Wait…how did that get there?

A case of mast cell tumors in a dog

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Introduction:

Canine mast cell tumors are the most common cutaneous tumor in the dog and account for over 20% of reported skin tumors (4). Breeds such as Labrador retrievers, Golden retrievers, Boston terriers and pugs are commonly predisposed, but any dog can be at risk for their formation (4). Middle aged to older dogs are typically the age range affected by MCTs (4). Currently there is not a specific etiology for the formation of MCTs but there is thorough debate on how they form. Some researchers believe there is an underlying genetic component based on previously described breed predilections and genetic mutations in the c-kit tyrosine kinase receptor. The specific nature of c-kit mutations and their relevance will be discussed later.

Mast cell tumors can be found in any region of the body, including mucocutaneous areas (vulva, prepuce, conjunctiva, oral cavity), inguinal region, on the muzzle/oral cavity and also the dermis, or the subcutaneous tissue (4). MCTs can also be found in the spleen, liver, GI tract, the lungs on very rare occasions and lymph nodes. Additionally, while mast cells can also be found in normal lymph nodes, the presence of mast cells in lymph nodes with those diagnosed with MCTs is of concern for metastasis. Some factors that differentiate normal mast cells from metastasized mast cells in lymph nodes include the presence of clusters of mast cells, poorly differentiated mast cells and increased visualization of mast cells in the lymph nodes. MCTs can vary from raised and superficial, to deep and fixed through multiple layers of the skin with a soft, firm or fluctuant nature about them (4). Commonly, mast cell tumors can look like lipomas, meaning any mass of suspicion should be evaluated by your veterinarian. Diagnosis of MCTs can be achieved by fine needle aspiration (FNA) of the mass. For staining and adequate visualization after FNA, typically a diff-quik cytology will lead to a proper diagnosis. On occasion, a Wrights stain may be needed due to the diff-quiks inability to stain the mast cell granules (4).

Many MCTs can be cured with surgical excision, being sure to have 2-3 cm lateral margins on each side and going 1 fascial plane deeper than the tumor (4). This is to be sure that all the tumor margins are removed, and no residual disease remains. However, while most MCTs are cured with surgery, some are more aggressive and require chemotherapy, radiation or both depending on the tumor behavior. Additional things to investigate are regional draining lymph node involvement and staging/grading of these MCTs as this will affect treatment and prognosis. Staging gives us an idea of tumor burden, other organs affected and lymph nodal involvement. Grading helps to determine tumor features, recommended treatment and future prognosis (4).

History and Presentation to MSU CVM:

Bailey is an approximately 11-year-old female spayed Corgi mix dog that presented to MSU-CVM Oncology department for a mass on her vulva. This mass was first noticed on September 25, 2018 and a veterinarian at an emergency hospital performed an excisional biopsy that night. The pathology report sent to us indicated the mass was a low grade 2, mast cell tumor. Bailey's owners reported that she was still high in energy and was eating, drinking, urinating and defecating normally. She is up to date on vaccines. She receives Bravecto and Sentinel for flea, tick and heartworm prevention. She also was started on 20 mg of famotidine at the time of diagnosis. Famotidine was prescribed to prevent any side effects from the mast cells tumor. These side effects include GI ulceration and allergic type reactions. Bailey then presented to MSU-CVM Oncology service for subsequent staging and grading before presenting to MSU-CVM surgery department on 10/19/18 for the removal of the tumor.

On presentation to MSU-CVM, Bailey was bright and alert, with a weight of 14.6 kg (32.1 pounds) and body condition score of 5/9 (normal is a 4 or 5). Her mucous membranes were pink and moist, with a capillary refill time of less than 2 seconds (normal and hydrated). Her teeth had generalized moderate plaque buildup. There was a mass, as previously noted, that was 13 mm by 13 mm on the right lip of the vulva. There was also a mass on her head that is approximately 0.5 cm by 0.5

cm, that is firm, but moveable and is located just medial to the base of the right ear. Her vitals were within normal limits (pulse of 80, temperature of 99.0 and Respiratory rate of 20). The rest of her physical exam showed no other abnormal findings.

Diagnostic Approach/Considerations

Her RDVM collected baseline diagnostics pertinent to mast cell tumors prior to workup at MSU-CVM. These diagnostics obtained included a CBC/chemistry panel. These panels revealed no clinically significant findings. A urinalysis was performed by MSU-CVM upon presentation and revealed no significant findings. Fine needle aspirates of popliteal lymph nodes revealed small amounts of blood with low numbers of small, medium and large lymphocytes and a moderate number of well-granulated mast cells. Additionally, fine needle aspirates of the mass on her head revealed fat droplets and a small amount of blood, this was nondiagnostic of anything at this time.

