

Clinical Cytology of Companion Animals: Part 2. Cytology of subcutaneous swellings, skin tumours and skin lesions

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INTRODUCTION

Subcutaneous swellings, skin tumours, and skin lesions are extremely well suited for cytological examination via FNAB (Fine needle aspiration biopsy). Aspiration can be performed without difficulty, and causes little or no pain except with processes on the feet or the nose, and even in these cases, anaesthesia is seldom required. An impression smear or scraping can easily be made of open lesions, but it is advisable to perform FNAB from the edges of the wound as well. Cytological examination can in many cases lead quickly to the correct diagnosis and be of decisive importance to the choice of therapy and to the prognosis.

The cytological examination of FNAB of skin tumours and subcutaneous swellings often makes histological examination unnecessary. Histological examination costs more time, is more invasive, and more expensive. However, it should be emphasized that follow-up histological examination almost always offers a solution in the event that cytological examination is insufficient, and some processes can only be determined by histological examination.

Cytological examination of skin tumours and to a lesser extent also skin lesions and subcutaneous swellings forms a good start for veterinarians who wish to become familiar with cytology. Many diagnoses are rather easy to make and to verify subsequently via a second opinion by an experienced cytologist or by histological examination.

This paper is organized in a way to help the inexperienced cytologist quickly on the way to the first diagnosis but also to help recognize as such the preparations that are difficult to interpret. The evaluation of the latter can be left to an experienced cytologist. The reader is further advised to consult one of the many cytology books that are available, as in this article only condensed information can be given due to publication limits.

The following advice is offered to the inexperienced cytologist in order to avoid erroneous diagnoses:

1. Examine only cell-rich, well streaked-out preparations.
2. Interpret the cytology as far as possible in relation to the clinical information such as age of the patient, past history, and the macroscopic appearance, rate of growth, and location of the tumour.
3. For the time being have all diagnoses, including those made without difficulty, verified by an experienced cytologist or by histological examination.

It can be difficult to differentiate skin tumours and subcutaneous swellings. Skin tumours can spread subcutaneously and subcutaneous processes can infiltrate the dermis, and both can cause skin lesions. Usually it is possible by inspection and palpation to correctly localize a process. This is important in obtaining a FNAB because the origin can give specific indications of the nature of the process (Table 1).

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Causes of subcutaneous swellings

- Haematoma
- Fat
- Cysts
- Inflammation, abscess
- Mesenchymal tumours
 - lipoma, fibroma, haemangioma, haemangiopericytoma, haemangiosarcoma, (injection-site) fibrosarcoma, liposarcoma, leiomyosarcoma, chondrosarcoma, osteosarcoma
- Malignant lymphoma
- Glandular-epithelial tumours
 - adenomas and adenocarcinomas of sebaceous gland, sweat gland, thyroid, salivary gland, perianal gland, mammary gland, and anal sac

Skin tumours

- Round cell tumours
 - mast cell tumour, melanoma, malignant lymphoma, plasmacytoma, histiocytoma, condyloma
- Epithelial tumours
 - basal cell tumour, squamous cell carcinoma, adenoma and adenocarcinoma

Table 1

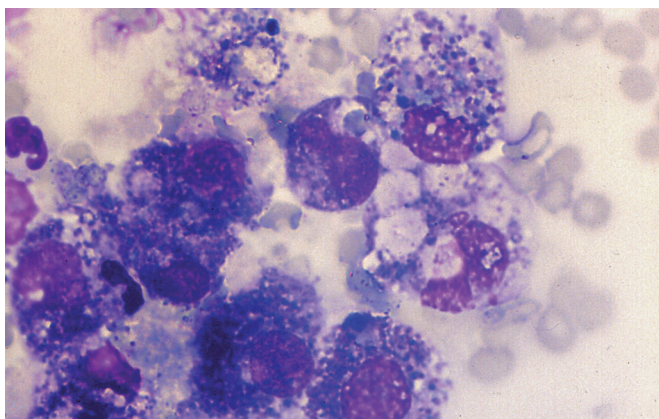
Subcutaneous swellings

Subcutaneous swellings can arise from structures of the subcutis as well as from other tissues located under the skin, such as salivary glands, lymph nodes, thyroid, mammary glands, cartilage, bone, etc. Mammary tumours and inflammations of the mammary glands will not be dealt with in this chapter. Cytology of the mammary gland of the cat and especially of the dog is one of the most difficult areas of clinical cytology in companion animals.

Haematoma

Preparations that contain many erythrocytes are searched

Fig. 1 Haematoma in a dog. Several erythrocytes are in the background, but also phagocytized by macrophages and broken down to dark granular material.



carefully for the presence of other cells. If only a few epithelial cells and/or mesenchymal cells are found and by examination under the 100x objective these are seen to have no malignancy criteria, then i) a blood vessel may have been penetrated, or ii) there may be a tumour of cells of vessel walls (e.g. haemangioma/haemangiosarcoma), or iii) the blood is from a haematoma. The latter can usually be confirmed by the fact that blood from a slightly older haematoma contains no thrombocytes but does contain brown bilirubin crystals and macrophages that show erythrophagocytosis and accumulation of iron pigment. A few inflammatory cells, like neutrophils and lymphocytes, may also be present (Fig. 1).

