

Cytologic comparison of the percentage of mast cells in lymph node aspirate samples from clinically normal dogs versus dogs with allergic dermatologic disease and dogs with cutaneous mast cell tumors

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OBJECTIVE

To compare percentages of mast cells in lymph node (LN) aspirate samples from clinically normal dogs, dogs with allergic dermatologic disease (ADD), and dogs with cutaneous mast cell tumors (MCTs).

DESIGN

Prospective cross-sectional study.

ANIMALS

20 healthy dogs (group 1), 20 dogs with ADD (group 2), and 20 dogs with an MCT on the head or limbs (group 3).

PROCEDURES

LN aspirate samples were obtained from easily accessible LNs in group 1, affected skin regions in group 2, and the likely draining LN or LNs of the MCT in group 3; the percentage of mast cells was manually determined for each LN. For group 3, LNs were cytologically categorized with a modified version of a published metastasis categorization scheme.

RESULTS

Median (range) percentage of mast cells in aspirate samples was 0% (0% to 0.1%) for group 1, 0.05% (0% to 0.55%) for group 2, and 0.4% (0% to 77.4%) for group 3. In group 3, 16 LNs (13 dogs) were palpably normal in size; 6 of these had evidence of possible or certain metastasis. Seven LNs (7 dogs) in group 3 were palpably enlarged, and 5 of these had evidence of certain metastasis.

CONCLUSIONS AND CLINICAL RELEVANCE

This study provided evidence to support the use of a uniform cytologic grading system to further define nodal metastasis in dogs with MCTs as well as estimates of the percentage of mast cells in LN aspirate samples for healthy dogs and dogs with ADD. Palpably normal LNs in dogs with cutaneous MCT may contain metastasis. (*J Am Vet Med Assoc* 2017;251:421–428)

Mast cell tumors are the most common cutaneous tumors of dogs, representing 7% to 21% of all skin tumors.^{1–5} The biological behavior of these tumors is highly variable. Several potential prognostic factors for local tumor recurrence and metastasis have been examined, including histologic grade,^{6–8} clinical stage,^{4,9} anatomic location,^{4,5,10} growth rate,¹¹ dog breed,^{11–13} various cell proliferation indices,^{8,14–21} and presence of *c-KIT* mutations.^{22,23}

After a diagnosis of neoplasia has been established, the extent of disease should be determined by presurgical examination and testing and categorized by the clinical stage.¹ A modified version of the World Health Organization clinical staging scheme for MCTs

includes 6 stages of metastasis,^{4,24–26} which are further categorized by whether systemic signs are present. The role of metastasis to the LNs (stage 2 disease) in the prognosis for dogs with MCTs remains undefined. The prognosis for dogs with Patnaik grade 2, stage 2 MCT treated with surgery, radiation therapy, and chemotherapy has been evaluated, revealing that this combination approach prolongs the time to local tumor recurrence or metastasis and can provide a median survival time > 40 months.²⁷

Mast cell tumors metastasize most commonly to the regional LN, spleen, and liver. In a retrospective study²⁸ of 220 dogs, all dogs with distant metastasis also had metastasis to the regional LN, suggesting that metastasis to those LNs preceded the development of distant metastasis in all of those dogs. Therefore, accurate staging to determine the extent of any metastasis to the regional LNs in dogs with MCT is of utmost importance to guide treatment recommendations and predict overall prognosis.

ABBREVIATIONS

CI	Confidence interval
LN	Lymph node
MCT	Mast cell tumor

Assessment of LNs for metastasis is usually first done via cytologic examination of LN aspirate samples. This technique is easily performed, safe, non-painful, and reportedly sensitive and specific for evaluating metastasis to regional LNs in dogs and cats with solid tumors.²⁹ No set cytologic criteria exist for determining MCT metastasis to LNs. Reported criteria for evidence of metastasis include the presence of clusters or sheets of mast cells, large numbers of well-differentiated mast cells, or atypical (pleomorphic and poorly granulated) mast cells within an organ of interest.^{5,9,28,30-33} In an attempt to better define criteria for metastasis in dogs, Krick et al⁹ reported more specific criteria for interpretation of mast cells in LN aspirate samples, including the categories normal, reactive lymphoid hyperplasia, possible metastasis, probable metastasis, and certain metastasis. To further confound the issue, no clear consensus exists on the number of mast cells typically present in healthy or reactive LNs in dogs. Although verification of metastasis through histologic examination of the resected node is considered the gold standard test, this may not be practical in all patients owing to costs and adverse effects associated with surgery.^{29,34}

