Canine Lymphoma: Clinical Overview and Current Therapeutic Options for Veterinarians

Cancer is a major cause of morbidity and mortality in dogs. Approximately 25% of dogs will die as a result of cancer. Lymphoma is one of the most common cancers in dogs and is the most common one treated with chemotherapy. It is estimated that 8% of all cancers in dogs annually are diagnosed as lymphoma or lymphosarcoma (LSA). Certain dog breeds have an increased incidence of lymphoma. The incidence generally increases as dogs age with middle age and older age dogs most commonly affected. Conditional license has been granted by the USDA, for canine-specific monoclonal antibodies as aids in the treatment of B-cell and T-cell lymphoma in dogs, bringing new beginnings for these dogs and their owners.

Clinical Presentation of Lymphoma

The clinical signs associated with canine lymphoma are variable and depend in part upon the extent and location of the tumor. Dogs are often presented with one or more lymph nodes enlarged (lymphadenopathy). Feeling a lump under the chin or near a shoulder is often the first sign that an owner will notice. Involvement of other organs may indicate a more advanced disease. Many dogs exhibit few, if any, clinical signs of illness early in the course of the disease. Owners may report reduced exercise tolerance, fatigability or mild inappetence. If the dog has hypercalcemia of malignancy it may present for polydipsia and polyuria. As the disease progresses, dogs may show weakness, depression, anorexia and vomiting. Dogs may become dyspneic due to respiratory tract obstruction from lymph node enlargement. If lymphoma invades the gastrointestinal tract, signs may include vomiting, diarrhea, weight loss, and malabsorption.

Types of Lymphoma

The term lymphoma actually describes a diverse group of cancers derived from the white blood cells called lymphocytes. Anatomically, lymphoma is often categorized by location. Approximately 80% of lymphoma cases involve multiple lymph nodes. In some cases, lymphoma may involve other tissues, such as the skin. In later stages of
disease, the cancer cells become more numerous and are found in multiple organs not usually associated with lymphatic tissue, such as the liver. Classification of malignant lymphoma in the dog is based on anatomic location, histologic criteria, and immunophenotypic characteristics. With increasing knowledge of the maturation and differentiation of lymphoid cells, new classification systems have been developed; which are extensive and list more than 30 subtypes of lymphoma.4

Histologic systems have been developed to classify non-Hodgkin’s lymphoma (NHL) in humans and some of these systems have been adapted to be used in dogs. Classification systems such as the National Cancer Institute Working Formulation and an updated Kiel system from human medicine have been adapted to canine tumors. The World Health Organization (WHO) publishes a system for human NHL that incorporates anatomic, histologic, and immunophenotypic criteria. This has been adapted to a WHO system for classification of canine lymphoma.4

Historically, classification systems were based entirely on the morphologic characteristics of the malignant lymphocytes and location in the body where the cancer is most prominent. (See Table 1) The ability to further differentiate cells immunophenotypically has led to additions and revisions of this historical classification system.

### Table 1: Classification Based on Anatomic Characteristics

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
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<tr>
<td>Multicentric Lymphoma</td>
<td>Mostly affects the external lymph nodes but may or may not involve other organs. Common clinical signs include loss of appetite, lethargy and weight loss. Accounts for 80% - 85% of cases.3</td>
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<td>Mediastinal Lymphoma</td>
<td>Develops in the lymphoid tissue in the chest, in tissues near the heart.</td>
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<tr>
<td>Cutaneous Lymphoma</td>
<td>Involves the skin and may cause extreme discomfort. Lesions appear as ulcers, nodules, plaques and red or flaky skin. Can be pruritic.</td>
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<tr>
<td>Gastrointestinal Lymphoma</td>
<td>Involves the stomach and/or intestines. The clinical signs include vomiting, diarrhea, weight loss and malabsorption.</td>
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<tr>
<td>Central Nervous System Lymphoma</td>
<td>May be primary, but is most commonly the result of the spread of multicentric lymphoma.</td>
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To be clinically useful, classification systems should yield information about response to therapy, maintenance of remission, and survival. Not all pathologists use the same system, however, most diagnostic reports will differentiate whether or not the tumor is high-grade (diffuse large cell, centroblastic, and immunoblastic) or low-grade (small lymphocytic or centrocytic lymphoma), and the histologic structures involved. Recommendations for determination of cell origin are given. High-grade lymphomas are more common and are characterized as being biologically aggressive and progressing more rapidly. Low-grade lymphomas are less common and are characterized by a slow growth rate and indolent biological behavior. Biopsy and histopathology are necessary to confirm a diagnosis of indolent lymphoma.