Fat/lipoma

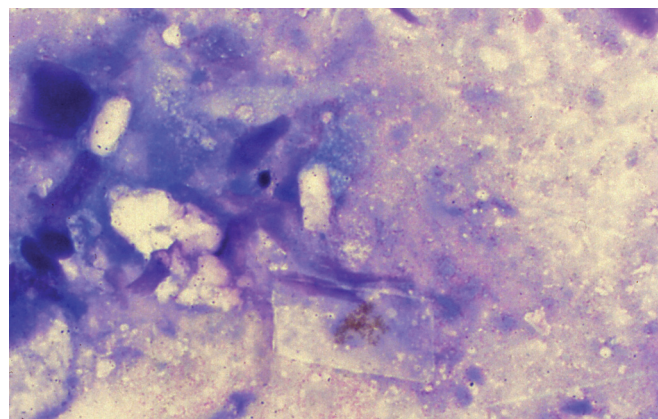
Preparations that before fixing/staining contain a good amount of glassy, fatty material but after staining contain only a vague background with some empty round spots of different sizes probably contained only fat that has been dissolved by the alcohol. Sometimes there are few free pyknotic nuclei of the fat cells and/or intact three-dimensional clusters of empty fat cells. The presence of pure fat indicates an unsuccessful aspiration (subcutaneous fat) or an aspiration from a lipoma. The cells of a lipoma look the same as normal fat cells.

Cysts

Smears that contain large, blue, epithelial cells without nuclei are usually contaminated with flakes of keratin from the skin. If the smear contains almost exclusively large numbers of keratin flakes or amorphous blue or black material, then probably a *dermoid (epidermoid)* cyst has been aspirated (Fig. 2). Such cysts also often contain cholesterol crystals. These can cause secondary inflammation. If in addition to keratin flakes there are also immature nucleated epithelial cells, these should be examined carefully for malignancy criteria.

Preparations can also contain a homogenous pink layer of protein in which there are sporadic epithelial cells and/or mesenchymal cells or intermixed blood cells. This can indicate aspiration from 1) oedematous tissue, 2) a very poorly exfoliating tumour, or 3) a *serous cyst (seroma, hygroma)*. Aspiration from a cyst is usually recognized during aspiration. By microscopic examination one

Fig. 2 Content of an epidermoid cyst. Several keratin flakes are present with their characteristic sky blue colour. A large cholesterol crystal is also present.



also usually finds a few mononuclear cells (macrophages, cyst wall cells) and cholesterol crystals.

Salivary cysts are equally easy to recognize (Fig. 3). The characteristic findings, apart from the location of the cyst and the mucoid consistency of the contents, are the presence of clumps and strings of amorphous blue material (mucus) and many foamy macrophages, which develop very strong phagocytic activity and can contain erythrocytes, bilirubin crystals, and haemosiderin, because there is always some blood in salivary cysts. Because of the high viscosity of the saliva the erythrocytes are often stretched out in rows. Preparations from salivary cysts also often contain a few groups of cells from the salivary gland. These are easy to recognize. The clumps of cells, which have a clearly acinar structure and a great amount of cytoplasm, are often enclosed in dark blue strings of mucus.

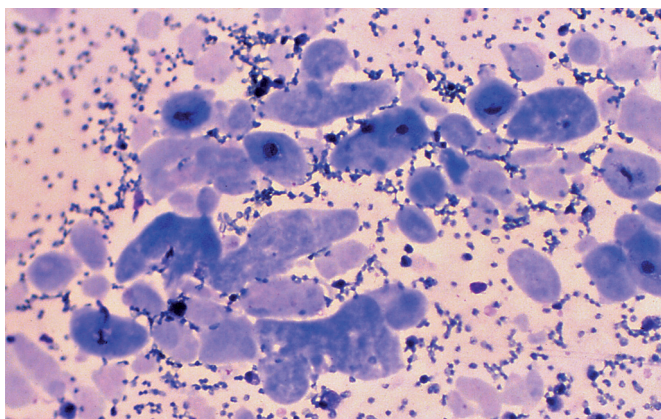
Smears from abscesses have large numbers of neutrophils. A single lymphocyte, plasma cell, or macrophage may also be found. Dependent upon the cause of the abscess the neutrophils may show many degenerative signs. One should always carefully look for intracellular bacteria.

Inflammation

General inflammatory processes have been described in Part I. Some special types of inflammatory processes can be recognized on cytological evaluation:

- *Plasmacytic pododermatitis*
Plasmacytic pododermatitis starts as a soft swelling of one or more footpads. Aspirates demonstrate large numbers of plasma cells. Some lymphocytes and neutrophils may also be present. Most plasma cells are well differentiated.
- *Nodular panniculitis*
In nodular panniculitis sterile subcutaneous inflammation of fat tissue results in the formation of one or more subcutaneous nodules, which may ulcerate. A pyogranulomatous inflammation with non-degenerated neutrophils, foamy macrophages and multinucleated giant cells is characteristic of the disease. A fatty background and lipocytes are present. No microorganisms can be found.
- *Eosinophilic inflammations*

Fig. 3 A salivary cyst with some secondary haemorrhage. Many large clumps of amorphous blue mucus are to be seen, with macrophages on the background and sometimes on top of these clumps.



Biopsy samples contain large numbers of eosinophilic granulocytes. This type of inflammation occurs in the eosinophilic granuloma in the cat, the lick granuloma in the dog, and also in parasitic infections and allergic reactions. Eosinophilic granulocytic infiltrates are also a characteristic finding in many mast cell tumours.

Mesenchymal cells in FNABs

Mesenchymal cells occur in all organs, as connective and supporting tissue and as a component of the blood vessels. They are also the elementary building blocks of many different tissues (connective tissue, cartilage, bone, muscle, vascular wall, adipose tissue). Mesenchymal cells are also almost always involved in repair of damaged organs, in which they can partly replace the original tissues (mesenchymal hyperplasia, scar tissue). During inflammatory processes mesenchymal cells are stimulated to cell division and proliferation to aid in tissue repair. FNABs from inflammatory processes thus also usually contain fibroblasts. These can be so strongly stimulated that they give the impression of being malignant. Hence the cytoplasm can be strongly basophilic staining and there can be remarkable large or multiple nucleoli. Giant cells with more than one nucleus, sometimes varying in size, can also be present. The presence or absence of inflammatory cells is thus of great importance in the interpretation of mesenchymal proliferations.

