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Review article

Prognostic indicators for mast cell tumors

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Abstract

Mast cell tumors are among the most common canine cutaneous neoplasms. These tumors are routinely graded with regard to malignancy based on histomorphological features which are related to the post-surgical course of the disease. This classification is the main criterion for clinicians and surgeons to direct the therapy. However, mast cell tumors biologic behavior is extremely variable, leading to frequent failure in treatment. This occurs, for the most part, in tumors with moderate differentiation and can be explained by the fact that they share histomorphological features with the well-differentiated and the poorly-differentiated tumors. Because of these difficulties, several methods have been studied with the objective of predicting tumor behavior and the evolution of the cases in a more precise and trustful manner. The present work reviews the main aspects of this important neoplasm, as well as the available literature about the prognostic indicators, commenting their advantages, disadvantages and the results obtained by various authors.

Key Words: Mast cell tumor, mastocytoma, neoplasia, pathology, diseases of dogs

Introduction

The mast cells

It was because of their "well-nourished" appearance, due to the presence of cytoplasmic granules, that Paul Ehrlich, in 1877, baptized mast cells with the name "mastzellen", from the German word "mast", that means food (EHRLICH, 1956 apud MACY, 1985, p. 783)[1]. The first canine mast cell was described in 1905 by Bashford et al. (apud MACY, 1985, p. 783)[2] and the word "mastocytoma" was first mentioned by Bloom, in 1952 (apud MACY, 1985, p. 783)[3].

Mast cells are pleomorphic cells on histological evaluation: spherical, stellate or spindle-shaped. They have round nuclei and show cytoplasmic granules which contain acidic proteoglycans. This intracellular material assumes violaceous tonality when stained with Romanowsky-type stains or Toluidine blue and are called "metachromatic". The granules are composed by several biologically active substances such as histamine, proteolytic enzymes and heparin. Mast cells are found in different locations such as

[1] EHRLICH P. Beitrage zur theorie und praxis der hischen farbung hemmelweit's collected papers of Paul Ehrlich. Elmsford, New York: Pergamon Press, 1956: 65-98.

[2] BASHFORD EF., MURRAY JA., CRAMER W. Comparison between the transmission of an effective granuloma of the dog and carcinoma of the mouse. *Sci. Rep. Cancer Res. Fd.*, 1905, 2, 33-37.

[3] BLOOM F. Effect of cortisone on mast cell tumors (mastocytoma) of the dog. *Proc. Soc. Exp. Biol. Med.*, 1952, 79, 651-654.

lungs, gastric mucosa, dermis and perivascular region. They vary in distribution depending on the animal species and the number of mast cells tends to increase with age (2, 11, 22, 40, 46, 58). Neoplastic mast cells also have granules which vary in number according to tumor differentiation (58, 81).

Mast cells originate from CD34 pluripotent hematopoietic cells and, after migration to peripheral tissues, undergo *in situ* differentiation (27). In rodents, mast cells can assume two distinct phenotypes: "mucosal-type", in the gastrointestinal tract, which contains chondroitin sulfate as the main granular proteoglycan; and "connective tissue-type", found in the lungs and serosal membranes, which contains heparin and histamine. In humans, mast cells phenotypes are not as clearly defined as in mice, but it is known that the main differences reside in granule contents (2). In dogs, three types of mast cells have been described according to their protease, chymase and tryptase content (6, 10, 32). Basophils share similarities with mast cells, but must be considered a distinct cell type because they undergo differentiation in the bone marrow, circulate and emigrate from blood vessels when recruited by inflammatory responses, just like other granulocytes (2, 52).

Mast cells stimulation results in three types of biological responses: (1) degranulation, in which the preformed granule content is released by exocytosis; (2) synthesis of membrane-derived chemical mediators, such as arachidonic acid; and (3) transcription, translation and secretion of cytokines. Degranulation may be triggered by physical, chemical, neurological and immunological stimuli. The released substances cause vasodilation, increased vascular permeability, smooth muscle cells contraction, mucus secretion, neutrophils and eosinophils chemotaxis, pruritus, anticoagulation and tissue destruction. Mast cells are also capable of producing leukotrienes and TNF-α when stimulated by IgE. It was verified, by means of electron microscopy, that mast cells have cytoplasmic membrane excess after degranulation due to exocytosis (2, 11, 33, 40, 52).

