INSIGHTS IN CLINICAL PATHOLOGY

Cytology of Lymph Nodes

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Lymph node cytology submissions account for a large proportion of all cytology submissions to a veterinary pathologist. Lymph node cytology can also be performed in-house to evaluate the quality of a smear before sending it to a pathologist for final interpretation or to make a preliminary diagnosis if rapid therapeutic intervention is indicated. Common clinical indications to perform lymph node cytology include[1,2]:

1. **Lymphadenopathy** (localized or diffuse)
2. **Staging for metastatic disease.** Draining lymph node(s) in the region of a primary neoplasm should be evaluated, especially for tumors known to metastasize more frequently. In these cases, lymph node fine-needle aspiration (FNA) should be attempted even if the lymph node is not enlarged. Tumors that tend to metastasize to lymph nodes include mast cell tumors, melanomas (especially in oral and digit locations), histiocytic sarcoma, and most carcinomas. Most sarcomas metastasize to the lung and/or visceral organs first, but exceptions exist.
3. **Suspected infectious disease** (e.g., fungal)
4. **Screening for occult disease** (e.g., fever of unknown origin, hypercalcemia with suspected paraneoplastic origin)

SLIDE PREPARATION

A detailed description of how to obtain a good sample for cytology, including supplies, collection methods, smear preparation techniques, and processing, has been published elsewhere.[3] Every cytology interpretation starts with a good-quality diagnostic specimen that, ideally, is a thin, highly cellular preparation of intact

Abstract

Lymph node fine-needle aspiration and cytology is a noninvasive, rapid, and cost-effective diagnostic tool. This article highlights a step-by-step approach to lymph node cytology after sample acquisition, explains the most common cytologic findings, and describes how to recognize nondiagnostic and poor-quality specimens. A thin, highly cellular smear with predominantly intact cells is crucial for accurate interpretation; making an interpretation from a thick smear or one with predominantly ruptured cells can be misleading. Every smear should be screened on low power to evaluate if the sample is adequate, to recognize unevenly distributed cells (i.e., metastatic cells), and to identify the area of interest for detailed high-power microscopy.

Take-Home Points

- In-house lymph node cytology can provide rapid preliminary diagnostic information.
- The most common cytologic findings in an enlarged lymph node are reactive hyperplasia, lymphoma, and metastatic neoplasia.
- A thin, highly cellular smear with intact cells is crucial for accurate interpretation.
- Thick, poorly stained areas and areas with predominantly ruptured cells should not be used for interpretation.
- Screening the smear on low power is essential to the correct approach and to evaluate if the sample is adequate.
- It is critical to recognize nondiagnostic aspirates.
- If the sample is not adequate, resampling or performing a different test (i.e., histopathology) is recommended.

cells. Smears can be made from an FNA sample and/or a touch imprint.[3-6] Lymphoid cells generally exfoliate well. Techniques vary, and aspiration or nonaspiration techniques can be used. The nonaspiration technique may be preferred for vascular and/or firm lesions in which aspiration tends to produce hemodiluted specimens (e.g., metastatic lymph node) and especially with delicate cell populations such as lymphocytes.[3-6]