Sporadic, well-differentiated mesenchymal cells such as fibrocytes are usually from the normal connective tissues that are also aspirated in the FNAB. If fibrocytes are found in large numbers, they can be an indication of a **fibroma**. In this case, there are few inflammatory cells. Malignant mesenchymal tumours can have histological characteristics of the tissue of origin (e.g. **fibrosarcomas, osteosarcomas, liposarcomas, chondrosarcomas, haemangiosarcomas, leiomyosarcomas**). It is not always possible to differentiate the different types of mesenchymal tumours cytologically and it can even be difficult to recognize that a malignant tumour is of mesenchymal origin. It is essential that the diagnosis of malignant tumour is only made if sufficient criteria of malignancy are confirmed (see Part I) and that, if the cells are of uncertain mesenchymal origin, that the proliferation of the "tumour" cannot be attributed to inflammation.

A few special types of mesenchymal tumours will be discussed: Biopsies of **osteosarcomas** are usually reasonable cellular and often show cellular necrosis. Mild to moderate amounts of an eosinophilic, osteoid-like, extracellular substance surrounding the osteoblasts can be seen. Depending on the histological subtype few to moderate amounts of fibroblasts can be found as well. Less frequent osteoclasts are present. The osteoblasts have a pale blue to blue cytoplasm with eccentric nuclei, often with a Golgi like elucidation in the cytoplasm. Slight to moderate eosinophilic granulation of the cytoplasm is a distinct characteristic of malignant osteoblasts (Fig. 4). The tumorous osteoblasts frequently have poor to moderately distinct cell borders. Distinct nucleoli, often more than 2 per nucleus, are present. Chromatin pattern is reticular to clumped. Additional malignancy criteria can be found, like angular nucleoli, anisonucleoliosis, macronucleolisation, nuclear moulding and

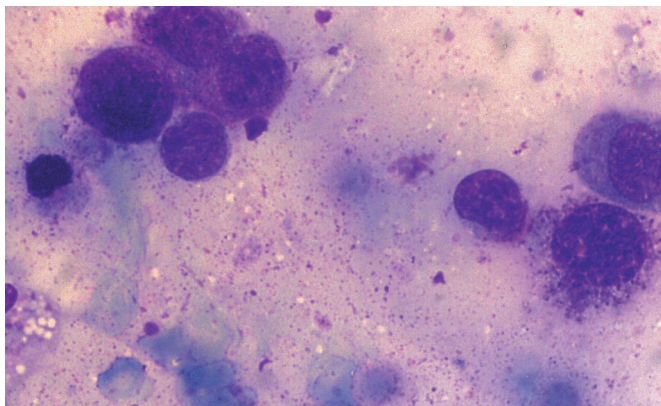


Fig. 4 Aspirate of an osteosarcoma in a dog. Some poorly differentiated osteoblasts are present with in some cells moderate eosinophilic granulation.

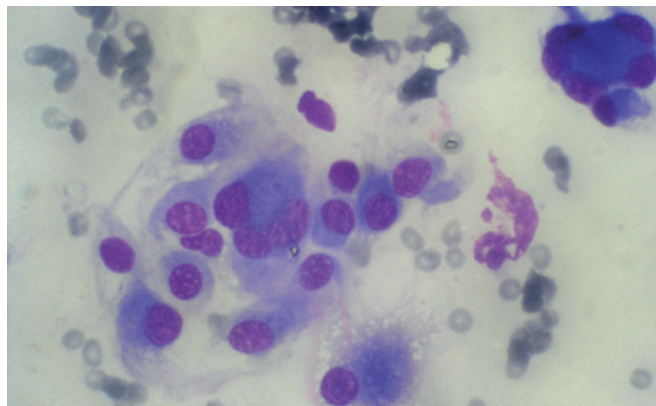


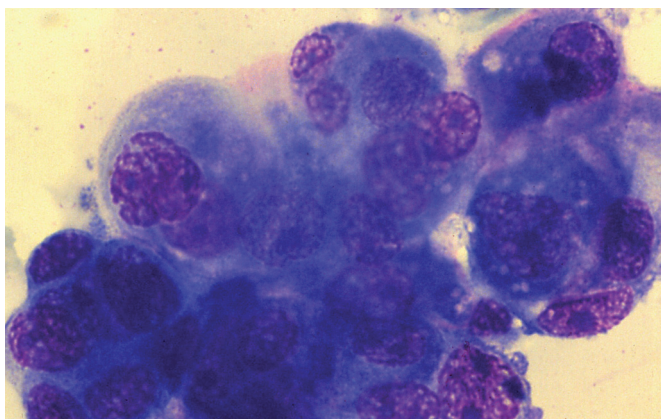
Fig. 5 Hemangiopericytoma. Several individual mesenchymal cells can be seen with whirling like protrusions, round nuclei and sometimes multinucleated cells. On the right top a typical crown-form is present.

aberrant mitoses. Additional staining with alkaline-phosphatase may differentiate between osteosarcoma and other types of sarcomas.

Haemangiopericytomas are vascular neoplasms thought to be derived from pericytes. They are classified within the group of peripheral nerve sheath tumours. Cytologically, they have very distinct characteristics. The cellularity can vary from moderate to abundant. The cells are individual, spindle shape formed and have whirling-like protrusions of the cytoplasm. The nucleus is round and the cells can be binuclear or even multinucleated, forming so-called insect-head or crown like cells, respectively (Fig. 5).

Injection-site sarcomas in the cat are located in the hypodermis and have great cellular pleomorphism and high mitotic rates. Many of these sarcomas are associated with inflammation. The inflammatory reaction is characterized by frequent aggregates of lymphocytes and smaller numbers of plasma cells. Large round macrophages with blue-grey cytoplasm, presumably associated with phagocytised adjuvant material, are commonly found within, around, or adjacent to these sarcomas. Tumours are

Fig. 6 Clusters of epithelial cells from a sweat gland adenocarcinoma. Several malignancy criteria like anisokaryosis, coarse chromatin pattern, abnormal nuclear forms and multiple nucleoli are present.



often contiguous with granulation tissue that surrounds areas of necrosis at the vaccine site. Multinucleated giant cells are a common finding in feline vaccine-associated fibrosarcoma.

