Lymphoma: Diagnostic and Prognostic Advances

Douglas H. Thamm, VMD, DACVIM (Oncology)
Colorado State University
Fort Collins, CO

Lymphoma (LSA) is a relatively common disease entity in veterinary medicine. Most small animal practitioners will encounter LSA in their practice, and will be asked to provide information and treatment recommendations for pets with this condition.

Diagnosis and staging – Dogs

The typical dog with LSA will present with generalized (or less commonly regional) lymphadenopathy. Differential diagnoses for generalized adenopathy can include Ehrlichiosis or other immune-mediated diseases, systemic mycosis, severe pyoderma or other skin disease, and reactive hyperplasia. The most simple way do discern the cause for lymphadenopathy is via needle aspiration cytology of an affected lymph node. If possible, the submandibular lymph nodes should be avoided due to the likelihood of some component of reactive hyperplasia being present due to drainage from the mouth and ears. Although many clinical pathologists are able to confirm a diagnosis of canine LSA cytologically, excisional biopsy of an affected lymph node provides the most information. It is critical that empiric prednisone therapy not be employed prior to diagnosis if lymphoma is a differential, as this may mask the signs of illness and has the potential to induce resistance to other forms of chemotherapy (See below).

Complete clinical staging helps to ascertain the extent of disease, ensures that other types of medical problems are not present, and can provide prognostic information for the client. Complete staging should include complete blood count, serum chemistry panel, urinalysis, thoracic radiographs, and a bone marrow aspirate. Imaging of the abdominal cavity is of limited use, unless abdominal palpation is extremely difficult, or if abnormalities other than cranial organomegaly are palpated or clinical signs consistent with primary gastrointestinal disease are present. The World Health Organization has developed a clinical staging system for dogs with multicentric LSA, which takes into account the number and location of involved lymph nodes, presence or absence of hepatosplenomegaly, and the presence or absence of disease in the bone marrow, central nervous system, or other extranodal sites. In addition, a substage is assigned, (a) representing a patient without clinical signs of illness, and (b) representing a patient with clinical signs (anorexia, lethargy/weakness/ depression, significant weight loss, vomiting, diarrhea, etc.) (See Table 1). Most dogs that present are WHO Stage IIIa or IVa.

Table 1: WHO staging criteria for canine lymphoma

Stage I:	Disease confined to a single lymph node.
Stage II:	Regional lymphadenopathy (confined to one side of diaphragm).
Stage III:	Generalized lymphadenopathy.
Stage IV:	Hepatosplenomegaly (with or without lymphadenopathy)
Stage V:	Bone marrow, CNS, or other extranodal site involvement
Substage a:	No clinical signs
Substage b:	Clinical signs of illness

Complete staging allows a thorough assessment of factors that may help to predict the outcome with treatment for an individual patient. Factors that have historically carried the most prognostic significance for remission duration and survival include presence of clinical signs at presentation (substage b), presence of hypercalcemia, mediastinal lymphadenopathy, and significant bone marrow infiltration. It is probable that both hypercalcemia and mediastinal lymphadenopathy are actually surrogate markers for LSA with a T cell immunophenotype, a very powerful predictor of outcome. Most veterinary pathology laboratories are capable of immunophenotyping lymphomas with the use of CD3 immunohistochemistry. Additionally, the University of California at Davis, Colorado State University and North Carolina State University can perform this evaluation on fine-needle aspirates using flow cytometry or PCR for antigen receptor rearrangement (PARR). These prognostic factors do not typically alter the likelihood that a patient will achieve a complete response (CR); they do however, affect the likely duration of that response.

Diagnosis and staging – Cats

Generalized lymphadenopathy is an uncommon presentation for cats with LSA. Clinical signs are dependent on the body system affected. Common anatomic sites include alimentary, mediastinal, nasal, renal, cutaneous and multicentric. Due to the changes in FeLV testing and vaccination, there has been a shift in the anatomic distribution of feline LSA over the past 20 years. Whereas the mediastinal form, occurring in young FeLV+ cats previously predominated, we are now seeing a great deal more of the alimentary form in older, FeLV- cats. Given the anatomic distribution in cats, diagnosis is more often achieved through histopathology after

exploratory laparotomy or endoscopy. Needle aspiration cytology of enlarged peripheral lymph nodes in cats can sometimes be difficult to interpret, as cats are subject to a variety of lymphoid hyperplastic conditions that can mimic LSA cytologically.

Clinical staging in cats with LSA is very similar to that in the dog. However, addition of FeLV and FIV serology is reasonable, due to its impact on prognosis and husbandry. A pre-treatment abdominal ultrasound can be helpful to establish a pre-treatment baseline in cats with alimentary LSA.

What's new – Diagnosis, staging, prognosis

Most veterinary pathology laboratories are now capable of immunophenotyping lymphomas with the use of immunohistochemistry. However, this does require a biopsy. Several laboratories in the US are capable of performing immunocytochemistry for T-cell and B-cell markers on air-dried fine-needle aspirates as well.

The University of California at Davis, Colorado State University and North Carolina State University can perform immunophenoptying on fresh fine-needle aspirates using flow cytometry. This method is useful for not only lymphoma immunophenotyping, but also for phenotyping and confirming diagnoses in animals with suspected leukaemias and distinguishing between lymphoma and thymoma in dogs with mediastinal masses. Additional information regarding prognosis may be obtained through flow cytometry; specifically, dogs with B-cell lymphoma whose tumour cells have low expression of MHC class II have a significantly worse outcome than those with higher class II expression.

A fourth method for establishing immunophenotype is through PCR for antigen receptor rearrangement (PARR). This molecular diagnostic test evaluates the presence or absence of a clonally expanded population of B cells or T cells, and is approximately 85% sensitive and 95% specific for canine lymphoid neoplasia. It is approximately 65% sensitive for feline lymphoid neoplasia. An advantage of this technique is that it can be performed on almost any type of sample, including air-dried or previously stained cytology slides, effusions, aspirates, cerebrospinal fluid, frozen tissue and peripheral blood. Formalin-fixed tissues generally cannot be used for this technique.

Several new publications have evaluated the utility of real-time PCR to quantify the amount of DNA possessing the lymphoma-specific clonal gene rearrangement in the blood. This correlates with remission status, and early evidence suggests that the amount of DNA in the blood following chemotherapy may correlate with remission length as well.

A recent publication suggests that immunostaining for the cellular survival protein survivin may be a useful prognostic factor in dogs with stage IIIa and IVa B cell lymphoma, a population for which there are no reliable prognostic factors currently. Another recent study has demonstrated that monocyte count may be an independent predictor of outcome in dogs with lymphoma: dogs with monocyte counts higher than 800 cells/dL had an outcome nearly 4 times worse than those with lower monocyte counts. These interesting preliminary findings need to be confirmed in additional studies.

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