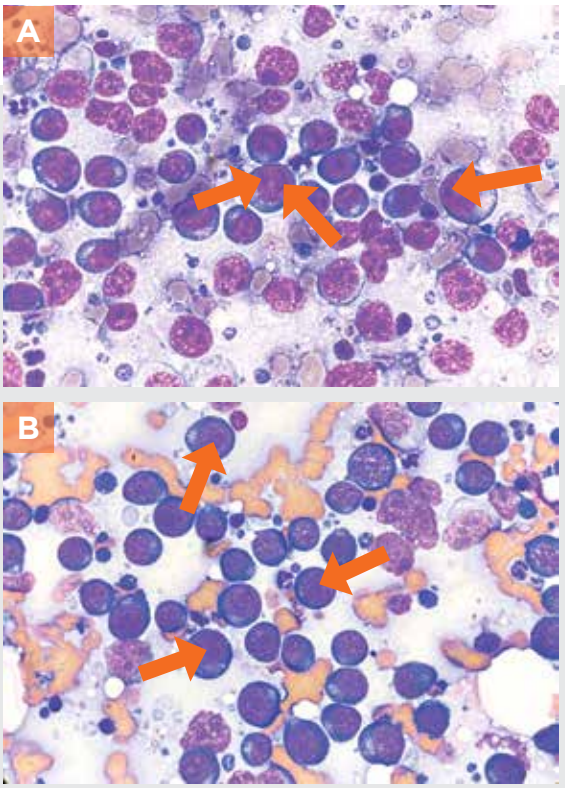

FIGURE 1. Lymph node aspirate from a dog with lymphoma with predominantly large lymphocytes. **(A)** Aqueous Romanowsky stain (Diff-Quik), 100× objective. Note the coarser texture of the chromatin and prominent nucleoli **(arrows)** compared with **B**. **(B)** Alcohol-based Romanowsky stain (modified Wright-Giemsa) from the same aspirate, 100× objective. The nuclear chromatin texture is finely stippled and smooth. Nucleoli **(arrows)** are visible but not as prominent as in **A**.

To make a good-quality smear, gentle pressure should be used to spread cells; applying too much pressure can cause excessive cell damage. For lymph node aspirates, especially lymphoma, the "roll preparation" technique has been successful at the author's institution in minimizing cell lysis that can occur with the traditional smear technique.[3] If FNA is performed using ultrasound guidance, the ultrasound gel must be wiped from the surface prior to inserting the needle through the skin. Even small amounts of ultrasound gel, like lubricant, can adhere to cells, causing damage and poor cell staining and resulting in nondiagnostic or suboptimal slides.[7]

Smears should be air dried, not heat fixed/dried, and stained with a Romanowsky-type stain (e.g., Wright-Giemsa). The water-based Romanowsky-type stains (Diff-Quik) are inexpensive, fast, and easy to use and work very well for lymphoid cells. Compared with alcohol-based Romanowsky-type stains, the nuclear chromatin can appear slightly coarser with Diff-Quik (**FIGURE 1**). The sample should be fixed properly in the fixation solution to ensure adequate staining. Thick, highly cellular smears may require more time for adequate fixation.

APPROACH TO LYMPH NODE SAMPLE EVALUATION

Step 1: Determine whether the sample is adequate and highly cellular.

Screening multiple areas on the slide using low power is essential in this stage of the evaluation. Do not rush to oil. Slide examination should begin by focusing on low power (4× and 10× objectives). The distribution of certain cell types, such as metastatic cells, can be patchy, and such cells can be easily missed when starting with the 40× objective and rushing to oil (50× or 100×). If metastatic neoplasia is suspected, a thorough low-power screen of all slides is necessary.

If the sample is not adequate (e.g., acellular, poorly cellular, all cells are broken, only blood is present),

