A Chip on His Shoulder

A case of lymphoma in a canine patient

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Introduction

Lymphoma is the uncontrolled proliferation of neoplastic lymphoid cells arising in lymph nodes or other organs of the body (such as the skin). Lymphoma may remain localized in the organ of origin or it may spread systemically.[12] The use of glucocorticoids prior to treatment of lymphoma in the canine patient is common in veterinary medicine, though this practice is contra-indicated for many reasons. Glucocorticoids function by binding to the glucocorticoid receptor, which is a ligand-inducible transcription factor belonging to the nuclear receptor super-family.[11] A study in human medicine revealed that human acute leukemic lymphoblasts from patients still responsive to steroid therapy contain a cytoplasmic protein capable of binding glucocorticoids with an affinity appropriate to the response of the cells to steroids.[7] Glucocorticoids, once bound to the glucocorticoid receptor, induce reactions that can lead to apoptosis (programmed cell death).[9] This cell death induced by glucocorticoids causes the cells to lyse, which can mask the morphology of lymphoma cells, leading to difficulty in diagnosis via cytology or biopsy.[9] This case outlines the effect of glucocorticoids on lymphoma patients and how it can complicate its diagnosis.

History and Presentation

Chip, a 5-year-old male neutered maltipoo, presented on September 26th, 2018, to MSU-CVM Dermatology Department for ulcerated skin lesions most severely affecting his caudal back and groin. Chip originally presented to his referring veterinarian on August 24, 2018, for skin issues where he received a Depomedrol injection and was sent home on cephalexin 250 mg twice daily. Chip returned on August 31, 2018, due to his skin condition worsening. A T4 test was performed and Chip was diagnosed with hypothyroidism. Chip was then started on levothyroxine (0.3 mg) twice daily and ciprofloxacin (250 mg) once daily. On September 11, 2018, Chip again returned to his veterinarian for tachypnea, pyrexia, worsening skin issues, and

leukopenia. At this visit, Chip's owners expressed concerns about Chip drinking more than usual. Chip was diagnosed with pneumonia and was hospitalized for three days. He was given a Convenia injection, penicillin injections on 9/11 and 9/12, and a dexamethasone injection (3 mg). Chip was also started on Baytril (136 mg) once daily and a lime sulfur dip was performed. When Chip's clinical signs had improved, he was discharged from the hospital and sent home with Baytril and levothyroxine. On September 18, 2018, Chip once again presented to his veterinarian with a fever of 104.7 degrees Fahrenheit and was depressed in mentation. A complete blood count was performed with a result of an increased leukocyte count. A tick panel was submitted at this time (results were negative) and Chip was started on Doxycycline (100 mg) administered twice daily. He received another injection of Dexamethasone (3 mg) and received another lime sulfur dip. At this time, Chip was noted to have severely enlarged lymph nodes in the areas of the inguinal, submandibular, and thoracic inlet regions. The owners noted that Chip was still drinking excessively. Chip was once again hospitalized from September 18 to September 21, 2018. Over his hospitalization, it was noticed that his lymph nodes began to decrease in size and his fever had reduced. He was continued on the doxycycline and Baytril that was previously prescribed. Additionally, Chip was sent home with oral prednisone (2.5 mg) administered twice daily. A skin scrape was performed during his stay, with no results reported. Though his clinical signs were improving while being hospitalized, his leukocyte count continued to increase. On Chip's day of discharge (9/21/18), Chip was vomiting. He was given an injection of Cerenia and sent home with doxycycline (100 mg) twice daily, prednisone (2.5 mg) twice daily, Pro-Pectalin, and Cerenia (16 mg) if needed. Chip was then referred to the MSU-CVM Dermatology Department with a consult from the Internal Medicine Department.

On September 26, 2018, Chip presented to MSU-CVM Dermatology Department for his unresolved skin lesions and his continued increased respiratory effort. Upon presentation, Chip was bright and alert. He had a heart rate of 120 beats per minute, a respiratory rate of 75 breaths per minute, and a rectal temperature of 102.6 degrees Fahrenheit. Chip weighed 6.8 kg and was given a body condition score of 7/9. His mucous membranes were pink with a capillary refill time of 2 seconds. There was mild dental tartar noted on his canines, premolar, and molar teeth, with small petechiations on his mucous membranes bilaterally around his maxillary canines. Mild serous ocular discharge was noted bilaterally. His ears contained a moderate amount of yellow, ceruminous debris with mild inflammation. On thoracic auscultation, harsh lung sounds were appreciated, but no crackles, wheezes, murmurs, or arrhythmias were heard. There were multiple ulcerated skin lesions, most severely affecting his dorsum, his right side, and his inguinal area. His skin was reddened, flaky, and non-pruritic, with partial to complete alopecia around the ulcerated lesions. All palpable peripheral lymph nodes were severely enlarged and firm. His abdomen was distended but did not seem painful upon palpation.

