Cytodiagnosis of Feline Lymphoma

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ABSTRACT:
Lymphoma is one of the most common tumors diagnosed in cats. Feline lymphoma can be difficult to diagnose definitively by cytology alone because of the increased prevalence of internal organ involvement and small cell morphology compared with canine lymphoma. This article discusses the common differences between feline and canine lymphoma, the basics of lymph node cytology, and the key cytologic features of each anatomic form of feline lymphoma.

Differences Between Feline and Canine Lymphoma

Lymphoma is the most commonly diagnosed neoplasm in cats and accounts for approximately 30% of all diagnosed tumors. Similarly, canine lymphoma is relatively common and accounts for up to 24% of all canine neoplasms. There are some differences in the presentation and cytologic appearance of feline lymphoma compared with canine lymphoma that make the feline condition diagnostically more challenging.

Most canine lymphomas are composed of large lymphocytes, with only a low incidence of well-differentiated small cell lymphomas. Feline lymphoma is most often an intermediate or large cell type, but small cell lymphomas are much more common in cats than in dogs. Small cell lymphomas are often seen in older cats, most commonly in the alimentary tract or liver, whereas large cell lymphomas are seen more frequently in younger cats (<6 years of age). Feline lymphoma is often more challenging to diagnose than canine lymphoma because small cell lymphomas are difficult to differentiate cytologically from a lymphoid inflammatory infiltrate or reactive hyperplasia. Definitive diagnosis of small cell lymphoma often requires histopathology, and the practicing cytologist must be aware of the cytologic appearance of feline lymphoma to accurately interpret cytologic findings and determine the necessity of surgical biopsy.

Classification of Lymphoma

Classification of lymphoma is based on the anatomic site, the morphology of the cells, and
immunologic and molecular testing. Most (80% to 85%) dogs with lymphoma have the multicentric form and usually present with generalized peripheral lymphadenopathy. Less common forms of canine lymphoma include those involving the gastrointestinal (GI) tract, skin, mediastinum, and other tissues. In contrast, feline lymphoma is more often located in the alimentary tract or mediastinum, and compared with dogs, fewer cats develop multicentric lymphoma. Also, multicentric lymphoma in cats very rarely manifests as peripheral lymphadenomegaly and almost always involves multiple internal lymph nodes and organs.

Most canine lymphomas are B cell in origin, with less than 30% being T-cell neoplasms. Feline lymphoma is also more commonly B cell in origin, with the anatomic location being a factor in the immunophenotypic presentation in both species. Immunophenotyping of lymphoma has become a useful tool to determine the cell type involved and provide prognostic information. These techniques use antibodies to label clusters of differentiation (CD) antigens on the surface of cells. Clinically important CD antigens include CD3 (T cell) and CD79 (B cell). Immunocytochemistry involves the use of special stains, particularly immunoperoxidase, to detect specific antigens on a cell’s surface. These stains can differentiate cell lines, such as lymphoid, epithelial, or mesenchymal, and can also be used to differentiate subtype neoplasms, such as the B- and T-cell subtypes of lymphoma. Flow cytometry can be used to determine the presence of surface markers on the neo-

**Lymphoma is the most common neoplasm in cats.**
Retroviral Involvement in Feline Lymphoma

Infections with the retroviruses FeLV\(^{1,6,17,21–25}\) and FIV\(^{4,15,26–29}\) have long been associated with development of lymphoma in cats. In a recent study, approximately 70% of cats with lymphoma tested positive for FeLV.\(^{15}\) FeLV infection significantly affects the age at which cats develop lymphoma, with FeLV-positive cats tending to be younger and FeLV-negative cats tending to be older at onset of disease.\(^\text{15,23}\) Introduction of the FeLV vaccine and effective testing for retroviruses have significantly reduced the incidence of lymphoma.\(^{17}\) Vaccination and increased detection have also shifted the incidence of lymphoma from young, FeLV-positive to older, FeLV-negative cats.\(^{30}\)

