

Cytology and the Diagnosis of Neoplasia

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KEY WORDS

- cytology
- neoplasia
- fine-needle aspiration biopsy
- impression smear
- malignancy

Cytology refers to the microscopic evaluation of cells. Cytologic evaluation can be very useful in the clinical diagnosis of neoplasia. Samples for cytology can be collected from a wide variety of sites and many different tissues. Sample collection is relatively noninvasive, and most samples can be collected on an outpatient basis. Both sample collection and specimen preparation can be performed using inexpensive equipment that is readily available in most veterinary practices. In-house interpretations can be made the same day, and interpretations from reference laboratories frequently are available within 24 hours. Complications of sample collection for cytologic evaluation are uncommon and usually are limited to minor hemorrhage. Infection, injury to adjacent structures, and dissemination of neoplastic cells are extremely uncommon.

Although cytology should be viewed as a screening tool, many reactions can be classified as inflammatory, hyperplastic, or neoplastic. For neoplastic processes, an experienced cytologist can definitively diagnose several specific neoplasms, make a tentative diagnosis of neoplasia for many types of tumors, identify sites of tumor metastasis, and monitor tumor regrowth following anticancer therapy. Information gained from cytology may be useful in establishing a diagnosis, determining a prognosis, and formulating a diagnostic or therapeutic plan. The most significant disadvantage of cytology is the absence of tissue architecture. The arrangement of neoplastic cells within tissues is critical in determining the diagnosis of many types of tumors, in evaluating surgical margins, and in establishing whether a tumor is benign or malignant.¹

SAMPLE COLLECTION AND SPECIMEN PREPARATION

Choosing an area from which to collect a sample for cytology and the method of collection depend on what abnormality is detected clinically. Neoplastic processes can result in discrete tumor masses, invade normal parenchyma and cause organ enlargement, induce inflammation or hemorrhage, or result in abnormal secretions or effusions. There are numerous references describing sample collection techniques from a wide variety of tissues, and specific techniques are not discussed in this article.

In general, samples for cytologic evaluation are collected by fine-needle aspiration, touch impression, or gentle tissue scraping. Superficial cutaneous and subcutaneous nodules and readily accessible tissues such as peripheral lymph nodes are easily sampled by fine-needle aspiration. Ulcerated masses can be sampled by impression smears, fine-needle aspiration, or gentle tissue scraping. Ultrasound is useful for guiding needle aspiration biopsies of focal or diffuse neoplastic infiltrations involving internal organs; using ultrasound increases the likelihood of obtaining a diagnostic sample and decreases the risk of complications. Specimen preparation and submission are addressed elsewhere in these Proceedings (see pp. 7–9).

CYTOLOGIC INTERPRETATION

Interpretation of cytologic specimens requires knowledge of normal cellular and tissue morphology, recognition of the limitations of cytology, and experience. To avoid unrealistic expectations and overinterpretation, cytologic findings should be correlated with other clinical and laboratory findings and with information about tumor incidence, site predilection, and gross morphology. For specimens submitted to a reference laboratory for interpretation, it is useful for the cytologist to know signalment, a brief history, relevant physical examination findings, previous therapy, a summary of results of pertinent diagnostic tests, the site from which the sample was collected, and the clinician's tentative diagnosis.

Evaluation of stained cytologic preparations requires a routine pattern that includes gross and microscopic examination. The cytologic smear or imprint should be observed grossly to evaluate the quality of the preparation and to locate cellular areas on the slide to be examined microscopically. The slide is then evaluated with the 10× objective to estimate cellulari-

ty of the sample, observe cell-to-cell associations, tentatively identify cell types, and locate areas to be examined with higher magnification. Scanning the entire slide with the 10× objective is important as many cytologic samples have cells that are distributed on only a small part of the slide.²

The 100× (oil immersion) objective is used to examine cellular detail. Changes in nuclear and cytoplasmic morphologic characteristics of neoplastic cells are best evaluated at this higher magnification. Only intact cells should be examined and interpreted. Disrupted cells reveal artifactually enlarged nuclei, pale-staining diffuse chromatin, and nucleolar prominence, all of which may be misinterpreted as cytologic characteristics of neoplastic cells.²

Inflammation and Hyperplasia

Many tumors elicit an inflammatory response that may mask the presence of neoplastic cells. Inflammation is characterized by a mixed population of cells including neutrophils, lymphocytes, plasma cells, eosinophils, monocytes, and macrophages.³ The presence of inflammation may be problematic in the cytologic diagnosis of neoplasia because of the small amount of tissue evaluated and the lack of architecture to define the demarcation between inflammation and neoplasia.

Inflammatory reactions often result in hyperplasia of surrounding tissues. Cells from hyperplastic tissue resemble normal cells except they appear more immature. Cytologic characteristics of hyperplastic cells include large nuclei with poorly condensed chromatin and prominent nucleoli. Cytoplasm often is basophilic. Hyperplastic cells have a relatively constant nuclear-to-cytoplasmic (N:C) ratio (nuclear size compared with the amount of cytoplasm present), an important feature in distinguishing hyperplastic from neoplastic cells.³ The distinction between hyperplasia and benign neoplasia often must be made histologically.

