DETERMINATION OF A REFERENCE INTERVAL FOR MAST CELLS IN PERIPHERAL LYMPH NODES OF HEALTHY DOGS AND COMPARISON TO DOGS WITH INFLAMMATORY SKIN DISEASE

by

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(Under the Direction of Pauline Rakich)

ABSTRACT

Reference intervals for mast cell (MC) counts in popliteal lymph node (LN) aspirates of 30 healthy dogs were established by evaluating the total MCs counted in 20 fields, the total MCs/ 500 lymphoid cells, and the total MCs/ entire smear. MC counts from superficial cervical and popliteal LNs were determined in 20 dogs with allergic skin disease and compared to the counts from the healthy dogs.

There were significantly more MCs in smears of LN aspirates of allergic dogs as compared to healthy dogs. Reference intervals based on counts from the total sample evaluated in healthy dogs was 0-13. Significantly more MCs were counted in the popliteal versus the superficial cervical LNs with the “Total” and “Fields” methods. Evaluating the MC counts of the entire smear found significantly more MC than other methods and is the preferred method to evaluate for the presence of MC in aspirates.

INDEX WORDS: mast cells, lymph node aspirates, mast cell tumor staging

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CHAPTER 1

INTRODUCTION

Mast cells (MC) play a central role in inflammatory and immune reactions and are normal components of connective tissues throughout the body. Skin is one of the sites containing the highest numbers of mast cells, and it is the tissue in which mast cell tumors (MCT) develop most frequently, where they comprise 7-20% of skin tumors in dogs.[1] Mast cells also play a critical role in the IgE- and IgG- dependent allergic responses and parasitic diseases associated with histamine release and inflammatory skin disease.[2]

Malignant forms of mast cell tumor grow rapidly and usually spread via the lymphatic system. Regional lymph nodes (LN) are one of the most common sites for metastasis of MCT and are involved in 76%-96% of dogs with mast cell tumors.[1] Therefore, evaluation of patients with MCT includes examination of regional lymph nodes for evidence of metastasis and is the first step in staging the tumor.[2] However, lymph nodes may normally contain mast cells[3] and canine neoplastic mast cells closely resemble normal canine mast cells, making them very difficult to distinguish from each other. Thus, until mast cells are present in large numbers in a lymph node aspirate, it is not possible to accurately detect lymph node metastasis by a MCT. Determining metastasis can be improved by defining reference intervals for mast cells in lymph nodes in animals without neoplasia.

In studies on mice involving immune and antigenic stimulation, mast cell activation and expansion of mast cell numbers in local lymph nodes was noted.[4,5] Lymph node size is often not an indicator of metastasis as numerous instances of metastasis have been documented in

normally sized lymph nodes; also, enlargement of lymph nodes may be caused by reactive hyperplasia rather than from tumor metastasis.[6] Little information is currently available for mast cell counts in lymph nodes of dogs who are either normal or have other disease conditions such as inflammatory skin diseases. When lymph node aspirates were taken from healthy research dogs, a range of 1-16 mast cells was counted.[7] In a study evaluating mast cell counts from lymph node aspirates using a computerized morphometric technique, dogs that had inflammatory or infectious skin diseases had a range of 0.0-0.1% of mast cells counted/2000 lymphoid cells. The purpose of this study is to establish reference ranges in healthy client-owned dogs and to compare them with mast cell counts from dogs with allergic skin disease.[8] The goal of this study is to determine reference values for mast cells in lymph node aspirates from normal healthy dogs to improve accuracy of staging canine mast cell tumors.

Objectives:

1. Establish reference ranges of mast cell counts in peripheral lymph nodes of clinically healthy dogs who presented to the University of Georgia Veterinary Teaching Hospital Community Practice Clinic for annual examinations or who were volunteered for the study.
2. Determine if there are differences in mast cell counts of dogs with allergic skin disease and healthy dogs.
3. Determine whether there is any difference in mast cell numbers in lymph nodes which drain different parts of the body in dogs with generalized allergic skin disease.

