Skin and Subcutaneous Lumps and Bumps: Cytology of Inflammation

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Introduction
Skin and subcutaneous lesions in animals are readily discovered by owners and are a common reason for presentation of animals to a veterinarian for examination. Cytology samples are relatively easy to collect and can provide useful diagnostic information in a relatively short time period. Inflammatory cells tend to readily exfoliate and will often outnumber the tissue cells in a cytology preparation. It may be difficult to collect diagnostic samples from lesions with a significant population of mesenchymal cells. In most animals, samples can be collected without sedation or anesthesia. Collection of high quality diagnostic samples may obviate the need for more invasive procedures (such as surgical biopsy), direct additional diagnostic steps or guide therapy. Some common lesions are readily identified in-house. More complex lesions can be sent to commercial or university laboratories for review by a board certified clinical pathologist.

Sample Collection and Handling
Collection of a quality sample is key to success in cytology. Most cutaneous and subcutaneous samples are collected by fine needle biopsy (FNB) using a needle (usually 20 to 22 gauge) without or without aspiration. An air filled syringe is used to expel the material in the needle onto a clean glass slide. The material is then spread using another glass slide, creating a monolayer of cells that is thin enough to allow the cells to spread. After the smear is allowed to air dry, it is stained with a Romanowsky-type stains such as Diff-Quik®. If the lesion is ulcerated, samples can be obtained by gently scraping with a scalpel blade or by gently rolling a moistened cotton swab over the surface of the lesion. Cytology samples collecting by scraping or swabbing may reflect a secondary superficial process and not be representative of a deeper, underlying disorder.

The tissue can be stabilized by palpation to allow collection of material from multiple regions of the lesion. If the lesion is fluid filled, fluid can be collected for analysis in addition to FNB from any solid regions in the lesion. If infection is suspected, an aliquot of material should be place into a sterile tube for culture. Direct smears of the fluid can be used to estimate the cellularity of the fluid. Concentrated smears should be also be made from a cell pellet following centrifugation of an aliquot of fluid.

Basic Approach to Microscopic Examination
The slides are first scanned using a 10x objective to determine the quality of the preparation and adequacy of staining. Poorly stained slides should be restained. If the smears are poorly cellular, considerations include inadequate tissue sampling or, if the lesion was fluid-filled, a poorly cellular cystic lesion. Resampling a poorly cellular sample using a different technique or
from different areas of the lesion may yield diagnostic samples. If the smear contains deteriorated or necrotic cells, then collection of additional samples from other regions of the lesion are recommended in an attempt to collect viable cells to evaluate. If the smears are of adequate cellularity, the initial step is to determine if the cells are inflammatory cells, non-inflammatory tissue cells or a combination of inflammatory cells and tissue cells.

Expected normal cells from skin or subcutaneous tissues include squamous epithelial cells, adipocytes from subcutaneous fat, glandular epithelium from adnexal sebaceous or sweat glands, and few mast cells. The mesenchymal components of the tissue (muscle cells or fibrocytes and associated collagen fibers from the connective tissue) do not usually exfoliate in significant numbers and are not found in samples from healthy animals. If there is significant blood contamination secondary to sample collection, leukocytes such as neutrophils, lymphocytes and eosinophils may be present.

Cystic or Fluid Filled Lesions
Cysts form when fluid produced by glandular epithelial cells (such as sweat glands) accumulates under the skin. The fluid is usually poorly cellular, containing few macrophages and erythrocytes. Protein in the fluid causes the background to stain grainy blue or pink. The cells that line a cyst define the type of cyst but are usually not found in cytologic preparations. Histologic examination may be required for definitive characterization.

Epidermal inclusion cysts or follicular cysts are lined by squamous epithelial cells. Because the cysts do not open to the surface, mature keratinized epithelial cells drop off and become trapped under the surface of the skin. Microscopic review reveals keratin debris, anucleate squames, nucleated keratinocytes, cholesterol crystals and possible melanin granules. If the cyst ruptures, it may become infected or inflamed with influx of neutrophils and macrophages intermixed with the keratin and squames.

Hematomas occur secondary to trauma or in animals with a hemostatic disorder. Microscopic examination reveals variable amounts of blood and macrophages. Erythropagocytosis is expected and hematoidin crystals, a breakdown product of hemoglobin, may also be present. Platelets are not expected unless the hemorrhage is recent or hemorrhage also occurred secondary to sample collection. Seromas and hygromas also occur with trauma. Fluid is typically clear to hazy. Cytologic preparations are poorly cellular and contain few erythrocytes and macrophages.

