New Diagnostic and Prognostic Tests for Lymphoma in Dogs

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Lymphoma is a very common cancer of dogs. The most common form in dogs is high grade, rapidly progressing, large cell lymphoma which is easy to diagnose with cytology. Fine needle aspiration cytology of an enlarged lymph node with a 22 g. needle and 6 cc syringe is simple to perform and almost always diagnostic. A needle aspirate of the typical untreated canine lymphoma consists of a monomorphic population of immature [large] lymphocytes. These cells are large size (2-6 times the diameter of a red blood cell), have a high nuclear to cytoplasmic ratio. The nuclei contain a coarse (lacy) nuclear chromatin pattern with prominent nucleoli and deep blue cytoplasm. A normal LN contains 75 to 95% small, mature lymphocytes; a reactive LN can have up to 50% lymphoblasts along with plasma cells. The presence of greater than 50% lymphoblasts is diagnostic for lymphoma. A definitive diagnosis can be adversely affected by poor cellularity, easily lysed cells, small numbers of neoplastic cells, and the presence of other cells (inflammation, blood contamination) obscuring the neoplastic cells. Deciding the significance of many small cell lymphoid cells seen on an aspirate is challenging because the cells are often indistinguishable from normal lymphocytes. Histopathology is one way to determine if small cell proliferation is neoplastic

Although not necessary to diagnose most cases of lymphoma, histopathology is the gold standard for diagnosis of lymphoma. Evaluation of a tissue sample can be used to separate large cell lymphoma (usually involving the entire lymph node) from less common small cell lymphoma arranged in focal patterns throughout the node. This distinction has clinical significance, because dogs with large cell lymphoma respond well to the typical multidrug intravenous chemotherapy and require it; whereas, small cell (indolent) low grade lymphoma is less responsive to chemotherapy and, in some instances, does not require any chemotherapy. A Tru-cut^{R} biopsy is inferior to excising the entire lymph node as the node architecture in often distorted in smaller samples and may not be representative of the entire lymph node. Fortunately, lymphoma can now be diagnosed and characterized with less invasive, less expensive sampling not requiring general anesthesia or heavy sedation. Cell identification (even small malignant lymphoid lymphomas) and malignant cell characterization can now be made using DNA PCR (PARR assay), immunocytochemistry, and flow cytometry on samples obtained from fine needle aspiration sample of solid tissue like a lymph node or blood/bone marrow.

PARR---Polymerase Chain Reaction for Detection of Antigen Receptor Rearrangement

The finding of a malignant lymphoma clone (i.e., the cells all have the same genetic defect) with the PARR test strongly supports a diagnosis of lymphoma when coupled with a cytology suggestive of

lymphoma even if the clinical presentation is atypical. The PARR assay allows detection of a clonal DNA (lymphoma) from samples obtained from a wide variety of tissues and fluids. The PARR assay is a PCR assay in which a specific area of DNA is amplified and separated by size. The specific area evaluated is the unique genes coding for the antigen-binding region found in B cells (Ig genes) and T cells (T cell receptor genes). The results tell us if the majority of cells in the sample are derived from the same original clone (most consistent with neoplasia), or from multiple clones (most consistent with a reactive process). This molecular diagnostic test evaluates the presence or absence of a clonally expanded population of B cells or T cells. It has relatively high specificity (94% canine and 65% feline cases). It is sensitive enough to detect 1 malignant cell in approximately 100 lymphocytes with a sensitivity of 75% in dogs and a sensitivity of 65% in cats. The only nonneoplastic disease reported to cause a clonal lymphocyte expansion of T cells and thus would be detected with the PARR test, *is E. canis* infection. It is recommended to run a 4 Dx test for *E. canis* prior to PARR testing. It is sufficiently sensitive to allow detection of 1 malignant cell in approximately 100 lymphocytes. Perhaps the most important advantage is that it can be performed on almost any sample including lymph node aspirates, whole blood, organ aspirates, body cavity effusions, CSF, bone marrow aspirates, and even aqueous humor. The cells can be nonviable or viable, previously fixed and stained for cytologic examination, or from an impression smear. It cannot be performed on paraffin-embedded, formalin fixed samples, on glass slides with a previously glued on cover slip, or on tissue samples. PARR can reveal immunophenotype. It can indicate whether the malignant clone is of T or B cell lineage. While it is an important, noninvasive diagnostic tool, it unfortunately does not correlate with clinical staging, but can be used for early detection of relapse.