Estrogen and progesterone cytoplasmic receptors were identified in canine neoplastic mast cells, and estrogen administration in primates inhibited mast cell degranulation by chemical stimuli (17). In dogs, the presence of estrogen receptors did not correlate to the histopathological grading, suggesting no relation between this characteristic and tumor aggressiveness (34). Cytoplasmic glucocorticoid receptors were also identified in murine mast cells, helping to explain the responses of some mast cell tumors to glucocorticoid therapies (40). A recent study detected higher expression of PGE2 in grade III mast cell tumors by immunohistochemical methods (61).

In humans, mast cell proliferation, with accumulation in one or more organs, is called "mastocytosis". This disease is considered a hematopoietic disorder that may assume cutaneous or systemic forms. Mastocytosis has several behaviors and clinical manifestations, from simple cutaneous lesions that involute spontaneously to aggressive and systemic disease. Unlike canine mast cell tumors, the incidence is low and it is considered a rare condition (23, 33, 83).

General aspects of canine cutaneous mast cell tumors

Incidence and Etiopathogenesis

Mastocytomas are the most common skin neoplasm in dogs, representing 7-25% of all skin tumors and 11-27% of the malignant skin neoplasms in this species. Sexual predilection was not identified and the incidence increases with age, affecting animals about 8.5 years old. Boxer, Boston Terrier, English Bulldog, Labrador, Golden Retriever, Shar Pei and Pug are reported as the most susceptible breeds (8, 21, 22, 25, 26, 38, 48, 52, 53, 60, 69, 76, 78, 79).

Although mast cells are abundantly found in the lungs and gastrointestinal tract, the majority of mast cell tumors in dogs arise from the dermis and subcutis. Primary mast cell tumors in other locations are considered uncommon (8, 38). The occurrence of mast cell leukemia is rare, more frequently occurring as a consequence of systemic dissemination of the cutaneous mast cell tumors (38, 47).

Among the cutaneous lesions, the most common sites are: trunk and perineal region (50-63%), extremities (33-40%) and head and neck (10-15%). Multiple tumors were observed in 3-14% of the cases (1, 21, 38, 69, 76). Strefezzi (2007) reported the extremities as the most common location (36.5%), followed by the thorax (22.6%), inguinal/perineal region (17.2%), abdomen (15%) and head and neck (6.5%). Multiple lesions were diagnosed in 18.9% of the animals in this research.

These neoplasms show extremely variable macroscopic characteristics, which makes them difficult to diagnose using just these features, as well as to predict their behavior. Dermal tumors are well-defined raised masses, frequently alopecic and erythematous. Ulceration and pruritus may be present. Most of the mast cell tumors are less than 3 cm in diameter, but some may reach 10 cm or more. They can also occur as diffuse skin masses. Subcutaneous tumors are soft ill-defined haired masses without erythema and pruritus, often mistaken for lipomas (25, 38, 87).

Mast cell tumors are composed by round cells, arranged in rows or groups, with variable amounts of cytoplasmic granules. The nuclei may vary in shape and size, depending on the grade of tumor differentiation. Bi- or multinucleated cells are occasionally found, in addition to eosinophils, areas of hyalinization and necrosis. Mitotic figures are common in poorly-differentiated tumors (8, 25, 60).

This neoplasm has already been experimentally transmitted using tumor extracts, suggesting viral etiology.

Nevertheless, no viral particles have been demonstrated (43, 52). Hereditary transmission in some breeds like Boxer, Bulldog and Boston Terrier was also investigated as a possible etiology. Instabilities in chromosomes 3, 4 and 15 have been detected, but these abnormalities were interpreted as consequences of aging, not a racial or familiar disease (21, 77).

Mutations in the proto-oncogene KIT (c-kit/CD117), that encodes stem cell factor receptor (SCR), seem to be involved in mast cell tumor pathogenesis. KIT is a transmembrane receptor with tyrosine-kinase activity and induces cellular proliferation and differentiation. It is expressed in mast cells and hematopoietic cells and has been used as an immunohistochemical marker for mastocytomas. Duplications and deletions of KIT juxtamembrane domain have been described and related to aggressiveness of the tumors (36, 37, 52).

Diagnosis

Mast cell tumors are diagnosed by means of fine needle biopsy or imprint of the lesions on microscopy slides. The most appropriate staining methods are Romanowsky-type or New Methylene Blue (38, 40, 58). Mastocytomas are included in round cell tumors, along with lymphoma, plasma cell tumor, histiocytoma, melanoma and transmissible venereal tumor (15, 38).

