T-cell Lymphoma in the Feline Patient

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Introduction

In the feline patient, lymphoma the most commonly diagnosed malignancy, accounting for roughly 30% of all reported malignant tumors and 50-90% of all hematopoietic neoplasms. [1] [2] Lymphoma in cats, a multi-etiological disease with a lack of definitive causation, is believed to be caused by three main links: 1) chronic inflammation, particularly in association with nasal and intestinal lymphoma, 2) diet, in association with intestinal lymphoma, and 3) viral agents, particularly that of C-type retroviruses such as feline leukemia virus (FeLV). [2] [3] Lymphoma in dogs and cats is diagnosed and classified according to the disease's anatomical location, cell morphology classification, and immunophenotype. [2] Often, the disease is typically categorized into alimentary, cutaneous, mediastinal, multicentric, nasal, nodal, extranodal, and combined forms. [2] [3] The most prominent etiological component known is the FeLV retrovirus, accounting for approximately 70% of all lymphoma cases in cats under 5 years of age. [3] Although FeLV-positive cats still account for the highest prevalence of lymphoma, with mediastinal and multicentric forms being the most common, other forms of lymphoma have been reported in increasing numbers over the past several years. [3] [4] In recent years, the alimentary form of lymphoma has developed into the most common form due to decreasing numbers of cats affected by FeLV, associated with a general increase in regular veterinary screening and vaccination. [5] Feline lymphoma has a bimodal age distribution, with a mean age of 3 years in FeLV-positive cats and 10 years in FeLV-negative cats. [2] [6] Once a feline patient has been initially diagnosed with lymphoma, several factors, such as immunophenotype, histological grading, granular cell association (mast cells, basophils, eosinophils), paraneoplastic syndromes (PTH-rP-associated hypercalcemia, hypergammaglobulinemia), and disease staging, are taken into consideration to determine appropriate treatment course and prognosis.

Additionally, clinical signs, which are often exacerbated by the stage of disease, are taken into consideration for appropriate level of medical care. The clinical signs associated with lymphoma varies according to what anatomical region is affected, but often include anorexia, weight loss, lethargy, vomiting, gastrointestinal signs, and regional lymphadenopathy. Treatment of feline lymphoma, while not curable, is focused predominantly on the characterization of the disease and anatomical location. Treatment often involves systemic control through a chemotherapeutic protocol, localized control of a primary tumor through radiation or surgery, and supportive care. One of the most principal factors in determining prognosis is response to therapy, which cannot be assessed before initial treatment. Additionally, feline lymphoma is always considered a systemic disease, regardless of primary anatomical classification, and the disease does not often stay isolated to its primary growth location, creating a significant challenge in both diagnosing and treating the disease.

Patient History

Jupiter, an 11.5-year-old male neutered Domestic Shorthair, presented to MSU-CVM Internal Medicine Services on August 29, 2016 after referral for a suspected neoplasia from a fine needle aspirate obtained from the right submandibular lymph node. Jupiter presented to the referring veterinarian when the owners started to notice that he was increasingly lethargic, began having an unkempt haircoat, and was hiding more often at home. Bloodwork was performed by the referring veterinarian, revealing a mild anemia (29%), leukocytosis (~33,000/μL), and elevated liver enzyme values (ALT 176 U/L, ALP 234 U/L). The referring veterinarian initiated a drug course of prednisolone along with supportive care, consisting of subcutaneous fluids, a single antibiotic injection, and additional pain medication. The referring veterinarian performed a fine needle aspirate of the right submandibular lymph node, which was noted to possess

numerous reactive lymphocytes. The referring veterinarian discussed the likelihood of lymphoma versus a reactive lymph node with the owners, emphasizing on the possibility of lymphoma, and sought referral from MSU-CVM.

