Canine Mast Cell Tumor

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Introduction

Mast cells are leukocytes that are derived from hematopoietic progenitor cells in bone marrow under the control of stem cell factor, also known as KIT ligand via the KIT receptor (CD117) on mast cells [8, 14, 15, 17, 19]. Mast cells present foreign molecules to immune system cells, and recruit macrophages and other cells capable of phagocytosis to engulf foreign or invading material. They originate in the bone marrow, but mature in other tissues. Cell precursors move from the bone marrow, through circulation, then to the tissues where they mature into effector mast cells [15]. These cells are not usually in circulation; however, their immature form circulates in the blood before migrating to vascularized tissues. Once in vascularized tissues, they undergo a final differentiation and maturation stage with the assistance of stem-cell factors and other cytokines secreted by endothelial cells and fibroblasts. Mast cells are found throughout the body, but are concentrated in locations that are in close association with the external environment, such as skin, respiratory tract, and the digestive tract. So, these cells are ideally placed to participate in the early recognition of pathogens, as well as, mediation of inflammation. Mast cells respond to stimuli by releasing a variety of substances from their cytoplasmic granules such as vasoactive amines (histamine, serotonin), enzymes (acid hydrolyses, cathepsin G, phospholipase A, chymase, tryptase, carboxypeptidase), proteoglycans (heparin, chondroitin sulfate), and cytokines [15]. Mast cell-induced inflammation is mediated mainly by histamine, followed by heparin and proteolytic enzymes. Mast cells are best known for their role in mediating allergic diseases, modulating immune responses by stimulating T-cell activation, and promoting leukocyte migration during both acute and chronic inflammation [11,15]. However, studies highlight the important role these cells play in the protection against infection with a variety of organisms [11].

In addition to being a cellular barrier to external agents, mast cells have a regulatory

function on nerves in the skin, blood circulation, fibrous tissue, and other immune cells [11]. They are of specific importance in allergic responses, tissue remodeling, wound healing, and non-allergic skin diseases. Mast cells in hair follicles also help to regulate the growth cycle of those follicles [20].

The mechanism of activation of mast cells is via stimulation of the high-affinity immunoglobin E (IgE) receptor FcεRI [3,11]. This high-affinity receptor is specific for the Fc region of the epsilon (ε) heavy chain of IgE [11]. Activation of mast cells results in the release of a variety of soluble factors. Within seconds of stimulation, mast cells undergo degranulation, rapidly releasing pre-formed mediators present within cytoplasmic granules, including histamine, neutral proteases (tryptase and chymase), as well as, pre-formed tumor necrosis factor-alpha (TNF- α) [3,5,7,10,18]. Shortly after the initiation of degranulation, mast cells can produce lipid-derived eicosanoids such as prostaglandin D2 and leukotriene [11]. Finally, over the course of hours, the transcriptional upregulation of cytokines and chemokines, including TNF- α and interleukin-4, can be observed. Each of these responses may occur alone or in combination depending on the stimulus. Because of their location, plasticity and the various mediators they produce, mast cells are important immune effectors and modulatory (environmentally adaptable) cells.

History and Presentation

Rip is a seven-year-old intact, male Boykin Spaniel who originally presented to MSU-CVM Animal Health Center on June 27, 2016 for persistent draining, pain and swelling over his scrotum for a duration of approximately a week. The owner stated that he constantly licked at it and was very painful in that area; therefore, they could not fully assess it.

Upon initial presentation, Rip was bright, alert and responsive; however, he was very anxious, keeping his back in the corner with his tail tucked, guarding his scrotum. A full physical

examination was performed, except for testicular and scrotal examination, since Rip would become aggressive when attempting to palpate that area. He was assigned an ideal body condition score of 5/9. His heart and respiratory parameters were within normal limits along with normal auscultation. His mucous membranes were pink with a capillary refill time of less than two (< 2) seconds.

