the fluid, obstruction of the needle, inappropriate needle length, or chronic pleural effusion with fibrous adhesions. Aspiration at different sites, use of ultrasound guidance, and changing the position of the patient may help facilitate fluid retrieval.

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CHAPTER 21

Respiratory Tract Cytopathology

Patricia M. McManus

Introduction to Cytopathology

Diagnosis of respiratory disorders commonly requires cytological evaluation of tissues collected from the upper or lower respiratory tract or the pleural space. Although cytological evaluation is usually done by clinical pathologists in large referral diagnostic laboratories or academic institutions, time constraints often mandate that clinicians examine slides and act on their own observations prior to receiving the pathologist's report. Cytopathology has the potential to provide useful information regarding inflammation, infectious agents, and neoplasia, but knowledge of what to expect in normal tissue is a prerequisite for evaluation of the abnormal. In addition, an understanding of the techniques used in preparation and staining of smears is imperative to avoid

misidentification and misinterpretation. This chapter addresses some of these issues.

Techniques

Detailed discussion of smear and staining techniques is best found in texts specifically addressing this subject,[1,2] but a few general points are worth clarifying. Successful microscopic examination of cells in a cytological preparation depends on creation of an artifact, in that spherical cells must be flattened in order to visualize interior cytoplasmic and nuclear structures. Identification of cells and infectious agents is difficult to impossible if cells are not flattened adequately (Figure 21-1). The quality of spreading within any one smear tends to be highly variable; therefore, skill in recognition of adequately spread cells must be developed.

Solid tissues can be sampled by direct impression, fine needle aspiration, nonaspiration fine needle biopsy, surface scrapings, swabs, or brushings. Of these, direct impressions tend to be the least helpful, in that generally only easily exfoliated cells (e.g., blood and inflammatory cells) are found. Tissue cells (e.g., spindle and epithelial cells) are more efficiently obtained using slightly more aggressive techniques.

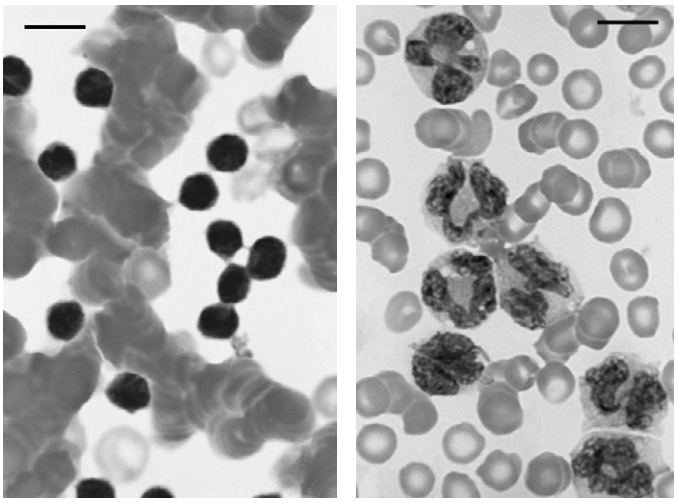
Pleural fluids are processed according to their cellularity. Fluids with greater than 30,000 cells/μl can be handled without concentration by preparing direct smears. Concentration techniques are advised when nucleated cell counts are less than 30,000 cells/μl. In fluids that have between 2000 and 30,000 cells/μl, simple centrifugation and smearing of the sediment is generally adequate. Cytocentrifugation is advised when cell counts are less than 2000/μl. Buffy coat preparations are recommended for very bloody fluids in which the packed cell volume (PCV) is greater than 5% to 10%.

Slides should always be labeled as they are being prepared to prevent confusion later. Labeling should include the name of the owner, case number, date, and site. Labeling tools can include lead pencils, diamond pencils, or fine-tip black permanent markers. Green, blue, and red permanent markers are not really very permanent and should be avoided.

The next step in preparation is fixation and staining. Quick staining methods should be applied to effect rather than according to the manufacturer's timing suggestions, which only apply to thin blood smears. There is no danger of overstaining, but understaining can result in nondiagnostic preparations. The preferred method of staining is a more complex Romanowsky hematological stain (e.g., Wright-Giemsa). The processing is longer (approximately 12 minutes) but results in greater cytological detail (Box 21-1).

Generally it is best to dedicate a very good microscope for evaluation of blood and cytology smears and use a separate microscope for urine and fecal analysis. Recommended objectives for cytological evaluation include 4×, 10×, 20×, 50× oil, and 100× oil. Using a 50×-oil lens simplifies processing of slides because a coverslip is not necessary to examine cells and there is no fear of getting oil on a 40× dry lens.

Storage of slides after examination is just as important as storage of radiographs. Slides used as part of medical decision making are part of the medical record and should not be discarded. Oil can be removed by simply wiping it off using a tissue (e.g., Kleenex). Kimwipes and lens paper will scratch the smears. Using tissues that are impregnated with lotions or aloe vera is not recommended because they leave a film on the slide that is incompatible with immersion oil. Slides should then be stored, either in empty, cardboard slide boxes or slide file cabinets (Figure 21-2). Labeling of boxes or drawers by date is generally adequate, although a system using accession numbers can also be implemented. Coverslipping is not necessary prior to storing. Smears stored back to front will not be dam-



A B

Figure 21-1. *Two sites within the same blood smear demonstrating the effect of smear thickness on cell identification.* ***A,*** *The nucleated cells are too thick to identify as monocytes.* ***B,*** *Improved smear technique allows easy identification of the cells. Bars = 10 μm. Wright-Giemsa stain.*

BOX 21-1
Protocol for Wright-Giemsa Stain* (Harleco®)

- Place dried smears on staining rack
- Cover entire smear with methanol using Pasteur pipette; leave on 1 minute
- Decant methanol
- Cover entire smear with filtered Wright-Giemsa stain; leave on 3 minutes
- Pipette an equal amount of deionized (or distilled) water on top of Wright-Giemsa stain; leave on 6 minutes
- Wash smears with deionized (or distilled) water and let dry

*Stain should be filtered using No. 42 Whatman filter; prepare a moderately large amount as a stock for staining.

