At the time of cancer diagnosis, the clinician’s first task is to determine disease extent within the body, that is, to stage the cancer. Stage describes the extent of the tumor, lymph node involvement, and spread of disease, measuring the scope of metastasis. Grade describes the appearance of the cells upon histopathology. Higher grade tumors are more likely to present at a higher stage. Staging is performed by the clinician, whereas grading is performed by the pathologist.

With practice, enough information can be gained from lymph node cytology to allow the general practitioner to begin a dialogue with pet owners about concern for metastatic cancer and its impact on prognosis.

This article reviews normal and abnormal lymph node cytology in both solid and round/discrete cell (hemolymphatic) tumors from the perspective of an oncologist.

ROUTES OF METASTASIS
Cancer can metastasize via lymphatics or blood vessels (hematogenously):
- Mesenchymal tumors (sarcomas) predominantly metastasize via blood vessels, but can occasionally travel by lymphatics, which is typically a sign of more aggressive disease—one that metastasizes more readily, resulting in shorter survival times.
- Epithelial (carcinomas) and round/discrete cell tumors metastasize via lymphatics more often than mesenchymal tumors.

Regardless of tumor type, regional lymph node cytology should be included in the first wave of diagnostics for most cancers.

LYMPH NODES TO SAMPLE
While metastasis to an unexpected lymph node is always possible, the important lymph nodes to aspirate at time of staging are:
- The primary draining lymph node
- Any enlarged lymph nodes (even if distant from the primary tumor).

For several tumors, including melanoma and mast cell tumor, metastasis can be identified even if lymph nodes are of normal size. In addition to screening for metastasis of solid tumors, lymphoma is often easily diagnosed using lymph node aspiration and cytology.

FINE-NEEDLE ASPIRATION
Location Considerations
When evaluating metastatic solid tumors, the primary draining lymph node that should be sampled depends upon location (eg, popliteal node for a hind foot digital tumor). Tumors located in areas of the body where lymphatic drainage is not clear, such as on the lateral thorax, can be challenging. In this situation, the nearest major lymph node should be aspirated if it can be isolated, though at times this is not possible and lymph nodes are noted as within normal limits without cytologic interpretation.

When diagnosing lymphoma, the popliteal lymph nodes are the most accessible lymph nodes, followed by the prescapular nodes. Mandibular lymph nodes should be avoided if other nodes are enlarged, because reactive lymphoid cells (due to changes in the ears or mouth) may cloud a diagnosis of lymphoma. However, if the mandibular nodes are the only enlarged lymph nodes, they should be aspirated. A cytologic diagnosis of reactive or equivocal lymph node should be interpreted with caution: if lymphoma is suspected, further testing, such as biopsy, should be pursued if suspicion is high.

Isolation of Node
When isolating a lymph node:
1. Place the forefingers in an anatomic location just beyond the node.
2. Use the thumb to isolate and steady the node.
   For example, to isolate the prescapular/superficial cervical node:
   1. Place the fingers in, or just above, the thoracic inlet.
   2. Sweep the thumb down the front of the shoulder where the supraspinatus muscles meet the neck muscles.
3. The gesture above will guide the node between the thumb and forefinger.

While each practitioner will develop their own feel for lymph node palpation, this technique may help isolate the deeper and more elusive lymph nodes.

Normal sized lymph nodes can sometimes be difficult to palpate and properly aspirate, especially in overweight or heavily muscled (e.g., Staffordshire terrier, some Labrador retriever) dogs. To increase the chance of success, before isolating the node itself:
1. Use a reference point as described above.
2. Make a mental note of normal structures that are palpated near the node.

Fine-Needle Aspiration
1. Use a needle without a syringe attached; any gauge is acceptable, but my preference is to use a 22-gauge needle to avoid discomfort.
2. Once a node is trapped between thumb and forefinger, introduce the needle.
3. Redirect the needle by moving it in and out through the node several times, until—when looking into the needle hub—a tiny bleb is apparent within the needle’s inner circumference; this avoids unnecessary hemodilution.

Slide Preparation
The following technique provides high-quality diagnostic slides for needle aspiration cytology:
1. Attach an air-filled syringe to the needle and expel only ½ drop from the needle onto each of 2 to 3 slides, which keeps each slide’s sample the right consistency, avoiding preparations that are too thick (Figure 1A).
2. Gently lay a clean slide crosswise on the droplet, allowing it to break the surface tension (Figure 1B).
3. While holding the slide on both ends with the free hand, gently pull the spreading slide across the aspirate slide, which allows good smearing of the droplet (Figure 1C and 1D). This technique avoids applying too much pressure on the sample and traumatizing the cells.