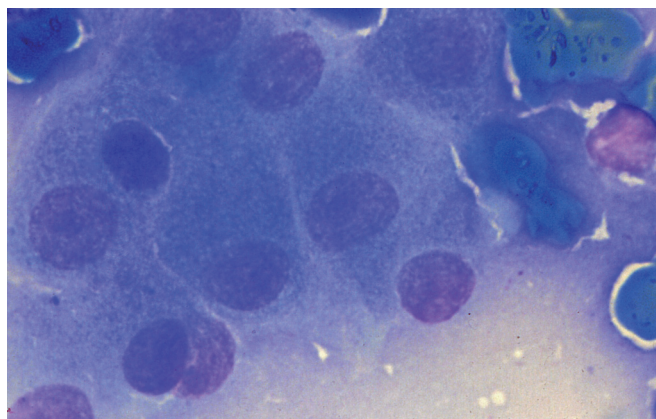
Adenomas and adenocarcinomas

Various glandular structures in and under the skin can give rise to benign tumours (adenomas) or malignant tumours (adenocarcinomas). The endocrine or exocrine gland of origin of many adenomas and adenocarcinomas is difficult to determine but a few tumours carry characteristic features, which reveal their origin.

A general diagnostic feature of a glandular epithelial tumour is of course the occurrence of tumour cells in clusters, as is true of all epithelial tumours. More specifically in cell clusters of glandular origin, an acinar structure can still be recognized. In very malignant carcinomas, however, this is lost.

Adenomas scarcely differ cytologically from normal gland tissue. The round to cuboid cells show little variation in cell size, nuclear size, and N/C ratio. The macroscopic appearance of tumour formation in combination with the cytological finding of clusters

Fig. 7 Cluster epithelial cells of a perianal gland tumour. Typical hepatoid cells with abundant somewhat granular/foamy cytoplasm and round nuclei, which sometimes have a large nucleolus.



of uniform epithelial cells which may have a slightly high cell density and slightly chaotic arrangement is consistent with the diagnosis of adenoma.

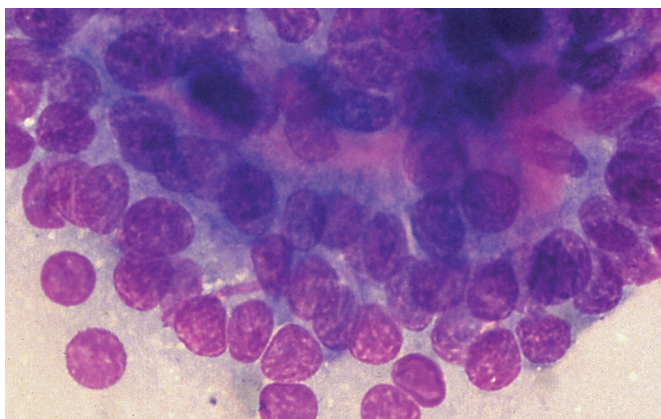
Adenocarcinomas can still show some acinar formation here and there but also have a number of criteria of malignancy (see Part I). In general at least four such criteria should be found for the diagnosis of "malignant" to be made (Fig. 6). In well-differentiated adenocarcinomas, that can sometimes be difficult.

Perianal gland adenoma/carcinoma are tumours of the perianal glands that occur usually in male dogs and less frequent in bitches. They are located in the immediate surroundings of the anus, occasionally on the tail, on the prepuce, in the flank, and on the back. They are usually benign but can become malignant. The tumour is sometimes called a hepatoid tumour because the tumour cells resemble hepatic parenchymal cells. The cells lie mainly in three-dimensional clusters, but on the edge of the clusters the cells can be examined more closely. They are large cells, often egg-shaped, with an eccentric nucleus that contains one or two obvious nucleoli. The cytoplasm is usually somewhat granular/foamy (Fig. 7). Often remnants of vascular structures can be found in the smear. Perianal tumours often ulcerate and can then be infiltrated with inflammatory cells. Sometimes there are malignancy criteria.

Anal sac adenocarcinoma is a malignant tumour that, like the thyroid carcinoma in the dog, does not always show obvious characteristics of being malignant. Very often the biopsy reveals large numbers of monomorphous cells, frequently without any apparent cytoplasm. On careful examination, however, acinic structures can be recognized. Also some anisokaryosis, as one of the few malignancy criteria, is usually present. Morphologically the tumour cells resemble the thyroid adenocarcinomas in the dog.

Mammary tumours are not always recognizable as such without cytological or histological examination. The cytology of mammary tumours, however, is one of the most difficult in veterinary cytology and will not be described in this paper.

Fig. 8 Aspirate of a thyroid gland carcinoma. Groups of, often naked nuclei, in acinic structures are present. The nuclei are round, very uniform, and lacking obvious malignancy criteria.



Thyroid tumours are composed of epithelial cells that are very rich in cytoplasm. The cells exfoliate easily and also rupture quickly. The preparations are often very bloody. If, however, little blood is aspirated, the obvious acinar clusters as well as primarily loose nuclei will be found in an homogenous protein-rich background. The nuclei are about the size of lymphocytes and must not be confused with them. A FNAB from a thyroid contains in any case no lymphoglandular bodies. Here and there is usually an intact cluster of cells with an acinar structure. The nuclei show usually only sporadic malignancy characteristics such as mild anisokaryosis and multiple nucleoli (Fig. 8). In dogs these tumours are nevertheless always considered to be malignant. In cats they are usually benign hyperplasia (adenoma).

Skin tumours and skin lesions

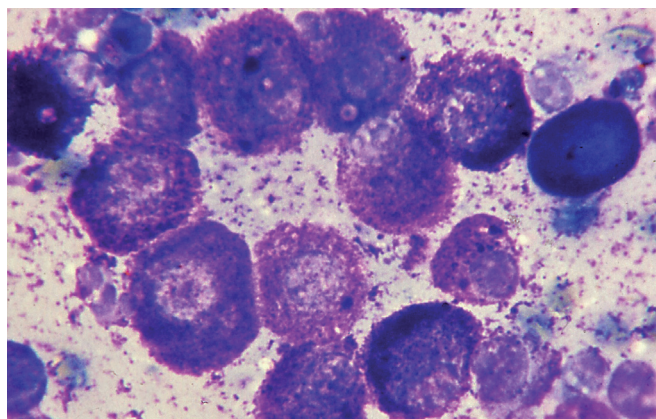
Skin tumours are usually recognizable as such. Sometimes the subcutis is included in the process or an inflammation or tumour from the underlying tissues is infiltrated in the skin. The differential diagnosis then becomes more extensive and more difficult. Usually cytological examination can still arrive at the diagnosis, provided that there are enough characteristic cells present.

Tumours of individualized round cells

Several skin tumours are characterized cytologically by a uniform population of round tumour cells that have little or no apparent connection with each other. The FNAB from such tumours is usually cell rich because they, in contrast to mesenchymal tumours, release cells easily during aspiration. To this group of so-called "discrete cell neoplasms" belong the mast cell tumours, malignant lymphomas, cutaneous plasmacytomas, histiocytomas, melanomas, and the transmissible venereal tumours (TVT).

Mast cell tumours are usually immediately recognizable because of the presence of many purple cytoplasmic granules. The cells are large and round. The nucleus of the cell is often difficult to see because it is poorly stained and covered by granules that absorb so much stain (Fig. 9). Sometimes large

Fig. 9 Several large discrete cells are present in this aspirate of a mast cell tumour. The cells have many purple granules in their cytoplasm.



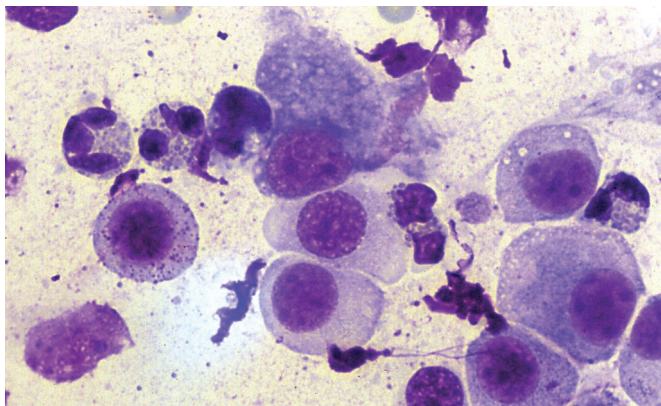


Fig. 10 The tumour cells of this mast cell tumour lack the characteristic purple granules. Only in one cell on the left some granules are present. Among the cells several eosinophils can be seen.

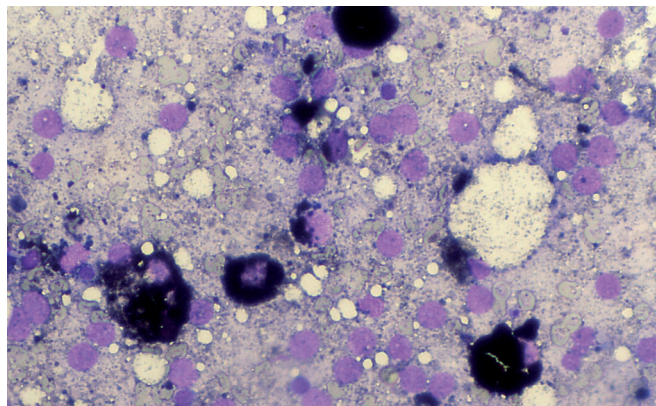


Fig. 11 Aspirate of a melanoma tumour in a dog. Apart from several naked nuclei some large melanoma cells filled with black melanine granules are seen.

numbers of eosinophils and/or fibroblasts can be seen. In poorly differentiated mast cell tumours the purple granules are often less frequently present or even lacking. Cytology cannot, however, be used for grading the tumours.

Differential diagnosis: There are mast cell tumours that contain very few granules (Fig. 10). These can be difficult to diagnose. The presence of many eosinophils can give support to the presumptive diagnosis of mast cell tumour. Inflammatory processes can give differential diagnostic problems because they can also contain mast cells and eosinophils. In an inflammation, however, the number of other inflammatory cells is considerably greater than the number of mast cells. Melanomas could also be mistaken for mast cell tumours. Melanoma cells can, like mast cells, be round to oval and contain pigment granules. However, most melanomas also contain spindle-shaped tumour cells. In addition, the pigment granules in melanomas are variable in size and irregular in shape and they stain, depending on the thickness of the pigment layer, greyish-blue to greenish-black.

Melanomas are tumours composed of cells that produce melanin. The melanomas belong to the group of "round cell

tumours without cell interconnections" because they largely meet the characteristic cytological features of these tumours. In addition to round-oval cells, however, there are usually some spindle-shaped cells and sometimes these dominate the picture. Very bizarre cell forms and giant cells can also occur. The amount of pigment in melanoma cells can vary markedly. The nucleus of the melanoma cell is sometimes barely visible because it is covered by melanin granules (Fig. 11). These granules are blue to greenish-black, irregular in shape and variable in size. Melanomas can be malignant or benign. If the nucleus is visible it also may show definite malignancy criteria. The melanomas that hardly contain any pigment are almost always malignant. Such amelanotic melanomas are difficult as such to diagnose but a careful search of the preparation will often still reveal a few melanin-containing cells. Macrophages with phagocytized melanin granules may give an indication to the origin of the tumour.

Cutaneous malignant melanomas in cats can also be melanotic or amelanotic. Five types of melanomas can be distinguished: epithelioid, spindle, mixed, signet-ring, and balloon cell. Whereas all epithelioid, spindle, and mixed epithelioid/spindle cell types

Fig. 12 Signet-ring cell type melanoma in the cat. Many amelanotic cells, often with multiple nuclei are present.

