

## **Canine lymphoma: Different faces with different treatment recommendations**

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### **CANINE LYMPHOMA**

The malignant transformation of any lymphocyte population, termed lymphoma, is the most common hematologic malignancy observed in dogs. Like many diseases, lymphoma is not a singular cancer; rather, divergent categories of lymphoma behave differently and require specific therapies. Canine lymphoma bears a similarity to the non-Hodgkin's lymphomas (NHL) in humans and both exhibit similar responses to treatment with chemotherapy. Treatment of lymphoma in dogs can be extremely rewarding. Standard chemotherapeutic protocols can provide many dogs with prolonged survival times and high quality of life scores. Additionally, newer therapies have been developed and validated in pet dogs, and provide pet owners with different and effective ways to control disease for prolonged periods of time. Given the prevalence of this malignancy and the potentially rewarding outcomes associated with systemic chemotherapeutic treatment and other therapies, the focus of this presentation is to highlight the important clinical facts known about canine lymphoma.

### **Clinical Findings**

Clinical findings for canine lymphoma can be quite variable and are often dependent upon anatomic form, clinical stage and substage of disease. In dogs, four recognized anatomic forms of lymphoma exist and include multicentric, alimentary, mediastinal, and extranodal (renal, central nervous system, and cutaneous). Multicentric lymphoma is the most common form and may account for up to 84% of all canine patients diagnosed with this hematopoietic malignancy. Most dogs with multicentric lymphoma present for a chief complaint of non-painful, generalized, peripheral lymphadenopathy. In addition to peripheral lymphadenopathy, malignant lymphocytes may infiltrate into other organs including the spleen, liver, bone marrow, and other extranodal sites; constitutional signs including lethargy, anorexia, and depression may be observed due to significant tumor burden.

Alimentary lymphoma is much less common and accounts for approximately 5-7% of all canine lymphomas. Dogs with alimentary lymphoma may manifest with significant gastrointestinal signs, including anorexia, vomiting, diarrhea, and profound weight loss secondary to severe malabsorption and maldigestion of nutrients. Mediastinal and extranodal forms of lymphoma account for the remaining portion of lymphoma observed in the dog. Mediastinal lymphoma is characterized by enlargement of the cranial mediastinal lymph nodes and/or thymus. Because the thymus serves as the central lymphoid organ for maturing T lymphocytes, many mediastinal lymphomas are a malignancy of T lymphocytes. Dogs with mediastinal lymphoma may manifest with respiratory signs associated with pleural fluid accumulation, direct compression of adjacent lung lobes, or superior vena cava syndrome. A complaint of primary polyuria with compensatory polydipsia in a dog with suspect neoplasia should alert the clinician to the possibility of a hypercalcemia of malignancy, a paraneoplastic syndrome seen in up to 40% of dogs with mediastinal lymphoma.

The clinical findings associated with extranodal lymphoma, including involvement of the skin, lungs, kidneys, eyes and central nervous system, can be quite variable. Cutaneous lymphoma can manifest with a wide spectrum of clinical presentations ranging from solitary, raised, ulcerative nodules to generalized, diffuse, scaly lesions. Cutaneous lymphoma can be categorized as either epitheliotropic or nonepitheliotropic. Epitheliotropic lymphoma tends to be a malignancy of T lymphocytes, while nonepitheliotropic lymphoma tends to be a neoplastic expansion of B lymphocytes. Mycosis fungoides is a variant of epitheliotropic lymphoma and is reported to be the most common form of cutaneous lymphoma observed in the dog.

## **Diagnosis**

The diagnosis of lymphoma is many times straightforward. Definitive diagnosis can be obtained by either cytologic or histopathologic evaluation of the affected organ system. Because multicentric lymphoma accounts for the majority of cases seen in the dog, fine-needle aspiration (FNA) of an enlarged peripheral lymph node is often adequate to make a definitive diagnosis. Cytologically, lymph node aspirates will usually identify a monomorphic population of large lymphoblastic cells. Although cytologic diagnosis of lymphoma can be performed with ease, cytology is unable to differentiate and to categorize the wide spectrum of lymphomas with regard to morphologic pattern (diffuse versus follicular) and immunophenotype (B versus T lymphocyte). Due to these constraints of cytology, histopathologic tissue evaluation has proven invaluable for the further classification of lymphomas. Immunophenotyping using monoclonal antibodies against cell surface glycoproteins, termed cluster of differentiation (CD), allows the identification of lymphoid malignancies of either B lymphocyte origin (CD79+) or T lymphocyte origin (CD3+).

### Diagnostic tests

Immunophenotyping (cytology, histopathology, or flow cytometry): Using antibodies against specific cell surface markers (ex. B cell CD 79a/CD20, T cell CD3/CD4/CD8), this test is primarily used to determine if the lymphoma is B or T cell in origin. However, it can also be helpful to support a diagnosis of lymphoma by documenting a homogenous population of the same immunophenotype within a tissue.

Flow cytometry: This test allows immunophenotyping of cells in suspension (blood, effusions, and aspirates of LNs or organs). Flow cytometry can also provide information regarding cell size and expression of other CD molecules that may correlate with prognostic information.

PARR (PCR for antigen receptor rearrangement): Theoretically, a malignant cell population should be derived from expansion of a single clone. PARR amplifies the variable regions of the T cell receptor or Immunoglobulin (Ig) receptor gene to detect the presence of clonal lymphocyte populations. When it is not possible to differentiate between malignant and benign lymphocytes based on cytology or histopathology alone, PARR may be helpful to confirm a diagnosis (especially useful when the lymphocyte population is heterogeneous). PARR can be used to detect minimal residual disease but investigations are ongoing to determine if this is a useful clinical marker of early recurrence.

