Skin and Subcutaneous Lumps and Bumps: Cytology of Neoplasia

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Introduction
Skin and subcutaneous lesions are readily discovered by veterinarians during routine physical examination and may be detected by owners, prompting a visit to the veterinarian. It is often difficult from physical examination alone to determine the cause. Cytology is a non-invasive technique that can often provide useful diagnostic information. Cytology samples from normal skin and subcutaneous tissues are typically of low cellularity and contain a mixture of keratin bars, anucleate squames, and well-differentiated superficial squamous epithelial cells (keratinocytes). Mature keratinized squamous cells are large, individualized, angular shaped cells that have a large amount of glassy blue cytoplasm and a small dark staining nucleus. Squames are similar in appearance but lack a nucleus. Adipocytes, glandular epithelial cells (e.g., sebaceous cells, apocrine cells), melanocytes, and mast cells may be found in low numbers.

Cytology can often differentiate non-neoplastic cysts, inflammatory lesions secondary to tissue damage/infection, or proliferative lesions. Proliferative lesions can then be characterized as benign (hyperplasia or benign neoplasia) or malignant neoplasia. Neoplasia is easiest to recognize when cytology reveals a monomorphic or monotypic population of non-inflammatory cells. Although it is sometimes difficult to determine specific types of neoplasia using cytology, morphologic criteria can be used to differentiate benign and malignant proliferations and to classify tumors into general categories. Benign neoplastic cells are uniform in appearance while malignant cells lack uniformity. Caution is warranted when diagnosing malignant neoplasia in an inflamed lesion because inflammation causes dysplastic changes in non-inflammatory tissue cells that may mimic neoplastic changes. In these cases, biopsy and histologic examination may be required to make a definitive diagnosis.

General Classification of Tumor Type
Neoplasms are classified into 3 general categories (epithelial, mesenchymal, or discrete round cell) based upon morphologic appearance (e.g., size, shape and distribution on a smear), not function or embryonic origin. These features are often best assessed by scanning the slide on low magnification (10x).

Epithelial cells generally exfoliate well and yield cellular preparations. In the skin, they arise from glands (apocrine or sebaceous), hair follicles or epidermis. The cells are cohesive and distributed in clusters or sheets, though individualized cells may be present. Within the clusters, the cells have tight cell junctions and often display distinct cytoplasmic borders. Cohesive clustering of cells may be difficult to differentiate from cell crowding in densely cellular smears.
Be careful to assess for cohesion in well-spread areas. Epithelial cells tend to be medium to large. Nuclei are oval to round while the cells are round to polygonal.

Mesenchymal cells in the skin and subcutaneous tissues arise from fibroblasts in connective tissue, adipocytes, muscle cells, bone or cartilage, or blood vessels. As a general rule, mesenchymal cells do not exfoliate well so fine needle biopsy or impression smear techniques may yield poorly cellular preparations. In some cases, scrapings of a biopsy sample may be required to get enough cells to yield a diagnostic sample. With the exception of adipocytes, mesenchymal cells lack cohesion and are individualized. Aggregates of cells may be found if the cells are entrapped in an extracellular matrix. Within these aggregates, the cells are loosely distributed and lack obvious cell junctions. Cytoplasmic margins may be indistinct and blend into the background. Mesenchymal cells are oval to stellate to spindle in shape. Nuclei range from round to oval. The cells tend to be smaller than epithelial cells.

Cytology samples from discrete round cell tumors contain cells that are round to oval and individualized. Cytoplasmic boundaries tend to be distinct. Nuclei are round to indented. The cells tend to be smaller than epithelial cells. Cells exfoliate well so the cellularity is generally moderate to high. Neoplasms in this category include plasmacytoma, lymphoma, mast cell tumor, histiocytoma, histiocytic sarcoma, and transmissible venereal tumor. Undifferentiated sarcomas and carcinomas as well as some melanomas may have a round cell appearance.

