VCS/ACVP Oncology-Pathology Working Group
Summary and Subgroup Recommendations for
Grading of Canine Cutaneous Mast Cell Tumors (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 divulgated in 2013. Since then, some key manuscripts have been published expanding and improving our knowledge on the subject. Some studies validated the use of the two-tier grading system and other supported the prognostic value of the mitotic index. A new innovative field is the development of cytological grade and we report the initial findings. This current document takes into account new and relevant literature since the time of the initial document (2013) 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.

Overall Summary / Recommendations:

Based on review of the recent literature, the ***Canine Cutaneous Mast Cell Tumor*** subgroup has concluded and recommends the following regarding *grading of canine cutaneous mast cell tumors.*

Histological Grading

The Patnaik grading system has been the foundation for determining the prognosis of canine cutaneous mast cell tumors (MCTs) since 1984[1]. This grading system divides cutaneous MCTs into three different grade categories (grade I (GI), grade II (GII), and grade III (GIII) based on extent of tissue involvement, cellularity, cellular and nuclear morphology, mitotic activity, stromal reaction, and edema/necrosis. Numerous studies have proven its validity in the clinical setting[2]. 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 rate[3]. It is more difficult, however, to predict the behavior of GII MCTs; in fact the majority of them have a benign clinical progression, while about 20-50% are characterized by an aggressive clinical behavior[2]. Another limitation of the Patnaik system is the subjectivity of its application by different pathologists with the resultant variability in the grade assignment[4-6].

In order to overcome these two limitations, a new two-tier grading system was developed by Kiupel and colleagues in 2011[4]. This system divides cutaneous MCTs into two categories (low grade and high grade) based only on nuclear morphological criteria including mitotic figures, multinucleation, bizarre nuclei, and karyomegaly. The Kiupel grading system has been validated in multiple studies[5,7-10] as an independent prognostic factor in canine cutaneous MCTs being able to predict local recurrence[8], metastatic propensity[9] and overall survival[5,7,10]. An advantage of the Kiupel grading system is that the criteria for assigning the grade are more objectively defined, increasing the concordance between pathologists[5,7].

Few studies have applied both grading systems (Patnaik and Kiupel) to the same population of dogs[5,8-10]. 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 18%) being HG[5,8-10]. 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 disease[5]. The evidence is still weak, but it appears that the two systems could be complementary so the Kiupel system might help to redefine the prognosis of GII Patnaik MCTs[4,10], while the Patnaik system clarifies the behavior of HG MCTs[9].

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

- **GI/LG**. The prognosis is excellent with virtually no tumor-related deaths. One study[9] reports a low risk of lymph node metastasis (6%) and distant metastasis (2%) stressing the importance of staging even in this group of patients.
- **GII/LG**. Only two manuscripts describe this category of patients[9,10]. The prognosis is good with 6% of dogs dying for causes related to MCTs[10]. At initial staging, the metastatic rate to the lymph node was 16%, while distant metastases were seen in 2% of the cases[9].
- **GII/HG**. Only two manuscripts describe this category of patients[9,10]. The prognosis is guarded with 50% of dogs dying for causes related to MCTs[10]. At initial staging, the metastatic rate to the lymph node was 15%, while distant metastases were seen in 2% of the cases[9].
- **GIII/HG**. The prognosis is guarded to poor with 67-75% tumor-related deaths in studies with adequate number of cases[3,10]. At initial staging, the metastatic rate to the lymph node was 46-49% and 21% in distant sites[9,11].

According to the current data available it appears that the difference in survival seen between GII/LG and GII/HG is due to local recurrence rather than metastatic disease. In fact, metastatic rates between these two groups appear similar[9]. This hypothesis could in part be corroborated by the study of Donnelly and colleagues, which showed that removal of MCTs with histologically 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 margin[8].