A morphometric approach to predicting micro-metastatic load in regional LNs has been evaluated in dogs with MCTs.³⁵ Nuclear morphometry allows quantitative analysis of nuclear variations and was deemed a useful predictor of LN metastases in a study³⁵ involving dogs with MCTs. In that study,³⁵ the number of mast cells in aspirate samples from the LN to which the MCT drained was reported as a percentage of the total cell count. Lymph node aspirate samples from dogs with MCTs were further subcategorized cytologically as no metastasis, inconclusive for metastasis, and obvious metastasis. A limitation of that study was the lack of a clear definition of metastasis. Dogs in which MCTs were categorized as inconclusive for metastasis had only occasional mast cells detected in regional LN node aspirate samples, with no evidence of distant metastasis. Although cytologic findings for most dogs with MCTs were consistent with metastatic disease, a group of dogs remained with occasional mast cells in aspirate samples that were difficult to interpret.

Estimates of numbers of mast cells in peripheral LNs of clinically normal dogs versus dogs with allergic skin disease have been reported.^a In the associated study,^a LN aspirate samples from 5 of 30 healthy dogs and 15 of 20 dogs with allergic skin disease contained some mast cells. Numbers of mast cells were significantly higher in the allergy group on the basis of several criteria (number of mast cells/20 microscope fields, number of mast cells/500 lymphoid cells, and total number of mast cells in the entire smear).

The objective of the study reported here was to compare percentages of mast cells in peripheral LN aspirate samples from clinically normal dogs with those from dogs with allergic skin disease and dogs with cutaneous MCTs. Our hypothesis was that the

samples from dogs with MCT would contain a higher median percentage of mast cells than samples from dogs in the other groups. Use of this information, along with clinical assessment and LN palpation and results of previous studies, would hopefully allow further elucidation and classification of metastasis to the LNs in dogs with MCTs.

Materials and Methods

Animals

Canine patients of the Louisiana State University Veterinary Teaching Hospital between July 2011 and February 2013 were eligible for inclusion in the study. These patients included dogs referred to the teaching hospital as well as staff- and student-owned dogs. Three groups of 20 dogs each were enrolled. Dogs were included in group 1 (clinically normal) if they were ≥ 1 year of age and had unremarkable physical examination findings and no evidence of dermatologic disease as deemed by a Dermatology Service clinician, no prior history of dermatologic disease as reported by the owner or in the medical record, and unremarkable results of CBC, serum biochemical analysis, and urinalysis in the past year; were currently receiving a heartworm preventive; and had negative results of heartworm antigen testing within the past year.

Dogs included in group 2 were enrolled through the Dermatology Service as patients with active allergic dermatologic disease and were required to be ≥ 1 year of age and receiving a heartworm preventive, with negative results of heartworm antigen testing within the past year. No dogs were excluded from group 2 on the basis of medications they could have been receiving (2 were receiving an orally administered antihistamine-corticosteroid medication^b at the time of study enrollment); however, to be enrolled, their dermatologic disease was required to be deemed active, which for most patients represented an acute flare-up of their chronic allergic disease.

Dogs in group 3 were enrolled through the Oncology Service as patients with a cytologic or histologic diagnosis of MCT on the head, limbs, or both. These locations were chosen to ensure that the LN to which the tumor most likely drained was accessible for fine-needle aspiration. Dogs that had received any prior treatment except surgery (including chemotherapy, radiation therapy, or glucocorticoids within 3 weeks before enrollment) were excluded from the study. Additionally, dogs in this group were required to be ≥ 1 year of age, have no evidence of dermatologic disease as deemed by a Dermatology Service clinician, have no prior history of dermatologic disease as reported by the owner or in the medical record, and be receiving a heartworm preventive, with negative results of heartworm antigen testing within the past year. Clinical staging in addition to the bloodwork and urinalysis required for group 1 was recommended for all dogs in group 3, including thoracic radiography and abdominal ultrasonography.

