“Lumps and Bumps: Cytologic Evaluation of Masses”
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Introduction:

Cutaneous lesions are easily noticed by owners and are one of the top reasons patients are brought into veterinary clinics. During physical examination, if mass/masses are found in cutaneous or subcutaneous areas, cytologic samples are often obtained from those lesions. Although you cannot become an expert on cytology in just a few hours, with practice and some basic guidelines, you can make a confident diagnosis in many cases. Based on the cell morphology, nucleated cells usually can be classified into categories and recognizing common features of the basic cell types will help to narrow down differentials. Even if a definitive diagnosis cannot be made, cytologic samples may provide important information and/or suggest additional diagnostic tests. The following information provides guidelines for evaluation of cytologic samples.

Quality Assessment:

The first step to evaluating any cytologic sample is scanning at low magnification to determine if it is cellular enough, has cells that are intact, well-spread, and well-stained. When we talk about “cellularity” for these purposes, we mean nucleated cells, and generally ignore the background erythrocytes from blood. How highly cellular a sample needs to be depends a lot on the tissue sampled, the lesion present, and what the goals of submission are. For example, if a just few inflammatory cells containing a definitive infectious organism are present, that sample is sufficient for diagnosis. On the other hand, if one is trying to determine if there is metastasis within a lymph node, many more cells may be required to feel confident that there is no evidence of neoplasia. Whether cells are well-stained or not is another important consideration. Both under-staining and over-staining can make interpretation difficult. The type of stain can also make a big difference. Rapid, water-based Diff-Quik type stains can have problems such as not staining mast cell granules as well as alcohol-based Wright stains (Figure 1). Finally, as overspread or ruptured cells generally can’t be interpreted, it is important to make sure there are enough intact cells to make a diagnosis.
Determining the Major Cell Types, Categorizing the Lesion:

After determining a sample is diagnostic, it is important to identify if the nucleated cells present are inflammatory cells, tissue-type cells, or a mix. Which cell categories and specific types are present will tell you a lot about the disease process going on. After learning this basic schematic approach, you should be able to discriminate numerous lesions, from abscesses to granulomas to common round cell tumors, as well as identify suspected carcinomas and sarcomas.

Inflammatory

The main inflammatory cells are neutrophils, macrophages, lymphocytes, plasma cells, eosinophils, basophils, and mast cells. Because these cells respond to different stimuli and arrive to damaged tissue at different times, which cells are present and in what proportion can provide clues as to the cause of inflammation, where allergic, infectious, foreign body, etc. When neutrophils are the main inflammatory cell present the lesion is called purulent/suppurative or simply neutrophilic inflammation. Neutrophils are characterized as degenerate or non-degenerate based on cell morphology. Degenerate neutrophils have nuclei that are swollen and less lobulated, have less condensed chromatin, and stain lighter than the nuclei of nondegenerate neutrophils (Figure 2). Degenerate change is commonly induced by infectious organisms or damaging enzymes released during tissue necrosis. Non-degenerate neutrophils appear similar to the neutrophils from peripheral blood, and can purulent inflammation not due to necrosis or infection can be caused by trauma, chemical irritants (urine, bile), keratin, immune-mediated diseases and more.

If macrophages are predominant, it is referred to as granulomatous or macrophagic inflammation. If the inflammation is composed of both macrophages and neutrophils, it is referred to as pyogranulomatous inflammation. Macrophages are recruited because the etiologic agents are hard for neutrophils to eliminate, and typically signify chronic inflammation. Examples of etiologic agents are Mycobacterium spp., foreign bodies (e.g. vaccine), fungi and protozoa. Macrophages may look epithelioid (activated macrophages that morphologically resemble epithelial cells) and multinucleated giant macrophages may be present (Figure 2). Lymphocytes (and/or plasma cells) may also be present with or without macrophages in chronic inflammation. Lymphoid populations due to chronic inflammation/antigenic stimulation are heterogeneous and composed primarily of small lymphocytes (i.e. smaller than a neutrophil) with increased numbers of medium-sized lymphocytes (i.e. about the same size as a neutrophil) and large lymphocytes (i.e. larger than a neutrophil).
If the lesion contains greater than 10% eosinophils in addition to other inflammatory cell types, it is referred to as eosinophilic inflammation. Differentials for eosinophilic inflammation include allergy/hypersensitivity, parasitic infections, eosinophilic granuloma, paraneoplastic syndrome (e.g. mast cell tumors). Eosinophilic inflammation may be accompanied by mast cells and/or basophils.

**Tissue**

The three main categories of tissue-type cells are epithelial cells, mesenchymal/spindle cells, and discrete round cells. These types of tissue differ from each other morphologically, functionally, and suggest different diagnoses. Epithelial cells are adherent to each other and generally exfoliate fairly well. The cellularity of the smears is usually good. Epithelial cells are often found in cohesive clusters (Figure 3). The cells are aligned, organized and arranged next to each other and are not just pushed together by sample preparation. Epithelial cells vary in shape depending on the tissue of origin. They may be round, irregularly round, cuboidal, columnar, angular or polygonal and have round to irregularly round nuclei.

