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Review

Canine cutaneous mast cell tumors: A combined clinical and pathologic approach to diagnosis, prognosis, and treatment selection

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ABSTRACT

In view of the varied biologic behavior and the costs of treatment for canine cutaneous mast cell tumors, development of appropriate treatment plans for individual affected dogs can be difficult, but decisions regarding treatment should be made using a systematic, evidence-based approach. This manuscript reviews the current state of diagnostics and prognostication of canine cutaneous mast cell tumors, and suggests a combined approach based on clinical and pathologic assessment for decision making regarding treatment choices. The current state of histologic grading, evaluation of proliferation indices, evaluation of mutations in the c-*kit* gene and KIT expression, evaluation of excision and clinical staging are examined. On the basis of the current understanding of prognostication and treatment response, algorithms for selection of local and systemic therapy are presented.

Introduction

In view of the prevalence and variable biologic behavior of canine cutaneous mast cell tumors (MCTs), and the cost of and variable response to therapeutics, it is important to accurately prognosticate cutaneous MCTs and to correctly select the most appropriate therapeutic approach. Cutaneous MCTs are one of the most common neoplasms in dogs, reportedly having a prevalence of up to 0.27% in the total dog population and representing up to 21% of all canine cutaneous neoplasms (Brodey, 1970; Priester, 1973; Finnie and Bostock, 1979; Rothwell et al., 1987; Misdrop, 2004; Shoop et al., 2015). In most cases, MCTs are benign and solitary, and can be cured by surgical excision. However, a subset of MCTs is locally invasive and progress to fatal metastatic disease (Priester, 1973; O'Keefe, 1990; Misdrop, 2004).

Whereas the diagnosis of canine cutaneous MCTs rarely poses a diagnostic challenge, and can often be achieved through cytologic evaluation (Barrett, 1976; Duncan and Prasse, 1979; Macy, 1986), the differentiation of malignant from benign neoplasms can be challenging (O'Keefe, 1990). While histologic features alone may suggest a less aggressive biologic behavior, approximately 5% of dogs with histologically low grade MCTs will die due to MCT-associated disease (Kiupel et al., 2011). Up to 15% of dogs with low grade cutaneous MCTs have regional lymph node spread at the time of presentation (Stefanello et al., 2015). Moreover, almost 20% of dogs diagnosed with a low grade MCT will develop additional MCTs that either represent metastases or de novo masses (Kiupel et al., 2011).

On the basis of current understanding, accurate histologic grading remains a cornerstone of MCT prognostication, but supplementation of histologic grading with modern molecular tests and interpretation of results with respect to clinical findings is recommended. With these combined tools, there is a greater likelihood of identifying MCTs that pose a high risk of aggressive behavior. Furthermore, the results of such evaluation can aid in selection of the best treatment plan for a given dog and owner by a veterinary oncologist. This is particularly important for owners who wish to treat beyond primary surgical excision.

In this article, we review the current understanding of prognostically significant and therapeutically predictive markers in canine cutaneous MCTs, including histologic grading, proliferation activity, mutations of the c-*kit* proto-oncogene, and KIT expression patterns, along with clinical features that have prognostic significance.

Histologic grading of cutaneous mast cell tumors

Regardless of advances in molecular analysis, histologic grading remains a cornerstone of MCT prognostic assessment and determination for inclusion of additional therapy following biopsy. The histologic grading systems that historically have been most commonly used to grade MCTs have been described by Bostock (1973) and Patnaik et al. (1984). Both of these systems classified MCTs into three grades: well-differentiated tumors, intermediately differentiated tumors and poorly differentiated tumors. While both systems have been correlated with prognostic outcomes, the application of

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these systems has been shown to be inconsistent in practice (Northrup et al., 2005a and b; Kiupel et al., 2011).

To decrease inter-observer variation, a two-tier histologic grading system was proposed by Kiupel et al. (2011). This two-tier classification decreases inter-observer variation and provides 96.8% inter-observer consistency, while providing strong correlations with overall survival, MCT-associated mortality and risk of metastasis (Kiupel et al., 2011; Takeuchi et al., 2013; Vascellari et al., 2013; Sabattini et al., 2015). As such, the two-tier classification system is considered to be the current standard for MCT grading.

Prior to the description of the two-tier system, the Patnaik classification system was the most widely used grading system. Under this system, well-differentiated MCTs were designated grade 1, intermediately differentiated MCTs were designated grade 2, and poorly differentiated MCTs were designated grade 3 (Patnaik et al., 1984). While the Bostock grading system used similar morphologic criteria to classify canine MCTs, grades were applied in reverse order of the Patnaik classification, making its use less intuitive (Bostock, 1973).

According to the Patnaik classification system, grade 1 MCTs are confined to the superficial dermis, separated by collagen bundles and located in interfollicular spaces, and are composed of distinct, round, monomorphic neoplastic mast cells that have a round nucleus and no nucleolus (Patnaik et al., 1984). Edema and/or necrosis are absent or minimal. There are no or only rare mitoses, defined as <2 mitoses/10 high power fields (HPF). Grade 2 MCTs are located in the superficial and/or deep dermis, may also infiltrate the subcutis and subjacent skeletal muscle, and are more cellular than grade 1 MCTs. The neoplastic cells of grade 2 MCTs are often pleomorphic, less basophilic than their normal counterpart, and have indented nuclei, with a single nucleolus. Diffuse edema and necrosis are common, and mitoses are infrequent (0–2/HPF). Grade 3 MCTs are more cellular than grade 1 and 2 MCTs, and are composed of closely packed populations of pleomorphic cells that have irregularly shaped nuclei and multiple nucleoli. Multinucleate and bizarre cells are common, and mitotic figures are frequent (3–6/HPF). Criteria for grading under the Bostock classification system are comparable, but the Bostock system does not utilize tumor depth and focuses in more detail on cell morphology, which is reflected by the incorporation of the nucleus to cytoplasm ratio into grading (Bostock, 1973).