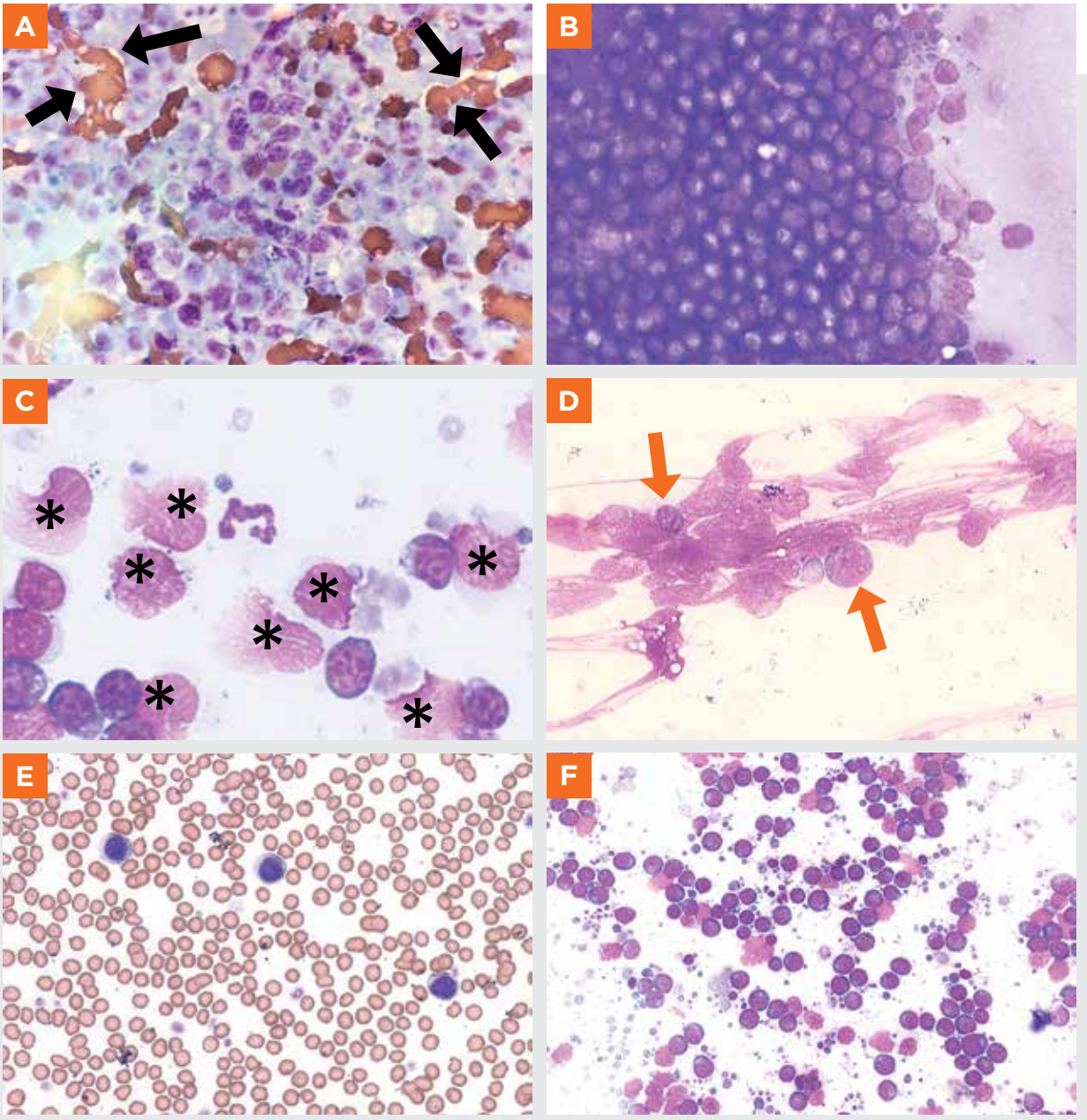

FIGURE 2. Identifying the most appropriate area for microscopic evaluation of a lymph node aspirate is crucial. **(A AND B)** Areas that are too thick will be understained. In **A**, red blood cells appear in dense, thick aggregates **(arrows)** and are piled on top of each other. **(C)** Many ruptured cells **(asterisks)** are present along with a few small, intact lymphocytes and a neutrophil. Ruptured cells should not be falsely interpreted as large lymphocytes. **(D)** Pink streaming nuclear material from ruptured cells. Rare intact lymphocytes **(arrows)** are present. **(E)** Markedly hemodiluted lymph node aspirate. Too few lymphocytes are present for interpretation. **(F)** Adequate area characterized by a thin monolayer with predominantly intact cells that are nicely spread out. All images, Wright-Giemsa stain; **A-E**, 100× objective; **F**, 50× objective.

resampling or performing a different test (i.e., surgical biopsy) is recommended.

Step 2: Identify the area of interest for high-power microscopy. Avoid examining poorly stained or overtly thick regions. Thick or poorly stained areas can make cell size difficult to judge (**FIGURES 2A AND 2B**). Additionally, pale staining results in pale blue nuclei that lack sharp chromatin detail and nucleoli that can appear very prominent; therefore, an understained lymph node sample can mimic lymphoma.

When moving to high-power microscopy, if only a 40× objective (no 50×) is available, a thin coverslip should be placed on the stained smear. A smear without a coverslip will look blurry (**FIGURE 3**). Oil should never be used with the 40× objective.

Step 3: Confirm cells are intact (**FIGURES 2C AND 2D**).

Step 4: Size the lymphocytes. A thin area with intact cells should be used. Thin areas can be identified by red blood cells in the background of the slide that are not overtly piled up.

