“Lexie’s New, Old Nose”

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Introduction:

Mast cell tumors (MCTs) are a common skin tumor in dogs with approximately 20% of all skin tumors being mast cell tumors.[1,6,12,13] Though mast cell tumors can occur in any breed, several breeds, such as Boxers, Boston Terriers, Labrador Retrievers, Beagles, and Schnauzers, are known to be predisposed.[2,6,13] Of the predisposed breeds, brachycephalic breeds, particularly Boxers, are at a higher risk of developing these tumors, although they tend to be a low grade.[2,13]

MCTs can generally be further classified into two categories: cutaneous and subcutaneous, although a visceral form has been documented.[1,17] The diagnosis of tumor type can be made via fine needle aspiration and cytology, but histopathology of a tissue biopsy is needed for grading. Other than location, cutaneous and subcutaneous tumors differ greatly in how they are graded, which will later be discussed. In addition to grading the neoplasia, staging should be performed. Possible metastasis can be checked via lymph node aspiration and cytology, abdominal ultrasound with aspiration and cytology of the liver and spleen, and abdominal and thoracic radiographs.[2,17]

History and Presentation:

Lexie, an approximately 4-year-old, spayed female Doberman Pinscher, was presented to her primary veterinarian on August 1st, 2019 for a growth noticed on her left nostril by her owner. Before presenting at their veterinarian, her owner would remove the growth and it would grow back shortly after. A biopsy of the mass was performed by her primary veterinarian on August 7th, 2019 and sent to Idexx for evaluation. The biopsy came back as a grade II/low grade cutaneous mast cell tumor, so Lexie was then referred to the Memphis Veterinary Specialists. Lexie's owners were given the options of excision with reconstructive surgery or radiation therapy. At that point, Lexie's owners elected an additional referral.

Lexie was presented to the MSU-CVM Oncology service on September 4th, 2019. On physical examination, Lexie was bright, alert, and responsive. She had a heart rate of 108 beats per minute, respiratory rate of 84 breaths per minute, and a temperature of 101.7°F. She weighed 35.9 kgs and had a body condition score of 7 out of 9, with 4 or 5 being ideal. Her mucous membranes were pink and moist with a capillary refill time of less than two seconds. Mild dental disease was appreciated on oral exam. No ocular, nasal or auricle discharge was appreciated. A rostral mass was located in her left nostril. Her peripheral lymph nodes palpated normally. On thoracic auscultation normal bronchovesicular sounds were appreciated bilaterally with no crackles or wheezes heard. Lexie's abdomen was tense but non-painful on palpation. Two ecchymotic spots were noted in her left inguinal region. The remainder of her physical exam was unremarkable. Following her visit with oncology, Lexie was placed on diphenhydramine and omeprazole to protect against the side effects of mast cell tumors. At this time, Lexie underwent staging followed by a consultation with small animal surgery.

On September 23, 2019, Lexie was presented to the MSU-CVM Small Animal Surgery service for consultation on the removal of her nasal mast cell tumor. At this presentation, Lexie's owner informed us that her appetite had decreased since starting diphenhydramine, and although she was not overly lethargic, she had slowed down since the last visit. Since returning home, Lexie had a few episodes of vomiting and diarrhea. The diphenhydramine was briefly stopped, but then recontinued with no further complications other than a decreased appetite. On physical exam, Lexie was bright, alert, and responsive. She weighed 33 kg and had a body condition score of 6 out of 9, with 4 or 5 being ideal. She had a heart rate of 120 beats per minute, a temperature of 103.4°F, and she was panting. Her eyes, ears, and nose were free of discharge. On cardiothoracic auscultation, there were normal bronchovesicular sounds bilaterally with no

crackles, wheezes, murmurs, or arrhythmias heard. Her abdomen was tense, but non-painful on palpation. There was no peripheral lymphadenopathy noted. The mass in the left naris had not significantly changed in size since her visit with the oncology service.

