Woolie Bully: Living with Lymphoma

Canine Multicentric Lymphoma

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Lymphoma, known alternately as lymphosarcoma (LSA), is a malignant neoplasia that plagues a variety of species at a variety of ages with many different presentations. For the purposes of this discussion, the focus will be the presentation, diagnosis, and front line therapy of canine multicentric lymphoma affecting B-cell lineage. A patient from the Mississippi State University College of Veterinary Medicine Oncology Service will be used to illustrate the clinical process of diagnosing and managing canine lymphoma.

The exact mechanism for the development of lymphoma in patients is unknown. There are multiple theories that have been found while using genomic study of affected patients, and their greatest use has been in characterizing the aggressiveness of the disease [9]. The disease occurs due to "pathologic clonal expansion" [2] of B or T cell immunophenotype lymphoid cells, which can explain lymphoma's presence in the primary and secondary lymphoid tissues of the bone marrow, thymus, lymph nodes, and spleen. Extranodal sites such as the skin, intestinal tract, liver, eyes, central nervous system, and bone can also be affected [2]. The causes of lymphoma have been studied, and though no distinctive causes have been isolated, hypothesized causes include genetic breed predisposition, environmental contamination, magnetic field exposure, chromosomal abnormalities, and immune dysfunction [2, 9].

Canine lymphoma commonly occurs in middle age to older patients, with some reports stating that intact females have a reduced risk [9]. In a review article by Zandvliet, breed predispositions were reported to include Basset hounds, Bernese Mountain Dogs, Boxers, Bulldogs, Bullmastiffs, Cocker Spaniel, Doberman Pinchers, German Shepherds, Golden Retrievers, Labrador Retrievers, Rottweilers, Saint Bernards, and Scottish Terriers [9]. The disease is less likely in breeds such as Chihuahuas, Dachshunds, Pomeranians, Poodles, and Yorkshire terriers [9].

The most common form of lymphoma found in canine patients is the multicentric form [9]. The hematopoietic neoplasm, lymphoma, has been found to represent up to 24% percent of all canine neoplasms, and 80% of those patients are diagnosed with multicentric lymphoma [5]. The form of this disease that affects the B cell lineage, as opposed to T cell lineage, has great similarity with human non-Hodgkin lymphoma [1]. For this reason, the canine had been used as a model to explore the clinical presentation, molecular biology, treatment, and response [9].

The patient, Woolie Bully, mentioned previously will now be used to further illustrate the initial clinical signs, diagnosis, and treatment of a canine multicentric B-cell lymphoma case. Woolie Bully presented to MSU CVM Internal Medicine service on September 26, 2019 for initial diagnostics. Woolie Bully was an eleven-year-old male neutered medium size mixed breed dog. On September 18th, 2019 Woolie Bully presented to his primary veterinarian with severe lymphadenopathy, lethargy, and a fever. He was prescribed amoxicillin/clavulanic acid (Clavamox) and referred to MSU CVM Internal Medicine for the probable diagnosis of lymphoma.

Upon presentation to the internal medicine service, Woolie Bully was dull, alert, and responsive and weighed 12.9 kilograms, with a body condition score of 6/9. He had a fever denoted by a temperature of 104.1 degrees Fahrenheit. His pulse was 136 beats per minute with strong and synchronous pulses. Cardiac auscultation revealed a grade I/VI left apical systolic murmur. His respiratory rate was 28 breaths per minute with normal bronchovesicular sounds and no auscultation of crackles or wheezes. There was severe enlargement of the mandibular, prescapular, inguinal, and popliteal lymph nodes. The thorough history and physical examination is an essential part of the diagnostic process, as it will be used as a part of the staging process to be discussed later [4]. Following the history and physical examination, diagnostics were performed

to confirm lymphoma as well as rule out any infectious etiologies that could be responsible for lethargy, fever, and peripheral lymph node involvement. Differential diagnosis at this stage included primary lymphoid neoplasia, systemic infection, and lymphoid reactive hyperplasia. An occult heartworm 4dx test was used to rule out the rickettsial diseases caused by *Ehrlichia canis* and *ewingii*, *Borriella burgdoferi*, and *Anaplasma phagocytophilum* and *platys*, as well as *Dirofilaria immitis*. The 4Dx test was below detectable limits for *Ehrlichia*, *Borriella*, and *Anaplasma* antibodies, as well as *Dirofilaria immitis* antigen. An initial complete blood count revealed a white blood cell count within reference intervals, a mild hyperchromasia, moderate neutrophilia. A serum chemistry profile resulted in values within reference intervals.

Diagnostic imaging was then performed. Thoracic radiographs were interpreted to contain a bronchial pulmonary pattern, presumptive tracheobronchial and mediastinal lymphadenopathy. They showed no evidence of nodular pulmonary neoplasia. Abdominal radiographs interpretation revealed mild splenomegaly and suspected lymphadomegaly of a structure ventral to the L6 and L7 vertebrae. An abdominal ultrasound and fine needle aspirates of the spleen and liver were performed. Lymphoma was diagnosed in the spleen and multifocal lymphadenopathy was found throughout the abdomen. Fine needle aspirates of the peripheral lymph nodes supported the presumptive diagnosis of lymphoma. The presumptive diagnosis became a definitive one when the fine needle aspirates of the peripheral lymph nodes were reported by Matthew Williams, DVM, DACVP to contain medium to large lymphocytes with scant basophilic cytoplasm, finely stippled chromatin, prominent nucleoli, and high numbers of mitotic figures. The result of flow cytometry was B cell lymphoma.

