Neoplasia involving cutaneous and subcutaneous tissues occurs frequently in dogs and cats. Fine needle aspirates (FNA) can be helpful in differentiating these tumors, and in some cases, can provide a definitive diagnosis. Collection of FNA is relatively non-invasive, the risk of complications is low, and there is a short turnaround time for results. For tumors involving cutaneous and subcutaneous tissues, cytology based on FNA has a relatively high diagnostic sensitivity, specificity, and positive predictive value, and a high negative predictive value compared to histologic evaluation. However, important limitations for cytology include inability to evaluate surgical margins, vascular invasion, and organization of the cells within the lesion, which may be important in establishing a definitive diagnosis or determining clinical behavior.

Sample collection and processing

Cutaneous and subcutaneous lesions are sampled using a 21-25 gauge needle coupled to a 12-20 ml syringe. The skin is cleansed using an alcohol swab, the mass is immobilized with one hand, and the other hand is used to introduce the needle into the lesion and apply slight negative pressure on the syringe. Depending on the size and firmness of the mass, the needle can be redirected several times to ensure adequate sampling. Suction is released prior to withdrawing the needle to minimize contamination with blood or cells from surrounding tissues. For small skin masses, sometimes only the needle is used to prick the lesion several times, and the syringe is attached prior to making smears. If only a small amount of material has been collected by either technique, the needle is detached, air is aspirated into the syringe, the needle is replaced, and a small amount of material is carefully expelled onto the glass slide. A spreader slide and a pull or push technique is used to disperse the cells. Common errors during slide preparation include failure to disperse the cells on the slide, resulting in smears that are too thick, and applying too much pressure, resulting in broken cells.

Alternately, impression smears from ulcerated masses or from a small portion of a biopsy sample prior to formalin fixation for histopathology can be evaluated. Blood, tissue fluid, and superficial cells should be gently blotted from ulcerated lesions or the cut surface of a biopsy, taking care not to damage the tissue or disrupt surgical margins. Clean glass slides are touched to the surface of the ulcerated lesion or biopsy surface using minimal pressure. Firm, dense lesions may not exfoliate readily when sampled by FNA or impressions smear. For excised firm masses, a scalpel blade can be used to make a crosshatch pattern or gentle scrapes across the surface of these lesions prior to making impression smears. This may improve cell exfoliation but sometimes causes cell breakage.
Smears made from FNA or impression smears should be clearly labeled with the owner’s name, patient’s name or identification number, and date. Slides are dried in ambient air prior to staining. Slides should not be stored in a refrigerator or exposed to formalin, because water condensation from refrigeration will lyse cells and formalin fumes will prohibit adequate staining. Slides can be sent unstained to a reference laboratory or stained for in-house interpretation. Romanowsky-type stains (Wright's stain, Wright-Giemsa stain, or commercially available quick stains such as Diff-Quick®) are most commonly used. These stains provide good color contrast, acceptable cytoplasmic and nuclear detail, stain most infectious agents, and are relatively inexpensive and easy to use. Slides stained with these types of permanent stains can be sent to a reference laboratory for a second opinion if needed. Special stains used to determine cell lineage or identify etiologic agents are available at some commercial reference laboratories or academic institutions.

If slides are mailed to a reference laboratory for interpretation, the laboratory should be contacted for information on requirements for sample submission. In general, the following information should be included: identification name or number, species, age, sex, a brief history, relevant physical examination findings, previous therapy, a summary of results of previous pertinent diagnostic tests, a description of the lesion, differential diagnoses, and the site from which the sample was collected. The description of the lesion should include size, and whether it is superficial or deep, firm or soft, ulcerated or inflamed, and freely moveable or firmly attached to surrounding tissue. This information is very helpful for the clinical pathologist to adequately evaluate the sample and provide the most complete information for optimal patient care.

**Cystic lesions**

Cystic lesions are non-neoplastic, but sometimes resemble neoplasms based on clinical findings. The most common cutaneous cystic lesions in dogs and cats are epidermal inclusion cysts (follicular cysts), apocrine cysts, and sebaceous cysts.

**Epidermal inclusion cyst:** Epidermal inclusion cysts arise from hair follicles and are relatively common in older dogs. Aspirated material often appears very thick. Smears are characterized by numerous anucleate keratinized squamous epithelial cells that appear eosinophilic, basophilic, or poorly stained. There may be abundant amorphous cellular debris and occasional cholesterol crystals. These cysts may become inflamed and occasionally infected. The cytology of epidermal inclusion cysts and other similar benign epithelial tumors can appear similar. Histologic evaluation may be helpful for a definitive diagnosis.