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CHAPTER 2

LITERATURE REVIEW

Mast Cell Tumors

Mast cells (MC) are involved in a variety of functions such as protection against gastro-intestinal nematodes, some bacterial infections, and wound healing but they have a long recognized role in mediating hypersensitivity reactions.[1] In addition to regulating hypersensitivity reactions, mast cells can also undergo malignant transformation and become neoplastic, and a dysregulation of the expression of stem cell factor receptor (kit, SCFR) is suspected to contribute to neoplastic formation.[2] Cutaneous mast cell tumors represent 7-20% of all cutaneous tumors in the dog. Breeds that are predisposed to forming MCTs are dogs of bulldog descent, Labrador Retrievers, Golden Retrievers, Schnauzers, Chinese Shar Peis, and Cocker Spaniels.[3] Mast cell tumors occurring in the skin of the extremities comprise 40% of the mast cell tumors seen and 10% arise in the skin of the head and neck.[4] Prognostic factors of MCT include location, clinical appearance of the tumor, growth rate, size, presence of systemic paraneoplastic signs, breed, sex, and clinical stage; however, the most valuable factor is histologic grade.[5] Although multiple grading systems have been developed all use a system to divide MCT into well-differentiated (low-grade, mature), intermediate-grade, and undifferentiated (poorly differentiated, anaplastic, high grade) groups.[5]

Staging canine mast cell tumors is based on the World Health Organization clinical staging protocol which utilizes detection of mast cells in lymph nodes.[4] One of the problems with MCTs is that histological evaluation does not accurately differentiate clinically benign tumors

from malignant tumors.[1] Most neoplastic mast cells in the dog resemble normal mast cells (MC).[2] Furthermore, there are no standardized guidelines for obtaining lymph node aspirates, method of smear examination, or interpretation of the number of mast cells seen. There is only a single publication reporting mast cell counts in lymph nodes and it evaluated only normal dogs and a single lymph node, the popliteal.[6]

Mast Cells and Hypersensitivity

Mast cells reside in connective tissues and naturally occur in parts of the body which interact with the environment.[2] MCs originate from hematopoietic stem cells, their precursors circulate in the blood and they then infiltrate connective tissue to differentiate into mature mast cells. [1] Mast cells are key effector cells in type I hypersensitivities. Their main function is the release of inflammatory mediators and cytokines triggered by a number of stimuli, the most important of which is aggregation of surface-bound immunoglobulin E (IgE) by specific antigens. [1,2] They are regarded as key in the pathogenesis of allergic diseases including atopic dermatitis, urticaria, anaphylaxis and food allergy. Once activated they release stores of inflammatory mediators (heparin, histamine, and eosinophilic chemotactic factor) as well as regulate immune responses by producing cytokines(interleukin 4 (IL-4), IL-1, IL-3, IL-5, IL-6, and IL-8).[1,2]

Animals with hypersensitivities causing inflammatory skin diseases are well documented. Atopy accounted for 21.6% of dogs diagnosed with any “skin or ear disease” in one study; and 8.7% of all 31,484 dogs examined in 52 private practices were diagnosed with atopic/allergic dermatitis, allergy or atopy.[7] Another report indicates that, flea allergy dermatitis and atopy are the most common causes of pruritus in dogs.[8] Breeds of dogs that are predisposed to develop atopy which include dogs of bulldog descent, Labrador Retrievers, Golden Retrievers,

Schnauzers, and Cocker Spaniels, are reported to be predisposed to developing MCTs.[3,9] The mast cell is critical in the hypersensitivity reaction of dogs that are sensitive to various triggers including fleas, food, and inhalant allergens. Antigenic stimulation in rats and hypersensitivity reactions induced in the skin of mice showed an influx of mast cells in regional lymph nodes post stimulation.[10,11] Also, dogs with inflammatory skin diseases were found to have variable numbers of mast cells, ranging from a few too many, in buffy coat preparations .[12] These findings indicate that dogs with inflammatory skin disease have circulating mast cells, which may also increase the number of mast cells in lymph nodes of dogs with inflammatory skin conditions.

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CHAPTER 3

DETERMINATION OF A REFERENCE INTERVAL FOR MAST CELLS IN PERIPHERAL LYMPH NODES OF HEALTHY DOGS AND COMPARISON TO DOGS WITH INFLAMMATORY SKIN DISEASE[1]

[1] C.L.Bauer, P.M.Rakich, K.S. Latimer, and P. Hensel. To be submitted to *Veterinary Pathology*

Abstract

Background: Clinical staging of dogs with mast cell tumors (MCT) involves cytological examination of regional lymph nodes (LNs) for evidence of metastasis. LNs may normally contain mast cells (MC) and there are no reference values for MC counts in the LNs of healthy dogs or dogs with allergic skin diseases.

Objective: The aim of this study was to establish cytologic reference intervals for MC counts in LNs of healthy dogs and to compare the counts to dogs with allergic skin disease.

Methods: Reference intervals were determined using aspirates of the prescapular and popliteal LNs from 30 healthy dogs. These values were compared to MC counts from 20 allergic dogs. Three methods were used to assess the MC counts: the number of MCs counted in 20 fields at 20X magnification; the number of MCs/500 lymphoid cells; and the total number of MCs/entire smear. A CBC, urinalysis (UA), fecal examination, canine heartworm antigen test, and complete dermatological examination were performed to eliminate other disease processes.