Note: If you stain a slide in-house using rapid fixation stains in preparation for laboratory evaluation, always send at least one unstained slide, which allows the clinical pathologist to apply his or her own stain.

CYTOLOGIC EVALUATION
Lymphocyte Size
Lymph nodes are predominantly comprised of small, mature lymphocytes (80%–90%). Lymphoid cells typically have high nuclear to cytoplasmic ratios.

Size is important when determining whether the lymphoid population is of concern. Cell size is typically compared to a red blood cell (RBC), but neutrophils are less likely to fold and pile up, and are slightly larger than RBCs.

Therefore, if possible, the nucleus should be compared to a RBC, and the whole cell to a neutrophil, if any are present (Table 1, page 20).

Findings That Complicate Diagnosis
Reactive Lymph Nodes
Reactive lymph nodes are characterized by increased numbers of plasma cells, notable due to their deeply basophilic cytoplasm and perinuclear clear zone.
- Increased numbers of lymphoblasts are often seen in reactive lymph nodes, but they should not total more than 50% of the lymphoid population.
- Increased numbers of neutrophils are also seen, and other inflammatory cells, such as eosinophils may also be present.

Inflammatory Cells
When inflammatory cells are present, they can sometimes obscure metastatic cells or make cytologic changes difficult to interpret. The presence of mast cells in a lymph node near a mast cell tumor can be especially challenging. While some mast cells may represent cytokine signaling and chemotaxis attracting normal mast cells, a high proportion as well as clustering of mast cells may represent true metastatic disease.

Infectious Organisms
Other findings that do not belong in lymph nodes include infectious organisms, which may be seen against a background of reactive lymphocytes and inflammatory cells. Fungal and bacterial causes of lymphadenopathy can stimulate increased numbers of macrophages and neutrophils.

Figure 1. Optimal lymph node aspirate slides are not too thick, and the smear has a smooth, oval appearance.
Categories of Neoplasms
After evaluating lymphocyte cell size, the next step in successful cytologic evaluation is to determine whether any other types of cells that represent metastasis to the node are present. If present, these cells can be categorized as mesenchymal, epithelial, or round/discrete. In addition, uncommon categories, such as neuroendocrine or histiocytic, may be relevant.

Criteria of Malignancy
Finding cells where they do not belong, such as the presence of epithelial cells in a lymph node, is a sign of neoplasia. Malignant cells are bizarre, and may not immediately resemble their cell of origin. While atypia can be seen in diseased tissue, the presence of cytologic changes outlined in Table 2, combined with lack of inflammation, is a strong indication of malignancy:
• When inflammation is prominent, it can be difficult to commit to a diagnosis of cancer.
• However, very bizarre cells support a diagnosis of malignancy even if some inflammation is present.

Fibroblasts can be misleading—tumors can elicit a scirrhous response in which reactive fibroblasts are present among an atypical population, potentially creating the impression of a mesenchymal neoplasm, when the primary malignant process has a different origin, such as carcinoma or mast cell tumor.

Mitotic figures can be seen in both normal and reactive lymph nodes as part of the normal renewal of lymphocytes. Mitotic figures that are concerning include those:

TABLE 1. Lymphocyte Size & Appearance

<table>
<thead>
<tr>
<th>TYPE</th>
<th>SIZE</th>
<th>APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small Mature</td>
<td>Same size or smaller than</td>
<td>• Dense chromatin in nucleus (more deeply basophilic appearance)</td>
</tr>
<tr>
<td></td>
<td>neutrophil or RBC</td>
<td>• High nuclear:cytoplasm ratio (very little cytoplasm)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Similar to, or only slightly</td>
<td>• Interpret in light of total cell population (Table 3)</td>
</tr>
<tr>
<td></td>
<td>larger than, neutrophil</td>
<td></td>
</tr>
<tr>
<td>Lymphoblasts:</td>
<td>Larger than neutrophil or</td>
<td>• Looser chromatin in nucleus (lighter blue appearance)</td>
</tr>
<tr>
<td>Large Immature</td>
<td>RBC</td>
<td>• Lower nuclear:cytoplasm ratio (more cytoplasm)</td>
</tr>
</tbody>
</table>

DIAGNOSES BASED ON LYMPH NODE CYTOLOGY
Lymphoma
The most common finding on fine-needle aspiration and cytology of lymph nodes that are enlarged due to lymphoma is a monomorphic population of lymphoblasts (Figure 2). Characteristics of lymphoma identified by lymph node cytology are listed in Table 3.

