

## Diagnostic Cytology of Neoplastic Lesions in Dogs

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### ABSTRACT

Cytology is a reliable and minimally invasive method of obtaining tissues for diagnosis. It is a rapid method to be used for differentiating inflammatory from neoplastic disease. Inflammation is easily differentiated from neoplasia based on the presence of inflammatory cells. Ulcerative neoplasm is always secondarily infected by bacteria, and thus inflammatory cells could also be observed. Cytologically, neoplastic cells are classified into three major categories based on certain common cytological features (Meinkoth *et al.*, 2008), namely round, epithelial, and mesenchymal cells (Meinkoth *et al.*, 2008). Cytological images and specific features of neoplastic lesions in dogs from cytology smear, which were obtained from the Veterinary Haematology and Clinical Biochemistry Laboratory, Universiti Putra Malaysia (UPM), were illustrated and briefly described. Round cell tumour was identified as round and discrete neoplastic cells with specific cytoplasmic features, whereas epithelial tumour was identified as clusters of neoplastic cells with closed cytoplasmic attachment, and mesenchymal tumour was identified as spindle neoplastic cells with indistinct cytoplasmic border.

**Keywords:** Cytology, dog, neoplasia

### INTRODUCTION

Cytology is a quick diagnostic tool to be used to differentiate between inflammatory and neoplastic conditions. Inflammatory conditions are easily diagnosed by examining the presence of inflammatory cells. Chronicity of the inflammatory conditions is then evaluated through evaluating the percentage of polymorphonuclear (PMNs) and mononuclear cells (MNC). In acute inflammation, the percentage of PMNs is approximately 70%. For sub-acute or chronic active inflammation, the percentage of PMNs is more than 50%, and for chronic inflammation, more than 50% of the inflammatory cells are

MNC. Neoplastic conditions are diagnosed by examining the presence of neoplastic cells. Malignant neoplastic cells are easily differentiated from benign or hyperplastic cells through identification of the criteria of malignancy. It is important to note that the differentiation of benign and hyperplastic cells can only be performed via histopathological examination. Cytologically, neoplastic cells are divided into three groups, namely round or discrete cell, epithelial, and mesenchymal tumour. The specific criteria of each group of tumours will be briefly described and illustrated.

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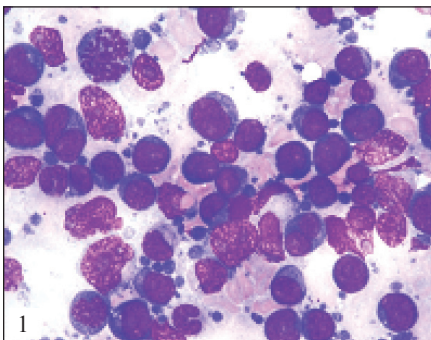
## MATERIALS AND METHODS

Cytology samples, submitted to Veterinary Haematology and Clinical Biochemistry Laboratory, Universiti Putra Malaysia (UPM), were examined. The samples were stained with Wright's stain and examined using a light microscope. Images were captured using a digital camera and ACT2U Imaging Software, and they were compared to the images illustrated and described by Cowell and co-workers (2008).

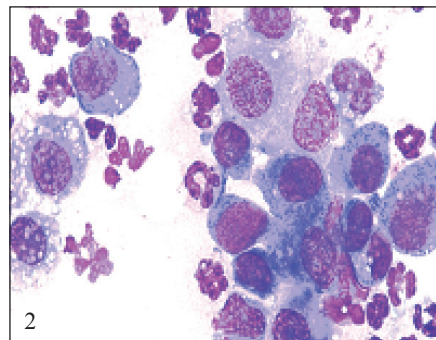
## RESULTS AND DISCUSSION

Round cell tumour, such as lymphosarcoma, mast cell tumour, melanoma and transmissible venereal tumour (TVT), is identified as a group of discrete cells with specific cytoplasmic features. The cytoplasmic features are used to differentiate the different types of round cell tumours. For instance, lymphosarcoma (Fig. 1) has a scanty basophilic cytoplasm as compared to histiocytoma and malignant histiocytosis (Fig. 2) with abundant pale to basophilic and vacuolated cytoplasm. Differential diagnosis of lymphosarcoma is always included in dogs with clinical signs