Results: A reference intervalfor MCs in fine-needle aspirates of LNs of clinically healthy dogs was determined to be 0-13. Significantly more mast cells were present in fine needle aspirates of lymph node aspirates of allergic dogs (should include mean +/-SD here) as compared to healthy dogs.

Conclusions: MC numbers in LN aspirates from allergic dogs are significantly higher than in normal dogs. This finding indicate that interpretation of LN aspirates in clinical staging of MCTs may be complicated in dogs with MCTs and concurrent allergic skin disease.

Introduction:

Cutaneous mast cell tumors (MCTs) represent 7-20% of all cutaneous neoplasms in the dog.[1] Malignant MCTs grow rapidly and usually spread via the lymphatic system. Regional lymph nodes (LNs) are one of the most common sites for metastasis of MCT and are involved in 76%-96% of cases.[1] Differentiation of metastatic nodes from LNs that are merely reactive is difficult because LN size is not an indicator of metastasis as numerous instances of metastasis have been documented in normally sized nodes.[2] Therefore, evaluation of patients with MCT involves examining regional LNs cytologically for evidence of metastasis.[3] Previous studies have been published evaluating MC presence in LN aspirates of dogs without metastasis and their presence in LNs is considered normal.[4,5] So the significance of a small number of MCs in LN aspirates is difficult to interpret.[6] Thus, until MCs are present in large numbers in a LN aspirate, it is not possible to diagnose MCT metastasis with certainty.[7] Therefore, knowledge of MC numbers in LNs of dogs without neoplasia is important.

MC numbers may be increased in other conditions besides MCTs. The MC is an important mediator in the pathogenesis of cutaneous hypersensitivity reaction of dogs that are sensitive to various antigens including fleas, food, and inhalant allergens.[8] Their role in allergic reactions are to release inflammatory mediators such as histamine and cytokines like IL-1, IL-4, and IL-8 causing typical clinical signs of erythema and vascular leakage and inflammatory cellular chemotaxis.[9] Antigenic stimulation in rats and hypersensitivity reactions induced in the skin of mice showed an influx of MCs into regional LNs post stimulation.[10,11] In a study examining MC numbers in buffy coat preparations, dogs with inflammatory skin diseases had variable numbers of MCs ranging from a few to many.[12] This finding indicates that dogs with inflammatory skin disease have circulating MCs, which may increase their numbers in LNs.

Currently, no reference intervals for MCs in LN aspirates in client owned healthy dogs have been established. Furthermore, the effect of allergic skin disease on MCs in LNs has not been evaluated to our knowledge.

The objectives of this study were to establish reference ranges for MCs in smears from peripheral LN aspirates in normal dogs and to compare them to MC counts in dogs with allergic skin diseases. We hypothesized that MC numbers from aspirates of LNs in dogs with allergic dermatoses will be significantly higher than clinically healthy dogs. This information will be useful in interpreting LN aspirates for staging dogs with MCTs.

Materials and Methods

Thirty clinically-healthy dogs and 20 dogs with a history and clinical signs consistent with allergic dermatitis which were presented to the Veterinary Teaching Hospital of the University of Georgia were enrolled into this study. Dogs accepted for inclusion in the study were required to be at least one year of age or older to allow maturation of their immune systems and exposure to all seasons. Animals in the healthy study group were also required to have an absence of seasonal or non-seasonal pruritus, chronic skin infections, ectoparasite infestation, or any other causes of inflammatory dermatitis. Animals included in the allergic dermatitis group had a history of seasonal or non-seasonal pruritus, flea allergy dermatitis, and at presentation clinical signs of dermatitis with or without secondary infections. Animals were excluded from either group if they had any history of steroid use four weeks preceding taking the aspirates or antihistamine use two weeks preceding taking the samples or if there was evidence of neoplastic disease.

All animals considered for inclusion in the study received a complete physical examination, including a detailed dermatologic examination. Each dog was tested for heartworm infection with the canine antigen test (Canine Heartworm Antigen Test, IDEXX Laboratories, Inc., Westbrook, Maine). All animals were tested for intestinal parasites using routine fecal floatation techniques. A complete blood count (CBC) and urinalysis (UA) were performed on all animals to look for any evidence of other systemic infections and diseases. Urine was collected by cystocentesis. If animals were positive for heartworm disease, intestinal parasites, or if evidence of infection was found that could cause mastocythemia the dogs would be excluded from the study.