Abdominal radiographs were then taken and had no significant findings. Bailey was then taken to abdominal ultrasound and showed mild renal mineralization bilaterally (not clinically significant). Fine needle aspirates of the liver and spleen were taken as a precaution and both revealed large amounts of blood, but no presence of mast cells at this time. She was then transferred to MSU-CVM surgery department on 10/18/18.

Surgical findings:

The mast cell tumor on the vulva was removed and did not appear to be invasive into the surrounding tissue. The mast cell tumor was sent for biopsy and it revealed complete excision with no residual disease remaining in the vulva. The popliteal lymph nodes were both removed intraoperatively and underwent analysis via histopathology. Metastasis to the popliteal lymph nodes were determined with clusters of mast cells present. This is a strange finding as usual lymphatic drainage from the vulva

goes to the superficial inguinal lymph nodes (9). The resulting popliteal lymph node finding could reveal a more systemic MCT, even though the liver and spleen aspirates came back as non-diagnostic.

Oncology visit:

We discussed with her owners that her mast cell tumor was completely excised, was an intermediate grade, and showed evidence of metastasis (to the popliteal lymph nodes). These considerations prompted MSU-Oncology to worry for potential for this cancer to act in a more aggressive manner. As it had already shown us that it can metastasize, we recommended to follow up Bailey's surgery with chemotherapy to slow down its progression. We discussed with the owners that without chemotherapy, we would worry about the potential for this cancer to recur and may spread systemically in months. With chemotherapy to help control and slow the progression, there was hope to extend the survival time to close to a year.

Pathophysiology of disease:

Mast cells are common to find throughout the body and are important cells of the immune system that are derived commonly from a hematopoietic lineage. Hematopoiesis is known as the process by which immature blood cells develop into mature cells (1). Hematopoietic stem cells give rise to myeloid and lymphoid progenitors which then differentiate into different cell lineages. Mast cells are of myeloid origin, originate in the bone marrow and mature from these hematopoietic stem cells. (1). Mast cells then mature from the c-kit ligand and by other environmental factors present in the differing tissues (1). Common organs of origin include the skin, gastrointestinal tract and the respiratory tract (upon antigen-induced inflammation) (1). They are commonly found in the mucosal and epithelial tissues and primarily assist with inflammatory, allergic and immune mediated conditions. They mediate inflammation by their cytoplasmic granules. Mast cell functions physiologically include vasodilation, angiogenesis (formation of blood vessels) and bacterial/parasitic elimination (1).

The main mechanism of action of mast cells are from IgE-mediated allergic reactions (1). After antigen induced activation of the mast cell and subsequent release of IgE, degranulation occurs. This event results in a direct toxic effect on foreign things like parasites/bacteria. The cytoplasmic granules inside mast cells release histamine, heparin and proteolytic enzymes. When these events occur in excess, such as an event of a mast cell tumor, where there are clusters of mast cells, they begin to pose health hazards. Large amounts of the substances released can affect heart rate, blood pressure and other body functions including the cause of gastrointestinal ulceration (1).

The complete underlying cause of mast cell tumors is unknown but there are hypothesized reasons as to how they proliferate. There seems to be a connection in a proto-oncogene mutation (5). The c-kit proto-oncogene encodes the tyrosine kinase receptor KIT, which consists of a binding domain with immunoglobulins, a few different essential membrane domains and a kinase domain (5). The KIT ligand is commonly found to be a mast cell growth factor which provides a connection to mast cell growth and proliferation (5). KIT is known to be expressed by a few cell types including hematopoietic progenitors, germ cells, interstitial cells of cajal, melanocytes and mast cells (5). The thought is that c-kit plays a role not only with MCTs but other neoplastic diseases in humans such as small lung cancer, prostate cancer and acute myeloblastic leukemia (5). This is accomplished by means of a c-kit mutation. This mutation leads to a constitutively (always turned on) activated KIT product with absence of the KIT ligand described previously (5). Studies are still determining the relevance to the Kit mutation, as some dogs with MCTs have these mutations and other do not (5).

Grading MCTs:

A histologic well-known grading scheme(I-III) called the Patnaik scheme was developed for classification of MCTs and is a very reliable indicator of the tumor behavior (2). Another grading scheme commonly implemented is the Kiupel, with either a low, or a high grade given. Histological assessment

can be variable however, as each pathologist may be more aggressive in their diagnosis or have a differing opinion. The grade/degree of malignancy can be determined effectively histologically with a sample from the tumor tissue (2). The pathologist will look for cellular, nuclear and cytoplasmic features. Certain nuclear features can make it more likely that a tumor is malignant. Nuclear criteria looked for by the pathologist can range from anisokaryosis, dispersed or coarse chromatin, thickened or indented membranes, macrokaryosis, increased mitotic figures and nuclear molding (2). On the Patnaik grading scheme, a grade one MCT is well-differentiated with many granules, has few mitoses, involves just the subcutaneous tissue, no necrosis present and is unlikely to metastasize (2). A grade II has intermediate differentiation (what Bailey has), rare mitoses (0-2 per high power field), involves the dermis and subcutaneous tissue, and has variable granules with necrosis/edema. These may metastasize depending on if they are a low or a high grade II (2). Even if you have a low grade II MCT, they can still metastasize. Grade III MCTs are poorly differentiated histologically with frequent mitoses (3-6 per hpf), invading deeper tissues, poorly granulated with marked necrosis/edema and the likelihood is that they will metastasize (2).

