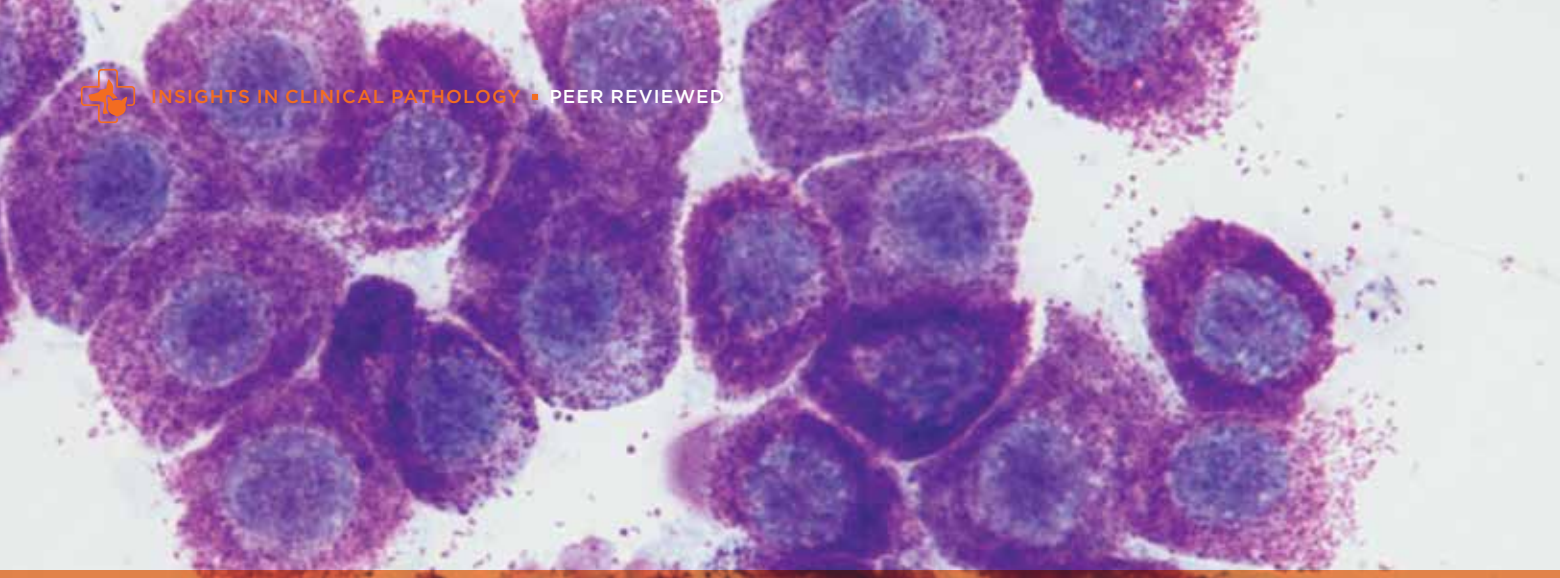

INSIGHTS IN CLINICAL PATHOLOGY

Cytologic Grading of Mast Cell Tumors

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Mast cell tumors (MCTs) are common in dogs, making up approximately 20% of canine skin tumors. Their biological behavior is variable, and grading provides significant prognostic information for use in making treatment decisions.[1]

HISTOPATHOLOGIC GRADING

Currently, histopathologic evaluation is considered the gold standard for grading MCTs. These grading systems have evolved over the years. Historically, a 3-tier system (Patnaik system) has been used, defining MCTs as grade I, II, or III. Grade I tumors are well differentiated with a low metastatic rate (7% mortality from tumor-related disease) and grade III are poorly differentiated with a high metastatic rate (94% mortality from tumor-related disease).[2] Patnaik grade II tumors tend to be overrepresented, generally accounting for 42% to 78% of MCTs, but sometimes cited as comprising 91% of MCTs.[3-5] Reports have shown significant interobserver differences in grading MCTs with the Patnaik system.[3,6] The biological behavior of grade II tumors is variable, with up to 20% being aggressive,[7] and together, these factors lead to uncertainty in the appropriate management of many patients using the Patnaik system.

In 2011, a 2-tier classification system was proposed by Kiupel et al, defining MCTs as either low grade or high grade.[6] In this system, criteria for a high-grade MCT include the presence of any of the following characteristics:

- ≥7 mitotic figures in 10 high-power fields (HPF)
- ≥3 multinucleated cells (at least 3 nuclei) in 10 HPF
- ≥3 bizarre nuclei in 10 HPF

Abstract

Mast cell tumors are common skin tumors of dogs. These tumors have variable biological behavior, and tumor grading can provide prognostic information and help determine an individual treatment plan. This article briefly outlines the histologic grading schemes in use and reviews several recent studies that attempt to apply grading criteria to cytologic samples from primary mast cell tumors. These studies have shown that cytologic grading is promising for predicting patient outcome. Potential limitations are also discussed.