Each dog included in the study was evaluated for evidence of pyoderma or yeast dermatitis using acetate tape imprints obtained from the face, dorsum, ventrum, and interdigital space. The tapes were stained with a modified Wrights stain (Protocol HEMA 3®, Fisher Diagnostics, Middletown, VA) and evaluated cytologically at 4x for neutrophils; if the cells were found, the specimens were examined at 100x. At least ten fields of view were evaluated at 100x oil immersion, and the number of microorganisms (*e.g.* cocci, rods, yeast) counted and averaged. The presence of more than 5 organisms per high power oil immersion field was considered evidence of skin infection. Ear cytologies were also taken using a cotton swab. The sample was rolled onto a glass slide, heat fixed, stained, and evaluated as described for skin cytologies. All animals were flea combed and a Wood's lamp was used to look for evidence of dermatophytosis.

A deep skin scraping was taken from the ventrum and hind leg of each dog using a number 10 blade. The material obtained with the blade was transferred on to a microscope slide, mixed with a drop of mineral oil, and coverslipped. The slide was evaluated at 10x for the

presence of mites. If *Demodex* or *Sarcoptes spp.* mites were observed, the dog was excluded from the study.

The popliteal LN was aspirated from all dogs; the prescapular LNs were also aspirated from the allergic dogs. The prescapular LNs were not aspirated in normal dogs due to the inability to identify and isolate the non-reactive nodes. A 22 gauge needle was inserted into the LN and then redirected at a 45 degree angle in two directions to obtain a representative LN sample. The material was expelled onto slides, smears was prepared, allowed to air dry, and then stained with modified Wright's stain (Diff Quik®). In order to insure adequate fixation of the smears, the slides were fixed for 5 minutes in the first solution prior to being placed in the second and third solutions for 15 dips.

MC counts from the LN samples were determined in three ways: the total number of MCs was counted in twenty fields evaluated at 20x ("Fields"), total number of MCs were determined per 500 lymphoid cells counted at 40x ("Cells"), and the total number of MCs was determined for the entire slide evaluated at 10x ("Total").

Statistical analysis of the MC count data was performed using SAS statistical software (Version 9.2, Cary, NC, USA). Reference ranges were calculated for aspirates from LNs of healthy dogs by calculating 5 and 95 percentiles. The presence or absence of MCs was compared between allergic and healthy dogs with a chi-square test. MC counts were also compared between the popliteal and superficial cervical LN counts in allergic dogs with a Wilcoxon signed-rank test.

To compare the three counting methods, a simple Pearson test was used to detect correlations between the three measurements. A repeated measures model which included a fixed factor of measurement and a random factor of dog was used to test for differences between the

three measurement methods. Multiple comparisons were adjusted for using Tukey's test. An unstructured covariance structure was used in the repeated measures model.

Results:

Thirty healthy dogs were included in the study. The ages ranged from one to eleven years with an average age of 4.26 years. Body weights ranged from 2.2 to 40.0 kg with an average weight of 20.59 kg. The healthy breeds included in the study are presented in Table 1. There was one intact male, thirteen neutered males, and sixteen spayed females.

Twenty allergic dogs were included in the study and the ages ranged one to eleven years with an average age of 4.02 years. Body weights ranged from 5.08 to 39.4 kg with an average weight of 20.07 kg. The allergic breeds included in the study are presented in Table 1. There were two intact males, ten neutered males, and eight spayed females.

Dogs in the healthy group did not have any significant findings on CBC, UA, skin cytology, ear cytology, fecal examination, heartworm examination, or physical examination. The flea comb, skin scrape, and Wood's lamp evaluations were all negative on healthy dogs. There was no evidence of infection or inflammation in healthy dogs. Abnormalities seen on CBC and UA from the allergic dogs included mild mature neutrophilia (3), monocytosis (1), eosinophilia (2), and anemia of chronic disease (1).

Of the twenty allergic dogs, six had infectious otitis externa with three having primarily yeast otitis and three with primarily bacterial otitis. Fourteen dogs had infectious dermatitis. Of the fourteen dogs, four had yeast dermatitis and twelve had pyoderma. Gross cutaneous lesions seen in the allergic dogs were multiple and included erythema (15), alopecia (14), crusts (11), papules (10), lymphadenomegaly (6), pododermatitis (5), epidermal hyperplasia (4), aural

discharge (4), seborrhea sicca (4), pustules (3), salivary staining of the haircoat (3), pruritus (3), lichenification (2), hyperpigmentation (2), stenotic aural canal (2), epidermal collarettes (2), macules (1), excoriation (1), and depigmentation (1). Fleas or evidence of flea dermatitis was found on two dogs.