Since Rip's scrotal area could not be fully assessed while he was awake, he was sedated to accurately evaluate his scrotum. Scrotal swelling and reddening were present, but it seemed to have no testicular involvement. The swelling was firm to the touch and two small puncture-like wounds were present. We decided to attempt to drain the swelling. We administered a dose of diphenhydramine hydrochloride (1-2 mg/kg) for precautionary measures; and proceeded with the plan. However, no fluid was rendered from the site. While sedated, we also collected a blood sample for a complete blood count (CBC) and chemistry panel, as well as, urine for a urinalysis. CBC revealed a mildly decreased hematocrit level of 32.6% (normal range: 34-60%) and an increased mean corpuscular hemoglobin concentration (MCHC) of 38.7 g/dl (normal range: 32-37). The chemistry panel revealed a mild hyperglycemia (132 mg/dl), a mildly elevated blood urea nitrogen (28 mg/dl**)**, and a mild hypoalbuminemia, hyperphosphatemia, hypocholesterolemia, and hypomagnesemia. Urinalysis rendered no significant findings. We provided the owner with a list of potential rule outs (possible gastrointestinal ulceration, parasites, early renal disease, tick borne disease, anemia of chronic disease, neoplasia) since these correlated with the clinical signs, presentation, and bloodwork abnormalities. We also discussed the importance of neutering Rip with a scrotal ablation technique to resolve all sources of irritation and minimize the chance of delayed healing, but the owner declined.

We diagnosed Rip with abscessation of the scrotum with concurrent cellulitis and bacterial

infection due to a presumptive penetrating wound. He was prescribed half of a 75 mg carprofen tablet every 12 hours for ten days, 150 mg cefpodoxime tablet every twenty-four hours for seven days, and topical silver sulfadiazine paste to be applied twice daily to his scrotum, for the bacterial infection, until healed. The owner was instructed to keep Rip in strict confinement for seven days and to keep him in his e-collar to prevent licking of the affected area. The owner was also informed about potential persistent infection, dehiscence, or poor/delayed wound healing that may require surgical intervention if Rip was allowed to lick his scrotum. Rip was scheduled for a recheck on July 5, 2016 for reassessment.

Rip presented again on July 12, 2016 for excess swelling of his scrotum with a blackened area and pus-like discharge oozing underneath the black spot. The owner stated that the antibiotics previously prescribed cleared the infection and swelling completely within the week; however, the lesion reappeared about 2-3 days afterwards. The owner also stated that in the past few days, the area would get bigger, then become smaller. A full physical examination was performed again and Rip seemed to be bright, alert, yet anxious. He was assigned an ideal body condition score of 5/9. His heart rate and respiratory rate parameters were within normal limits. His mucous membranes were pink with a capillary refill time of < 2 seconds. His heart and lungs auscultated normally. Again, due to anxiousness and pain, we could not assess Rip's scrotum while awake, so sedation was elected to accurately evaluate his scrotum once again.

Genital evaluation revealed scrotal swelling and reddening with necrosis of scrotal tissue. When the scrotum was palpated, no testicular involvement was suspected; however, it was about two to three times the size it was two weeks ago. It was firm to the touch and the two, small puncture-like wounds that were present two weeks prior formed a full opening with black, necrotic surrounding tissue. The rest of the physical exam was within normal limits.

With the aforementioned information, we decided to perform a CBC, small animal chemistry profile, coagulation profile, and, speak to the owner once again in regards to potential neoplasia and need to neuter Rip. The CBC and coagulation profile were within normal limits. The small animal chemistry profile revealed a mild hypoalbuminemia – possibly due to the continuous leakage of fluid from his scrotum, and a mildly decreased alanine aminotransferase (ALT). We attributed these finding to scrotal disease or another underlying disease process.