Cytologic Features of Neoplasia

Neoplasia usually is recognized cytologically by the presence of cells that are neither inflammatory nor normally expected from the site of collection.⁴ For example, the presence of squamous epithelial cells in a lymph node aspirate is highly suggestive of metastatic neoplasia. Because neoplasms are clonal expansions of a particular type of cell, cells from a specific tumor often appear similar cytologically. This is described as a uniform or monomorphic population of cells, even though the cells may be morphologically dissimilar. The cytologic features of neoplastic cells vary considerably with cell type. In general, neoplastic cells are

CYTOLOGIC FEATURES OF MALIGNANT CELLS

Cellular Features

- Pleomorphism
- Anisocytosis
- Macrocytosis
- Variation in nuclear to cytoplasmic ratio
- Increased nuclear to cytoplasmic ratio (except lymphoid cells)
- Variation in stages of differentiation

Nuclear Features

- Anisokaryosis
- Macronuclei
- Multinuclearity with abnormal nuclei
- Abnormal mitotic figures
- Finely dispersed or coarsely clumped chromatin
- Thickened, angular, or indented nuclear membrane
- Nuclear molding
- Macronucleoli
- Multiple nucleoli
- Irregularly shaped nucleoli
- Anisonucleoliosis

Cytoplasmic Features

- Increased basophilia
- Abnormal vacuoles or granules
- Phagocytosis of other cells

large and pleomorphic in size and shape when compared with normal cells of the same type (see box above).

Benign neoplastic lesions yield cells that are relatively uniform in size and appearance. Nuclei have a similar chromatin pattern, and nucleoli usually are small and regular in outline or inconspicuous.¹ Rarely do nuclei of benign cells exceed two to three times the size of homologous red blood cells in diameter. There is minimal variation in N:C ratio in cells from most benign tumors. The cytoplasm from benign neoplastic cells appears to be at a similar stage of differentiation.¹

In contrast to benign neoplastic lesions, cells from most malignant tumors are very pleomorphic in appearance. Although the degree of pleomorphism may directly correlate with clinical behavior for some tumors, there are exceptions. For example, cells from some tumors that behave in a relatively benign manner may appear very pleomorphic (e.g., histiocytoma) and cells from some clinically aggressive malignant

tumors are not very pleomorphic (e.g., canine thyroid carcinomas).

Malignant cells often exhibit moderate to marked variation in cell size, which is referred to as *anisocytosis*. Cells from malignant tumors usually are macrocytic compared to nonneoplastic cells of the same type. The N:C ratio often varies markedly from cell to cell. For most tumors, the N:C ratio increases with malignant transformation. Lymphoid cells, however, normally have a high N:C ratio. The N:C ratio appears decreased in many lymphoid neoplasms, compared to normal lymphocytes.⁵ Malignant cells from some tumors appear to be at different stages of differentiation and may exhibit asynchrony between nuclear and cytoplasmic maturation. For example, some cells from a squamous cell carcinoma may appear to be very immature, some may appear relatively mature, some may appear to have a mature nucleus with immature cytoplasm, and some may appear to have an immature nucleus with mature cytoplasm.

Nuclear changes are of primary importance in determining malignancy of a neoplasm. In the absence of inflammation or other causes of dysplasia, the presence of more than three nuclear criteria of malignancy in the majority of the cells is considered highly suggestive of malignant neoplasia.^{6,7} Malignant cells may have nuclei as large as 10 to 12 microns in diameter (macronuclei), which is about the size of a neutrophil. There may be variation in nuclear size (anisokaryosis) among malignant cells. Multinuclearity is not a criterion of malignancy unless the nuclei within the cell exhibit nuclear criteria of malignancy.⁸ Nuclei that vary in size within the same cell and cells that have odd numbers of nuclei are indications of malignancy. Frequent normal mitotic figures do not necessarily indicate neoplasia.⁴ However, abnormal mitotic figures usually are associated with malignant neoplasms.^{3,4}

Nuclear chromatin patterns vary, depending on the type of tumor. Chromatin may appear less clumped, as in neoplastic lymphocytes, or may be coarsely clumped and unevenly distributed, as in some carcinomas.⁶ The nuclear membrane may appear irregularly thickened, angular, or indented. Some malignant cells show nuclear molding around adjacent nuclei or cytoplasmic vacuoles.^{1,8} Nucleoli may be large (macronucleoli) in malignant cells. Nucleoli greater than 5 microns in diameter (red blood cells are 5 to 7 microns in diameter) are highly suggestive of malignancy. Nucleoli also may be irregularly shaped, and more than one nucleolus per cell may be present. Anisonucleoliosis (variation in nucleolar size) occurs relatively frequently in malignant cells.

Cytoplasmic changes in neoplastic cells may aid in

determining the degree of differentiation but are not as useful as nuclear criteria for distinguishing between benign and malignant cells. The cytoplasm of neoplastic cells may be more basophilic than the cytoplasm of the normal cell counterpart. Some neoplastic cells may have abnormal cytoplasmic vacuolization and/or granulation.^{4,9}

NEOPLASTIC EFFUSIONS

Recognition of neoplastic cells in body cavity effusions depends on the cytologist's ability to identify an abnormal cell type and to recognize the criteria of malignancy. This frequently is difficult because neoplasms involving the thoracic or abdominal cavity can cause inflammation and mesothelial cell hyperplasia. Reactive mesothelial cells may closely resemble cells from malignant neoplasms and are characterized by increased basophilia, variation in cell size, binucleation or multinucleation, and frequent mitoses.¹⁰⁻¹² Reactive mesothelial cells may occur in large clusters, which also is characteristic of some malignant neoplasms. Unfortunately, there are no morphologic criteria that clearly distinguish between reactive mesothelial cells and cells from malignant neoplasms.