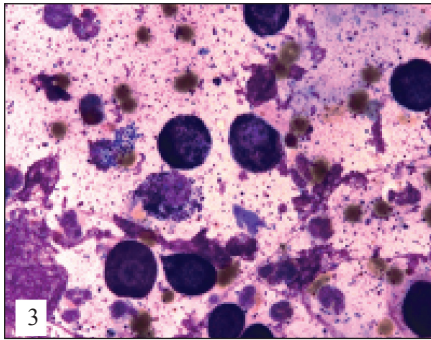
of generalised lymphadenopathy. Mast cell tumour is usually and easily differentiated from other types of round cell tumours based on the presence of purplish granules in the cytoplasm (Figs. 3, 4, and 5). Meanwhile, well to poorly differentiated mast cell tumour (Fig. 4) is usually diagnosed from a cutaneous ulcerated mass. The one with intermediate to poorly differentiated (Fig. 5) is usually involved or metastasised to the lymph nodes and/or internal organs, such as spleen. Diagnosis of intermediate to poorly differentiated mast cell tumour is challenging when a rapid stain, such as Diff quick, is used. Mast cell granules are poorly stained using these types of stain. Melanoma is usually diagnosed from ulcerated lesions in the oral cavity and limbs (Fig. 6). It is a greater imitator which yields cell populations that may appear discrete, epithelial, or mesenchymal. The cytoplasmic features of this tumour, however, made it easily distinguished from other types of round cells tumours. The dusky blue-black pigments or granules are presence in the cytoplasm and/or in the background of the smear. Another type of round cell tumour which is usually diagnosed in dogs is TVT (image not shown). It is usually present in external genital organs.



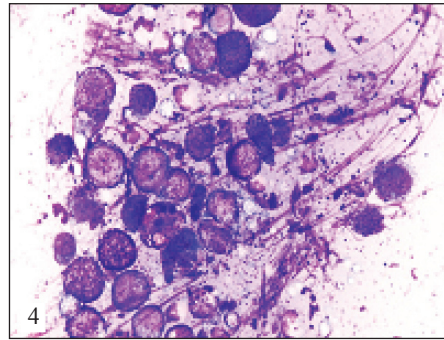
*Fig. 1: Fine-needle aspiration (FNA) of a lymph node from a dog with lymphosarcoma containing numerous lymphoblasts and lymphocytes with prominent criteria of malignancy. The neoplastic cells have a scanty basophilic cytoplasm. One abnormal mitotic figure is present. Numerous cytoplasmic fragments (lymphoglandular bodies) are present in the background of the smear*



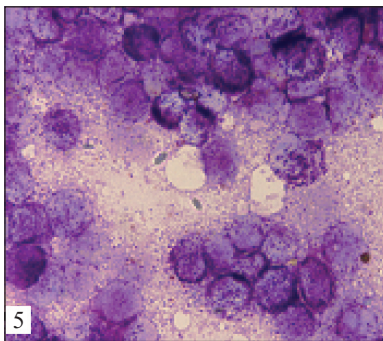
*Fig. 2: Pleural fluid from a dog with malignant histiocytosis. The pleomorphic neoplastic cells characterised by marked anisocytosis, cytoplasmic vacuolation, anisokaryosis, multiple nucleoli, and irregular chromatin clumping. Numerous neutrophils are present*



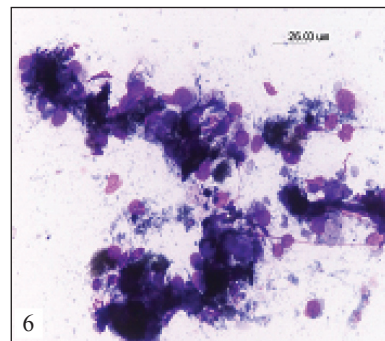
*Fig. 3: Impression smear of an ulcerated wound from a dog with well-differentiated granulated mast cell tumour. The neoplastic cells are uniform and the nuclei are almost obscured from view because of the many coarse purple granules*



*Fig. 4: Fine-needle aspirations (FNA) of a cutaneous mass from a dog with mast cell tumours. The smears are highly cellular, containing a mixture of well to intermediately differentiated granulated mast cells, and inflammatory cells*



*Fig. 5: Fine-needle aspirations (FNA) of an enlarged spleen from a dog with mast cell tumours. The smears are highly cellular, and contain a mixture of intermediate to poorly differentiated granulated mast cells. The neoplastic cell nuclei are stained very pale blue because of the heavy degree of granulation and lack of stain penetration to the nucleus*



*Fig. 6: Fine-needle aspiration (FNA) from an oral cavity of a dog with melanoma. The cellular detail in most of the intact cells is obscured by the pigmentation, preventing evaluation of these cells. Nuclei can be seen in some cells which appear partially ruptured. Numerous free melanin granules are present in the background of the smear*

The cytoplasm of the neoplastic cells contains numerous clear vacuoles and smoky grey in colour. The vacuoles may also be freely present in the background.