**Definitive Diagnosis**

Currently, no single test exists to diagnose, classify and stage lymphoma in dogs, so several tests are often recommended. Dogs with lymphoma often present with acutely enlarged lymph nodes.

There are other causes of enlarged lymph nodes that need to be ruled out. These include:

1) systemic infections — bacterial, fungal, parasitic, viral, and rickettsial;
2) immune-mediated disease;
3) reactive lymphadenopathy due to dermal disease;
4) nodal metastatic solid tumors.

Many tests are available to aid in the diagnosis of canine lymphoma. Multiple tests are often conducted, either in conjunction with each other or in sequence, to enhance the accuracy of identifying the type of lymphoma and possibly assist in predicting prognosis and response to therapy.

**Fine-Needle Aspirate (FNA)**

Fine-needle aspirates are often the first tests to be conducted. Sample collection and slide examination can be performed in-house with minimal resources. Romanowsky stains such as Diff Quick or Wright Giemsa are used on the sample; monotonous populations of large- or medium-sized lymphoblasts are typical of lymphoma. Occasionally, FNA of a diffuse lymphoma may yield a population of small or intermediate cells that are difficult to differentiate from benign lymphocytes.

In cytologic samples of high-grade lymphoma, most of the lymphoid cells are large and often have visible nucleoli and basophilic cytoplasm. Patients with low-grade or indolent lymphoma may have been diagnosed cytologically as lymphoid hyperplasia. Small-cell and intermediate-cell lymphoma is difficult to determine from cytology alone, and may require
additional supportive evidence. Early lymphoma is also often difficult to differentiate from reactive hyperplasia. Immunocytochemistry may be utilized to further classify cells into phenotype (B-cell vs. T-cell).

**Histopathology**

Histopathologic examination provides diagnostic information, as well as additional information about tissue architecture, cell distribution, and the number of mitotic figures. Excisional or Tru-Cut biopsy samples are the best to obtain definitive information. Other tests can also be conducted on the paraffin-embedded tissue to further classify the lymphoma. Diagnostic studies using immunohistochemistry further classify lymphomas. With this better understanding of maturation and differentiation of lymphoid cells, more diagnostic capabilities have evolved. Histologically, effacement of normal nodal architecture by neoplastic lymphocytes and capsular disruption is characteristic of lymphoma rather than reactive hyperplasia.

**Flow Cytometry**

The types of lymphocytes can also be identified using flow cytometry. Neoplastic cells in blood and nonhemorrhagic fluid samples, such as peritoneal fluid and pleural fluid, can be evaluated. Solid tissue aspirates can be processed to obtain cell suspensions, which can then be tested. Specific antibodies are used to detect cell surface proteins. The patterns of expression or specific markers have diagnostic and prognostic value. For example, CD-21 is a marker commonly found on B-cells and CD-3 is found on T-cells.

**Polymerase Chain Reaction to Detect Clonal Receptor Gene Rearrangement (PARR)**

A newer test in veterinary medicine, polymerase chain reaction to detect clonal receptor gene rearrangement (PARR) is a promising technique. Fewer than 10 veterinary laboratories around the world are conducting PARR testing at this time and clinicians are advised to contact their individual laboratories to determine the sample needed and the nature of test interpretations. In the future, PARR may become more readily available. Using PARR allows one to examine whether there is one foundational cell to a population or whether the population is heterogenous; in other words, it allows one to look at whether the proliferating cells are cancer (one founder) or reactive (lots of cells responding to an infection). It is a very sensitive technique and may be useful in detecting early lymphoma cases, or determining if dogs with cells that appear mature on cytology are lymphoma cells (e.g., follicular lymphoma).