The cytoplasmic granules, an important diagnostic feature (Fig. 1), may not be stained with Diff-Quik™, unless the smears have been fixed for several minutes. Moreover, in poorly-differentiated tumors, the quantity of granules is frequently insufficient to visualize (Fig. 2). In these situations, Giemsa or Toluidine blue are better histochemical methods, identifying granules in purple (38). Immunohistochemistry with anti-KIT, anti-tryptase or anti-chymase antibodies may be helpful in the diagnosis of the poorest differentiated tumors (28). In a retrospective study, the concordance for cytopathological and histopathological diagnosis reached 94% (15). Some authors affirm that poorly granulated cells are more easily recognized in cytopathology than in histopathology because of the resolution of cytoplasmic granules (15, 40). The evaluation of every nodule in the same animal is considered important because mast cell tumors may be multiple or occur simultaneously with other different pathologic processes (38).

Mast cell tumors may also be diagnosed by histopathology when samples are collected by surgical excision, “punch” or “tru-cut” needles (15, 38). The main contribution of histopathology is the possibility of grading the lesions, discussed further in the text, besides the confirmation of cytopathological diagnosis.

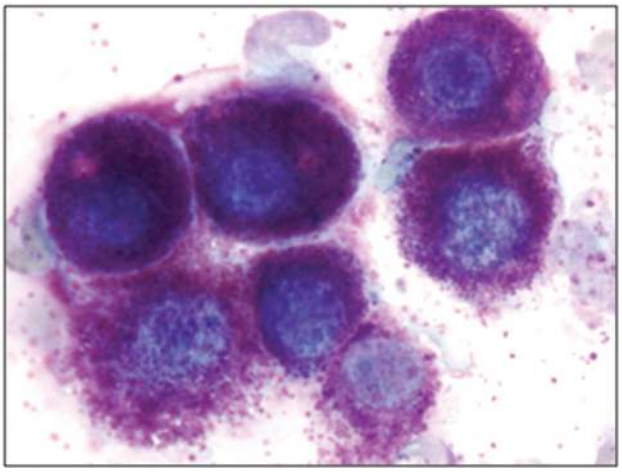

Figure 1 – Photomicrograph of a well-differentiated mast cell tumor on cytological smear, showing abundant cytoplasmic granules. Panoptic stain, Obj. 100x.

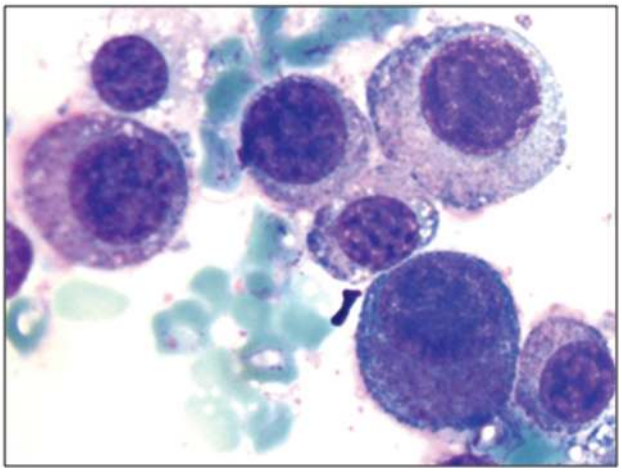

Figure 2 – Photomicrograph of a poorly-differentiated mast cell tumor cytological smear, showing scant cytoplasmic granules. Panoptic stain, Obj. 100x.

Biological behavior and metastasis

Mast cell tumor behavior is extremely variable, which makes clinicians and surgeons consider this neoplasm as potentially malignant. Approximately 50% are aggressive tumors, with recurrence and/or metastasis rates reaching 80% of the cases. Metastases are more frequently found in regional lymph nodes, spleen and liver (40, 58, 76, 87).

Other reported complications comprehend gastroduodenal ulceration, coagulation disorders and delayed wound healing (22, 40, 58, 68, 87). Ulceration is more common in the stomach than in the duodenum, with a reported frequency of 25-83% of the necropsied animals (40, 58, 87). Hypotensive shock may occur as a consequence of excessive degranulation of mast cells during manipulation or cryosurgery (68, 87). Multifocal glomerulitis was observed in 70% of the dogs, and was

characterized by plasma cell infiltration, focal thickening of the glomerular capillary basal membrane and Bowmann capsule (22).

Prognostic indicators

Cutaneous mast cell tumors have caused many therapeutic frustrations. Due to the difficulties in predicting its biological behavior and, consequently, defining the treatment protocols, the search for more precise prognostic indicators have intensified. Several methods have been tested and used, as described bellow.

Clinical and Epidemiological Parameters

Location of the masses, age, breed, sex, presence of multiple nodules, macroscopic appearance, size and growth rate are the clinical factors already tested as prognostic indicators. None of these characteristics was an independent predictor for the neoplasm. They act just as complementary parameters.