The study protocol was reviewed and approved by the institutional clinical protocol committee. Additionally, owner consent was obtained prior to study enrollment.

Data collection

For all dogs, data were collected regarding breed; body weight; sex; age; results of CBC, serum biochemical analysis, and urinalysis; heartworm preventives received; and identities of aspirated LNs, including size in 2 dimensions. For dogs in group 2, additional data included the type of flea preventive used, dermatologic history (including documentation of food trial or allergy testing [blood or intradermal testing]), physical examination findings, current medications, diet, current working diagnosis, evidence of concurrent infection, and other diagnostic testing performed on the day of initial evaluation. For dogs in group 3, additional data included physical examination findings (including size of the primary tumor or tumors if known), imaging results (thoracic radiography and abdominal ultrasonography), cytologic examination results for the liver or spleen if performed, buffy coat or bone marrow aspirate results when available, place where tumor excision was completed (referring veterinarian or Louisiana State University), whether the draining LN was removed and related histologic findings, postoperative complications, type of adjuvant treatment provided if applicable, date of last follow-up (determined by medical record review or by contacting the patient's referring veterinarian), cause of death when applicable, and whether a necropsy had been performed and applicable results.

Information obtained from reports of histopathologic findings for tumors included tumor grade, mitotic index, and completeness of excision. Primary tumors were histologically graded in accordance with the previously established Patnaik grading scheme,⁶ as this was the most commonly used grading scheme at the time of study enrollment. According to this scheme, grade 1 MCTs are confined to the dermis and contain well-differentiated mast cells that lack mitotic activity. Grade 2 MCTs infiltrate or replace the lower dermal, subcutaneous, or skeletal tissues and contain mast cells that vary moderately in cell or nuclear size or shape (pleomorphic). Mitotic cells are rare. Grade 3 MCTs replace the subcutaneous and deeper tissues and contain highly pleomorphic mast cells. Mitotic cells are common.⁶

To improve concordance among veterinary pathologists, a 2-tier grading system based on quantitative cytologic criteria was proposed in 2011.⁷ However, this grading system was not used in the present study because the retrieved biopsy reports were graded by use of the Patnaik system. Tumors were deemed completely excised if ≥ 5 mm of histologically normal tissue was present between the tumor and surgical margin,³⁶ narrowly excised if tumor cells were noted within 1 to 5 mm of the surgical margin, and incompletely excised if tumor cells were < 1 mm from the

surgical margin. Mitotic index was recorded as the number of mitotic figures/10 hpfs (40X). Histologic slides from outside institutions were not routinely reviewed as part of the study.

Sixteen board-certified veterinary anatomic pathologists were represented, including 5 from the study institution. A stage and substage were assigned to each dog in accordance with the modified World Health Organization clinical staging scheme.^{25,26} These stages were as follows: stage -1, single tumor removed with clean margins; stage 0, 1 tumor incompletely excised from the dermis, identified histologically, and without regional LN involvement; stage 1, 1 tumor confined to the dermis without regional LN involvement; stage 2, 1 tumor confined to the dermis with regional LN involvement; stage 3, multiple dermal tumors or large, infiltrating tumors with or without regional LN involvement; and stage 4, any tumor with distant metastasis, including blood or bone marrow involvement. For patients with multiple tumors, the highest clinical stage was recorded. Substages were assigned on the basis of whether systemic signs were not (substage A) or were (substage B) present.

LN evaluation

Percutaneous fine-needle aspirate samples were obtained from LNs in all dogs with a 22-gauge needle and a so-called needle-off or woodpecker technique (ie, only a needle was used, with no syringe attached). For dogs in group 1, aspiration of a popliteal or prescapular LN was preferred; however, if aspiration was unsuccessful, a submandibular LN was used. Unsuccessful LN aspiration was most often attributable to a large amount of perinodal fat. For dogs in group 2, choice of LN was based on the body region most affected by the dermatologic condition. For dogs in group 3, choice of LN was made on the basis of the LN to which the MCT most likely drained. In some dogs, > 1 LN was aspirated (ie, dogs with multiple tumors or a primary tumor located near 2 accessible LNs, such as an MCT on the ear or an MCT in the center of the inguinal area).

