

INTRODUCTION TO CYTOLOGY: A CASE-BASED APPROACH

Cytologic specimens can be obtained by fine needle aspiration of enlarged organs and masses, or impression smears of draining tracts or biopsy specimens. FNAs are performed using a needle attached to a syringe. 20-22 gauge needles are large enough to get a diagnostic sample, but small enough to be non-invasive. The needle is inserted into the tissue or mass and redirected several times. The aspirated material is sprayed on a slide and smeared. Once the sample dries the slide is ready to be stained. No fixation is necessary. Stains commonly used in practice include Dif Quik® which is readily available and easy to use. Limitations for this stain are that mast cell granules and basophil granules do not readily stain. Most clinical pathologists will use Wright-Giemsa stain which stains mast cell granules vibrantly.

Once the slide is stained it is ready for microscopic examination. Scan the slide on low power (10x) first to get an overall feel for the cellularity of the sample and then go to high power to evaluate the cell types and individual cytologic features of the cells. Inflammatory processes usually have a mixed population of cells including inflammatory and tissue cells. The inflammatory process is characterized by the cell types present. When neutrophils predominate (>85%), this is considered a suppurative response. The character of the neutrophils should be closely evaluated. If there is a bacterial infection, the neutrophils will often undergo karyolysis and the nucleus will appear pale and swollen. These cells are often called degenerate neutrophils. Also, the cells should be closely examined for intracytoplasmic bacteria. A pyogranulomatous response consists of a mixture of neutrophils, epithelioid macrophages and multinucleated giant cells. This type of inflammatory response is commonly observed with fungal infections or responses to foreign material, though sterile pyogranulomas are also possible. Granulomatous inflammation consists primarily of macrophages with multinucleated giant cells and lesser numbers of other inflammatory cells. This type of inflammatory response does not always exfoliate well and may be of low cellularity and can have considerable fibrosis. Eosinophilic inflammation is more subtle. Cytologic specimens containing >12% eosinophils are considered eosinophilic inflammatory lesions. Common causes include allergic or hypersensitivity response, parasitic infestation or paraneoplastic response.

Tissue cells, more specifically neoplastic cells, are classified as epithelial, mesenchymal (connective tissue) or round cells. Epithelial cells are cohesive and are often observed in tight clusters. This feature can be observed on low power evaluation. The cells are generally round and the sample is often highly cellular.

Mesenchymal cells do not exfoliate nearly as well as epithelial tissue. In benign lesions, it is not uncommon to see only 1-2 cells on a slide. The cells are commonly spindle shaped with wispy cytoplasmic borders. The nuclei are often oval in shape. Round cells are a specific group of neoplasms, which will be discussed in greater detail in the section on skin and subcutaneous tissue. The cells themselves exfoliate readily, resulting in highly cellular samples. The cells are round with a scant to moderate rim of basophilic cytoplasm. They are not cohesive but can occur in sheets which may be difficult to distinguish from epithelial cells. Once the cell type has been characterized, the next step is to determine if the process is hyperplastic or neoplastic and if it is neoplastic, if it is benign or malignant. It is important to evaluate the cells for criteria of malignancy. These criteria include: prominent and multiple nucleoli, anisocytosis, anisokaryosis, multiple nuclei, mitotic figures and nuclear molding. There are others, but these are the most common. When these criteria are present it is fairly easy to identify a malignant tumor; however, not all tumors are malignant and not all malignancies express these criteria. Therefore, on cytology we cannot always rule out a malignant process.

The final step in cytologic evaluation is examination of the material in the background. Proteinaceous material is eosinophilic and granular and is usually observed evenly distributed throughout the sample. Granules from ruptured mast cells or melanocytes must be distinguished from bacteria. Most cytologic samples contain some level of peripheral blood contamination. With the red blood cells come white blood cells. It is important to remember this when trying to distinguish inflammation from peripheral blood contamination. It may be necessary to do a CBC on the patient to determine the WBC.

Skin and subcutaneous tissue

Normal skin is predominated by mature, anucleated, keratinized squamous epithelial cells with lesser numbers of glandular and basal epithelial cells and few stromal connective tissue cells. The subcutaneous tissue is primarily composed of mature adipose tissue. Blood is almost always observed in cytology specimens. It is important to understand what cells are present in normal skin before trying to interpret abnormal tissue. Skin lesions may present as a mass, or an alopecic or ulcerated lesion. Subcutaneous lesions usually present as a mass. Cytology may only lead to a diagnosis of inflammation or neoplasia; however, there are many lesions of skin and subcutaneous tissues that have unique features, allowing for definitive diagnosis with cytology.