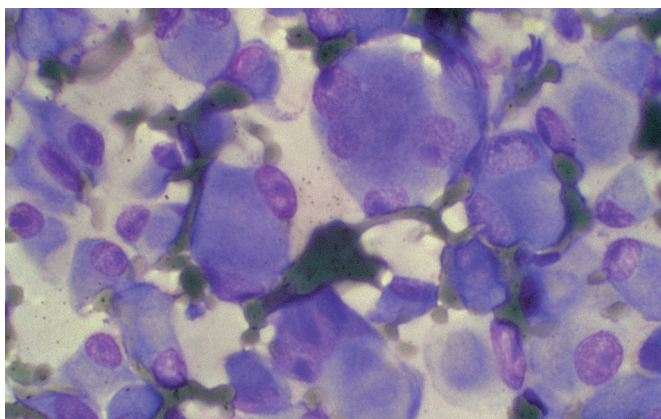
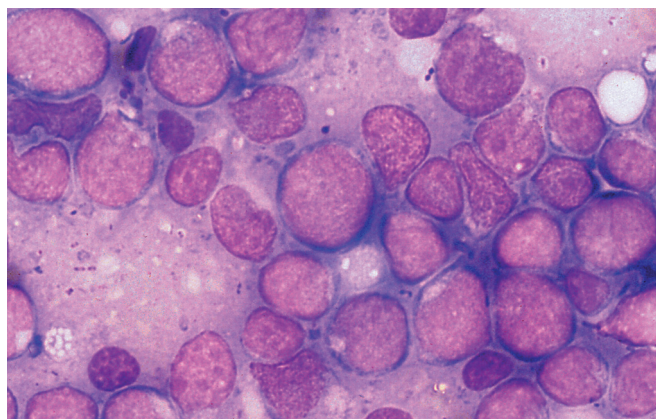


Fig. 13 Cutaneous malignant lymphoma in a cat. There are several large, immature lymphoid tumour cells, and a few mature reactive lymphocytes.



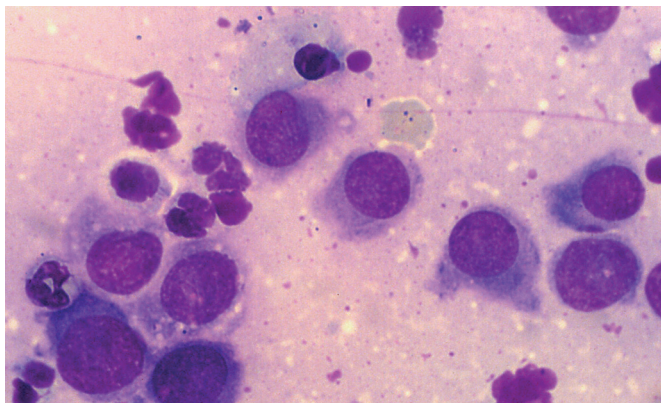


Fig. 14 Mixture of several histiocytoma tumour cells with some neutrophils and lymphocytes. The histiocytes are the large cells with pale grey-blue cytoplasm.

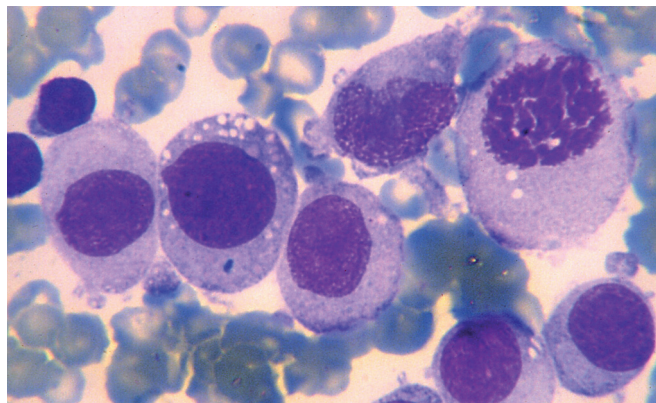


Fig. 15 The transmissible venereal tumour cells are big cells, with distinct cytoplasmic borders, sometimes with fine vacuoles. On the right a mitosis can be seen and on the left some reactive lymphocytes are present.

show pigmentation, signet-ring and balloon cell types are often amelanotic (Fig. 12).

The pigmented pathological cells of melanomas are easy to differentiate from normal pigmented epithelial cells. Melanocytes and pigmented squamous cells have very uniform, rod-shaped granules. The nucleus may be degenerated or may have disappeared, but will certainly have no malignancy criteria. The difference between melanomas and mast cells has been discussed in the section on mast cell tumours. Melanocytes must also be differentiated from macrophages that have phagocytized melanin (melanophages) or contain haemosiderin. Melanophages usually contain coarse conglomerates of melanin as well as vacuoles. They are encountered especially in melanomas but also in inflamed lymph nodes and in some disorders of the skin. Haemosiderophages are found in old haematomas. These are macrophages having vacuoles in which iron pigment is stored. Like the pigment of melanoma cells, the color of this hemosiderin is blue to greenish-black. The presence of cells that contain bilirubin crystals and show erythrophagocytosis helps in the differentiation.

Malignant lymphomas can be primary or secondary tumours in the skin. The primary, often epitheliotrophic lymphomas are usually characterized by the presence of large number of T-lymphoid tumour cells. These cells may look more differentiated, with some nuclear indentations, and with pale cytoplasm. This in contrast to the B-lymphoid tumours, which are more often characterized by the presence of large, blastic cells, with dark blue cytoplasm and round nuclei with prominent nucleoli (Fig. 13).

(Muco)cutaneous plasmacytomas are in principle benign tumours. In contrast to their malignant counterpart, the multiple myeloma, they are not combined with paraneoplastic syndromes. Aspirates normally yield large amount of tumour cells. The cells sometimes look like typically plasma cells, while in other cases they are less well differentiated. Most often there are discrete cells with distinct cytoplasm borders and a blue cytoplasm. The nucleus can be eccentric, a Golgi apparatus can sometimes be seen, and there can be several cells with two or more nuclei.