Take-Home Points

- Grading of mast cell tumors (MCTs) provides significant prognostic information about expected biological behavior and helps devise appropriate treatment plans.
- Two histologic grading systems are currently in use for cutaneous MCTs. The Patnaik system classifies MCTs into 1 of 3 grades (grade I, II, or III) and the Kiupel system is a 2-tier system (low grade and high grade). Often, both are reported.
- Cytologic grading systems have been examined and show promise, both in predicting the histologic grade of the tumor following removal and in independently predicting clinical outcome.
- Cytologic features that are evaluated when attempting to grade MCTs include the degree of granulation of the cells and the presence of mitotic figures, variation in nuclear size, nuclear pleomorphism, the presence of binucleated or multinucleated cells, and the number of fibroblasts/collagen fibrils.
- Granules of MCTs may not stain well with aqueous Romanowsky stains such as Diff-Quik, and this must be considered when staining slides from these tumors in clinic.

- Karyomegaly (nuclear diameters vary by at least twofold in ≥10% of cells)

Classification is based on the histologic area with the most mitotic figures or nuclear variation. Kiupel et al found that there is good interobserver consistency in categorization with this system and a high correlation between grade and clinical outcome.

In the 2013 Oncology-Pathology Working Group (OPWG) consensus statement on grading of cutaneous MCTs, the application of both the Patnaik and Kiupel grading schemes was suggested. In 2021, the OPWG suggested incorporation of the 2 grading systems to better predict behavior of some tumors and study survival rate.[7] MCTs are thus categorized as:

- Grade I/low-grade
- Grade II/low-grade
- Grade II/high-grade
- Grade III/high-grade

CYTOLOGIC GRADING

Cytology is a quick, inexpensive method routinely used to diagnose MCTs. Generally, these tumors exfoliate high numbers of cells that typically contain large numbers of small, round, purple granules, making diagnosis straightforward.

The cellular features that compose the Kiupel grading system can be evaluated on cytologic preparations. Several recent studies investigated how well cytologic features correlate to histologic grade. Two of these studies directly applied the Kiupel system to cytologic preparations.[8,9] Histopathology was considered the gold standard for grading, and overall, both studies found good correlation between

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(florfenicol, terbinafine, mometasone furoate)
Otic Solution for use in dogs only

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See full product insert for complete prescribing information, a summary of which follows.
CAUTION: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.
DESCRIPTION: CLARO® contains 16.6 mg/mL florfenicol, 14.8 mg/mL terbinafine (equivalent to 16.6 mg/mL terbinafine hydrochloride) and 2.2 mg/mL mometasone furoate. Inactive ingredients include purified water, propylene carbonate, propylene glycol, ethyl alcohol, and polyethylene glycol.
INDICATIONS:
CLARO® is indicated for the treatment of otitis externa in dogs associated with susceptible strains of yeast (*Malassezia pachydermatis*) and bacteria (*Staphylococcus pseudintermedius*).
DOSAGE AND ADMINISTRATION:
CLARO® should be administered by veterinary personnel.
Wear eye protection when administering CLARO®.
(see Human Warnings, PRECAUTIONS, POST APPROVAL EXPERIENCE).
Splatter may occur if the dog shakes its head following administration. Persons near the dog during administration should also take steps to avoid ocular exposure.
Shake before use.
Verify the tympanic membrane is intact prior to administration. (see CONTRAINDICATIONS, PRECAUTIONS, POST APPROVAL EXPERIENCE).
Administer one dose (1 dropperette) per affected ear.

1. Clean and dry the external ear canal before administering the product.
2. Verify the tympanic membrane is intact prior to administration.
3. Remove single dose dropperette from the package.
4. While holding the dropperette in an upright position, remove the cap from the dropperette.
5. Turn the cap over and push the other end of the cap onto the tip of the dropperette.
6. Twist the cap to break the seal and then remove cap from the dropperette.
7. Screw the applicator nozzle onto the dropperette.
8. Insert the tapered tip of the dropperette into the affected external ear canal and squeeze to instill the entire contents (1 mL) into the affected ear.
9. Gently massage the base of the ear to allow distribution of the solution. **Restrain the dog to minimize post application head shaking** to reduce potential for splatter of product and accidental eye exposure in people and dogs (see **POST APPROVAL EXPERIENCE**).
10. Repeat with other ear as prescribed.
11. The duration of the effect should last 30 days. Cleaning the ear after dosing may affect product effectiveness.