**Staging Lymphoma**

When lymphoma is diagnosed, the extent of the disease should be determined, along with any unrelated or secondary conditions treated or controlled, before starting the appropriate therapy. Staging is based on the results of physical examination, clinical laboratory testing, histopathology, and imaging procedures.
In general, dogs in lower stages have higher remission rates, longer remission durations, and longer survival times than dogs in Stage V disease. However, staging is inconsistent with regard to predicting treatment outcome/prognosis, and other factors like anatomic location, immunophenotype, and histopathologic grade are stronger predictors.

One of the most clinically relevant predictors of survival is diagnosing the substage (a or b), with substage b having a more guarded prognosis.

The World Health Organization has described different stages of lymphoma in humans based on its degree of metastasis and invasiveness. The WHO staging system has also been adapted for use in canine lymphoma.³

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<th>Stages of Canine Lymphoma</th>
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<td>Stage I:</td>
<td>Involvement restricted to a single lymph node or lymphoid tissue in a single organ (excluding the bone marrow).</td>
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<tr>
<td>Stage II:</td>
<td>Involvement of many lymph nodes in a regional area.</td>
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<tr>
<td>Stage III:</td>
<td>Generalized lymphadenopathy.</td>
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<tr>
<td>Stage IV:</td>
<td>Liver and/or spleen involvement (with or without lymphadenopathy).</td>
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<tr>
<td>Stage V:</td>
<td>Bone marrow, central nervous system, or involvement of other extranodal sites.</td>
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Each stage is sub-classified into:
- **Substage a** – Without systemic signs of disease.
- **Substage b** – With systemic signs of disease.

![Canine Lymphoma Image]

In Order to Stage Canine Lymphoma, the Following Tests Should be Considered.

1. **Complete Blood Count (with platelet count)** – The blood smear should be screened for the presence of circulating lymphoblasts.

2. **Biochemistry Profile** – Baseline values are important to evaluate organ function and determine if paraneoplastic hypercalcemia is present. Hypercalcemia is more common with mediastinal lymphoma or T-cell lymphoma.

3. **Urinalysis** – Urinary tract infections should be treated to decrease the risk of sepsis when chemotherapy is started.

4. **Thoracic Radiographs** – Masses and evidence of pulmonary infiltrate or pleural effusion should be evaluated.

5. **Abdominal Imaging** – Abdominal ultrasound is usually more sensitive than abdominal radiographs. This is recommended as baseline when the dog presents with gastrointestinal signs or if kidney and/or liver changes are noted on the baseline screening.

6. **Bone Marrow Aspiration** – Even dogs with normal CBC may have bone marrow involvement with lymphoma.
**Current Approaches to Cancer Treatment**

Advances in therapy have extended not only length of life, but also the quality of life for dogs with lymphoma. Chemotherapy is the most commonly recommended treatment, and combinations of drugs are currently the most common protocols followed. It should be noted, lymphoma is likely to return even when treated aggressively.

With multiagent chemotherapy, the median survival time for a dog with lymphoma is approximately one year with 25-percent of dogs living two years or longer. Along with clinical stage and substage being important prognostic factors, phenotype is an important prognostic factor. In general, with current therapies, B-cell has a longer remission duration and longer survival times; while T-cell has a shorter remission duration and shorter survival times.

Although dogs generally do not experience the hair loss that affects humans undergoing chemotherapy, there are definitely risks and common adverse events associated with treatment. The most common adverse effects of cancer chemotherapy in dogs are gastrointestinal (loss of appetite, vomiting, diarrhea) or a decreased white blood cell count which may increase the risk of secondary infections.

#### Current Treatment Options

- **No Treatment** – Survival is dependent upon several factors including stage of disease at diagnosis as well as type of lymphoma.
- **Prednisone Alone** – Palliative option - Caution - Prior use of steroids may affect the response to chemotherapy.
- **Single Agent Therapy** – Doxorubicin alone in dogs with B-cell lymphoma achieve a median survival time of 5 months.
- **Multidrug Protocols** – Adjustments to the protocols may be needed depending upon the patient.

**Common Protocols Include:**

- **VELCAP** – Vincristine, Elspar® (asparaginase), Cyclophosphamide, Adriamycin, Prednisone.
- **CHOP** – Cyclophosphamide, Hydroxydaunorubicin (doxorubicin), Oncovin® (vincristine), Prednisone.
- **ACOPA II** – Cyclophosphamide, Vincristine, Prednisone, Doxorubicin, and L-asparaginase.