The diagnostic process followed throughout Woolie Bully's case is supported in the literature as the gold standard approach. The diagnostic process is centered on ruling out

infectious and inflammatory etiologies, and staging the severity of the resulting disease for appropriate treatment and prognosis using bloodwork, diagnostic imaging, cytological and histologic examination, and immunophenotyping [3]. Initial bloodwork in patients with presumptive lymphoma often results in a mild to moderate non-regenerative anemia, often with an accompanying hypercalcemia as a common paraneoplastic syndrome, which is a common negative prognostic indicator in these patients [4, 9]. Bone marrow biopsies are controversial because while there is bone marrow involvement in a majority of cases, the presence of bone marrow infiltration often does not change the therapeutic protocol for the patient [4, 9]. Diagnostic imaging should be performed to identify abnormalities, though they may be non-specific, to aid in supporting a diagnosis of lymphoma and staging to guide further treatment and prognosis [9].

Perhaps the most important diagnostics performed by a clinician are cytological examination of fine-needle aspirates and flow cytometry. These diagnostics allow the clinician to determine a definitive diagnosis and immunophenotype of the patient, helping to determine the prognosis and guide treatment protocols. Cytologic examination of lymph node fine-needle aspirates is the method of choice for most clinicians when diagnosing high-grade lymphoma because of its efficiency and cost-effectiveness [9]. Histological biopsy, through excisional or tru-cut methods, may aid in diagnosis of low-grade lymphoma through characterization of morphologic criteria such as growth pattern, nuclear size, nuclear morphology, mitotic index, and immunophenotype [9]. The characterization of lymphoma as either B-cell or T-cell in origin is important to know because of how it affects prognosis, which will be discussed later in this paper [6]. Most commonly, immunophenotyping using either biopsy samples or flow cytometry has been the method of choice for characterization of the tumor as either B-cell or T-cell [1]. Another method that has been applied and will be briefly mentioned is PCR assay for antigen receptor

rearrangement (PARR). This method uses amplification of the immunoglobulin genes and T-cell receptors to detect clonality, looking for monoclonal populations to support a diagnosis of lymphoma [1]. Understanding and pursuing characterization of the cell types involved in a specific case of lymphoma is important for a clinician to pursue appropriate treatment and communicate with the client regarding and accurate prognosis.

The importance of immunophenotyping the lymphoma is to provide an accurate prognosis for the patient to communicate about appropriate treatment with the owner. A majority of multicentric lymphoma patients are B-cell in origin, with prevalence as high as 70% in a review article by Zandvliet [9]. In general, T-cell lymphomas have shorter remission and survival times than B-cell lymphomas. A study by *Mutz et al* found that patients with B-cell lymphoma had a progression free survival of 264 days compared to 33 days for dogs with T-cell lymphoma [5]. Taking into account the cost of specific treatment options with typical response to treatment can provide owners with the information necessary to make an informed decision for their dogs.

Because lymphoma is a disease with such variety in clinical presentation and disease progression, unified staging systems are an important part of the diagnostic process to help provide guidelines for the treatment and prognostic decisions necessitated by clinicians. There are different options regarding staging systems, but because the World Health Organization (WHO) staging system was used in Woolie Bully's diagnosis, that is the classification system that will be discussed in this paper. The WHO staging system is based on histopathological and immunophenotypic features [6]. The basis of the WHO classification system considers the location of the disease and presence or absence of clinical signs [9]. Stage I patients have disease limited to a single lymph node or lymphoid tissue in a single organ, where stage II patients have regional lymph node involvement of multiple lymph nodes, possibly including the tonsils. Stage III

patients have generalized lymph node involvement, and stage IV have involvement of the liver and or spleen, with or without lymph node enlargement. Stage V includes patients with involvement of the blood or bone marrow most commonly, though patients can have involvement of any other organ not previously mentioned. Patients can further be classified into substage a or b depending on their systemic signs. A patient with an absence of systemic signs is considered substage a, while those with systemic signs such as fever, weight loss of greater than 10%, or hypercalcemia are classified as substage b [9]. Using this staging system, Woolie Bully's multicentric B-cell lymphoma was classified as a stage IV b, meaning that there was involvement of his spleen and systemic signs of fever present.