CONTRAINDICATIONS:
Do not use in dogs with known tympanic membrane perforation (see **PRECAUTIONS**). CLARO® is contraindicated in dogs with known or suspected hypersensitivity to florfenicol, terbinafine hydrochloride, or mometasone furoate.
WARNINGS:
Human Warnings: **CLARO® may cause eye injury and irritation** (see **PRECAUTIONS, POST APPROVAL EXPERIENCE).** If contact with eyes occurs, flush copiously with water for at least 15 minutes. If irritation persists, contact a physician. Humans with known hypersensitivity to any of the active ingredients in CLARO® should not handle this product.
PRECAUTIONS:
For use in dogs only. Do not use in cats (see **POST APPROVAL EXPERIENCE**).
Wear eye protection when administering CLARO® and restrain the dog to minimize post application head shaking. Reducing the potential for splatter of product will help prevent accidental eye exposure in people and dogs and help to prevent ocular injury (see **DOSAGE AND ADMINISTRATION, Human Warnings, POST APPROVAL EXPERIENCE**).
Proper patient selection is important when considering the benefits and risks of using CLARO®. The integrity of the tympanic membrane should be confirmed before administering the product. CLARO® has been associated with rupture of the tympanic membrane. Reevaluate the dog if hearing loss or signs of vestibular dysfunction are observed during treatment. Signs of internal ear disease such as head tilt, vestibular signs, ataxia, nystagmus, facial paralysis, and keratoconjunctivitis sicca have been reported (see **POST APPROVAL EXPERIENCE**) with the use of CLARO®.
Do not administer orally.
Use of topical otic corticosteroids has been associated with adrenocortical suppression and iatrogenic hyperadrenocorticism in dogs (see **ANIMAL SAFETY**).
Use with caution in dogs with impaired hepatic function (see **ANIMAL SAFETY**).
The safe use of CLARO® in dogs used for breeding purposes, during pregnancy, or in lactating bitches, has not been evaluated.
ADVERSE REACTIONS:
In a field study conducted in the United States (see **EFFECTIVENESS**), there were no directly attributable adverse reactions in 146 dogs administered CLARO®. **POST APPROVAL EXPERIENCE (2019)**: The following adverse events are based on post-approval adverse drug experience reporting for CLARO®. Not all adverse events are reported to FDA/CVM. It is not always possible to reliably estimate the adverse event frequency or establish a causal relationship to product exposure using these data.
In **humans**, accidental exposure leading to corneal ulcers and other ocular injuries such as eye irritation and redness have been reported. Exposure occurred when the dog shook its head after application of CLARO®. Skin irritation has also been reported. In **dogs**, the adverse events reported are presented below in decreasing order of reporting frequency: Ear discharge, head shaking, ataxia, internal ear disorder (head tilt and vestibular), deafness, emesis, nystagmus, pinnal irritation and ear pain, keratoconjunctivitis sicca, vocalization, corneal ulcer, cranial nerve disorder (facial paralysis), tympanic membrane rupture.
CLARO® is not approved for use in **cats**. The adverse events reported following extra-label use in **cats** are presented below in decreasing order of reporting frequency: Ataxia, anorexia, internal ear disorder (head tilt and vestibular), Horner's syndrome (third eyelid prolapse and miosis), nystagmus, lethargy, anisocoria, head shake, emesis, tympanic rupture, and deafness.
To report suspected adverse drug events and/or obtain a copy of the Safety Data Sheet (SDS) or for technical assistance, contact Elanco at 1-800-422- 9874.
For additional information about adverse drug experience reporting for animal drugs, contact FDA at 1-888-FDA-VETS or online at http://www.fda.gov/reportanimalae.
Information for Dog Owners:
Owners should be aware that adverse reactions may occur following administration of CLARO® and should be instructed to observe the dog for signs such as ear pain and irritation, vomiting, head shaking, head tilt, incoordination, eye pain and ocular discharge (see **POST APPROVAL EXPERIENCE**). Owners should be advised to contact their veterinarian if any of the above signs are observed. Owners should also be informed that splatter may occur if the dog shakes its head following administration of CLARO® which may lead to ocular exposure. Eye injuries, including corneal ulcers, have been reported in humans and dogs associated with head shaking and splatter following administration. Owners should be careful to avoid ocular exposure (see **PRECAUTIONS, POST APPROVAL EXPERIENCE**)

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MCT grades determined cytologically versus histologically. Cytologic grading matched histologic grading in 94% of cases in both studies. Some cytologic limitations were identified, including fewer numbers of cells for evaluation and lower occurrence of mitotic figures on cytologic preparations compared with histologic preparations.

