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)

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Objective—To compare the Kiupel (2 categories) and Patnaik (3 categories) histologic grading systems for predicting the presence of metastasis at the time of initial examination in dogs with cutaneous mast cell tumors (MCTs).

Design—Retrospective case series.

Animals—386 client-owned dogs with cutaneous MCTs.

Procedures—Medical records of dogs with newly diagnosed, histologically confirmed cutaneous MCTs that had undergone complete clinical staging were reviewed for clinical and histopathologic data.

Results—All Patnaik grade 1 MCTs (n = 52) were classified as Kiupel low-grade MCTs, and all Patnaik grade 3 MCTs (43) were classified as Kiupel high-grade MCTs. Of the 291 Patnaik grade 2 MCTs, 243 (83.5%) were classified as Kiupel low-grade tumors, and 48 (16.5%) were classified as Kiupel high-grade MCTs. Dogs with Patnaik grade 3 MCTs were significantly more likely to have metastases at the time of initial examination than were dogs with grade 1 or 2 MCTs (OR, 5.46), and dogs with Kiupel high-grade MCTs were significantly more likely to have metastases than dogs with Kiupel low-grade MCTs (OR, 2.54). However, 3 of 52 (5.8%) dogs with Patnaik grade 1 tumors, 48 of 291 (16.5%) dogs with Patnaik grade 2 tumors, and 44 of 295 (14.9%) dogs with Kiupel low-grade tumors had metastatic disease.

Conclusions and Clinical Relevance—Findings indicated that in dogs with cutaneous MCTs, prognostication should not rely on histologic grade alone, regardless of grading system used, but should take into account results of clinical staging. (J Am Vet Med Assoc 2015;246:765–769)

In dogs, cutaneous MCTs are characterized by highly variable biological behavior, ranging from low malignant potential to local invasiveness and high metastatic risk.1 Because of the high incidence and heterogeneity of cutaneous MCTs, management of affected dogs is challenging. Several prognostic factors that can potentially be used to predict the biological behavior of MCTs have been described, with histologic grade being the most important.2–5 Historically, canine cutaneous MCTs have been graded according to the Patnaik system, with grade 1 tumors defined as well-differentiated tumors confined to the interfollicular dermis, grade 2 tumors defined as intermediate-differentiated tumors extending to the deep dermis and subcutis, and grade 3 tumors defined as poorly differentiated tumors with infiltration of the subcutis and deep tissues.1 Although the biological behavior of Patnaik grade 1 and 3 cutaneous MCTs can generally be anticipated, the prognosis for Patnaik grade 2 MCTs is variable. Histologically, Patnaik grade 2 cutaneous MCTs may appear heterogeneous, and there can...
be some histopathologic variation among and within tumors.\(^6,7\) Hence, Patnaik grade 2 MCTs likely include some tumors that may behave more aggressively and for which a multimodal therapeutic approach would be beneficial. The Patnaik grading system underwent modifications in 2011, when a new grading system was proposed, triggered by changes in clinical practice and a better understanding of MCT biology and aimed at improving concordance among pathologists.\(^8\)

In contrast to the Patnaik grading system, the Kiupel histologic grading system consists of only 2 categories, with high-grade Kiupel MCTs characterized by at least 7 mitotic figures, 3 multinucleated cells, or 3 bizarre nuclei in 10 hpf or karyomegaly in 10% of cells (with assessment of the most mitotically active fields or the fields with the highest degree of anisokaryosis) and all other MCTs classified as low grade. This 2-category histologic grading system was demonstrated to be more accurate at predicting metastasis development and death than the Patnaik system.\(^9\)

The purpose of the study reported here was to retrospectively analyze a large series of cases to compare the 2-category Kiupel histologic grading system with the 3-category Patnaik histologic grading system in predicting the presence of metastasis at the time of initial examination in dogs with cutaneous MCTs.

### Materials and Methods

**Case selection criteria**—Members of the Italian Society of Veterinary Oncology were asked to search their records to identify dogs examined between 2009 and 2014 with previously untreated, histologically confirmed cutaneous MCTs that had undergone complete clinical staging. Dogs with multiple concurrent MCTs or with subcutaneous MCTs were excluded from the study.