Diagnostic Approach/Consideration

On presentation, a minimum database consisting of a complete blood count and serum chemistry panel should be performed on the patient. Additionally, staging, with the methods that have been previously mentioned, should be completed.[2,17]

As the nasal mast cell tumor had been previously diagnosed, Lexie underwent staging at MSU-CVM. A complete blood count, neuro chemistry panel, urinalysis, and abdominal ultrasound and radiographs were performed. On CBC, there was a mild lymphopenia (641 /ul with normal being 1200-6500 /ul). On serum chemistry, there was a mild decrease in CO_2 (14.1 mEq/L with normal being 20.0-28.0) and a mild increase in total bilirubin (0.8 mg/dl with normal being 0.2-0.6). Her urinalysis was relatively unremarkable. On the abdominal radiographs, the spleen was enlarged with rounded margins, the colon was distended with a fluid opaque material and gas, there were multifocal new bone formation along the lumbar spine (spondylosis deformans), as well as transitional thoracolumbar vertebra. On the abdominal ultrasound, hyperechoic debris was noted in the urinary bladder along with echogenic fluid within the colon. Fine-needle aspirated were taken of the liver and spleen during the ultrasound. On cytology, the spleen had mild amounts of extramedullary hematopoiesis, which could explain the enlarged spleen seen on radiographs, with no overt evidence of a mast cell tumor. The cytology of the liver aspirates also came back with no obvious evidence of a metastatic mast cell tumor. In addition to the cytology of these two locations, a cytology of the left and right mandibular lymph

node was performed. These aspirates came back with the right mandibular lymph node being compatible with lymphoid hyperplasia, but no evidence of neoplasia was seen. The left mandibular lymph node had significant eosinophilic lymphadenitis, but there was not a high enough number of mast cells to indicate that it was a metastatic process; however, it could not be ruled out that an early metastatic process was occurring.

Following the return of the cytology results, computed tomography of the head was performed for surgical planning. On CT, there was an irregularly shaped mass within the subcutaneous soft tissues of the rostral most aspect of the left naris, which caused doming of the lateral cutaneous margin. It was at this time that the Oncology service consulted with MSU-CVM Small Animal Surgery service. Following an additional consultation by the MSU-CVM Small Animal Surgery service with a surgical oncologist at Colorado State University College of Veterinary Medicine, it was determined that a partial nasal planum resection and reconstruction would be possible for Lexie's mass. Due to Lexie's breed and previous ecchymotic spots noted, a buccal mucosal bleeding time was checked when Lexie returned to MSU-CVM for her surgery. The results were within normal limits with a time of 2 minutes and 45 seconds. Additionally, a small animal anesthesia profile was performed and had no abnormalities. Surgery was scheduled for the following day.

Pathophysiology:

Cutaneous MCTs arise from mast cells within the tissue of the dermis and subcutis and can vary in clinical appearance.[17] Subcutaneous MCTs are only located in the subcutis and are typically surrounded by fat.[8] Because of the large variety in clinical presentation for these tumors, they can be mistaken for a non-neoplastic lesion. For cutaneous MCTs, the exact events involved in the development of these tumors unknown, but a genetic component is suspected.[1,13]

Receptor tyrosine kinase (RTK) KIT is normally expressed on multiple cell types, including mast cells. This protein is encoded by the c-KIT oncogene, which has been noted to play a role in some human neoplasms. When there is a mutation present in this oncogene, it allows for the RTK KIT to be activated. This activation leads to uncontrolled growth and progression of a tumor.[10,14] This mutation has been found in some canine MCTs, which has led to researching various chemotherapeutic drugs targeting RTKs for the treatment of canine MCTs.[10] The only limitation is that this mutation is only known for cutaneous tumors. The research for subcutaneous MCTs has really only occurred within the last 15 years.[6,8-10,12] In 2 different studies of subcutaneous tumors, there was no association yet made regarding c-KIT mutations and the development of the tumor;[6,8] however, in a more recent study, a single case of subcutaneous MCTs did express a KIT mutation.[12] Though a c-KIT mutation still has to be further researched, the expression of KIT within the tumor has been demonstrated.[6,8] In one study, there was a statistically significant risk factor for KIT that was localized diffusely within the cytoplasm to be associated with a more aggressive subcutaneous tumor.[8] In another study, an intermediate grade of a subcutaneous type was associated with a lesser stippled cytoplasmic localization.[6]