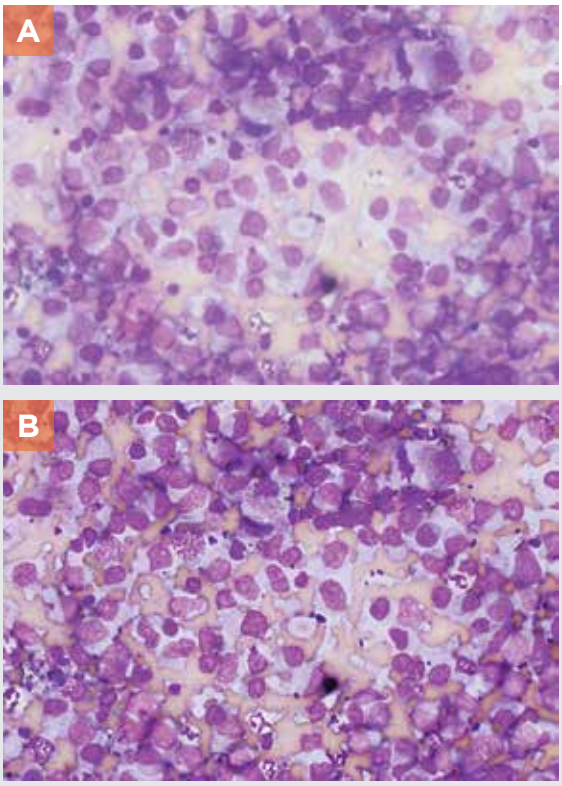

FIGURE 3. Lymph node aspirate from a cat. **(A)** Without a coverslip, the field appears blurry, which prevents evaluation of cellular details. **(B)** Same area as **A**, with a coverslip on top of the slide. Cellular details are clearly visible. Wright-Giemsa stain, 40× objective.

If the sample is not adequate (e.g., acellular, poorly cellular, all cells are broken, only blood is present), resampling or performing a different test (i.e., surgical biopsy) is recommended.

There are different ways to size lymphocytes; a helpful measuring stick is the neutrophil. A small lymphocyte will be smaller than a neutrophil and will have coarse, dark chromatin and just a small amount of cytoplasm, often visible on only one side of the cell. Intermediate-sized lymphocytes are approximately neutrophil-sized. They have a little more cytoplasm, and the chromatin is slightly more "open" (dispersed, stains paler, and is less clumped). Large lymphocytes are larger than a neutrophil (up to 4 times the diameter of a red blood cell); have pale, lacy chromatin and a thin rim of cytoplasm; and may or may not have visible nucleoli.

Step 5: Evaluate the relative percentages of small, intermediate, and large lymphocytes (**FIGURES 2E AND 2F**). Also look for plasma cells, histiocytes/macrophages, granulocytes, mast cells, and other cell types (e.g., atypical cells). The proportion of nondegenerate neutrophils should be interpreted in light of the amount of hemodilution as peripheral blood contamination can increase the number of leukocytes, especially neutrophils, if leukocytosis is present.

Step 6: Interpret findings.

POSSIBLE LYMPH NODE CYTOLOGY FINDINGS

The 3 basic findings for lymph tissue samples are (1) normal or unremarkable, (2) reactive/hyperplastic, and (3) lymphoma. Additional lymph node findings include inflammation/infection, metastatic neoplasia, and nondiagnostic specimens (e.g., acellular, aspiration of perinodal fat, salivary tissue).[8]

Normal Lymph Node

In a normal lymph node, small, mature lymphocytes compose the majority of cells (approximately 75% to 95%; **FIGURE 4A**).[8,9] The remaining cells are primarily intermediate lymphocytes and a few large lymphocytes. Rare plasma cells may be seen; these cells have moderate amounts of deeply basophilic cytoplasm with a perinuclear clear zone (Golgi zone) and a round, eccentrically located nucleus with very coarse chromatin. Mott cells are plasma cells distended with large vacuoles of immunoglobulin (Russell bodies). Rare histiocytes/macrophages and/or mast cells may be seen; however, mast cell granules may not stain with Diff-Quik. Granulocytes should be proportional to the degree of hemodilution. Small basophilic fragments of lymphocyte cytoplasm (lymphoglandular bodies) are often present in the background. They are highly characteristic of lymphoid tissue but are not specific for lymphoma.