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**Figure 2**: A = Degenerate neutrophils with intracellular cocci bacteria from an abscess. B = multiple “epithelioid” macrophages (one of which is multinucleated) that have phagocytized difficult-to-kill Mycobacteria organisms. (Right image courtesy Pete W. Christopherson, DVM, PhD, DACVP)
Mesenchymal cells are not cohesive to each other but are often held together by extracellular matrix (e.g. collagen) that they produce. Aspirates of normal or reactive mesenchymal tissues, or classic benign mesenchymal tumors are usually sparsely cellular due to the tight adherence to extracellular matrix. Mesenchymal cells may be found singly or in accumulations that are sometimes associated with extracellular fibrillar pink matrix. The cells are often spindle, fusiform or stellate and may have wispy or poorly defined cytoplasmic borders (Figure 4). They often have oval to elongated nuclei.

Figure 4: Cells from a feline injection site sarcoma. Note the individually-arranged cells, spindle to stellate shape, and wispy cytoplasm.

Discrete round cells are not adhered to each other or extracellular matrix, so they tend to exfoliate very well during fine-needle aspiration. The cellularity of the resulting smears is usually very...
The cells are round and have crisply defined cytoplasmic margins. Six cutaneous tumors are included in this category, and can usually be differentiated based on cell appearance: canine cutaneous histiocytoma, mast cell tumor, lymphoma, plasmacytoma, transmissible venereal tumor, and melanoma (Figure 5).

Figure 5: Examples of common round cell tumors. A = Lymphoma, B = Mast cell tumor, C = Histiocytoma, D = Plasma cell tumor.

Criteria of Malignancy:

When evaluating cytologic samples that contain tissue-type cells, it is important to discern if they are normal, hyperplastic, or neoplastic, and if possible, benign or malignant. To do this, pathologists typically look for cellular clues called "criteria of malignancy." These are a variety of abnormal cellular features (sometimes called “atypia” in pathology reports) that tend to be prominent in neoplastic tissues. Normal, hyperplastic, and benign lesions tend to contain uniform populations of cells that lack significant criteria of malignancy (notable exceptions include several of the round cell tumors, such as lymphoma or mast cell tumor). While no single feature is consistently reliable, in general, the more of these features that can be identified, the higher the probability the sample is cancerous (malignant neoplasia). Some pathologists use the rule of thumb that three or more criteria of malignancy in most of the cell population indicates malignancy.

There are two broad categories of criteria of malignancy: general features and nuclear features. Nuclear features of malignancy are more strongly associated with cancer than general/cytoplasmic features, however both can be helpful. General features of malignancy include hypercellularity (particularly for mesenchymal tumors, as these cells normally do not exfoliate well on cytology), variation in cellular shape (cellular pleomorphism), variation in cellular size (anisocytosis), and gigantic cells (macrocytosis). The main nuclear features of malignancy include gigantic nuclei (karyomegaly), variation in nuclear size (anisokaryosis), multiple nuclei and increased nuclear-to-cytoplasm ratio (excluding lymphoid cells and small basal-type epithelial cells). Nucleoli that are large, prominent, multiple in number, or variably shaped are also strong nuclear criteria of malignancy. Additional nuclear features of cancer include coarse or clumped chromatin; increased numbers of mitotic figures (particularly if these figures have lagging or abnormal numbers of chromosomes); and nuclear molding (nuclei folding against each other due to exuberant growth). A number of these examples can be seen in Figure 6.
Caution must be used when applying these cytologic criteria in several situations. First, when a significant amount of inflammation is present in a sample, the cytologist must consider the possibility of dysplasia or other reactive changes to the cells. Often, dysplastic cells will show more general than nuclear criteria of malignancy, but differentiating between neoplasia and dysplasia is often difficult even for experienced pathologists, and a biopsy with histopathology is recommended. Another scenario when these criteria do not apply is with lymph node cytology, because normal and reactive lymphoid populations have variation in cellular and nuclear size, while lymphoma is diagnosed based on a monomorphic population of lymphocytes. Some select “atypical” features may be normal in certain cells or tissues (for example, macrophages can become binucleate or turn into multinucleate giant cells). Finally, aggressive tumors do not always show significant criteria of malignancy, and a biopsy may be needed to provide clarification when cytology is equivocal.

Summary:

Cytologic samples of cutaneous masses can be obtained with multiple techniques that affect yield and quality. Determining if a sample is diagnostic requires looking at the cellularity, level of intact cells, and staining quality. Once a sample is deemed diagnostic, one needs to categorize the nucleated cells present into inflammatory, tissue type, or mixed. Inflammatory samples should be further classified into purulent, granulomatous, pyogranulomatous, lymphocytic-lymphoplasmacytic, and organisms should be searched for as appropriate. Tissue cells should be classified as epithelial, mesenchymal or discrete round cells. Looking for criteria of malignancy can help determine is a sample is normal, hyperplastic, or neoplastic, and whether the neoplasm is likely benign or malignant.

References & Further Reading:
