

VCS/ACVP Oncology-Pathology Working Group Summary and Subgroup Recommendations for Grading of Canine Cutaneous Mast Cell Tumors (2020) - [Updated from 2017]

Species/Tumor: CANINE CUTANEOUS MAST CELL TUMOR SUBGROUP

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Introduction: The cornerstone for the prognosis of canine cutaneous mast cell tumor has been the histological grade. A critical appraisal of the available literature can help pathologists and clinicians better understand the values and limitations of histological grading. The first consensus document of the OPWG on the histological grading of cutaneous mast cell tumors was divulged in 2013 and, thereafter, updated in 2017. Since then, additional manuscripts have been published, continuing to expand and improve our knowledge on the subject. Some studies validated the use of the two-tier grading system and others supported the prognostic value of the mitotic index. A new innovative field has been the development of cytological grade. These findings reported in the literature have also been reviewed. This current document takes into account new and relevant literature since the time of the initial document and subsequent document (2013 and 2017 respectively) to ensure currency of the VCS/ACVP OPWG consensus on the topic of CCMCT. Similarly, it is expected this document will be updated regularly as additional research and associated data come to light. This consensus does not address the literature related to canine subcutaneous MCTs, though this topic may be incorporated into future updates of this document or in a separate and independent consensus solely focused on canine subcutaneous MCTs.

Overall Summary / Recommendations:

Based on review of the literature, the **Canine Cutaneous Mast Cell Tumor (grading)** subgroup has concluded and recommends the following:

Histological Grading

The Patnaik grading system has been the foundation of the prognosis of canine cutaneous mast cell tumors (MCTs) since 1984¹. This grading system divides cutaneous MCTs in three different grade categories (GI, GII, and GIII) based on extent of tissue involvement, cellularity, cellular and nuclear morphology, mitotic activity, stromal reaction, and edema/necrosis (**Table 1**). Numerous studies have proven its validity in the clinical setting². GI MCTs have an excellent long-term prognosis, while GIII are associated with a guarded to poor prognosis because of a higher recurrence and metastatic rate³. More difficult is to predict the behavior of GII MCTs; in fact the majority of them present a benign clinical progression, while about 20-50% are characterized by an aggressive clinical behavior². Another limitation of this system is the subjectivity of its application by different pathologists with the resultant variability in the grade assignment⁴. This problem is more relevant for GI and GII MCTs, while it seems to be less of an issue for GIII MCTs^{5,6}.

In order to overcome these two limitations a two-tier grading system was developed by Kiupel and colleagues in 2011⁵ (**Table 2**). This system divides cutaneous MCTs into two categories (low grade and high grade) based only on cellular morphological criteria including mitotic figures, multinucleation, bizarre nuclei, and karyomegaly. The Kiupel grading system has been validated in multiple studies⁶⁻¹⁰ as an independent prognostic factor in canine cutaneous MCTs able to predict local recurrence⁸, metastatic propensity⁹ and overall survival^{6,7,10}. An advantage of the Kiupel grading system is that the criteria for assigning the grade are more objectively defined, increasing the concordance between pathologists^{6,7}.

Few studies have applied both grading systems (Patnaik and Kiupel) to the same population of dogs^{6,8-11}. In those studies, all GI tumors were low grade Kiupel (LG) and all GIII tumors were high grade Kiupel (HG), while GII split with the majority being LG and a smaller proportion (ranging from 7.5% to 21%) being HG^{6,8-11}. Both systems were able to predict outcome in dogs with cutaneous MCTs, but interestingly the Patnaik system was more sensitive, while the Kiupel system more specific, in detecting dogs with aggressive disease⁶. The evidence is still weak, but it appears that the two systems could be complementary in that the Kiupel system might help refine the prognosis of GII Patnaik MCTs^{5,6}, while the Patnaik system might clarify the behavior of HG MCTs^{9,10}.

Using the Patnaik and Kiupel grading system together, cutaneous MCTs can be divided into four categories with different prognoses:

- **G1/LG**. The prognosis is excellent with virtually no tumor-related deaths^{10,11}. One study reports a low risk of lymph node metastasis (6%) and distant metastasis (2%) at initial staging, stressing the importance of staging even in this group of patients⁹.
- **G2/LG**. Three manuscripts describe this category of patients⁹⁻¹¹. The prognosis is supposedly good with 3-17% of dogs dying of causes related to MCTs^{10,11}. In one study¹⁰ the median survival time for this group of patients was not reached after 92 months, and 94% were alive at 1 year. At initial staging, metastatic rate to the lymph nodes was 16%, while distant metastases were seen in 2% of the cases⁹.
- **G2/HG**. Three manuscripts describe this category of patients⁹⁻¹¹. The prognosis is fair to guarded with 14-56% of dogs dying of causes related to MCTs^{10,11}. The median survival time^{10,11} was between 7.5 and 23.3 months and only 46% of dogs alive at 1 year^{10,11}. At presentation, the metastatic rate to the lymph nodes was 15%, while distant metastases were seen in 2% of the cases⁹.
- **G3/HG**. The prognosis is guarded to poor with 67-75% tumor-related deaths in studies with adequate numbers of cases^{3,10,11}. The median survival time in two manuscripts^{10,11} was 3.6 and 6.8 months. The metastatic rate to the lymph nodes was 46% , with distant site metastasis in 21%⁹.