Camus Grading System

To overcome these limitations, Camus et al developed a unique cytologic grading system that did not directly apply the histologic grading criteria to cytologic samples.[10] They statistically evaluated various combinations of cellular features to create a grading scheme that correlated with not only histologic grade but also—and more importantly—patient survival. The cytologic feature that best correlated with 2-year survival was degree of granulation of tumor cells, followed by anisokaryosis, multinucleation, binucleation, and mitotic figures. In this cytologic grading scheme, tumors are classified as high grade if they meet either or both of 2 criteria:

1. Poor granulation of the entire cell population
2. The presence of 2 of the following features of atypia:
    - **a.** Mitotic figures
    - **b.** Binucleated or multinucleated cells
    - **c.** Nuclear pleomorphism (shapes not including round and oval)
    - **d.** Anisokaryosis (twofold variation in nuclear size, termed karyomegaly in Kiupel system)

Granulation of mast cells is categorized as heavily granulated (**FIGURE 1**), mixed granulation (a mixture of heavily granulated and poorly granulated cells), and poorly granulated (**FIGURE 2**). Only tumors in which all cells show poor granulation are categorized as high grade based on this criterion.

In contrast to the quantitative metrics of the Kiupel system, the Camus cytologic grading system considers the presence of *any* mitotic figures (**FIGURE 3**), binucleated (**FIGURE 4**) or multinucleated (**FIGURE 5**) cells, atypically shaped nuclei (bizarre nuclei or nuclear pleomorphism; **FIGURE 6**), or cells with twofold variation in nuclear size (**FIGURE 7**) a positive finding for that feature. The presence of any 2 of these features classifies a tumor as high grade. Cytologic and

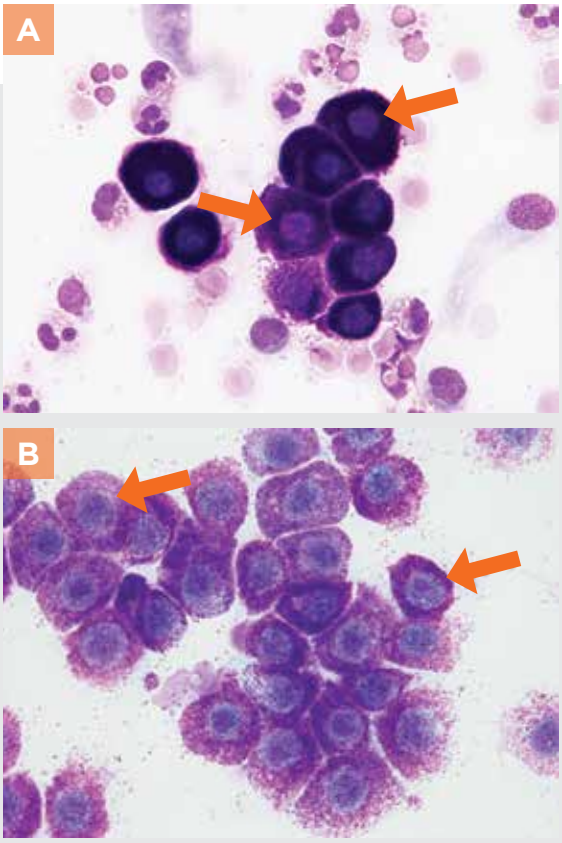

FIGURE 1. Heavily granulated cells from a low-grade mast cell tumor (MCT). **(A)** Cytoplasmic granules often nearly completely obscure the nucleus and limit evaluation of nuclear detail, particularly if the cells are not well spread out. Sometimes, the nucleus can be seen only as a lighter circle within the darkly stained mass of cytoplasmic granules **(arrows)**. Aqueous Romanowsky stain, 100× magnification. **(B)** In areas where the cells are well spread out, the nucleus can often be visualized even in heavily granulated MCTs. Note the uniform size and round shape of the nuclei and lack of binucleated or multinucleated cells. Aqueous Romanowsky stain, 100× magnification.

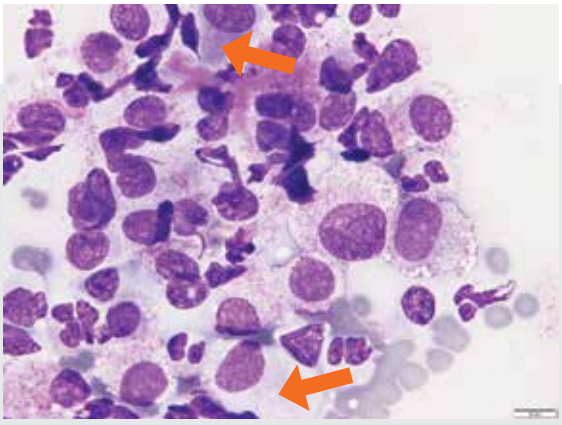

FIGURE 2. Poorly granulated cells in a high-grade mast cell tumor. While there are visible granules, they are relatively sparse compared to the cells in **FIGURE 1**. Some cells contain almost no granules **(arrows)**. Aqueous Romanowsky stain, 100× magnification.

histologic grading matched in 94% of cases.[10] Of the cytologic low-grade MCTs, 98.5% were also called low-grade by histology. However, of the tumors cytologically classified as high grade, approximately one-third (31.8%) were determined to be low grade by histology. Thus, there is a tendency to overestimate tumor grade compared with histology, likely resulting from less stringent criteria (e.g., any mitotic figures seen versus ≥7 per 10 HPF). The authors point out that as a screening test, this is preferable to underestimating tumor grade, which might result in aggressive tumors being undertreated.

