Proceeding of the NAVC
North American Veterinary Conference
Jan. 8-12, 2005, Orlando, Florida

Reprinted in the IVIS website with the permission of the NAVC
http://www.ivis.org/
Do not aspirate normal sized lymph nodes. Enlargement may be generalized in the case of systemic diseases, or a localized enlargement when the lymph node is draining an area with the primary problem. Cytologic or histologic evaluation is required to differentiate the most common causes of “lymphadenomegaly” (big nodes) = lymphoid hyperplasia (“reactive” lymph node), lymphadenitis, lymphoma and metastatic neoplasia.

The critical question is: Does the animal have lymphoma? Lymphoma (or lymphosarcoma) in dogs commonly presents as generalized (peripheral) lymph node enlargement, but can occur as regional cluster or individual lymphadenomegaly. Differentials for lymphadenomegaly are: lymphoma, reactive hyperplasia, lymphadenitis, or metastatic neoplasm (table) and cytology should identify which is present.

NORMAL LYMPH NODE CYTOLOGY

Although you should not aspirate normal sized nodes there are two primary situations when you might do this. One, is there a metastasis to a lymph node that is “draining” an area where a tumor is located? Two, you are suspicious that an animal has lymphoma but cannot locate the tumor, and want to determine if it may be in one or more lymph nodes that are not detectably enlarged (rarely to never is the diagnosis lymphoma if the node is not enlarged).

Cytologic sampling of normal lymph nodes yields 70-90% small mature lymphocytes. These cells are similar to the lymphocytes observed in peripheral blood: small (approximately 10-15 microns in diameter) round cells with small amounts of basophilic cytoplasm and a single, round nucleus with homogenous, dark purple chromatin. The nuclei are rounded, fill the majority of the cytoplasm, and, occasionally, have a slight indentation on one pole.

Intermixed with the small lymphocytes are low numbers of larger mononuclear cells, consisting of two populations: 1) prolymphocytes - these cells are 15-25 µm and have large, rounded to slightly indented nuclei with open chromatin that stains less intensely than the small lymphocytes. There is usually more cytoplasm than in small lymphocytes, and it is pale basophilic, and 2) lymphoblasts – few in number; larger (20-35 µm in diameter) more abundant and more basophilic cytoplasm and, large nuclei with open chromatin (light-violet) and 1-3 prominent nucleoli. It is okay to see immature cells in a normal node, but they must be in the minority (few!).

Plasma cells may be present; they are medium size, with moderate to abundant amounts of deeply basophilic cytoplasm. They have a round, eccentric nucleus with coarsely clumped chromatin and may have a pale zone next to their nuclei (Golgi apparatus used to package the immunoglobulins). In tissues that are constantly exposed to antigenic stimulation, i.e., tonsil and mesenteric lymph nodes, plasma cells will be numerous. A rare eosinophil and even mast cells can be seen in lymph nodes draining the GI tract. Therefore, before over interpreting a specimen, know what normal is for that particular tissue. Plasma cells are great cells to see, they are easy to recognize and the more you see the more likely the lymph node has hyperplasia and not lymphoma!

There also will be low numbers of macrophages, even in a normal lymph node. This should be expected since there is such close interaction between the lymphoid system and the monocyte/macrophage system for the processing of antigens. Like plasma cells, macrophages are seen in greater numbers in lymphoid tissue such as tonsil and mesenteric lymph nodes. Macrophages are 2-3X the size of lymphoid cells and they are often confused with neoplastic cells.

Numerous red blood cells without erythrophagocytosis indicates trauma to blood vessels during the aspiration technique. If erythrophagocytosis is present it indicates prior hemorrhage or the removal of senescent RBCs or immune mediated extravascular hemolysis. If platelets are present, then a blood vessel was entered, and intravascular blood was aspirated. You don’t see platelets in a hematoma or in an area of hemorrhage; if platelets are present, then blood was aspirated from a vessel.