According to data available, it appears that the difference in survival seen between G2/LG and G2/HG might be due to local recurrence rather than metastatic disease, because metastatic rates between these two groups appear similar. This hypothesis requires further investigation, although the study by Donnelly and colleagues⁸ on grade and margins as predictors of local recurrence provided corroborative findings. In this study the removal of MCTs with histological clean margins, even if narrow (≤ 3 mm), appeared adequate to prevent local recurrence in 96% of LG MCTs, while 36% of HG MCTs recurred regardless of the width of the histologically tumor-free margins⁸.

One single study¹² describes the value of incisional biopsies prior to curative intent surgery and found an overall concordance rate of 96% based on the Patnaik grading system and of 92% based on the Kiupel grading system. Discordance in grade was more likely to underestimate tumor grade. While the study did not directly evaluate concordance of incisional and curative-intent excisional biopsies using combined grading schemes (as proposed above), extrapolating the study's data in this manner suggested pre-treatment biopsies underestimated up to 44% of G2/HG MCTs with < 6% discordance for all other combined grades. Moreover, the majority of biopsies in the study were large (wedge or punch) raising the concern of increased expense and morbidity.

Mitotic Index

An independent prognostic indicator, as evidenced in the literature reviewed, is the mitotic index (MI).^{6,13-17} Recently, the appropriateness of the term *mitotic index* (defined as the number of cells in mitosis out of a known total number of cells) has come into question and the adoption of *mitotic count* (number of cells in mitosis in a given area), has been proposed. The mitotic count (MC) (reported as MI, in the literature reviewed) is obtained by counting the absolute number of mitoses in 10 high-power fields (400x magnification/40x objective) in the region with highest mitotic activity, as determined initially on a low power scan (100x magnification/10x objective) of the specimen¹³. In four studies evaluating the MI (MC) as a prognostic factor with a threshold of 5^{6,13,15,17}, the sensitivity of MI (MC) to predict tumor-related death ranged between 39 and 55% and the specificity between 86 and 99%. The median survival time for cutaneous MCTs with a MI (MC) ≤5 was >70 months, while for MCTs with a MI (MC) >5 varied between 2 and 5 months^{6,13,15,17}. Mitotic index (mitotic count) has been also significantly associated with metastatic rate but not with recurrence rate¹³. Some authors^{11,14,16} suggest that the stratification of the MI (MC) in three categories might be superior, but these methods are less easily applicable in the clinical setting. Future studies on MC should also better standardize the view area in which mitoses are counted since the actual size of the field seen with a high power (40x) objective can vary between microscopes depending on the type of ocular. Recently, it has been proposed the view area in veterinary histopathology reports to be of a standardized size of 2.37 mm²¹⁸.

Cytological Grading

The application of a cytological grading system based on the Kiupel histological grading system has been investigated in three studies¹⁹⁻²¹. The Kiupel grading system is very appealing for clinical pathologists because it relies on cellular morphological features rather than on tissue architecture, as opposed to the Patnaik system. Morphological features used in cytological grading include presence of mitotic figures, nuclear pleomorphism, binucleation/multinucleation, and anisokaryosis. In one study²¹, another important criterion for assigning the cytological grade was mast cell granulation, which is not assessed in the Kiupel grading system, but was one of the criteria used in the Patnaik grading system.

According to these studies¹⁹⁻²¹, cytological grading, using histopathology as gold standard, has a sensitivity of 85-88%, a specificity of 95-97% and an overall accuracy of 94%, which is comparable with the performance of a Incisional biopsy prior to curative intent surgery¹². The cytological grade was significantly associated with median survival times and tumor-related deaths in one study²¹. The

criteria applied to assign a high cytological grade were poor granulation, or at least two of the following features: presence of mitotic figures, binucleation/multinucleation, nuclear pleomorphism, or anisokaryosis (**Table 3**).

Despite these promising findings, cytological grading presents some limitations and challenges, which are difficult to resolve and require further investigations.

The first limitation is the difficulty for cytology to differentiate between cutaneous and subcutaneous MCTs, potentially leading to subcutaneous MCTs being graded too. For this aspect the clinical pathologist should rely on the information provided by the attending clinician in the submission form, but the provision of a clinical history can be quite variable.