For cutaneous MCTs, there are two methods of grading: the Patnaik system and the Kiupel system. The Patnaik system gives a grade of I, II, or III, with a grade III being linked to a poorer prognosis . The Kiupel system classifies MCTs as either low-grade or high-grade. Due to a varying of opinion among pathologists, these are frequently reported together with a grade I being low-grade and a grade III being high-grade.[7] Historically, subcutaneous MCTs were given a grade II on the Patnaik scale due to their location in the subcutaneous tissues, which gives them a variable prognosis; however, the Patnaik scale did not include this type of MCT in the original research.[6,9] Recent studies have shown that the subcutaneous type does have similar features with

a grade II cutaneous type, but the expression of proliferation markers suggest that those that fall within the subcutis alone have a lower level of proliferation and ultimately are not as aggressive leading to a better prognosis than the higher cutaneous grades.[6,9]

In addition to grading, other methods, such as proliferation activity and molecular markers, should be used as additional prognostic indicators.[7] Research has shown that the mitotic activity has the most impact on clinical outcome of the subcutaneous type. In dogs that have a subcutaneous MCT with a mitotic index greater than 4, there were shorter survival times, decreased time to local reoccurrence, and a decreased time to metastasis.[9] In addition to mitotic index, Ki67, argyrophilic nucleolar organizing regions (AgNOR), and KIT expression via immunohistochemistry are useful at determining the prognosis of the subcutaneous type.[8] AgNOR and Ki67 are examples of cellular proliferation assays that have been used as prognostic indicators in cutaneous MCTs. AgNOR plays a role in RNA transcription and the amount of AgNOR per cell is correlated to the rate of proliferation. Ki67 is a protein that is not expressed in noncycling cells, but it is expressed in all stages of the cell cycle. The expression of this protein has been used to assess the growth fraction of a tumor.[8,15] Dogs that developed recurrence or metastasis following surgery were seen to not only have a higher mitotic index, but also a higher count of Ki67, AgNOR, and cytoplasmic KIT localization. The results from this research indicate that these proliferation markers, especially mitotic index and Ki67, could play an important role in predicting biological behavior.[8]

Most commonly, these patients only present for a mass and rarely for any clinical signs; however, clinical signs of MCTs can be complicated due to the secondary effects of the release of histamine, heparin, and other vasoactive amines associated with mast cell granules such as erythema, edema, and pruritis.[2,16,17] When an animal has larger tumors or metastatic neoplasia,

they are more likely to have systemic signs such as vomiting, diarrhea, fever, melena, and peripheral edema. These GI signs can commonly occur because histamine is thought to act on gastric parietal cells increasing the secretion of hydrochloric acid.[2]

Treatment and Management Options:

The treatment of mast cell tumors is dependent upon the stage, grade, and clinical presentation. The mainstay treatment for most MCTs is surgery.[2] The most commonly accepted excision has margins that are 2 to 3 centimeters from the edge of the mass and 1 fascial plane deep for both cutaneous and subcutaneous types. Whenever margins are incomplete, a re-excision is warranted as well as the potential for radiation therapy. By using surgical excision and radiation therapy in combination, a 2 year control rate of 85% to 95% has been reported in low/intermediate grade tumors. [2,17] When a patient has a high grade MCT or metastasis, a combination of surgery and chemotherapy is warranted. Toceranib and masitinib are two chemotherapeutics that have been made to treat canine MCTs as they are receptor tyrosine kinase inhibitors.[1] Masitinib, in particular, has been shown to have an improved long-term survival in dogs with MCTs that are unable to be resected regardless of their KIT mutation status.[5]