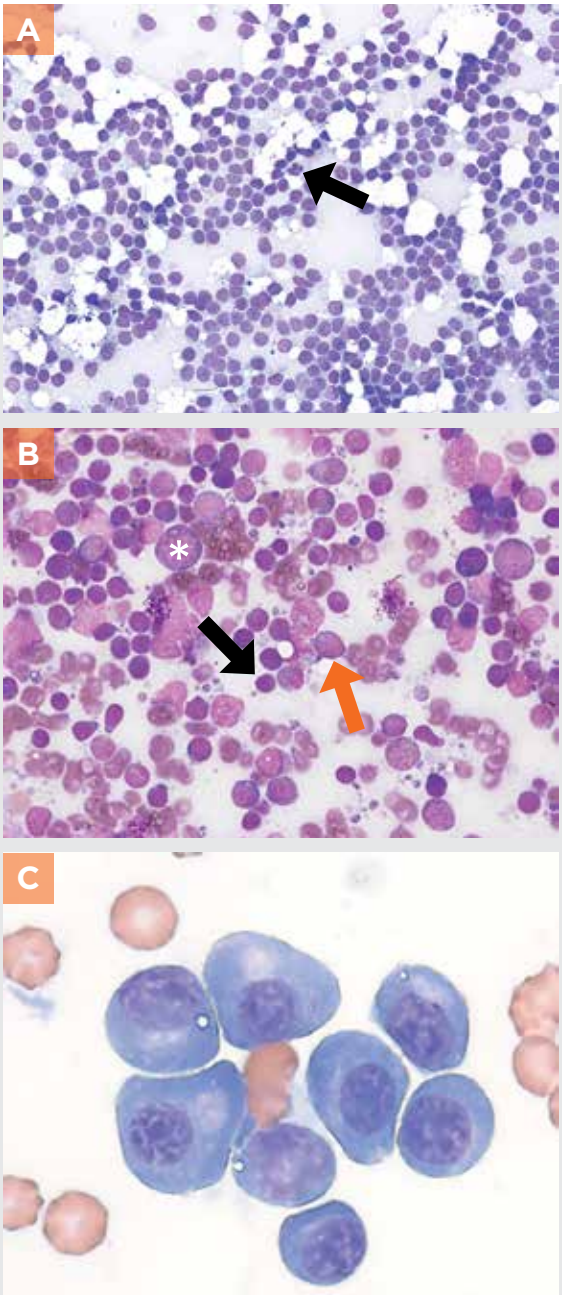

FIGURE 4. (A) Lymph node aspirate from a cytologically unremarkable lymph node. Small lymphocytes predominate and are smaller in diameter than a neutrophil **(arrow)**. 50× objective. **(B)** Lymph node aspirate from a reactive lymph node. Small lymphocytes **(black arrow)** still predominate, but the lymphoid population is mixed, with intermediate **(orange arrow)** and large lymphocytes **(asterisk)** also present. 100× objective. **(C)** A group of plasma cells, which is a common finding in reactive lymph nodes. Wright-Giemsa stain, 100× objective.

A "normal" cytologic appearance in a lymph node that is grossly increased in size can be consistent with either hyperplasia (reactive) or small-cell lymphoma. Small-cell lymphoma usually requires additional testing for diagnosis, such as histopathology, flow cytometry, and/or polymerase chain reaction for antigen receptor rearrangement. Discussion of these advanced diagnostics can be found elsewhere.[10,11]

Hyperplastic/Reactive Lymph Node

A reactive, hyperplastic lymph node has a mixed appearance on low magnification, with significant

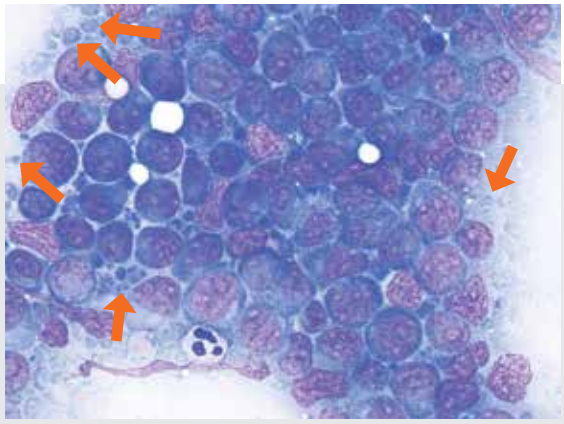

FIGURE 5. Aspirate from an enlarged lymph node from a dog with lymphoma. Large lymphocytes predominate throughout the smear. A neutrophil is shown at the bottom for size comparison. Lymphocytes have small amounts of deeply basophilic cytoplasm and a nucleus with fine chromatin and multiple visible nucleoli. Small, deeply basophilic cytoplasmic fragments (lymphoglandular bodies) **(arrows)** are scattered in the background. Wright-Giemsa stain, 100× objective.