The Bostock and Patnaik studies both found significant correlations between histologic grade and animal survival (Bostock, 1973; Patnaik et al., 1984). Results of these studies were based on similar, relatively large study populations (83 and 114 dogs, respectively), which included only dogs in which the tumor was excised as assessed by histologic examination. There was no clinical evidence of metastasis at the time of diagnosis, and complete follow-up data were available (Bostock, 1973; Patnaik et al., 1984). Furthermore, both studies followed dogs for relatively long periods after initial diagnosis (1500 and 910 days, respectively) (Bostock, 1973; Patnaik et al., 1984). In the Patnaik study, almost all dogs (93%) with well-differentiated (Patnaik grade 1) MCTs survived the study period of 1500 days, whereas only 77% of dogs with well-differentiated (Bostock grade 3) MCTs survived >210 days in the Bostock study (Bostock, 1973; Patnaik et al., 1984). In both studies, dogs with poorly differentiated MCTs (Patnaik grade 3, Bostock grade 1) had a poor prognosis, with only 6% and 13% of dogs surviving >1500 and >210 days, respectively (Bostock, 1973; Patnaik et al., 1984).

Criticisms of the Bostock and Patnaik studies include the lack of measured prognostic outcomes other than total survival time and lack of multivariate survival analysis. Neither study reported disease free interval, time to progression nor cause of death, whether due to MCT-related disease or other reasons. In view of the lack of multivariate analysis, the potential effects of variables including, but not limited to, age of the animals at diagnosis, breed and treatment on survival time were not assessed.

Inconsistencies in application of the Patnaik and Bostock classifications have been documented (Northrup et al., 2005a and b; Kiupel et al., 2011). Such inconsistencies result in high inter-observer variance in grading and arise in great part due to differences in assessment of mitotic index and inconsistent inclusion of tumor depth in the histopathologic classification. In two subsequent studies, 10 pathologists at a single institution, grading 60 canine cutaneous MCTs according to their own sets of histologic criteria or according to the Patnaik system, had only 50.3% and 62.1% agreement in grading, respectively (Northrup et al., 2005a and b). Similarly, for 95 canine cutaneous MCTs, there was only a 63.1% concordance for grade 1 MCTs, 63% concordance for grade 2 MCTs and 74% concordance for grade 3 MCTs among 31 pathologists from 16 different institutions (Kiupel et al., 2011).

Neither the Patnaik nor Bostock grading systems clearly defined the methods by which mitotic index should be assessed and neither original study employed an evidenced based method of determining the cutoffs for mitotic index for each defined grade, such as by receiver-operating characteristic analysis (Bostock, 1973; Patnaik et al., 1984). As an example, a Patnaik grade 2 tumor could have a range of 0–20 mitoses/10 HPF and maintain the criteria of only having 0–2 mitoses/average HPF. In one study that analyzed only the mitotic index, as defined by the number of mitoses/10 HPF, seven dogs with Patnaik grade 2 MCTs with a mitotic index >5 had a median survival time of 5 months, while 72 dogs with Patnaik grade 2 MCTs with a mitotic index ≤5 had a median survival time of 70 months (Romansik et al., 2007). Independent of grading, 19 dogs with a mitotic index >5 had a median survival time of 2 months and 80 dogs with a mitotic index ≤5 had median ST of 70 months; furthermore, an increasing mitotic index was associated with an increased rate of death due to MCT and metastasis (Romansik et al., 2007). This suggests that there is a significant difference in biologic behavior of MCTs with low and high mitotic indices, regardless of grade under the Patnaik system.

In respect of the criteria of depth of infiltration, many pathologists use only cellular features to grade MCTs following Bostock's classification and do not consider tumor depth, as required in Patnaik's classification, but assign grades based on the numbering system proposed by Patnaik (Bostock, 1973; Patnaik et al., 1984). As a result, a well-differentiated MCT may be assigned a grade of 1, regardless of tumor depth, which is in contradiction to the system Patnaik actually described. Interestingly, a recent study investigated tumor depth of canine cutaneous MCTs as an independent parameter and found no associated prognostic significance (Kiupel et al., 2005). Therefore, tumor depth should not be included in the histologic grading of canine cutaneous MCTs.

To decrease inter-observer variation, a two-tier histologic grading system was established that has a 96.8% inter-observer consistency and is not only predictive of overall survival, but, more importantly, MCT-associated mortality and risk of metastasis (Kiupel et al., 2011; Takeuchi et al., 2013; Vascellari et al., 2013; Sabattini et al., 2015). The two-tier system divides canine cutaneous MCTs into high and low grade MCTs (Figs. 1A–E). The diagnosis of a high-grade MCT is based on the presence of any of the following criteria: (1) seven or more mitotic figures in 10 HPF; (2) three or more multinucleate cells that have three or more nuclei in 10 HPF; (3) three or more bizarre nuclei in 10 HPF; and (4) karyomegaly and anisokaryosis, as defined by nuclear diameters of at least 10% of neoplastic cells varying by at least two times (Kiupel et al., 2011). Fields with the highest mitotic activity, or with the highest degree of anisokaryosis, are selected to assess the different parameters. A low grade is based on the absence of each of these features. While not a discrete criteria of grading, low grade MCTs are also often well