**Medical records review**—Information obtained from the medical record for each dog included signalment, primary tumor description (location, size, presence of ulceration, and histologic grade according to the Patnaik and Kiupel systems\(^3,5\)), clinical stage and substage, and site of metastasis, if present.

Clinical staging consisted of hematologic and serum biochemical analyses, cytologic evaluation of fine-needle aspirates from the cutaneous nodule and regional lymph node (ie, the first lymph node in the expected lymphatic drainage basin), thoracic radiography, abdominal ultrasonography, cytologic evaluation of fine-needle aspirates from the liver and spleen, and, in dogs with metastatic disease, cytologic examination of a bone marrow aspirate.

Depending on clinician preference, fine-needle aspirates of the liver and spleen were always obtained (4 centers) or were only obtained when ultrasonographic abnormalities were seen or when clinical behavior of the MCT was particularly aggressive (2 centers), as previously described.\(^3,9\)

The regional lymph node was identified by either palpation or ultrasonography. Metastasis to the lymph node, liver, or spleen was diagnosed if mast cells appeared in clusters or sheets, occurred in very large numbers, or were morphologically atypical, consistent with previous descriptions.\(^8\)

### Histologic evaluation and classification

—After resection, all specimens were fixed in neutral-buffered 10% formalin and embedded in paraffin. Five-micrometer-thick sections were cut and stained with H&E. Special histochemical stains (Giemsa or toluidine blue) were used when necessary (eg, to identify poorly granulated mast cells in primary tumors and to ascertain metastatic involvement in lymph nodes). Grading was determined on the basis of the Patnaik and Kiupel grading systems.\(^3,5\) All tumor samples (including the primary cutaneous MCT and, for some cases, the regional lymph node) were examined by experienced pathologists unaware of the results of clinical staging. For dogs examined prior to the introduction of the Kiupel grading system, MCTs were reviewed by the same pathologist who had made the initial diagnosis, and Kiupel grades were assigned. Pathologists were blinded to follow-up data while grading MCTs and strictly followed the Patnaik and Kiupel guidelines.\(^3,5\)

**Statistical analysis**—When appropriate, data were tested for normality with the D’Agostino and Pearson omnibus normality test. Continuous values that were normally distributed are expressed as mean ± SD; values that were not normally distributed are expressed as median and range.

The \(\chi^2\) test (categorical variables) and Mann-Whitney U test (continuous variables) were used to test for associations between various clinical variables and the presence of lymph node metastases. Variables that were assessed consisted of breed (most represented breeds [Boxer, Labrador Retriever, Golden Retriever, American Staffordshire Terrier, and Shar-Pei] vs all other breeds), body weight, tumor location (head and neck, trunk [including abdominal wall and proximal portions of the limbs to the elbow or knee], inguinal [including perineal] region, distal portions of the limbs excluding the digits, and digits), macroscopic tumor diameter (<3 or ≥3 cm), ulceration, and substage.

The proportions of dogs with metastases were compared among histologic grades according to the Patnaik and Kiupel systems with the \(\chi^2\) test. The likelihood of metastatic disease at the time of initial examination according to tumor grade was assessed by means of logistic regression.

All statistical analyses were performed with standard software.\(^9\) Values of \(P \leq 0.05\) were considered significant.

### Results

**Patient and tumor characteristics**—A total of 386 dogs fulfilled the criteria for inclusion in the study. Mean ± SD age was 7.7 ± 2.8 years. Two hundred twelve dogs were females (of which 92 were spayed), and 174 dogs were males (of which 29 were castrated). Eighty-six dogs were of mixed breeding, with the remaining 300 dogs representing 51 breeds, including Boxer (n = 79), Labrador Retriever (65), Golden Retriever (30), English Setter (20), American Staffordshire Terrier (13), Shar-Pei (8), Beagle (7), French Bulldog (6), Bernese Mountain Dog (4), Epagneul Breton (4), Shih-Tzu (4), and 40 other breeds each represented by 1 to 3 animals.