FeLV has a single-stranded RNA genome that is reverse transcribed into a DNA provirus that can be incorporated into the cellular genome.\(^{25,31,32}\) In general, one proviral integration is not enough for malignant transformation of the cell. However, sufficient DNA damage can occur after multiple integrations over a period of time, leading to malignant transformation of the cell.\(^{33}\) FeLV may also be associated with development of lymphoma in serologically FeLV-negative cats.\(^{10,22,34}\) Proviral DNA has been detected in the cellular genome of FeLV-negative cats with lymphoma; therefore, an inactive infection without viral replication may cause malignant transformation without positive results of a serologic FeLV test.\(^{10,22,33}\)

GENERAL PRINCIPLES OF LYMPH NODE CYTOLOGY

Cytology is generally a very rewarding and relatively noninvasive method of evaluating lymph nodes. A normal lymph node is predominantly composed of well-differentiated small lymphocytes,\(^{12,34–36}\) which have dense, smooth chromatin, scant cytoplasm, and no distinct nucleoli.\(^{36}\) Small lymphocytes are approximately one to one and a half times the size of an erythrocyte (Figure 1). Much lower numbers of medium and large lymphocytes make up the remaining cells.\(^{34,35}\) Occasional plasma cells, neutrophils, macrophages, and mast cells are usually also present.\(^{34}\) Samples from neoplastic lymphoid tissue most often contain predominantly immature, large lymphocytes that are three to five times the size of an erythrocyte.\(^{12,34}\) These cells have more abundant cytoplasm and a round to slightly cleaved nucleus that has pale chromatin and one or more nucleoli\(^{12,34}\) (Figure 2). Lymphomas containing a population of small neoplastic lymphocytes are rare, however, and clinicians should suspect
small cell lymphoma when tissue aspirate from an enlarged lymph node(s) results in a homogenous population of small lymphocytes with few plasma cells or intermediate or large lymphocytes. Because these lymphocytes morphologically appear normal, histopathology is needed to recognize the architectural disruption of the tissue by the neoplastic population. 

Distinctive Peripheral Lymph Node Hyperplasia

Although multicentric lymphoma is commonly seen in cats, it rarely manifests as generalized peripheral lymphadenomagaly. More commonly, peripheral lymphadenomagaly that mimics multicentric lymphoma in cats is due to distinctive peripheral lymph node hyperplasia, which is also known as atypical follicular lymphoid hyperplasia. This syndrome is proposed to be due to a host immune response and may be associated with retroviral infection. The syndrome is a nonneoplastic disorder of usually young cats that most commonly manifests as an episode of fever, generalized lymphadenomagaly, and polyclonal gammopathy that generally resolves in a variable length of time (5 to 120 days). Diagnosing this syndrome can be difficult because of the similar cytologic and histologic appearance to lymphoma, with samples containing a population of large lymphocytes in numbers typically seen only in lymphoma (Figure 3). Therefore, for accurate diagnosis of the cause of generalized lymphadenomagaly in a cat, the signalment, history, and clinical signs along with appropriately interpreted cytology and histopathology must be the primary diagnostic tools.

ANATOMIC LOCATIONS OF FELINE LYMPHOMA

Alimentary Lymphoma

The GI tract is most commonly affected by feline lymphoma, with solitary, multifocal, and diffuse lesions being reported. Alimentary lymphoma is most commonly reported in older FeLV-negative cats, with most lymphomas arising from B-cell lymphocytes within the gut-associated lymphoid tissue. However, T-cell alimentary lymphomas have also been reported in cats. Many cases of alimentary lymphoma are composed of large lymphocytes. The cytodiagnosis of lymphoma is possible because neoplastic lymphocytes are a monomorphic population of large, immature cells (Figure 4). In some cases, a mixed lymphoid population with increased numbers of blast cells may be seen if a germinal lymphoid follicle is aspirated. However, the overall pleomorphic appearance of the lymphocytes helps exclude the diagnosis of lymphoma. Histopathology of the lesion may be required if the lymphoid population is pleomorphic but contains many large lymphocytes.
Small cell alimentary lymphomas are frequently seen in cats. In these cases, the neoplastic infiltration of lymphocytes does not have cytologic features of malignancy and the diagnosis often requires concurrent histopathologic evaluation (Figure 5). Although inflammatory bowel disease is a separate lesion from lymphoma, the cytologic appearance of the two diseases is very similar. It has been suggested that lymphoplasma-cytic inflammatory bowel disease can progress to alimentary lymphoma.