CYTOLOGY OF REACTIVE LYMPHOID HYPERPLASIA

This is a common and nonspecific finding in lymphoid tissue. Marked lymph node enlargement can be seen with this problem, especially if the primary problem is a dermatitis. There are numerous causes of lymphoid hyperplasia because this is a defensive reaction by the body’s immune system, i.e., dermatitis, septicemia, regional inflammation, metastatic tumor, etc., all stimulate lymphoid hyperplasia. Cytologic evaluation is very helpful in differentiating between neoplasia and lymphoid hyperplasia. Rarely is the inciting agent present in the reactive node.

In the majority of the cases, even with extreme reactive lymphoid hyperplasia, the small lymphocyte is still the predominant cell type seen. The key to the diagnosis is a greater proportion of plasma cells. Numerous plasma cells lead you away from lymphoma. Macrophages, as well as prolymphocytes and lymphoblasts, will also increase. Relative numbers of the various cell types are quite variable, but generally the small lymphocyte is still greater than 70% of the total cellularity. Other cells that you may identify in a reactive node, and that are not compatible with a diagnosis of lymphoma are: mast cells, eosinophils, a few neutrophils and “Mott” cells - these are plasma cells filled with packets of immunoglobin that are visible as eosinophilic globules (Russel bodies). A heterogeneous cell population indicates that lymphoma is not present and that reactive hyperplasia is present. You still may need to determine why the lymphoid system is hyperplastic, but at least lymphoma is ruled out.

Immature lymphoid cells will be increased because there is hyperplasia of lymphocytes and therefore cell division. These large, immature cells will catch your attention and may cause you to consider lymphoma. However, estimate the mature lymphoid cells compared to these large immature cells and you’ll see that the mature cells still greatly outnumber immature cells. Don’t consider lymphoma until
the immature cells are greater than 50% of the mature cells. Fortunately, in most cases of lymphoma, the immature cells will be 80-100% of the cell population. Prolymphocytes and lymphoblasts should be present in a node with reactive hyperplasia.

CYTOLOGY OF LYMPHADENITIS

Inflammatory disease involving the lymphoid tissue is classified based on the type of lymphadenitis (suppurative, eosinophilic, granulomatous, etc.). Do not include lymphocytes when trying to classify the type of inflammation (i.e., "lymphocytic lymphadenitis" is not in my dictionary).

If eosinophils are numerous (>10% of total nucleated cells), then it is an eosinophilic lymphadenitis; if neutrophils predominate, then it is a purulent lymphadenitis, etc. When these cells are seen, search for an etiologic agent. Admixed with these inflammatory cells, there will also be numerous plasma cells and immature lymphocytes. It is characteristic to have some degree of reactive hyperplasia with the inflammation. In some purulent nodes there may be 100% neutrophils in the aspirate (absscess of lymph node).

ETIOLOGY

eosinophils: parasitic, immune, dermatitis, some tumors – (Produce IL3 and IL5 and stimulate eosinophilopoiesis)

neutrophils: if degenerate = septic : Staphylococcus, Streptococcus, etc. if nondegenerate = nonseptic: necrosis, tumor, viral, post antibiotic therapy of a bacterial infection

granulomatous: fungal : blastomycosis, other yeast and hyphal organisms, foreign material mycobacteriosis – some cases may be 100% macrophages- they are large and filled with organisms