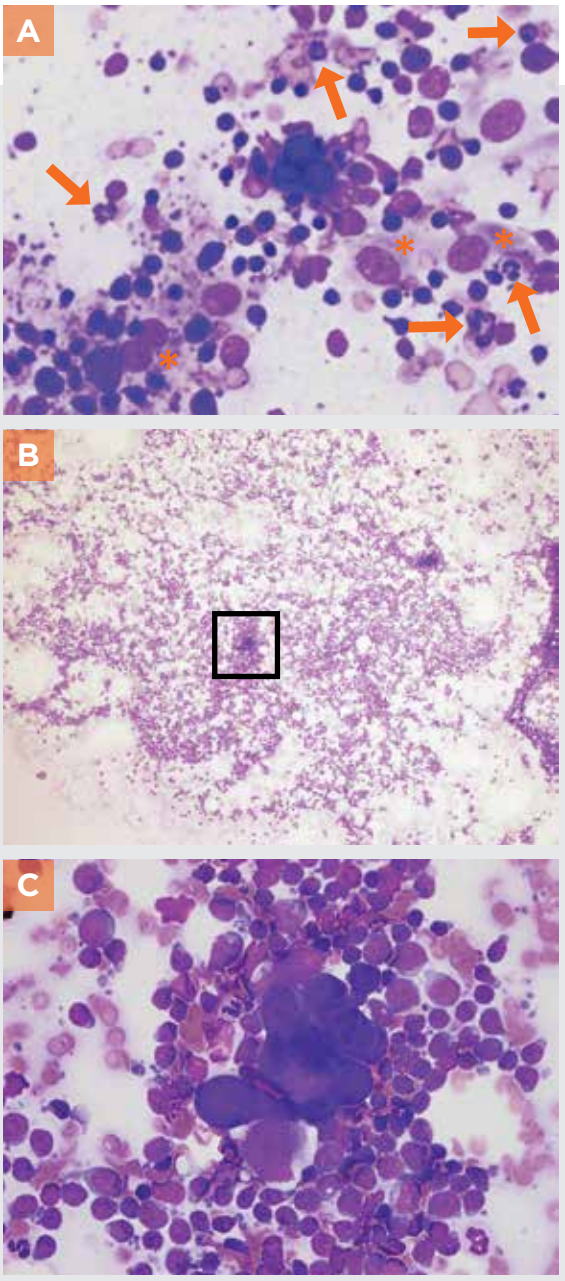

FIGURE 6. (A) Lymph node aspirate from a dog with fever, cough, and mild peripheral lymphadenopathy. A group of 3 *Blastomyces* species yeast cells is present in the center of the image, surrounded by mixed lymphocytes, some macrophages **(asterisks)**, and mildly increased neutrophils **(arrows)**. Wright-Giemsa stain, 100× objective. **(B)** Submandibular lymph node aspirate from a dog with chronic nasal discharge and recent onset of epistaxis. Rare small clusters of large cells **(black box)** are identified on low-power examination. Wright-Giemsa stain, 10× objective. **(C)** Same case as **B**. On high-power examination, these cells are pleomorphic epithelial cells surrounded by mixed lymphocytes. The diagnosis was metastatic carcinoma. The primary tumor was a nasal adenocarcinoma. The metastatic cells in the lymph node could have been easily missed without a thorough low-power screen. Wright-Giemsa stain, 100× objective.

variation in cell size and chromatin texture and/or color (dark and coarsely clumped to pale and open). In contrast, "normal" and "lymphoma" nodes appear more homogenous. Small lymphocytes still predominate in a reactive lymph node, but more intermediate and large lymphocytes are present (**FIGURE 4B**), and there may be an increased number of plasma cells and/or Mott cells (**FIGURE 4C**). Increases in other cell types (macrophages, mast cells) are variable.[8,9] Neutrophils and eosinophils may also be increased but represent less than 5% and 3% of the nucleated cell count, respectively (if greater, then the node is inflamed).

A single reactive lymph node or region of nodes can be due to any source of inflammation and/or antigenic stimulation in the draining area, such as skin disease, an abscess, injury, or an inflamed neoplasm. Generalized reactions affecting most or all peripheral and/or internal lymph nodes can be seen with systemic infections (e.g., Rocky Mountain spotted fever, ehrlichiosis/anaplasmosis, feline leukemia virus, feline immunodeficiency virus), immune-mediated disease, or any other systemic inflammatory condition (e.g., atopic dermatitis).