Tumors were located on the head and neck (n = 74 [19.2%]) or trunk (209 [54.1%]) in the inguinal region.
(36 [9.3%]), or on the distal portions of the limbs (58 [15.0%]) or digits (9 [2.3%]). Tumor diameter ranged from 0.4 to 20 cm (median, 3 cm); 332 (86%) MCTs were not ulcerated, and 54 (14%) were. Three hundred sixty-four (94.3%) dogs were subclinically affected (substage a), whereas the remaining 22 (5.7%) dogs had systemic signs (eg, vomiting, diarrhea, pruritus, and regional edema; substage b).

**Histopathologic findings and staging**—Of the 386 dogs, only 33 did not undergo fine-needle aspiration of the liver and spleen, either because there were no ultrasonographic abnormalities or because there were no signs of particularly aggressive biological behavior. Overall, 319 (82.6%) dogs underwent complete clinical staging, including fine-needle aspiration of the liver and spleen. Seventy-two (18.7%) dogs underwent bone marrow evaluation.

On the basis of the Patnaik grading system, 52 (13.5%) dogs had grade 1 MCTs, 291 (75.4%) had grade 2 MCTs, and 43 (11.1%) had grade 3 MCTs (Table 1). On the basis of the Kiupel grading system, 295 (76.4%) dogs had low-grade MCTs, and 91 (23.6%) had high-grade MCTs.

All Patnaik grade 1 MCTs were classified as Kiupel low-grade MCTs, and all Patnaik grade 3 MCTs were classified as Kiupel high-grade MCTs. Of the 291 Patnaik grade 2 MCTs, 243 (83.5%) were classified as Kiupel low-grade MCTs, and 48 (16.5%) were classified as Kiupel high-grade MCTs. On the basis of results of clinical staging, 70 (18.1%) dogs had regional lymph node metastasis, and 316 (81.9%) did not. Fifty dogs had lymph node metastasis diagnosed on the basis of results of both cytologic and histologic evaluation, with complete agreement between the 2 methods, and 20 had lymph node metastasis diagnosed on the basis of results of cytologic evaluation alone. Sixteen (4.1%) dogs had distant metastasis, and 370 (95.9%) did not. Of the 16 dogs with distant metastasis, 6 had metastasis to the spleen and liver; 5 had metastasis to the spleen; 2 had metastasis to the liver; 1 had metastasis to the spleen, liver, and bone marrow; 1 had metastasis to the lymph nodes, and 1 had cutaneous metastasis characterized by multiple satellite nodules around the primary MCT. Notably, 2 of the 16 dogs with distant metastasis had no regional lymph node involvement; 1 had a Patnaik grade 3 MCT classified as a Kiupel high-grade tumor, and 1 had a Patnaik grade 2 MCT classified as a Kiupel low-grade tumor. Overall, 72 of the 386 (18.7%) dogs had metastatic disease.

When considering the Patnaik grading system, 3 of 52 (5.8%) dogs with grade 1 MCTs and 48 of 291 (16.5%) dogs with grade 2 MCTs had metastatic disease. All 3 dogs with Patnaik grade 1 MCTs had nodal metastasis. Of the 48 dogs with Patnaik grade 2 MCTs that had metastatic disease, 42 had nodal metastasis alone, 5 had nodal and distant metastasis, and 1 had distant metastasis alone. Of the 43 dogs with Patnaik grade 3 MCTs, 21 (48.8%) had metastatic disease, including 12 with nodal metastasis alone, 8 with nodal and distant metastasis, and 1 with distant metastasis alone. Percentage of dogs with metastatic disease was significantly (*P* < 0.001) different between Patnaik grades 3 and 1 and between Patnaik grades 3 and 2, but not between Patnaik grades 2 and 1.

