CYTOLOGY OF LYMPH NODES
Maxey L. Wellman, DVM, PhD, DACVP (Clinical Pathology)
Professor, Department of Veterinary Biosciences, College of Veterinary Medicine,
The Ohio State University, Columbus, OH, USA

SUMMARY

Enlarged peripheral lymph nodes (lymphadenopathy or lymphadenomegaly) can occur with lymphoid and plasma cell hyperplasia, inflammation, or lymphoid or metastatic neoplasia. Fine-needle aspiration (FNA) cytology of enlarged lymph nodes is relatively non-invasive and inexpensive, and with experience, often provides an accurate diagnosis. However, in some cases, special stains, biopsy, immunophenotyping by flow cytometry, or polymerase chain reaction for antigen receptor rearrangement (PARR) may be helpful for further evaluation.

Sample collection and processing

Clinical history and physical findings are helpful in the cytologic evaluation of lymphoid tissue. For example, recent immunization or local or systemic inflammatory diseases may cause mildly to moderately enlarged lymph nodes associated with lymphoid hyperplasia. Dogs with multiple, markedly enlarged peripheral lymph nodes are more likely to have lymphoma than dogs with a single enlarged lymph node. Young cats with enlarged peripheral lymph nodes may have non-neoplastic proliferative disease instead of lymphoid neoplasia. In some cases, lymph nodes that appear normal in size are aspirated to determine if there is metastatic disease. If multiple lymph nodes are enlarged, aspirates of popliteal and prescapular lymph nodes are preferred because mandibular lymph nodes often appear hyperplastic and mildly inflamed from constant exposure to normal oropharyngeal flora. Extremely enlarged lymph nodes also should be avoided because necrosis or hemorrhage may preclude adequate sampling and evaluation.

The skin overlying the lymph node can be cleaned using an alcohol swab, the lymph node is immobilized with one hand, and the other hand is used to introduce a 21-25 gauge needle coupled to a 6-12 ml syringe needle into the lymph node. Slight negative pressure is applied, and the needle can be redirected several times to ensure adequate sampling. Suction is released prior to withdrawing the needle to minimize contamination with blood or cells from surrounding tissues. If only a small amount of material has been collected, the needle is detached, air is aspirated into the syringe, the needle is replaced, and a small amount of material is carefully expelled onto several clean glass slides. A spreader slide and a pull or push technique is used to disperse the cells. Common errors during slide preparation include failure to adequately disperse the cells on the slide, resulting in smears that are too thick, and applying too much pressure, resulting in broken cells.

Smears made from FNA should be clearly labeled with the patient’s name or identification number and date, and allowed to dry in ambient air prior to staining. Slides should not be stored in a refrigerator because water condensation from will lyse cells, or exposed to formalin because formalin fumes prohibit adequate staining. Slides can be sent unstained to a reference laboratory or stained for in-house interpretation. Romanowsky-type stains (Wright's stain, Wright-Giemsa stain, or commercially available quick stains such as Diff-Quick®) are most commonly used because these stains provide good color contrast, acceptable cytoplasmic
and nuclear detail, and stain most infectious agents. Most commercially available quick stains are inexpensive and easy to use, and like Wright's or Wright-Giemsa stain, are permanent so previously stained slides can be sent to a clinical pathologist at a reference laboratory for a second opinion. Additional tests to determine cell lineage, assess clonality, or identify etiologic agents are available at some commercial reference laboratories or academic institutions, so in some cases it may be helpful to save several unstained slides.

If slides are mailed to a reference laboratory for interpretation, the laboratory should be contacted for information on requirements for sample submission. In general, the following information should be included: identification name or number, species, age, sex, a brief history, relevant physical examination findings, previous therapy, a summary of results of previous pertinent diagnostic tests, a description of the lesion, differential diagnoses, and the lymph node from which the sample was collected. The description should include whether the lymph nodes are mildly, moderately, or markedly enlarged, and whether there is enlargement of a single lymph node or multiple lymph nodes. Lesions involving the surrounding tissue also should be described. This information is very helpful for the clinical pathologist to adequately evaluate the sample and provide the most complete information for optimal patient care.