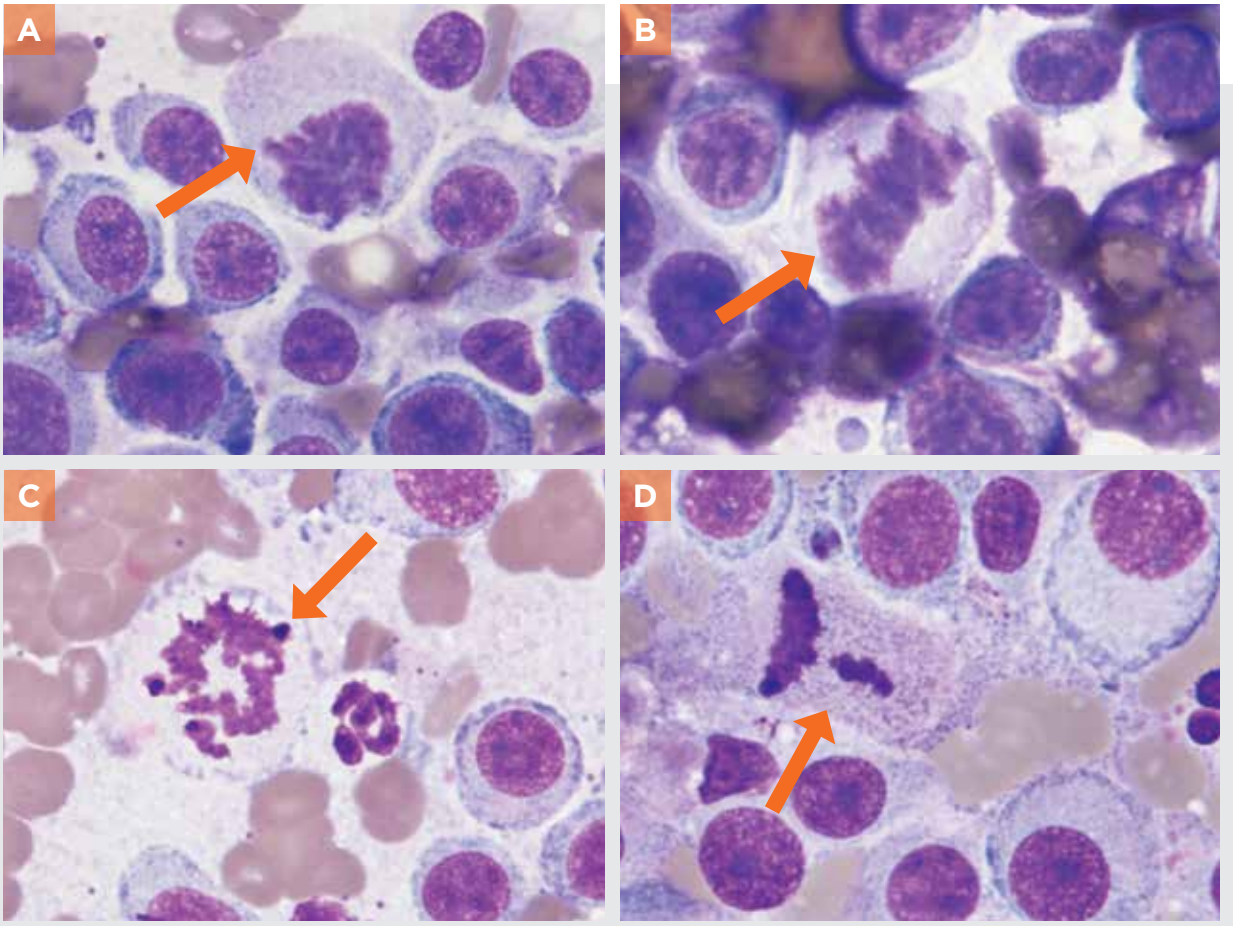

FIGURE 3. Mitotic figures in a high-grade mast cell tumor. Mitotic figures may have a variety of appearances. **(A)** Normal prophase mitotic figure **(arrow)**. **(B)** Normal metaphase mitotic figure **(arrow)**. **(C AND D)** Atypical mitotic figures **(arrows)** in which some chromosomes are not associated with the rest (lagging chromosomes). Aqueous Romanowsky stain, all images taken on 100× magnification.