Diagnostic Approach

On the day of presentation, blood was taken for a complete blood count and a serum chemistry, with results displaying a moderate leukocytosis (44.4 K/ul), a thrombocytopenia (109 K/ul), an increase in segmented neutrophils (37,570 /ul), decreased blood urea nitrogen (4 mg/dl), an increased ALT (355 U/L), and an increased ALP (2066 U/L).

Thoracic radiographs revealed a severe, unstructured interstitial pulmonary pattern coalescing to alveolar within the right middle lung lobe characterized by air bronchograms and a lobar sign. The sternal lymph node was enlarged, measuring at 2.0 x 1.4 cm. Abdominal radiographs revealed severe enlargement of the liver with rounded ventral margins causing

caudal displacement of the gastric axis and splenic head. Enlargement of the sub lumbar lymph nodes, located at the level of L6 and L7, was causing mild ventral displacement of the descending colon. The inguinal lymph nodes were enlarged bilaterally. There was a smoothly marginated, ovoid, soft tissue opaque nodule, which was confluent with the caudal right lateral abdominal body wall that measured approximately 3.2 x 2.0 cm.

An abdominal ultrasound revealed a small volume of peritoneal effusion between the liver and the diaphragm and dissecting between the liver lobes. The liver was diffusely enlarged and mildly hyperechoic as compared to the echogenicity of the spleen. The spleen was enlarged, hyperechoic, heterogenous, and diffusely moth eaten. The hepatic, medial iliac, jejunal, and inguinal lymph nodes were markedly enlarged, irregularly shaped, irregularly marginated, hyperechoic, and heterogenous. The urinary bladder wall was diffusely thickened and irregularly marginated. The region of the pancreas near the body and proximal right limb was thickened, hyperechoic, heterogenous, and mottled. There were occasional pin point, hyperechoic foci within the mucosal layer of the duodenum. Caudal to the kidneys, there was a large aberrant vein located just leftward of the aorta. Numerous B-lines were noted at the caudal lung-pleural interface adjacent to the diaphragm while imaging the liver. B lines are artifacts that arise from the pleural line when there is a loss of aeration and an increase in lung parenchyma.

Aspirates of the left and right popliteal and left prescapular lymph nodes were done. The results revealed high cellularity with the majority of the lymphoid cells being medium to large lymphocytes. The lymphocytes varied in size and nuclear to cytoplasmic ratio. The cells had scant to moderate amounts of pale to moderate basophilic cytoplasm and round to indented to convoluted nuclei. The chromatin was finely stippled, and some of the cells had multiple prominent nucleoli. Small lymphocytes were present in lower numbers and plasma cells were

rarely seen. Aspirates from the left popliteal lymph node also appeared to have increased numbers of nondegenerate neutrophils and low numbers of macrophages.

Aspirates of the spleen revealed high cellularity with moderate numbers of splenic stroma clusters, which often had moderate numbers of siderophages. The majority of the lymphoid cells were medium to large lymphocytes. These lymphocytes varied in size and nuclear to cytoplasm ratio. They had scant to moderate amounts of pale to moderate basophilic cytoplasm and round to indented convoluted nuclei. The chromatin was finely stippled and some of the cells had multiple prominent nucleoli. Small lymphocytes were present in lower numbers and plasma cells were rarely seen. There were also low to moderate numbers of megakaryocytes and erythroid precursors.

Aspirates of the liver were of moderate cellularity with moderate amounts of blood and lysed cells. There were moderate numbers of hepatocyte clusters. These hepatocytes often had mild to moderate amounts of cytoplasmic rarefaction typical of glycogen accumulation and some hepatocytes had mild amounts of cytoplasmic vacuolation typical of lipid accumulation. There were moderate numbers of medium to large lymphocytes, containing scant amounts of basophilic cytoplasm, finely stippled chromatin, and often multiple prominent nucleoli.

Flow cytometry was performed on the aspirates from lymphoid tissue, revealing a cell population that consisted of 95% CD8+ cytotoxic T cells. The importance of this is that it demonstrates that there indeed was a monomorphic population of cells but could not be appreciated cytologically. Cytologically, the majority of the cells seen in all aspirate samples were medium to large lymphocytes, leading to the presumption that a proliferative phenomenon such as lymphoma was taking place. A definitive diagnosis could not be made due to the lack of a monomorphic or homogenous population of lymphocytes.