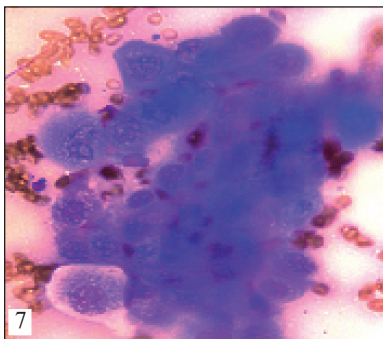
The second category of neoplastic lesion which can be cytologically diagnosed is epithelial neoplasm. It is identified as clusters of epithelial cells characterised by large pleomorphic

neoplastic cells with a closed cytoplasmic attachment (Figs. 7, 8, 9, 10, 11, and 12). Examples of these epithelial cells tumours are squamous cell carcinoma (SCC) (Figs. 7 and 8), nasal carcinoma (Fig. 9), metastatic carcinoma (Fig. 10), mammary gland adenoma (Fig. 11), and Sertoli cell tumour (Fig. 12). Squamous cell carcinoma is usually seen in the oropharyngeal

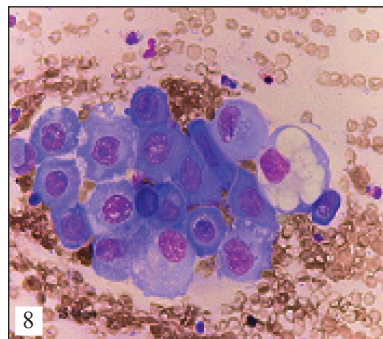
organs. Two types of SCC, well- and poorly differentiated, are generally diagnosed in dogs. Well-differentiated SCC (*Fig. 7*) is characterised by fully cornified superficial cells with prominent perinuclear vacuolation. In the presence of inflammatory cells, biopsy is necessary to rule out epithelial hyperplasia or dysplasia. Meanwhile, poorly differentiated SCC is characterised by large round neoplastic epithelial cells with abundant, pale to basophilic cytoplasm (*Fig. 8*). Perinuclear vacuolation and cytoplasm showing angular borders could also be observed. Cytology of malignant neoplastic epithelial cells, which were obtained from the nasal cavity (nasal carcinoma), is easily distinguished from benign neoplastic epithelial cells (nasal adenoma). *Fig. 9* clearly demonstrates the malignancy criteria of nasal epithelial cells including coarse granular chromatin, macronuclei, marked multinucleolar nuclei, high nuclear to cytoplasmic ratio, anisokaryosis, and poikilocytosis. Similar malignancy criteria are used to distinguish metastatic carcinoma (*Fig. 10*) from other types of metastatic neoplastic cells, such as lymphosarcoma and mast cell tumour, obtained from the body cavity fluids. The example of benign epithelial neoplastic cells or hyperplasia is shown in *Fig. 11*. The cells obtained from an

enlarged mammary gland are lacking cytologic criteria for malignancy. Histopathology of the enlarged mammary gland must be performed for a definitive diagnosis. Sertoli cell tumour (*Fig. 12*) is the tumour of the epithelial cells of testes. It can be mistaken from the round cell tumour, particularly TVT. Based on the location of the affected organs, and the presence of highly pleomorphic neoplastic cells with abundant small and very distinct vacuolated cytoplasm, finely reticulated nuclear chromatin and small multinucleolar nuclei, Sertoli cell tumour, is easily identified.