A specific type of alimentary lymphoma in cats can arise from a subset of lymphocytes known as large granular lymphocytes. Large granular lymphocytes have been identified in humans as natural killer cells and cytotoxic T cells. One report of large granular lymphoma in a cat describes the neoplasm as a T-cell-rich B-cell lymphoma. In general, cats with large granular lymphoma are older and test negative for FeLV. The cells are morphologically distinct lymphocytes that are identified by the presence of variably sized azurophilic or brightly magenta granules in their cytoplasm (Figure 6). The large size of the neoplastic lymphocytes and the characteristic cytoplasmic granules allow definitive cytologic identification. These granules may stain poorly when using rapid, Romanowsky-type stains (Figure 7). Alternatively, widespread infiltration can occur. Abdominal and thoracic effusions that contain neoplas-
tic large granular lymphocytes can also be seen in cats with large granular lymphoma.\textsuperscript{30,42}

**Renal Lymphoma**

Renal lymphoma is a relatively common tumor in cats\textsuperscript{5,15} and is the most common type of tumor that affects feline kidneys.\textsuperscript{1} Cats that develop renal lymphoma are usually older and test negative for FeLV.\textsuperscript{4,16} Infection with FeLV is often associated with a poorer prognosis.\textsuperscript{4,49} Renal lymphoma can be a primary tumor or part of a multicentric or alimentary lymphoma.\textsuperscript{1,50} Both kidneys are usually affected and are large and irregular when palpated.\textsuperscript{5,15,33} A connection has been postulated between renal and central nervous system (CNS) lymphoma because infiltration of the CNS has been seen during recurrence of renal lymphoma.\textsuperscript{1,15} The clinical signs are generally vague and are often associated with chronic renal failure, which may include weight loss and nonregenerative anemia.\textsuperscript{35,33} Most renal lymphomas are composed of large lymphocytes and are easily identified cytologically using preparations made from fine-needle aspiration samples. These neoplastic lymphocytes generally have relatively abundant cytoplasm and large nuclei that contain pale chromatin and one or more prominent nucleoli (Figure 8). Neoplastic, large lymphocytes can usually be distinguished from renal tubular epithelial cells. Renal epithelial cells have more abundant, frequently vacuolated cytoplasm and round nuclei with indistinct nucleoli and tend to exfoli-
of thymic or mediastinal lymphoma include dyspnea, coughing, regurgitation, and potentially Horner’s syndrome.\textsuperscript{15,51} Chylous and nonchylous thoracic effusions may be seen\textsuperscript{53} (Figure 9). Because the thymus is the site of T-cell maturation,\textsuperscript{47} thymic lymphoma is most commonly T-cell in origin.\textsuperscript{1,33} Most are composed of large lymphocytes with round nuclei that have smooth chromatin and one to multiple nucleoli. Small cell thymic lymphoma has also been reported but is uncommon.\textsuperscript{51} The more challenging cytodiagnosis would be the distinction between thymic lymphoma, which involves a neoplastic population of lymphocytes, and thymoma, which is a neoplasm composed of neoplastic epithelial cells and a nonneoplastic lymphocyte population (Figure 10). This distinction is very important because of the difference in treatment modalities for these diseases.