MCs were seen in samples from 5 of the 30 healthy dogs and 15 of the 20 allergic dogs. (Table 2 and 3) Reference intervals for healthy dogs were as follows: 0-2 for the “Fields” method, 0-1 for the “Cells” method, and 0-13 for the “Total” method. MC counts for allergic dogs were as follows: 0-3 for the “Fields” method, 0-1 for the “Cells” method, and 0-84.5 for the “Total” method. (Table 4)

When comparing the number of MCs between groups of dogs, there were significantly more MCs in LNs from allergic dogs than healthy dogs when counted based on “Fields” method (healthy) 7%; allergic: 30%, $p=0.0275$) and the ”Total” method (healthy) 17%, allergic 65%, $p=0.0005$).

Comparing the different methods of MC counting with the repeated measures analysis indicated that the ”Total” counts were significantly higher than “Fields” ($p=0.0051$) or ”Cell”($p=0.0040$) counts. The simple Pearson test indicated significant fair correlation between “Fields” and “Cells” methods ($r=0.46$, $p<0.0001$), and between “Total” method and “Fields” ($r=55$, $p<0.0001$) or “Cells” ($r=0.33$, $p=0.0060$) methods of counting MCs in the LN aspirates.

When evaluating the differences between LNs sampled in the allergic dogs, the mean counts from the “Field” method ($p=0.0313$) and “Total” method ($p=0.0286$) were significantly higher in the popliteal LNs versus the superficial cervical LNs , but there were no significant differences in the MC counts using the “Cell” method.

Discussion:

MCTs are one of the most common cutaneous tumors in dogs and their diagnosis can be relatively easily confirmed by either cytology or histopathology. However, distinguishing clinically benign tumors from malignant tumors cannot reliably be established by either method.[1] Currently, staging canine MCTs is based on the World Health Organization (WHO) clinical staging protocol which utilizes only the presence or absence of MCs in LNs to identify metastasis.[13] It has been established that MCs can be found in dogs without MCTs. [4,5,6] and our study agrees that MCs can be found in LN aspirates from healthy dogs. The reference interval we established (0-13) using the "total" method is similar to that found in another study in which a range of (1-16) was determined. Based on our findings, counts of <14 mast cells per total sample should not immediately be considered metastatic.

Client owned dogs were used in this study, rather than research dogs, to mimic the typical population that would present as either clinically-healthy dogs or dogs will allergic skin disease. No dogs in either group had any apparent age, breed, or sex predilections. Breeds that are predisposed to developing MCTs include dogs of bulldog descent, Labrador Retrievers, Golden Retrievers, Schnauzers, and Cocker Spaniels, breeds that are also predisposed to allergic skin disease.[14,15] Some of these breeds were included in this study. The overlapping predisposition of these diseases in such breeds should be considered if the diagnosis of MCT is made. The finding presented in this paper can be especially useful in these dogs when both diseases are present.

The significance of the healthy dog that had 66 MCs counted is unclear. No abnormalities were recognized in any examinations or tests performed on this dog which was presented as a healthy volunteer for the study with no known health issues. The tests run in our study were not

exhaustive and certainly other subclinical conditions could have been present to account for the high value.

MCs are stimulated in allergic reactions and our study showed that dogs with allergic disease had significantly more MCs in their LNs than normal dogs. MC counts using the "Total" method ranged from 0-84.5 MCs. Two dogs had counts greater than 80. The presence of increased numbers of MCs in these dogs could potentially confuse interpretation of results from LNs when staging dogs with MCTs. In a dog with a MCT and concurrent allergic dermatitis findings of significant numbers of MCs in the regional LN should be carefully considered. This is particularly important in breeds of dogs which are predisposed to both allergies and MCTs. It is interesting to note that no MC clumping was seen in any of the samples. This feature has been recognized in dogs which have metastasis to regional LNs and perhaps should be further evaluated as a diagnostic feature of metastasis.[13]A limiting factor of our study was the low number of healthy and allergic dogs utilized, but reference ranges could be established and showed that both healthy dogs and especially allergic dogs may have large numbers of MCs in LNs.