When considering the Kiupel grading system, 44 of 295 (14.9%) dogs with low-grade tumors had metastatic disease, including 38 with nodal metastasis alone, 5 with nodal and distant metastasis, and 1 with distant metastasis alone. Of the 91 dogs with Kiupel high-grade tumors, 28 (30.8%) had metastatic disease, including 18 with nodal metastasis alone, 9 with nodal and distant metastasis, and 1 with distant metastasis alone. There was a significant (*P* = 0.001) difference in the percentage of dogs with metastatic disease between Kiupel high-grade and low-grade MCTs.

Forty-one of the 243 (16.9%) dogs with Patnaik grade 2 MCTs classified as Kiupel low-grade tumors had metastatic disease, including 36 dogs with nodal metastasis alone, 4 dogs with nodal and distant metastasis, and 1 dog with distant metastasis alone. Seven of the 48 (14.6%) dogs with Patnaik grade 2 MCTs classified as Kiupel high-grade tumors had metastatic disease, including 6 dogs with nodal metastasis alone and 1 dog with nodal and distant metastasis.

The prevalence of metastasis did not differ significantly (*P* = 0.833) between dogs with Patnaik grade 2 MCTs classified as Kiupel low-grade tumors and dogs with Patnaik grade 2 MCTs classified as Kiupel high-grade tumors. Similarly, the prevalence of metastasis did not differ significantly (*P* = 0.068) between dogs with Kiupel low-grade MCTs classified as Patnaik grade 3 MCTs and dogs with Kiupel high-grade MCTs classified as Patnaik grade 2 MCTs.

### Table 1—Prevalence of metastatic disease in 386 dogs with cutaneous MCTs classified according to the Patnaik and Kiupel histologic grading systems.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of dogs</th>
<th>Absence of metastases</th>
<th>Metastatic disease</th>
<th>Lymph node metastases</th>
<th>Distant metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>386/386 (100)</td>
<td>314/386 (81.3)</td>
<td>72/386 (18.7)</td>
<td>70/386 (18.1)</td>
<td>16/386 (4.1)</td>
</tr>
<tr>
<td>Patnaik grade</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>52/386 (13.5)</td>
<td>49/52 (94.2)</td>
<td>3/52 (5.8)</td>
<td>3/52 (5.8)</td>
<td>1/52 (1.9)</td>
</tr>
<tr>
<td>2</td>
<td>291/386 (75.4)</td>
<td>243/291 (83.5)</td>
<td>48/291 (16.5)</td>
<td>47/291 (16.2)</td>
<td>6/291 (2.1)</td>
</tr>
<tr>
<td>3</td>
<td>43/386 (11.1)</td>
<td>22/43 (51.2)</td>
<td>21/43 (48.8)</td>
<td>20/43 (46.5)</td>
<td>9/43 (20.9)</td>
</tr>
<tr>
<td>Kiupel grade</td>
<td></td>
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</tr>
<tr>
<td>Low</td>
<td>295/386 (76.4)</td>
<td>251/295 (85.1)</td>
<td>44/295 (14.9)</td>
<td>43/295 (14.6)</td>
<td>6/295 (2.0)</td>
</tr>
<tr>
<td>High</td>
<td>91/386 (23.6)</td>
<td>63/91 (69.2)</td>
<td>28/91 (30.8)</td>
<td>27/91 (29.7)</td>
<td>10/91 (11.0)</td>
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<tr>
<td>Patnaik grade</td>
<td></td>
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<tr>
<td>2</td>
<td></td>
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</tr>
<tr>
<td>Kiupel low grade</td>
<td>243/291 (83.5)</td>
<td>202/243 (83.1)</td>
<td>41/243 (16.9)</td>
<td>40/243 (16.5)</td>
<td>5/243 (2.1)</td>
</tr>
<tr>
<td>Kiupel high grade</td>
<td>48/291 (16.5)</td>
<td>41/48 (85.4)</td>
<td>7/48 (14.6)</td>
<td>7/48 (14.6)</td>
<td>1/48 (2.1)</td>
</tr>
</tbody>
</table>
and dogs with Kiupel low-grade MCTs classified as Patnaik grade 2 (4/243 [16.9%]). Conversely, among dogs with Kiupel high-grade MCTs, those with Patnaik grade 3 MCTs had a significantly (P < 0.001) higher prevalence of metastatic disease (21/43 [48.8%]) than did those with Patnaik grade 2 MCTs (7/48 [14.6%]).