Tumors in the muzzle tend to be more aggressive, showing higher metastatic rates then mastocytomas in other locations (19, 29). On the other hand, inguinal, preputial and perineal lesions are usually considered more malignant, but these observations were not scientifically verified and are just based on clinical impressions (14, 52).

Al-Sarraf et al. (1996) compared age, sex, breed, tumor location and number of surgical interventions with survival and did not obtain statistically significant differences. Kiupel et al. (2005) reported that older dogs and Boxers have higher risk of recurrence at distant sites, and that older and male dogs had significantly shorter survival times. Animals with tumors in the trunk had shorter survival and disease-free intervals when treated with radiotherapy (82).

The presence of multiple masses is related to worse prognosis by some authors (29, 64), but this feature was not significant for others (4, 48, 73). Lesion depth, one of the criteria included in Patnaik's classification (60), was not considered an efficient indicator by Kiupel et al. (2005), as well as tumoral volume at the moment of the diagnosis. In a recent research (64), the multivariate analysis of the parameters analyzed in the histopathological grading method of Patnaik et al. (1984) revealed that invasiveness was the only with prognostic value in predicting disease-free intervals and survival. Surgical techniques with different margins of safety could be the reason for the differences observed. This criterion remains controversial, because Michels et al. (2002) and Murphy et al. (2004) did not detect significant differences between the survivals of mast cell tumor cases with completely or incompletely resected lesions.

The objective of clinical staging is to classify the disease according to the extent of the lesions. It is a very important procedure because it helps to formulate the prognosis and establish the adequate treatment. The most commonly used method is the World Health Organization staging system for mast cell tumors. This proposal includes four stages: stage I, when the neoplasm is confined to the dermis, without regional lymph node involvement; stage II, one tumor confined to the dermis, with regional lymph node involvement; stage III, multiple masses confined to the dermis or one large infiltrating tumor, with or without regional lymph node involvement; and stage IV, any tumor with metastasis, mast cell leukemia and/or bone marrow involvement, or recurrence with metastasis (14, 26, 40, 58). The higher clinical stages correlate to the decreases in disease-free interval and survival time (26). Turrel et al. (1988) added to this staging system the stage 0, in which one incompletely resected tumor was present in the dermis, without lymph node involvement. Dogs with stage 0 had better prognosis than dogs with stage I, II and III.

Histopathological grading

Histopathological grading is the method of choice in the prediction of mast cell tumors behavior (38, 52, 64, 76). Three histomorphological classifications for this neoplasm were proposed: Hottendorf and Nielsen (1967), Bostock (1973) and Patnaik et al. (1984). The most frequently used system is the Patnaik et al. (1984) grading method, which is considered more logical and complete (38, 40, 52, 58, 64, 69, 70, 79). In this proposal, the tumors were divided in three grades: grade I or well-differentiated tumors (Fig. 3); grade II or moderately-differentiated (Fig. 4); and grade III, the poorly-differentiated neoplasms (Fig. 5). This grading system is based on several histomorphological features, which are listed in Table 1.

According to this report (60), 46% of the dogs died due to the disease in a period of 1500 days, reinforcing the malignant potential of mast cell tumors. Survival time was related to the grade of cellular differentiation, since 93% of the dogs with grade I tumors, 44% with grade II and 6% with grade III were alive at the end of the study.

The most common comment upon the histopathological grading is that the method is based on subjective parameters like mitotic index, amount of cytoplasmic granules and cellular pleomorphism, generating intra- and interobserver variations, mainly in respect of grade II tumors (1, 21, 29, 44, 52, 56, 79, 87). Interobserver variations in mast cell tumor grading were detected by Strefezzi et al. (2003), Northrup et al. (2005) and Pinczowski et al. (2008). These authors described significant differences between pathologists, demonstrating the need for more objective methods. Preziosi et al. (2007), by means of multivariate analysis, verified that invasiveness, cellular distribution patterns, cellular differentiation and mitotic index are the most important parameters in histopathological evaluation. Despite the critiques, histopathological grading is still considered indispensable and has not been overcome by any other prognostic indicator (38, 52, 76, 84).