On July 12, 2016, an aerobic culture and sensitivity and anaerobic culture were performed. Culture swab of the area revealed heavy growth of mixed bacteria including probable Staphylococcus aureus, possible *Enterococcus or Alpha Streptococcus, Escherichia coli, and Bacteroides eggerthii*. Although the cultured organisms were sensitive to (cefpodoxime) Simplicef, no evidence of healing was noticed and neither testicle was a decrease in size; so, a new differential diagnoses list was formed. Given the history and clinical signs, we placed neoplasia at the top of our differential diagnoses list followed by fungal abscess and granuloma. Since neoplasia was now at the top of our list, we decided to report to the owner and proceed with surgery.

On July 13, 2016, a modified closed technique orchiectomy with a scrotal ablation was performed, using an elliptical incision with wide margins. The testicles and scrotum were completely excised in conjunction with one another (en bloc) as to not disrupt the potential infected/cancerous site. The scrotum along with testicles were placed in a sterile container and sent to the lab for biopsy. Biopsy results revealed high grade (Patnaik grade III), mast cell tumor. Histopathology results determined the dermis was infiltrated by large numbers of neoplastic mast cells with moderate pale staining cytoplasm that contained poorly staining cytoplasmic granules and large, round to ovoid nuclei with marginated to lightly stippled chromatin and one or two small nuclei. Mitotic figures were three in ten high power fields. There was moderate anisocytosis and

moderate to marked anisokaryosis. Bizzare nuclei were greater than three in ten high power fields, and multinucleated cells were greater than seven in ten high power fields. The tumor also contained numerous eosinophils. This tumor met the high-grade criteria based upon bizarre, karyomegalic nuclei, and multinucleated cells, which placed it in the high-grade category with a poorer prognosis. Surgical excision was confirmed to be complete, but metastatic disease was of major concern.

Pathophysiology/Anatomical Considerations

The underlying cause promoting the development of mast cell tumor is not known. However, it is presumed that a mast cell tumor results from a mutation in those cells which leads to unregulated reproduction and growth. There are many factors included in the development of mast cells, but there is a strong suspicion for dysregulation of the KIT receptor in provoking mast cell tumor development and progression [15]. Mast cells are able to release their granular contents in response to various stimuli, inducing an inflammatory reaction. These granules are filled with substances, mainly histamine, which can be released into the bloodstream potentially causing serious systemic issues. The release of histamine causes an increase in plasma histamine which leads to an increase in gastric acid production and increased GI motility, ultimately resulting in gastroduodenal ulceration and bleeding. Granular release also causes swelling and redness at and around the tumor site and potentially life-threatening complications, such as a dangerous drop in blood pressure and a systemic inflammatory response leading to shock. Care must be taken not to palpate a suspicious tumor too vigorously because mechanical manipulation may cause degranulation and excess secretion of histamine, producing erythema and wheal formation [1,7]. It may also cause the skin growth become more inflamed, increasing in size, a phenomenon known as Darier's sign [7]. Darier's sign is considered of diagnostic significance. In the worst case, it will

cause the patient to go into an allergic reaction and shock if excess histamine is released from the mast cell tumor.

According to multiple research studies, mast cell tumors in dogs are very common and is the most common skin tumor. There are two forms of mast cell tumors. Cutaneous, being the most common form, and visceral, being the most aggressive form. Mast cell tumors account for approximately seven to twenty-one percent (7-21%) of all skin tumors in dogs and eleven to twenty-seven percent (11-27%) of malignant skin tumors. Mast cell tumors can arise from any skin site on the body; and can have a variety of appearances. These tumors can range from being a raised lump or bump on or just underneath the skin to multiple, small to large, poorly marginated masses. They may be red, ulcerated, and/or swollen. Owners may report a "waxing and waning" size of the tumor. This size variation can occur spontaneously, or it can be induced by agitation of the tumor site, such as excessive licking and gnawing, which in turn causes degranulation. Certain dogs are predisposed to mast cell tumors, including brachycephalic breeds and retriever breeds; however, any breed of dog can develop this tumor.