For the Patnaik grading system, dogs with grade 3 MCTs were significantly more likely to have metastases at the time of initial examination than were dogs with grade 2 or 1 MCTs (OR, 3.46; 95% confidence interval, 2.80 to 10.66; P < 0.001). For the Kiupel grading system, dogs with high-grade tumors were significantly more likely to have metastases at the time of initial examination than were dogs with low-grade tumors (OR, 2.54; 95% confidence interval, 1.46 to 4.39; P = 0.001).

Variables other than histologic grade significantly associated with nodal metastasis at the time of initial examination included tumor diameter ≥ 3 cm (P < 0.001), digit location (P = 0.002), ulceration (P = 0.01), Shar-Pei breed (P < 0.001), and substage b (P < 0.001). Dogs with MCTs located on the trunk had a significantly (P < 0.001) lower prevalence of metastatic disease than did dogs with MCTs located elsewhere.

Discussion

The purpose of the present study was to compare the 2-category Kiupel histologic grading system with the 3-category Patnaik histologic grading systems in predicting the presence of metastasis at the time of initial examination in dogs with cutaneous MCTs. While dogs with Patnaik grade 3 MCTs were significantly (OR, 5.46) more likely to have metastases than were dogs with grade 2 or 1 MCTs and dogs with Kiupel high-grade MCTs were significantly (OR, 2.54) more likely to have metastases than were dogs with low-grade MCTs, substantial proportions of dogs with grade 2 (16.5%) and grade 1 (5.8%) tumors and dogs with low-grade tumors (14.9%) had metastases. Therefore, we concluded that in dogs with cutaneous MCTs, prognostication should not rely on histologic grade alone, regardless of grading system used, but should take into account the results of clinical staging. Lymph node status and histologic grade are reported by among the most important prognostic indicators for dogs with cutaneous MCTs, and detection of lymph node metastasis or a high histologic grade is a key factor in recommending systemic treatment. In clinical practice, some clinicians may not suggest any further staging in dogs with Patnaik grade 1 and Kiupel low-grade MCTs, on the basis of the assumption that the likelihood for metastasis is low. On the basis of the findings of the present study, this assumption does not apply as a whole. A proportion of dogs will have metastatic disease despite histologic grade, thereby requiring a multimodal therapeutic approach.

Various studies have shown histologic grade to be an independent prognostic indicator in dogs with cutaneous MCTs and have shown better interobserver agreement with the Kiupel grading system, compared with the Patnaik grading system. However, histologic grading remains somewhat subjective, and incorrect grades may be assigned for individual MCTs, which may result in inappropriate treatment decisions. Also, histologic grade does not take into account other factors with possible prognostic importance, such as tumor size and location and the presence or absence of metastases.

It is well accepted that Patnaik grade 3 MCTs have an aggressive biological behavior and a high metastatic potential (> 80%). Conversely, Patnaik grade 1 MCTs only rarely metastasize (< 10%). This is in agreement with the findings of the present study. Dogs with MCTs classified as Patnaik grade 1 had a significantly lower prevalence of metastasis than did dogs with MCTs classified as grade 3, and dogs with MCTs classified as Kiupel low grade had a significantly lower prevalence of metastasis than did dogs with MCTs classified as Kiupel high grade. On the other hand, the biological behavior of Patnaik grade 2 MCTs is difficult to predict, with Patnaik grade 2 MCTs reported to have an intermediate metastatic potential (5% to 22%). In the present study, 16.5% of 48/291 of dogs with Patnaik grade 2 MCTs had nodal or distant metastases. Interestingly, adding the Kiupel grading system did not seem to overcome the issue of indeterminate biological behavior for Patnaik grade 2 MCTs, in that the prevalence of metastasis did not differ significantly between dogs with Patnaik grade 2 MCTs classified as Kiupel low-grade tumors (16.9%) and dogs with Patnaik grade 2 MCTs classified as Kiupel high-grade tumors (14.6%). Among dogs with Kiupel high-grade MCTs, those with Patnaik grade 3 MCTs had a significantly higher prevalence of metastatic disease (48.8%) than did those with Patnaik grade 2 MCTs (14.6%). Nevertheless, our findings suggested that histologic grading on its own is not reliable enough to allow treatment decisions for dogs with cutaneous MCTs.