Clinical Presentation

Upon presentation at MSU-CVM Internal Medicine Services, Jupiter was noted to have a depressed mentation, but still responsive. Jupiter weighed 5.3 kg with a body condition score of 3/9, possessing widespread cachexia. Jupiter possessed normal vital parameters, with a rectal temperature of 101.3°F, a pulse rate of 216 beats per minute, and a respiratory rate of 44 breaths per minute. On physical examination, Jupiter was noted to possess an unkempt haircoat, a small amount of mucoid ocular discharge bilaterally, and was approximately 5-8% dehydrated. Jupiter possessed a severe amount of tartar buildup with severe periodontal disease. Auscultation of Jupiter's thoracic cavity revealed an increase in bronchovesicular lung sounds diffusely, but without abnormalities.

Diagnostics and Case Summary

The previously collected aspirate performed by the referring veterinarian was examined, revealing a normal lymphocyte population with a moderately increased number of mast cells. A complete blood count was performed on August 29, 2016, which revealed a normocytic, normochromic mild anemia (25%) and leukocytosis (25,400/µL), characterized as a segmented neutrophilia (19,050/µL), basophilia (254/µL), and eosinophilia (1524/µL). A blood chemistry panel was performed, revealing a mild increase to liver enzyme values (ALT 118 U/L, ALP 147 U/L) and a severe hypercalcemia (15.1 mg/dL) and azotemia (BUN 83 mg/dL, creatinine 4.77 mg/dL). Urinalysis performed on August 29, 2016 revealed isosthenuria and casts. Thoracic and abdominal radiographs performed on August 29, 2016 revealed a moderate-to-severe

bronchointerstitial pattern diffusely throughout the lungs and hepatomegaly. An abdominal ultrasound performed on August 29, 2016 revealed hepatomegaly with hyperechoic foci, hypoechoic iliac lymph nodes, enlargement of the jejunal lymph nodes, mineralization of the kidneys, and mild thickening of the intestines. Ultrasound-guided fine needle aspirates were obtained from the liver and spleen for cytological evaluation. FNA cytology of the liver and spleen revealed moderate hepatic lipidosis and mild mastocytosis. Jupiter was hospitalized to address the hypercalcemia and azotemia.

On August 30, 2016, Jupiter appeared depressed and lethargic, and possessed mild enlargement of the left submandibular lymph node. A repeat blood chemistry was performed, revealing improvement of the hypercalcemia (11.7 mg/dL), but worsening of the azotemia (BUN 105 mg/dL, creatinine 5.15 mg/dL). A malignancy profile was performed, revealing a moderately elevated ionized calcium (1.81 mmol/L) and a low 25-hydroxyvitamin D level (53 mmol/L); interestingly, there was no evidence of parathyroid hormone-related protein (PTH-rP) found at this time. Additional testing, involving an FeLV/FIV SNAP test and Baermann sedimentation, were performed, revealing no abnormalities.

On the morning of August 31, 2016, Jupiter mentation status declined significantly, and he developed facial and forelimb edema. Jupiter's respiratory effort was markedly increased, displaying open-mouth breathing intermittently. A venous blood gas, complete blood count, blood chemistry panel, and coagulation profile were run at that time. The blood gas revealed a severe metabolic acidosis (pH = 7.165). Clotting times from the coagulation profile were markedly increased. Complete blood count revealed a severe leukocytosis (52,400/µL), moderate thrombocytopenia (100 K/µL), and anemia (15%). Blood chemistry revealed a mild improvement of the azotemia (BUN 95 mg/dL, creatinine 4.77 mg/dL), but worsening of the

hypercalcemia (12.1 mg/dL). Emergency care was provided, however, due to the rapid decompensation in Jupiter's health, he was euthanized that morning.

Pathological Findings

At the time of necropsy on August 31, 2016, Jupiter was of fair body condition, with moderate sarcopenia and minimal decomposition. Mild ecchymosis was present on the abdomen, with marked ecchymosis and subcutaneous swelling at the intravenous catheter site of the left forelimb. All palpable lymph nodes were of roughly 5 times that of normal size; the tissues bulged markedly when sectioned and were of a homogenous tan, soft consistency. The retroperitoneal, perinephric tissues were moderately expanded due to hemorrhaging. The spleen was approximately 3 times that of normal size, with hypertrophied white pulp corpuscles. The liver was 3 times that of normal size and markedly yellow, with an accentuated lobular pattern diffusely. Thoracic evaluation revealed enlarged sternal lymph nodes, similar in size and texture to that of the external lymph nodes.