A minimum of 1 attempt and a maximum of 4 attempts were made to obtain an aspirate sample from each selected LN. Aspirate samples were air-dried and stained with May-Grünwald-Giemsa stain.^c The number of slides submitted for cytologic examination varied among dogs, depending on the amount of material recovered from the LNs. All slides were reviewed by the same board-certified veterinary clinical pathologist (AR). The best slide was subjectively identified through cursory evaluation of overall cellularity, cell preservation, and staining quality. Slides that contained only blood or only a few stromal cells were excluded from evaluation and considered nondiagnostic.

On each slide, 2,000 cells were manually counted, and the percentage of mast cells per total cell count was determined for each LN. In some situations, 2 slides were assessed to achieve the 2,000 to

tal cell count. The amount of time needed to perform a 2,000 cell count varied, depending on sample cellularity, cellular preservation, and cell types present, for a mean of approximately 20 minutes. In addition, for the dogs in group 3, metastasis was cytologically classified by use of a modified version of the criteria reported by Krick et al.⁹ Categories included normal (no mast cells visible), reactive lymphoid hyperplasia (> 50% small lymphocytes with a mixed population of intermediate and large lymphocytes as well as plasma cells; few numbers of macrophages, neutrophils, and eosinophils; and rare individual mast cells), possible metastasis (2 to 3 incidences of mast cells in aggregates of 2 to 3 cells), and certain metastasis (effacement of lymphoid tissue by mast cells or the presence of aggregated, poorly differentiated mast cells with pleomorphism, anisocytosis, anisokaryosis, or decreased or variable granulation or > 5 aggregates of > 3 mast cells).⁹

Statistical analysis

Summary statistics were calculated for all evaluated variables. Data regarding percentages of mast cells were examined for normality of distribution. Because these data were not normally distributed, logistic regression was used to compare among the 3 groups the odds of having mast cells or not having mast cells in any LN aspirate sample. For dogs with > 1 sample, only the overall finding was used. Two such analyses were performed. In the first, mast cells were considered to be present if the percentage exceeded 0.5%, and in the second if the percentage exceeded 0% (ie, ≥ 1 mast cell was identified). A statistical software program^d was used for all analyses. Values of $P < 0.05$ were considered significant.

Results

Clinically normal dogs

Dogs in group 1 had a median age of 3 years (range, 1 to 13 years) and median body weight of 21.9 kg (48.2 lb; range, 4 to 69.5 kg [8.8 to 152.9 lb]). Twelve dogs were castrated males, 7 were spayed females, and 1 was a sexually intact male. There were 9 mixed-breed dogs, 3 Dachshunds, 2 Labrador Retrievers, 2 Welsh Corgis, and 1 each of Boxer, Great Dane, Doberman Pinscher, and Golden Retriever. All LNs were palpably normal in size, and 1 LN was aspirated/dog. Aspirated LNs included the left popliteal ($n = 5$), right popliteal (5), left prescapular (2), right prescapular (2), left mandibular (2), and right mandibular (4) LNs. All LN aspirate samples were cytologically normal with no evidence of neoplasia. Median percentage of mast cells/2,000 total cell count was 0% (range, 0% to 0.1%), and the median total mast cell count was 0 (range, 0 to 2).

Dogs with allergic dermatologic disease

Dogs in group 2 had a median age of 6 years (range, 1 to 12 years) and median body weight of 26 kg (57.2 lb; range, 7.5 to 39.5 kg [16.5 to 86.9 lb]). Seven dogs were spayed females, 4 were sexually intact

females, 7 were castrated males, and 2 were sexually intact males. There were 3 mixed-breed dogs, 3 Golden Retrievers, and 2 English Bulldogs. Twelve other breeds were represented by 1 dog each. Ten dogs had a previous diagnosis of atopy that was confirmed with intradermal testing. Nine dogs had a tentative diagnosis of suspected combined food allergy and atopy. One dog had a diagnosis of flea allergic dermatitis, and 1 dog had contact dermatitis. Sixteen dogs had concurrent active bacterial infection at the time of LN aspiration; 6 of these dogs had otitis externa, 9 had bacterial pyoderma, and 5 had a combination of otitis externa and bacterial pyoderma. Aspirated LNs in this group (1/dog) included the left popliteal ($n = 4$), right popliteal (9), left prescapular (2), right prescapular (4), and right mandibular (1) LNs. These LNs ranged from normal in size to 3 X 3 cm. Median percentage of mast cells/2,000 total cell count was 0.05% (range, 0% to 0.55%), and median total mast cell count was 1 (range, 0 to 110). All LN aspirate samples were cytologically normal, with no evidence of neoplasia.