CYTOLOGY OF PRIMARY LYMPHOID NEOPLASIA

Lymphoma is the number one differential diagnosis for peripheral lymphadenopathy (enlargement) without a known cause. In the majority of these cases, cytologic evaluation can give a specific diagnosis before histopathologic evaluation is even begun. The primary thing to remember is that in all of the non-neoplastic situations noted above, the cytology has presented with a mixture of various cell types, and the small lymphocyte was the most common cell seen. With lymphoma, unless in the very early stages of development (which is rarely seen in clinical settings in which lymph nodes are enlarged), there is replacement of the lymph node by a monomorphic population of large immature lymphocytes. The small lymphocyte is now an uncommon cell and lymphoblasts are the number one cell population. These cells are bigger than neutrophils, have small to moderate amounts of blue cytoplasm, and a large nucleus with fine chromatin and prominent nucleoli. Plasma cells and other cells are not present or are rare. The key to the diagnosis is replacement of small lymphocytes by large lymphoblasts. If you need a reference point for size, find a neutrophil (~15µ) or a red blood cell (7µ) for comparison. At this point, you are probably done, but if you want to further classify the lymphoma, you can try yourself or you can send slides to a reference laboratory: lymphoblastic - most common, large cell, light violet/pink/amphophilic chromatin, nucleoli common; prolymphocytic - next most common, medium size, clumpy chromatin - but not dark like a small lymphocyte, nucleoli uncommon; histiocytic - 3rd on your list, largest cell type, most abundant cytoplasm, open chromatin = very light, indentations in nuclei, nucleoli not obvious; lymphocytic - rare, small cell, dense chromatin, nucleoli not visible. Try not to make a diagnosis of lymphocytic lymphoma because this type of lymphoma is rare in veterinary medicine. It is probably just a "normal" lymph node with many small lymphocytes. If you are convinced there is lymphocytic lymphoma then send slides from at least two lymph nodes to a reference laboratory for their interpretation. These forms do exist, of course it will be in "cats" and to add to the diagnostic difficulty it will be in mesenteric lymph nodes. There are other classifications based on cytology, histology, and distribution. The key is to diagnose lymphoma. T and B lymphoid neoplasms cannot be identified via routine cytology or histology. This requires the use of flow cytometry or immunohistochemistry. However, the single best predictor of response to therapy and survival is B vs T cell lymphoma. B-cells respond better to chemotherapy and have a longer survival than do T-cell neoplasms in dogs. In cats the criterion to predict survival and/or response to treatment are not well established. There is a suggestion that lymphocytic lymphomas in cats respond better to some forms of therapy.

"Lymphoglandular" bodies are pinched off pieces of cytoplasm that appear as anuclear, basophilic structures scattered in between cells. They are more prominent with lymphoma but can be seen with lymphoid hyperplasia in reactive lymph nodes. Sometimes, lymphoma cells can look plasmacytoid. These types have more abundant basophilic cytoplasm, an eccentric round nucleus and a prominent Golgi. This pattern is especially easy to see in body cavity fluids that contain exfoliated lymphoma cells.

Another type of lymphoma is the large granular cell lymphoma (LGL). The cells and nuclei are round, they look lymphoid but the cytoplasm contains a few to many eosinophilic granules. The granules are usually small and inconspicuous but occasionally can be prominent. When granules are observed one of the differentials is a mast cell neoplasm. LGL have less cytoplasm, fewer granules, smaller size and no or few eosinophils as compared to mast cell neoplasms. LGL stain positively with PTAH, and negative with toluidine blue, mast cell tumors stain just the opposite. LGL’s are more common in cats than dogs, they often involve mesenteric lymph nodes and they are not associated with FeLV. I am aware of definitive techniques, or if they even exist, to distinguish LGL from globular leukocyte cell tumors.

As always, correlate the laboratory data with the patient, i.e. age, hypercalcemia, weight loss, nonregenerative anemia, mediastinal mass, leukemia is actually rare, and lymphopenia is common. In veterinary medicine, the majority of cases of lymphoma are lymphoblastic.