Comparison to RBCs or neutrophils can help with size determination. It is tempting to suspect lymphoma when a large population of lymphoblasts is seen; however, use caution and spend some time evaluating all the cells—there may be more small lymphocytes present than appreciated initially.

If an enlarged lymph node is reactive, there should be a population of plasma cells and inflammatory cells, and more variety to the lymphoid population (Figure 3). Biopsy may be needed to confirm the diagnosis if only lymphoid cells—most of similar size (and intermediate or small)—are seen; however, lymphoma should be strongly suspected. This is especially true when multiple peripheral

TABLE 2. Malignancy Criteria

• Mitotic figures, especially if bizarre, with haphazardly arranged, instead of orderly, chromatin
• Multiple nuclei, especially if an odd number
• Multiple nucleoli, especially if they vary in size
• Anisocytosis
• Anisokaryosis

TABLE 3. Lymphoma Characteristics Identified by Lymph Node Cytology

• All (or almost all) cells in the aspirate will be lymphoblasts characterized by:
  » High nuclear:cytoplasmic ratio
  » Loose chromatin pattern
  » Discrete cell pattern: Abundant cellular yield, round cells and nuclei, and no obvious clustering (although number/close proximity of cells may initially resemble clustering)
  • Anisocytosis and anisokaryosis may be present
  • The sample often lacks any indication of inflammation
  • Monomorphous cells (roughly the same size and shape) that are intermediate or small in size should arouse suspicion of lymphoma—a normal or reactive lymph node contains lymphocytes and lymphoblasts of many different sizes, though smaller in proportion to mature lymphocytes
Lymph Node Cytology

When using flow cytometry, the laboratory that will be processing the sample should be contacted before sample collection, as special handling (suspending the aspirate in a specific medium, and overnight shipping on ice) is required.

nodes are enlarged and the dog is asymptomatic, because infectious causes of lymphadenopathy more often lead to clinical signs of illness.

Histopathology can identify marginal and T-zone lymphomas that are indolent; biopsy specimens should ideally include the entire node and should be sent to a pathologist with expertise in this area. These lymphomas may not require treatment; if treated, they have a lower response rate to treatment but longer survival times.

Flow cytometry can determine the immunophenotype of a labeled cell. Immunophenotype of lymphoma affects prognosis; median survival for T-cell lymphoma is typically one-half the median survival for B-cell lymphoma for a given chemotherapy protocol. Flow cytometry is performed on a needle aspirate sample suspended in a special medium.

Immunohistochemistry (biopsy samples) or immunocytochemistry (fine-needle aspirate samples) can also be used to determine immunophenotype.

While knowledge of immunophenotype may provide an improved understanding of expected outcome, and while some clinicians favor certain protocols based on immunophenotype (some clinicians prefer alkylator-heavy protocols for T-cell lymphoma), it has never been shown that modifying the chemotherapy protocol based on knowledge of immunophenotype improves outcome, and multidrug (CHOP-based) protocols are still effective for all high-grade lymphomas.

PCR for Antigen Receptor Rearrangement (PARR) can be performed on aspirate slides when a diagnosis of lymphoma is elusive. With this technique, DNA of the variable regions encoding the immunoglobulin and T-cell receptors is amplified.

- In a normal immune system, there is great variety to protect the body from as many antigens as possible.
- When a lymphocyte or lymphoblast undergoes a malignant transformation, then clonally expands, there is great redundancy in the lymphoid population of a sample.

• In other words, all malignant lymphocytes are programmed to make the same receptor because they all came from the same progenitor; PARR detects this monotony.

Thus, detection of monoclonal population of cells by PARR confirms the presence of neoplasia. False positive tests are rare, but may be seen with ehrlichiosis.