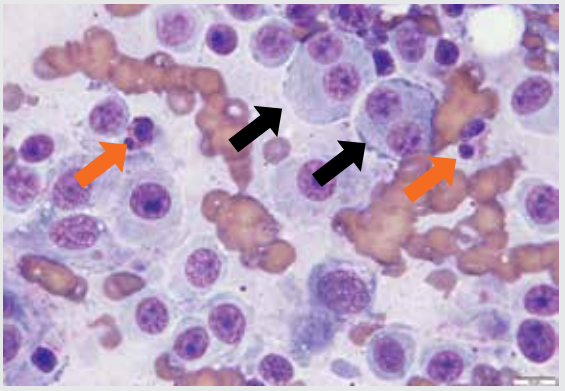

FIGURE 4. Binucleated cells **(black arrows)** in a high-grade mast cell tumor (MCT). Note the near-complete absence of any granules within the mast cells. Alone, this would make diagnosis of an MCT challenging, but some granules were found in other fields in this case. Also note the presence of eosinophils **(orange arrows)**, which should prompt consideration of a poorly granulated MCT when seen in a round cell tumor. Binucleated cells are included in the Camus scale as a criterion of a high-grade tumor but are not included in the Paes scale, which evaluates only multinucleated (3 or more nuclei) cells **(see Paes Grading System section)**. However, Paes et al noted that binucleated cells were most often seen when multinucleated cells were also present.[11] Identification of binucleated cells should therefore prompt a search for multinucleation. Aqueous Romanowsky stain, 100× magnification.

Paes Grading System

Cytologic grading schemes are likely to continue to evolve. A 2022 study by Paes et al modified the Camus cytology scale by including evaluation of the number of fibroblasts (**FIGURE 8**) and collagen fibrils (**FIGURE 9**) and eliminating binucleated cells and nuclear pleomorphism.[11] Fibroblasts and collagen fibrils are both common findings in MCTs and are more common in low-grade tumors. In this study, finding only low numbers of these elements correlated with a reduced 1-year survival.

In all studies, variable numbers of cases had discordant results between cytologic and histologic grades. The overall agreement varied from 77% to 94%. Both the Camus and Paes systems tended to classify more tumors as high grade than did histology. However, the main goal of tumor classification is to predict patient outcome, and both systems correlated with patient survival approximately as well as, or better than, histologic grading.[10,11] In fact, the study by Paes et al showed that in 11 cases called high grade on cytology but low grade on histology, there were 5 tumor-related deaths (45%), so it is possible that cytologic grade may be predictive of outcome even in histologically low-grade tumors.

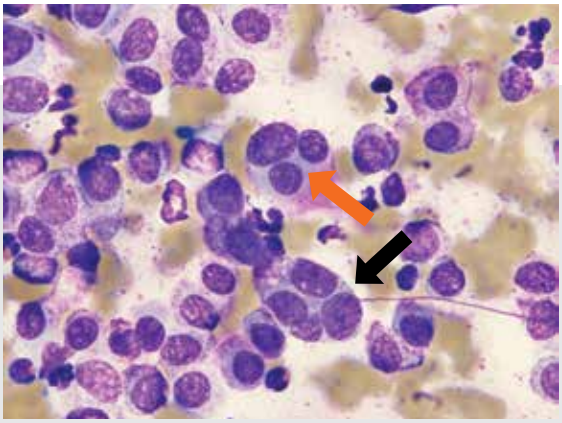

FIGURE 5. Multinucleation in a high-grade mast cell tumor. Note the presence of relatively poorly granulated mast cells. There are 2 multinucleated cells, one with 3 nuclei **(orange arrow)** and one with 4 nuclei **(black arrow)**. Note the variation in nuclear size within the same cell in both multinucleated cells. Aqueous Romanowsky stain, 100× magnification.

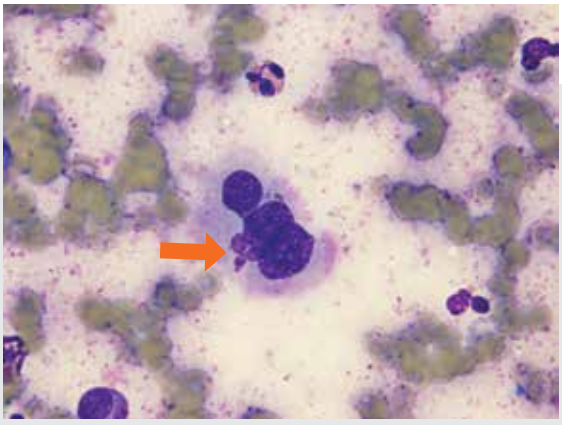

FIGURE 6. Nuclear pleomorphism (variation in nuclear shape) in a high-grade mast cell tumor. Note the bizarrely shaped nucleus with multiple lobes of varying size. This cell may also be binucleated or multinucleated, as it can be difficult to distinguish between nuclear lobes and separate nuclei. However, smaller, connected lobes **(orange arrow)** are clearly seen. Any nucleus shape other than round or oval is considered atypical. This is one of the least commonly seen atypical features and is not included in the Paes system **(see Paes Grading System section)**. Aqueous Romanowsky stain, 100× magnification.