- **Radiation or Surgery** – Radiotherapy or surgery may be indicated when there is a solitary tumor other than in the lymph node. These patients may or may not require systemic chemotherapy initially, but are at risk for systemic disease in the future. Irradiation has also been used in adjunct with chemotherapy between cycles or following chemotherapy induced remissions.

#### New Treatment Options

- **MAb** – Monoclonal Antibody therapies are currently being used on a limited basis, in selected clinics to collect additional data under a conditional license issued by the USDA. Additional studies are in progress to support the safety and effectiveness of this new therapeutic option.
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Treating Lymphoma in Dogs

Some of the more aggressive lymphomas are unresponsive to any available treatment, and current options to treat lymphoma in dogs are limited to mostly chemotherapy. The most common multi-agent chemotherapy, commonly known as CHOP, is the University of Madison-Wisconsin 19-week combination chemotherapy protocol.*

Rescue Therapy

Although many dogs achieve initial remission with standard chemotherapy, most will eventually relapse. Cancer cells become increasingly resistant to chemotherapeutic agents during the course of treatment. If the relapse is more than six months after the original treatment, the original protocol used for induction is typically attempted. If the relapse occurs less than six months after induction, novel drugs or protocols are often used.

Typically, improvement is only achieved with the rescue chemotherapeutic treatments during the treatment period and, with every cycle, their effectiveness decreases over time, while their toxicity increases.

Prognosis

The prognosis for dogs with lymphoma is variable. Although rarely curable, many dogs favorably respond to therapeutic protocols. Multiple factors influence progression and response to therapy including anatomic location, histopathology (i.e., high-grade vs. indolent), immunophenotype, and clinical stage and substage of disease at time of diagnosis. More accurate diagnostic techniques and better therapeutic approaches will continue to improve outcomes for these patients.

Because most of the treatments available for dogs with lymphoma are adaptations from human medicine, the need for a treatment approach specifically dedicated to dogs represents a significant unmet medical need.

Future Options

Improvements in chemotherapy protocols have increased the longevity and quality of life for cancer patients. Lymphoma is the most common hematologic cancer of dogs, but it is also one of the most treatable canine cancers. Lymphomas are a diverse group of neoplasms and new protocols are being developed and evaluated to help refine treatment recommendations.

As stated earlier, most of the current lymphoma treatment protocols are based only on chemotherapy and seem to have reached their limitations when it comes to improving overall survival times with a reasonable quality of life. Treatment of lymphoma in humans faced a similar limitation in the late 1990’s. At that time, monoclonal antibodies were introduced to target B-cell and T-cell lymphomas. After their introduction, monoclonal-based therapies became the standard in treating humans either in conjunction with chemotherapy during the induction phase, following relapse, or as monotherapy during maintenance.

Aratana Therapeutics Delivers First

A conditional license has been granted by the U.S. Department of Agriculture (USDA), for B-cell and T-cell canine-specific monoclonal antibody therapies as aids in the treatment of lymphoma in dogs.
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AT-005, the first T-cell biological therapeutic, is a caninized monoclonal antibody engineered using the Aratana technology platform. AT-005 provides a targeted immunotherapy that specifically recognizes, with high affinity, the target cell-surface antigen CD-52, expressed on the cancer (lymphoma) T-cells in dogs. Upon binding to the target, the dog’s immune system is better able to identify and help to eliminate the lymphoma cells.

AT-005 received, in January 2014, a conditional license from USDA as an aid for the treatment of T-cell lymphoma in dogs. Additional studies are in progress to further support the safety and efficacy of this new therapeutic option.

Delivering New Beginnings

Using monoclonal antibody therapy in combination with chemotherapy may be less stressful on the dog’s system than only the chemotherapeutic drugs used today. And, it may lead to new beginnings for treatment and quality of life for canine lymphoma patients in the future.

Safety: MAb safety has been demonstrated by administration of the recommended dose to dogs with T-cell lymphoma.

Precautions and Contraindications: There are no known contraindications for the use of monoclonal antibodies in dogs with T-cell lymphoma. In instances of hypersensitivity types of reactions, therapeutic administration should be suspended. Treatment may include antihistamines and/or anti-inflammatories.

References:

*While not approved specifically for veterinary use, these drugs are recommended by veterinary oncologists and are routinely used in their practice.*