

BRIEF COMMUNICATIONS

Variation among pathologists in histologic grading of canine cutaneous mast cell tumors

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Abstract. Ten veterinary pathologists at 1 veterinary institution independently assigned histologic grades to the same 60 canine cutaneous mast cell tumors (MCTs). There was significant variation among pathologists in grading the MCTs ($P < 0.001$). The probability of assigning a low grade was significantly higher for the pathologists in this study who use a published reference for histologic grading of canine cutaneous MCTs that allows subcutaneous MCTs or MCTs with mitotic figures to be included in the low-grade category ($P < 0.0001$ and $P < 0.0001$, respectively).

Key words: Dog; grade; histopathology; mastocytoma.

Mast cell tumors (MCT) are the most common cutaneous neoplasm of the dog.¹⁰ These tumors vary widely in their behavior from nearly benign to highly invasive and metastatic. It has been recognized for more than 30 years that histologic grading is prognostic for the behavior of canine cutaneous MCTs.^{2,4,7} The 2 most widely recognized grading systems classify MCTs into 3 grades based on histologic characteristics that include: cellularity, cell morphology, invasiveness, mitotic activity, and stromal reaction.^{2,7} Both grading systems correlate with the survival rate of canine patients with MCT. Well-differentiated, low-grade (Patnaik Grade I, Bostock Grade 3) MCTs are generally benign in behavior and are usually cured by surgical excision. Poorly differentiated, high-grade (Patnaik Grade III, Bostock Grade 1) MCTs are generally locally invasive, more likely to metastasize, and associated with a poor survival rate. The histologic appearances and clinical courses of intermediate-grade MCTs range between those of high- and low-grade MCTs.

Histologic grade is the most important criterion in determining the staging tests and adjunctive therapy that will be recommended for a dog with cutaneous MCT.^{6,10} Chemotherapy is routinely recommended for dogs with high-grade MCTs and not for dogs with low-grade MCTs. For patients with intermediate-grade MCTs, the recommendation of adjunctive therapies depends on other prognostic indicators, completeness of excision, or evidence of nodal or distant metastasis. Because of the importance of histologic grade in the decision-making process involving staging and treatment of canine cutaneous MCTs, it is of value for veterinarians to know whether variability exists among pathologists in assigning grades to MCTs. The goal of this study was to evaluate variability in histologic grading of canine cutaneous MCTs among veterinary pathologists at 1 academic institution.

The University of Georgia College of Veterinary Medicine (UGA-CVM) patient database was searched to identify dogs diagnosed with cutaneous MCT from 1995 to 2002. Histology reports were reviewed to identify 20 cutaneous MCTs that were originally reported as low, intermediate, and high histologic grade, for a total of 60 tumors. Hematoxylin and eosin (HE)-stained slides were obtained for each tumor. All veterinary pathologists in the UGA Department of Pathology and at the UGA Athens Veterinary Diagnostic Laboratory (also located at the CVM) were invited to participate in this study. Information collected from pathologists included: years practicing as a pathologist, residency program where trained, and references used in grading MCTs.

Pathologists were provided with an HE stained slide from each of the 60 MCTs for examination. All pathologists evaluated the same set of slides. Slides were reviewed before randomization by 1 pathologist (EWH) to confirm the adequacy of sections and staining for evaluation. If granules were not easily visualized on HE staining, the diagnosis of MCT was confirmed with toluidine blue staining before in-

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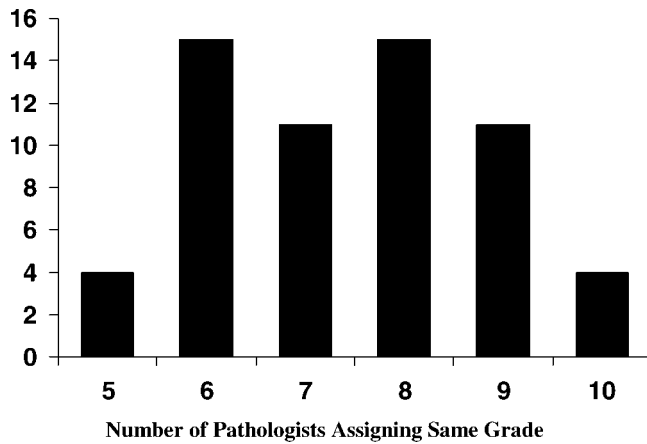