Potential Limitations

There are potential limitations of applying cytologic grading schemes to MCTs. One is that nuclear features are often obscured in heavily granulated tumors, making evaluation of nuclear morphology difficult and potentially masking atypical features. Despite this potential challenge, cytologic grading classified more tumors as high grade than histology in the studies by Camus and Paes and grading by both methods significantly correlated with survival data.

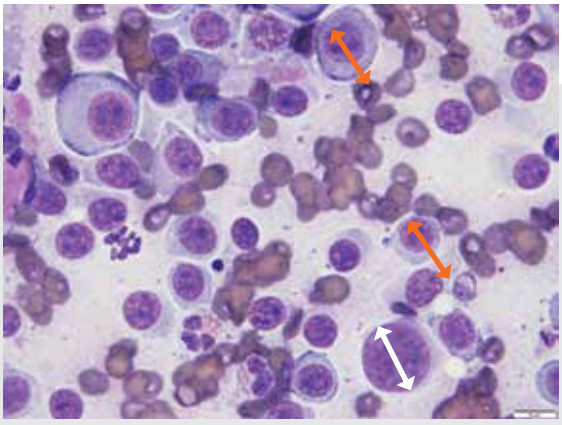

FIGURE 7. Anisokaryosis (variation of nuclear size) in a high-grade mast cell tumor (same case as **FIGURE 5**). Note the near-complete absence of granules in the neoplastic cells and presence of eosinophils, as in **FIGURE 5**. Also note the marked variation in nuclear size. The size of the large nucleus **(white arrow)** has been placed over other cells **(orange arrows)** to show relative nuclear sizes. Finding any areas where some cells have nuclei that are 2× larger than other nuclei is considered abnormal. Aqueous Romanowsky stain, 100× magnification.

A second concern is that cytology cannot distinguish cutaneous from subcutaneous MCTs. The current histologic grading systems are applied only to cutaneous MCTs.[1,7] One study did include both cutaneous and subcutaneous tumors and still showed 85% agreement with histologic grading.[12]

A third potential issue is the type of stain used. Aqueous Romanowsky stains (e.g., Diff-Quik) may not stain mast cell granules as well as methanolic Romanowsky stains such as May-Grünwald-Giemsa (MGG). As granularity of mast cells is used in cytologic grading, the lack of granularity with aqueous stains may falsely indicate a high-grade tumor. In a recent study, approximately 18% of cytologic samples from primary MCTs showed less granulation with aqueous stains than with a standard methanolic stain.[12] Notably, hypogranularity was significantly correlated with other cytologic evidence of malignancy. In this study, cytologic samples stained with both aqueous stains and MGG stain were graded using the Camus scheme and compared with histologic grading. While there were some differences in grading of individual tumors with different stains, the diagnostic accuracy was 85% with both types of cytologic stain when compared with histologic classification. This study included both cutaneous and subcutaneous MCTs.

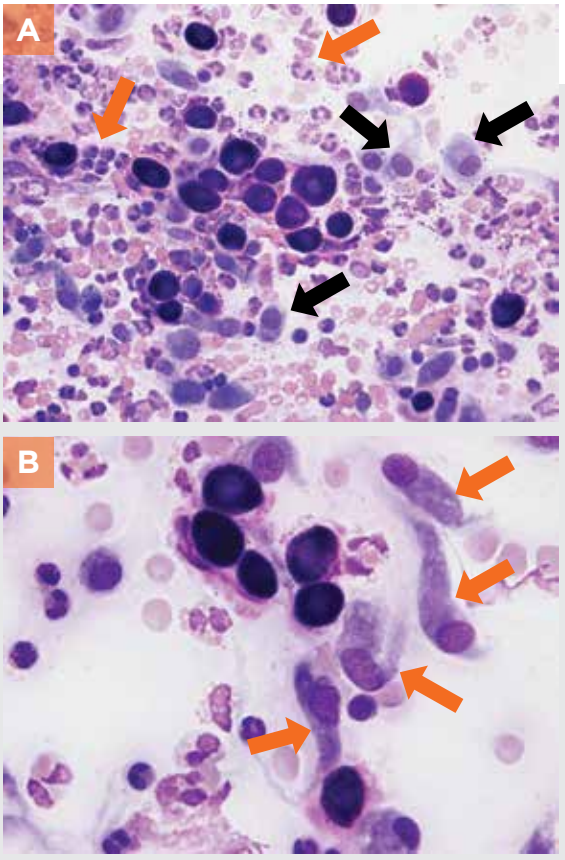

FIGURE 8. Fibroblasts in a low-grade mast cell tumor (MCT). **(A)** Note several heavily granulated mast cells surrounded by numerous eosinophils **(orange arrows)**. Numerous spindle-shaped fibroblasts are present **(black arrows)**. Fibroblasts are typically spindle shaped, but some are nearly round. Fibroblasts are an expected finding with low-grade MCTs, and their absence or rarity (only 1 to 2 per slide) is considered a criterion of a potentially high-grade tumor. Aqueous Romanowsky stain, 50× magnification. **(B)** Higher magnification; same case. Fibroblasts **(arrows)** have an elongated shape and often have indistinct cytoplasmic borders. Aqueous Romanowsky stain, 100× magnification.