**Apocrine cysts:** Apocrine cysts occur from secretory accumulations from apocrine sweat glands, and these cysts sometimes resolve after aspiration of accumulated fluid. The aspirated fluid is clear and smears are minimally cellular. Typically only a few well-differentiated epithelial cells or large mononuclear cells are present.

**Sebaceous cysts.** Sebaceous cysts are secretory accumulations from sebaceous glands that occur most often on the head or in the ears. The aspirated fluid is brown and
oily and minimally cellular in a background of amorphous, basophilic, proteinaceous material.

**Inflammation**
Cytology can be useful in differentiating between inflammatory and neoplastic lesions, and in some cases provides an etiologic diagnosis. FNA from inflammatory lesions often are very cellular, and can include neutrophils, eosinophils, macrophages, lymphocytes, plasma cells, and mast cells, depending on the cause of the inflammation. Many etiologic agents have a characteristic appearance with routine staining, although a definitive etiologic diagnosis may require additional testing. It is important to remember that inflammation may be associated with hyperplasia or dysplasia of epithelial cells and mesenchymal cells, which often appear similar to neoplastic cells. Distinguishing epithelial and mesenchymal hyperplasia from neoplasia often requires histologic evaluation.

**Neoplasia**
Neoplasia is characterized by the presence of a uniform population of cells from the same tissue of origin. It may be difficult to distinguish benign neoplasia from hyperplasia using cytology. Hyperplastic cells and benign neoplastic cells are characterized by minimal variation in cell size, nuclear size, and nuclear to cytoplasmic ratio. Nucleoli may be present, but should be similar in size, shape, and number. In contrast, malignant neoplastic cells often have marked variation in cell size (anisocytosis); nuclear size (anisokaryosis) and number; nuclear to cytoplasmic ratio; and the size, shape, and number of nucleoli. Nuclear chromatin often appears fine or irregularly condensed, nuclear molding may be present, and there may be increased numbers of mitotic figures.

**Round cell tumors**
Cytology is useful in the diagnosis of several round cell tumors involving the skin and subcutaneous tissues. These tumors also are called discrete cell neoplasms because the cells occur individually in FNA. Round cell tumors include mast cell tumors, histiocytomas, transmissible venereal tumors, plasmacytomas, lymphoma, and melanoma.

**Mast cell tumors (MCT)**
MCT are one of the most common cutaneous neoplasms in dogs and occur less frequently in cats. Cutaneous MCT usually occur in middle aged animals, but can occur in younger or older animals. Some breeds of dogs (boxers, Boston terriers, Labrador retrievers, beagles, and Schnauzers) have a higher incidence of MCT. Most cutaneous MCT occur as a single, well-circumscribed nodule that usually involves the dermis, but can extend into the subcutis and underlying musculature.

FNA of MCT usually are highly cellular. Neoplastic mast cells are large (10-35 microns in diameter), round cells with round to oval nuclei that have finely stippled or aggregated chromatin. Nuclei may stain poorly or be obscured by the presence of round or oval, fine to course, variably sized granules, which are a characteristic feature of mast cells.
With Wright-Giemsa stain, the granules are purple, but the granules may stain poorly with some commercial stains. This is important because these tumors may be misdiagnosed by cytology if only commercial quick stains are used. There also may be variable numbers of eosinophils and spindle-shaped cells that resemble fibroblasts in FNA of MCT in dogs. Eosinophils are less commonly observed in FNA of MCT in cats.

The cytologic appearance of FNA of MCT does not always correlate with clinical behavior, so biopsy with histologic grading is recommended. In dogs, all cutaneous MCT should be considered potentially malignant. Staging may include an FNA of regional lymph nodes (> 5 aggregates of more than 3 mast cells/aggregate = metastasis), spleen (overlap between normal and dogs with metastasis), and liver (< 1 mast cell/100 hepatocytes= normal); a CBC to determine if there are circulating mast cells (may be increased in dogs with inflammatory disease); and evaluation for c-kit mutations (exon 11 mutations are associated with poorer outcome; exon 8 mutations may respond to tyrosine kinase inhibitors). Cutaneous MCT in cats occur most often on the head and neck and usually are benign. Recurrence is uncommon and metastases are rare, in contrast to MCT involving hematopoietic or gastrointestinal tissues in cats, which often are aggressive.

**Histiocytomas**

Canine cutaneous histiocytomas (CCH) are relatively common benign tumors of dendritic cells that occur most often in young dogs and less commonly in older dogs. Some breeds (boxers, dachshunds, cocker spaniels, Great Danes, and Shetland sheepdogs) are predisposed to CCH. CCH usually occur as a single, relatively small (<2-3 cm in diameter) “button-like” mass, most commonly involving the dermis and subcutis on the head, ears, neck, or distal portions of the limbs. They may be rapidly growing but most often spontaneously regress over weeks to months.