Figure 1. Agreement among 10 pathologists at the University of Georgia, College of Veterinary Medicine in the histologic grading of the same 60 canine cutaneous MCTs. The x-axis shows the number of pathologists assigning the same grade and the y-axis shows the number of MCTs (out of the 60 MCTs graded) for which this number of pathologists was in agreement.

clusion in the study. Pathologists were informed that all tumors were confirmed to be MCTs. Pathologists were blinded with regard to patient identity, previously reported tumor grade, and percentages of study slides originally assigned to each grade. No information regarding patient history, tumor size, or tumor site was provided with the slides. Two weeks were allotted for each pathologist to evaluate the slides.

Pathologists were provided with identical instructions for evaluating the slides. They were instructed to evaluate the slides in the same manner as routinely submitted histologic samples in the randomized order provided by the UGA Statistical Consulting service. Grade was to be reported as I (low), II (intermediate), or III (high). Additional instructions included 1) do not return to a slide once it has been evaluated and 2) avoid allowing previous slides to influence the grading of a particular tumor. Resources normally used in evaluating slides (for example, textbooks and publications in veterinary journals) were allowed only to the extent that a given pathologist routinely used these resources. No communication concerning the slides was allowed among pathologists.

Statistical analysis of data was performed by the UGA statistical consulting service using SAS software.^a A cumulative logit model¹ was used to compare the proportion of MCTs assigned to each grade by each pathologist. This method was also used to calculate the association between histologic grade and pathologists' characteristics. Variables examined for relationship to histologic grading included: site of pathology residency training, years of experience (categorized as <5 years or ≥5 years), and the published reference(s) for histologic grading of canine cutaneous MCTs used by each pathologist. Specifically, the analysis examined the association between the probability of the pathologists assigning an MCT a low, intermediate, or high grade and whether the reference(s) they used in grading allow MCTs located in the subcutis or MCTs with any mitotic figures to

Table 1. Numbers of canine cutaneous MCTs classified as low, intermediate, and high histologic grade by 10 pathologists grading the same 60 MCTs.

| Pathologist | No. Low grade | No. Intermediate grade | No. High grade |
|-------------|---------------|------------------------|----------------|
| 1 | 3 | 39 | 18 |
| 2 | 7 | 32 | 21 |
| 3 | 8 | 36 | 16 |
| 4 | 9 | 41 | 10 |
| 5 | 10 | 29 | 21 |
| 6 | 10 | 33 | 17 |
| 7 | 19 | 32 | 9 |
| 8 | 24 | 20 | 16 |
| 9 | 32 | 15 | 13 |
| 10 | 37 | 15 | 8 |

be included in the low grade or whether a subcutaneous location or the presence of a mitotic figure dictates that a tumor be classified as intermediate grade. A *P* value of ≤0.05 was considered statistically significant.

Ten of 12 pathologists at the UGA-CVM participated in this study. Participating pathologists completed residency/PhD programs at UGA (*n* = 6), Michigan State University (*n* = 1), Texas A&M University (*n* = 1), University of California–Davis (*n* = 1), and University of Florida (*n* = 1). Pathologists had practiced in the specialty of pathology for 1 year (*n* = 3), 3 years (*n* = 1), 8 years (*n* = 1), 12 years (*n* = 1), 15 years (*n* = 1), 16 years (*n* = 1), 21 years (*n* = 1), and 23 years (*n* = 1). Eight pathologists were board certified and 2 were board eligible. Six references were used by pathologists in the histologic grading of MCTs.^{2,3,7,8,11,12} Four of these references allow subcutaneous MCTs and MCTs with mitotic figures to be included in the low-grade category.^{2,8,11,12}

Each pathologist evaluated 60 canine cutaneous MCTs. Although for every MCT, at least 5 pathologists agreed on the tumor grade, and for 50% of the MCTs, at least 8 pathologists agreed on the tumor grade, there was significant variation among all pathologists in grading all 60 MCTs (*P* < 0.001). There was agreement of all 10 pathologists on the grade of only 4 of the 60 tumors (3 high grade and 1 low grade). Six of the 60 tumors were assigned all 3 grades by different pathologists. A detailed description of the pathologists' concurrence in the histologic grading of these MCTs is provided in Figure 1 and the numbers of MCTs assigned low, intermediate, and high grade by each pathologist are provided in Table 1.

