INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Fine Needle Aspiration Cytology (FNAC) as a Diagnostic Technique in the Study of Effusions of Thoracic and Abdominal Cavities in Dogs and Cats

Rashmi Choudhary, Supriya Shukla, Nitesh Kumar*, Danveer Singh Yadav and Sarita Mangal

College of Veterinary Science and Animal Husbandry, MHOW, Indore, Madhya Pradesh, India.

ABSTRACT

Fine needle aspiration (FNA) is a relatively non-invasive, less painful and quicker method when compared to other methods of tissue sampling such as surgical biopsyand has a significantly shorter recovery time. It is a quicker method of diagnosis of diseases like cancer, giving more options for treatment, or that benign lumps are diagnosed without the need for surgery. FNA biopsies do require some expertise to perform and interpret. To ensure that an accurate result is achieved, it is important that the general practitioner, radiologist, surgeon, pathologist or oncologist who performs procedure has experience in FNA biopsy. FNA is being increasingly utilized in clinical diagnosis in order to avoid invasive investigations, surgical biopsies and costly exploratory surgeries.The technique allows cost-effective and efficient tissue sampling and has the potential to provide cells and fluid for molecular, genetic and proteomic analyses. In this review papers FNA analyses may become very important in the future for diagnosis and prognosis of tumor disease for establishing the effectiveness of therapy.

Keywords: Fine needle aspiration, cancer, cytology, diagnostic techniques.

INTRODUCTION

In pathology cytology refers to the study under a microscope of individual cells to establish the cause of a disease, most commonly for a pre-malignant or malignant condition. Cells are stained with a number of tinctorial or other stains that enable the nuclear and cytoplasmic features of cells to be examined.

The cells that are examined in cytological samples usually originate from epithelial, or epithelial like tissues, and are most simply obtained as the epithelium exfoliates. These cells may be naturally exfoliated as in sputa or urine, or the cells may be mechanically obtained by brushing or scraping. Sometimes cells may be obtained by the use of fine needle aspiration (FNA) that enables more deep-seated tissues from the live subject, human being or animals that would not necessarily exfoliate to be sampled.

The sample of cellular material taken during an FNA is sent to a pathology laboratory for analysis. The samples taken are examined by a pathologist under a microscope. A detailed report will then be provided about the type of cells that were seen, including any suggestion that the cells might be cancer. It is important to remember that having a lump or mass does not necessarily mean that it is cancerous; many fine needle aspiration biopsies reveal that suspicious lumps or masses are benign(non-cancerous) or cysts.

Aspirate samples may be described as one of the following types

1. Inadequate/insufficient type
   The sample taken is not adequate to exclude or confirm a diagnosis.
2. Adequate/Sufficient type:It is of again three types.

a) Benign

There are no cancerous cells present. The lump or growth is under control and has not spread to other areas of the body.

b) Atypical/indeterminate, or suspicious of malignancy: The results are unclear. Some cells

appear abnormal but are not definitely cancerous. A surgical biopsy may be required to adequately sample the cells.

c) Malignant

The cells are cancerous, uncontrolled and have the potential or have spread to other areas of the body. Fine-needle aspiration (FNA) is a biopsy technique that uses aspiration to obtain cells or fluid from palpable or ultra sound-detected masses i.e. the technique is applied for diagnosis of palpable as well as non-palpable lesions.

Palpable Mass Lesions includes lymph nodes, mammary gland, thyroid, salivary glands, soft tissue masses, bones Non-palpable Mass Lesions includes Abdominal cavity, thoracic cavity and retroperitoneum Non-palpable lesions require some form of localization by radiological aids for FNA to be carried out. Plain x-ray is usually adequate for lesions in bones and chest. Ultrasonography (USG) allows direct visualization of needle in intra-abdominal and soft tissue masses. CT scan can be used for lesions in chest and abdomen.

Effusions are small amount of fluid present in the body cavities in different disease conditions. In this topic we mainly focus on FNAC in the study of effusions of thoracic and abdominal cavities in dogs and cats.

History

The first report on the use of needles for therapeutic purposes can be found in Arab medicine, in the writings of Albucasis or Abu al-Qasim, court physician (936-1013A.D) in medieval period. He first time described the therapeutic punctures of the thyroid gland, using instruments resembling modern aspiration needles.

Albucasis' description resembles a modern FNA of the thyroid gland (Kaadan, 2004).The earliest report of a needle technique to obtain material for microscopy was employed by Kun in 1847 who described a "new instrument for the diagnosis of tumors". There followed sporadic reports of this technique, by clinicians including Leydon who in 1883 used needle aspiration to obtain cells to isolate pneumonic microorganisms and Greig and Gray in 1904 diagnosed trypanosomiasis in cervical lymph node aspirates from patients with sleeping sickness in Uganda (webb, 2003).

Hayes Martin, Edward Ellis and Fred Stewart gave brief rebirth to this technique in 1930's. In the early 20th century, Martin and Ellis are considered to be the founder of modern needle aspiration techniques. After 20th century FNAC has emerged as a sophisticated diagnostic technique both in the field of medical and veterinary science.