FNA from CCH contain round, oval, or irregularly shaped cells that resemble monocytes or histiocytic cells. Neoplastic cells are 12-26 microns in diameter and have a moderate amount of pale blue cytoplasm with relatively distinct borders. Nuclei are often are eccentric and have a round, oval, indented, or irregular shape. Chromatin is finely stippled and nucleoli usually are inconspicuous. In CCH that are ulcerated, inflamed, or infiltrated with lymphocytes and plasma cells, the cytology resembles chronic inflammation and the definitive diagnosis may require histopathology.

**Transmissible venereal tumor (TVT)**

TVT are rarely observed in dogs in the United States. They usually involve mucous membranes of the external genitalia. Smears of FNA from TVT usually are very cellular and are characterized by large (14-30 microns in diameter), round to oval cells with distinct cytoplasmic borders. Abundant pale blue or moderately basophilic cytoplasm often contains distinct vacuoles. Nuclei are round or oval and have coarsely granular chromatin and a single, prominent nucleolus. Plasma cells, lymphocytes, and macrophages also may be present. The unique location of most TVT is helpful in cytologic interpretation but differentiation from lymphoma and canine cutaneous histiocytoma may require histologic evaluation.
**Plasma cell tumor (PCT)**
Cutaneous PCT have been described in cats, but occur more commonly in middle-aged to older dogs. PCT typically are small, hairless solitary masses involving skin of the digits, lips, face, and ears, or the mucous membranes of the oral cavity. PCT involving skin and mucous membranes usually are benign and are cured by surgical excision.

FNA from cutaneous PCT usually are very cellular. The cells typically are round to oval and 12-30 microns in diameter. Variable amounts of basophilic cytoplasm often contain a perinuclear clear area. Nuclei are round or oval and often eccentric. Binucleated and multinucleated cells are relatively common. Nuclei have fine to moderately clumped chromatin and may have visible nucleoli. There may be minimal to marked variation in cell size, nuclear size, and nuclear to cytoplasmic ratio. Histologic confirmation may be necessary when the cells are anaplastic. Immunophenotyping by flow cytometry or immunohistochemistry to detect surface expression of immunoglobulin light chains also may be helpful in establishing the diagnosis.

**Lymphoma**
Primary cutaneous lymphoma in dogs and cats is rare. Lesions may be single or multiple, and often appear as cutaneous plaques or nodules. FNA are characterized by a uniform population of intermediate to large round cells with a relatively high nuclear to cytoplasmic ratio. These cells have a moderate amount of basophilic cytoplasm. Nuclei may be round, indented, or very irregularly shaped, and have finely stippled chromatin and one to several nucleoli. Although cytology is useful in the provisional diagnosis of cutaneous lymphoma, histologic confirmation is recommended.

**Melanoma**
Cutaneous melanomas are relatively common in dogs and rare in cats. Melanomas occur most often in middle-aged to older dogs, especially dogs with heavily pigmented skin. Most cutaneous melanomas in dogs are benign, but interdigital and oral cavity melanomas uniformly are malignant. Malignant melanomas may appear very anaplastic or well-differentiated cytologically, so histologic evaluation is recommended.

Melanocytes from FNA of melanomas usually occur singly, but aggregates of cells may be present. Round, stellate, and spindle-shaped cells, 12-30 microns in diameter, often occur in the same aspirate. Moderate to abundant cytoplasm often contains brown to greenish-black melanin granules that resemble small rods of relatively uniform size. Melanomas may be sparsely or heavily pigmented. Poorly pigmented melanomas (amelanotic melanomas) are more difficult to recognize cytologically, and biopsy may be necessary for a definitive diagnosis. Nuclei are round or oval and have fine to coarsely condensed chromatin and single or multiple prominent nucleoli. Mitotic figures often are present, especially in malignant melanomas. Melanophages often accompany melanomas. Melanophages usually are larger than melanocytes and have vacuolated cytoplasm and more variably sized granules, but this distinction sometimes is difficult.
Epithelial Tumors of the Skin

In contrast to the round cell tumors described above, most other cutaneous neoplasms require histopathology for definitive diagnosis. However, there are some general criteria that may be useful in the tentative diagnosis of epithelial or mesenchymal tumors involving skin. Epithelial tumors usually exfoliate relatively easily. There may be organized clusters of cells with intercellular junctions. Epithelial cells are round or polyhedral and have abundant cytoplasm with well-defined cytoplasmic borders. The cytoplasm of epithelial cells differs among tumor types with respect to amount, color, granularity, and vacuolation. Benign epithelial tumors often end with “oma” whereas malignant epithelial tumors are called carcinomas.