Dogs with MCTs

Dogs in group 3 had a median age of 7 years (range, 3 to 13 years) and median body weight of 28 kg (61.6 lb; range, 3.8 to 37.9 kg [8.4 to 83.4 lb]). Nine dogs were spayed females, 10 were castrated males, and 1 was a sexually intact male. There were 7 mixed-breed dogs, 6 Boxers, 3 Labrador Retrievers, and 1 each of Dachshund, Italian Greyhound, Miniature Poodle, and English Bulldog. These 20 dogs had 25 MCTs; 15 dogs had 1 tumor, 3 dogs had 2 tumors, 1 dog had 3 tumors, and 1 dog had multiple variably sized nodules on the right ear pinna that were too numerous to count. Primary tumor location included the hind limb ($n = 10$; 2 localized to the paw), forelimb (6; 2 localized to the paw), thorax (3), head (3; 1 each on the lip, base of the ear, and multiple coalescing nodules on the pinna), and perineum or inguinal region (3). The 3 dogs with an MCT on the thorax also had an MCT on the head or limbs. Sixteen dogs had had their tumors excised prior to initial evaluation at the teaching hospital, and 4 had tumors at the time of referral. For all dogs, the median interval from diagnosis to study enrollment was 15 days (range, 0 to 118 days).

Histologic grade was available for all tumors in group 3. There were 2 grade 1 tumors, 18 grade 2 tumors, and 5 grade 3 tumors. Information pertaining to completeness of excision was available for all tumors. Six tumors had been completely excised, 15 tumors had been incompletely excised, and 4 tumors had been narrowly excised. Median mitotic index of all tumors was 1/10 hpfs (range, 0 to 22/10 hpfs). Median mitotic index for grade 1 tumors only was 0/10 hpfs, for grade 2 tumors was 1/10 hpfs (range, 0 to 15/10 hpfs), and for grade 3 tumors was 20/10 hpfs (range, 6 to 22/10 hpfs). Metastasis stage was determined for all dogs (not tumors) and included stage -1

($n = 1$), stage 0 (7), stage 1 (1), stage 2 (5), stage 3 (5), and stage 4 (1). Of the 5 dogs with multiple tumors (stage 3), 3 dogs had confirmed metastasis to the LNs. The dog with stage 4 disease had confirmed LN and splenic metastasis. All dogs were in substage A of disease (ie, without systemic signs).

Aspirate samples were obtained from 23 regional LNs in group 3 for cytologic evaluation. Aspirated LNs in this group included the left inguinal ($n = 1$), left popliteal (6), right popliteal (4), left prescapular (3), right prescapular (5), left mandibular (1), and right mandibular (3) LNs. Sixteen of these LNs were palpably normal in size, with 9 categorized as reactive lymphoid hyperplasia, 3 as possible metastasis, and 3 as certain metastasis. The aspirate sample from 1 LN was nondiagnostic. The remaining 7 LNs were palpably enlarged, ranging in size from 1 X 1 cm to 5 X 5 cm; 2 were categorized as reactive lymphoid hyperplasia and 5 as certain metastasis. None of the 23 aspirated LNs were categorized as cytologically normal. Median percentage of mast cells/2,000 total cell count was 0.4% (range, 0% to 77.4%), and median total mast cell count was 8 (range, 0 to 1,548). For the grade 1 tumor with an accessible LN, the percentage of mast cells was 0.05%. Median percentage of mast cells for grade 2 tumors was 0.1% (range, 0% to 77.4%) and for grade 3 tumors was 4% (range, 0.5% to 12.4%).