Solid Tumor Metastasis

When lymph nodes are completely effaced with tumor cells, the diagnosis of metastatic neoplasia is often straightforward. However, in the absence of background lymphoid population, treatment may be delayed as the diagnosis of lymphoma is pursued.

| TABLE 4. Appearance of Metastatic Cells Found in Lymph Nodes |
|---------------------------------|-----------------------------|-------------------------------|
| **METASTATIC CELLS** | **APPEARANCE** | **CELLS ORIGINATE FROM:** |
| Mesenchymal Cells | • Round to ovoid nuclei | Sarcoma |
|                   | • Indistinct cell borders | |
|                   | • Trailing/wispy cytoplasm | |
| Epithelial Cells  | • Round nuclei | Carcinoma |
|                   | • Abundant, angular cytoplasm | |
|                   | • Tend to occur in clusters or sheets* | |
|                   | • Background population of mostly small lymphocytes | |
| Round Cells       | • Round nuclei | Histiocytoma |
|                   | • Cytoplasm of varying amounts | Lymphosarcoma |
|                   | | Mast cell tumor |
|                   | | Melanoma** |
|                   | | Plasmacytoma |
|                   | | Transmissible venereal tumor |

* These may be infrequent, requiring that the entire slide be carefully examined.
** Melanoma may occasionally be categorized differently, depending on the characteristics of individual tumors.
Lymph nodes draining in the region of a mast cell tumor can be particularly troubling, because some mast cells are normally found in reactive lymph nodes. When reactive mast cells are recruited to local lymph nodes by cytokines produced by the mast cell tumor (ie, nonmetastatic mast cells), they are expected to infiltrate the node individually. In addition to metastatic mast cells found in lymph nodes meeting cytologic criteria of malignancy (Table 2), clustering of these cells (into aggregates) can also signify metastatic disease.1 When in doubt, the lymph node should be surgically removed and submitted for histopathology, perhaps at the time of initial mast cell surgery.

Lymph node involvement in mast cell tumors impacts both treatment and prognosis of these tumors. Locoregional treatment of a mast cell tumor with surgery, with or without radiation therapy to include a local lymph node, can be very successful unless metastasis has spread beyond the regional lymph node.

Melanocytes, characterized by their granules, should not be present in regional lymph nodes and typically represent metastatic disease (Figure 6). However, lymph nodes can contain regional melanophages—large round cells with abundant, vacuolated cytoplasm—and this may not represent metastatic disease.

IN SUMMARY

Lymph node cytology can be performed quickly, as a minimally invasive procedure, and results can strongly influence treatment and outcome for cancer patients. Like any other skill, evaluation of cytology from a lymph node takes time and practice. ■

PARR = PCR for antigen receptor rearrangement; RBC = red blood cell

References


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cytos, it can sometimes be difficult to determine with certainty whether the aspirated structure actually was a lymph node. In most cases, the sample contains small numbers of metastatic cells mixed with lymphocytes (Table 4, page 21).

Diagnosis based on cytology of a metastatic lymph node—even before the primary tumor is located—is not uncommon. One example is oral tumors: because owners do not routinely look inside their dogs’ mouths, they may present the pet because a mass was noted in the mandibular region (Figure 4).

Early metastasis can be challenging, because its presence can cause some degree of reactivity in the node (Figure 5) or, if metastasis to lymph nodes has occurred, in regions of the node that are not sampled by fine-needle aspiration.

Lymph node biopsy or removal for histopathology should be performed if there is concern for metastasis based on size or shape of a lymph node that cannot be confirmed on cytology.

Figure 4. Squamous cell carcinoma metastasis to a lymph node: the abundant population of small lymphocytes in the background confirms lymph node origin, but the large, angular epithelial cells with abundant cytoplasm do not belong in a lymph node under any circumstance; note the occasional lymphoblast and plasma cell. Courtesy Dr. Natalie Hoepp

Figure 5. View (2×) of a sectioned histologic specimen of a lymph node showing the limitations of needle aspirates: On the left side of the image, the node is effaced with a neoplastic cell population, but the right side of the specimen shows a reactive lymph node population. Needle aspiration can miss a neoplastic population, and histopathology is recommended if there is clinical concern. Courtesy Dr. Tamara Hancock

Figure 6. Melanophages, containing abundant dark pigment, can be seen in areas of chronic inflammation and other nonneoplastic pathology in draining lymph nodes. However, the more lightly granulated cell on the lower right (arrows) is a melanocyte, which should not be present in a lymph node and, therefore, is consistent with lymph node metastasis. Courtesy Dr. Erin Burton