Another controversial element is the stain used to prepare the sample. Stains routinely used in clinical pathology such as May-Grünwald-Giemsa or Wright's stains allow rapid and accurate diagnosis of MCTs because of the intense coloration of the cytoplasmic granules. However, the abundance of granules in well-differentiated mast cells might obscure some nuclear criteria, especially pleomorphism, making the assignation of a cytological grade based only on Kiupel criteria difficult. With the evidence available at this time, de-staining and re-staining cytological samples to allow a better visualization of the nucleus, using specific stains such as hematoxylin and eosin, does not appear to be cost effective and could lead to staining artifacts²⁰. Moreover, some nuclear features have been associated with inaccurate grading; for example karyomegaly is the main reason for assigning a false positive cytological high grade to a histological low grade tumor^{19,20}, whilst nuclear pleomorphism (bizarre nuclei) is consistently poorly correlated to the histological grade across all three studies¹⁹⁻²¹. Aqueous-based rapid cytological stains should also be critically evaluated in the future, due to notably more variable staining of cytoplasmic granules, as well as its more general use in practice²². For now, there is no available evidence of their utility for grading purposes.

The last challenge is the difficulty of assessing the mitotic activity in cytology, which is likely in part due to the heterogeneous distribution of mitoses within the tumour and variation in the quality of aspirated samples, but also to the poor visualization of the nucleus in well-granulated mast cells.

Recommendations

1. Considering the available evidence, we suggest histopathological reporting of canine cutaneous MCTs include both histological grading systems (Patnaik and Kiupel). Additionally, as recommended in the initial consensus on grading (2013), all diagnostic pathologists reporting on canine cutaneous MCTs should have the criteria for each^{1,5} respective system (Table 1 and 2) available for reference at the time of grading.

2. All histological reports of canine cutaneous MCTs should also include the MI (MC), obtained counting the absolute number of mitoses in 10 high-power fields (400x magnification/40x objective) in the regions with highest mitotic activity as determined initially on a low power scan of the specimen. Future studies which evaluate MC as a prognostic parameter, should also adopt this methodology, while also defining and standardizing the microscopic area evaluated to 2.37 mm².

3. It is important to highlight that a subset of tumors with a histologically benign appearance (GI/LG or GII/LG with MI≤5) can still manifest an aggressive clinical behavior. Regardless of the grading system used, grade must be considered as only one prognostic factor and used in conjunction with the overall clinical picture: age, clinical progression, size, site, stage, completeness and quality of surgical margins, and other prognostic markers (e.g. proliferative markers, kit expression, *c-kit* mutation).

4. Cytological grading is promising. The Camus grading system (Table 3) should be further validated, but may provide valuable pre-operative information. Based on current information²¹, a cytologic diagnosis of low grade MCT correlates well with histology and outcome. However, a diagnosis of high grade MCT should be received with caution if only based upon two morphological criteria, such as binucleation and anisokaryosis, because of the risk of false positives when compared to histological grading. Until more fully evaluated, other factors including clinical presentation and signs should be included to support a more aggressive MCT when guiding therapy and staging.

5. Incisional biopsies prior to curative intent are moderately accurate to grade MCTs prior to definitive treatment, but at the risk of underestimating the grade, particularly in GII/HG. Members of the OPWG rely on cytology as initial screening test, but rarely recommend incisional biopsies to guide staging or treatment planning because of their limited clinical benefit and morbidity.

Canine Cutaneous Mast Cell Tumor Grading Systems

Table 1. Patnaik Histological Grading Criteria, 1984

	Tumor Grade		
	I (low)	II (intermediate)	III (high)
Location	Dermis and interfollicular spaces	Infiltrate lower dermal and subcutaneous tissue; some extend to skeletal muscles or surrounding tissues	Replace subcutaneous and deep tissues
Cell morphology	Round, monomorphic, ample distinct cytoplasm with medium-sized granules	Round to ovoid, moderately pleomorphic, with scattered spindle and giant cells; distinct cytoplasm with fine granules in most cells, but indistinct cytoplasm and large/ hyper-chromatic granules in some	Round, ovoid, or spindle shaped, pleomorphic, medium sized; indistinct cytoplasm with granules that are fine or not obvious; many giant cells and scattered multinucleated cells
Nuclear morphology	Round, condensed chromatin	Round to indented with scattered chromatin and single nucleoli; some binucleated cells	Indented to round vesiculated, with one or more prominent nucleoli; common binucleated cells
Architecture, cellularity, stromal reaction	Arranged in rows or small groups, separated by mature collagen fibers of the dermis	Moderately to highly cellular, arranged in groups with thin, fibrovascular stroma (sometimes thick and fibrocollagenous with areas of hyalinization)	Cellular, arranged in closely packed sheets; stroma fibrovascular or thick and fibrocollagenous with areas of hyalinization
Mitotic figures	None	Rare (0-2/high-power field)	Common (3-6 high-power field)
Edema and necrosis	Minimal	Area of diffuse edema and necrosis	Common, edema, hemorrhage, and necrosis