Cytology of normal lymph nodes

Knowledge of normal lymph node cytology is important for accurate evaluation of abnormal lymphoid tissue. Normal lymph nodes are characterized by a heterogeneous population of 80-95% small lymphocytes and 5-20% intermediate and large lymphocytes. Small lymphocytes resemble those seen in peripheral blood. They are ~9 μm in diameter, which is smaller than neutrophils (~12 μm in diameter). Small lymphocytes have scant light blue cytoplasm and a high nuclear to cytoplasmic (N:C) ratio. Nuclei are round and have dense, coarsely clumped chromatin and inconspicuous nucleoli. Intermediate lymphocytes are similar in size to neutrophils and have moderately condensed chromatin and a moderate amount of basophilic cytoplasm. Nucleoli may be visible in some cells. Large lymphocytes are larger than neutrophils (~13-18 μm in diameter) and have a relatively high N:C ratio, finely stippled chromatin, 1-2 nucleoli, and variable amounts of basophilic cytoplasm. Occasional plasma cells may be present and are characterized by abundant basophilic cytoplasm, eccentric nuclei, and a clear region near the nucleus. Occasional neutrophils, macrophages, and mast cells are present in most normal lymph nodes.

Lymphoid hyperplasia

In hyperplastic lymph nodes, intermediate and large lymphocytes may be mildly, moderately, or markedly increased, but these comprise less than 50% of all of the lymphocytes that are present. If more than 50% of the lymphoid cells are intermediate or large, lymphoma should be considered as a differential diagnosis. Hyperplastic lymphoid tissue also often has increased numbers of plasma cells, which sometimes is referred to as a reactive lymph node. Plasma cells in reactive lymph nodes sometimes contain clear, basophilic, or eosinophilic inclusions which are secretory vesicles (Russell bodies) filled with immunoglobulin. These plasma cells sometimes are called Mott cells. Lymphoid and plasma cell hyperplasia usually cause mild to moderate enlargement of a single lymph node, although multiple lymph nodes can become hyperplastic with systemic disease.
**Lymphadenitis**

Inflammation involving lymph nodes is sometimes called lymphadenitis. Inflammatory cells can include neutrophils, eosinophils, macrophages, and mast cells, depending on the cause of the inflammation. Many etiologic agents have a characteristic appearance with routine staining, although a definitive etiologic diagnosis may require additional testing. Neutrophilic (purulent, suppurative) inflammation is characterized by increased neutrophils (>5%). Bacterial infections typically are associated with neutrophilic inflammation, although some bacteria may be associated with inflammation characterized by neutrophils and macrophages (pyogranulomatous or mixed inflammation). Some fungal and protozoal diseases are associated with neutrophils and macrophages and others are associated with predominantly macrophages (granulomatous inflammation). Mycobacterial infections also can be associated with granulomatous inflammation. Eosinophilic lymphadenitis (>3% eosinophils) can occur with parasite infections, allergic or hypersensitivity reactions, some fungal infections, mast cell tumors in dogs, and lymphoma and certain carcinomas.

**Lymphoid neoplasia**

Lymphoma most commonly arises from lymph nodes, the spleen, the thymus, or the bone marrow, but other sites include the gastrointestinal tract, skin, kidney, eye, and central nervous system. Morphology of neoplastic lymphocytes may vary depending on the tissue involved, the type of lymphocyte, stage of differentiation, and species or breed in which the lymphoma occurs.

**Lymphoma in dogs**

Lymphoma is the most common hematopoietic neoplasm in dogs. Most dogs present with multicentric lymphoma, which is characterized by non-painful, generalized peripheral lymphadenopathy. Other less common forms include gastrointestinal lymphoma, mediastinal lymphoma, and lymphoma arising in the skin, lungs, kidneys, eyes, and central nervous system. The normal heterogeneous population of lymphocytes is replaced by a monomorphic population of neoplastic lymphocytes. Neoplastic lymphocytes usually are > 90% of the lymphoid cells in involved tissue. Plasma cells and inflammatory cells typically are rare. Most lymphoid neoplasms in dogs involve large lymphocytes with abundant basophilic cytoplasm and round or slightly to moderately irregular nuclei with finely stippled chromatin and single or multiple, prominent nucleoli. Although uncommon, the presence of binucleated or multinucleated cells may be associated with a shorter remission and decreased survival in dogs treated with chemotherapy.