Prognosis in MCTs is difficult to determine. While surgery is usually curative as mentioned previously, others can behave in a more aggressive manner, metastasizing to lymph nodes or other organ systems before they are removed. In a study done, prognosis was tested for regional lymph nodes with and without metastasis in MCTs, and conclusively those without metastasis at the time of surgical removal carried a more favorable prognosis for remission of the cancer (3). In those dogs with lymph node metastasis, removal of the affected lymph nodes showed an increase in survival time as the mast cells no longer had a place to proliferate (3). Additionally, tumor location had a more significant effect on longer term survival as some tumors behave more aggressively in different locations, like those found on the vulva, inguinal regions and the muzzle/oral cavities (3). As mentioned in the next section, for grade II intermediate MCTs, it is also useful to see the stage of disease as well. Some grade II MCTs

can have a more favorable prognosis for long term survival, but others can manifest systemically and can lower survival time (3).

Staging MCTs:

Tumor staging assesses the volume and location of the patient tumor burden. Staging evaluates the extent of the local tumor (T) through a TNM system, spread to regional lymph nodes (N)and metastasis to lymph nodes/organs (M). Staging is very important as it will also guide future therapy and is very frequently a good prognostic indicator of long-term survival (4). Prior knowledge of tumor behavior helps guide where to look as many MCTs will metastasize to similar locations. The most often positive test is regional lymph node aspiration. As mentioned before mast cells can be a normal finding in lymph nodes (7), but in those dogs with diagnosed MCTs, aspiration of mast cells from lymph nodes is indicative of metastasis (4). Other staging tests are rarely positive (such as with abdominal ultrasound and subsequent FNA of the liver/spleen), and rarely find evidence of MCT metastasis with higher rates of false positives (4). MCTs in this case are difficult to diagnose as mast cells can be found in normal spleens and livers (4). However, those dogs found with MCT infiltration to the spleen and liver had significantly shorter survival times than those dogs without (4).

Treatment and Management:

Surgery is the mainstay of therapy for MCTs and is curative depending on the grade/ stage of the MCT (4). The goal of surgery is to have 2-3 lateral margins and going 1 fascial plane deep. It is important to mention that MCTs can and will recur with clean margins and it varies case to case (4). MCTs are also radiosensitive and some sources report definitive radiation therapy can assist with post-operative local residual disease (4). Additionally, if the tumor can only be minimally resected due to its anatomical location, radiation can be used as a follow up treatment (4).

Additionally, chemotherapeutics are commonly implemented, especially in areas where radiation therapy cannot be performed and in dogs with poor prognostic indicators (more aggressive/malignant) even after removal of the local tumor (4). Protocols for more aggressive treatment are initiated based on the surgical biopsy results, and the likelihood of systemic disease. Common chemotherapeutics include the use of agents such as vinblastine/prednisone and lomustine. Lomustine is non-cell cycle specific but has preferential killing of cells in the early S and G1 stages of replication, while Vinblastine will kill cells in the M phase of cell replication (8). Side effects of lomustine (CCNU) include dose dependent hepatotoxicity and thrombocytopenia, and side effects of vinblastine include bone marrow suppression and extravasation (8). Prednisone use is also commonly implemented along with these therapeutics to help mediate inflammation and there is some thought that prednisone can be effective as an anti-neoplastic agent.

Tyrosine kinase inhibitors (Palladia) is another chemotherapeutic that is labeled in the US for recurrent grade II/III recurrent MCTs (8). Palladia is commonly use due to the KIT mutations mentioned previously (8). This chemotherapeutic can also provide some benefit in the microscopic setting, including treatment in MCTs with poor prognostic indicators such as a high mitotic index, high grade and poor location (4). Side effects can include GI upset (most common) and dose dependent myelosuppression (6). Adding Palladia to the already discussed treatment regimen has shown success when dosed every other day. Some studies also report those dosed once daily along with other recommended chemotherapeutics use can assist with a slower disease progression and have longer survival times (4). Another known TKI such as masitinib used to be commonly used for the KIT mutation. However, it lost its approval in the US and has not been readily available for the past few years.