Figure 21-2. *Slides can be stored in either file cabinets designed for slides or in empty slide boxes. Labeling can be by accession number or date.*

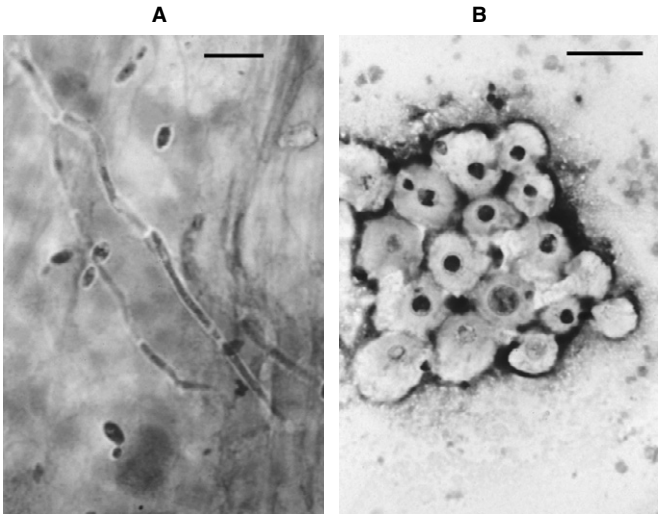


Figure 21-3. A, *Fungal hyphae and spores, typical for opportunistic fungal infections (bar = 10 μm).* ***B,*** *Yeast forms* (Cryptococcus neoformans) *from the nose of a cat (bar = 25 μm). Wright-Giemsa stain.*

aged when reasonable care is practiced during filing and retrieval.

Nasal Cytology

PURPOSE/SAMPLING

Evaluation of nasal specimens can result in determination of an inflammatory profile, identification of a specific infectious agent, and/or the diagnosis of neoplasia. Sampling of nasal passages is best done via lavage, aspirates, swabs, brushings, or scrapings.[3-5] Direct impressions of almost any lesion, regardless of the underlying cause, generally result in collection of superficial mucosal cells, inflammatory cells, and secondary bacterial agents. This type of profile may not reflect the primary disease process.

NORMAL

Normal elements detected in a nasal sample include nonkeratinized squamous epithelial cells or ciliated columnar cells, normal oropharyngeal flora such as *Simonsiella* spp., and a small amount of light pink–stained mucus. With traumatic procedures there may also be blood and nucleated cells appropriate to blood.

COMMON PROFILES

Common inflammatory profiles include acute (neutrophilic), chronic active or subacute (mixed neutrophils and macrophages), chronic (macrophages), lymphocytic-plasmacytic, and occasionally eosinophilic. Lymphocytic-plasmacytic inflammation is commonly mixed with acute inflammation and a secondary bacterial infection.

A bacterial infection should be suspected with acute inflammation, although bacteria are rarely the primary pathogen. Bacteria stain dark blue in Romanowsky-stained smears, regardless of Gram-staining characteristics. A monomorphic population of intracellular and extracellular organisms suggests a bacterial infection. Extracellular or squame-associated bacteria, which are mixed in type, suggest oropharyngeal contamination.

Fungal infections common to the upper respiratory tract include hyphal forms of *Aspergillus* spp. and *Penicillium* spp., which are both branching, septate, and 2 to 4.5 μm wide (Figure 21-3, *A*). Common yeast forms include *Cryptococcus neoformans* (Figure 21-3, *B*), primarily in cats, and *Rhinosporidium seeberi* in dogs.

Neoplasms of the upper respiratory tract likely to be encountered in cytological evaluations include adenocarcinoma, squamous cell carcinoma, lymphosarcoma (LSA), fibrosarcoma, chondrosarcoma, and osteosarcoma. Nasal adenocarcinomas, the most common tumor type,[6] can be either highly anaplastic, with marked pleomorphism, or consist of small uniform cells, with high nuclear to cytoplasmic ratios. The latter form can be difficult to distinguish from LSA (Figure 21-4). Chondrosarcomas are often distinguished by the presence of a bright purple- to magenta-stained extracellular matrix. The staining intensity of this matrix can result in poor staining of the chondroblasts themselves. This same material can be present in aspirates of osteosarcomas but is generally less abundant.

CONFUSION

Dysplastic and hyperplastic epithelial tissue should not be confused with neoplasia. Furthermore, squamous metaplasia can occasionally be observed in association with long-standing severe inflammation. In cases of lymphocytic-plasmacytic inflammation, lymphocytes can be

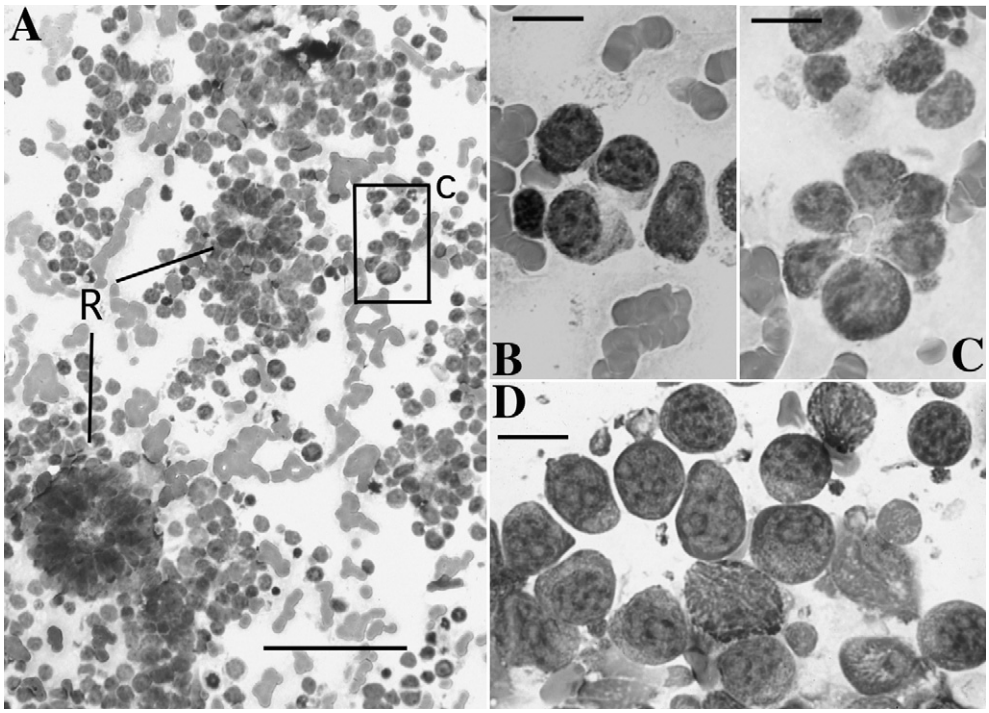


Figure 21-4. *Nasal carcinomas can be difficult to distinguish from lymphosarcomas in cytological preparations.* ***A, B, C,*** *Cells taken from a cat with nasal carcinoma confirmed by biopsy.* ***D,*** *Cells from a dog with lymphosarcoma. The key to identification of carcinomas is demonstration of cell:cell adhesion and rosetting of clustered cells (*R *and* ***C****).* ***A,*** *Bar = 75 μm;* ***B, C,*** *and* ***D,*** *bars = 10 μm. Wright-Giemsa stain.*