For 7 dogs in group 3, 1 LN each was submitted for histologic examination as part of a separate study or because of suspected metastasis. Six LNs cytologically categorized as having certain metastasis were confirmed as metastatic through this histologic examination. Three of these LNs were normal in size (2 associated with a grade 2 tumor and 1 associated with a grade 3 tumor). The LN associated with a grade 3 tumor was categorized as reactive lymphoid hyperplasia on cytologic and histologic examination. The associated dog had a palpably normal LN.

For dogs in group 3, the median interval from diagnosis to follow-up was 173 days (range, 7 to 555 days). Only 4 dogs were known to be deceased at the time of last follow-up. Cause of death for 1 dog was unknown, and the other 3 dogs were euthanized because of progressive cutaneous mast cell disease at 149, 150, and 236 days after diagnosis. The only dog with stage 4 mast cell disease was in this group and survived for 150 days after diagnosis. Of the remaining 16 dogs, 7 were lost to follow-up. At the time of last follow-up, no dog had developed recurrence or metastatic disease. At last follow-up, 9 dogs were alive with no evidence of recurrence or metastatic disease during a median follow-up period of 326 days (range, 77 to 555 days). Of the 8 dogs with certain metastasis to the LNs (stage 2 disease), 3 were lost to follow-up. Of the remaining 5 dogs, 2 died of progressive mast cell disease, with survival times of 149 and 150 days after diagnosis. At last follow-up, 3 dogs were still alive with no evidence of recurrence or metastasis at 344, 495, and 555 days after initial evaluation.

Comparisons of percentages of mast cells among groups

One (5%) dog in group 1, 9 (45%) dogs in group 2, and 12 (60%) dogs in group 3 had $> 0.05\%$ mast cells in LN aspirate samples. Group 3 was significantly more likely to have $> 0.05\%$ mast cells in LN aspirate samples than groups 1 and 2 combined (OR, 4.5; 95% CI, 1.2 to 16.6; $P = 0.008$) or group 1 alone (OR, 28.5; 95% CI, 3 to 1,280.0; $P < 0.001$), but not group 2 alone (OR, 1.8; 95% CI, 0.4 to 7.7; $P = 0.34$). Four (20%) dogs in group 1, 12 (60%) dogs in group 2, and 15 (75%) dogs in group 3 had > 0 mast cells in LN aspirate samples. Compared with group 1, group 3 was significantly ($P < 0.001$) more likely to have > 0 (ie, at least 1) mast cells in LN aspirate samples (OR, 12; 95% CI, 2.2 to 70.5), but not when compared with group 2 (OR, 2.0; 95% CI, 0.4 to 9.8; $P = 0.31$).

Discussion

The purpose of the study reported here was to cytologically evaluate and compare peripheral LNs of clinically normal dogs, dogs with allergic dermatologic disease, and dogs with cutaneous MCTs. Dogs with cutaneous MCTs were significantly more likely to have $> 0.05\%$ mast cells in LN aspirate samples than clinically normal dogs, but not when compared with dogs with allergic dermatologic disease. The LNs to which the MCTs likely drained were also significantly more likely to contain any mast cells than peripheral LNs in clinically normal dogs. This did not hold true when dogs with MCTs were compared with dogs with allergic dermatologic disease. Nineteen percent (3/16) of palpably normal LNs were classified as metastatic on cytologic examination (as defined by Krick et al).⁹ Additionally, 3 of 10 LNs considered metastatic were palpably normal in size.

Mast cells play an important role in immunologic, inflammatory, and allergic reactions. It is intuitive that they would be present in LNs of dogs with various disease conditions, and it is widely accepted that mast cells can be present in regional LNs because of nonneoplastic, reactive conditions. Despite this, literature^{37,38} exists in which metastasis is described as the presence of any mast cells in an organ. Further complicating the issue, no standardized cytologic or histologic criteria exist for determination of MCT metastasis to the LNs. Smears of LN aspirate samples from clinically normal dogs in 1 study³⁹ contained between 1 and 16 well-differentiated mast cells/slide, for a mean of 6.4 cells/slide. In that study,³⁹ only 1 slide/dog was evaluated and no description was given as to which LNs were chosen for evaluation. It was later suggested that MCT metastasis to the LNs can be diagnosed by cytologic examination if mast cells represent $> 3\%$ of the cell population in LN aspirate samples.⁴⁰ If that criterion were used, metastatic MCT would be diagnosed in up to 25% of clinically normal dogs,⁴¹ emphasizing again that more standardized criteria are needed for determination of MCT metastasis

to the LNs. In the study reported here, clinically normal dogs and dogs with allergic skin disease (primarily atopy) had a median of 0 to 1 mast cell/2,000 cells counted in LN aspirate samples. Dogs with MCTs had a median of 8 mast cells/2,000 cells counted, which would represent 0.4% of the cell population evaluated for these dogs.