Table 2. Kiupel Histologic Grading Criteria, 2011

High-grade if any one of the following criteria is present:	
≥ 7 mitoses/10 high-power fields	In regions with the highest mitotic activity
≥ 3 multinucleated cells/10 high-power fields	Multinucleated cells defined as cells with 3 or more nuclei
≥ 3 bizarre nuclei/10 high-power fields	Highly atypical with marked indentations, segmentation, and irregular shape
Karyomegaly	At least 10% of neoplastic cells vary by 2-fold

Table 3. Camus Cytologic Grading Criteria, 2016

High-grade if there is:	
Poor granulation	Or
	At least two of the following features: - presence of mitotic figures - binucleation/multinucleation - nuclear pleomorphism ('non circular') - anisokaryosis (>50% difference)

References

(References specifically and critically reviewed by the subgroup members are denoted with an asterisk (*). Additional summaries from these reviews are provided below. Remaining references were either reviewed at the time the original consensus document was generated (**) or are included relative to their significance regarding background or which arose during subgroup discussions.)

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22. *Sabattini S, Renzi A, Marconato L, et al. Comparison between May-Grünwald-Giemsa and rapid cytological stains in fine-needle aspirates of canine mast cell tumour: Diagnostic and prognostic implications. *Vet Comp Onc.* 2018 Dec;16:511–517.

Specific Literature Critically Reviewed by the Subgroup

Schultheiss et al. Association of histologic tumor characteristics and size of surgical margins with clinical outcome after surgical removal of cutaneous mast cell tumors in dogs. *JAVMA 2011*

Study Objective: The purpose of the study reported here was to evaluate the relationship between width and depth of surgical margins, amount of edema within and around the tumor, and degree of demarcation between the tumor and surrounding tissues with the clinical outcome following surgical removal of MCTs in dogs.

Study Design: Retrospective cohort study.

Materials & Methods: Consecutive cases of MCT submitted to CSU over a 2 months period. Follow up by questionnaire. Histopathology reviewed by two pathologists (only Patnaik grading system used). No adjuvant treatment.

Conclusions Drawn: Most GI and GII MCTs in dogs can be successfully treated by complete surgical removal with margins smaller than those currently recommended. Edema and degree of demarcation were not correlated with outcome.

Statistical Soundness: Ok, but analysis performed only on GI and GII because insufficient number of GIII. Number of events was very low (no recurrences and only 4 patients developed metastatic disease, 3 of which were GIII). Follow up was ok.

Subgroup conclusions: The conclusions drawn are probably true for this cohort of samples submitted by general practitioners; MCTs were mostly GI or GII (96%), located in the trunk (~70%), and of small dimension (12±8mm). However, the study presents information about histologic margin, which can't be translated into recommendations for surgical margins. In addition, tumors were not stratified according to the Kiupel system. For the above reasons, not much information with regard to this subgroup's goals is provided by this article, which has not been included in the list of the selected reference.

Donnelly et al. Evaluation of histological grade and histologically tumour-free margins as predictors of local recurrence in completely excised canine mast cell tumors. VCO 2013.

Study Objective: The purpose of the study is to evaluate associations among the width of the histologically tumor-free margins, tumor grade, tumor size and local recurrence rate in canine MCTs.

Study Design: Retrospective

Materials & Methods: Study included completely removed GII and GIII MCTs. Systemic adjuvant treatment allowed, but not revision surgery or radiotherapy. All cases reviewed by a single pathologist, both grading schemes applied.

Conclusions Drawn: HG tumors were more likely to recur than LG tumors (36% versus 4%, $P < 0.0001$), with no association between histologically tumor-free margins width and local recurrence. Twenty-nine percent of LG tumors had histologically tumor-free margins less than 3 mm; none recurred. HG tumors have significant risk of recurrence regardless of histologically tumor-free margins width.

Statistical Soundness: Good. Tumor location and distribution between low and high grade was good as well as the follow up. Allowing inclusion of cases that received systemic treatment might have confounded the results.

Subgroup conclusions: The study shows a good correlation between Patnaik system and Kiupel system, with all GIII classified as HG and most of GII included in the LG group. The study also validates the Kiupel system as an independent prognostic factor for local recurrence. HG MCTs benefit of local adjuvant treatment regardless of the histologically tumor-free margins width, while narrowly excised (≤ 3 mm) LG MCT can be successfully treated with surgery alone.

Takeuchi et al. Validation of the prognostic value of histopathological grading or c-kit mutation in canine cutaneous mast cell tumours: A retrospective cohort study. Vet J 2013.

Study Objective: Investigating the prognostic value of the Patnaik and Kiupel histopathology grading systems and c-kit mutations for canine MCTs by a retrospective evaluation of the correlation between tumor status and clinical course in dogs diagnosed with cutaneous MCTs.

Study Design: Retrospective cohort

Materials & Methods: Cases seen by one institution, standard work-up, and histopathology reviewed by 3 pathologists. Follow-up standardized.