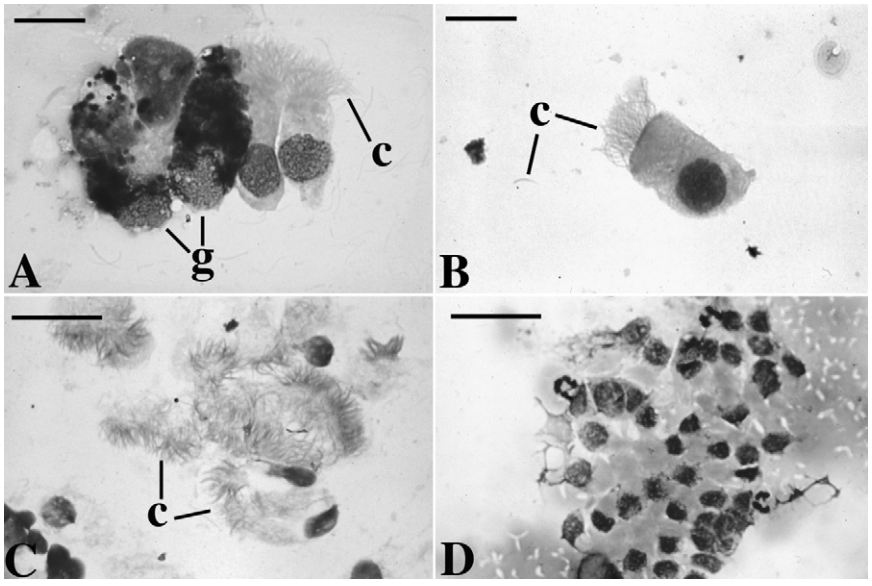


Figure 21-5. *Tracheal wash with ciliated columnar epithelial cells.* ***A, B,*** *Extremely well-preserved cells, something unusual in a TW. Goblet cells* (g) *contain dark purple mucus granules that may be mistaken for large cocci following rupture of these cells. Intact goblet cells that become isolated from other columnar cells are sometimes mistaken for mast cells. Columnar cells are usually more difficult to identify, as in* ***C*** *and* ***D.*** *Free cilia* (c) *should not be misidentified as thin, rod-shaped bacteria. Bacteria generally stain dark blue in Romanowsky stained smears, whereas mucin granules are purple and cilia are light pink. Bars = 10 μm. Wright-Giemsa stain.*

numerous; therefore, criteria for a diagnosis of lymphosarcoma must be strictly applied (i.e., a homogeneous population of immature lymphocytes).

Trachea

PURPOSE/SAMPLING

Tracheal washes (TW) are used to delineate the type of inflammatory profile present in the trachea and bronchi (and possibly, bronchioles and alveoli) and potentially identify a specific infectious agent. TW are rarely helpful in the diagnosis of neoplasia. Typically, the trachea is sampled either by transtracheal or endotracheal techniques.[3]

NORMAL

Normal wash cytology contains low to moderate numbers of ciliated columnar epithelial cells, occasional goblet cells and macrophages, and a small amount of mucus (Figure 21-5). It can be difficult to distinguish a normal wash from missampling. If a normal TW interpretation conflicts with clinical findings, a second wash should be considered.

COMMON PROFILES

Inflammatory profiles common to tracheal washes include acute, chronic active or subacute, chronic or granulomatous (epithelioid macrophages and multinucleate giant cells), and eosinophilic. The cytologist cannot as-

sume that the location of the inflammation is alveolar, in that inflammation limited to the trachea or even the oropharynx may result in an inflammatory TW. For this reason interpretations generated by a clinical pathologist are not anatomically specific (e.g., acute bronchopneumonia). This diagnosis is best reserved for histopathology, where the inflammation is seen in the context of anatomic structures. When histopathology is not available, a diagnosis of pneumonia is based on a combination of the cytological and radiographical interpretations, coupled with the physical examination.

Acute inflammation is typically associated with a bacterial infection. As with nasal specimens, a monomorphic population of intracellular and extracellular organisms suggests a bacterial infection, which may be primary or secondary. Mixed types of bacteria may indicate contamination, perforating wounds, or aspiration.

Chronic inflammation can be seen in animals with neoplasia; parasitic, fungal and protozoal infections; chronic congestive processes; lung lobe torsion; and pulmonary embolism. Although fungal infections are included in this list, many fungal infections of the lung are opportunistic and are likely to be seen in association with acute inflammation and bacterial infections. Opportunistic infections include the same hyphal forms that can be seen in nasal infections. Their presence should be anticipated in animals with aspiration pneumonia. On the other hand, the systemic mycoses (e.g., *Blastomyces dermatitidis, Cryptococcus neoformans, Coccidioides immitis,* and *Histoplasma capsulatum*), detected as yeasts, are more likely to result in chronic or granulomatous inflammation, which is characterized by epithelioid and multinucleate macrophages.

Eosinophilic inflammation is associated with hypersensitivity, parasitism, neoplasia (e.g., bronchogenic carcinoma), idiopathic eosinophilic infiltrative disorders, and occasionally fungal infections. Likely parasites include lungworms such as *Filaroides* spp., *Aelurostrongylus abstrusus, Crenosoma vulpis*,[7] and heartworms *(Dirofilaria immitis)*. Fortunately, distinguishing the two broad groups of worms is simple. Lungworm infections are generally diagnosed by detection of tightly coiled larvae that usually stain turquoise blue (Figure 21-6, *A*). Heartworms are seen as thin, straight to slightly curvy microfilaria approximately 200 to 300 μm in length (Figure 21-6, *B*). Very small, dark blue nuclei may be evident (Figure 21-6, *C*). Lungworm larvae are almost twice as long as heartworm microfilaria and are much thicker. Parasitic infections may or may not be associated with a strong eosinophilic response, but if eosinophils are consistently noted, especially within a pyogranulomatous setting, a careful low-power scan of all areas of several slides is advised. In addition, fecal analysis for nematode larvae, using the Baermann technique, is recommended.[7]

Other cells occasionally seen in TW include lymphocytes, mast cells, basophils, and plasma cells. These cell types are always low in frequency, and a high proportion of any of these cells would be a very abnormal profile. For example, many lymphocytes within a tracheal wash are suggestive of lymphosarcoma.