Histopathological samples were obtained of multiple anatomical sites. Multiple lymph nodes were effaced by a monomorphic population of lymphocytes that expand the paracortex, displacing germinal centers and infiltrating surrounding soft tissues. The cells viewed had relatively small lymphoblastic nuclei with a low mitotic rate. Those cells exhibited strong membranous staining for CD3 with no affinity for CD79, verifying that they were T-cells. Additionally, the medullary sinuses of several lymph nodes possessed large numbers of clustering mast cells. All sections of the liver possessed a moderate-to-severely infiltrative population of monomorphic, neoplastic small lymphocytes that centered on the portal areas. The spleen appeared hypercellular, possessing large numbers of small lymphocytes that appeared similar to those described in the lymph nodes. Additionally, clusters of mast cells could be

visualized, surrounding the varying populations of lymphocytes. Similar neoplastic lymphocytes were found in the lungs, stomach, small intestine, and colon. Bone marrow histopathology revealed a high cellularity, with a M:E ratio of 2.5:1, accompanied by many large lymphomyeloid cells.

The necropsy findings, in correlation with the antemortem diagnostics and procedure performed, lead to the conclusion of widespread multicentric T-cell lymphoma, assumed as stage IV. The widespread cancer created drastic paraneoplastic changes in the forms of hypercalcemia, clotting cascade abnormalities, peripheral basophilia, and mast cell accumulation within the lymph nodes and spleen. Perimortem diagnosis was initially malignant mastocytosis, attributed as a form of leukemia, but could not explain the relationship of the neoplasm and hypercalcemia. Additionally, the malignancy panel that was performed antemortem revealed no evidence of PTH-rP. Although the referring veterinarian made an initial diagnosis of lymphoma based on lymph node cytology, evaluation of the same slide was inconclusive, and high numbers of mast cell accumulates were present in tissues examined by cytology of other lymph nodes and the spleen. Because of these findings, and the presence of a peripheral basophilia, a mastocytosis event occurring with a secondary cause to hypercalcemia were concluded as the diagnosis prior to necropsy.

Pathophysiology

Feline lymphoma, the most common hematopoietic neoplasia seen in cats, is comprise of a clustering of neoplasms that all originate from lymphoreticular cells. [3] Typically, these cancerous cells arise from lymphoid tissue, such as the spleen or lymph nodes, but can originate and develop in almost any tissue type within the body. [3] [6] As stated previously, several forms of lymphoma exist, depending on their anatomical site of development and behavior. In the case

of FeLV-negative cats, such as Jupiter, the alimentary form is the most prevalent, with multicentric lymphoma following behind. [5] In cats with FeLV infection, which are 62 times more likely to develop lymphoma than those that are negative, the lymphocytes that become malignant contain a provirus and surface FeLV structural antigens. [8] [9] Once the lymphocyte is infected, the FeLV virus will then integrate into the *myc* oncogene, leading to unregulated proliferation and eventual tumor formation. [10] In cats without FeLV infection, the exact mechanism behind lymphoma development remains unknown; however, mutations of the tumor suppressor gene *p*53 have been identified in roughly 40% of reported cases. [11]

Lymphoma is further broken down into specific cell lineage, or immunophenotyped: B-cell or T-cell; rarely, both cell lineages can be neoplastic simultaneously. In the feline species, B-cell lymphomas have been typically diagnosed more often than T-cell lymphomas, although there are recent reports of increasing incidences of T-cell lymphoma. [12] Although not well documented, research performed in human medicine has shown that T-cell lymphomas often carry a poorer prognosis than B-cell lymphomas. [13] [14]

Treatment and Disease Management

Treatment in small animals diagnosed with lymphoma falls into 4 main categories: surgical, chemotherapeutic, radiation, and supportive. Determination of the proper treatment requires properly determining the form and grading of the disease, stage of the disease, and willingness and compliance from the owner. Treatment also depends on the neoplastic characteristics and aggressive behavior of the tumor, clinical signs of the patient at the start and throughout treatment, and the patient's response to treatment. In general, treatment of lymphoma in cats is not aimed to curing, or removing all evidence of disease, but set at increasing quality of life and extending life through the possibility of remission.

