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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 Grieco, DVM, PhD; Selina Iussich, DVM; Massimiliano Tursi, DVM; Timothy Scase, BVM&S, 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

From the Department of Veterinary Sciences and Public Health, Faculty of Veterinary Medicine, University of Milan, 20133 Milan, Italy (Stefanello, Giudice, Grieco, Ferrari); the Department of Veterinary Sciences, Faculty of Veterinary Medicine, University of Turin, 10124 Turin, Italy (Buracco, Iussich, Tursi, Martano, Gattino); the Department of Veterinary Medical Sciences, 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); Idexx 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 Torinese, Lungo Dora Colletta 147, 10153 Turin, Italy (Vasconi); Clinica Veteri- naria Tibaldi, Viale Tibaldi 66, 20136 Milano, Italy (Annoni); and Centro Oncologico Veterinario, Via San Lorenzo 1/4, 40037 Sasso Marconi, Italy (Marconato).

Address correspondence to Dr. Marconato (marconato@centroncologicovet.it

ABSTRACT

Objective—To compare the Kiupel (2 categories) and Patnaik (3 categories) histologic grad- ing 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 cu- taneous 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 sig- nificantly 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 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

In dogs, cutaneous MCTs are characterized by highly variable biological behavior, ranging from low malig- nant 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 poten- tially 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 intermediately 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.3 Although the biological be- havior 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.5

In contrast to the Patnaik grading system, the Kiu- pel 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.5

The purpose of the study reported here was to ret- rospectively 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 So- ciety 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.[8,9]

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]

98 Statistical analysis—When appropriate, data were tested for normality with the D'Agostino and Pearson
99 omnibus normality test. Continuous values that were normally distributed are expressed as mean ± SD;
100 val- ues that were not normally distributed are expressed as median and range.

101 The χ2 test (categorical variables) and Mann-Whitney U test (continuous variables) were used to test for as-
102 sociations between various clinical variables and the presence of lymph node metastases. Variables that
103 were assessed consisted of breed (most represented breeds [Boxer, Labrador Retriever, Golden Retriever,
104 American Staffordshire Terrier, and Shar-Pei] vs all other breeds), body weight, tumor location (head and
105 neck, trunk [in- cluding abdominal wall and proximal portions of the limbs to the elbow or knee], inguinal
106 [including peri- neal] region, distal portions of the limbs excluding the digits, and digits), macroscopic
107 tumor diameter (< 3 or ≥ 3 cm), ulceration, and substage.The proportions of dogs with metastases were
108 compared among histologic grades according to the Patnaik and Kiupel systems with the χ2 test. The
109 likelihood of metastatic disease at the time of initial examination according to tumor grade was assessed by
110 means of logistic regression. All statistical analyses were performed with standard software.a,b Values of P
111 ≤ 0.05 were considered significant.

112 RESULTS

113 Patient and tumor characteristics—A total of 386 dogs fulfilled the criteria for inclusion in the study.
114 Mean ± SD age was 7.7 ± 2.8 years. Two hundred twelve dogs were females (of which 92 were spayed), and
115 174 dogs were males (of which 29 were castrated). Eighty- six dogs were of mixed breeding, with the
116 remaining 300 dogs representing 51 breeds, including Boxer (n = 79), Labrador Retriever (65), Golden
117 Retriever (30), English Setter (20), American Staffordshire Terrier (13), Shar-Pei (8), Beagle (7), French
118 Bulldog (6), Bernese Mountain Dog (4), Epagneul Breton (4), Shih-Tzu (4), and 40 other breeds each
119 represented by 1 to 3 animals. Tumors were located on the head and neck (n = 74 [19.2%]) or trunk (209
120 [54.1%]), in the inguinal region (36 [9.3%]), or on the distal portions of the limbs (58 [15.0%]) or digits (9
121 [2.3%]). Tumor diameter ranged from 0.4 to 20 cm (median, 3 cm); 332 (86%) MCTs were not ulcerated,
122 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 Pat- naik 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 clin- ical 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 com- plete 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 dis- ease. 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, in- cluding 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 1 (3/52 [5.8%]) and dogs with Kiupel low-grade MCTs classified as Patnaik grade 2 (41/243 [16.9%]). Con- versely, 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, 5.46; 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 ini- tial 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 reportedly 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.2–5,10 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 pres- 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 thera- peutic approach.