Methods to aspirate LNs and the method of counting MCs are not standardized. The lack of standardized cytological criteria to determine metastasis makes it difficult to establish the stage of disease and, therefore, prognosis.[7] There was variability in the samples obtained, despite using the same technique with each animal, so the samples were slightly different in shape, consistency, and cellularity. It is widely believed that larger cells are distributed in greater numbers at the feathered edge of the sample, but cellular distribution will change depending on the viscosity of the sample. The material obtained from aspiration of LNs from the healthy dogs in this study was thick and viscous. In contrast, aspiration of LN from the dogs with allergic skin

disease which had hyperplasic LNs yielded a thin, non-viscous sample. There is also no standardized method to evaluate LN aspirates for presence of MCs. If the mere presence of MCs in a LN aspirate suggests metastasis the entire smear should be evaluated. In the few studies that specified the method used to determine MC numbers in LN's, the total number of MC's were counted in the smear at10 or 20x.[5] In another study, a computerized morphometric technique was used on slides at 40x until 2000 cells were counted and the number of MCs were expressed as a percentage of the total population.[6] In other studies evaluating MCT metastasis in regional LNs no specifics were given on how the samples were cytologically evaluated to count MCs.[7,16] Three methods of counting MC in LN aspirates were compared in this study to determine the most accurate method. Our goal was to find a standardized method to enumerate MCs in fine-needle aspirates in a clinical setting.

Three different methods ("Fields", "Cells", and "Total") were considered and outcomes were compared to determine how well the methods correlated to each other. The three methods used were based on trying to find one that was clinically relevant, reliable, quick, and/or reproducible regardless of sample size obtained. It was felt that the "Fields" method would be the least time consuming, a benefit in the clinical setting. The "Cells" method was attempted to minimize the variability of cellularity of the samples taken. This method could allow the data to be presented as a percentage of the total number of cells counted and would more accurately represent the findings regardless of the cellularity. Finally the "Total" method was used as it represented the method typically used to evaluate aspirates from dogs with MCTs when looking for metastasis. While slightly more time consuming it would give the best idea of the "presence" of mast cells as is suggested by the WHO staging protocols.

None of the methods used met all the criteria. Our study indicates that the "Total" method was more sensitive in finding significantly more mast cells per sample than the other two methods. Evaluating the total sample at a lower magnifications (10x objective), while taking longer than the other methods, was still as timely as the other methods and could be used clinically. We also found that the "Fields" and "Cells" methods showed some correlation (r = 0.46), and the "Total" method had fair correlation(r = 0.55) to the other methods. This demonstrates that some correlation exists between the "Total" method and others used but the ability of the other tests to detect the true number of MCs present was not significant.

The "Total" method was similar to the one used in a study that evaluated MC counts in healthy research dogs, and the findings in that study are similar to the present study performed on healthy client owned animals (1-16 and 0-13 respectively).[5] The recent study which utilized a morphometrical approach for predicting regional LN micrometastatic load found an average percentage of 0.0% MCs.[6] Only four healthy dogs were evaluated in that study and the number was determined by calculating the percentage of total number of MCs counted per 2000 lymphoid cells. This is similar to the "Cells" method used in the present study in which the total number of MCs was counted per 500 lymphoid cells. The range of MCs counted in our healthy dogs was 0-1 MCs and is similar to the findings in the previous study. This type of method would eliminate the variable cellularity from sample to sample and results in a more consistent approach when evaluating samples. However, this method did not provide an accurate indication of the number of MCs present in a LN when compared to the "Total" method. Perhaps counting MCs per more lymphoid cells in the "Cells" method would have yielded a better correlated result between the "Cell" method and "Total" method counting the number, but it would have been time prohibitive without more advanced equipment which is not widely available. Therefore, it is

felt using the “Total” counting method yields the most significant results and may be the best method for clinical evaluation.

MCTs occurring in the skin of the extremities comprise 40% of the MCTs in dogs and 10% arise in the skin of the head and neck.[2] An original goal of this study was to aspirate two lymph nodes which drain different body regions in order to determine whether MC numbers would be significantly different in normal dogs and dogs with inflammatory skin disease. Unfortunately, adequate samples could not be obtained from the superficial cervical LNs of normal dogs because their small size precluded isolation and aspiration of the node. In contrast, the LNs of allergic dogs tended to be larger and easier to isolate; and adequate samples were easy to obtain from their LN. Results indicated that measurements were significantly higher in popliteal LNs than superficial cervical LNs using the “Fields” and “Total” methods. Based on our findings the “Fields” method did not count a significant number of the total mast cells present and should not be used to evaluate samples. The reason for this difference is unknown. One explanation would be an increased cellularity in the samples obtained from the popliteal LNs. The best method to differentiate between the cellularity of the two samples would be the “Cells” method, but this method did not significantly represent MC presence. Also, in dogs with inflammatory skin diseases where MCs were found in the popliteal LN, they could often be found in either LN sampled. This may be important when trying to determine if the MCs seen in samples from allergic dogs with MCTs are evidence of metastasis or a reflection of the skin disease. By evaluating distant LNs not associated with the tumor, the presence of increased MCs could help indicate a reactive pattern associated with inflammatory skin disease, and may mean that the MCs seen in the sample of the regional LN of the tumor could also represent the dog’s inflammatory disease and not metastasis.