The biological behavior of mast cell tumors can vary widely; some may be present for many months without growing much, while others may appear suddenly and grow very quickly. Most mast cell tumors are considered to be locally invasive, and can be difficult to remove completely because of the extent of local spread. The behavior of these tumors is governed by their stage/grade and is determined mainly by the *Patnaik grading system* or the *Kiupel System*.

The *Patnaik grading system* is the classic system commonly used to assign biopsied mast cell tumors as Grade 1, 2, or 3. The *Kiupel system* is a two-tier histologic grading system that was developed by pathologists to evaluate the consistency of microscopic grading among veterinary pathologists and the prognostic significance of the Patnaik Grading System [1,7,10]. Although 74%

of pathologists agreed on the diagnosis of grade III MCTs, only 63% agreed on the diagnoses of grade I and grade II MCTs, suggesting that this degree of variation deems the current histologic grading system (Patnaik Grading System) sometimes unreliable [10]. The researchers concluded that a two-tier system with clear histologic grading criteria eliminated the uncertainty of intermediate grade mast cell tumors. However, both the *Patnaik grading system* and *Kiupel system* are used as dependable systems by many pathologists.

With respect given to the Patnaik Grading System, mast cell tumors are ranked between grades one through three, with grade I being the least aggressive and least likely to metastasize, and grade III being highly aggressive tumors with a higher likelihood of metastasis [1,4,6,9,10,13,14,15,19].

Grade I tumors are confined to the dermis, well differentiated, minimally necrotic and are very unlikely to metastasize. They also have low cellularity, monomorphic nuclei, no mitoses, and have ample granulated cytoplasm. Grade II tumors can be in either the dermis or subcutaneous tissues. They have moderate cellularity, moderate pleomorphic cells, minimal nucleolar pleomorphism, 0-2 mitoses per high power field, minimal edema and necrosis, a variably granulated cytoplasm, and a low possibility of metastasis. Most low grade II tumors do not metastasize, although the potential for spread is still likely. Low grade II mast cell tumors have a much lower rate of local recurrence and longer survival rate [1]. In contrast, high grade II mast cell tumors are highly aggressive and metastatic. Grade III tumors are deeply invasive. They have high cellularity, high cellular pleomorphism, 3-6 mitoses per high power field, marked edema and necrosis, poorly granulated cytoplasm, and are multi-nucleated [19].

According to the *Kiupel system*, diagnosis of "high grade" mast cell tumors are based on the presence of any one of the following criteria: at least seven to ten mitotic figures in ten high

power fields; at least seven multinucleated cells in ten high power fields; at least three bizarre nuclei in ten high-power fields; and karyomegaly (abnormal nuclear enlargement of a cells) [1,7,10].

In the present case, the nuclear diameters of at least ten percent of neoplastic cells varied by at least two-fold, meaning this tumor – as with the vast majority of Grade III tumors – was highly likely to metastasize. The most common sites mast cell tumors metastasize to are regional lymph nodes (most common site), liver, spleen, and bone marrow.

Diagnostic Approach/Considerations

Dogs are frequently asymptomatic and non-painful. Owners may report a frequent wax and wane effect (due to local histamine release), bruising, enlargement caused by manipulation, and multiple physical variations of the tumor –fluctuant, diffuse, discrete, ulcerative, small, large, focal, or multiple locations. Mast cell tumors can look like anything! Systemic clinical signs include, but are not limited to gastrointestinal ulceration, abdominal discomfort, vomiting, melena, anemia, and coagulation abnormalities.

Diagnosis is based off fine needle aspiration, commonly referred to as FNA. FNA is utilized to objectify the presence/severity of mast cell infiltration via cytology once an aspirate is collected. FNA is the initial diagnostic tool of choice because mast cell tumors are round cells that shed well with characteristic granules. Presence of these granules allows for a diagnosis, but not a grade without histopathology. Occasionally, higher grade mast cell tumors lose their granules and certain stains, such as Romanowsky stains (Giemsa, Wright Stain, etc) are better at revealing the granules compared to the traditional Diff-Quick stain. Prior to collecting an aspirate, it is common to pre-treat the patient with an anti-histamine such as diphenhydramine HCl (Benadryl) @ 1-2 mg/kg.

