows that this was possible in 84.3% of cases, and it created a mild agreement with the histological grading system of Kiupel et al. (2011).

This paper shows a higher number of high-grade MCTs in cytology than histology, in other words, 40.9% of the cases were false positive. Similarly, Kiupel & Camus (2019) once noted that the positive predictive value of the cytology grade is low, and like Camus et al. (2016) described that 31.8% of their samples were false positive. In this way caution must be exercised before the decision-making of the clinical conduct of these animals, since there is a possibility of the high-grade tumors in cytology actually being low-grade tumors in histology.

Still, as cytology is a screening examination, false positive results can happen without greater harm to the animals, since the main inconvenience of a high-grade tumor in cytology is that the animal may go through an invasive surgery with no need, while the false negative can allow a more aggressive tumor to remain untreated (Camus et al. 2016).

The data shown in this paper reinforce the observations of Camus et al. (2016), since low-grade neoplasia were the majority (73.5%) in the cytology grade. Other papers that also adapted the grading system of Kiupel et al. (2011) for cytology showed a prevalence of low-grade tumors (Scarpa et al. 2014, Hergt et al. 2016).

Furthermore, while different authors reported that grade I MCT animals showed a better prognosis (Patnaik et al. 1984, Sabattini et al. 2015, Stefanello et al. 2015, Willmann et al. 2021), in this paper the animals with grade I did not reach the average survival time. However, few animals with grade I were clinically followed in this paper. One of the disadvantages of the grading system of Patnaik et al. (1984) is that most of the tumors receive grade II classification (Northrup et al. 2005, Sabattini et al. 2015, Stefanello et al. 2015, Camus et al. 2016). That way, the high number of animals with grade II tumors, which was 66.3% in this paper, and consequently the low number of animals with grade I and II tumors harmed the prognostic evaluation of this grading system.

Regarding the grading system of Kiupel et al. (2011), most of the tumors were low grade (79.5%), a higher result than that shown by Horta et al. (2018) and Sabattini et al. (2015). These low-grade MCT animals had an average survival time of a little longer than two years, similar to what Kiupel et al. (2011) reported. Horta et al. (2018) and Sabattini et al. (2015) affirm that animals with high-grade tumors have a higher risk of death when compared to animals with low-grade MCTs, but the limited number of high-grade tumors made it difficult to evaluate the clinical follow-up of these animals in this paper. Even though there was no statistical difference between the survival times of animals with low-grade and high-grade tumors, the animals with low-grade MCTs reached the median survival time, whereas those with high-grade tumors did not, which shows a tendency of animals with high-grade tumors to have a shorter survival time.

Even though Strefezzi et al. (2003) described one of the advantages of the cytopathological examination being the possibility of having a better cellular description, once there is loss of details in the material section, like in the histopathological examination, Berlato et al. (2021) say that the staining used in cytology may stain the granules in a very intense way, which harms the evaluation of nuclear pleomorphism. Once the cytology classification based on Camus et al. (2016) considers both nuclear pleomorphism and granulation, it becomes necessary to use stains that favor both features. According to the second consensus of diagnosis, prognosis, and treatment of MCT (De Nardi et al. 2022), the Romanowsky stains (Panoptic Fast and Giemsa), which were the stains used in this study, are efficient to stain the mastocyte granules. In a similar way, Barbosa et al. (2014) described that Diff-Quick is suitable for nuclear morphometric analysis. That way, in this paper, in both staining methods, the identification and quantification of the granules of the mastocytes were possible, as well as the nuclear pleomorphism.

Berlato et al. (2021) noted some limitations of grading using the cytopathological examination, among them the differentiation of cutaneous and subcutaneous MCT frequent in the cytology routine. However, this paper included only cutaneous MCT confirmed by histology. Another limitation according to Berlato et al. (2021) could be the evaluation of mitotic activity, also shown by this paper. Even with all the limitations of the technique, in this paper the cytology grading was an excellent tool to add to the routine of the oncological patient as a form of evaluation of the prognosis of MCTs. This is because the animals with high-grade tumors in cytology did not reach the average and showed a higher chance of dying than the dogs with low-grade tumors in cytology. Camus et al. (2016) also observed that animals with high-grade tumors showed a risk of death 25 times greater than those with low-grade tumors, and stated that the cytology grade is a useful predictor for treatment planning and prognosis. Nonetheless, the high number of false positive animals (high grade in cytology and low grade in histology) reinforces that the gold standard method for grading of MCT is histology, and its usage is necessary even in animals that undergo a cytological examination prior to surgery. Kiupel & Camus (2019) state that the cytological grade helps in the initial decision-making, but the histological grade in two grades, proposed by Kiupel et al. (2011) is the best way to identify high-grade neoplasia.

This paper declares as one limitation the small number of animals that could be followed, which is a reality in research using survival time. However, this is the first paper in Brazil to use the cytology grade according to Camus et al. (2016) with patient follow-up.

CONCLUSIONS

As far as the authors are concerned, this is the first Brazilian study to compare the cytology grade to the histology grade of mast cell tumors (MCTs), and also to present a great number of dogs with follow-up.

Canine MCT have been diagnosed frequently at HOVET-UFU, and involve mainly adult to elder dogs, females, mixed breeds and Labradors.

The canine mast cell tumor grading through cytology is useful to provide information about prognosis that precedes the surgical removal of the tumor, besides showing a moderate agreement with the histological grade.

Furthermore, the cytology grade is related to survival time, showing that dogs with a high grade at cytology have a shorter survival time than dogs with a low grade at cytology.

Conflict of interest statement.- The authors have no competing interests to declare that are relevant to the content of this article.

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