Mucus production increases with almost any type of inflammation or chronic irritation; therefore, pink- to purple-stained mucus is a common constituent of TW. Fine to thick spirals of mucus, termed Curschmann's spirals, may represent mucus from small airways although they do not add specificity to the interpretation (Figure 21-7, *A*).

Oropharyngeal squames with associated oral flora (Figure 21-7, *B*) usually indicate oral contamination of the sample, which can either be procedural or pathological. Appropriate interpretation requires inspection for a neutrophilic response targeting the bacteria. The presence of inflammation, with mixed intracellular bacteria and squamous cells, is supportive of the clinical diagnosis of aspiration pneumonia, although the inflammation

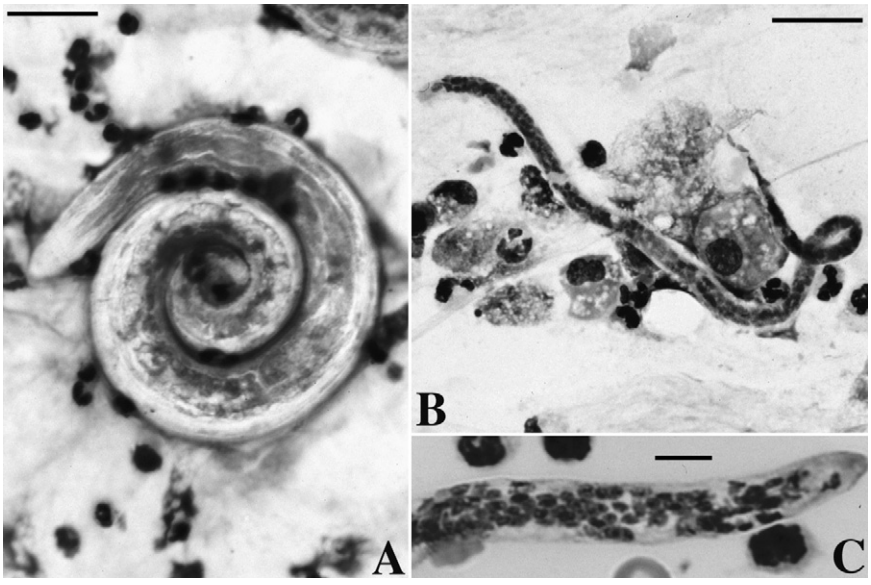


Figure 21-6. *Lung nematodes in a tracheal wash.* ***A*** *is a larval form of* Aelurostrongylus abstrusus *from a cat.* ***B*** *and* ***C*** *are Dirofilaria immitis microfilariae from a dog.* ***A*** *and* ***B*** *are taken at the same magnification, but notice the size difference between the two larval forms.* ***C*** *is a close-up of the small nuclei visible in stained* D. immitis *microfilariae. Nuclei are not as distinct in* A. abstrusus *larvae. Morphology of canine lungworm larvae is similar.* ***A*** *and* ***B*** *bars = 25 μm.* ***C*** *bar = 5 μm. Wright-Giemsa stain.*

could also be within the oropharynx. Again, findings should be interpreted in the context of other diagnostic clues such as clinical signs and radiographs.

Hemorrhagic or congestive processes can result in the presence of erythrophagocytosis or hemosiderosis. Hemosiderin is classically golden-brown, refractile granular material; however, in TW macrophages it can be gray-blue and non-refractile. If unsure, a Prussian blue stain for iron can help identify hemosiderin within macrophages. Associations include trauma, neoplasia, congestive heart failure, foreign bodies, pulmonary embolism, coagulopathies, lung lobe torsion, eosinophilic inflammation, and chronic infection. Significance should be considered in context of other changes found. Blood, without evidence of erythrophagocytosis and hemosiderin, suggests procedure-related contamination, which is more likely to be a complication of transtracheal collection than endotracheal sampling.

CONFUSION

Columnar epithelial cells are often numerous in inflammatory TW, but they are usually poorly preserved and difficult to identify (see Figure 21-5, *D*). Smudged epithelial cells and free cilia should not be mistaken for lymphocytes and bacteria, respectively. Cilia stain light pink, whereas bacteria are more consistently dark blue. Smudged cells are represented by only bare nuclei. Nuclei of the epithelial cells approximate the size of lymphocyte nuclei, but the chromatin tends to be less coarse and more homogeneous and the nucleoli are very tiny. As a general precautionary strategy, it is wise not to infer an identity to any cell represented by only the nucleus.

Small to moderately large clusters of hyperplastic epithelial cells, displaying increased anisocytosis and cytoplasmic basophilia, may occasionally be observed in association with inflammation. These hyperplastic cells may be cuboidal to low columnar with moderately large and prominent nucleoli. Detection of carcinoma cells in TW is extremely rare; therefore, low to moderate numbers of mildly to moderately atypical epithelial cells, in the context of severe inflammation, should be interpreted as reactive. A definitive diagnosis of carcinoma should be reserved for profiles featuring markedly pleomorphic epithelial cells with a minor inflammatory component.

Eosinophilic infiltrates can occasionally create confusion. First, regional variations within lavage samples are possible, so that some areas may be predominantly eosinophilic, whereas other areas contain mostly neutrophils or macrophages. Finding collections of eosinophils, even with many neutrophils and bacteria, suggests that eosinophilic inflammation is concurrent or possibly the primary cause for the animal's clinical signs. Secondly, eosinophil granules in sight-hound breeds such as greyhounds and whippets often do not stain pink, and appear more like vacuoles (Figure 21-8, *A*). This is problem enough in blood smear evaluations but could potentially completely misdirect a differential diagnosis if the cells in a TW are misidentified as degenerate neutrophils or macrophages. Finally, eosinophil granules from cats are rod shaped, resembling pink- to red-stained, rod-shaped bacteria; however, bacteria stain dark blue (Figure 21-8, *B*). Context and staining characteristics will help the examiner distinguish the structures.