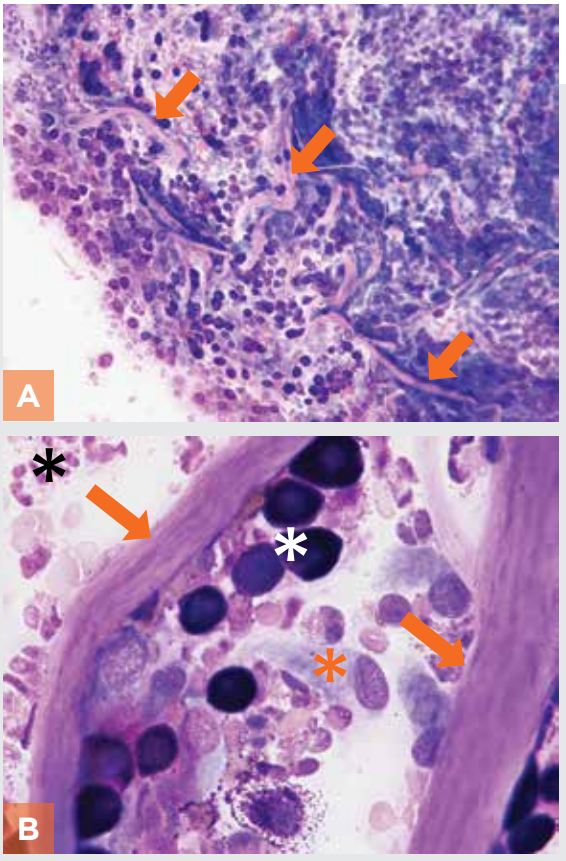

FIGURE 9. Collagen fibrils in a low-grade mast cell tumor (MCT). **(A)** Note the dense population of heavily granulated mast cells within which numerous elongated, pink collagen fibrils can be seen **(arrows)**. Like fibroblasts, these are an expected finding with low-grade MCTs and their absence anywhere on the slide is considered indicative of a potentially high-grade tumor. Aqueous Romanowsky stain, 20× magnification. **(B)** Higher magnification; same case. The fibrillar nature of elongated collagen fibrils is visible **(arrows)**. Heavily granulated mast cells **(white asterisk)**, eosinophils **(black asterisk)**, and fibroblasts **(orange asterisk)** are seen in the background. Aqueous Romanowsky stain, 100× magnification.

BOX 1 How To Evaluate a Cytology Slide

Scan the slide at low magnification to evaluate cellularity and find a spread-out, well-stained area with intact cells. Then determine if the mast cells are heavily granulated (**FIGURE 1**) or poorly granulated (i.e., all cells have minimal granulation; **FIGURE 2**). If the mast cells are poorly granulated, the tumor is considered high grade and further evaluation is not required.

If the cells are highly granulated, look for the presence of ≥2 of the atypical features in **TABLE 1**. Mitotic figures, binucleated or multinucleated cells, variation in nuclear size, and nuclear pleomorphism each need to be found only once on the slide for the sample to be considered abnormal. The number of collagen fibrils and fibroblasts are evaluated together. For the sample to be considered abnormal, these must be absent or rare (1–2 per high-power field) throughout the entire slide.

TABLE 1 Atypical Features Used to Grade Mast Cell Tumors[a]

CAMUS ET AL[10]	PAES ET AL[11]
Mitotic figures (**FIGURE 3**)	Mitotic figures (**FIGURE 3**)
≥50% anisokaryosis (variation in nuclear size; **FIGURE 7**)	≥50% anisokaryosis (**FIGURE 7**)
Binucleation (**FIGURE 4**) or multinucleation (**FIGURE 5**)	Multinucleation (**FIGURE 5**)
Nuclear pleomorphism (**FIGURE 6**)	Low collagen/fibroblasts (associated with high grade; **FIGURES 8 AND 9**)

[a]*Other than granulation*

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SUMMARY

Grading of MCTs provides significant prognostic information and can be useful in determining a treatment plan. Histologic evaluation is currently the gold standard for grading; histology also allows for evaluation of tumor margins. However, several studies show that cytologic grading schemes may be useful for predicting the histologic grade and independently correlate well with clinical outcome (**BOX 1**). Cytologic findings associated with poor outcome include poor granularity of the tumor cells or the presence of 2 or more atypical features, including anisokaryosis, mitotic figures, binucleation/multinucleation, nuclear pleomorphism, and reduced numbers of fibroblasts/collagen fibrils. TVP

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