Mediastinal lymphoma is a common cause of neoplastic effusion in small animals, especially in cats.⁴ Effusions from animals with mediastinal lymphoma often contain large numbers of neoplastic lymphocytes, which most frequently are larger than normal, mature lymphocytes. The nucleus usually is round but may be indented or irregular in shape. There is a fine chromatin pattern, and one to several nucleoli may be present. The cytoplasm is variable in amount and staining intensity.¹²

In addition to mediastinal lymphoma, the most commonly diagnosed tumors in small animals involving pleural and peritoneal cavities are adenocarcinoma (mammary, bronchiolar, thyroid, pancreatic, sweat gland), transitional cell carcinoma, and squamous cell carcinoma. Neoplastic cells from mast cell tumors and mesotheliomas also can be seen in body cavity effusions.^{4,10,13} These tumors often exfoliate readily, and numerous neoplastic cells are evident cytologically. However, in many tumor-induced effusions, neoplastic cells are not detected by cytologic examination.^{10,14} Tumors that may cause neoplastic effusions but usually exfoliate poorly include hemangiosarcoma, melanoma, chemodectoma, and pheochromocytoma.¹⁰

Pericardial effusions also can be evaluated cytologically. Although neoplasia is a relatively common cause of pericardial effusion in dogs, cytologic evaluation of pericardial fluid has been shown to be unreli-

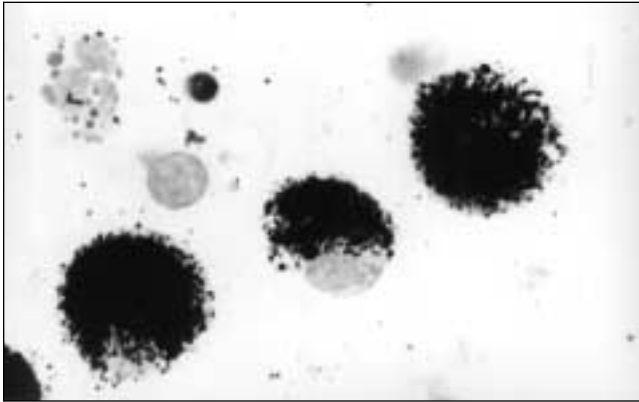


Figure 1. Aspirate from a mast cell tumor. Three mast cells and one eosinophil are present. (Wright's-Giemsa stain, $\times 1000$).

able in distinguishing neoplastic from nonneoplastic pericardial effusions.¹⁵

SKIN TUMORS

Tumors involving the skin and subcutaneous tissue occur frequently in dogs and cats. Samples from cutaneous and subcutaneous masses are obtained easily for cytologic evaluation and can be used to differentiate between neoplastic and nonneoplastic processes. Cytologic evaluation resulted in the correct diagnosis for 74% of skin and subcutaneous tumors.¹⁶ The cytologic appearance of some of the more common skin tumors in dogs and cats is presented below.

Round Cell Tumors

Several skin tumors can be definitively diagnosed with confidence using cytology. Most of these are referred to as *round cell tumors* or *discrete cell tumors* because of their characteristic appearance both cytologically and histologically.² Cells from round cell tumors microscopically are round and have well-defined cytoplasmic margins. These cells do not have cell-to-cell attachments and therefore appear as separate cells. Most exfoliate well when sampled by fine-needle aspiration or impression.^{6,9} Round cell tumors include mast cell tumors, lymphosarcoma, plasmacytomas, histiocytomas, and transmissible venereal tumors. Cells from basal cell tumors and melanomas also may appear as discrete round cells cytologically, but histologically these tumors are not considered to be round cell tumors.⁷

Mast Cell Tumor

Mast cell tumors are common skin tumors in dogs¹⁷; in cats these tumors more commonly involve internal organs but also can occur as cutaneous masses. Cytologic evaluation is an accurate method for the diagnosis of mast cell tumors.⁴ In fact, some are more readily

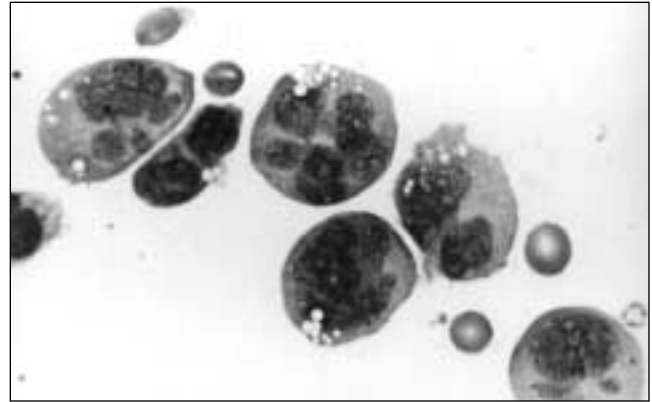


Figure 2. Neoplastic lymphocytes with convoluted nuclei typical of some cutaneous lymphomas. (Wright's-Giemsa stain, $\times 1000$).

diagnosed cytologically than histologically.¹⁸ Aspirates from mast cell tumors usually are very cellular. Mast cells predominate and are usually round to oval in shape; numbers of eosinophils and/or fibroblasts are variable.^{3,4,18} The round to oval nucleus has aggregated chromatin but may stain poorly or may be obscured by the presence of distinct cytoplasmic granules (Figure 1).¹⁸ These granules are round or oval in shape and fine to coarse in size and are the striking feature that allows the specific identification of mast cells. With most Wright's-Giemsa stains, the granules are blue-black to purple-red. Diff-Quik^{®a} may stain the granules poorly or not at all.

Canine mast cell tumors can be graded according to their degree of granulation and cellular anaplasia. Well-differentiated mast cells contain numerous granules, whereas poorly differentiated ones contain few granules. Although dogs with anaplastic mast cell tumors have significantly shorter survival times than those with more well-differentiated tumors, these tumors are highly unpredictable. Even well-differentiated mast cell tumors can be widely metastatic.⁶ The primary skin mass may have well-differentiated mast cells, whereas metastatic sites in the same animal may contain poorly differentiated tumor cells.¹⁹ Mast cell tumors must be differentiated from inflammatory processes containing mast cells. The presence of other inflammatory cells such as neutrophils and macrophages usually makes this distinction relatively easy, but histologic evaluation may be required in some cases.