Summary: Lymphoma is easily recognized when the neoplastic process is fully developed. Key to the diagnosis is to find 80-100% of the lymphoid cells immature and "blastic." If there are numerous mature lymphocytes and clearly visible plasma cells don’t diagnose lymphoma. The more plasma cells visible and especially if neutrophils or eosinophils are seen, then the diagnosis is not lymphoma. What makes lymphoma so easy to diagnose is that the neoplastic...
population is generally the only cell present in the preparations. Don't diagnose a mature lymphocytic lymphoma because they are very rare in veterinary medicine. Lymphoma is rare, and it does not exist as the only place lymphoma could be found. However, nasal tumors (poorly differentiated sarcomas and carcinomas) can look lymphoid because of the round shape to the nuclei, little visible cytoplasm and prominent nuclear chromatin. This is a characteristic pattern for nasal carcinomas that are undifferentiated and they are not lymphomas. Cutaneous lymphoma can be difficult to differentiate from histiocytoma.

**Location:** Lymph nodes, skin, liver, spleen, GI, eyes, CNS, reproductive, heart, anyplace. Usually by the time the tumor is causing problems it can be located on both sides of the diaphragm, cats tend to have more visceral involvement (thoracic and abdominal) and dogs tend to have more peripheral lymph node involvement. The concept of nervous, ocular, gastrointestinal, cutaneous, etc. forms of lymphoma is slightly misleading. In “all” these cases the lymphoma is also located in lymph nodes, someplace!! The primary clinical problem may be located in one of these systems, but if the diagnosis is lymphoma then neoplastic cells are present in lymph nodes and neoplastic cells can be found elsewhere in the body.

**CYTOLOGY OF SECONDARY NEOPLASIA = METASTATIC TUMOR**

Cytology is as accurate as histology in identifying this problem. The discovery of a metastatic tumor in a lymph node is entirely dependent on features out of your control, i.e., how much of the lymph node has tumor, is the needle in an affected area? The way to combat these odds is to take multiple aspirates from the suspect lymph node. Should you aspirate the right area, you will see cytologic abnormalities compatible with a reactive lymph node, plus variable numbers of the tumor cells. The tumor cells are usually huge, compared to small lymphocytes (3-10 x their size) and may occur as singles or clusters. Some tumors have nuclei larger than leukocytes. You are just as likely to find a carcinoma as a sarcoma in a lymph node; neither has preferential pattern of metastases in lymphatics vs. blood vessels. If the suspected tumor cells are in clusters, rafts, acini, spheres etc. It is probably a carcinoma. Anytime you diagnose a metastatic tumor consider granulomatous inflammation as a differential diagnosis (and vice versa). Macrophages can be large and easily misinterpreted as neoplastic cells. Search for phagocytosis in the large cells = large vacuoles, abundant cytoplasm and an admixture of other inflammatory cells – all of which favor granulomatous inflammation rather than metastatic tumor, and correlate cytologic abnormalities with clinical suspicion.

A draining lymph node is sometimes aspirated to determine if a tumor has metastasized. If the primary tumor is a melanoma or a mast cell neoplasm then the preparation must contain numerous cells with granules and preferably the cell will be in groups or clusters. Melanophages and melanocytes can be found in normal lymph nodes or especially in nodes draining areas of inflammation (dermatitis). So, simply finding pigmented laden cells is insufficient (but highly suspicious) to confirm a melanoma. Furthermore, hemosiderin (green, golden brown, yellow, blue) must be differentiated from melanin. Mast cells are often in hyperplastic lymph nodes, especially if the node is draining a dermatitis (allergic or parasitic) or is a gastrointestinal lymph node. The latter tend to have numerous plasma cells and other inflammatory cells (mast cells included) due to the tissue they are responding to. If mast cells are numerous or in clusters it will increase your confidence of a metastatic pattern.

<table>
<thead>
<tr>
<th></th>
<th>“Normal”</th>
<th>Hyperplasia (reactive)</th>
<th>Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small lymphocytes</td>
<td>&gt;90%</td>
<td>&gt;50%</td>
<td>&lt;50%</td>
</tr>
<tr>
<td>Large lymphocytes</td>
<td>&lt;10%</td>
<td>&lt;50%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>Few</td>
<td>Many</td>
<td>Few to none</td>
</tr>
<tr>
<td>Other cells</td>
<td>Few</td>
<td>Increased</td>
<td>Rare to none</td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>