Given that additional treatment options are increasingly available, the clinical management of dogs with cutaneous MCTs should be based on results of both clinical and histopathologic evaluations. As shown in the present study, metastasis may be present at the time of initial examination even in dogs with Patnaik grade 1 or Kiupel low-grade MCTs. We believe that combining histologic grade with clinical stage data would provide a more accurate predictor of biological behavior than either parameter alone.

In agreement with the published literature, the present study also found significant associations between nodal metastasis and tumor diameter ≥ 3 cm, digit location, ulceration, Shar-Pei breed, and substage b. This highlights the suggestion that these variables may be useful adjunctive tools for predicting metastasis and more aggressive biological behavior.

A limitation of the present study was that the presence of distant metastasis was mainly documented by means of cytologic evaluation, rather than histologic examination. This was a multi-institutional retrospective study, and staging procedures were not uniform among centers, with some clinicians performing fine-needle aspiration of the liver and spleen only in the case of ultrasonographic abnormalities or signs of aggressive biological behavior of the tumor. Although most (82.6%) dogs underwent complete clinical staging, it is
possible that distant metastases may have been missed in some dogs. Overall, distant metastases were detected in 4.1% of the dogs, which is in agreement with recent findings.\textsuperscript{11} Notably, 2 dogs with distant metastasis did not have nodal involvement, further suggesting that complete clinical staging is necessary to predict prognosis and drive treatment.

Regional lymph nodes were evaluated in all the dogs in the present study, but only 50 of 70 lymph nodes with cytologic evidence of metastasis were subsequently surgically removed and submitted for histopathologic confirmation. Despite the concordance between results of cytologic and histologic evaluation in these 50 dogs, it is possible that in some of the 20 dogs with cytologic evaluation alone, accumulations of reactive mast cells in the lymph node were misinterpreted as neoplastic. Importantly, the presence of mast cells in a draining lymph node may reflect increased trafficking of reactive cells, rather than true metastasis. The finding of well-differentiated mast cells in a lymph node aspirate is not necessarily sufficient to determine the anatomic location of the mast cells within the lymph node; hence, distinguishing metastasis from increased mast cell trafficking may be impossible on the basis of cytologic results alone. Nevertheless, the presence of several aggregates of mast cells and detection of mast cells with atypical morphology in some cases rendered the hypothesis of reactive mast cells unlikely in these cases. As a further confounding factor, the regional lymph node may not reflect the lymph node actually receiving the draining tumor lymph, as recently described.\textsuperscript{22}

Finally, Ki-67 expression and the presence of c-kit mutations were not evaluated in the present study. Future research should be directed at determining the possible clinical impact of Ki-67 expression in predicting the behavior of Patnaik grade 2 MCTs classified as Kiupel low-grade tumors.

In conclusion, determining the optimal combination of histopathologic and clinical information to develop a therapeutic plan in dogs with cutaneous MCTs is an evolving challenge. Although many studies indicate the usefulness of histologic grading in predicting the benefit of chemotherapy, given the financial constraints of many owners and limited access to molecular testing, studying the importance of clinical staging (along with other parameters) continues to be relevant. It is the authors’ opinion that histologic grade, when assessed by means of both grading systems, is a valuable prognostic factor in dogs with cutaneous MCTs that can be assessed cost-effectively in clinical practice. However, at present, results of histologic grading should always be integrated with results of clinical staging to provide reliable therapeutic decisions.

\textsuperscript{a} SPSS Statistics, version 19, IBM, Somers, NY.
\textsuperscript{b} Prism, version 5.0, GraphPad Software Inc, San Diego, Calif.

References