Histiocytomas are usually benign. They occur primarily in young dogs and can disappear spontaneously. A population of cells with somewhat variable shapes characterizes them cytologically. Small histiocytes resemble lymphocytes but have finer nuclear chromatin and more cytoplasm (Fig. 14). The larger histiocytes resemble epithelial cells but have no tissue organization. The cytoplasm is grey to light blue; a few cells are clear. The nuclei are mainly round but may be indented. The nuclei contain a few small, not very obvious nucleoli.

Differential diagnosis: The FNABs of histiocytomas usually contain few tumour cells, in contrast to the FNABs of other "discrete round-cell tumours". Because histiocytomas often ulcerate and become secondarily inflamed, it can be difficult to recognize the tumour cells, which resemble lymphocytes and epithelioid cells, among the inflammatory cells. Histiocytomas can also slightly resemble a transmissible venereal tumour (see below).

Transmissible venereal tumours (TVT) in the dog occur in the genital area, but on the head as well. They are seldom seen in the northern parts of Europe. The cells resemble histiocytes but the tumour exfoliates more easily and thus the preparations are richer in cells. The cytoplasm is also more sharply outlined and sometimes contains readily visible vacuoles (Fig. 15). The round to oval nucleus is eccentric, seldom indented, and can have large, noticeable nucleoli. The cell size, nuclear size, and the N/C ratio vary much more than in histiocytomas. Usually many mitotic figures are seen. TVTs can contain remarkably many plasma cells and macrophages in addition to tumour cells.

Differential diagnosis: apart from histiocytomas, basal cell tumours in particular must be considered (see below).

Epithelial tumours

Different types of epithelial cells can be encountered in cytological preparations from skin tumours, skin lesions, and subcutaneous swellings. They may be squamous cells but they can also be of glandular origin.

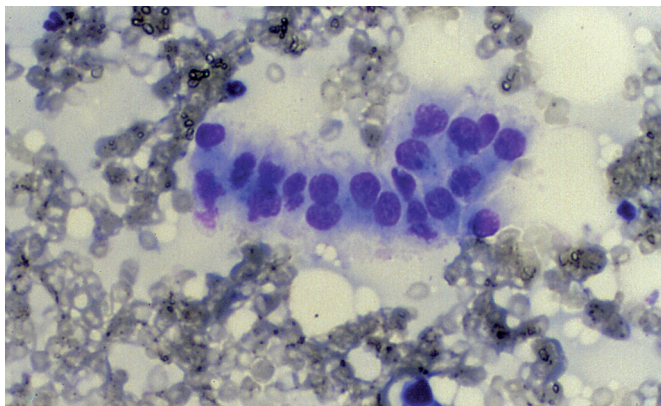


Fig. 16 A typical linear cell aggregate in an aspirate of a basal cell tumour. The basal cells are very monomorphic, with small round nuclei and few or no malignancy criteria.

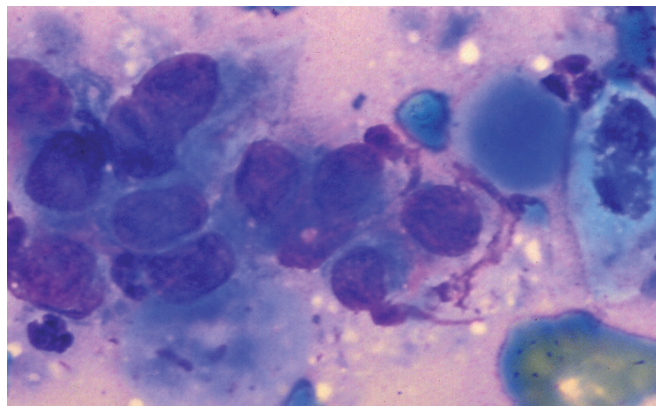
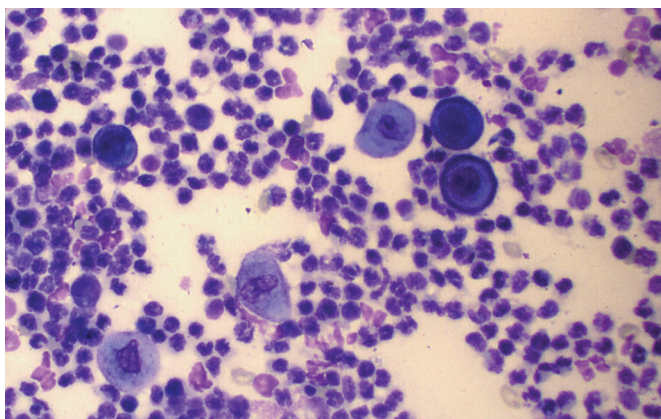


Fig. 17 Fully cornified, but round squamous cells without a nucleus as well as a cornified cell with a nucleus are present. In addition, a group of poorly differentiated cells with anisokaryosis, abnormal nuclei forms and multiple and abnormal nucleoli can be seen.

FNABs of lesions in the skin and the mucosa contain squamous cells in all stages of development, in addition to inflammatory cells. A cell-rich preparation can contain many individualized epithelial cells, but what is characteristic of normal epithelial cells is their appearance in groups or clusters that are often composed of a single layer of cells (so-called "monolayers"). Depending on the depth of the lesion, more or less immature epithelial cells will be seen. These basal cells and parabasal cells are round, deep blue, small in relation to mature epithelial cells, and have a higher N/C ratio. The mature squamous cells, which are usually much more numerous, appear in different stages of keratinization. The largest cells have often already lost the nucleus (keratin flakes or dandruff) or still contain a shrunken, pyknotic nucleus. The N/C ratio is very low and the cells are rectangular and often folded double. As the cell becomes more keratinized the cytoplasm staining changes from dark blue to sky blue. The cells can also contain vacuoles as signs of keratinization. Mature but not yet keratinized epithelial cells are lightly basophilic, round or oval, and have a centrally located nucleus with a well-defined chromatin structure that resembles a fine network.