Layering of inflammatory cells and infectious agents, especially lungworm larvae, can occur in wash fluids. This layering can result in all the larvae being on only one of several slides prepared from sediment or zones of

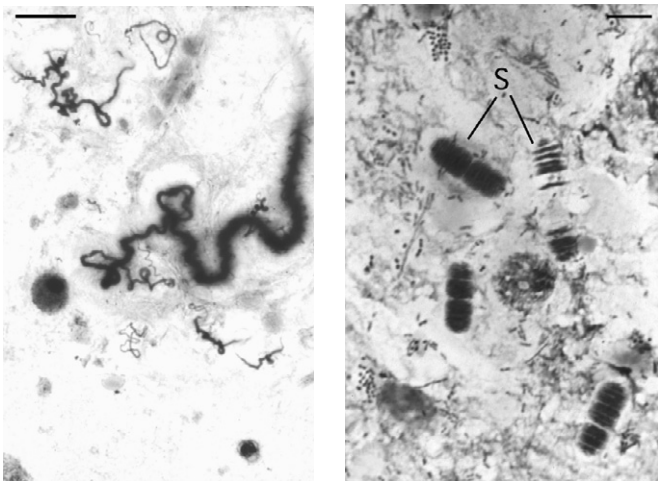


Figure 21-7. *Tracheal wash. Curschmann's spirals* ***(A)*** *may represent mucus from small airways but they do not add specificity to a cytological interpretation (bar = 25 μm). The large rod-shaped bacteria in* ***B*** *are* Simonsiella *organisms, which are normal oropharyngeal flora. The organisms adhere to each other side to side, which results in their appearance as giant bacteria. Bar = 10 μm. Wright-Giemsa stain.*

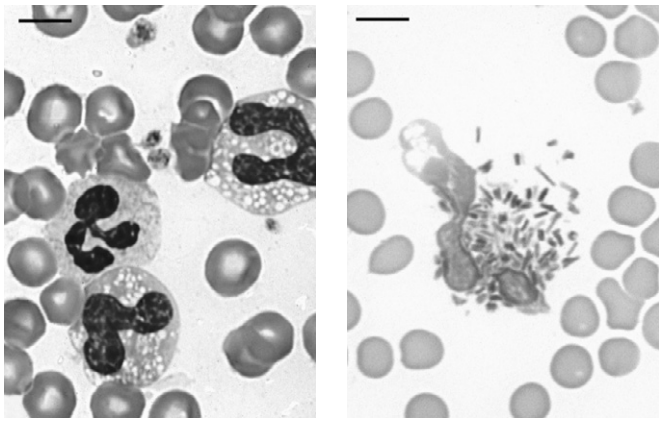


Figure 21-8. *Canine and feline eosinophils. The two vacuolated cells in* ***A*** *are eosinophils from a greyhound. Their granules fail to pick up the eosin stain, so they appear vacuolated. These cells can be difficult to distinguish from macrophages or degenerate neutrophils in cytological preparations.* ***B,*** *A partially ruptured feline eosinophil. Cat eosinophilic granules are rod-shaped and can be misidentified as bacteria; however, bacteria generally stain dark blue, whereas the eosinophil's granules stain pink. Bars = 10 μm. Wright-Giemsa stain.*

inflammation, which contain differing cell distributions. This tendency toward layering can be compensated for by evaluation of several areas of a few slides.

Finally, it seems likely that the tracheal wash profile does not always represent alveolar patterns. A review of bronchopneumonia in 42 dogs reported that in 11 of these dogs the TW was not inflammatory.[8] A retrospective study of over 100 cats infected with *Toxoplasma gondii* found that the alveolar infiltrate seen in these cats is primarily histiocytic.[9] However, the few *Toxoplasma gondii* infections detected in tracheal washes by the author have been associated with acute inflammation accompanied by a large amount of necrotic cellular debris and moderate numbers of macrophages.

Bronchi

PURPOSE/SAMPLING

Sampling of the bronchi, similar to that of the trachea, can provide information regarding the type of inflammation and possibly the etiological agent. Sampling is done via either bronchoalveolar lavage or bronchial brushings. Bronchoalveolar lavage (BAL), compared with TW, may be more predictive of alveolar histological patterns.[10] There is also a slightly greater likelihood that sampling could aid in the diagnosis of neoplasia, although it is still a poor choice for this purpose.[11,12] Bronchial brushings are used for selective sampling of bronchial lesions.

NORMAL

Bronchoalveolar lavage results in a semiquantitative evaluation of the contents of airways below the trachea.[13-15] Aliquots from the left bronchus are generally distinguished from the right and submitted as separate samples. A consistent dosage of lavage fluid should be instilled so that comparisons regarding nucleated cells per microliter are possible. Usually counts are low, necessitating the use of a cytocentrifuge to prepare smears.[16] In addition to the routine cytological description, a BAL report includes nucleated cells/μl, red blood cells (RBC)/μl, and the cell differential. Quantitative analysis yields normal cell counts of about 200 to 500 nucleated cells/μl. Normal findings include mostly mononuclear cells and columnar epithelial cells, and occasional goblet cells, mast cells, neutrophils, and eosinophils.[17] Mucus is scant.

Bronchial brushings from healthy animals contain moderate to many low to moderately high columnar epithelial cells, rare small clusters of mature lymphocytes, and a very small amount of mucus.

COMMON PROFILES

The types of inflammation and disease associations are the same as for TW, but BAL findings do not always duplicate TW findings, with up to 68% discordance reported.[10] In staging lymphosarcoma, studies show a greater sensitivity in detection of neoplasia using BAL compared with TW.[11,12]

Bronchial brushings can be cytologically unremarkable even in the face of inflammatory TW and BAL. Bronchial brushings are most useful if carefully applied to visible lesions.

CONFUSION

Clinically healthy cats are reported to have high percentages of eosinophils in quantitatively normal BAL fluids.[18] In this study, the mean nucleated cell count for 24 cats, after instillation of 50 ml of fluid, was 301 ± 126 cells/μl (80 to 665/μl), with a mean eosinophil count of 25% ± 21% (2% to 83%) in the differential. High nucleated cell counts should be used to confirm the potential for a true eosinophilic inflammatory response.

Romanowsky-stained goblet cells contain many dark purple granules, creating the potential for misidentification as distorted mast cells in a cytocentrifuged BAL smear. In addition, if there is a large amount of mucus in a BAL sample, quantitative analysis is likely to be inaccurate.