Lymphoma

Primary cutaneous lymphoma in dogs and cats is rare. Most cutaneous lymphomas typically exfoliate cells well and consist of a uniform population of poorly differentiated lymphoid cells.^{7,20} Cytologically,

^aAmerican Scientific.

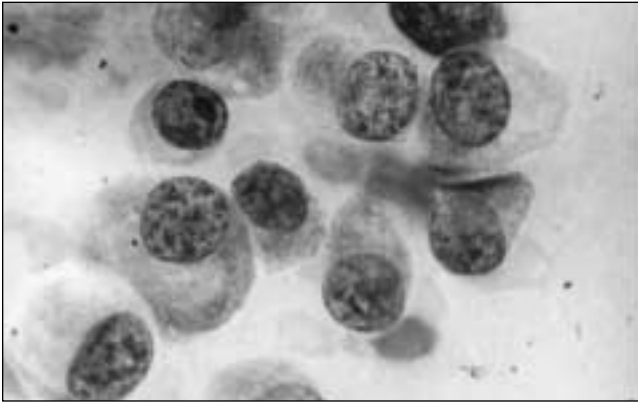


Figure 3. Aspirate from a plasma cell tumor. Note the perinuclear clear area in some cells. (Wright's-Giemsa stain, $\times 1000$).

these cells are larger than neutrophils and have a moderate amount of blue-staining cytoplasm. The nucleus is round or irregularly shaped and has a finely stippled chromatin pattern. One to several nucleoli may be present. Nuclei may be strikingly convoluted in some cutaneous lymphomas (Figure 2). Cutaneous lymphoma composed of small lymphocytes occasionally may be present. These lymphocytes are smaller than neutrophils and cannot be distinguished cytologically from hyperplastic lymphoid tissue. Although cytology is useful in tentatively diagnosing cutaneous lymphoma, histologic confirmation is recommended.

Cutaneous Plasmacytoma

Cutaneous plasmacytomas are benign neoplasms that should be included in the differential diagnosis of cutaneous round cell tumors. These tumors occur at solitary sites and most commonly involve the skin of digits, lips, and ears in dogs. Cells from cutaneous plasmacytomas are round to oval and have predominantly round nuclei with coarsely clumped chromatin and a single, small nucleolus. There is a variable amount of amphophilic to pale basophilic cytoplasm. A perinuclear clear area representing the Golgi zone is typical of plasma cells (Figure 3). Mitotic activity is low to moderate, and there may be binucleate and multinucleate cells.²¹

Histiocytoma

Benign histiocytomas are very common tumors of the dermis and subcutis of young dogs.¹⁷ Cytologic samples from histiocytomas contain a uniform population of round, oval, or irregularly shaped cells morphologically resembling monocytes or epithelioid cells.^{3,18} The eccentric nuclei of these cells are variable in size and shape.¹² The chromatin is dispersed and nucleoli usually are not prominent (Figure 4).³ There is a moderate amount of pale blue-staining cytoplasm

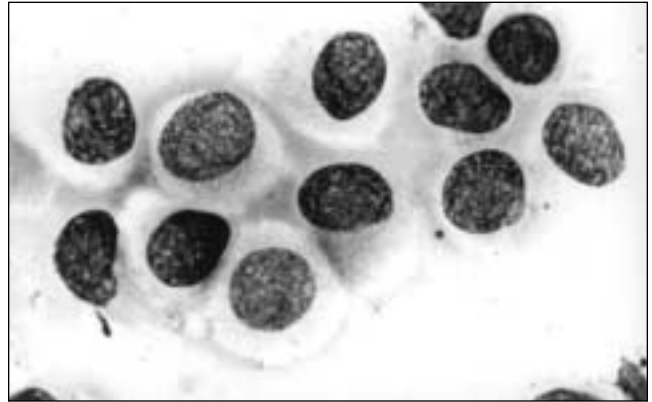


Figure 4. Aspirate from a histiocytoma. (Wright's-Giemsa stain, $\times 1000$).



Figure 5. Aspirate from a transmissible venereal tumor. (Wright's-Giemsa stain, $\times 1000$).

with distinct borders that may appear scalloped.⁹ Histiocytomas may be infiltrated with lymphocytes and plasma cells. Histiocytomas also may be ulcerated and secondarily inflamed; in these cases the cytology resembles chronic inflammation and the definitive diagnosis may require histopathology.³ Differentiation among histiocytoma and large cell lymphoma, histiocytic lymphoma, transmissible venereal tumor, basal cell tumor, and anaplastic, agranular mast cell tumor also may be difficult without histologic evaluation.^{3,6,18}

Transmissible Venereal Tumor

Transmissible venereal tumor (TVT) is a discrete cell tumor of dogs. Such tumors most commonly involve mucous membranes of the external genitalia, but extragenital tumors also occur.¹⁷ Cytologic samples from TVTs usually are very cellular and contain round to oval cells with round or oval nuclei.⁶ The nucleus often is eccentric and varies in size. The chromatin is coarsely granular, and there is usually a single prominent nucleolus.^{4,9} TVT cells have abundant cytoplasm with distinct cytoplasmic borders.³ The cy-