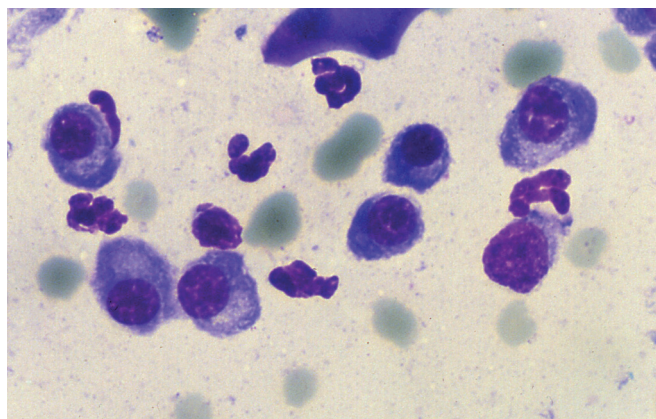
Fig. 18 An aspirate of a vesicle in a dog with pemphigus foliaceus. Among the large number of neutrophils several acantholytic cells are situated. These cells are round, have a nucleus and sometimes a perinuclear halo.



Just as for normal epithelial cells, the arrangement of the cells in clusters is characteristic of epithelial tumours. Although in FNAB preparations from epithelial tumours many loose tumour cells can be seen, usually several definite clusters can be found. If the epithelial tumour is of glandular origin, the cell clusters also have an acinar structure (arrangement in a group or in a circle around a usually invisible duct). In some preparations there are predominantly clusters or monolayers of normal epithelial cells but careful searching also reveals definitely malignant cells.

Basal cell tumours are infrequent epithelial tumours that arise from the basal cell layer of the epidermis. Histologically, they can be divided into different varieties. The cytological diagnosis can be difficult due to the variable cytological features. Basal cells are round or elongated cells with one nucleus in a central or basal position and fine granular chromatin with a single and, in most cases, poorly detectable nucleolus. The N/C ratio is 1:1, with a uniform size of cells within cell aggregates. The basal cells can be organized in fragments of tissue, often with a typical linear or palisade-like arrangement (Fig. 16). In some cases well-differentiated fibrocytes and fibroblasts can be found, as well as pigmented basal cells or melanocytes. In addition, the

Fig. 19 Mature looking plasma cells in a brush smear of a cat with plasmacytic gingivitis.



presence of some squamous cells in some basal cell tumours with basosquamous areas can confuse the examiner even further. In most tumours also some inflammatory cells, such as neutrophils, can be detected. Despite the fact that in several tumours a significant proportion of the basal cells might reveal a few malignancy criteria, such as anisokaryosis, anisonucleoliosis and clumped chromatin pattern, giving the tumour a less differentiated appearance, basal cell tumours are considered benign.

Squamous cell carcinomas are quite common tumours, both in the cat and the dog. Preparations from squamous cell carcinomas are usually cell rich and contain loose tumour cells as well as clusters. The characteristics of malignancy can be very pronounced or not very obvious.

Squamous cell carcinomas easily become ulcerated and then contain many inflammatory cells. Because aspirates from squamous cell carcinomas usually contain neoplastic as well as normal, non-neoplastic squamous cells in all stages of development, the malignancy criteria of well-differentiated squamous cell carcinomas can be less obvious, the diagnosis of these tumours is sometimes difficult. One of the characteristic features of the squamous cell carcinoma is the discrepancy between maturation of the nucleus and the cytoplasm (Fig. 17). The tumour cells can contain a large amount of vacuolated cytoplasm and at the same time a completely intact, non-pyknotic nucleus having a detailed structure that sometimes also has malignancy criteria. Sometimes the vacuoles have become confluent into one large vacuole that causes a noticeable clear space around the nucleus, so that the cell resembles a bull's-eye. This is a strong indication of malignancy. Also, the occurrence side by side in a single cluster of mature and immature cells, or cells of markedly different basophilia, is very suspicious.

Skin lesions

It must be emphasized that skin lesions can become secondarily infected and inflamed. The confirmation of septic inflammation is thus not very informative in case of a skin lesion and certainly not if this is demonstrated by an impression smear, unless a very specific infective agent is demonstrated. The microorganisms that can play a role in inflammatory processes will be discussed in a later chapter. Inflammation and necrosis can make it very difficult to determine the primary cause of a process. Hence it is advisable not to rely on an impression smear but also to obtain a FNAB from a peripheral, not yet inflamed, part of the swelling. If the cytology of a skin lesion does not lead to a diagnosis, a dermatologist should be consulted and/or histological examination should be performed.

Pemphigus foliaceus is an autoimmune process that is directed against keratinocyte desmosomal cadherins, and in which interference occurs with the adhesive function of these molecules. When the epidermal cells lose their cohesion due to degeneration of the intercellular bridges, intra-epidermal clefts, vesicles, and bullae are formed. The isolated epidermal cells are called *acantholytic cells*. They are characterized by a basophilic cytoplasm and have well defined, round borders (Fig. 18). Several

cells have a perinuclear halo. The nucleus is somewhat enlarged and has a coarse, irregular chromatin pattern. Often a clear nucleolus can be seen. Acantholysis is most often associated with the pemphigus complex. The acantholytic cells are usually surrounded by many neutrophils and/or eosinophils. No bacteria are present, unless the lesion has been ulcerated.

(Lympho)plasmacytic gingivitis/stomatitis, a disease with an unclear aetiology can present itself in many different ways in cats of all ages. Cytologic specimens can be obtained by brush methods and might reveal a combination of plasma cells, lymphocytes and sometimes also neutrophils (Fig. 19). The morphology of the cells is normal. The ratios between the different cell types can vary greatly among cases.

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