There were insufficient numbers of pathologists in each group to evaluate the effect of site of residency training on histologic grading of MCTs. Of the remaining factors evaluated for association with grading, only those related to the reference(s) used in grading MCTs were significant. On the basis of the results of the statistical model, the pathologists in this study who use a reference allowing inclusion of subcutaneous MCTs in the low-grade category had a 39% probability of assigning a low grade to an MCT, whereas pathologists using a reference that does not allow subcutaneous MCTs to be considered low grade had a 20% probability of

assigning a low grade. This difference was significant, $P < 0.0001$. In addition, the results of the statistical model suggest that pathologists in this study who use a reference that allows inclusion of MCTs with mitotic figures in the low-grade category had a 41% probability of assigning a low grade to an MCT, whereas pathologists using a reference that does not allow this inclusion had a 21% probability of assigning a low grade. This difference was also significant, $P < 0.001$.

This study reveals that there is variation among pathologists in histologic grading of canine cutaneous MCTs. Significant differences were observed in the histologic grades assigned to the same canine cutaneous MCTs by 10 pathologists at one institution, and there was consensus among all 10 pathologists on less than 7% of the tumors. Current MCT grading systems were designed to allow an objective assignment of a numerical grade to an MCT based on established histologic criteria.^{2,3,7,8,11,12} Defined numerical grading systems may give clinicians the impression that histopathologic grading is completely objective and absolute. This study suggests that this is not the case. A single MCT may be assigned different grades by different pathologists for reasons that include differences in published grading systems, heterogeneity of tumors (so that some MCTs may exhibit characteristics of more than 1 grade), and subjectivity in applying grading schemes. Another factor which, if present, might contribute to variation among pathologists in histologic grading is the consistency of grading by an individual pathologist. This was not evaluated in this study but would limit the degree of grading consensus possible.

To allow comparison of the pathologists participating in this study, the numbers of MCTs assigned low, moderate, and high grade by each pathologist are presented in Table 1. Although some pathologists have similar numbers in each grade, there are large differences when the entire group is considered. Because 20 tumors of each grade (based on the original histology report) were selected for this study, it would be expected that the proportions of tumors assigned to each grade would be similar. Interestingly, that is not what was observed. Because there is no gold standard for histologic grading of MCTs, it is not possible to provide a "correct" grade for each tumor.

Because this study included pathologists at only one institution, the results may not be representative of other groups of veterinary pathologists. In particular, greater agreement in grading the MCTs might be expected in this group than in pathologists practicing at different institutions, especially because 6 pathologists completed their residency training at the UGA-CVM. Although the pathologists in this study practiced at one institution, they were trained at 5 different institutions and had a wide range of years of experience (1–23 years). It is likely that the differences in site of training and years of experience among the veterinary pathologists at UGA-CVM are similar to those among pathologists at other institutions suggesting similar variations in grading would occur at other institutions. Unfortunately, sample sizes were too small to give this study sufficient power to identify differences in grading that might have been associated with years of experience or site of residency training.