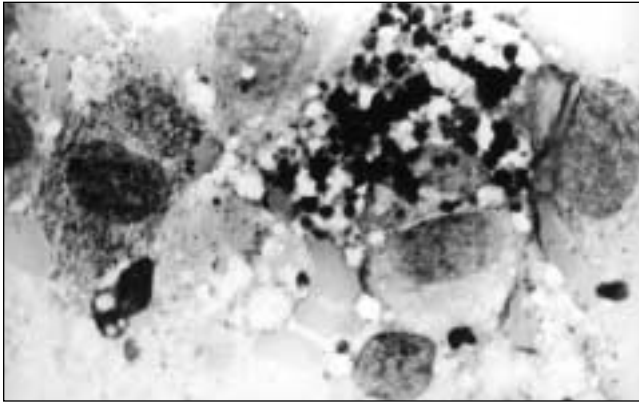


Figure 6. Aspirate from a malignant melanoma. A melanocyte with fine pigment granules and a melanophage with coarse pigment granules are present. (Wright's-Giemsa stain, $\times 1000$).

toplastm may appear pale blue or moderately basophilic and often contains distinct vacuoles (Figure 5). Cytologic samples from TVTs also may contain plasma cells, lymphocytes, and macrophages.^{9,18} Although the unique location of most TVTs is helpful in cytologic interpretation, definitive differentiation of TVT from lymphoma and histiocytoma may require histologic evaluation. TVT cells usually have more abundant cytoplasm than most neoplastic lymphoid cells and are more uniformly round in nuclear and cellular shape than cells from a histiocytoma.³

Melanoma

Melanomas are relatively common in dogs and rare in cats. Melanomas often are not classified as discrete cell tumors as described above nor do they fit neatly with the epithelial and mesenchymal tumors described below. Melanomas are of neuroectodermal origin, and the cells may have characteristics of both mesenchymal and epithelial cells in the same tumor.⁶ Most cutaneous melanomas are benign whereas oral and distal extremity melanomas most often are malignant.²²

Melanocytes produce melanin pigment, which cytologically appears as brown to greenish black cytoplasmic granules that are very fine and of uniform size. Melanin granules often are more obvious cytologically than histologically, and their presence is the most important criterion used to identify melanocytes. Melanomas may be very sparsely or heavily pigmented.³ If the tumor is heavily pigmented, the mass appears dark grossly and is easily recognized cytologically. The sample may appear brown even on unstained slides. In general the less pigment, the more anaplastic the cells appear cytologically.⁶ However, the amount of melanin present does not necessarily indicate the degree of malignancy.¹⁷ Poorly pigmented

melanomas may be difficult to recognize cytologically. In most cases, careful observation reveals a few pigmented melanocytes. If no melanin granules are found, melanomas are difficult to distinguish from other mesenchymal tumors cytologically.^{6,9}

Cytologically, cells from melanomas usually occur singly or in small clusters.^{4,9,19} Both round and spindle-shaped cells often are found in the same neoplasm. Animals with melanomas in which the predominant cell type is round have shorter survival times than those with melanomas of predominantly spindle-shaped cells.²³ Nuclei in melanocytes are round to oval. There is a moderate amount of cytoplasm with ill-defined cytoplasmic borders.⁹ Melanocytes from a melanoma must be differentiated from pigmented macrophages. Macrophages may contain large coarse granules of hemosiderin or melanin and, when compared with melanocytes, usually are larger and have vacuolated cytoplasm. The hemosiderin and melanin pigment granules in macrophages are more coarse and variably sized than those in melanocytes (Figure 6).⁶

In contrast to the discrete cell tumors and melanomas described above, most other neoplasms require histopathology for definitive diagnosis. However, cytologic evaluation often can distinguish neoplasia from inflammation and may be useful in differentiating neoplasia from hyperplasia. If the cytologic diagnosis is neoplasia and the cells do not resemble those from a discrete cell neoplasm or melanoma, there are some general criteria (discussed below) to tentatively diagnose the tumor as epithelial or mesenchymal in origin.

Epithelial Tumors of the Skin

There are several characteristics of epithelial tumors in general that are useful cytologically in tentatively diagnosing the cell of origin. Epithelial tumors exfoliate relatively easily. There may be organized groups of cells and evidence of cell-to-cell adherence. Individual epithelial cells are round or polyhedral and have abundant cytoplasm with well-defined cytoplasmic borders. The cytoplasm of epithelial cells differs greatly among tumor types with respect to amount, color, granularity, and vacuolation.⁹ Many epithelial tumors involving the skin are of low grade cytologic malignancy, making the cytologic distinction between benign and malignant neoplasms difficult. Tumors with many characteristics of malignancy can be called malignant; however, if few malignant characteristics are present, the confirmation that the tumor is benign must be made histopathologically.⁶

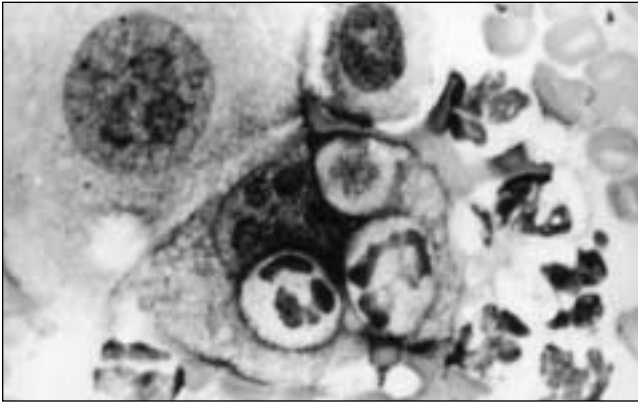


Figure 7. Aspirate from a squamous cell carcinoma. Two large neoplastic squamous epithelial cells are present. The neutrophils are typical of the inflammatory response that often accompanies squamous cell carcinomas. (Wright's-Giemsa stain, $\times 1000$).

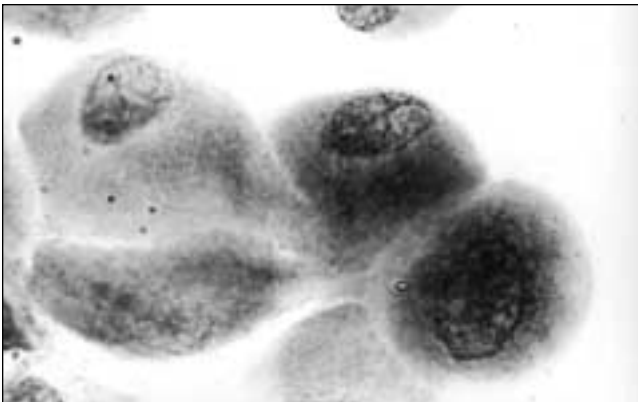


Figure 8. Aspirate from a basal cell tumor. (Wright's-Giemsa stain, $\times 1000$).

Squamous Cell Carcinoma

Squamous cell carcinomas usually exfoliate well. Those that are poorly differentiated are characterized by large, anaplastic cells with giant, polymorphous nuclei. The cytoplasmic borders may appear angular due to keratin production (Figure 7). Small, keratinized squamous cells and keratin debris usually are present and aid in the diagnosis of squamous cell carcinoma.⁶ Keratin may induce a chronic inflammatory reaction. Chronic inflammation often is associated with epithelial cell dysplasia, which may be difficult to differentiate cytologically from a well-differentiated squamous cell carcinoma.² The definitive diagnosis of squamous cell carcinoma should be made histologically.

Basal Cell Tumor

Basal cell tumors arise from multipotential germinal epidermal cells and are common in the skin of cats and dogs.^{6,17} The tumors may be pigmented, especially in cats, and often contain cystic spaces.⁶ Basal cell tumors usually are benign, but the biologic behavior is difficult

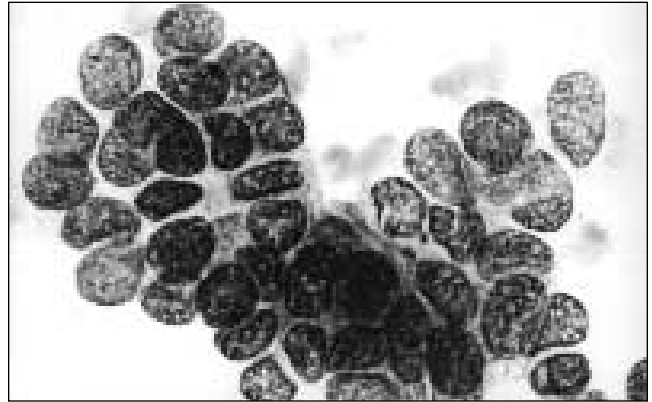


Figure 9. Aspirate from a perianal gland adenoma. (Wright's-Giemsa stain, $\times 1000$).

to predict either cytologically or histologically. Aspirates from these tumors often show cords or ribbons of small, uniform epithelial cells. In some samples there may be only individual cells. The cells have a high N:C ratio with scant basophilic cytoplasm (Figure 8). The cells are not distinctive and appear benign. Histologic confirmation is recommended because similar-appearing basilar reserve cells can be seen cytologically with other cutaneous tumors.⁹ Pigmented basal cell tumors contain melanocytes and may be difficult to distinguish cytologically from melanomas.⁶

Sebaceous Gland Tumor

Sebaceous gland tumors occur commonly in old dogs,¹⁷ and most are benign. Cells from sebaceous gland tumors often exfoliate in clumps, so it may be difficult to distinguish individual cells cytologically. The cells have small, centrally located nuclei and abundant foamy cytoplasm. There are occasional reserve cells that appear smaller and have more basophilic cytoplasm and a higher N:C ratio.⁹ Sebaceous gland adenocarcinomas cytologically appear as clusters of highly basophilic reserve cells with malignant characteristics. There are occasional cells with cytoplasmic secretory material, which sometimes may be quite abundant and displace the nucleus to the periphery of the cell (signet ring cell).⁶

Perianal Gland Tumor

Perianal gland tumors, most of which are adenomas, are common in intact, aged male dogs.¹⁶ The differentiation of nodular hyperplasia of the perianal glands from adenoma is difficult cytologically or histologically.⁶ Cells from perianal gland tumors exfoliate readily and appear as variably sized clusters or individual cells that are polygonal in shape. The cells have round, central nuclei that frequently contain nu-

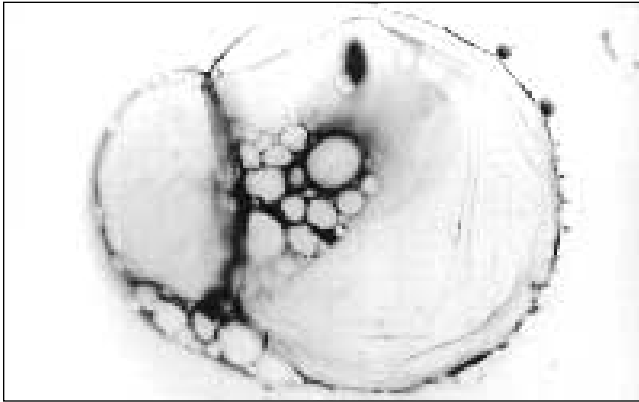


Figure 10. Aspirate from a lipoma. Two adipocytes are present. (Wright's-Giemsa stain, $\times 1000$).

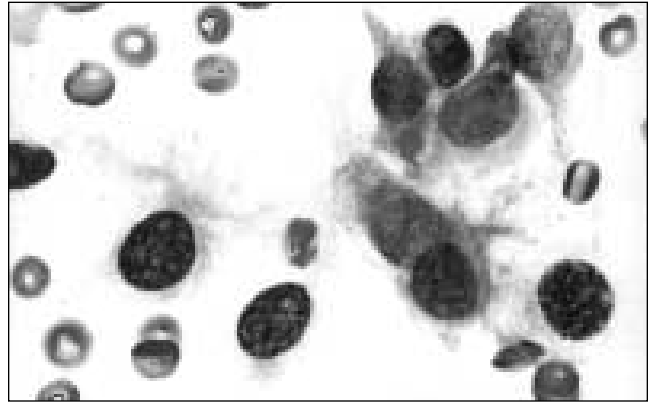


Figure 11. Aspirate from a hemangiopericytoma. (Wright's-Giemsa stain, $\times 1000$).

cleoli. There is abundant pinkish-blue cytoplasm that is distinctly granular (Figure 9). The cells resemble hepatocytes, and these tumors often are referred to as hepatoid gland tumors. Perianal adenocarcinomas are rare. Cells from perianal adenocarcinomas usually exhibit variation in nuclear size and number and size of nucleoli; well-differentiated perianal adenocarcinomas are difficult to differentiate from adenomas cytologically or histologically.⁶

Mesenchymal Tumors of the Skin

Cytologic characteristics of mesenchymal tumors are different from those described for epithelial neoplasms. In general, mesenchymal tumors exfoliate poorly because the cells are embedded in extracellular matrix such as fibrous connective tissue, cartilage, or bone. Cytologically, the cells present are individual cells or unorganized clusters. Cells from most mesenchymal tumors are spindle shaped, with cytoplasmic extensions and ill-defined cytoplasmic borders. Nuclei may be fusiform or oval.¹ Even if the cell of origin cannot be determined cytologically, a tentative diagnosis of mesenchymal tumor usually can be made. Benign or well-differentiated malignant mesenchymal tumors may resemble actively proliferating granulation tissue.⁶ Definitive diagnosis and determination of biologic behavior of most mesenchymal tumors require histologic confirmation.

Lipomas

Lipomas occur commonly in the subcutaneous tissues of dogs and cats. Grossly, aspirates from lipomas appear oily and smears do not dry on microscope slides.¹ The cells often rupture, and the alcohol fixative used in some stains dissolves the fat; thus the slide often appears acellular cytologically. Intact cells from lipomas cannot be distinguished from normal

adipocytes cytologically. Adipocytes are very large cells that are distended with fat (Figure 10). Slides from lipomatous tumors should always be examined microscopically because many malignant tumors invade subcutaneous fat and neoplastic cells can be detected cytologically.⁶

Liposarcomas occur rarely.¹⁷ Aspirates from liposarcomas usually contain numerous cells. These cells have abundant cytoplasm histologically, but the cell membrane may be difficult to appreciate cytologically. Usually there are some cells that contain cytoplasmic vacuoles, which vary in size and number. Often there are fat vacuoles in the background.⁶ The diagnosis of lipomas frequently is made cytologically.

Hemangiopericytoma

Hemangiopericytoma is a tumor of questionable origin that may arise from cells around blood vessels.¹⁷ The tumor most commonly occurs on the extremities of older dogs. Cells exfoliate relatively well compared with other mesenchymal tumors. Usually there are individual cells with round, uniform nuclei. The cytoplasm varies in amount and cytoplasmic membranes are indistinct (Figure 11). In thick areas of the smear, there may be whorls of cells, which represent the characteristic swirling pattern seen histologically. There may be occasional multinucleate cells.⁶ Cytologically, it may be difficult to distinguish hemangiopericytomas from fibrosarcomas, and the diagnosis should be confirmed histologically.

LYMPHOID NEOPLASIA

Lymphadenopathy may be associated with lymphoid hyperplasia, inflammation, or hematopoietic or metastatic neoplasia. Fine-needle aspiration cytology of enlarged lymph nodes often can provide a quick diagnosis with minimum inconvenience and expense.

With experience, the cytologic diagnosis of lymphadenopathy, including that caused by lymphoid neoplasia, can be highly accurate.^{16,24,25} Knowledge of history and physical examination findings is useful in the cytologic evaluation of lymphoid tissue, and it is important for the clinician to communicate this information to the cytologist. For example, immunization may cause diffuse paracortical hyperplasia to the extent that the lymphocytes may appear neoplastic.²⁶ A dog with multiple peripheral lymph node enlargements is more likely to have lymphoma than a dog with a single enlarged lymph node. Young cats with enlarged peripheral lymph nodes may have nonneoplastic proliferative disease instead of lymphoma.²⁷

Cytology of Normal Lymph Nodes

The cytologist must be familiar with normal lymph node morphology. Normal lymph nodes consist of a heterogeneous population of cells. The predominant cells are mature lymphocytes, which cytologically appear smaller than neutrophils. The nucleus is similar to red blood cells in size.^{28,29} Mature lymphocytes have a very high N:C ratio, coarsely aggregated chromatin, and scant cytoplasm. Nucleoli usually are absent, but there may be one small nucleolus.^{28,29} Prolymphocytes, less mature lymphoid cells, are slightly larger than neutrophils. The nuclear chromatin in prolymphocytes is moderately clumped, and there is a moderate amount of cytoplasm. Mature lymphocytes and prolymphocytes constitute 90% to 95% of the normal lymph node population.⁴ Lymphoblasts are large cells (two to four times the size of mature lymphocytes) with fine nuclear chromatin and one to two nucleoli. Cytoplasm may be scant or abundant. Lymphoblasts represent less than 15% of the cells in normal lymph nodes.^{28,29}