Surgical treatment can be useful in treatment and diagnosis, particularly in cases where cytological aspirates or biopsies are unrewarding. In forms such as alimentary or mediastinal solitary masses, tumors can be removed to great extent and aid in the prevention of tumor-related sequela. However, surgical removal of a tumor in these cases, or for the improbable case of a solitary tumor, such as in multicentric lymphoma, does not prevent reoccurrence of disease, and should be followed with chemotherapy. [2]

Chemotherapeutic treatment remains the mainstay of treatment for lymphoma, as cats typically tolerate chemotherapy well and chemotherapy has been shown to improve life expectancy from 6 months to 2 years post-diagnosis, depending on the form and staging. [15] Treatment protocols typically include administration of cyclophosphamide, vincristine, prednisolone, doxorubicin, and L-asparaginase in combination over several weeks. In terms of drug choice, protocols including L-asparaginase and doxorubicin have resulted in prolonged survival in cats with different forms of lymphoma. [16] [17] In general, however, treatment protocols will differ based on disease response, drug tolerance, severity of side effects, and financial constraint.

Lymphoma is a very sensitive neoplasia to radiation therapy, and is successful in human medicine, but animals typically possess increased side effects to whole-body treatment. Because of this, radiation therapy is typically applied in a localized manner in the treatment of certain forms of lymphoma, such as localized cutaneous, nasal, and mediastinal lymphomas. Because radiation treatment is not applied in a systemic method, chemotherapy is often recommended as a combined therapy. [3]

Supportive therapy remains a crucial aspect for treatment of lymphoma. Many side effects from radiation or chemotherapeutic treatment exist, and combating these side effects

through supportive care is necessary for maintaining a good quality of life and aiding in the success of treatment. Supportive care is applied from the use of appetite stimulator in cats that appear anorexic, placement of feeding tubes to assure nutritional support and prevent hepatic lipidosis, treating secondary diseases associated to lymphoma, and counteracting paraneoplastic syndromes, such as hypercalcemia or hyperglobulinemia. In general, the prognosis without therapy is 6 to 8 weeks once a lymphoma diagnosis is made. [2] With the use of specified treatment protocols and supportive care, cats may respond well to treatment therapy and have significantly improved outcomes.

Paraneoplastic Considerations

Several paraneoplastic syndromes have been identified in patients diagnosed with lymphoma. Hypercalcemia, although having little evidence of prognostic significance, are most often seen in T-cell lymphomas and can cause debilitating disease. [2] T-cell lymphoma is capable of causing hypercalcemia in multiple ways such as through the production or PTH-rP, manipulating tissue macrophages to abnormally synthesize excess 1,25-dihydroxyvitamin D, destruction of bone sites, and some methods that are not yet fully understood. [3] [18]. Other common paraneoplastic responses seen are anemia, which are the most common in both veterinary and medical oncology, hypergammaglobulinemia, often monoclonal, erythrocytosis, leukemoid reactions, thrombocytopenia, especially in conjunction with chemotherapy, and coagulopathies, such as disseminated intravascular coagulation (DIC). [3] [12] In the aforementioned case of Jupiter, antemortem diagnosis of multicentric T-cell lymphoma remained very difficult, as multiple cytology samples concluded large numbers of mast cells and basophils. These reports, with the negative result of both 1,25-dihydroxyvitamin D and PTH-rP, drew a conclusion with mastocytosis and a secondary hypercalcemia of unknown origin. Albeit rare, and

not well documented in the feline patient, generalized mastocytosis has been characterized in human patients with non-Hodgkin's lymphoma. [19] The infiltrative mast cells in these few cases appears to originate from a myeloproliferative event caused by cytokine release from basophils, predominately through stimulation from IL-3, IL-5, and IL-9. [20] Immunological research has been able to describe the interactions that basophils and mast cells have in proliferating lymphocytes, but this case presents the perplexing issue of whether the malignant T-cells were capable of causing a myeloproliferation of either the mast cells or basophils or if two malignancies were simultaneously interacting with one-another.

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