Bronchial brushing may result in the collection of aggregates of lymphocytes. These represent bronchus-associated lymphoid tissue and must be distinguished from lymphosarcoma. The presence of predominantly small lymphocytes supports an interpretation of normal or possibly hyperplastic lymphoid tissue.

Lungs

PURPOSE/SAMPLING

Direct aspiration of lung tissue is generally performed when other procedures have failed to provide a specific diagnosis. The most common type of lesion to aspirate is a mass suspicious for neoplasia that is detected by diagnostic imaging.[19] Compared with the above procedures, this technique is the most likely to yield specific information regarding neoplasia.

NORMAL

The term "normal profile" does not apply for this procedure because it is highly unlikely that a lung viewed as normal radiographically would be aspirated. Nondiagnostic smears, consisting of mostly blood, a few macrophages, and a small amount of mucus, are common. Mechanically exfoliated mesothelial cells are occasionally detected due to inadvertent sampling of the pleura (Figure 21-9).

COMMON PROFILES

The same inflammatory profiles previously described for tracheal washes are possible in samples obtained by direct aspiration of the lung. Typically, lung aspirates are performed when neoplasia is suspected; therefore, acute inflammation and bacterial infections are usually not diagnosed in this manner.

The most common tumor type to be diagnosed via direct aspiration is carcinoma (Figure 21-10).[19] Bronchogenic carcinomas can be well differentiated, consisting of colum-

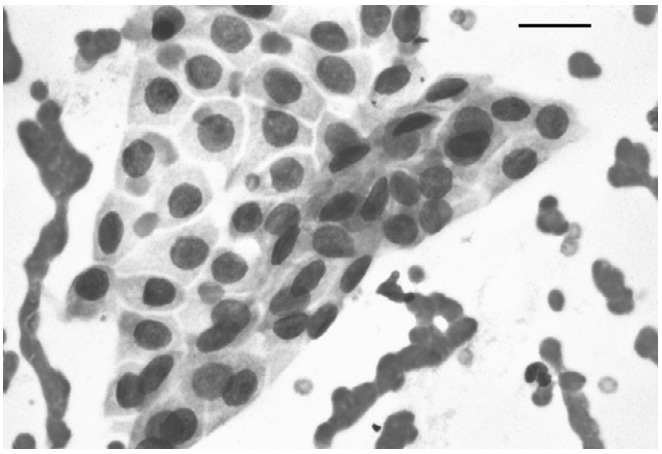


Figure 21-9. *Lung aspirate: Mechanically exfoliated, nonreactive mesothelial cells appear as sheets of flat, uniform, squamous cells. Bar = 25 μm. Wright-Giemsa stain.*

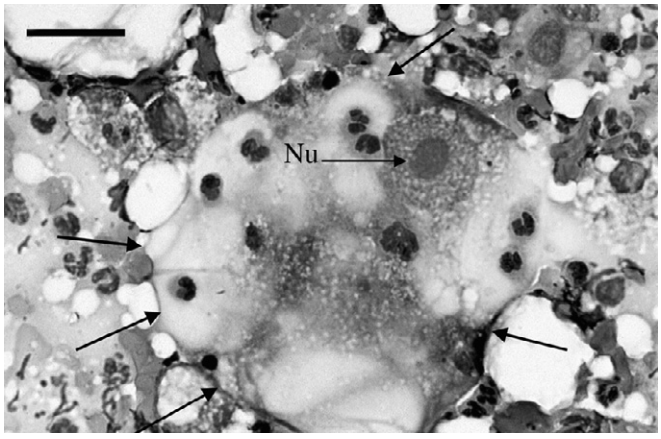


Figure 21-10. *Lung aspirate showing feline metastatic mammary gland adenocarcinoma. The arrows point to the cell boundaries of a single, giant carcinoma cell. Inside the cell are neutrophils displaying the phenomenon known as* emperipolesis, *where inflammatory cells migrate into or through another cell. Nu = nucleolus. Bar = 25 μm. Wright-Giemsa stain.*

nar epithelial cells, making them difficult to distinguish from hyperplastic tissue. Malignant histiocytosis is another neoplasm that exfoliates readily with direct aspiration. The neoplastic histiocytes must clearly display marked pleomorphism beyond that expected for reactive macrophages in order for a cytological diagnosis to be made; otherwise, histological evaluation is needed to confirm this diagnosis. Most spindle cell tumors do not exfoliate readily and are difficult to diagnose using direct aspiration methods.[20] Hemangiosarcomas, in particular, are almost never represented in smears, although evidence of chronic inflammation, hemosiderosis, and hemorrhage are common in these tumors. Regardless of the specific diagnosis, neoplasia is typically accompanied by a mix of inflammatory cells (e.g., macrophages and neutrophils), as well as evidence of hemorrhage, necrosis, and possibly epithelial cell hyperplasia.

CONFUSION

Tissue reacting to the presence of severe inflammation can be mistaken for neoplasia. Hyperplastic cells that are most often mistakenly interpreted as neoplastic include epithelial cells and fibroblasts. Macrophages can also be misleading when they become large, foamy, and multinucleate.

Inadvertent sampling of liver tissue via the diaphragm occasionally occurs. Hepatocytes are generally easily identified as contaminants because they have a specific uniform cuboidal morphology; however, if the liver is abnormal, missampling may not be obvious.

Pleural Effusions

PURPOSE/SAMPLING

Sampling and analysis of pleural fluid is always indicated when an effusion is perceived to exist clinically or radiographically.[21] Fluid analysis potentially offers diagnosis of a specific cause of the effusion (e.g., neoplasia or infection). Contradictory reports in the human literature question whether cytological evaluation is necessary if the quantitative analysis indicates a transudative process.[22-24] This controversy has not been debated in the veterinary literature, but based on the author's experience, cytological evaluation of most fluids, regardless of quantitative analysis, is advised. Smear inspection often offers insight that the quantitative analysis misses. Clumping of cells can spuriously decrease nucleated cell counts and result in counts typical of transudates. This is fairly common in carcinoma-induced malignant effusions where large clusters of pleomorphic adherent cells can result in inaccurate cell counts. On the other hand, the need for cytological evaluation of acute hemorrhagic effusions is questionable. When the quantitative analysis is basically consistent with blood, with a nucleated cell count proportionate to that of the peripheral blood, cytological evaluation rarely allows determination of a specific cause.