Dermal lesions (non-neoplastic and non-inflammatory)

These consist primarily of cysts and hyperplastic tissue. Epidermal inclusion cysts are common masses on older dogs. Cytologically they consist of mature squamous epithelial cells and thick aggregates of keratinaceous debris. Occasionally cholesterol crystals are identified. If these cysts rupture, they elicit a tremendous inflammatory response, suppurative to pyogranulomatous. The inflammatory cells are observed surrounding the aggregates of keratinaceous material. Apocrine cysts are cytologically unremarkable. They are low cellular samples, and are more of a gross diagnosis confirmed with cytology. Sebaceous cysts and sebaceous hyperplasia consist of clusters of sebaceous epithelial cells. These cells are cohesive and consist of a moderate rim of cytoplasm filled with distinct cytoplasmic vacuoles. Cytologically it is difficult to differentiate sebaceous hyperplasia from adenoma.

Inflammatory lesions

Suppurative inflammation of the skin and subcutaneous tissue is common. Immune-mediated diseases such as pemphigus and nodular panniculitis are suppurative. The lesions are sterile and the neutrophils appear nondegenerative. Bacterial infections of the skin and subcutaneous lesions usually result in suppurative inflammation. Intracellular bacteria are fairly easy to find and the neutrophils are often degenerative. Pyogranulomatous inflammation is often caused by fungal organisms, though sterile pyogranulomatous inflammation does occur. Dimorphic fungal organisms such as *Blastomyces*, *Cryptococcus*, *Histoplasma* and *Coccidioidomycosis* are more likely to cause pyogranulomatous inflammation. Granulomatous inflammation of the skin is fairly uncommon but can be observed with atypical bacterial infections such as *Mycobacteria*. These organisms are negative staining rods with most of the common cytologic stains.

Neoplasia

There are many more tumors than will be discussed in this hand-out, but the most common ones will be discussed. Common epithelial tumors of the skin include squamous cell carcinoma, basal cell tumors, hair follicle tumors, sebaceous adenomas, perianal gland tumors and apocrine anal sac adenocarcinomas. Individual features of each tumor type will be discussed in more detail. It is difficult on cytology to distinguish the different mesenchymal tumors. Often a diagnosis of sarcoma or benign mesenchymal tumor is made based on the morphology of the cells. Histopathology, commonly with

immunohistochemical staining, is necessary to make a more specific diagnosis. Lipomas are a mesenchymal tumor; cytologically they appear consistent with mature adipose tissue and are difficult to differentiate from underlying subcutaneous fat. Histopathology or history of a fluctuant mass can be helpful in making a definitive diagnosis. Melanomas may be characterized as mesenchymal or round cell tumors. The majority of melanomas are benign; however from certain locations, such as the mouth or nail bed, malignancy is likely. Benign melanomas contain so much pigment in their cytoplasm that it is difficult to examine specific cytologic features. Malignant melanomas often have little to no pigment making it easy to identify the criteria of malignancy. Other round cell tumors include lymphoma, mast cell tumor, plasma cell tumor, histiocytoma and transmissible venereal tumors (TVT). Each of these cell types has distinct features making it fairly easy to differentiate each tumor. Mast cell tumors contain red to purple granules in their cytoplasm, TVTs have multiple, discrete, cytoplasmic vacuoles, histiocytomas have peripheral clearing of their cytoplasm whereas plasma cells have a perinuclear clearing of their cytoplasm. Occasionally, histiocytomas and plasma cell tumors look very similar and the signalment of the patient becomes very important. Histiocytomas are generally a tumor of young dogs and will often resolve on their own. Plasma cell tumors occur in middle aged to older dogs and surgical excision is often curative.

Suggested Reading:

Raskin RE, Meyer DJ. Canine and Feline Cytology, A Color Atlas and Interpretation Guide, 2nd ed. Saunders Elsevier, St. Louis, MO. 2010.

Cowell RL, Tyler RD, Meinkoth JH and DeNicola DB. Diagnostic Cytology and Hematology of the Dog and Cat, 3rd ed. Mosby Elsevier, St. Louis, MO 2008.