Clinical follow-up of canine mast cell tumour cases diagnosed by cytology and histopathology

Sebastián Cifuentes-Arias, Lina Osorio-Morales and Francisco Pedraza-Ordóñez*

Abstract

Canine mast cell tumours (MCT) are malignant neoplasms, and are possibly the most common skin cancers in dogs. A precise diagnosis is fundamental in making appropriate therapeutic decisions and thus avoiding a poor prognosis, which frequently ends in rapid death. In this study, data from 59 dogs diagnosed with mast cell tumours of three malignancy grades were selected and clinical follow-up was carried out over 47 months to determine the relationship between the decision to perform surgery, the degree of tumour malignancy and the survival status of the animals. The results showed that the majority of affected animals died as a result of the disease, some of which had undergone surgical treatment. The surviving dogs were diagnosed mostly with mast cell tumours of low malignancy. There was no significant association between surgery and survival. We conclude that cytological analysis is fundamental for an early diagnosis of canine MCTs and enable a more accurate prognosis to guide subsequent treatment. We discuss the need for clear malignancy criteria allowing for adequate cytological classification of these lesions.

Key words: *cancer; cytology criteria; diagnosis*

Introduction

A mast cell tumour is a malignant skin neoplasm that can affect dogs of any age, although it occurs often in animals from 8 to 10 years old. It can develop anywhere on the body surface but most commonly arises on the extremities (especially the thigh), ventral abdomen, and thorax (O'Keefe, 1990). The external appearance of an MCT corresponds to a raised and nodular mass, soft or solid, that is generally well circumscribed with a diameter ranging from 1–10

Sebastián CIFUENTES-ARIAS, DVM, Researcher, Veterinary Pathology Research Group, Universidad de Caldas, Manizales, Colombia; Lina OSORIO-MORALES, DVM, MSc, Professor, Veterinary Pathology Research Group, Universidad de Caldas, Manizales, Colombia, Veterinary Medicine Program, Corporación Universitaria Santa Rosa de Cabal – UNISARC, Santa Rosa de Cabal, Colombia; Francisco PEDRAZA-ORDÓÑEZ*, DVM, PhD, Titular Professor, (Corresponding author, e-mail: fpedraza@ucaldas.edu.co), Animal Health Department, Universidad de Caldas, Manizales, Colombia

centimetres (occasionally larger), although these lesions can also present as an oedematous, poorly defined swelling (Dobson and Scase, 2007). Histologically there has been an evolution in the interpretation of morphological changes in an MCT. Initially, they appear in three grades (Lee Gross et al., 2005). Grade I refers to a solitary tumour confined to the dermis without lymph node involvement, round or ovoid mast cells of uniform size and well-defined cytoplasmic borders, uniformly spherical nuclei, large granules in the cytoplasm, and uniformly distributed cells separated by collagen fibres. An intermediate grade II MCT is a solitary tumour with regional lymph node involvement and neoplastic cell infiltration in the deep dermis. These lesions are similar to those of grade I but have variable cell sizes, cytoplasmic borders that are not always well defined, and large and slightly vesicular nuclei. Grade III or anaplastic MCTs are characterized by multiple dermal tumours that can affect the lymph nodes. These cells are distributed in a way that forms large masses of neoplastic tissue with marked pleomorphism and have large nuclei with irregular internal vesicles and generally one to three prominent nucleoli. Sometimes very numerous mitotic figures appear in grade III MCTs, and the cytoplasm has indefinite edges (Patnaik et al., 1984).

In some cases, it is difficult to distinguishing grade II MCTs from the other categories. Taking the biological behaviour of these neoplasms into account, Kiupel et al. (2011) proposed a new classification involving two stages of malignancy, *i.e.* a low degree or a high degree of malignancy. This grading system has since been adopted by a number of pathologists, although the old classification is still used. Cytologically, there is no specific classification for the different grades of MCT (Pedraza et al., 2011), though in this regard Kiupel

and Camus, (2019) proposed that their two histopathological stage approach has utility since many of its parameters coincide between histology and pathology. Cytologically, a canine MCT is a round cell neoplasm that can also be staged as a well, moderately or poorly differentiated lesion (Raskin, 2010). The behaviour of canine MCTs varies from animal to animal, though these lesions should be considered malignant in all cases. These tumours generally start to spread to the regional lymph nodes, spleen, liver, kidneys, lungs, and heart. Dogs affected by MCTs also develop gastroduodenal ulcers at a relatively high frequency due to the release of vasoactive amine from malignant mast cells (Dobson and Scase, 2007). The most effective treatment is surgery, though only if conducted by an experienced surgeon, as a lack of expertise with this procedure will likely result in a very poor outcome (Simpson et al., 2004). Some authors have reported that chemotherapy can maintain animals in a reasonably stable condition for a few months, though this will not be curative (Grier et al., 1995). A clinical follow-up of the survival outcomes of dogs affected by MCTs of different malignancy grades, diagnosed by cytology and histopathology, is described in this study.