47 dogs, MCTs classified as follows:

- GI (n = 3), GII (n = 37) and GIII (n = 7)
- LG (n = 28) and HG (n = 19). Genetic alterations of c-kit were found in 34/47 (72.3%);

13 dogs (27.7%) had an activating c-kit mutation. TD-Exon11 was observed in eight dogs (17%).

Conclusions Drawn: The present study indicated a superior prognostic value of the Kiupel histopathology grading for canine cutaneous MCTs compared to the Patnaik system. Patnaik grade III was correlated with poorer prognosis, whereas no difference of mortality between GI and GII was detected. Furthermore, ITDExon11 appeared to be a useful predictor for PFS, while activating c-kit mutations were insufficient prognostic tools among cases of canine cutaneous MCT.

Statistical Soundness: ok, but low number of cases (47). No exclusion for dogs treated with local or systemic adjuvant treatment. The margin of excision was not considered.

Subgroup conclusions: GIII and HG MCTs are associated with a shorter PFS and OS and *ckit* ITD of exon 11 is also associated with a shorter PFS, but might not be independent from tumor grade. The Kiupel grading scheme, which is here validated as an independent prognosticator of OS, shows a greater inter-observer consistency compared to the Patnaik system. Considering the limitations of the study (various adjunctive therapies, relatively small sample size with few GI, staging not included) the results are recognized and considered in conjunction with other studies in the literature evaluating similar parameters.

Vascellari et al. Expression of Ki67, BCL-2, and COX-2 in Canine Cutaneous Mast Cell Tumors: Association With Grading and Prognosis. *Vet Path.* 2014.

Study Objective: Ki67, BCL-2, and COX-2 protein and gene expression have been investigated in canine cutaneous MCTs by immunohistochemistry (IHC) and quantitative real time RT-PCR (qPCR), in order to evaluate their prognostic significance and their association with other well-accepted prognostic markers, such as the histologic grading and the MI.

Study Design: Prospective with follow-up of at least one year.

Materials & Methods: Cutaneous MCTs excised and available for review by 3 pathologists and for evaluation of the molecular markers Ki-67, BCL-2 and COX2. These were evaluated for impact on survival and for independence from grade.

Conclusions Drawn: Both grading systems were significantly associated with prognosis. The Patnaik grading was of limited prognostic value for GII MCTs, with 23% being associated with mortality. The concordance among pathologists was strongly improved by the application of the 2-tier grading system, and 71% of HG MCTs died of mast cell disease. MI and Ki67 protein expression were significantly associated with grading and survival.

Statistical Soundness: Ok, but low number of events (16%, 8/51). The distribution between grades was suboptimal with only few Patnaik GIII and few Kiupel HG MCTs. Definition of end-points, and how they were determined, was lacking as well as the description of follow-up protocol as it would be expected in a prospective study. Dogs treated with chemotherapy were allowed and this could be a confounder.

Subgroup conclusions: Both grading systems are significantly associated with prognosis. Patnaik system is more sensitive, while Kiupel system is more specific in detecting dogs with aggressive disease. Kiupel system causes less discordance amongst pathologists (the most significant inter-observer variation using the Patnaik system was in the distinction between GI and GII). All GI are classified as LG, all GIII are classified as HG and most of GII are LG. MI and Ki67 remain valuable prognostic factors. The method of performing MI and/or Ki67 index in daily diagnostic practice needs to be standardized or emulate that performed in the study from which the pathologist references / draws conclusions from.

Sabattini et al. Histologic Grading of Canine Mast Cell Tumor: Is 2 Better Than 3? *Vet Path* 2015.

Study Objective: To evaluate the Patnaik and Kiupel grading systems comparatively in the same cohort of dogs with MCT to determine which is the best predictor with regard to tumor mortality. To describe the behavior of subcutaneous MCT compared to dermal MCT.

Study Design: Retrospective cohort

Materials & Methods: Cases with surgery as only treatment modality and at least 12 months of follow up were included in the study. Kiupel and Patnaik grade was assigned by the agreement of three pathologists.

Conclusions Drawn: Both grading systems were prognostic for OS. All GI were LG, all GIII were HG and most GII were LG. Statistically significant differences in survival were detected for GII/LG vs GII/HG and GII/HG vs GIII/HG. Most subcutaneous MCTs behaved in an indolent way.

Statistical Soundness: ok, high number of cases (162).

Subgroup conclusions: This study confirms the prognostic value of Patnaik system (GIII vs GII+GI) and validates the Kiupel system as an independent prognosticator of OS. The Kiupel system helps to redefine the prognosis of GII MCTs.

Stefanello et al. Comparison of 2-and 3-category histologic grading systems for predicting the presence of metastasis at the time of initial evaluation in dogs with cutaneous mast cell tumors: 386 cases (2009-2014). JAVMA 2015.