For a tumor of this size, surgical margins of 2 cm are generally what is recommended; however, there are currently no published guidelines for a nasal planum mast cell tumor. At this time, Lexie's owners were more interested in only pursuing radiation therapy with the thought that a significant portion of her nose would be resected if 2 cm margins were pursued. Following the consultation with a surgical oncologist at Colorado State University with our CT images, she confirmed that 1 cm margins would be sufficient for excision in her experience. By having smaller margins, a more cosmetically appealing approach to removing the entire tumor could be achieved. It also allowed for planned reconstruction of the nasal planum using a nasal rotational

flap. The reconstruction of the nasal planum was based off of two case studies that had successfully closed and reconstructed defects of the nasal planum with cosmetically appealing results.[4,11]

For Lexie's case, her owners had originally contemplated the idea of radiation therapy after discovering how much of Lexie's nose may have to be resected. After speaking with the Small Animal Surgery service at MSU-CVM and learning that smaller margins could be taken with a more cosmetic result, they agreed to proceed with surgery. On September 24, 2019, Lexie underwent a partial nasal planum resection and reconstruction. A sterile skin marker was used to draw 1 cm margins around the mast cell tumor. An elliptical marginal incision was made around the mass to provide proper margins. The subcutaneous tissue and the lateral and dorsal nasal cartilage were excised. The excision of the cartilages was one facial plane deep to the mass. The resected mass was marked with suture in the rostral and ventral areas and submitted for histopathologic evaluation. After copious lavage of the surgical site, the subcutaneous tissues along the lateral and ventrolateral aspects of the left rostral nasal cavity were apposed to the remaining nasal cartilage using 3-0 PDS in a simple interrupted pattern to reconstruct the rostral nasal cavity. A sliding advancement flap harvested from the caudal aspect of the nose to close the caudal part of the defect and a modified rotational flap was used to reconstruct the nasal planum. A sliding advancement flap is a type of subdermal plexus flap that is used because they can be stretched forward over the wound, as they are parallel to lines of least tension, without bringing additional loose skin.[3] The rotational flap that was used does not provide a direct cutaneous artery for survival of the flap, so with the use of the subdermal plexus from the sliding advancement flap, the blood supply should be sufficient for the flap to survive.[3,4,11] The rostral aspect of the naris and muzzle were closed by primary apposition of the local tissues. An 8

French red rubber catheter was placed as an intra-nasal stent in the left naris and was secured to the nasal planum.

Unfortunately, the biopsy report showed that the mass had an incomplete excision, so a revision was scheduled for September 27, 2019. This biopsy also gave a definitive diagnosis of a subcutaneous mast cell tumor. The reason for the difference in tumor depth could be because the original diagnosis of the cutaneous mast cell tumor was made based off of an incisional biopsy rather than excisional, which may not have determined the origin of the mass. Alternatively, the tumor cells may have been introduced into the subcutaneous tissues by the previous biopsy procedure. The postoperative histology results showed that the neoplastic cells extended to the surgical margins between the epidermal and mucosal areas. Based on consultation with pathology on the location of the contaminated margin, about 4 mm of the luminal aspect of the alar fold was excised. Additional tissue was removed around this area as extra precaution. The resected tissues were submitted for histopathologic evaluation. Following this submission, Lexie's biopsy was determined to have complete margins.

Case Outcome:

Lexie was discharged on September 28, 2019. At that time, she was sent home on Tylenol 4, trazodone, diphenhydramine, and omeprazole. The nasal stent that was placed was to remain there until Lexie's 2 week recheck. Lexie returned to MSU-CVM on October 14, 2019 for her incision recheck. At that time, her skin flap appeared to be healthy and viable. Her skin sutures and nasal stent were removed.

As of May 4, 2020, Lexie is doing incredibly well! At her 6-month recheck, there were no signs of additional growth. Lexie's owner was proud to report that her appetite has returned, and she is back to jogging. Though it is tweaked, everyone is very happy with her new, old nose.