Lymphoma

Lymphoma is the top differential in any dog with generalized lymphadenopathy. Lymphoma of the lymph node (large-cell lymphoma) can be diagnosed when large lymphocytes represent more than 50% of the cell population, but most cases have more than 80% to 90% of these cells (**FIGURES 1 AND 5**).[8,9] Unlike many malignant neoplastic populations, lymphoma tends to be relatively uniform on cytology (less anisocytosis and anisokaryosis). Lymphoglandular bodies are often numerous in the background, and mitotic figures may be seen. However, the last 2 findings can also be observed in reactive lymph nodes. Other, less common, variants than large-cell lymphoma also exist (e.g., T-zone lymphoma, small-/intermediate-cell lymphoma, large granular lymphocyte lymphoma, lymphoma with a mixed-cell appearance).[8,12]

In cats, peripheral lymphadenopathy is much more commonly reactive than lymphoma. An in-house diagnosis of lymphoma in the peripheral lymph node of a cat should always be confirmed by a pathologist.

Other Important Findings

Lymphadenitis

Inflammation within the lymph node is characterized by increased numbers of neutrophils (>5%), eosinophils (>3%), macrophages, or multinucleated giant cells. Reactive hyperplasia is usually also present. Neutrophilic or pyogranulomatous inflammation should trigger a thorough screening for etiologic agents (e.g., bacteria, protozoa, fungi, algae; **FIGURE 6A**). Some neoplasms can induce neutrophilic inflammation if metastatic to the lymph node (e.g., squamous cell carcinoma).[8,9]

Metastatic Neoplasia

This is characterized by the presence of a foreign cell population (e.g., epithelial cells in metastatic carcinoma) or high numbers of a cell type that should only be present in very low numbers (e.g., mast cells, melanocytes; **FIGURES 6B AND 6C**).[8,9] The absence of metastatic cells on cytology does not exclude the possibility of metastatic disease, due to sample collection bias (e.g., minimally sampled metastatic lymph node, sample collected during early metastasis from area of a metastatic lymph node that did not contain metastatic cells).

Adipose Tissue

Perinodal adipose tissue appears similar to normal subcutaneous fat. Popliteal lymph nodes are often surrounded by prominent adipose tissue (**FIGURE 7**).

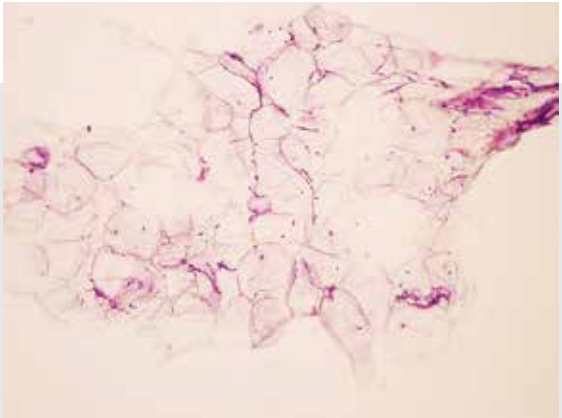

FIGURE 7. Aspirate of presumptive popliteal lymph node. Large aggregates of uniform, well-differentiated adipocytes are present, indicating aspiration of perinodal fat instead of lymphoid tissue. Wright-Giemsa stain, 20× objective.

Salivary Tissue

Salivary gland tissue is occasionally aspirated instead of a mandibular lymph node sample. On cytology, it has a streaming, pink, mucinous background with prominent windrowing of red blood cells (rowing up of cells). Glandular epithelial cells are often arranged in small clusters, but they can easily rupture, resulting in "naked nuclei" that can be mistaken for small lymphocytes. It is also important to not mistake salivary tissue for metastatic neoplasia (**FIGURE 8**).

Artifacts

Only intact cells should be evaluated for interpretation. Interpretation of ruptured cells will give erroneous results. Cells in thick areas take up stain poorly, making nuclear morphology difficult to assess. Dense/thick regions prevent cells from spreading out, making sizing difficult (**FIGURES 2A AND 2B**).