## **Clinical Staging**

Once a diagnosis of lymphoma has been made the extent of neoplastic involvement should be determined. The World Health Organization (WHO) 5-tier staging system is routinely used to stage dogs with lymphoma. Most dogs are presented with advanced disease and are categorized as stage III or IV. In addition to the WHO 5-tier staging system, dogs with lymphoma can be further categorized by clinical substage. Dogs manifesting without systemic signs of illness are classified as substage A, and dogs with systemic signs of illness as substage B.

For most dogs with multicentric lymphoma, diagnostic evaluation should include a complete blood count (CBC), serum chemistry panel, urinalysis, thoracic radiographs, and abdominal ultrasound. Thoracic radiographic changes consistent with lymphoma may include diffuse or localized pulmonary infiltrates, thoracic lymphadenopathy, and cranial mediastinal mass effects. Suspected neoplastic infiltration of peripheral lymph nodes, liver or spleen should be evaluated with FNA cytology. Dogs with anemia, thrombocytopenia, and/or leukopenia could be evaluated with a bone marrow aspirate to confirm or deny neoplastic infiltration. Dogs manifesting with neurologic abnormalities may require

advanced imaging studies, including computed tomography or magnetic resonance imaging with cerebrospinal fluid analysis. Cerebrospinal fluid analysis from dogs with central nervous system lymphoma typically will have elevated protein and cell counts, with identification of the malignant population of lymphocytes in a high percentage of affected dogs.

### **Prognostic Factors**

Despite the high response rate of canine lymphoma to systemic treatment, small subsets of dogs fail to benefit from even aggressive combination chemotherapy. The reason behind treatment success and failure is likely to be multifactorial. However, several prognostic factors have been identified in predicting a patient's response to therapy and survival time. Traditionally accepted prognostic factors include WHO clinical substage, histologic grade, immunophenotype, and anatomic location. Dogs manifesting with constitutional signs of systemic illness, categorized as substage B, tend to have a worse prognosis than substage A dogs not manifesting with systemic illness. Histologic grade or subtype of lymphoma appears to have an influence on response to chemotherapy and overall length of survival. Dogs with lymphoma histologically classified as being either intermediate- or high-grade tend to be highly responsive to chemotherapy, but early relapse is common with shorter survival times. Contrarily, dogs with lymphoma histologically classified as being low-grade have a lower response rate to systemic chemotherapy, but experience a positive survival length advantage when compared to intermediate- or high-grade tumors. In addition to histologic classification, the type of malignantly transformed lymphocyte appears to affect prognosis. Dogs with T-cell lymphomas have a shorter survival time when compared with dogs with B-cell malignancies. The anatomic form of lymphoma appears to affect survival times. Dogs with diffuse alimentary, central nervous system, or cutaneous lymphoma are afforded shorter survival times when compared to dogs with other anatomic forms of lymphoma. Recently, it has been shown that B-cell lymphomas expressing low levels of class II MHC or lower than normal levels of B5 antigen also had a poorer prognosis. Presence of anemia is also associated with a worse prognosis. Alternatively, it appears that dogs with indolent lymphoma experience prolonged survival times.

### **Standard treatment options**

Multiagent chemotherapy is the mainstay of treatment for lymphoma. For intermediate to high grade lymphomas, CHOP-based protocols are typically advised as first line therapy and provide the best response rates (80-95%) and treatment outcomes. At this time, long term maintenance chemotherapy does not appear to improve remission times. Additionally, dogs that do not receive maintenance therapy appear to be more likely to achieve a second remission following relapse. Several studies suggest that inclusion of L-asparaginase in the protocol does not significantly improve outcome (remission rates or duration of remission). Individual response and remission durations vary depending on prognostic factors. Overall median survival times are 12-14 months with approximately 20-25% of dogs alive at 2 years. Alternative protocols are offered if clients need less costly or more convenient options.

Rescue chemotherapy is associated with lower response rates and shorter remission times. Chemotherapy agents that are commonly used in the rescue setting include lomustine (CCNU), doxorubicin, mitoxantrone, MOPP (mustargen, vincristine, procarbazine and prednisone), actinomycin-D, and dacarbazine (DTIC).

### **Novel treatment options**

Monoclonal Antibodies (Mab): Outcome improvements in people with non-Hodgkin's lymphoma have been due in large part to Mab therapies such as rituximab (anti-CD20 antibody used to treat B-cell

lymphomas). However, rituximab is not effective in dogs. Currently, clinical studies are ongoing to evaluate two conditionally approved monoclonal antibodies (Aratana Therapeutics) for use in the treatment of canine lymphoma. These promising canine-specific antibodies are directed against CD20 (AT-004) for B-cell lymphoma and CD52 (AT-005) for T-cell lymphoma.

CD20 Vaccine: This strategy tries to trick the immune system into attacking all cells with the expression of CD20, which is found on B lymphocytes. While vaccination appears to be clinically safe, the results of a pivotal trial are still pending and it cannot be stated whether this treatment option is effective in controlling lymphoma through harnessing the immune system.

Bone Marrow/ Stem cell transplantation: Ablative total body irradiation and/or chemotherapy combined with bone marrow or stem cell transplantation is available for dogs with lymphoma. However, these treatments are not widely accessible, are costly, and are associated with increased morbidity in dogs undergoing treatment. While these treatments present a potential for increased cure rates, results of a large number of treated cases have yet to be reported.

Adoptive T cell therapy: Expanded autologous T cells infused after CHOP chemotherapy has been shown to significantly improve overall and disease-free survival in a small number of dogs with B cell lymphoma. While quite promising, this therapy is currently available to client-owned dogs only through clinical trials.