Bringing the case back to Bailey, after surgical healing, she was brought back to MSU-CVM on October 30th, 2018 to go over the results of the surgical biopsies and its metastasis to the popliteal lymph nodes. A course of eight (8) treatments were administered to Bailey during this protocol

beginning on November 7th and going to January 16th. She was treated with a standard vinblastine/prednisone protocol. She received vinblastine at 2mg/m2 weekly for four weeks then every other week for another 4 doses. This was combined with prednisone at 40mg/m2 for the first week and then 20mg/m2 every other day for the remainder of the protocol. She was restaged at the midpoint of the treatment and at the end of the protocol on January 17th, 2019. She was also prescribed metronidazole and ondansetron for an as needed basis for diarrhea/vomiting.

Case outcome:

Bailey presented for restaging and for her last dose of chemotherapy on January 17th, 2019. Her thoracic radiographs (chest x-rays) and abdominal ultrasound showed no evidence of cancer spread (metastasis). Bailey received her dose of vinblastine 2 mg/m2 (1.1 mL) in the left lateral saphenous (left back leg) without complications. Her spleen and liver aspirates (cell sampling) returned the next day and was unfortunately suspicious of cancer spread to the liver and that systemic spread of the MCTs were likely occurring. For palliative care, Bailey was also prescribed Diphenhydramine 25 mg tablets and Omeprazole 20mg tablets to help with any future gastrointestinal ulcer formation from her MCTs. At this time, this is the last visit and the owners have indicated not to pursue further treatment. They will let her live out her days as happy as she can before succumbing to her illness.

Mast cell tumors are unique in the fact that they can be very aggressive or can be benign. Bailey's initial MCT was removed but only after metastasis to the popliteal lymph nodes. The draining tract of the vulva commonly goes to the superficial inguinal lymph nodes and these were not evidently suspect of metastasis. There is evidence out there to support that with heavy tumor burden, alteration of lymphatics can be an explainable cause of Bailey's mast cell metastasis to the popliteal lymph nodes (9). When tumor burden overwhelms the lymphatic system locally, the flow resistance in the lymphatics increases (9). This resistance in lymphatic flow could have resulted in the diversion of lymph and the carrying of the cancer cells to Bailey's popliteal lymph nodes. In conclusion, Bailey's case was very

unique and the ways that it manifested raises many future questions about the pathophysiology, lymphatic draining tracts, diagnostics and future treatment of systemic MCTs.

Reference list

1. Krystel-Whittemore, Melissa, et al. "Mast Cell: A Multi-Functional Master Cell." *Frontiers in Immunology*, Frontiers Media S.A., 6 Jan. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC4701915/.
2. C Northrup, N & Howerth, Elizabeth & G Harmon, B & Brown, CA & Carmichael, Karen & Garcia, AP & S Latimer, K & Munday, John & Rakich, Pauline & Richey, Lauren & Stedman, Nancy & L Gieger, T. (2005). Variation among Pathologists in the Histologic Grading of Canine Cutaneous Mast Cell Tumors with Uniform Use of a Single Grading Reference. Journal of veterinary diagnostic investigation: official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 17. 561-4. 10.1177/104063870501700606.
3. Baginski, Heather, Davis, Garrett. "The prognostic value of Lymph Node Metastasis with Grade 2 MCTs in Dogs: 55 cases." AAHA, JAAHA. 2014, https://jaaha.org/doi/pdf/10.5326/JAAHA-MS-5997.
4. Garrett, Laura D. "[Full Text] Canine Mast Cell Tumors: Diagnosis, Treatment, and Prognosis | VMRR." *Veterinary Medicine: Research and Reports*, Dove Press, 12 Aug. 2014, www.dovepress.com/canine-mast-cell-tumors-diagnosis-treatment-and-prognosis-peer-reviewed-fulltext-article-VMRR#.
5. Webster, Joshua D, et al. "The Role of c-KIT in Tumorigenesis: Evaluation in Canine Cutaneous Mast Cell Tumors." *Neoplasia (New York, N.Y.)*, Neoplasia Press Inc., Feb. 2006, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1578516/
6. Marquardt, Taya. "Round Cells I." Small animal medicine and surgery. 2019, Mississippi State, College of Veterinary Medicine, Mississippi State, College of Veterinary Medicine.
7. Marquardt, Taya. "Cancer therapeutics." Small animal Medicine and Surgery. 2019, Mississippi State, College of Veterinary Medicine, Mississippi State, College of Veterinary Medicine.
8. Aquino, José Ulcijara, et al. "Experimental Canine Model for Sentinel Lymph Node Biopsy in the Vulva Using Technetium and Patent Blue Dye." *Acta Cirurgica Brasileira*, U.S. National Library of Medicine, Feb. 2012, www.ncbi.nlm.nih.gov/pubmed/22378363.
9. Padera, Timothy P, et al. "The Lymphatic System in Disease Processes and Cancer Progression." *Annual Review of Biomedical Engineering*, U.S. National Library of Medicine, 11 July 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC4946986/.