All of the allergic dogs with mild mature neutrophilia had pyoderma, accounting for the neutrophilia. The dog with mild anemia (34.5%, 36.6-59.6) has almost a life-long history of chronic skin infections and dermatitis associated with allergic disease. The chronic infectious and allergic dermatitis was interpreted to be the cause of the anemia; however, the dog was not evaluated for evidence of covert blood loss or other causes of anemia. The two allergic dogs with eosinophilia did not have evidence of external or internal parasites and no other tests were performed to determine other causative agents of the eosinophilia, other than the presence of the dog's allergic disease.

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CHAPTER 4

CONCLUSIONS

Mast cell tumors are one of the most common cutaneous tumors in dogs and their diagnosis can be relatively easily confirmed by either cytology or histopathology. However, distinguishing clinically benign tumors from malignant tumors cannot reliably be established by either method.[1] Currently, staging canine mast cell tumors is based on the World Health Organization clinical staging protocol which utilizes only the presence or absence of mast cells in lymph nodes to identify metastasis.[2] It has been established that mast cells can be found in dogs without mast cell tumors. [3,4,5] and our study agrees that mast cells can be found in lymph node aspirates from healthy dogs. The reference interval we established (0-13) using the "total" method is similar to that found in another study in which a range of (1-16) was determined. Based on our findings, counts of <14 mast cells per total sample should be interpreted with caution as normal dogs may have this number of mast cells in smears of their lymph nodes.

Mast cells are stimulated in allergic reactions and our study showed that dogs with allergic disease had significantly more mast cells in their lymph nodes than normal dogs. Mast cell counts using the "Total" method ranged from 0-84.5 MCs. The presence of increased numbers of mast cells in these dogs could potentially confuse interpretation of results from lymph nodes when staging dogs with mast cell tumors. In a dog with a mast cell tumor and concurrent allergic dermatitis findings of significantly increased numbers of mast cells in the regional lymph node should be carefully considered.

It is interesting to note that no mast cell clumping was seen in any of the samples. This feature has been recognized in dogs which have metastasis to regional lymph nodes and perhaps should be further evaluated as an important diagnostic feature of metastasis.[6] A limiting factor of our study was the low number of healthy and allergic dogs evaluated, but reference ranges could be established and confirmed that healthy dogs have mast cells in their lymph nodes and that allergic dogs have large numbers of mast cells in peripheral lymph nodes.

Three methods of counting MC in LN aspirate smears were compared. The goal of this study was to establish a standardized method to enumerate mast cells in fine-needle aspirate smears that was accurate, reproducible, and yet easily done in a clinical setting. Three different methods ("Fields", "Cells", and "Total") were done and the results compared to determine how well the methods correlated to each other. These three methods were chosen in an attempt to find one that was clinically relevant, reliable, quick, and/or reproducible regardless of sample size obtained. We found that none of the methods used met all the criteria. Our study indicates that the "Total" method was more sensitive in finding significantly more mast cells per sample than the other two methods. Evaluating the total sample at lower magnifications (10x objective) was no more time consuming as the other methods. The "Fields" and "Cells" methods showed some correlation, and the "Total" method had fair correlation to the other methods. This demonstrates that some correlation exists between the "Total" method and others used but the ability of the other tests to detect the true number of mast cells present was not significant.

The "Total" method was similar to the one used in a study that evaluated mast cell counts in healthy research dogs, and the findings in that study are similar to ours performed on normal client owned animals (1-16 and 0-13 respectively).[4] The recent study which utilized a morphometrical approach for predicting regional lymph node micrometastatic load found an

average percentage of 0.0 MCs.[5] Only four healthy dogs were utilized in that study and the number was determined by calculating the percentage of total number of MCs counted per 2000 lymphoid cells. This is similar to the "Cells" method used in our study in which the total number of mast cells was counted per 500 lymphoid cells. The range of mast cells counted in our healthy dogs was 0-1 MCs and is similar to the findings in the previous study. This type of method would eliminate the variability in cellularity between samples and would result in a more consistent approach. However, this method did not provide an accurate indication of the number of mast cells present in a LN when compared to the "Total" method. Perhaps counting mast cells with respect to a larger number of lymphoid cells in the "Cells" method would have provided better correlation between the "Cells" and "Total" methods, but it would have been time prohibitive without equipment which is not widely available. Therefore, the "Total" counting method yields the most significant results and is the best method for clinical evaluation of mast cells in smears of lymph node aspirates.