Mitotic index

An independent prognostic indicator is mitotic index[5,12-15]. The mitotic index (MI) is obtained by 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 (100x magnification/10x objective) of the specimen[12]. In three studies evaluating MI as a prognostic factor with a threshold of 5[5,12,14], the sensitivity of MI to predict tumor-related

death ranged between 44 and 55% and the specificity between 86 and 98%. The 1-year survival rate for cutaneous MCTs with a MI≤5 ranged from 83 to 92%, while for MCTs with a MI>5 the survival rate varied from 20 to 25%. Mitotic index has been also significantly associated with the metastatic rate, but not with recurrence rate[12]. Some authors[13,15] suggested that the stratification of the MI into three categories might be superior, but these methods are less easily applicable in the clinical setting. Future studies on MI should also better define the number of sections evaluated to find the areas with highest mitotic activity and 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[16].

Cytological Grading

Cytological grading based on number of mitoses, multinucleated cells, bizarre nuclei and presence of karyomegaly correlated well with the Kiupel histological grade with a reported accuracy of 94%[17]. A limitation of this cytological grading is the risk of misdiagnosing a subset of HG as a LG MCT. A secondary limitation is that it is impossible to differentiate cutaneous from subcutaneous MCTs in cytological specimens. 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.

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 respective system (as published)[1,4] available for reference at the time of grading (See Appendix p. 6).

2. All histological reports of canine cutaneous MCTs should also include the MI, 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.

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: size and possibly site of the mast cell tumor, presence of metastasis (stage), completeness and quality of surgical margins, and prognostic markers (e.g. proliferative markers, kit expression, *c-kit* mutation).

4. Cytological grading should be further validated in combination with the above grading schemes before its routine application to the clinical practice.

Canine Cutaneous Mast Cell Tumor Grading Systems

Patnaik Grading Criteria, 1984

Table 1. Summary of the Patnaik morphologic grading classifications for canine cutaneous mast cell tumors.[8]

	Tumor grade		
	I	II	III
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; most cells distinct cytoplasm with fine granules, but some with indistinct cytoplasm and large/hyperchromatic granules	Round, ovoid, or spindle shaped, pelomorphic, medium sized; cytoplasm indistinct 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 with double nuclei	Indented to round vesiculated, with 1 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, fibrosvascular 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	Areas of diffuse edema and necrosis	Edema, hemorrhage, and necrosis common

Two-Tier Grading Criteria, 2011

High-grade MCT is characterized by any one following criteria:

- ≥ 7 MFs/10hpf

  Evaluated in regions with highest mitotic activity

- ≥ 3 multinucleated cells / 10hpfs

  Where ≥ 3 nuclei constitutes a multinucleated cell

- ≥ 3 bizarre nuclei / 10hpf

  Highly atypical with marked indentations, segmentation, and irregular shape

- Karyomegaly

  Where at least 10% of neoplastic cells vary by 2-fold

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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4. **Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. Vet Path. 2011 Jan 25;48:147–155.

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9. * Stefanello D, Buracco P, Sabattini S, 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 Apr 1;246:765–769.

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13. **Elston LB, Sueiro FAR, Cavalcanti JN, Metze K. Letter to the Editor: The importance of the mitotic index as a prognostic factor for survival of canine cutaneous mast cell tumors: a validation study. Vet Path. 2009 Mar 1;46:362–364.

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prognostication of canine cutaneous mast cell tumours. VCO. 2015 Jun;13:143–150.

15. * van Lelyveld S, Warland J, Miller R, et al. Comparison between Ki-67 index and mitotic index for predicting outcome in canine mast cell tumours. JSAP. 2015 May;56:312–319.

16. Meuten DJ, Moore FM, George JW. Mitotic count and the field of view area: time to standardize. Vet Path. SAGE Publications; 2016 Jan;53:7–9.

17. * Scarpa F, Sabattini S, Bettini G. Cytological grading of canine cutaneous mast cell tumours. VCO. Blackwell Publishing Ltd; 2014 Apr 9; DOI: 10.1111/vco.12090.

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 moths 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 prospectic 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 kariomegaly correlated well with the Kiupel grade with an overall accuracy of 94%. Kariomegaly 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.