Immunophenotyping

Immunophenotyping is one of the main prognostic indicators for lymphoma in dogs and has become the standard of care. It can be used to not only help determine prognosis, but also, is now being used to guide treatment selection. In a 2009 study of veterinarians who evaluate and treat dogs with lymphoma (about 50% were Board-certified Oncologists), almost one-third treated B and T cell lymphomas differently. Results of a few small studies suggest that for large cell lymphoma that T and B cell lymphoma responds differently to the same drug protocols. For example, dogs with T cell have decreased remission duration and overall survival time when treated with CHOP-based protocols (vincristine, cyclophosphamide, doxorubicin and prednisone) compared with dogs with B cell lymphoma (6 mos vs 14 mos. median survival time). In another study, where dogs were treated with doxorubicin only, 86% of 29 dogs with B cell lymphoma went into complete remission, while only 17% of dogs with T

cell lymphoma had a complete response. In that study, the overall response rate (complete plus partial responses) of a T cell lymphoma was found to be ½ the response rate of a B cell lymphoma. It was hypothesized that T cell lymphoma is associated with more chromosomal abnormalities than B cell lymphoma which may increase ability to acquire drug resistance more readily, because of the P-glycoprotein (ABCB1 transporter). Protocols containing alkylating agents like Mustargen, lomustine, or procarbazine might be an appropriate drug to add to a standard protocol when treating dogs with T cell, because these drugs are not substrates for the ABCB1 transporter, so do not exhibit drug cross resistance like vincristine, prednisone, and doxorubicin. Although PARR testing can establish malignancy and when positive can demonstrate immunophenotype, other methods provide more information once the lymphoma has been confirmed. Flow cytometry, immunohistochemistry, and immunocytochemistry are available tests. Although few studies compare these methods, flow cytometry is the most sensitive method for subclassification of lymphoma/leukemia.

Immunocytochemistry

Immunocytochemistry (ICC) involves the use of specific antibodies to detect antigens within nonviable cells. ICC is usually not used to obtain a diagnosis and is best interpreted when paired with additional routinely stained slides (Wright-Giemsa). Cytology identifies the lymphoma and it is further characterized into B and/or T cell lymphoma using ICC. The advantages of ICC include rapid turn-around time for tests (often same or next day), noninvasive sampling via fine needle aspiration of affected organs, sampling obtained without general anesthesia or sedation in most patients, ability to repeat sampling if cellularity is insufficient for ICC, and reasonable cost. Costs may be reduced overall, because unstained glass slides submitted for cytology can be submitted simultaneously for immunocytochemistry. This allows cytologic diagnosis and immunophenotyping on the same fine needle aspiration sampling visit. ICC requires 4 unstained glass slides which would be in addition to the slides submitted for examination. Disadvantages are most frequently insufficient good-quality smears. Poor cellularity caused by lysed cells and/or contamination with blood or other cell populations (blood/mixed cell background) will prevent adequate staining.

Flow cytometry

Flow cytometry involves binding fluorescent antibodies to cell surface antigens of live cells. This assay can be performed on a lymph node aspirate or a blood/bone marrow sample with increased lymphocytes. The cells are sorted for identification with laser technology. At ISU, this assay is performed on aspirates from lymph nodes from almost every dog with cytologically confirmed lymphoma. One advantage to flow cytometry over other methods of cell characterization is that many different

characteristics of clinical significance can be determined simultaneously. The number of cells stained with a particular antibody can be counted and it can be used to further characterize cells by the absence or presence of certain surface proteins, cytoplasmic characteristics, and cell size. Typical antibodies used are the following: for T cells, CD3 (pan T cell), CD4 (helper T), and CD8 (cytotoxic T) and for B cells, CD 79a, CD 20, and CD 21. The flow cytometer counts the cells of each type and helps identify unusually large numbers of a single cell subtype (like increased numbers of CD3 expressing lymphocytes consistent with a homogenous proliferation of CD3 positive T cells) confirming a diagnosis of T cell lymphoma. The information obtained with flow cytometry helps subtype lymphomas which has clinical significance. For example, B cell lymphomas express the B cell marker class II MHC, which correlates with prognosis and can be reliably detected using flow cytometry. Dogs with medium-sized cells and high MHC class II expression had a median survival of 330 days whereas large-sized cells and low MHC class II expression were poor prognostic indicators (Williams, 2008) It is useful because B cell lymphoma expressing low levels of class II MHC have a worse prognosis than B cell lymphoma with high class II MHC surface protein. Avery et al. and others have shown that T cell lymphomas are also heterogenous and flow cytometry can be used to accurately separate aggressive T cell lymphoma from indolent T cell lymphoma. The estimated prevalence of T-zone disease (one of the indolent lymphomas) is 3% to 13% of all canine lymphomas and the overall median survival times ranged from 21.2 to 33.5 months. T-zone disease affects older dogs, with a median age of 10 years with 40% of all cases being Golden retrievers (Seelig, 2014). Disadvantages of flow cytometry are the following: cells must be intact and alive when received so that the surface proteins are intact, they need to be sent suspended in a small amount of saline/serum, and they are sent cooled via overnight mail. Because of the influence of cell characteristics in addition to immunophenotyping, flow cytometry becomes an important part of providing the best treatment selection for patients and the most accurate prognosis for owners.

Many labs perform PARR, flow cytometry, and immunocytochemistry. Three are listed below with current information. We use CSU, but are developing our own flow cytometry which is currently available for in house cases only.

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