**Multicentric Lymphoma**

Multicentric lymphoma is much less common in cats than in dogs and is rarely associated with peripheral lymphadenomegaly.\textsuperscript{1} In cats, multicentric lymphoma usually involves the mesenteric lymph nodes and potentially the liver and spleen.\textsuperscript{1,35} Eventually, bone marrow can be infiltrated.\textsuperscript{1} Large, T-cell lymphocytes are the most common type involved.\textsuperscript{33} Clinical signs of multicentric lymphoma include enlarged visceral lymph nodes, weight loss, anorexia, and lethargy as well as clinical signs associated with organ infiltration, such as hepatic or renal disease.\textsuperscript{15}

A rare form of multicentric lymphoma has been described in cats and referred to as \textit{feline Hodgkin’s-like lymphoma} because of its similarity to the human form of the disease.\textsuperscript{54} This form of lymphoma affects only the lymph nodes of the head and neck.\textsuperscript{1,54,55} In this condition, typically only one or two lymph nodes are affected.\textsuperscript{14,53} These lymphomas are phenotypically heterogeneous: They either can have a lymphocytic predominance or can be a heterogenous population of lymphoid and inflammatory cells.\textsuperscript{54,55} Although few reports of feline Hodgkin’s-like lymphoma have been published, the prognosis of these cases is good compared with that of other forms of feline lymphoma.\textsuperscript{55,56} Removing the affected lymph node(s) and/or chemotherapy frequently results in no recurrence of clinical disease.\textsuperscript{55,57}

**Hepatic Lymphoma**

Lymphoma is the most common hepatic tumor in cats.\textsuperscript{57} Although hepatic lymphoma can be associated with multicentric lymphoma, primary hepatic lymphoma is more common.\textsuperscript{57} Hepatic lymphoma is usually associated with hepatomegaly, and the clinical signs generally result from liver dysfunction. Hepatic lymphoma in dogs is usually composed of large lymphocytes (Figure 11), but small cell lymphomas are commonly seen in cats\textsuperscript{56,57} (Figure 12). As with small cell lymphomas in other sites, histopathology is generally required to distinguish between inflammatory and neoplastic cellular infiltrates.\textsuperscript{56,57} In addition, the number of lymphocytes present and the signalment of the patient can be useful, assuming an adequate sample is obtained. Small cell hepatic lymphoma is usually associated with large numbers of lymphocytes infiltrating the liver, whereas low to moderate numbers of lymphocytes are associated with inflammatory infiltrates. Lymphocytic inflammation is often present in young adult males and
generally causes only mild hepatomegaly, whereas hepatic lymphoma is usually diagnosed in older cats with marked hepatomegaly.

**Cutaneous Lymphoma**

Although cutaneous neoplasia is common in cats, cutaneous lymphoma is relatively uncommon. Cutaneous lymphoma is usually seen in older FeLV-negative cats. However, there may be a link between FeLV and cutaneous lymphoma because FeLV provirus has been isolated from neoplastic cutaneous cells. Cutaneous lymphoma can present as either a solitary lesion or generalized skin disease and can manifest as either chronic pruritic exfoliative skin disease or chronic inflammatory disease. Two forms of cutaneous lymphoma have been described in cats: epitheliotropic and nonepitheliotropic. Epitheliotropic lymphoma, which is sometimes called mycosis fungoides, is usually a T-cell lymphoma with intraepidermal nests of five to 10 cells. These cells are usually large lymphocytes with round nuclei that have smooth chromatin and one to multiple nucleoli. The cytoplasm is relatively scant and basophilic. The large size of the lymphocytes allows the cytodiagnosis of lymphoma to be made relatively easily. Small cell lymphoma can also be seen, which frequently requires histopathology to distinguish the lesion from an inflammatory lymphocytic infiltrate. The nonepitheliotropic form of cutaneous lymphoma is usually composed of B cells that are present deeper in the dermis. These lymphocytes are generally large and readily distinguished on cytology from small lymphocytes.

**Central Nervous System Lymphoma**

Tumors of the CNS are rare in cats; however, the most commonly reported tumor is lymphoma. Lymphoma of the CNS can be primary but is more commonly associated with either multicentric or renal lymphoma. Cats with primary CNS lymphoma are most commonly young, and many test positive for FeLV. Clinical signs of CNS lymphoma include general neurologic abnormalities such as weakness, paralysis, and peripheral nerve dysfunction. Hindlimb paralysis is particularly common with spinal lymphoma. Although cerebrospinal fluid analysis is a relatively simple and quick method of diagnosing CNS disease, cerebrospinal fluid findings in patients with lymphoma are generally supportive of but not necessarily diagnostic of lymphoma because neoplastic and reac-
Cytodiagnosis of Feline Lymphoma

Differentiating lymphocytes can be difficult to distinguish and low cellularity is frequently present. A fine-needle aspiration sample or tissue imprints obtained during surgery are usually of greater diagnostic value (Figure 13).

