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)

Damiano Stefanello, DVM, PhD; Paolo Buracco, DVM; Silvia Sabattini, DVM, PhD; Riccardo Finotello, DVM, PhD; Chiara Giudice, DVM, PhD; Valeria Greco, DVM, PhD; Selina Iussich, DVM; Massimiliano Tursi, DVM; Timothy Scase, BVMS, PhD; Stefano Di Palma, DVM; Giuliano Bettini, DVM; Roberta Ferrari, DVM; Marina Martano, DVM, PhD; Francesca Gattino, DVM; Mary Marrington, MA, VetMB; Monica Mazzola, DVM; Maria Elisabetta Vasconi, DVM; Maurizio Annoni, DVM; Laura Marconato, DVM

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 were 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 296 (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 intermediatedifferentiated 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

ABBREVIATIONS

| CI | Confidence interval |
| MCT | Mast cell tumor |

From the Department of Veterinary Sciences and Public Health, Faculty of Veterinary Medicine, University of Milan, 20133 Milan, Italy (Stefanello, Giudice, Greco, Ferrari); the Department of Veterinary Sciences, Faculty of Veterinary Medicine, University of Bologna, Bologna 40064, Italy (Sabattini, Bettini); the Small Animal Teaching Hospital, School of Veterinary Science, University of Liverpool, Liverpool, Merseyside, L69 3BX, England (Finotello, Marrington); Bridge Pathology Ltd, 637 Gloucester Rd, Bristol, BS7 0BJ, England (Scase); Ilexx Laboratories Italy srl, Via Antonio Canova 27, 20145 Milano, Italy (Di Palma); Clinica Veterinaria Gran Sasso, Via Donatello 26, 20131 Milano, Italy (Mazzola); Centro Veterinario Tormese, Lungo Dora Colletta 1/4, 10153 Turin, Italy (Vasconi); Clinica Veterinaria Tibaldi, Viale Tibaldi 66, 20136 Milano, Italy (Annoni); and Centro Oncologico Veterinario, Via San Lorenzo 1/4, 40037 Sasso Marconi, Italy (Marconato). Presented in part at the Annual European Society of Veterinary Oncology Congress, Vienna, May 2014. Address correspondence to Dr. Marconato (marconato@centroncologevet.it).
be some histopathologic variation among and within tumors.8,9 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.9

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.3

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.

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 χ² 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 χ² 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 ≤ 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 (63), 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...
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>
<td></td>
<td></td>
</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>
<td></td>
<td></td>
<td></td>
<td></td>
</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>
</tr>
<tr>
<td>Patnaik grade 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</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>

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 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 (14.6%) 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 MCT classified as a Kiupel high-grade tumor, and 1 had a Patnaik grade 2 MCT classified as a Kiupel low-grade tumor.
SMALL ANIMALS/AVIAN

peutic approach. histologic grade, thereby requiring a multimodal thera-
 proportion of dogs will have metastatic disease despite 
grading remains somewhat subjective, and incorrect 
agreement with the Patnaik grading system. However, histologic 
be an independent prognostic indicator in dogs with 
aggressive biological behavior and a high metastatic 
grade 2 or 1 MCTs (OR, 3.46; 95% confidence inter-
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 
were 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, prog-
nostication should not rely on histologic grade alone, 
regardless of grading system used, but should take into 
account the results of clinical staging. Lymph node sta-
tus and histologic grade are reported among the most 
important prognostic indicators for dogs with cutane-
ous MCTs, and detection of lymph node metastasis or 
a high histologic grade is a key factor in recommend-
ing systemic treatment.2-3,10 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 me-
tastasis is low.11 On the basis of the findings of the pre-
ent study, this assumption does not apply as a whole. 
A proportion of dogs will have metastatic disease despite 
histologic grade, thereby requiring a multimodal ther-
apeutic approach.

Various studies3-5,12,13 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.6,7 Also, 
histologic grade does not take into account other fac-
tors with possible prognostic importance, such as tu-
or 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%).2,3,12 Conversely, Patnaik grade 1 
MCTs only rarely metastasize (< 10%).2,3,14 This is in 
agreement with the findings of the present study. Dogs 
with MCTs classified as Patnaik grade 1 had a significa-
cantly 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 clas-
sified as Kiupel high grade. On the other hand, the bi-
ological 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%).2,3,14 In 
the present study, 16.5% (48/291) of dogs with Patnaik 
grade 2 MCTs had nodal or distant metastases. Interest-
ingly, adding the Kiupel grading system did not seem 
to overcome the issue of indeterminate biological be-
havior 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 preva-
ience of metastatic disease (48.8%) than did those with 
Patnaik grade 2 MCTs (14.6%). Nevertheless, our find-
ings 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 increasin-
gly available,15-21 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.
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. 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. 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.

References