In the study by Krick et al⁹ in which cytologic categories of MCTs were derived, a significant difference in survival time was identified between dogs with stage 1 (6.2 years) versus stage 2 (0.8 years) disease, and stage was identified as a prognostic factor independent of grade on multivariate analysis. No difference was detected in survival times between dogs with possible metastasis (n = 14) and those with normal and reactive LNs (75). In addition, there was no difference between dogs with probable (5) and certain (55) metastasis.⁹ Because of this, we chose to eliminate the category of probable metastasis in the present study. Although the previous study⁹ was useful for establishing specific cytologic criteria for evaluation of LNs in dogs with MCTs, a major limitation was that only 1 dog had regional LN excision for histologic confirmation of metastatic disease.

Physical examination alone (palpation) is not a reliable method for determining regional metastasis to the LNs in dogs and cats with solid tumors.²⁹ In only 13 of 21 dogs with documented metastasis to the LNs in 1 study,²⁷ the draining LN was palpably abnormal. In the present study, 16 of the draining LNs in the MCT group were deemed palpably normal; however, on cytologic evaluation of LN aspirate samples, 3 were categorized as having possible metastasis and 3 as having certain metastasis. Further, on histologic examination of LNs, 3 of the 6 dogs confirmed to have metastasis to the LNs had a palpably normal LN.

Cytologic examination of LN aspirate samples has been shown to be a sensitive and specific method for evaluating the extent of metastasis to the regional LNs in dogs and cats with solid tumors, although only 7 of the 44 tumors in that study were MCTs.²⁹ Excisional biopsy of the entire LN remains the standard of care for confirming metastasis to the LNs, given that agreement between a cytologic and histologic diagnosis can vary.^{29,42}

Although the present study was prospective in design, it had inherent limitations and should be considered preliminary. The main limitation was that only 7 LNs from dogs with MCTs were evaluated via the gold standard test, histologic examination. A novel histologic classification system for the evaluation of nodal metastasis was recently published and is based on the number of, distribution of, and architectural disruption by nodal mast cells.⁴³ Four categories (HN0 through HN3) were proposed.⁴³ The authors noted that although this system was correlated with clinical outcome, the number of categories may require modification for future studies. In the present study, a correlation between cytologic and histopathologic findings would have helped to support the use of the proposed modified version of the cytologic criteria reported by Krick et al⁹ and should be a focus of future studies.

A cytologic grading system that correlates with a histologic grading system would be of great benefit in staging not only LNs but also the primary tumor prior to surgical removal. The applicability of the Kiupel histologic grading system for fine-needle aspirate samples from MCTs has been evaluated.⁴⁴ Cytologic criteria included the total number of mitoses, multinucleated cells, bizarre nuclei, and presence or absence of karyomegaly. This system was associated with an accuracy of 94%.⁴⁴ Investigators in that study⁴⁴ noted that although grading of poorly differentiated canine MCTs posed no difficulty, the system failed to detect high-grade tumors in 4% of cases. Evaluation of LN aspirate samples via this cytologic system was not performed in the present study. Furthermore, if the cytologic criteria used in the present study were found to predict which dogs went on to develop distant metastatic disease, cytologic characteristics may provide support as to which dogs may benefit from adjunctive treatment after surgery.