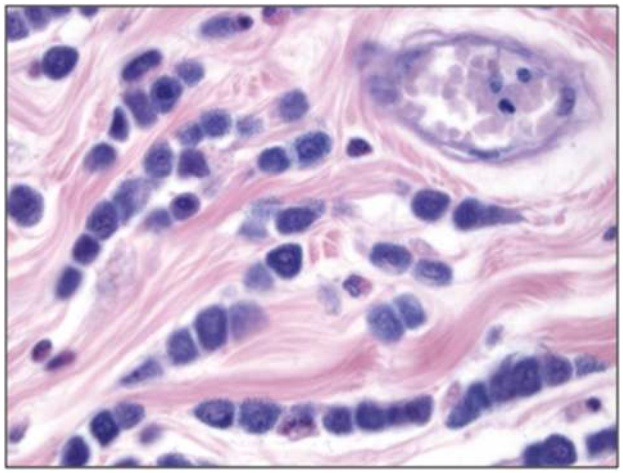

Figure 3 – Photomicrograph of a grade I (well-differentiated) mast cell tumor. Low cellularity, cells arranged in rows or small groups, with distinct cytoplasm full of granules and round nuclei. H&E, Obj. 40x.

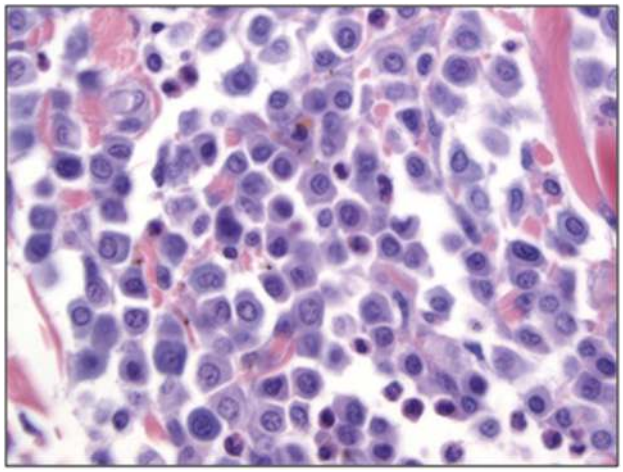

Figure 4 – Photomicrograph of a grade II (moderately-differentiated) mast cell tumor. Moderate to high cellularity, cells arranged in groups, most of them with distinct cytoplasm. Granules are not evident in some of the neoplastic cells. H&E, Obj. 40x.

Morphometry

Computerized image analysis can reduce inherent intra- and interobserver variations of morphological diagnosis (12, 16, 30, 45, 79). The great value of this technique is the possibility of establishing precise quantitative data, from optical or electron microscopy, aiming at the construction of data bases for normal, atypical or neoplastic tissue parameters (13, 16).

Strefezzi et al. (2003) demonstrated the correlation between nuclear parameters (area, perimeter and diameter), measured on cytopathology smears, to histopathological grading of mast cell tumors. Maiolino et al. (2005) corroborated these observations on histopathology slides, reinforcing the utility of morphometric analysis in helping mast cell tumor grading. Strefezzi et al. (2009) confirmed the advantages of nuclear morphometry as prognostic indicator for mast cell tumors after the clinical follow up of the previously studied cases, associating larger nuclei to worse prognosis and shorter post-surgical survival times.

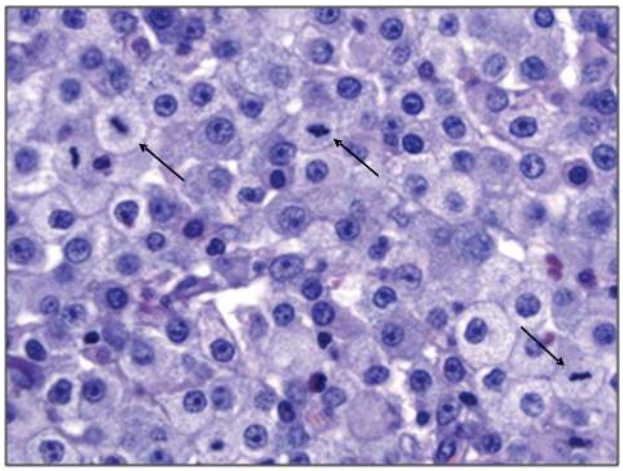

Figure 5 – Photomicrograph of a grade III (poorly-differentiated) mast cell tumor. High cellularity, cells arranged in closely packed groups, most of them with indistinct cytoplasm. Granules are fine or not evident. Mitotic figures are common (arrows). H&E, Obj. 40x.

Mitotic index

Mitotic index has been the main test to evaluate the proliferative activity of a variety of human and animal neoplasms (65, 70). This procedure is relatively simple because it does not require special histochemical techniques. However, it may lead to reproducibility problems due to selection of fields, interobserver variation, and size of microscope objective fields. Moreover, mitosis represents just the M phase of the cell cycle (65).