**Differentiation of Benign and Malignant Neoplasia**

Benign and malignant neoplasms are differentiated based upon the morphology of the cells. Benign neoplasia is difficult to differentiate from hyperplasia from cytology alone. Cells have consistent nuclear size and shape, uniform (though often increased) nuclear to cytoplasmic (N:C) ratios, and uniform nuclear features (e.g., shape, chromatin pattern and nucleolar features).

Malignant cells are characterized by variability in cellular morphology. Nuclear criteria are more reliable than cytoplasmic criteria in identifying malignancy. Generally, a minimum of 3 nuclear criteria are required to diagnosis malignant neoplasia in a non-inflamed lesion. Nuclear criteria of malignancy include: (1) abnormally large nuclei (macrokaryosis); (2) variable nuclear size (anisokaryosis) or shape; (3) variable N:C ratios; (4) atypical or variable chromatin patterns; (5) variable nucleolar number, size or shape; (6) nuclear molding; (7) multinucleation in cells that are not normally multinucleated (especially if there is variation in nuclear size within a single cell); and (8) increased mitotic figures (especially if atypical). Well-differentiated malignant tumors may not exhibit significant cellular atypia to make a cytologic diagnosis; diagnosis may require biopsy and histologic examination. Histology allows assessment of tissue architecture and invasiveness, features that cannot be evaluated on cytology.

**Epithelial Neoplasms of the Skin and Subcutaneous Tissues**

As a general rule, benign epithelial neoplasms are named by their cell of origin followed by adenoma and malignant tumors are named by their cell of origin followed by carcinoma or adenocarcinoma. Exceptions to this rule occur. Epithelial tumors of the skin include cutaneous basilar epithelial neoplasms, sebaceous gland tumors, sweat gland tumors, perianal gland tumors, anal sac tumors, ceruminous gland neoplasms, mammary gland tumors, squamous papillomas, squamous cell carcinomas, and hair follicle tumors.
Although it may be difficult to identify specific types of epithelial tumors based upon cytologic features alone, knowledge about location in the patient and physical properties of the mass help in differentiation. Tumors of glandular origin may have prominent vacuolization of the cytoplasm of the neoplastic cells. Perianal gland tumors are usually located around the anus but may be found in other locations (e.g., tail, thigh, back, or caudal abdomen). The cells resemble hepatocytes and have moderate to abundant granular cytoplasm. Anal sac tumors are also located around the anus. Compared to perianal gland tumors, anal sac tumor cells are generally smaller, have higher N:C ratios, and often lack well-defined cell borders. Basilar epithelial neoplasms are common on the head, neck or limbs. The cells are small, have high N:C ratios, contain monomorphic round nuclei and may have cytoplasmic melanin or keratohyalin granules. Hair follicle tumors contain a population of basal appearing epithelial cells in combination with keratin debris, squames, and cholesterol crystals. They may be difficult to differentiate from non-neoplastic epidermal cysts or follicular cysts. Squamous papillomas contain well-differentiated squamous cells with uniform appearing nuclei. Squamous cell carcinomas contain individualized squamous cells as well as clusters of cohesive cells that have significant nuclear atypia. The cells can range from small basal appearing cells to larger angular cells. Perinuclear vacuolation may be prominent.

**Mesenchymal Neoplasms of the Skin and Subcutaneous Tissues**

Benign mesenchymal neoplasms are named by their cell or origin followed by “oma” (e.g., hemangioma, fibroma). Malignant tumors are named by their cell of origin followed by “sarcoma” (e.g., hemangiosarcoma, fibrosarcoma). Mesenchymal tumors are often referred to as spindle-cell tumors because of their appearance. The different types of mesenchymal neoplasms are difficult to distinguish based upon cytologic criteria alone. Diagnosis may be limited to identifying the cells as mesenchymal and determining if there are enough cytologic criteria to suggest malignancy. Sarcomas are easily confused with reactive fibroplasia that occurs with inflammation or healing following tissue injury. Reactive fibroblasts may exhibit multiple cytologic criteria of malignancy, though the changes will generally not be as marked as seen with neoplasia. If tissue is inflamed or if a lesion is associated with an area of known injury, biopsy and histologic examination are warranted before a diagnosis of sarcoma is made.