Radiographically, enlargement of intra-thoracic, and intra-abdominal lymph nodes were appreciated. This, along with the enlargement of the spleen and the liver, suggests metastatic neoplastic disease. Thoracic radiographs show a pulmonary pattern that is suggestive of metastatic neoplasia and/or pneumonia.

In this case, a presumptive diagnosis was made based on the history, the physical exam, and information that came from the diagnostic procedures performed. The ulcerated skin, peripheral lymphadenopathy, the imaging, and the cytology that was highly suggestive but not confirmatory, lead to the presumptive diagnosis of lymphoma. It can also be concluded, from the thoracic radiographs and Chip's history, that Chip had pneumonia as well. Ideally, biopsies of the ulcerated skin lesions would have been performed, however, the condition of the patient and other constraints did not allow for samples to be taken for histopathology. These biopsies may have been more reliable in making a definitive diagnosis.

Pathophysiology of Disease

Lymphoma, the uncontrolled proliferation of lymphoid cells, is the most common neoplastic disorder in dogs.[8,12] The etiology of lymphoma is reported to be multifactorial, including viral infections, genetic predisposition, and environmental factors.[5] Lymphoma may remain localized in the organ of origin or may spread systemically.[12] Signs caused by lymphoma are primarily related to the organ(s) affected.[12] General signs include lethargy and decreased appetite.[11]

Diagnosis of lymphoma can be achieved in multiple ways. Fine needle aspirates of peripheral lymph nodes, masses, skin lesions, or organs may provide appropriate samples for diagnosis. In cases where FNA does not give a definitive diagnosis, histopathology, immunohistochemistry, flow cytometry, and polymerase chain reaction assay for antigen

receptor rearrangement may be options for diagnostics.[12] Other tests that can be performed in conjunction include a complete blood count, a serum chemistry panel, and/or a urinalysis.[12]

In veterinary medicine, glucocorticoids are commonly used, either alone or in conjunction with a chemotherapeutic agent, in the treatment of canine lymphoma. The current standard treatment for dogs with intermediate to large-cell lymphoma involves administration of a multiagent, CHOP-based protocol, that involves the use of cyclophosphamide, vincristine, doxorubicin, and prednisone.[8] In cases of T-cell lymphoma, protocols such has LOPP (lomustine, vincristine, procarbazine, and prednisone) have been shown to have greater effectiveness with decreased complications due to toxicity, when compared to CHOP.[4] In vivo administration of glucocorticoids has been shown to induce marked changes in the size of lymphoid organs as well as in lymphocyte circulation and to alter many immunological reactions.[6] In vitro, glucocorticoids are generally considered as catabolic agents that induce an inhibition of membrane transport and macromolecular synthesis leading to an arrest of cell growth, sometimes accompanied by cell lysis.[6]

Because of the high sensitivity of lymphoma cells to glucocorticoid-induced apoptosis, administration of glucocorticoids can mask the morphology and it has even been reported to cause tumor vanishing (complete remission).[9] This characteristic of glucocorticoids can make diagnosis of lymphoma in patients pre-treated with glucocorticoids difficult cytologically. A lot of the effects of glucocorticoids on lymphoma has to do with the number of glucocorticoid receptors in lymphoma cells and how exogenous glucocorticoids interact with these receptors. Administration of prednisone at an immunosuppressive dose to dogs caused marked decreases in the expression of phenotypic markers on lymphocytes after one day and persisted in some cases for as much as 38 days.[1] In a study by Ammersback et. al., glucocorticoids were shown to cause

a reduction in marker expression in vitro compared to saline and induced DNA fragmentation.[1] The conclusion was that glucocorticoids may affect immunophenotypic assessment for diagnostic purposes and suggests that apoptosis of lymphocytes is in part responsible for the immunosuppressive effects of glucocorticoids.[1]

In a study performed by Shipman et. al., it was found that the administration of glucocorticoids results in a measurable fall in the number of glucocorticoid receptors within malignant cells of patients with leukemia and lymphoma.[10] Glucocorticoids function by binding to their intracellular receptor, which is a ligand-inducible transcription factor.[11] Upon ligand binding, the glucocorticoid receptor complex changes its conformation and travels to the nucleus, where it interacts with co-regulators that assist glucocorticoid receptor transcriptional actions.[11] In the nucleus, the glucocorticoid receptor acts as a transcription factor mediating the up- and down-regulation of numerous genes.[11] Glucocorticoid receptors exert part of their anti-inflammatory effects by interacting with proinflammatory transcription factors, leading to the transcriptional regression of many genes.[11] In addition, membrane associated receptors for glucocorticoids affect prostaglandin synthesis and therefore the production of arachidonic acid-derived inflammatory mediators.[1]