NORMAL

Fluid analysis includes gross characteristics, total protein (TP), nucleated and red blood cell counts, a cell differential, a description of potentially neoplastic cells, and a search for infectious agents. Total protein is traditionally measured by refractometry rather than using a chemical analyzer; therefore, values of less than 2.5 gm/dl are not further quantified. Normal pleural fluid is scant and difficult to collect from healthy dogs and cats; it is transudative with a protein less than 2.5 gm/dl and a nucleated cell count less than 500 cells/μl.

COMMON PROFILES

Transudative effusions are characterized by low nucleated cell counts and few red blood cells. The most nu-

merous nucleated cells present are non-phagocytic macrophages and non-degenerate neutrophils, in approximately equal numbers. In addition, occasional mast cells, eosinophils, lymphocytes and mesothelial cells are detected, usually at frequencies less than 5%.

Pure transudates are characterized by a TP less than 2.5 gm/dl and a nucleated cell count less than 1500/μl.[2] Pure transudates generally reflect increased hydrostatic pressure or decreased oncotic pressure. Modified transudates, with protein greater than 2.5 gm/dl and nucleated cells between 3000/μl[25] and 7000/μl[2], are most likely to be seen secondary to liver or heart failure and neoplasia.[26] Feline infectious peritonitis-induced effusions may be characterized quantitatively as modified transudates, but the globulin content of the fluid is higher than with heart failure and neoplasia, and neutrophils predominate.[27]

Inflammatory exudates are characterized by elevations in both the nucleated cell count and the TP, although exceptions occur. Potential causes include bacterial, fungal, protozoal, and parasitic infections; neoplasia; lung lobe torsion; and immune-mediated pleuritis. Neutrophils usually predominate in exudative processes, although there are occasions when macrophages can almost match neutrophils in number. The most striking examples of high numbers of macrophages in an exudate seen by the author have been in association with lung lobe torsion. One patient with a lung lobe torsion seen at the Veterinary Hospital of the University of Pennsylvania had 45,500 nucleated cells/microliter, with 44% macrophages, 54% neutrophils, 1% lymphocytes, and 1% reactive mesothelial cells. Generally, a relative increase in macrophages suggests chronicity to the effusion (e.g., chronic bacterial or fungal infections) and neoplasia.

Chylous effusions are characterized by a triglyceride concentration greater than 100 mg/dl[28] but with highly variable nucleated cell counts.[29] Evaluation of serum triglyceride, serum cholesterol, and fluid cholesterol is not necessary to confirm this interpretation. Usually, small lymphocytes predominate (Figure 21-11), but with chronicity, neutrophils and macrophages may eventually exceed lymphocytes.[30] Chylous effusions most commonly reflect partial or complete obstruction of the thoracic duct, which then results in back-up of chyle within the lymphatic system and subsequent lymph seepage from intact lymphatics.[29] The obstruction can be either physical or functional. Potential causes include cardiomyopathy in cats,[31-33] constrictive pericarditis in dogs,[34] lung torsion in dogs,[35] granulomatous disease,[36] and neoplasia.[37] Occasionally the cause remains unknown. Traumatic rupture of the thoracic duct is an uncommon cause of chylous effusions.[29,30,38,39]

Occasionally lymphocytic effusions are observed that cytologically resemble chylous effusions. They contain mostly small, mature, morphologically unremarkable lymphocytes but have triglyceride concentrations less than 100 mg/dl. Despite the low triglyceride content, this type of effusion is still thought to result from lymphatic obstruction. The triglyceride content of the lymph arising from the gastrointestinal tract is diet related; therefore, a chronically anorexic animal may have peritoneal lymph with a low triglyceride concentration.[28] It is also possible that either lymphangiectasia or obstruction of pleural lymphatics is not impinging on or involving the thoracic duct; therefore, peritoneal chyle is not contributing to the effusion. Finally, nonchylous lymphocytic effusions can be seen with thymomas and well-differentiated lymphosarcomas, although the latter diagnosis is uncommon in the chest.

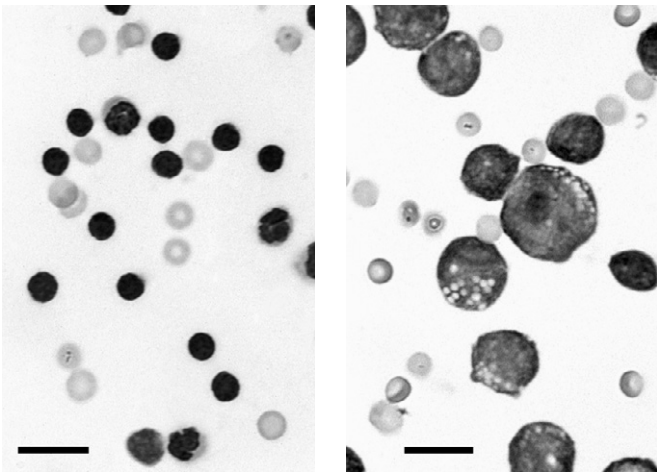


Figure 21-11. *Pleural effusions.* ***A,*** *Feline chylous effusion consisting of mostly small lymphocytes with very dense chromatin, no visible nucleoli, and a narrow rim of cytoplasm. Bar = 10 μm. Wright-Giemsa stain.* ***B,*** *Malignant effusion from an FeLV-infected cat with mediastinal lymphosarcoma. The neoplastic cells are moderately large to very large lymphoblasts with round or clefted nuclei, moderately coarse to fine chromatin, large nucleoli, and a small amount of variably vacuolated cytoplasm. Bar = 10 μm. Wright-Giemsa stain.*

Pseudochylous effusions are extremely rare in small animal medicine, although the term has been inappropriately applied to chylous effusions arising for reasons other than traumatic thoracic duct rupture. Pseudochylous effusions are milky and opaque, as are chylous effusions; however, they are characterized by high cholesterol content.[29] The cholesterol is derived from cell breakdown in a setting of severe chronic inflammation. Cytologically these effusions contain cholesterol crystals, necrotic cellular debris, and inflammatory cells (Figure 21-12). The most common causes reported in the human literature are tuberculosis and rheumatoid pleuritis.