Histology of the tumor is performed to confirm the diagnosis, grade, and evaluate surgical resection. In Rip's case, histology revealed round cytoplasmic borders with red to purple variably sized intracytoplasmic granules. Also, thoracic and abdominal radiographs are of importance to assess for any metastasis. Rip's thoracic radiographs revealed an unstructured interstitial pattern in the caudodorsal lung fields on both the right and left lateral view. Official report attributed these findings to possible diffuse non-nodular metastatic neoplasia or infectious/inflammatory etiology. There were multiple, small (2-3mm), metallic, circular ballistic masses seen within the subcutaneous tissues of the ventral, caudal, and dorsal thorax, as well as within the triceps musculature – presumed to be BB's. No evidence of nodular pulmonary metastasis was documented.

Once the tumor is removed, biopsy is warranted because it provides insight into the next stages of treatment. Biopsy helps determine whether additional intervention is needed, such as another surgery, radiation therapy, or chemotherapy. Factors taken into consideration when deciding whether additional treatment is indicated includes: grade, completeness of surgical margins, and stage (the presence of metastasis). Staging is important as it provides information about the extent of the disease, is a parameter for time to recurrence or survival, and is helpful in planning treatment regimens [9,12,15,17]. The World Health Organization (WHO) clinical staging system for mast cell tumors recognizes four stages of tumor disease. Stage I involves one tumor in the dermis with no involvement of regional lymph node(s) [12]. Stage II involved one tumor in the dermis with involvement of the regional lymph node(s) [12]. Stage III involves multiple dermal tumors or a large infiltrating tumor with or without regional lymph node involvement [12]. Lastly, stage IV involves any tumor with distant metastasis or recurrence with metastasis [12].

Additionally, ultrasonography is needed to assess for any lymph node involvement, as well as, for a guided fine needle aspirate (FNA), even if there is no lymphadenopathy, because this is an important step in staging the tumor. Ultrasonography in Rip's case revealed a sharply marginated, round to ovoid hypoechoic prostatic nodule that measured 0.42 cm. Official report considered probable benign prostatic hyperplasia with less consideration given to neoplasia or abscess. Both the left and right inguinal lymph nodes were hypoechoic and enlarged. The left measured 0.71 cm in thickness and the right measured 0.65 cm in thickness. Metastatic neoplasia and reactive lymphadenopathy were considered for the enlarged lymph nodes. An FNA was performed on the inguinal lymph nodes and revealed large amounts of mast cells, suggesting metastasis.

Additional fine needle aspirates of the liver and spleen may be collected via ultrasound as well, along with a bone marrow aspirate and buffy coat analysis to assess for systemic mastocytosis.

Treatment and Management

Most mast cell tumors are considered locally invasive, and can be difficult to remove completely because of the extent of local spread. Treatment of choice for mast cell tumors is surgical excision with or without adjuvant therapy depending on the histological grade and staging. Grade I tumors require surgical excision. There is a survival rate of 100% at twelve, eighteen, and twenty-four months. Grade II tumors require surgical excision and adjuvant therapy if margins are incomplete or if there is an increased chance or presence of aggressive/metastatic behavior. Studies have shown that there is a 71% survival rate at twelve months, a 56% survival rate at eighteen months, and 44% survival rate at twenty-four months with a median survival time of about 500 days. Grade III tumors require surgical excision followed by chemotherapy. There is a 24%

survival rate at twelve months, a 19% survival rate at eighteen months, and a 7% survival rate at twenty-four months. Strict adherence to surgical margins must be followed. There must be a two-centimeter margin around the area of grade I and grade II tumors or two centimeters laterally and one fascial plane deep. For grade III tumors, a three-centimeter area margin must be taken. As a general rule of marginal excision of mast cell tumors, there should be at least one centimeter for grade I tumors, two centimeters for grade II tumors, and three centimeters for grade III tumors.