Preziosi et al. (2007) demonstrated the correlation between the mitosis count proposed by Patnaik et al. (1984) and survival time, and therefore considered this an important criterion for mast cell tumors grading. Using a 5 mitosis/field-cutoff value, Romansik et al. (2007) observed found a correlation between the mitotic index and histopathological grades of Patnaik et al. (1984), metastasis rate and survival. Mitotic index was capable of identifying, among the grade II cases, the more aggressive ones. In the same work, it was described a grade III subgroup with low mitotic index which had a better prognosis. Despite the impressing results, this technique needs validation studies, as well as intra- and interobserver variation evaluations.

Table 1 – Main microscopic characteristics of mast cell tumors according to their grade of differentiation (60).

Characteristic	Grade I	Grade II	Grade III
Location	Dermis and interfollicular spaces	Dermis and/or subcutis, muscles or adjacent tissues	Dermis, subcutis and adjacent tissues
Cellularity	Low	Moderate to high	High
Distribution	Rows or small groups	Groups	Closely packed sheets of cells
Pleomorphism	Low	Moderate	Intense
Cytoplasmic characteristics	Distinct boundaries with granules	Most distinct, with fine granules; some indistinct with large and hyperchromatic granules	Indistinct with fine granules or not obvious granules
Nuclei	Round with condensed chromatin	Round to indented with scattered chromatin	Indented to round vesiculated
Nucleoli	Not evident	Single	Single or multiple, prominent
Edema and necrosis	Minimal	Diffuse	Common
Binucleation	Absent	Occasional	Common
Giant cells	Rare or absent	Diffuse	Common
Stroma	-	Thin fibrovascular; thick and fibrocollagenous with hyalinization in some areas	Fibrovascular or thick and fibrocollagenous with hyalinization
Mitosis/HPF	Absent	0-2	3-6

Histo- and Immunohistochemical Methods

Proliferative activity evaluation

Mensuration of the proliferative activity using histo- and immunohistochemical markers aims to complement or substitute mitosis counting, considered a subjective and arduous method (1, 18, 65). Other methods worthy of notice are the determination of the frequency of Nucleolar Organizer Regions stained with Silver (AgNORs) (9, 31, 76), positive cell counting for Proliferating Cell Nuclear Antigen (PCNA) (1, 76) and Ki-67 (1, 71).

AgNORs are DNA loops which transcribe ribosomal RNA and can be demonstrated by silver-staining methods. The number of AgNORs indicates the proliferative activity of a cell population and is related to the velocity of the cell cycle (49, 65). Great variations in the applied staining techniques impair the confrontation of the obtained results with literature data. However, the results have been found to be isolatedly consistent (72).

PCNA is a non-histone protein necessary for DNA synthesis and repair. It is detected from G1 phase until the transition G2-M, achieving its maximum expression in the S phase of the cell cycle (42). It is absent in G0 but, unlike Ki-67, its expression varies notably during the cycle. PCNA synthesis can be induced by DNA damage and, because of its prolonged half-life, may provoke proliferative fraction overestimation (1, 42).

Recently, immunohistochemical detection of Ki-67 became the best tool for proliferative activity assessment. Although its function is still unknown it appears to be related to nucleolar RNA and is present only during the active phases of the cell cycle. Ki-67's half-life is short, being degraded in approximately one hour after mitosis. Thus, even tenuous reactions can be considered positive, discarding the usually needed cutoffs for PCNA examination (1, 49, 65, 72).

Bostock et al. (1989) found significant differences in AgNORs counting between the histopathological grades (60) in mast cell tumors. Higher AgNORs counts represented worse prognosis, with mortality reaching 75% in the first four months after surgical treatment. Among the cases with intermediate counts, one-third of the animals died due to the mast cell disease, while none of the dogs with counts lower than 1.7 AgNORs/cell did. Kravis et al. (1996) obtained correlation between AgNORs counting in cytopathology smears and histopathological grading: grade III tumors had significantly higher AgNOR counts than grades I and II.

Simoes et al. (1994) compared histopathology, AgNOR count and the fraction of PCNA positive cells as prognostic indicators. The number of AgNORs/cell was the most precise indicator of recurrence in a 3-month period, followed by PCNA and histopathological grading. In a 6-month period, PCNA was the best predictor, followed by histopathological grading and AgNOR, and, finally, in a 9-month period the best was histopathological grading, followed by PCNA and AgNOR. A combination of the three methods predicted correctly 80% of the cases.