Study Objective: To compare the Kiupel and Patnaik histologic grading systems for predicting the presence of metastasis at the time of initial evaluation in dogs with cutaneous MCTs.

Study Design: Retrospective.

Materials & Methods: Dogs with cutaneous MCTs with complete clinical staging prior to treatment were included over a 5-year period. Variety of pathologists used.

Conclusions Drawn: All GI were LG, all GIII were HG and most GII were LG. Metastatic rates were as follows:

GI/LG: 6% lymph node mets and 2% distant mets GII/LG: 16% lymph node mets and 2% distant mets GII/HG: 15% lymph node mets and 2% distant mets

- GIII/HG: 46-49% lymph node mets and 21% distant mets GIII/HG were more likely to have mets than GII/HG. Prognostication should not rely on histological grade alone, as a low percentage of GI/LG had mets at diagnosis. Variables other than grade significantly associated with nodal metastases included tumor diameter (>3 cm), digit location, ulceration, Shar-Pei breed, and substage b.

Statistical Soundness: ok, large number of cases (386).

Subgroup conclusions: This study validates the Kiupel system as a predictor of metastatic disease at time of diagnosis. The Patnaik system clarifies the behavior of HG MCTs, as metastatic rates are significantly different between GIII/HG and GII/HG. GI/LG have a low risk of lymph node metastasis and distant metastasis, stressing the importance of staging even in this group of patients. GII/LG and GII/HG have similar metastatic rates.

Van Lelyveld et al comparison between Ki-67 index and mitotic index for predicting outcome in canine mast cell tumours. *JSAP* 2015.

Study Objective: To assess the correlation between Ki67 index and mitotic index and determine which more accurately predicts survival in dogs with MCTs.

Study Design: Retrospective.

Materials & Methods: Mitotic index and Ki67 index were calculated for 162 dogs from three referral hospitals. Different labs and cut-off values of Ki67 were used. Tumors were graded only with Patnaik system.

Conclusions Drawn: The mitotic index (cutoff value 5) was specific, but not sensitive to predict survival and a cut-off value of 2 was recommended to increase sensitivity. The authors suggested that a three-tier mitotic index assessment may more accurately predict death due to MCT. Ki-67 was sensitive, but not specific.

Statistical Soundness: Limited (different labs and cut-off values, no standardization between pathologists and follow-up).

Subgroup conclusions: This study overall confirms that mitotic index and Ki-67 are prognostic factors in dogs with MCTs. Specific data gained from this study need to be taken with caution due to poorly standardized pathology methodologies used.

Berlato et al. Comparison of mitotic index and Ki67 index in the prognostication of canine cutaneous mast cell tumours. VCO 2013

Study Objective: To compare the relative abilities of Ki67 and mitotic index to predict survival in the same cohort of dogs with cutaneous MCTs.

Study Design: Retrospective.

Materials & Methods: 95 MCTs were assessed for histologic grade (Patnaik system only), mitotic index, and Ki67 index. Clinical follow-up was performed.

Conclusions Drawn: Mitotic index (cut-off of 5) and Ki67 (cut off of 1.8) index were both able to significantly predict survival of dogs with MCT independently from the histological grade. Sensitivity, specificity and diagnostic accuracy of mitotic index and Ki67 index appeared similar for GII tumors, indicating that there might not be a real benefit in choosing one test instead of the other. Ki67 index should be requested when the history (tumour growth pattern or presence of paraneoplastic syndromes), characteristic of the tumour (size, ulceration or degree of local inflammation) and mitotic index are discordant.

Statistical Soundness: ok.

Subgroup conclusions: Mitotic index, grade (Patnaik) and Ki67 are independent prognostic factors. In this canine population, MI and Ki67 performed similarly.

Scarpa et al. Cytological grading of canine cutaneous mast cell tumours. VCO 2014.

Study Objective: To determine whether the Kiupel criteria may be successfully applied to cytologic samples resulting in pre-operative grading.

Study Design: Prospective.

Materials & Methods: 50 consecutive cases of cutaneous MCTs with cytologic diagnosis (with a minimum number of 1000 morphologically-intact mast cells assessed), surgical excision, and histologic examination were included.

Conclusions Drawn: Cytologic grading based on number of mitoses, multinucleated cells, bizarre nuclei and presence of karyomegaly correlated well with the Kiupel grade with an overall accuracy of 94%. Karyomegaly and multinucleation were the most sensitive individual indicators. This cytological grade may misdiagnose a certain subset of histologically HG as cytologically low grade tumors.

Statistical Soundness: ok.

Subgroup conclusions: The cytological grading scheme proposed by these authors correlate well with the histological Kiupel system, although a certain risk of underestimating the malignancy exist. Another limitation is that the cytological grade does not allow to differentiate between cutaneous and subcutaneous MCTs. Future studies might evaluate whether the accuracy of cytological grading can be improved with the addition of Romanowsky-type stained samples for non-nuclear features, such as variation in cytoplasmic granulation.