Some specific types of mesenchymal tumors can be identified on cytology. Lipoma is a very common benign tumor. Prior to staining, smears from a lipoma will appear wet and glistening. The alcohol fixatives in Romanowsky-type stains will dissolve lipids and may leave the slide acellular while in other cases adipocytes will remain. Adipocytes have large amounts of clear cytoplasm with a small, dark staining nucleus pushed to the edge of the cell. They may be individualized or in cohesive clusters. Aspirates from a lipoma will appear similar to aspirates from normal subcutaneous fat. Liposarcoma is a malignant tumor of fat that will contain clumps of pleomorphic mesenchymal cells that have cytoplasmic vacuoles. Caution is warranted when diagnosing liposarcoma because this tumor may be confused with granulomatous steatitis. Benign and malignant melanomas can have a spindle, epithelioid or round cell appearance. Well-differentiated melanomas have large numbers of fine black-green cytoplasmic granules that make it difficult to evaluate nuclear morphology. Poorly differentiated melanomas may contain few granules, making it easier to evaluate the cells for cytologic features of malignancy.
Round or Discrete Cell Neoplasms of the Skin and Subcutaneous Tissues

Round cell tumors are commonly found in the skin and subcutaneous tissues. Cytologic determination of the cell of origin can be challenging. Often the first step when evaluating round cell tumors is to look for cytoplasmic granules. Mast cell tumors contain variable numbers of reddish purple granules; however, some poorly differentiated tumors may have few to no recognizable granules. Occasionally, the granules do not stain with Diff-Quik® but will stain with Wright’s stain. Mast cell tumors may contain eosinophils and reactive fibroblasts. In contrast, melanomas contain black-green cytoplasmic granules.

If there are no granules, evaluate N:C ratios and chromatin patterns. Lymphoid cells have a high N:C ratio and smudged chromatin. If the majority of the lymphoid cells have prominent nucleoli and/or are similar in size or larger than a neutrophil, cutaneous lymphoma is likely. If the lymphoid cells are small lymphocytes, lymphoma must be differentiated from lymphocytic inflammation. With lymphoma, the cells are uniform in appearance. Lymphocytic inflammation is characterized by a mixed population of lymphocytes with or without accompanying plasma cells. Transmissible venereal tumor cells have a lower N:C ratio, moderate amounts of blue gray cytoplasm with small clear vacuoles that tend to line up along the cell margin, prominent nucleoli, and coarse nuclear chromatin. Histiocytoma cells have moderate amounts of clear to light blue cytoplasm, cytoplasmic margins that are variably distinct, fine nuclear chromatin, and generally indistinct nucleoli. Anisocytosis and anisokaryosis are mild. Regressing histiocytomas may contain significant numbers of lymphocytes. Histiocytic sarcomas contain cells that are more pleomorphic. Cells are round to occasionally spindle in shape. They have a round to oval to indented nucleus with prominent, variable nucleoli. Cytoplasm is often abundant and may be vacuolated or contain phagocytized material. Multinucleated cells are common. Plasmacytomas contain pleomorphic cells with frequent eccentric round nuclei, fine to coarse nuclear chromatin, and variably distinct nucleoli. The cells have moderate to abundant dark blue cytoplasm. Anisocytosis and anisokaryosis are prominent. Multinucleated cells are common.

If the cells do not exhibit any of the distinguishing characteristics listed above, then a definitive diagnosis may not be possible using routine cytology. Poorly differentiated carcinomas or sarcomas may have a discrete cell appearance. Agranular mast cell tumors or melanomas are also difficult to recognize. In these cases, biopsy and histologic examination and/or immunostaining are required for definitive diagnosis.

References