FNA of an enlarged peripheral lymph node, preferably the prescapular or popliteal lymph node, usually provides specimens of adequate quality to make a definitive diagnosis of lymphoma. If the lymph node is markedly enlarged, avoid sampling the center, which may be necrotic or hemorrhagic. Neoplastic lymphocytes rupture easily during specimen preparation. If only broken cells are present, the lymph node should be re-aspirated. Only intact lymphocytes should be evaluated. FNA smears from neoplastic lymphoid tissue often are very cellular. Lymphocytes in the thick portions of the smear may resemble small, mature lymphocytes. Only the portions of the smear where cells are intact and appropriately dispersed should be evaluated. Multiple smears from the same lymph node should be evaluated, and it may be helpful to evaluate FNA from multiple enlarged lymph nodes.
The classification of lymphoma may affect response to treatment and prognosis. The cytologic classification of lymphoma as high grade or low grade is based on nuclear size, shape, and chromatin pattern and nucleolar number and size. Although cytologic classification is not as reliable as histologic classification, it may be sufficient to recognize the common cell types. Low grade tumors often involve small lymphocytes with scant cytoplasm and round nuclei with condensed chromatin. Low grade tumors are less aggressive and have a better prognosis than high grade tumors. The majority of dogs have high-grade tumors, which usually involve intermediate to large lymphocytes with basophilic cytoplasm and round to irregularly shaped nuclei with fine chromatin and single or multiple nucleoli. Cell morphology does not have a high correlation with whether there is a neoplastic proliferation of B-cells, T-cells, or a non-B/non-T population of lymphocytes.

The cytologic diagnosis of lymphoid neoplasia may be difficult early in the disease; if there is atypical benign hyperplasia; if the neoplastic cells are small or intermediate, or otherwise atypical; or there also is proliferation of a non-neoplastic cell type. In these cases, histologic evaluation may be necessary for a definitive diagnosis. Histologic evaluation to determine mitotic index and argyrophil nucleolar organizer regions (AgNOR) may be useful prognostic indicators, although there is conflicting data on whether mitotic index is a prognostic indicator. Immunophenotyping by flow cytometry and polymerase chain reaction for antigen receptor rearrangement (PARR) may be helpful in determining whether the cells are B or T lymphocytes and if there is a clonal expansion of lymphocytes, respectively. B-cells express CD 20, CD21, and CD79a; T-cells express CD3 and CD4 or CD8. Although clonal rearrangement of B- and T-cell antigen receptor genes (cross-lineage rearrangement) can occur, co-expression of both B- and T-cell antigens is rare. In general, dogs with B-cell lymphoma have longer survival compared to dogs with T-cell lymphoma, but other factors such as clinical presentation, breed, anatomic site, and laboratory findings also may affect prognosis Expression of the p16 tumor suppressor gene also may be a prognostic indicator, but is not routinely evaluated.

**Lymphoma in cats**

Multicentric lymphoma is much less common in cats than in dogs. Young cats (2-4 years old) sometimes develop peripheral lymph node hyperplasia that clinically, cytologically, and histologically may resemble multicentric lymphoma. The lymphoid hyperplasia in young cats often spontaneously resolves. PARR for clonality may be helpful in differentiating lymphoid hyperplasia from lymphoma, but is not as reliable in cats as in dogs.

The most common form of lymphoma in cats involves the gastrointestinal tract. Neoplastic lymphocytes often are small, nuclear chromatin is condensed, nucleoli are inconspicuous, and there is minimal cytoplasm. In these cases, cytology may not be as helpful in the diagnosis of lymphoid neoplasia as in other forms of lymphoma. PARR may be helpful in supporting a diagnosis of lymphoma if a full thickness biopsy cannot be easily obtained. In some cats with gastrointestinal lymphoma, there is a neoplastic proliferation of large granular lymphocytes (LGL). LGL are a subset of T lymphocytes characterized by a moderate amount of relatively pale cytoplasm that often contains azurophilic granules. The granules usually are quite prominent when stained with Wright’s stain, but may be less apparent with commercial quick stains and the hematoxylin and eosin stain used for histopathology. The significance of immunophenotype and prognosis in cats with lymphoma is unclear.
Feline Hodgkin’s-like lymphoma has been described in older cats. Most affected cats present with a mass in the ventral cervical region, or enlarged mandibular or prescapular lymph nodes. The cytologic diagnosis is difficult because the neoplastic cells comprise only 15% of the cells in the affected lymph node. The remaining cells are non-neoplastic lymphocytes, macrophages, and granulocytes. Large, multinucleated Reed-Sternberg cells also may be present. The cytology may resemble mixed inflammation so the definitive diagnosis is made histologically.

**Metastatic neoplasia**

Lymph node aspirates sometimes are evaluated for evidence of metastasis from mast cell tumors, melanomas, histiocytic sarcoma, carcinomas, and sarcomas. If numerous metastatic cells are present, cytology is helpful. However, the absence of neoplastic cells on cytology does not preclude the presence of metastasis. Histopathology is more sensitive in the detection of metastatic disease.

**REFERENCES and RECOMMENDED READING**