The third category of neoplastic lesion which could be cytologically diagnosed is mesenchymal neoplasm, which includes fibrosarcoma (*Fig. 13*), osteosarcoma (*Figs. 14 and 15*) and chondrosarcoma (*Fig. 16*). It is characterised by oval to round neoplastic cells with indistinct cytoplasmic borders. Mesenchymal tumour is hardly diagnosed cytologically. In cases of advanced stage of neoplastic conditions, in which the neoplastic cells are able to aspirate from the affected organs or areas, rapid diagnosis could be made. Meanwhile, fibrosarcoma is identified as a spindle cell with oval to round nucleus and tapering long cytoplasmic tails. *Fig. 13* shows fibrosarcoma cells with prominent



*Fig. 7: Fine-needle aspiration (FNA) from an oral cavity of a dog with well-differentiated squamous cell carcinoma. Note the cluster of pleomorphic epithelial cells show perinuclear vacuolation and large nonpyknotic nuclei, despite a mature cornified appearance the cytoplasm*



*Fig. 8: Fine-needle aspiration (FNA) from an oral cavity of a dog with poorly differentiated squamous cell carcinoma. Note that the cluster of large pleomorphic epithelial cells showing perinuclear vacuolation and large nonpyknotic nuclei with a noncornified appearance of the cytoplasm*

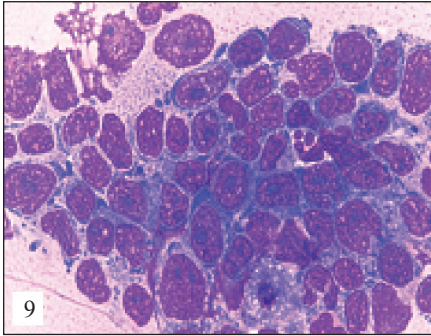


Fig. 9: Nasal wash of a dog with nasal carcinoma. There are a cluster of neoplastic epithelial cells with prominent criteria of malignancy with a few inflammatory cells

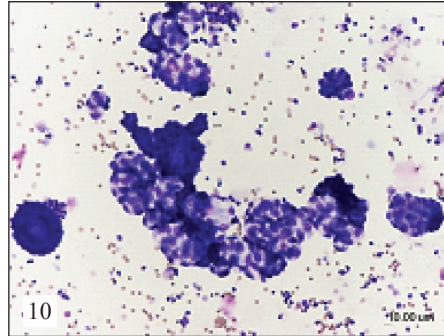


Fig. 10: Abdominal fluid of a dog with metastatic carcinoma. There are numerous aggregates of a homogenous population of large neoplastic epithelial cells with criteria of malignancy which show acinar-like structure

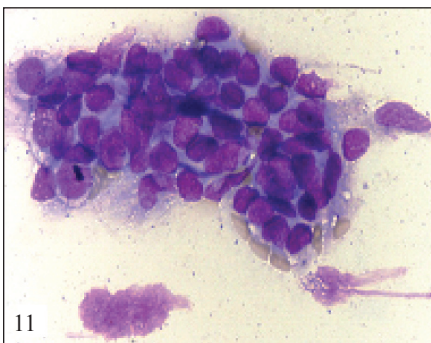


Fig. 11: Fine-needle aspiration (FNA) from enlarged mammary gland of a dog with hyperplasia or adenoma. These epithelial cells have little or no cytoplasmic vacuoles and minimal cellular atypia, emphasising the need for histopathologic confirmation when neoplasia is suspected

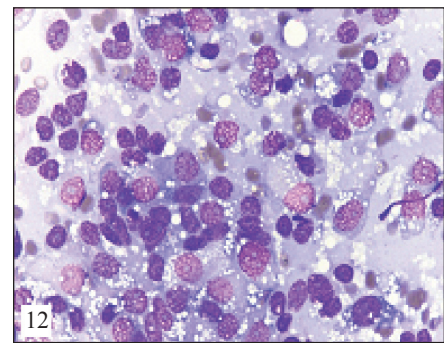
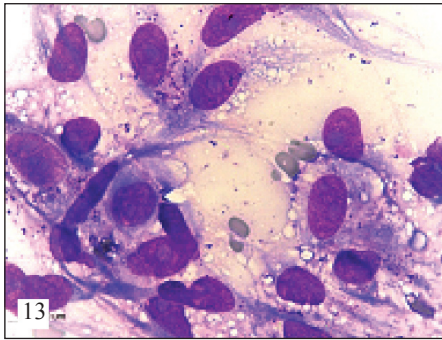