References:

1. Da Silva, L, Fonseca-Alves, CE, et al. Pilot assessment of vascular endothelial growth factor receptors and trafficking pathways in recurrent and metastatic canine subcutaneous mast cell tumours. Veterinary Medicine and Science 2017; 3:146-155.

2. Ettinger JS, Feldman, EC, and Côté, E. Mast Cell Disease. In: Ettinger JS, Feldman, EC, and Côté, E, eds. Textbook of Veterinary Internal Medicine. 8th ed. St. Louis: Saunders Elsevier, 2017; 2111-2114.3. Fossum, TW. Surgery of the Integumentary System. In: Dewey, CW, Horn, CV, Johnson, AL, MacPhail, CM, Radlinsky, MG, Schulz, KS, Willard, MD, eds. Small Animal Surgery. 4th edition. St. Loius Elsevier Mosby, 2013; 190-288

4. Haar, GT, Buiks SC, et al. Cosmetic Reconstruction of a Nasal Plane and Rostral Nasal Skin Defect Using a Modified Nasal Rotation Flap in a Dog. Veterinary Surgery 2013; 42: 176-179

5. Hahn, KA, Legendre, AM, et al. Evaluation of 12- and 24-month survival rates after treatment with masitinib in dogs with nonresectable mast cell tumors. AJVR 2010; 71:1354-1361.

6. Newman, SJ, Mrkonjich, L, et al. Canine Subcutaneous Mast Cell Tumor: Diagnosis and Prognosis. J. Comp. Path 2007; 136:231-239.

7. Sabattini, S, Scarpa, F, et al. Histologic Grading of Canine Mast Cell Tumor: Is 2 Better Than 3?. Veterinary Pathology 2015; 52(1):70-73.

8. Thompson, JJ, Yager, JA, et al. Canine Subcutaneous Mast Cell Tumor: Characterization and Prognostic Indices. Veterinary Pathology 2011; 48(1):156-168.

9. Thompson, JJ, Yager, JA, et al. Canine Subcutaneous Mast Cell Tumors: Cellular Proliferation and KIT Expression as Prognostic Indices. Veterinary Pathology 2011; 48(1):169-181.

10. Thompson, JJ, Morrison, JA, et al. Receptor Tyrosine Kinase Expression Profiles in Canine Cutaneous and Subcutaneous Mast Cell Tumors. Veterinary Pathology 2016; 53(3):545-558.

11. Veterinary Medical and Surgical Group of Orange County. Surgical Excision of a Nasal Mast Cell Tumor. Case Report by Brittany Steves, DVM. Available at: https://www.vmsg-oc.com/wp-content/uploads/2015/03/case-nasal-mast-cell-tumor.pdf. Accessed June 1, 2020.

12. Vozdova, M, Kubickova, S, et al. Mutation and methylation status of KIT and TP_{53} in canine cutaneous and subcutaneous mast cell tumours. Vet. Comp. Oncol 2019; 1-7.

13. Warland, J and Dobson, J. Breed predispositions in canine mast cell tumour: A single centre experience in the United Kingdom. The Veterinary Journal 2013; 197:496-498.

14. Webster, J, Yuzbasiyan-Gurkan, V, et al. The Role of c-KIT in Tumorigenesis: Evaluation in Canine Cutaneous Mast Cell Tumors. Neoplasia 2006; 8(2):104-111

15. Webster, J, Yuzbasiyan-Gurkan, V, et al. Cellular Proliferation in Canine Cutaneous Mast Cell Tumors: Associations with c-KIT and Its Role in Prognostication. Veterinary Pathology 2007; 44:298-308

16. Welle, MM, Bley CR, et al. Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. Veterinary Dermatology 2008; 19(6):321-339.

17. Withrow, SJ and Vail, DM. Mast Cell Tumors. In: Vail, DM, eds. Small Animal Clinical Oncology. 4^{th} ed. St. Louis: Saunders Elsevier, 2007; 402-416.