Occasional plasma cells may be present in aspirates from normal lymph nodes. Plasma cells have eccentric nuclei, abundant basophilic cytoplasm, and a perinuclear clear area.²⁸ Inflammatory cells such as neutrophils, macrophages, and mast cells are rare in most normal lymph nodes. However, the cell population in lymphoid tissue may vary according to location. Lymphoid tissue associated with the gastrointestinal tract may appear to have slightly increased numbers of plasma cells and inflammatory cells because of regular antigenic exposure.²⁹

Cytology of Hyperplastic Lymph Nodes

Lymphoid neoplasms must be differentiated from lymphoid hyperplasia. Hyperplastic lymph nodes contain lymphoid cells at all stages of differentiation whereas lymphoid neoplasms usually contain a

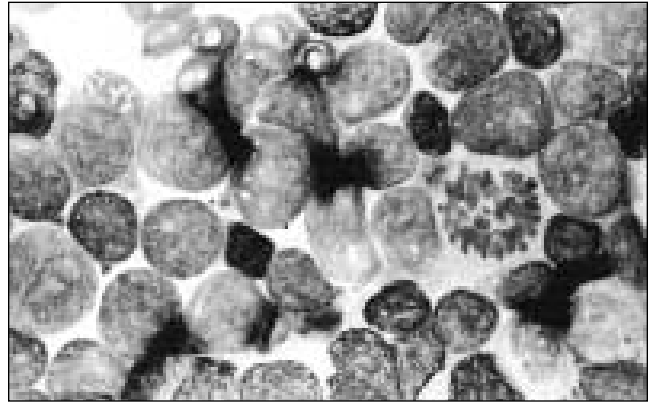


Figure 12. Aspirate from a lymph node from a dog with lymphoma. (Wright's-Giemsa stain, $\times 1000$).

monomorphic population of cells at the same stage of development.³ The mixed lymphoid population in hyperplastic lymph nodes may appear similar to normal nodes but with increased numbers of prolymphocytes and lymphoblasts. There may be increased numbers of plasma cells and macrophages, and an occasional neutrophil may be present. Numbers of mitotic figures may appear increased, but the figures are normal morphologically.^{3,26}

Cytology of Neoplastic Lymph Nodes

Lymphoid neoplasms are relatively common in dogs and cats and can involve multiple tissues including lymph nodes, intestine, thymus, and hematopoietic organs. In lymph nodes, the normal heterogeneous population of mature lymphocytes is replaced by a monomorphic (or occasionally dimorphic) population of lymphoid cells.²⁶ Plasma cells and inflammatory cells are rare. The morphology of the cells varies with the degree of differentiation and type of lymphocyte. Most lymphoid neoplasms involve immature lymphoid cells. Lymphoma involving immature cell types usually can be diagnosed with confidence cytologically. Lymphomas involving morphologically mature lymphocytes occasionally occur and are particularly difficult to diagnose cytologically.^{3,18,26}

The degree of differentiation and the histologic classification of lymphoma may affect response to treatment and prognosis. A classification system of canine lymphomas based on the National Cancer Institute (NCI) Working Formulation of Non-Hodgkin's Lymphomas in humans recently has been described.^{30,31} The histologic classification is based on tissue architecture, cell morphology, and mitotic rate; the cytologic classification is based almost entirely on nuclear characteristics including nuclear size, shape, and chromatin pattern and nucleolar number and size. While cytologic classification is not as reliable as his-

tologic classification, it is sufficient to recognize the common cell types.^{30,31} No classification of feline lymphomas has been published using this system.

The classification system groups tumors into low, intermediate, and high grade lymphomas based on clinical behavior. Low grade tumors are the least aggressive and have the best prognosis, whereas high grade tumors are the most aggressive and have the worst prognosis. The majority (66%) of canine lymphomas are classified as high grade types and include lymphoblastic, immunoblastic, and diffuse small non-cleaved lymphoma. Diffuse large lymphoma has a medium to high mitotic rate and also might be considered aggressive.³⁰ These more aggressive tumors are the most easily identified cytologically (Figure 12).

Metastatic Neoplasia

The presence of nonlymphoid, neoplastic cells in a lymph node indicates metastatic neoplasia. These cells can be identified as malignant using general cytologic criteria for malignancy. The cytoplasmic appearance can be used to determine whether the cells are from a carcinoma, sarcoma, or round cell tumor. Carcinomas are more likely to metastasize to lymph nodes than sarcomas and may be identified by the tendency of cells to remain in aggregates or clusters. With most metastatic neoplasms, the resident lymphoid population is not altered markedly. With metastatic squamous cell carcinoma, however, there may be frequent neutrophils from keratin-induced inflammation.²⁶ In advanced metastases, the entire node may be replaced by tumor cells. Absence of neoplastic cells does not exclude a diagnosis of metastases.

SUMMARY

Most veterinarians can use cytology to differentiate inflammation from neoplasia and thus provide useful information for the direction of further diagnostic testing. The experienced cytologist can definitively diagnose several specific neoplasms and make a tentative diagnosis of neoplasia for many other types of tumors. This information is useful in establishing a prognosis and in directing appropriate therapy. Cytologic findings should be correlated with other clinical and laboratory information. When the cytologic diagnosis of neoplasia is uncertain, the presence of tumor and tumor cell type should be confirmed histopathologically. As technology continues to advance, the cytologic diagnosis of neoplasia will be enhanced by the use of computerized imaging, cytochemical stains for intermediate filaments, immunophenotyping of hematopoi-

etic tumors, and evaluation of oncogenes and chromosomal abnormalities to determine prognosis.

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