Malignant effusions can have variable nucleated cell counts, red blood cell counts, and TP. Inflammation and hemorrhage are common complications. Based on a recent study, between 30% and 40% of effusions secondary to neoplasia contain no detectable neoplastic cells and are classed by other features such as modified transudates, inflammatory exudates, and hemorrhage.[40] Malignant effusions secondary to carcinoma and lymphosarcoma are the most likely to contain neoplastic cells (see Figure 21-11). Hemangiosarcoma-induced hemorrhagic effusions virtually never contain identifiable malignant cells. Mesotheliomas stimulate effusions, but the malignant mesothelial cells may not display enough atypia to warrant a cytological interpretation of

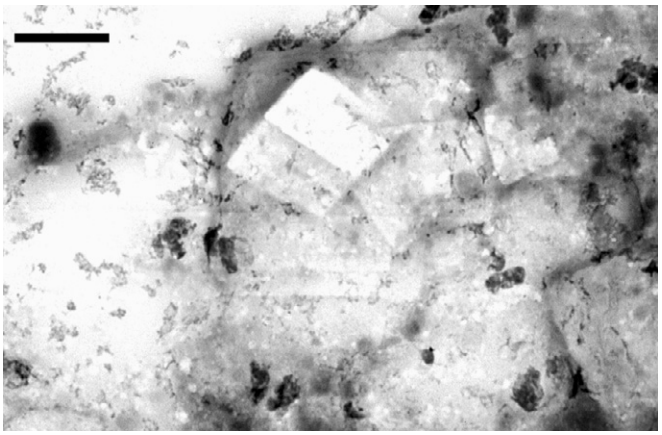


Figure 21-12. *Pseudochylous effusion. The relatively clear, angular structures are cholesterol crystals. The slightly smudged, poorly defined small dark structures are nuclei from disintegrating neutrophils. Bar = 25 μm. Wright-Giemsa stain.*

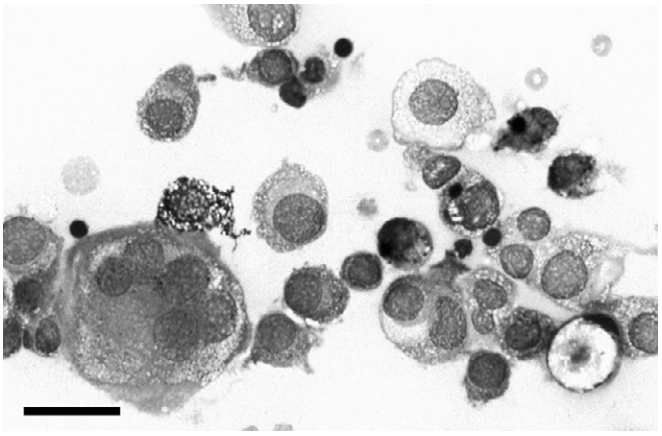


Figure 21-13. *Pleural effusion secondary to metastatic malignant melanoma in a dog. The primary tumor was located on an eyelid. Although the neoplastic cells are essentially amelanotic, there is a suggestion of very fine pigment granules in some cells (e.g., the multinucleate cell, bottom left). Cells containing large dense granules are melanophages. Bar = 25 μm. Wright-Giemsa stain.*

neoplasia, although occasional exceptions occur. Malignant melanoma is a rare cause for pleural effusions (Figure 21-13). Pigmentation of the neoplastic cells may or may not be apparent, although melanophages can occasionally be detected. Morphology of malignant melanoma cells is unpredictable, with the potential for spindle, epithelioid, and/or round cells.

Hemorrhagic effusions have packed cell volumes greater than 5%, with evidence of erythrophagocytosis or hemosiderosis. Causes include trauma, coagulopathies, and bleeding neoplasia. They are often complicated by an inflammatory component, which is detected by comparing the nucleated cell count with the peripheral blood count. A significant difference, favoring the fluid, indicates an inflammatory component to the hemorrhage.

Eosinophilic effusions contain greater than 10% eosinophils. They can be transudative or exudative. They are seen in association with the same disorders listed for nasal and tracheal specimens (i.e., hypersensitivities, parasitism, neoplasia, and idiopathic eosinophilic infiltrative disorders such as eosinophilic granulomatous disease or hypereosinophilia). Neoplasms reported to incite eosinophilic effusion in dogs and cats include carcinoma, lymphosarcoma, and hemangiosarcoma.[41] In dogs mast cell neoplasia should also be considered,[41] but mast cell neoplasia in cats seldom incites an eosinophilic response.

Pleural effusions are often multifactorial. For example, an animal can have a transudate with an inflammatory component, where the TP is low but the cell differential indicates a preponderance of neutrophils that cannot be explained by blood contamination. Both exudates and transudates can be complicated by a mild to moderate hemorrhagic component. The PCV may not be remarkably elevated, but erythrophages and hemosiderophages are found in the fluid.

CONFUSION

Reactive mesothelial cells can become numerous or pleomorphic in the presence of inflammation or chronic hemorrhage in canine pleural effusions. They can be difficult to distinguish from a neoplastic population. Reactive mesothelial cells are less evident in feline fluids. Adherent macrophages in both cats and dogs may be misinterpreted as carcinoma, especially if they are proliferative and multinucleate. Nucleated cell counts can be spuriously decreased by clumping or adhesion of cells (e.g., mesothelial cells and malignant epithelial cells).

Measuring TP by refractometry is subject to the same interferences as seen in the determination of plasma TP. Lipids, bilirubin, and hemolysis can all spuriously elevate TP. Lipid interference is particularly common with chylous effusions. Furthermore, if a sample is collected in an EDTA tube, but the quantity is very small, the EDTA can spuriously elevate the TP to extreme levels, even greater than 10 gm/dl. Interferences should be considered whenever receiving TP results that seem disproportionately high compared with plasma or serum levels.

Conclusion

Cytopathology of the respiratory tract can augment other diagnostic tools used in assessment of respiratory disorders. Sample collection tends to be easy, fast, and relatively noninvasive, although anesthesia is often required. Nasal tissue sampling can offer clues as to the type of inflammation and the potential cause, including neoplasia. Tracheal and bronchial sampling is useful for inflammatory patterns and infectious agents. Both direct lung aspiration and pleural fluid evaluation have the potential for diagnosis of neoplasia, in addition to inflammatory patterns and infectious agents. Quantitative and qualitative analysis of pleural fluids offers evidence regarding the mechanism for the effusion. On the other hand, cytological evaluation cannot determine invasive-

ness of a tumor within normal tissues or determine tissue distribution by inflammatory cell infiltrates. The lack of architectural relationships between cells and tissue result in a less definitive diagnosis as to the specific tumor identity compared with histopathology. Lastly, negative cytological findings must always be viewed as inconclusive because inability to detect an etiological agent or neoplastic cells does not rule them out.

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