Abadie et al. (1999) and Scase et al. (2006) verified that PCNA indices were higher in dogs that had their deaths related to the mastocytomas than in those that survived. Although they did not find significant differences between histopathological grades, other authors did using

Ki-67 (59, 71). Scase et al. (2006) demonstrated the utility of Ki-67 as an independent predictor, primarily in the subdivision of grade II mast cell tumors with regard to aggressiveness. Moreover, the usefulness of AgNOR count was reaffirmed with the failure of PCNA in predicting patient's survival. Webster et al. (2007) recommended the use of AgNOR and Ki-67, in association with the KIT immunodetection, as routine prognostic methods for mastocytomas.

Oncogenes

Oncogenes, the genes involved in neoplastic transformation, are derived from proto-oncogenes, which regulate normal cellular growth and differentiation. Several products of oncogenes – the oncoproteins - have been described. Among them are growth factor receptors, which are constitutively activated or overexpressed in cancer (33).

One of the extensively investigated gene expressions for mast cell tumors is the proto-oncogene *KIT*. This proto-oncogene encodes a transmembrane receptor with tyrosine-kinase activity that binds Stem Cell Factor (SCF, Mast cell growth factor or KIT-ligand), a cytokine that stimulates the development, maturation and survival of mast cells and other cell lineages like hematopoietic stem cells and melanocytes (36, 37). Point mutations and small deletions in the juxtamembrane domain were considered responsible for constitutive activation of the receptor, even in the absence of the ligand in humans and rodents (88). This constant activation causes excessive cell proliferation and tumor aggressiveness.

Mutations in canine mast cells were identified and characterized as juxtamembrane domain duplications (36, 39). Reguera et al. (2000) observed higher KIT levels as the tumor differentiation decreased and two patterns of immunohistochemical staining: membranous or cytoplasmic, usually around the nucleus. Finally, Zemke et al. (2002) identified mutations in grade II and grade III tumors, but not in grade I. These results suggest that KIT mutations and expression might be related to mast cell tumorigenesis and progression (38).

Kiupel et al. (2004) proposed a new classification for cutaneous mast cell tumors according to their immunohistochemical KIT-staining patterns. Three distinct patterns were described: (I) characterized by membrane-associated staining, with minimal cytoplasmic staining; (II) intense focal or stippled cytoplasmic staining; and (III) diffuse cytoplasmic staining, obscuring other cytoplasmic features. It was demonstrated that KIT-II and KIT-III patterns were significantly related to local recurrence and shorter survival. These results were later confirmed by the same group of researchers and considered important prognostic markers in association with AgNOR, PCNA and Ki-67 as complements of the histopathological grading. According to Webster et al. (2007), the best results in mast cell tumor behavior prediction was achieved with concurrent evaluation of histopathological grade, KIT-expression, AgNOR and Ki-67 counts. Additionally, an association was suggested between aberrant KIT localization and increased proliferation of mast cells. This hypothesis was investigated by Gil da Costa et al. (2007) who showed the correlation of the alterations in KIT expression patterns to the proliferation markers ki-67 and AgNOR, as well as to increased histopathological grades, the presence of necrosis and ulceration. However, there were no significant differences between KIT-II and KIT-III patterns for the variables tested, suggesting that they could represent similar changes or be the result of progressive accumulation of KIT in the cytosol.

The *MDM2* – mouse double minute-2 – is a proto-oncogene first identified in murine cell lines. Its amplification has been demonstrated in human and canine neoplasms like sarcomas and mammary tumors (74), in which MDM2 overexpression is associated with normal P53 (TP53) (54, 55, 74). The oncoprotein MDM2 negatively regulates TP53 action, catalyzing its ubiquitinization and exclusion from the nucleus, as well as its subsequent degradation. This allows the decrease of TP53 activity even in the absence of *TP53* gene mutation (50, 55, 57, 75). Overexpression of MDM2 in canine mast cell tumors has been detected in grade III tumors, but not every grade III tumor presents this alteration (86). It has been reported that a small proportion of MDM2-positive tumors (12.8%) are also positive for TP53. These results suggest that *MDM2* overexpression may occur independently of *TP53* mutation and be responsible for TP53 exclusion from the nucleus and degradation.

Antioncogenes or Tumor suppressor genes

Among tumor suppressor genes, responsible for inhibiting cellular growth and differentiation, the most frequently investigated is p53 (*TP53*) gene. Normal TP53 regulates the transition from G1 to S phase of the cell cycle, promoting or controlling RNAm synthesis. Its function is to warrant genomic fidelity by repairing damaged DNA or inducing apoptosis when these damages are irreparable (21, 26, 33, 55). The main interactions of this protein are with p21 (CDKN1A) and BAX: in the first, it inhibits progression to M phase of the cell cycle; and in the second, initiates apoptotic death (3, 35, 41, 55).