Material and Methods

This was a retrospective descriptive study of 59 dogs diagnosed with MCT over a two-year period from January 2016 and January 2018 at the Veterinary Pathology Laboratory of the Universidad de Caldas, Colombia. Survival monitoring was carried out until December 2019. The animals were presented at the veterinary hospital or other regional clinics as part of an outpatient care routine. In most cases, the neoplasia diagnosis was made by cytology (fine needle aspiration) in which slides were air-dried and stained

Table 1. Morphologic criteria currently used for grading canine mast cell tumours in cytopathology (Pedraza et al., 2011)

Cytologic Grade	Criteria Cell	Criteria Nucleus
Well differentiated	Mild anisocytosis, Mild pleomorphism, Hypercellularity	Mild anisokaryosis
Moderately differentiated	Moderate anisocytosis, Moderate pleomorphism, Hypercellularity.	Moderate anisokaryosis Moderate increase in nuclear-cytoplasmic ratio Moderate atypical mitotic figures
Poorly differentiated	Marked anisocytosis, Marked pleomorphism, Hypercellularity	Marked anisokaryosis Marked increase in nuclear-cytoplasmic ratio Increased atypical mitotic figures Multinucleated cells

with Kwik-Diff® and Giemsa. MCTs were staged as being of low-grade, moderate or high-grade malignancy. Table 1 summarizes the cytological criteria used for the animals, as described by Raskin, (2010) and Pedraza et al. (2011). Additionally, all cases were reviewed by histopathology, i.e. tissues were stained with haematoxylin-eosin (H&E) and then stained with Giemsa. Tumours were graded as grade I, II, or III, as suggested by Patnaik, (1984).

The cases were reviewed by light microscopy (both cytology and histopathology) by two pathologists, and only cases with no controversy regarding the diagnosis were included in this present study. The medical records of each animal were reviewed and data were collected on breed, sex, age, and history of presentation of MCT. We also collected the dates of diagnosis and surgery, as applicable, and whether the dog was alive at the cut-off date for the investigation.

Since cases were approved by different veterinarians and veterinary surgeons and animals were attended to at different times, it was not possible to obtain details of the chemotherapy protocols that some animals underwent or the surgical techniques used in some cases. It was only known that most cases were treated by surgical excision and regional adenectomy was not considered in any of these animals. For statistical analysis, an adjusted binary logistic regression model was used, where the dependent variable was survival and the independent variables were the performance of surgery and the MCT grade. SPSS version 23 software was used to conduct the statistical comparisons.

Results

Most of the dogs affected by MCT enrolled in this study (46/59; 78%) were of a defined breed, including Labradors, French poodles and the Pitbull terriers. Eleven dogs (18.6%) were mongrel breeds and no breeding data were available for the remaining 2 animals (3.4%). At the time of admission, the youngest dog was one year of age and the oldest was 14 years old. Most of the affected animals were of mid-age i.e. 15 (25.4%) were under 5 years, 35 (59.3%) were between 5 and 10 years and 9 (15.3%) were over 10 years. Almost half (29) of the animals were female.

Table 2. Survival outcomes among the canine subjects by the end of the study

Diagnosis	Status	With surgery	Without surgery	No Data	Total
MCT grade I	Alive	9	11	1	38
	Died	13	4		
MCT grade II	Alive	3	2	2	14
	Died	4	3		
MCT grade III	Alive	0	0	0	7
	Died	5	2		

With regard to the MCT diagnoses in our current canine series, the majority of cases (38/59; 64.4%) showed first grade malignancy (grade I), 14 (23.7%) showed grade II and 7 (11.9%) displayed grade III lesions (Table 2). It was determined that 31 of the animals (52.5%) died as a consequence of the disease and 25 (42.4%) were alive at the end of this study, with no survival data available for the remaining 3 cases (5.1%). Of the 38 animals affected by a grade I MCT, 17 (44.7%) died as a result of the disease, 20 (52.6%) were alive at the end of the study period and no data were available for one animal. Of the 14 dogs affected by grade II MCT, 7 (50%) died, 5 (35.7%) survived and 2 had no data. For the 7 animals affected by grade III MCT, the mortality rate was 100% during the study period (Table 2).