Berlato et al. Comparison of minichromosome maintenance protein 7, Ki67 and mitotic index in the prognosis of intermediate Patnaik grade cutaneous mast cell tumours in dogs. VCO 2018.

Study Objective: To determine if minichromosome maintenance protein 7 (MCM7) score was a prognostic factor in dogs with grade II MCT treated with surgery alone. Secondary objectives included to determine prognosis of MCM7 score in relation to the Kiupel grading system, mitotic index, and Ki67 index.

Study Design: Retrospective

Materials & Methods: 90 dogs with grade II MCTs were included in the study (82 with low grade MCTs and 8 with high grade MCTs). Patnaik and Kiupel histologic grade and mitotic index were determined histologically, and immunohistochemistry was done to determine MCM7 score and Ki67 index.

Conclusions Drawn: At the end of the study period, 72 dogs were alive (median 1136 days) and 18 dogs had died of MCT related reasons (median 116 days). Using a mitotic index threshold of 5, the sensitivity, specificity, and accuracy of predicting MCT-related death was 0.39, 0.99, and 0.87, respectively. Using a Ki67 threshold of 0.018, the sensitivity, specificity, and accuracy of predicting MCT-related death was 0.78, 0.83, and 0.86, respectively. Using a MCM7 threshold of 0.18, the sensitivity, specificity, and accuracy of predicting MCT-related death was 0.83, 0.86, and 0.86, respectively. The combination of mitotic index, Ki67 index, and MCM7 score had better accuracy of predicting MCT-related death than any single variable alone, but only when thresholds for Ki67 was increased from 0.018 to 0.05 and MCM7 was increased from 0.18 to 0.25.

Statistical Soundness: Good

Subgroup conclusion: The combination of Ki67 and MCM7 with mitotic index and the two histologic grading schemes was able to increase the ability to detect dogs likely to die of MCT-related reasons, not only in dogs with high grade II MCTs but, perhaps more importantly, dogs with low-grade II MCTs.

Shaw et al. Diagnostic accuracy of pre-treatment biopsy for grading cutaneous mast cell tumours in dogs. VCO 2018.

Study Objective: To determine concordance between preoperative biopsy techniques (wedge, punch, needle core) and postoperative specimen.

Study Design: Retrospective

Materials & Methods: Retrospective analysis of cases from a pathology laboratory from 2008 to 2016; cases included if they had a complete histopathology report for both a preoperative biopsy and postoperative specimen.

Conclusions Drawn: Concordance of 96% for Patnaik grading scheme (92%, 100%, and 100% for wedge biopsies, punch biopsies, and needle core biopsies, respectively) and 92% for Kiupel grading scheme (90%, 95%, and 100% for wedge biopsies, punch biopsies, and needle core biopsies, respectively). Concordance decreased with increasing age and decreasing tumor size. Discordant results tended to underestimate the histologic grade. Preoperative biopsies were sufficiently accurate to differentiate low and high grade MCTs prior to definitive treatment.

Statistical Soundness: Good

Subgroup conclusion: This study provides good evidence that preoperative biopsy is accurate, but most members would not recommend it.

Horta et al. Assessment of Canine Mast Cell Tumor Mortality Risk Based on Clinical, Histologic, Immunohistochemical, and Molecular Features. Vet Path 2018.

Study Objective: To develop a mortality risk classification system based on clinical, histologic, immunohistochemical, and molecular features of individual MCTs.

Study Design: Combined retrospective and prospective

Materials & Methods: 149 dogs with cutaneous and subcutaneous MCTs were included. Univariate and multivariate analyses were performed on a number of clinical, histologic, IHC, and molecular features of each MCT.

Conclusions Drawn: On multivariate analysis, an amended WHO clinical stage and a history of tumor recurrence (local or distant) were independent predictors of overall survival. The amended WHO clinical staging scheme was described; stage I: single non-metastatic MCT; stage II: ≥ 3 non-metastatic MCTs; stage III: single MCT metastatic to lymph node; stage IV: large, poorly circumscribed infiltrative MCTs or multiple (≥ 3) MCTs with lymph node metastasis; stage V: distant metastasis.

Statistical Soundness: Good, but too many uncontrolled variables

Group conclusion: Both cutaneous and subcutaneous MCT were included in the study. There was no standardized treatment. The mean follow-up was short (~1 year).

Hergt et al. Use of a 2-tier histologic grading system for canine cutaneous mast cell tumors on cytology specimens. Vet Clin Path 2016.

Study Objective: To determine whether the criteria of the Kiupel, 2-tier, grading scheme can be applied to cytology samples

Study Design: retrospective

Materials & Methods: inclusion of cases with a diagnosis of cutaneous MCT and both cytology and histopathology available for review. 141 MCTs meet the inclusion criteria and were graded histologically and cytologically according to the two-tier Kiupel grading scheme by one pathologist and one clinical pathologist. The Kiupel criteria was applied directly to both samples without modifications.