Other Sites of Lymphoma

Ocular lymphoma is relatively common in cats and can be either primary or a component of the multicentric form. The clinical signs of ocular lymphoma include a variety of ocular signs, such as blindness, exophthalmus, or an identifiable mass. Another relatively common form of feline lymphoma is nasopharyngeal lymphoma, which most commonly occurs in older FeLV-negative cats. It is usually associated with signs of upper respiratory disease, such as sneezing, nasal discharge, and difficulty breathing. Nasopharyngeal lymphoma is most commonly composed of intermediate to large lymphocytes with smooth chromatin and prominent nucleoli. Therefore, cytology can be useful in diagnosing these lesions because neoplastic lymphocytes can be easily differentiated from an inflammatory population.

Recommendations for Cytodiagnosis of Feline Lymphoma

Diagnosing feline lymphoma begins with compiling the history, signalment, clinical signs, physical examination findings, and retroviral status of the cat. This information can assist in the cytodiagnosis and prognosis. Cytology remains a rapid, noninvasive, and effective tool.
for diagnosing feline lymphoma. However, some of the unique features of feline lymphoma must be recognized to effectively use this tool to establish a “working” diagnosis. In cases in which a population of small lymphocytes is aspirated from an organ or tissue, histopathology is often needed for final confirmation of a diagnosis of lymphoma.

REFERENCES


2. Which statement regarding small cell lymphoma in cats is incorrect?
   a. It most commonly occurs in the GI tract or liver.
   b. It usually requires histopathology for definitive diagnosis.
   c. It usually occurs in young cats.
   d. It may be mistaken for an inflammatory lymphoid infiltrate.

3. The cell type most commonly involved in feline lymphoma is the
   a. T cell.
   b. B cell.
   c. Natural killer cell.
   d. Large granular lymphocyte.

4. Which cell type is predominantly found in a normal lymph node?
   a. Large lymphocyte
   b. Plasma cell
   c. Well-differentiated, small lymphocyte
   d. Macrophage

5. Which statement regarding FeLV is true?
   a. One proviral integration into the genome of a cat is sufficient to cause malignant transformation of the cell.
   b. There is no link between FeLV and lymphoma in cats that are serologically FeLV negative.
   c. Introduction of the FeLV vaccine and effective testing have not reduced the incidence of lymphoma in cats.
   d. Cats that test positive for FeLV usually develop lymphoma at a young age.

6. Which statement regarding multicentric lymphoma is incorrect?
   a. In cats, it commonly manifests as peripheral lymphadenomegaly.
   b. In cats, it is usually composed of T cells.
   c. In cats, it may be associated with hepatic or splenic infiltration with neoplastic lymphocytes.
   d. In dogs, it commonly manifests as peripheral lymphadenomegaly.

7. Which organ is most commonly affected by large granular lymphoma in cats?
   a. Kidneys
   b. Thymus
   c. Liver
   d. GI tract

8. Which statement regarding renal lymphoma in cats is incorrect?
   a. It is usually diagnosed in older, FeLV-negative cats.
   b. It usually affects only one kidney.
   c. Infiltration of the CNS with neoplastic lymphocytes has been seen in cats with renal lymphoma.
   d. Renal lymphoma is usually composed of large lymphocytes.

9. Which lymph nodes are usually affected by Hodgkin’s-like lymphoma?
   a. Popliteal
   b. Mediastinal
   c. Submandibular
   d. Mesenteric

10. Which statement regarding thymic lymphoma in cats is incorrect?
    a. Thymoma is more commonly diagnosed than thymic lymphoma.
    b. Thymic lymphoma is usually T cell in origin.
    c. Thymic lymphoma is usually composed of large lymphocytes.
    d. Clinical signs of thymic lymphoma may include dyspnea, coughing, and Horner’s syndrome.