Chronic administration of glucocorticoids can constitutively repress glucocorticoid receptor expression via an autoregulatory loop and thereby induce glucocorticoid resistance, thereby diminishing effectiveness of further glucocorticoid use.[11] A study by Gavazza et. al. also demonstrated that there was a significant correlation between pre-treatment with glucocorticoids and shorter survival times.[5] The proposed mechanism of this phenomenon was due to the up-regulation of P-glycoprotein, a transmembrane protein acting as adenosine triphosphate-dependent pump capable of forcing drugs out of the cell, and therefore, facilitating the

occurrence of chemoresistance because of a reduced penetration of chemotherapy drugs within the cell.[5]

While glucocorticoids affect diagnosis of lymphoma via cytology greatly, studies have shown that the adverse effects on biopsy are of lower incidence.[3,5] In a study investigating the effect of prebiopsy glucocorticoid administration on diagnosis of mediastinal lymphoma in children, it was found that in 22% of the cases in which glucocorticoids were administered, there was an adverse effect on diagnostic accuracy of the initial biopsy.[3] These adverse effects included non-diagnostic biopsy, a delay in diagnosis of more than 1 month, or the inaccurate staging of the disease.[3] The findings of this study, however, demonstrated that the use of prebiopsy steroids in symptomatic patients (patients who developed cardiorespiratory morbidity) is defensible considering that the incidence of diagnostic inaccuracy is low.[3] In another study investigating the accuracy of biopsy of primary central nervous system lymphoma in patients pretreated with corticosteroids, it was found that only one patient out of fourteen (7%) had a non-diagnostic biopsy after receiving 4 mg dexamethasone for three days prior to biopsy.[2] Considering this, there may be a benefit in performing biopsy over cytology as the primary diagnostic method in patients that have been pre-treated with glucocorticoids.

Glucocorticoids have been used traditionally as a treatment for lymphoma in dogs. In fact, glucocorticoids are a part of the current standard of treatment as prednisone is a main component of the CHOP protocol. Glucocorticoids cause apoptosis and cellular arrest of lymphoma cells, which is what makes them such a great treatment for lymphoma. Because of this, the onset of remission can occur quickly and lymphoid tissue can decrease in size drastically. Though glucocorticoids are a good option to include in the protocol for treating lymphoma, accurate diagnosis and a realistic assessment of staging the patient should be

performed prior to glucocorticoid administration. Glucocorticoids administration has been shown to cause apoptosis and cellular arrest, making diagnosis via cytology difficult. Administration in lymphoma cells has been shown to decrease the number of glucocorticoid receptors, leading to resistance in subsequent administrations of glucocorticoids. If a patient is pre-treated with glucocorticoids prior to diagnosis of lymphoma, biopsy of lymphoid tissue or other affected areas should be considered over cytology. If glucocorticoids are used in the treatment of lymphoma, it is also important to correctly stage the patient prior to treatment and to educate the client about how glucocorticoids are used to treat lymphoma and inform them of the possible outcomes when treating lymphoma with glucocorticoids.

Case Outcome

On September 28, 2018, fine needle aspirates were again obtained from the left and right pre-scapular lymph nodes and the right popliteal lymph node. Cytology results were similar to the previous, as there was high cellularity with the majority of cells being medium to mostly large lymphocytes. The diagnosis from this cytology was likely lymphoma. As with before, the lack of a monomorphic or homogenous population of cells hindered the ability to make a definitive diagnosis. While it cannot be confirmed, it can be hypothesized that glucocorticoid administration, prior to Chip's presentation to MSU-CVM, led to cellular arrest and glucocorticoid resistance due to a decrease in glucocorticoid receptors on the lymphocytes. Thus, leading to the difficulty in diagnosing lymphoma and the diminished effect appreciated by the referring veterinarian. Later, on the night of 9/28/18, Chip's health markedly declined. He was placed into the oxygen cage due to his inability to oxygenate sufficiently. After considering Chip's condition and poor prognosis, a conversation with the owners led to the election of humane euthanasia, which was performed on the night of 9/28/18.

Sources

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