199 Various studies3–5,12,13 have shown histologic grade to be an independent prognostic indicator in dogs
200 with cutaneous MCTs and have shown better interobserver agreement with the Kiupel grading system,
201 compared with the Patnaik grading system. However, histologic grading remains somewhat subjective,
202 and incorrect grades may be assigned for individual MCTs, which may result in inappropriate treatment
203 decisions.6,7 Also, histologic grade does not take into account other fac- tors with possible prognostic
204 importance, such as tu- mor size and location and the presence or absence of metastases.

205 It is well accepted that Patnaik grade 3 MCTs have an aggressive biological behavior and a high metastat- ic
206 potential (> 80%).2,3,12 Conversely, Patnaik grade 1

207 MCTs only rarely metastasize (< 10%).2,3,14 This is in agreement with the findings of the present study.
208 Dogs with MCTs classified as Patnaik grade 1 had a signifi- cantly lower prevalence of metastasis than did
209 dogs with MCTs classified as grade 3, and dogs with MCTs classified as Kiupel low grade had a significantly
210 lower prevalence of metastasis than did dogs with MCTs clas- sified as Kiupel high grade. On the other
211 hand, the bio- logical behavior of Patnaik grade 2 MCTs is difficult to predict, with Patnaik grade 2 MCTs
212 reported to have an intermediate metastatic potential (5% to 22%).2,3,14 In the present study, 16.5%
213 (48/291) of dogs with Patnaik grade 2 MCTs had nodal or distant metastases. Interest- ingly, adding the
214 Kiupel grading system did not seem to overcome the issue of indeterminate biological be- havior for
215 Patnaik grade 2 MCTs, in that the prevalence of metastasis did not differ significantly between dogs with
216 Patnaik grade 2 MCTs classified as Kiupel low- grade tumors (16.9%) and dogs with Patnaik grade 2

217 MCTs classified as Kiupel high-grade tumors (14.6%). Among dogs with Kiupel high-grade MCTs, those with
218 Patnaik grade 3 MCTs had a significantly higher preva- lence of metastatic disease (48.8%) than did those
219 with Patnaik grade 2 MCTs (14.6%). Nevertheless, our find- ings suggested that histologic grading on its
220 own is not reliable enough to allow treatment decisions for dogs with cutaneous MCTs.

221 Given that additional treatment options are in- creasingly available,15–21 the clinical management of
222 dogs with cutaneous MCTs should be based on results of both clinical and histopathologic evaluations. As
223 shown in the present study, metastasis may be pres- ent 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,1,2 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 pres- ence 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 aggres- sive 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.11 Notably, 2 dogs with distant metastasis did not have nodal involvement, further suggesting that complete clinical staging is necessary to predict prog- nosis 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 sub- sequently surgically removed and submitted for histo- pathologic confirmation. Despite the concordance be- tween 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 reac- tive 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 find- ing of well-differentiated mast cells in a lymph node as- pirate is not necessarily sufficient to determine the ana- tomic location of the mast cells within the lymph node; hence, distinguishing metastasis from increased mast cell trafficking may be impossible on the basis of cyto- logic results alone. Nevertheless, the presence of sev- eral 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.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 predict- ing the behavior of Patnaik grade 2 MCTs classified as Kiupel low-grade tumors.

In conclusion, determining the optimal combina- tion of histopathologic and clinical information to de- velop a therapeutic plan in dogs with cutaneous MCTs is an evolving challenge. Although many studies indi- cate the usefulness of histologic grading in predicting the benefit of chemotherapy, given the financial con- straints of many owners and limited access to molecu- lar 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 valu- able prognostic factor in dogs with cutaneous MCTs that can be assessed cost-effectively in clinical prac- tice. However, at present, results of histologic grading should always be integrated with results of clinical stag- ing to provide reliable therapeutic decisions.

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320 Table 1—Prevalence of metastatic disease in 386 dogs with cutaneous MCTs classified according to the

321 Patnaik and Kiupel histologic grading systems

Total	386/386 (100)	314/386 (81.3)	72/386 (18.7)	70/386 (18.1)	16/386 (4.1)
1	52/386 (13.5)	49/52 (94.2)	3/52 (5.8)	3/52 (5.8)	1/52 (1.9)
2	291/386 (75.4)	243/291 (83.5)	48/291 (16.5)	47/291 (16.2)	6/291 (2.1)
3	43/386 (11.1)	22/43 (51.2)	21/43 (48.8)	20/43 (46.5)	9/43 (20.9)
Kiupel grade					
High	91/386 (23.6)	63/91 (69.2)	28/91 (30.8)	27/91 (29.7)	10/91 (11.0)
Patnaik grade 2					
Kiupel high grade	48/291 (16.5)	41/48 (85.4)	7/48 (14.6)	7/48 (14.6)	1/48 (2.1)

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