With regard to the mortality rates and the decision to perform surgery, 13/38 (34%) dogs with a grade I MCT died between 120 and 180 days after surgery whereas 4 animals (10.5%) died of the disease, with some receiving cytotoxic drugs. There were no data available for one animal. The remaining animals with a grade I lesion (20/38; 52.6%) were living at the end of the study and the majority (11) did not undergo surgery or medical treatment (except for one case treated with glucocorticoids). Four of the animals (4/14; 28.6%) with a grade II MCT died (maximum 180 days) after surgery whilst 3 cases (21.4%) died from the disease without having undergone surgical

treatment. Two grade II cases had no data and the remaining 5 animals (35.7%) were still living at the end of the study (three with post-surgical follow-up between 690 and 750 days and two without surgery who had between 1260 and 1290 days of clinical follow-up). The dogs with a grade III MCT all died between 60 and 120 days after diagnosis, with 5 animals (71.4%) dying after surgery and the remaining 2 dogs (28.8%) succumbing to the severity of the disease without having the opportunity to receive any type of treatment (Figure 1).

The animal observed for the least time in our current series was a 10-year-old female Pointer that survived for 690 days
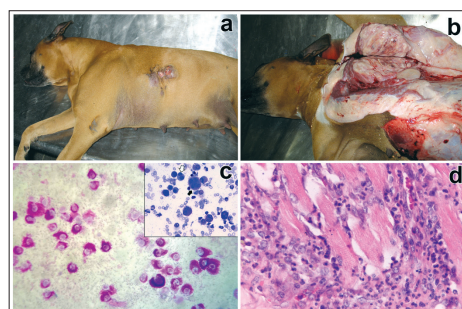
Figure 1a. Cutaneous MCT initially seen as solitary lump in underneath the trunk skin; **b**. Presence of axillary lymph node metastases in a grade II MCT; **c**. Cytological photomicrograph of undifferentiated mast cell tumour stained with Wright; in the upper right box, binucleated cells (white arrow) and multinucleate cells (black arrow) stained with Giemsa; **d**. Histological picture of muscle tissue underlying grade III MCT showing malignant cell infiltrate

after excision of a grade II MCT from the belly. The longest observation time was for a three-year-old female Pitbull that survived for 1290 days after excision of a soft mass, grade I MCT located in the skin of the right flank that had shown progressive growth of approximately one year. The longest surviving animal in our current cohort was a male Pinscher with a grade I MCT in the scrotum, who died as a consequence of systemic disease 31 months after having undergone an orchiectomy.

Among the dogs in our present series that did not undergo surgery, an 8-year-old female Labrador that presented with a small mass in the interdigital area of the left thoracic limb, a grade I MCT, had the shortest survival time (1230 days). Another female Labrador of 7 years of age who had masses in the mammary gland and a grade I MCT in the right pelvic limb was still living without having undergone surgery after 1320 days of clinical follow-up.
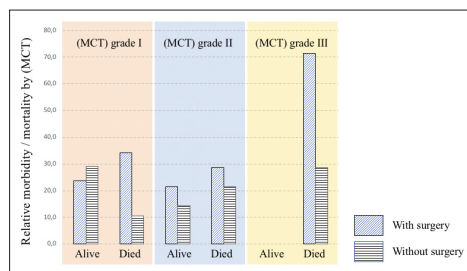
Figure 2. Survival outcomes (with and without surgery) for canine subjects with different grades of MCT. The mortality rate was 100% for grade III MCT cases

Necropsy was performed on two animals diagnosed with grade III MCT and revealed metastatic processes in the regional lymph nodes, and in the spleen in one case. No metastases to the lung or other organs were observed (Figure 2). Overall, there was no significant association found between surgery and survival outcomes ($P = 0.08$).

Discussion

Neoplasms of the cutaneous system are frequent in dogs, with MCT being the most common, followed by the transmissible venereal tumour and other round cell neoplasms such as histiocytoma and basal cell epithelioma (Duncan and Prasse 1979; Ferreira De La Cuesta, 2003). Canine MCT has a somewhat benign clinical appearance but is a highly aggressive tumour that almost always results in the death of the animal (Welle et al., 2008). In the present study of canine MCT cases, there appeared to be a disease predilection for dogs of a defined breed, regardless of sex, similar to reports by Shoop et al. (2015). We also observed that most animals affected by MCT in our current cohort died, with a 100% mortality rate seen for the grade III cases. In contrast, a number of animals with a grade I MCT (*i.e.* a well-differentiated tumour) were still living at the end of the study period. It is possible that the better survival rates in grade I cases may be related to the decision to not intervene surgically with some of the higher grade tumours, and to operate only in cases with a low degree of malignancy and at sites that enable wide surgical margins. However, this could not be fully elucidated in this study. Notwithstanding that issue, since MCT is an aggressive neoplasm that can invade underlying tissues and metastasize to other organs, a moderately invasive method for making an initial diagnosis such as fine needle aspiration cytology (FNAC) is essential in order not to stimulate the growth of these lesions and also to avoid surgery when the site of the tumour is difficult to access. This could be a decisive determinant of the animal's survival time.