There are multiple treatment options for dogs with mast cell tumors depending on the grade, margins of surgical completeness, and grade. Radiation therapy is an excellent and highly effective modality which is associated with a high rate of long-term local tumor control. It is used for incomplete surgical excision or in dogs where a second surgery is not feasible. Chemotherapy is usually used in conjunction with high doses of steroids when there is complete surgical excision and metastasis or poorly differentiated tumors. Antihistamines are commonly prescribed to suppress the release of histamine, as well as antacids, such as famotidine or omeprazole, to combat the side effects of high-dose steroid usage.

Prednisone alone or in combination with CCNU (lomustine) and/or vinblastine are used as Chemotherapeutical of choice for mast cell tumor treatment. Prednisone alone at a dose of 2 mg/kg/day for 2 weeks, then 1 mg/kg/day for 2 weeks, the n1 mg/kg every other day, can directly kill cancerous mast cells and decrease inflammation and the effects of degranulation associated with the tumor, has about a 20% response rate according to an article published by PennVet. Dogs that are tumor free after six months have a lower incidence of recurrence; therefore, therapy is usually discontinued [12]. A CCNU, at a dose of 90 mg/m2 every 4 weeks, and prednisone protocol has about a 37% partial response rate [12,19]. Vinblastine and prednisone have an approximately forty-seven percent response rate. According to an article published by the National Center for

Biotechnology Information, a CCNU and vinblastine protocol renders a 57% response rate in dogs, with an overall survival time of 35 to 48 weeks. This protocol appears to be well tolerated; however, mild toxicities were recorded in 54% of dogs treated [9]. For recurrent mast cell tumors, tyrosine kinase receptor inhibitors (RTK-inhibitors), toceranib phosphate (Palladia © or Kinavet ©), are used to disrupt the signaling pathway of the vascular endothelial growth factor receptor, platelet-derived growth factor receptor, and KIT [9]. Toceranib phosphate is the first anticancer drug approved by the FDA for veterinary use and is believed to have both direct antitumor and antiangiogenic activity [9].

Prognosis for these tumors depend on the histopathologic grade and in relevant cases, their location. If the location of the mast cell tumor is preputial, scrotal, inguinal, perineal, oral, associated with a nail bed (ungula), or associated with mucocutaneous junctions, – there is a poor prognosis and the grade is often increased by one. If the tumor is located viscerally or in the bone marrow, the prognosis is grave. Also, if there is evidence of rapid growth, there is a poor prognosis. Boxers with well-differentiated tumors have a better prognosis. If there are any systemic signs associated with the tumor, this signifies an aggressive disease process resulting in a poor prognosis. The ultimate goal of treatment is to assist in maintaining a good quality of life for as long as possible with palliative therapy aimed at controlling symptoms caused by the tumor.

Case Outcome

Rip was diagnosed with a high grade (Grade III) mast cell tumor and was transferred to Internal Medicine and Oncology department on July 20, 2016 for staging. A CBC was submitted to assess baseline white blood cell levels for possible future chemotherapy and was within normal limits. Thoracic and abdominal radiographs were performed and were also within normal limits. Abdominal ultrasound was performed and revealed a hypoechoic nodule in the prostate, enlarged

inguinal lymph nodes, a large amount of debris in the bladder, and debris in the gall bladder. Fine needle aspirates of the spleen, liver and lymph nodes were performed as well. Due to the large number of mast cells seen on cytology in the inguinal lymph nodes, metastasis was highly suspected. No signs of metastasis to the spleen or liver were seen. The Internal Medicine and Oncology department declared metastasis to the inguinal lymph nodes and recommended surgery as a next step to remove the metastatic lymph nodes. In addition, a chemotherapy protocol was also suggested. Surgery to remove the inguinal lymph nodes and chemotherapy was decline by the owner. The patient was unfortunately lost to follow-up.

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