In considering possible causes for variation among pathologists in histologic grading of MCTs, the primary investigator hypothesized that, despite the fact that all published references for histologic grading of MCTs describe low-, intermediate-, and high-grade classifications on the basis of similar histologic characteristics, differences might exist among the grading criteria described in these references. Consequently, pathologists using different references would not agree on the grade of certain MCTs. Two criteria were identified as varying among the references describing MCT histologic grading used by the pathologists participating in this study: 1) whether MCTs in the subcutis were allowed to be included in the low-grade category and 2) whether MCTs with mitotic figures were allowed to be included in the low-grade category. As expected, use of grading references that allow a greater variety of MCTs (including those with subcutaneous location or those with mitotic figures) in the low-grade category increased the likelihood that a pathologist would assign a low grade. As long as there are inconsistencies in references describing histologic grade of canine cutaneous MCTs, variation in grading must be expected. Despite this statement and the fact that 6 references were used in the grading of these MCTs, there was agreement of at least 80% of the pathologists in this study regarding the grade of 50% of the tumors. This level of agreement suggests that uniform application of a single histologic grading system with clearly defined classifications could result in greater consensus among pathologists in grading MCTs.

The authors of this study do not intend to suggest that veterinary pathologists are grading cutaneous MCTs incorrectly or that histologic grading of MCTs should be disregarded. Rather, they wish to suggest that strategies to improve consensus in grading MCTs should be investigated and that grade should be considered together with other known prognostic indicators when making staging and treatment recommendations for an individual patient. The American College of Veterinary Pathologists conducted a survey of Veterinary Cancer Society members and found that 94% of respondents agreed that the grading system for MCTs should be further developed and modified to better correlate morphologic, immunologic, or molecular characteristics (or all) with their biologic behavior and response to different modes of therapy.⁵ To this end, a future direction would be to conduct a follow-up study in which the same 10 veterinary pathologists would grade the same MCTs with uniform application of a single grading system to evaluate whether this improves consensus.

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Novel purification method for recombinant 3AB1 nonstructural protein of foot-and-mouth disease virus for use in differentiation between infected and vaccinated animals

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Abstract. An indirect enzyme-linked immunosorbent assay (ELISA) was developed for differentiation of animals infected with foot-and-mouth disease virus (FMDV) from vaccinated animals. The test was based on a highly pure and concentrated preparation of recombinant 3AB1 protein obtained by expression in a prokaryotic system, protein separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and electro elution. Experimental- and field-serum samples from naive, vaccinated, and infected cattle were tested for anti3AB1 antibody using the ELISA. A cutoff level was set at 35% of the maximum absorbance obtained with a positive control serum (FMDV-infected animal, 21 days postinfection [dpi]). This assay could detect antibodies from sera of animals experimentally infected by contact ($n = 118$) with a sensitivity of 97.5%. The specificity was 100%, based on negative test results obtained on 109 sera from naive animals. Remarkably, all sera from animals vaccinated either once ($n = 102$) or twice ($n = 30$) were negative. In addition, this 3AB1-ELISA could detect seroconversion at 7 dpi in animals inoculated intradermolingually. This assay constitutes an important tool for the rapid detection of FMDV outbreaks in a vaccinated population. In addition, it presents a reliable, economical, and simple method for testing large numbers of serum samples.

Key words: 3AB1-ELISA; foot-and-mouth disease; nonstructural protein; recombinant protein purification.

Foot-and-mouth disease (FMD) is one of the most devastating diseases of domestic ruminants because of the economic losses it generates (international trade embargoes), the decrease in productivity, and the highly expensive control programs. Foot-and-mouth disease affects all cloven-hoofed animals including cattle, sheep, pigs, goats, camellids, and more than 30 wildlife species. In countries where vaccination is practiced as a control measure, there is a need for

serological methods that can effectively discriminate infected animals among vaccinated herds, to track the presence or absence of the virus in any given area.

The FMD virus (FMDV), causative agent of FMD, is an aftovirus that belongs to the *Picornaviridae* family. Seven FMDV types (A, O, C, SAT1, SAT2, SAT3, ASIA1) exist. The virus contains a single-stranded RNA that codes for structural and nonstructural proteins. Nonstructural proteins (Lb, 2C, 3D, 3A, 3AB₁₂₃, 3ABC) are not present in vaccine formulations in large amounts and therefore provide a tool for differentiation of animals that have been exposed to live virus from noninfected (either vaccinated or susceptible unvaccinated) animals. Previous studies have shown that the nonstructural proteins 3AB₁₂₃ and 3ABC^{3,4,12} are the most probable potential markers for differentiation of infected from vaccinated or naive animals.

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