Conclusions Drawn: 141 MCTs were included in the study. Histologically 38 were HG and 103 LG. Cytologically 36 were HG and 105 LG. There was cyto/histo concordance in 133 cases (sensitivity 87%; specificity 97%; accuracy 94%; Kappa 0.85). 8 cases were diagnosed incorrectly: 3 were cytologically high grade, but histologically low grade (due to karyomegaly), and 5 were low grade on cytology, but high grade on histopathology.

Statistical Soundness: Good

Group conclusion: The histopathologic criteria of the Kiupel grading scheme does not translate directly into cytology and further evaluation is needed to determine which features are cytologically more important to provide an accurate grade. Interobserver variation is a reason of concern and the results of this study might not be reproducible. Histology is still the gold standard for grading MCTs.

Camus et al. Cytologic Criteria for Mast Cell Tumor Grading in Dogs With Evaluation of Clinical Outcome. Vet Path 2016.

Study Objective: to determine cytologic features which correlate with histologic grade and create a cytologic grading scheme which correlates with histopathologic grade and survival

Study Design: retrospective samples - cytologic and histologic evaluation repeated in blinded, prospective fashion with phone calls to evaluate outcome

Materials & Methods: cases were considered for the creation of a grading scheme and correlation with histopathology if a biopsy was available and obtained within 8 weeks of cytology. 152 cases met these criteria. Cases were considered for prognostic evaluation if follow up was available. 139 cases met these criteria. All samples were reviewed, blindly, by 3 pathologists and 3 clinical pathologists. Two different objectives were evaluated statistically, correlation with histopathology and prediction of survival, but there was no control for post-surgery treatment.

Conclusions Drawn: Histologic grades were GI 8%, GII 85.5%, GIII 6.5% and LG 89% and HG 11%. Different algorithms were evaluation to best predict survival. The cytological algorithm that most closely correlated with histological grade (K=0.73) classified a MCT as high grade if was poorly granulated or had at least two of the following features: presence of any mitotic figures, anisokaryosis, binucleation/multinucleation, or nuclear pleomorphism. With this system 32% of high grade MCTs were false positive, while only 1.5% of low grade MCTs were false negative. Both histological and cytological grading were significantly associated with outcome (median survival time and survival).

Statistical Soundness: Good.

Group conclusions: Cytologic grading is promising, inexpensive and available on samples already obtained for diagnosis. The cytologic diagnosis of low grade correlates well with histology and outcome and may be beneficial in limiting further diagnostics in cases where finances are limited. It should not be relied upon to determine adjuvant treatment or prognosis if high grade though, as 30% were false positives. Ideally, dogs with high grade cytology should receive staging and surgery, or an incisional biopsy prior if grade would change the surgical approach. Larger numbers of both samples and pathologists are needed to validate this particular grading scheme.

Sabattini et al. Comparison between May-Grünwald-Giemsa and rapid cytological stains in fine-needle aspirates of canine mast cell tumour: Diagnostic and prognostic implications. VCO 2016.

Study Objective: to determine the frequency of mast cell hypogranularity using rapid stains (RS) compared with May-Grünwald-Giemsa (MGG) and the possible effects on diagnosis, prognosis and recognition of nodal metastatic disease in cutaneous and subcutaneous MCTs.

Study Design: Prospective.

Materials & Methods: Cytological preparations of primary MCT and metastatic LNs were collected prospectively, stained with both RS and MGG, and assessed by two pathologists for granulation (scale 1-4). Before inclusion the diagnosis was confirmed histologically.

Conclusions Drawn: 60 MCTs in 50 dogs (40 cutaneous and 20 subcutaneous). With MGG, 48 MCTs (80%) were highly granular, 10 (16.7%) were moderately granular, and 1 (1.7%) tumour each was poorly granular and not granular. With RS, 44 MCTs (73.3%) were highly granular, 8 (13.3%) moderately granular, 4 (6.7%) poorly granular and 4 (6.7%) were not granular. Twenty-eight cases of histologically confirmed metastatic lymph nodes were included. With MGG, 12 MCTs (42.8%) were highly granular, 14 (50%) moderately granular and 2 (7.1%) poorly granular. With RS, 6 MCTs (21.4%) were highly granular, 10 (35.7%) moderately granular, 4 (14.3%) poorly granular and 8 (28.6%) were not granular. With RS, 13 cases (46.4%) were hypogranular compared with the corresponding MGG smear. The evaluation of the smears stained with MGG resulted in a diagnosis of certain or suspected metastasis in the 100% of cases by all 3 observers. With RS, the same observers did not identify metastatic lesions in up to 17.8% of cases.

Statistical Soundness: Good.

Group conclusions: This study confirms that RS can be ineffective in staining MCT granules, particularly in high-grade tumours. In dubious cases, methanolic stains should be applied. RS should not be used to assess lymph node specimens for staging.