None of the dogs in our present study series appeared to show any signs of a cutaneous mass as a underlying reason for their initial consultation. However, it is always important to investigate for possible non-cutaneous clinical signs, such as gastric ulcers caused by the release of histamine, leading to

vomiting, diarrhoea and anorexia, as well as alterations in the coagulation process (MACY and MACEWEN, 1989; Rogers, 1993; Lemarié et al., 1995). In our experience, cytology is essential for the rapid and effective diagnosis of canine MCT, and this may well improve survival outcomes by assisting with the decision regarding surgery or medical management. According to the current literature, once MCT is diagnosed, a new physical examination is recommended to determine whether there is any regional lymph node involvement and to rule out the presence of splenomegaly or hepatomegaly, which can be evidenced by radiography or ultrasound, thus excluding systemic neoplasia (O'Keefe, 1990). None of the animals in our current investigation showed lymph node involvement after a physical examination, and cytology samples were therefore not sent for this type of tissue. Although some authors have suggested that cytology is a science in itself with independent criteria (Thrusfield, 1995; Ghisleni et al., 2006), we contend that all diagnostic tests should be compared with a gold standard to confirm their accuracy. Thus, we believe that a less subjective and more reliable cytological classification system is required, including previously suggested additional criteria for malignancy that are commonly used in human medicine (Pedraza et al., 2011).

In our present investigation, some dogs with low-grade MCT that underwent surgery were still living at the time of verifying their clinical condition. This could be related to the prior recommendation of Martínez-De-Merlo (2000) that surgical excision remains the treatment of choice for canine MCT, as long as the masses are well defined and delimited. Other recommendations include removing the closest lymph node and skin three centimetres from each side of the tumour to a depth of one centimetre (Simpson et al., 2004). It must be noted that locally invasive MCT may be difficult or impossible to completely eliminate in some places, such as the oral mucosa, mouth, nose, thoracic and pelvic limbs due to the limited surgical margin (Davies et al., 2004). It is possible that some of the animals included in this study did not undergo surgery precisely due to the difficulty of the surgical approach. Although this could not be conclusively demonstrated, this could be related to a greater probability of survival.

Conclusions

Mast cell tumour is an aggressive neoplasm with variable biological behaviour. An early and non-invasive diagnosis facilitates decision-making regarding treatment. Cytology is a fast and inexpensive technique that showed great utility for the diagnosis in cases of canine MCT and possibly, the therapeutic decisions associated with its implementation. This was directly related to the increase in the survival period in some of the cases presented here.

Acknowledgment

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Kliničko praćenje slučajeva mastocitoma u pasa koji su dijagnosticirani citološki i histopatološki

Sebastián CIFUENTES-ARIAS, DVM, Researcher, Veterinary Pathology Research Group, Universidad de Caldas, Manizales, Colombia; Lina OSORIO-MORALES, DVM, MSc, Professor, Veterinary Pathology Research Group, Universidad de Caldas, Manizales, Colombia, Veterinary Medicine Program, Corporación Universitaria Santa Rosa de Cabal – UNISARC, Santa Rosa de Cabal, Colombia; Francisco PEDRAZA-ORDÓÑEZ, DVM, PhD, Titular Professor, Animal Health Department, Universidad de Caldas, Manizales, Colombia

Mastocitomi u pasa (MCT) maligne su neoplazije te vjerojatno najčešći oblik raka kože u pasa. Precizna dijagnoza ključna je za donošenje ispravnih odluka u svezi liječenja, a tako i za izbjegavanje loših prognoza koje često završavaju brzom smrću. U ovom istraživanju odabrani su podatci 59 pasa kojima je prethodno dijagnosticiran mastocitom tri različita stupnja malignosti te je provedeno kliničko praćenje tijekom 47 mjeseci za ustvrđivanje odnosa između odluke o operaciji, stupnja malignosti tumora i statusa preživljavanja životinja. Rezultati su pokazali da je većina oboljelih životinja, od kojih su neke bile podvrgnute operaciji, uginula uslijed bolesti. Psima koji su preživjeli uglavnom je dijagnosticiran tumor niske malignosti. Nije bilo značajne povezanosti između operacije i preživljavanja. Zaključujemo da je citološka analiza presudna za ranu dijagnozu MCT-a u pasa i za omogućavanje preciznije prognoze koja će voditi daljnje liječenje. Raspravljamo o potrebi uspostave jasnih kriterija malignosti da bi se omogućila prikladna citološka klasifikacija ovih lezija.

Ključne riječi: *rak, citološki kriteriji, dijagnoza*