An original goal of this study was to aspirate two lymph nodes which drain different body regions in order to determine whether MC numbers are significantly different in different lymph nodes in normal dogs and dogs with allergic skin disease. Unfortunately, adequate samples could not be obtained from the superficial cervical lymph nodes of normal dogs because their small size precluded isolation and aspiration of the node to obtain the sample, but adequate samples could be obtained in allergic dogs. Results in allergic dogs indicated that MC numbers were significantly higher in popliteal lymph nodes than superficial cervical lymph nodes using the "Fields" and "Total" methods. However, the "Fields" method was not found to give significant results and is not recommended. The significant difference the "Total" method yielded between the two lymph nodes could be due to where the allergic skin disease was in

relation to the body area drained by the lymph node. Evaluating this was not within the scope of this paper and could be evaluated in future studies. When evaluating the two lymph node samples using the “Cells” method there was no significant difference between mast cell counts. This would be the best method to differentiate between cellularity of two samples but the “Cells” method could not best represent mast cell presence. When MCs were present in an allergic dog they could typically be found in both the popliteal LN and the superficial cervical LN. This may be important when trying to determine if the mast cells seen in samples from allergic dogs with mast cell tumors are signs of metastasis or not. By evaluating distant lymph nodes not associated with the tumor, the presence of mast cells could help indicate a reactive pattern associated with inflammatory skin disease, rather than indicating metastasis.

All of the allergic dogs with a mild mature neutrophilia had pyoderma which accounts for the neutrophilia. The dog with mild anemia (34.5%, 36.6-59.6) has an almost life-long history of chronic skin infections and dermatitis associated with allergic disease. The chronic infectious and allergic dermatitis was interpreted to be the cause of the anemia; however, the dog was not evaluated for evidence of covert blood loss or other causes of anemia.

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Table 1. Dog Breeds

Healthy Dog Breeds	**Number**	**Allergic Dog Breeds**	**Number**
Labrador Retriever	3	Golden Retriever	2
Chow Chow	1	Springer Spaniel	1
German Shepherd Dog	1	Jack Russell Terrier	1
Miniature Schnauzer	1	Boxer	1
Peekapoo	1	Fox Terrier	2
Parson Russell Terrier	1	Standard Schnauzer	1
Rottweiler	1	Australian Shepherd	1
Dachshund	1	American Staffordshire Terrier	1
Boston Terrier	2	American Bulldog	1
American Staffordshire Terrie	1	Miniature Dachshund	1
Welsh Corgi	1	Labrador Retriever	1
Cocker Spaniel	1	English Bulldog	1
Italian Greyhound	1	Doberman Pinscher	1
Greyhound	1	Mixed Breed	2
Boykin Spaniel	1		
Boxer	1		
Australian Cattle Dog	1		
Mixed Breed	10		

Table 2. Mast cell counts from normal dogs.

Dog	Node	Cells/ 20 fields	Cells/ 500 lymphoid cells	Cells/ total sample
8	Popliteal	0	0	2
15	Popliteal	0	0	1
19	Popliteal	4	1	7
22	Popliteal	2	1	13
29	Popliteal	0	0	66

Table 3. Mast cell counts from allergic dogs.

Dog	Node	Cells/ 20 fields	Cells/ 500 lymphoid cells	Cells/ total sample
1	Pre-Scapular	0	0	0
	Popliteal	1	0	3
2	Pre-Scapular	0	0	0
	Popliteal	0	0	1
4	Pre-Scapular	0	0	0
	Popliteal	1	0	2
5	Pre-Scapular	0	0	8
	Popliteal	0	0	0
6	Pre-Scapular	0	0	1
	Popliteal	1	0	2
7	Pre-Scapular	0	0	0
	Popliteal	0	0	1
8	Pre-Scapular	0	0	2
	Popliteal	0	1	7
10	Pre-Scapular	0	0	0
	Popliteal	4	0	82
11	Pre-Scapular	0	0	0
	Popliteal	0	1	1
12	Pre-Scapular	0	0	1
	Popliteal	1	1	27
13	Pre-Scapular	0	0	15
	Popliteal	0	0	55
15	Pre-Scapular	0	0	3
	Popliteal	0	0	1
17	Pre-Scapular	0	0	1
	Popliteal	0	0	0
18	Pre-Scapular	0	0	1
	Popliteal	2	1	87
19	Pre-Scapular	0	0	0
	Popliteal	0	0	1

Table 4: Reference intervals, Mean, and Standard Deviation for Dogs

	Normal Dogs			Allergic Dogs		
Method	Interval	Mean	Standard Deviation	Interval	Mean	Standard Deviation
“Field”	0-2	0.20	0.81	0-3	0.50	1.00
“Cells”	0-1	0.07	0.25	0-1	0.20	0.41
“Total”	0-13	2.97	12.20	0-84.5	13.50	27.60