Sialoceles are fluid lesions found in the head and neck region that occur when saliva leaks into subcutaneous tissues. Grossly, the fluid is viscous and red to pink. Smears are usually poorly cellular and contain lakes of homogeneous mucoproteinaceous material, variable numbers of macrophages, and variable amounts of blood. Erythrocytes often line up in rows. The macrophages are often highly vacuolated and can be difficult to differentiate from salivary epithelial cells. The presence of phagocytized erythrocytes and/or hemosiderin is common with macrophages and not expected with salivary epithelial cells. Hematoidin crystals may be present. If traumatized or infected, neutrophils may also be found.
Abscesses may be sterile or infected. They are a localized, accumulation of neutrophils. Grossly, collected material is cloudy and varies from tan to red tinged to brown. Neutrophils range from nondegenerate (appear similar to neutrophils in the blood with well defined, densely stained nuclei) to degenerate (nuclei become swollen, lighter pink in color, irregular margins). Degenerate neutrophils support infection but may occur with noninfectious conditions. Nondegenerate neutrophils may be found with infections so the absence of degeneration does not rule out infection.

**Inflammatory Lesions From Solid Tissue**

Inflammatory lesions are usually classified based upon the make-up of the inflammatory cells. While the type of inflammation may help determine an underlying cause, there is overlap in the type of inflammatory responses to a variety of conditions so interpret cytologic findings in the context of signalment, history, and physical examination. Inflammation may be the primary process or may be secondary to neoplasia. Depending upon the sampling technique, neoplastic cells may not be present in the smears so knowledge of how the sample was collected and the collection site should be kept in mind. Inflammation may cause changes in the resident tissue cells that mimic neoplasia so caution is warranted when diagnosing neoplasia in inflamed lesions.

Neutrophilic (also called suppurative or purulent) inflammation contains primarily neutrophils. This type of inflammatory response is nonspecific but warrants careful examination for organisms such as bacteria or fungi. Even if an organism is not identified, culture may be warranted, particularly if the neutrophils are degenerate. Noninfectious causes of neutrophilic inflammation include trauma, chemical injuries, immune-mediated diseases (e.g., pemphigus complex), neoplasia, or foreign body reactions.

An eosinophilic component to inflammation occurs with arthropod bites, parasitic infections, allergic or hypersensitivity reactions, eosinophilic granuloma complex, fungal infections, collagen necrosis, or some types of neoplasia (e.g., mast cell neoplasia).

Macrophagic (granulomatous) inflammation is characterized by an influx of macrophages and may contain numerous multinucleated giant cells. Macrophages are often intermixed with neutrophils (pyogranulomatous inflammation), eosinophils, and/or lymphocytes/plasma cells and considerations are similar. A macrophagic component to inflammation is associated with infections (often more complex bacteria, protozoa, dermatophytes, or localized or systemic fungi), foreign body reactions, nodular panniculitis/steatitis, lick granuloma, sebaceous adenitis, calcinosis circumscripta, injection site reactions or cutaneous xanthoma. If the lesion is chronic, fibroplasia is common and reactive fibroblasts can be confused with neoplastic cells.

Lymphocytic or lymphoplasmacytic inflammation is relatively uncommon by itself and is usually found as part of a mixed inflammatory response. In inflammatory lesions, lymphocytes are heterogeneous but contain primarily small cells that have a round to oval, densely stained nucleus with a thin rim of cytoplasm. Cutaneous lymphoma should be considered if there is a monotypic population of atypical lymphocytes and plasma cells and other inflammatory cells are absent.
Specific Infections Found in Skin & Subcutaneous Lesions
Some bacteria are normal residents on the surface of the skin and should not penetrate into underlying tissues. Infection of the deeper tissues occur secondary to trauma, immune-mediated disease, immunocompromise or metabolic disorders (e.g., endocrinopathies). Simple bacteria typically cause a neutrophilic response while more complex bacteria (e.g., *Actinomyces* spp, *Nocardia* spp, or *Mycobacteria* spp.) often lead to a mixed response.

Fungal infections that infect skin or deeper subcutaneous tissues include dermatophytes, *Candida albicans*, *Malassezia pachydermatis*, *Sporothrix schenckii*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, or *Histoplasma capsulatum*. Protozoal or parasitic infections including leishmaniasis, protothecosis, toxoplasmosis, demodicosis, or dracunculiasis have also been reported.

References