Sebaceous Gland Adenomas: Sebaceous gland adenomas tumors occur commonly in older dogs. Cells from sebaceous gland tumors often exfoliate in clusters. Individual cells have abundant foamy cytoplasm and small, centrally located nuclei. Perianal adenomas are a kind of sebaceous gland tumor that occurs in dogs. Perianal gland tumors sometimes are called hepatoid tumors because the morphology of the cells resembles hepatocytes.

Basal cell tumor: Basal cell tumors arise from epithelium of the epidermis or adnexal structures and are common in middle-aged dogs and cats. They occur in the dermis and subcutis, usually as solitary masses on the head, neck, ears, or trunk. Basal cell tumors may be pigmented, especially in cats, and may contain cystic areas. FNA from basal cell tumors usually are moderately cellular. Typically there are clusters of relatively small (7 μ in diameter), uniform epithelial cells with scant cytoplasm, a high nuclear to cytoplasmic ratio, and minimal variation in cell size, nuclear size, and nuclear to cytoplasmic ratio. Histologic confirmation is recommended because similar appearing cells can be seen with several other cutaneous tumors of basal cell origin.

Squamous cell carcinoma: Squamous cell carcinoma is a malignant tumor of squamous epithelial cells, most commonly involving the skin of the ventral abdomen, medial stifles, scrotum, lips, and nail beds in dogs, and the pinnae, nasal planum, external nares, lips, or eyelids in cats. Tumors appear as papillary growths or shallow masses which may be ulcerated, inflamed, and sometimes infected. Neoplastic cells occur singly or in small clusters. Keratin production is characterized by angular cytoplasmic borders and small, clear vacuoles arranged around the nucleus. Nuclei are round to irregular in shape and have finely stippled to moderately clumped chromatin and multiple nucleoli that vary in size and shape. There may be marked variation in cell size, nuclear size, and nuclear to cytoplasmic ratio. Neutrophilic inflammation presents a diagnostic challenge because chronic inflammation often is accompanied by epithelial cell dysplasia, and this may be difficult to differentiate from SCC. The definitive diagnosis of SCC should be made histologically.
Mesenchymal Tumors of the Skin

In general, mesenchymal tumors exfoliate poorly and the cells occur as individual cells or in aggregates. Individual cells are spindle or irregularly shaped and have poorly defined cytoplasmic borders and round or oval nuclei. Mesenchymal cells such as fibroblasts from inflammatory lesions may appear anaplastic, and in these cases definitive diagnosis and determination of biologic behavior require histologic evaluation. Benign mesenchymal tumors often end with "oma" whereas malignant mesenchymal tumors are called sarcomas.

**Lipomas:** Lipomas are relatively common masses in the subcutaneous tissues of dogs and cats. They often are soft, non-painful, and freely moveable. FNA may appear oily grossly. There may be aggregates of well-differentiated adipocytes but often the FNA is too poorly cellular for adequate evaluation.

**Soft tissue sarcomas:** Soft tissue sarcomas arise from several different mesenchymal tissues and include fibrosarcoma, peripheral nerve sheath tumor, hemangiopericytoma, liposarcoma, leiomyosarcoma, and synovial cell sarcoma. Soft tissue sarcomas involving cutaneous tissues may have a similar cytologic appearance and the cell of origin often cannot be determined by cytology. Most are composed of large spindle to irregularly shaped cells that occur singly or in aggregates. Multinucleated giant cells and pleomorphic cells may be present. The neoplastic cells can be very large and have abundant, basophilic cytoplasm. Nuclei are large, oval, and have finely stippled chromatin and several nucleoli. There often is marked variation in cell size and nuclear size. The definitive diagnosis is based on histologic evaluation and determination of cell lineage often requires immunohistochemistry.

**Histiocytic sarcoma:** Histiocytic sarcoma is a malignant neoplasm of interstitial dendritic cells that occurs most often in middle-aged to older, large breed dogs, especially Rottweilers and Bernese Mountain dogs. Typically there is a single large, locally-invasive and rapidly growing subcutaneous mass, most frequently involving the hind limbs, often near large joints. FNA are characterized by large, round to irregularly shaped cells with abundant cytoplasm that may be vacuolated. Nuclei often are irregularly shaped, have fine to coarsely condensed chromatin, and multiple, prominent misshapen nucleoli. There frequently are multinucleated cells and there typically is marked anisokaryosis and anisocytosis. Erythrophagocytosis may be present. Immunophenotyping by flow cytometry or immunohistochemical staining may be helpful for a definitive diagnosis.
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