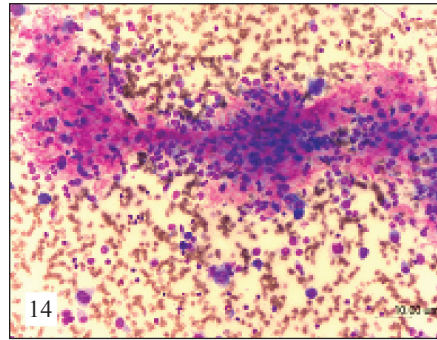
Fig. 12: Impression smear from the ulcerated testes of a dog with Sertoli cell tumour. There are clusters of neoplastic epithelial cells with abundant vacuolated cytoplasm showing prominent criteria of malignancy

criteria of malignancy which is as similarly described by Tyler *et al.* (2008), including multinucleolar nuclei, cytoplasmic basophilia, increased nuclear to cytoplasmic ratio, enlarged nucleoli, and mild to marked variation in cellular, nuclear, and nucleolar size and shape. In contrast to fibrosarcoma, spindle shape of osteosarcoma (Figs. 14 and 15) and chondrosarcoma (Fig. 16) cells is less prominent, mainly due to the lack of tapering long cytoplasmic tails. The

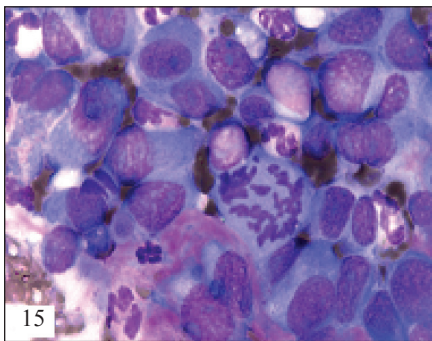
major characteristics of these malignant bone tumours are osteoid and chondroid, which are characterised by eosinophilic matrix in the background of the smears. Osteoid is identified as fibrillar eosinophilic matrix, in which the neoplastic mesenchymal cells are interspersed on the matrix (Fig. 14). Chondroid is identified as homogenous eosinophilic matrix, in which the neoplastic mesenchymal cells are embedded in the matrix (Fig. 16). Another example of



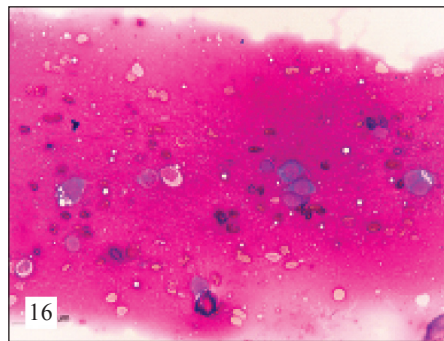
*Fig. 13: Fine-needle aspiration (FNA) from an oral cavity of a dog with fibrosarcoma. There are numerous neoplastic spindle cells with prominent criteria of malignancy. Note that most cells are extremely elongated with thin, tapered cytoplasm*



*Fig. 14: Fine-needle aspiration (FNA) from osteolytic bone of a dog with osteosarcoma. Low-magnification image shows numerous neoplastic mesenchymal cells which appear to be embedded in a brightly fibrillar eosinophilic matrix*



*Fig. 15: Fine-needle aspiration (FNA) from osteolytic bone of a dog with osteosarcoma. High-magnification image (magnification from Fig. 14) shows numerous neoplastic cells showing prominent criteria of malignancy. One abnormal mitotic figure is present*



*Fig. 16: Fine-needle aspiration (FNA) from osteolytic bone of a dog with chondrosarcoma. Low-magnification image shows a few individual neoplastic mesenchymal cells with prominent criteria of malignancy which appear to be embedded in a brightly homogenous eosinophilic matrix*

mesenchymal tumour which can cytologically be diagnosed is haemangiosarcoma (image not shown). The main constraint for diagnosis of haemangiosarcoma is the presence of iatrogenic blood contamination on the smear. However, one should be remembered, fine needle aspiration (FNA) of an enlarged spleen suspected with haemangiosarcoma is contraindicated.

## CONCLUSIONS

Cytology is a rapid tool for diagnosis of neoplastic lesions in dog. Neoplasia is easily distinguished from inflammation based on the types of cell. Further differentiation of malignant from benign neoplastic cells was performed via examination of the criteria of malignancy. The neoplastic cells are classified into three different groups

based on the origin of the cells including round, epithelial and mesenchymal cells. Meanwhile, the specific criteria of the tumour cells, which are either round cell, epithelial or mesenchymal tumours, are described.

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