SUMMARY

Lymph node FNA and cytology is a noninvasive, rapid, and cost-effective diagnostic tool. A good-quality smear and the ability to distinguish a diagnostic specimen from a nondiagnostic one are critical first steps in sample evaluation. Microscopy should never start with high power (oil). Every smear should be screened on low power to evaluate whether the sample is adequate, recognize patchily distributed cells (i.e., metastatic cells), and identify the area of interest for high-power

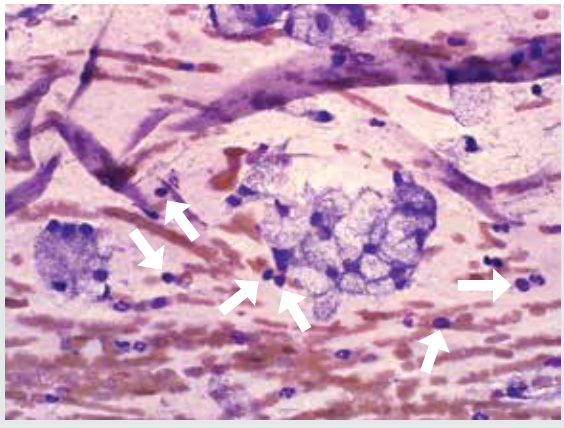

FIGURE 8. Accidental aspiration of salivary gland tissue instead of a submandibular lymph node. Note the clusters of uniform, foamy epithelial cells (salivary gland) and the linear arrangement (windrowing) of red blood cells and blood leukocytes in the background. The windrowing indicates the presence of viscous material (saliva). Free salivary gland nuclei **(arrows)** can mimic small lymphocytes. Wright-Giemsa stain, 100× objective.

microscopy. The most common pathologic cytologic findings in patients with lymphadenopathy are reactive hyperplasia, lymphoma, and metastatic neoplasia. TVP

References

1. Sharkey LC, Seelig DM, Overmann J. All lesions great and small, part 1: diagnostic cytology in veterinary medicine. *Diagn Cytopathol*. 2014;42(6):535-543. doi:10.1002/dc.23097
2. MacNeill AL. Cytology of canine and feline cutaneous and subcutaneous lesions and lymph nodes. *Top Companion Anim Med*. 2011;26(2):62-76. doi:10.1053/j.tcam.2011.02.004
3. Schlemmer SN. Obtaining a sample for cytology using fine needle biopsy. *Todays Vet Pract*. 2023;13(1):54-60.
4. Moore AR. Preparation of cytology samples: tricks of the trade. *Vet Clin North Am Small Anim Pract*. 2017;47(1):1-16. doi:10.1016/j.cvsm.2016.07.001
5. Arai S, Rist P, Clancey N, Gilroy C, Stryhn H, Amsellem P. Fine-needle aspiration of cutaneous, subcutaneous, and intracavitary masses in dogs and cats using 22- vs 25-gauge needles. *Vet Clin Pathol*. 2019;48(2):287-292. doi:10.1111/vcp.12751
6. Meinkoth JH, Cowell RL. Sample collection and preparation in cytology: increasing diagnostic yield. *Vet Clin North Am Small Anim Pract*. 2002;32(6):1187-1207. doi:10.1016/s0195-5616(02)00049-9
7. Meyer DJ. The acquisition and management of cytology specimens. In: Raskin RE, Meyer DJ, eds. *Canine and Feline Cytology*. 3rd ed. Saunders; 2016:1-15.
8. Raskin RE. Hemolymphatic system. In: Raskin RE, Meyer DJ, eds. *Canine and Feline Cytology*. 3rd ed. Saunders; 2016:91-137.
9. Cowell RL, Dorsey KE, Meinkoth JH. Lymph node cytology. *Vet Clin North Am Small Anim Pract*. 2003;33(1):47-67. doi:10.1016/s0195-5616(02)00055-4
10. Burkhard MJ, Bienzle D. Making sense of lymphoma diagnostics in small animal patients. *Clin Lab Med*. 2015;35(3):591-607. doi:10.1016/j.cll.2015.05.008
11. Keller SM, Vernau W, Moore PF. Clonality testing in veterinary medicine: a review with diagnostic guidelines. *Vet Pathol*. 2016;53(4):711-725. doi:10.1177/0300985815626576
12. Rout ED, Avery PR. Lymphoid neoplasia: correlations between morphology and flow cytometry. *Vet Clin North Am Small Anim Pract*. 2017;47(1):53-70. doi:10.1016/j.cvsm.2016.07.004

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