Woolie Bully's owners elected to pursue chemotherapy as treatment for his lymphoma at his initial visit on September 26, 2019. Chemotherapy is considered the treatment of choice due to lymphoma's systemic nature, and the goal of treatment is to balance a high complete response rate and long response duration with minimal appointments, drug administrations, and toxicities [9]. The initial protocol initiated in Woolie Bully's case was the CHOP protocol, which combines cyclophosphamide (250 mg/m^2), doxorubicin (hydroxydaunorubicin) (30 mg/m^2), vincristine (Oncovin) (0.7 mg/m^2), and prednisolone (1.5 mg/kg) tapering dose. He was treated supportively with as needed doses of metronidazole and ondansetron for gastrointestinal side effects. Prior to doxorubicin administration, his fractional shortening was measured due to doxorubicin's cumulative cardiotoxicity, and treatment was initiated as long as it was within normal limits. Aside from the cardiotoxic effects of doxorubicin, gastrointestinal disturbances may occur following administration and be managed with anti-diarrheals or antiemetics preemptively at subsequent treatments [8]. A complete blood count was performed before administration of any chemotherapeutic agent to ensure that there

were appropriate levels of white blood cells. The amount of neutrophils was closely monitored since they would be the first to respond. On October 4th, 2019, less than one month after the initiation of chemotherapy, Woolie Bully was in remission. He continued his chemotherapy protocol until January 21st, 2020, when he was discharged. One week after his administration of vincristine (dose 0.7 mg/m^2) he had a grade III neutropenia, so his cyclophosphamide dose was not administered, and instead amoxicillin/clavulanic acid (Clavamox) was prescribed to prevent any infection before his neutrophils could rebound. Chemotherapy-induced neutropenia has been shown in a study by Wang et. al. to be associated with increased remission and survival times [7]. This correlation is hypothesized to occur because it signifies that rapidly dividing cells, both neoplastic cells and bone marrow stem cells, are affected by the prevention of cellular replication and the induction of apoptosis. Therefore, a myelosuppressive response, characterized by neutrophils due to their short life spans, signifies that the chemotherapy protocol is working. Neutropenia is graded into four categories, with measurements of 1500-2999/uL, 1000-1499/uL, 500-999/uL, and <500/uL being grades 1-4, respectively [7]. Throughout his treatment, Woolie Bully's lymph nodes consistently measured less than 1 centimeter in diameter for the next six months, until he presented to MSU CVM Emergency Department for enlarged lymph nodes.

At this point, Woolie Bully had relapsed and was out of remission. Rescue chemotherapeutic agents were used over the next four and a half months. During this time, there was no notable response to the rescue chemotherapeutic agents used, including Lomustine (CCNU) (60mg/m^2), Tanovea (1mg/kg), CHOP, L-asparaginase (10,000 units/m^2, single dose doxorubicin (1mg/kg), and mitoxantrone (5.5mg/m^2). During the administration of his rescue protocols, Woolie Bully was diagnosed with osteosarcoma of his right scapula through cytological examination. He was able to ambulate well, so a palliative protocol for pain

medication of Tylenol 4 and Gabapentin was prescribed to be used as needed. On 7/23/2020, 306 days after his initial treatment, Woolie Bully's condition deteriorated and he was humanely euthanized.

Chemotherapy protocols often rely on cytotoxic effects to limit disease. However, it is not uncommon for these protocols to fail due to development of drug resistance over time. It is for this reason that cytotoxic drugs initially achieve response, but are unlikely to successfully control disease long-term [10]. Since chemotherapy protocols do not cure the disease long term, coupled with the cost of the protocols, some owners may elect for palliative care. For these patients, glucocorticoids are usually the drug of choice. They are found as part of chemotherapeutic protocols as well, since they induce lymphocyte and lymphoblast apoptosis [9]. Using glucocorticoids alone typically allows for 60-90 days of partial to complete response [9]. One main contraindication when utilizing glucocorticoids as a general practitioner, is that there are studies showing that patients treated initially with glucocorticoids prior to beginning a chemotherapy protocol have lower response rates and shorter remission times [9]. Therefore, clinicians should encourage clients to decide on a definitive course of action prior to beginning any therapy [9]. If the client chooses to pursue a palliative course of treatment for their dog, relapse will often occur after a few months due to resistance to apoptosis [10]. There are many appropriate therapies available, both palliative and chemotherapeutic, that warrant thorough discussion with canine patients diagnosed with multicentric B-cell lymphoma. If an owner elects to treat on a presumptive diagnosis, it is important to rule out any infectious causes of lymphadenopathy and systemic illness, as immunosuppression in the face of an undiagnosed infection could cause rapid deterioration of the patient.

In conclusion, lymphoma is a complex disease that can present in a variety of ways. Proper diagnosis of a canine patient with enlarged lymph nodes includes ruling out infectious causes before performing baseline bloodwork, diagnostic imaging of the thorax and abdomen with aspirates when appropriate, lymph node aspirates, and immunophenotyping. After these initial diagnostics, a patient should be classified using the WHO Classification system. Determining a patient's stage will help guide a discussion with the owners regarding prognosis for that specific patient's presentation. In the case of a typical canine multicentric B-cell lymphoma diagnosis, the CHOP chemotherapeutic protocol is considered the first line treatment of choice. Rescue protocols can be instituted when relapse of the patient occurs. At this time, there are a variety of medications that can be instituted at the discretion of the oncologist. If a client elects for a palliative course of therapy, glucocorticoids are most effective. Regardless of the type and duration of therapy decided on by the owner, there is a variety of options that veterinarians can utilize to maximize the comfort and quality of life of their patients affected by this disease.

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