Mutations in the TP53 gene have been considered as the most common alteration in human cancer, leading to decrease of loss of its product (21, 26, 55). They were described in canine tumors and their derived cell lines as, for example, rabdomyosarcomas, osteosarcomas, chondrosarcomas and myxosarcomas (74). Immunohistochemical detection of this protein is explained by the fact that the mutant variant is more stable and has a longer half-life than normal TP53 (21).

The correlation between TP53 expression and histopathological grading, recurrence rates and survival

time was investigated for canine mast cell tumors but, despite its great value in human neoplasms, the results were not similar for canine (21, 26, 86). However, TP53 function may be impaired by several non-mutational mechanisms such as: exclusion from the nucleus, complexes formations with viral proteins, and overexpression of the proto-oncogene *MDM2*, discussed earlier in this paper (55, 57, 86).

Cell cycle regulators

The cell cycle is regulated by several proteins such as cyclins and cyclin-dependent kinases (CDKs) (33). Altered CDK-inhibitors expression was assessed in mast cell tumors by means of immunohistochemical detection of p21 (CDKN1A) and p27 (CDKN1B), two of the regulators of G1-S phase-transition. CDKN1A expression increased with higher histopathological grades, while CDKN1B expression was independent of the tumor differentiation. It has been suggested that CDKN1B alterations are initial events in mast cell tumor development and that the expression of CDKN1A could be a tumor progression marker for this neoplasm. However, clinical follow up has not been performed, so the results are still speculative. Cyclin D1 expression was evaluated by immunohistochemistry in grade II mast cell tumors but no significant differences were detected between survival times of positive and negative tumors (59).

Tumor angiogenesis

Microcirculation development is considered an important requirement for expansion, dissemination and metastatization of tumors. It is known that vascularization correlates to metastatic potential (33, 85). Studies about angiogenesis in mast cell tumors were carried out with Factor VIII-related antigen, an immunohistochemical marker for endothelial cells, and detected higher vascular density in poorly-differentiated tumors compared to grade II and grade I lesions. It has been observed that cellularity also correlates to vascularization in grade III tumors (66). Preziosi et al. (2004) showed statistically significant differences in intratumoral vascular densities between moderately differentiated and highly invasive tumors. Moreover, it has been demonstrated that vascular intratumoral density has prognostic value in survival prediction, but not for disease-free interval.

DNA ploidy

The term ploidy refers to the quantity of DNA present in a cell. Abnormal DNA amounts (aneuploidy), due to duplication failure during mitosis, were related to worse prognoses in canine and feline tumors (7, 24). The majority of the mast cell tumors investigated by Ayl et al. (1992) were diploid, and multiple tumors of the same dog presented the same ploidy. In this study, no statistically significant differences were detected between survival time of animals with aneuploid and diploid tumors, but a trend to shorter survival of the animals with aneuploid mastocytomas.

Apoptotic mechanism defects

Growth is one of the most important criteria for prognostic evaluation in cancer but, besides cell proliferation rate, one must also know the neoplastic cell death rate. Alterations in the apoptosis mechanism were investigated by Scase et al. (2006) with immunohistochemical detection of surviving, an inhibitor of the caspases 9 and 3, but it was of no prognostic value for mast cell tumors.

Final comments

Mast cell tumors usually have extremely variable behavior and consist in one of the major therapeutic challenges for veterinary oncology. The most common neoplasm of dogs has been extensively investigated with respect to its etiology (probably multifactorial), biological behavior, treatment approaches and, principally, in search of more efficient prognostic indicators.

Among the indicators already tested, none of them overcame the accuracy of the histopathological grading proposed by Patnaik et al. (1984). The histomorphological features of these tumors are strongly correlated to disease evolution, as well as with post-surgical survival time. Auxiliary methods have been shown, with eminence for those which evaluated the proliferative activity: mitotic index, Ki-67 expression and AgNORs count. Recently, the detection of KIT mutations and the description of its immunohistochemical patterns have gained popularity in this diagnostic panel. KIT mutations seem to play an important role in mastocytoma oncogenesis, accelerating cell proliferation.

Further research is still needed to improve the knowledge about mast cell tumors behavior. Apoptosis-related genes expression, cell cycle regulators and multidrug resistance-associated proteins deserve attention and may contribute to the prediction of tumor evolution, minimizing failures in the available chemotherapies.

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