Of additional note was the inclusion of 2 dogs in group 2 that were orally receiving a corticosteroid drug at the time of study enrollment. These dogs were allowed to be enrolled because they had been receiving this medication for more than a month prior to enrollment and were deemed to still have active allergic disease despite this treatment. In vitro, glucocorticoids can primarily reduce the production of stem cell factor by fibroblasts and epithelial cells.⁴⁵ Stem cell factor, through binding of mast cell-associated KIT receptors, induces growth, differentiation, and chemotaxis of mast cells.⁴⁵ In dogs, the response rate of MCTs to prednisone is reportedly 20% to 75%.^{30,45,46} The duration of treatment in those studies^{30,45} differed (3 to 28 days), but in most dogs, an objective tumor response was obtained within 7 to 10 days after treatment began.

Findings of the study reported here support the use of a more uniform cytologic grading system for metastasis to the LNs in dogs with MCTs. We propose the use of modified cytologic criteria for the determination of MCT LN metastasis adapted from criteria initially proposed by Krick et al.⁹ Future studies should be directed toward comparing this method with histologic examination. The present study also provided estimates of the percentage of mast cells that may be present in the LNs of clinically normal dogs and dogs with allergic dermatologic disease. Findings highlighted the need for assessment of the regional LNs in dogs with MCTs, regardless of LN size.

Footnotes

- a. Bauer, CL, Rakich PM, Latimer KS, et al. Assessment and comparison of reference intervals for mast cells in peripheral lymph nodes of normal dogs and dogs with allergic skin disease (abstr), in *Proceedings*. North Am Vet Dermatol Forum 2011.
- b. Temaril-P tablets, Zoetis, Florham Park, NJ.
- c. Wescor Brand Wright-Giemsa stain for use with Wescor Aerospray 7150, Fisher Scientific, Pittsburgh, Pa.
- d. SAS, version 9.4, SAS Institute Inc, Cary, NC.

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From this month's AJVR

Effects of the α_2 -adrenoceptor agonist medetomidine on the distribution and clearance of alfaxalone during coadministration by constant rate infusion in dogs

R. C. Bennett et al

OBJECTIVE

To assess the possible impact of medetomidine on concentrations of alfaxalone in plasma, when coadministered as a constant rate infusion (CRI) to dogs, and to determine the possible impact of medetomidine on the cardiopulmonary effects of alfaxalone during CRI.

ANIMALS

8 healthy adult Beagles.

PROCEDURES

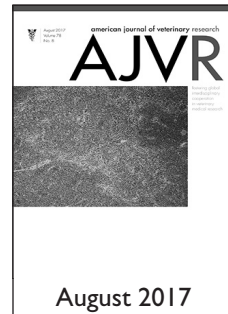
3 treatments were administered in a randomized crossover design as follows: 1 = saline (0.9% NaCl) solution injection, followed in 10 minutes by induction of anesthesia with alfaxalone (loading dose, 2.4 mg/kg; CRI, 3.6 mg/kg/h, for 60 minutes); 2 = medetomidine premedication (loading dose, 4.0 μ g/kg; CRI, 4.0 μ g/kg/h), followed by alfaxalone (as in treatment 1); and, 3 = medetomidine (as in treatment 2) and MK-467 (loading dose, 150 μ g/kg; CRI, 120 μ g/kg/h), followed by alfaxalone (as in treatment 1). The peripherally acting α_2 -adrenoceptor antagonist MK-467 was used to distinguish between the peripheral and central effects of medetomidine. Drugs were administered IV via cephalic catheters, and there was a minimum of 14 days between treatments. Cardiopulmonary parameters were measured for 70 minutes, and jugular venous blood samples were collected until 130 minutes after premedication. Drug concentrations in plasma were analyzed with liquid chromatography–tandem mass spectrometry.

RESULTS

The characteristic cardiovascular effects of medetomidine, such as bradycardia, hypertension, and reduction in cardiac index, were obtunded by MK-467. The concentrations of alfaxalone in plasma were significantly increased in the presence of medetomidine, indicative of impaired drug distribution and clearance. This was counteracted by MK-467.

CONCLUSIONS AND CLINICAL RELEVANCE

The alteration in alfaxalone clearance when coadministered with medetomidine may be attributed to the systemic vasoconstrictive and bradycardic effects of the α_2 -adrenoceptor agonist. This was clinically important because the use of α_2 -adrenoceptor agonists may increase the risk of adverse effects if standard doses of alfaxalone are used. (*Am J Vet Res* 2017;78:956–964)



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