Procedure for Fine Needle Aspiration Cytology

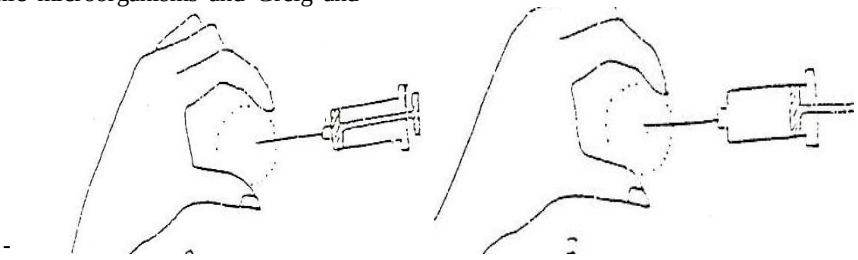
Materials required : Needle (22-25 guage), disposable syringe (3 - 20 ml), new glass slides, spirit swab, and suitable fixative is required.

Preparation of the site for aspiration

If microbiological tests are to be performed on a portion of sample collected, or a body cavity is to be penetrated, the area of aspiration is surgically prepared. An alcohol swab can be used to clean the area. If the samples are being collected using ultrasound guidance, it is important to avoid the use of ultrasound gel, substituting alcohol as a contact agent instead. Ultrasound gel stains pink with commonly used cytology stains. Even a small amount of ultrasound gel picked up as a contaminant when the needle passes through the skin and render a slide nondiagnostic.

Procedure

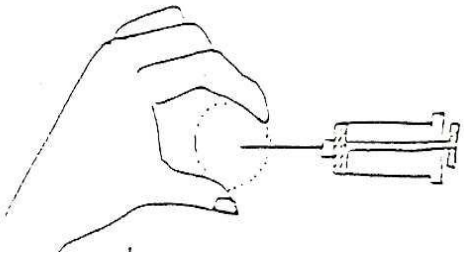
- To restrain and to avoid unwanted movements during procedure the tranquliser (siquil @ 2 to 4 mg/ kg, IM route) will be given 10 min. prior to the procedure.
- Palpate the target area, if it is palpable mass.
- Insert 22-25 guage needle into syringe depending upon suspected upon
- the suspected viscousness of sample material.
- Fix the mass by palpating hand and insert needle into target area. Apply suction while moving needle back and forth within the lesions and change the direction of the needle.



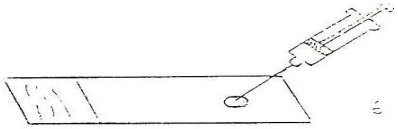
- Terminate the aspiration when aspirated material or blood is visible at the hub or base of the needle.
- The negative pressure created within the syringe by aspiration holds the tissue against the sharp cutting edge of the needle so that tissue will be cut by the

cutting end of needle and accumulates within the lumen of the needle/ syringe

- Release the suction before withdrawing the needle to equalize pressure within the syringe.



- After withdrawal of needle apply pressure for 2-3 min. at the site of puncture to arrest bleeding and prevent hematoma formation.
- Aspirated material from the needle is expelled on to clean glass slide by detaching the needle and filling the syringe with air and expelling it with pressure.



- Smears are prepared as for blood smears. If the material is semi-solid, it is 1st crushed by gentle pressure with a glass slide and smears prepared.

Fixatives & stains

1. Dried smear are stained by Romanowsky staining method, especially May Grunwald- Giemsa or its variations.
2. Other stains can be applied according to the need of diagnosis e.g. Gram's stain, Zeihl Nelson stain, Periodic Acid Schiff (PAS) stain, Alcian blue stain and Papanicolaou stain.
3. 1.5% glutaraldehyde fixative solution is used for electronmicroscopic (EM) study.
4. Specialized techniques are applied on immunohistochemistry for cancer markers.

Collection tips

1. Make and submit multiple slides

This is one of the most important thing that can be done to increase the diagnostic yield. Small gauge needle are used for collecting cytological specimens and prepare multiple slides. If possible, a minimum of four to five slides, from several sites within the lesion should be submitted from any lesion. If multiple masses are sampled, always use a new needle and syringe with each mass to avoid contamination from previous collection attempts.

There are many reasons why anyone slide may be nondiagnostic.

a) The needle missed the lesion during collection (geographic miss).
b) The needle is not in the area containing representative tissue of the lesion during sample collection. This is common in obese animals where the lesion may be surrounded by abundant subcut fat.
c) If cells are ruptured during smear preparation.
d) If slide prepared is too thick to evaluate.

1. Avoid blood dilution

Hemodilution is another common cause of nondiagnostic slides. The two major causes of blood contamination are the use of too large needle (<22-ga) and prolonged aspiration. Larger bore needles do not usually collect more cells but are more likely to rupture small blood vessels.

2. Don't be timid

Other reasons for poor cellularity of a sample are inadequate negative pressure and slow or shallow needle passages. (needle passages should be quick and of sufficient length)

Effusions are commonly encountered in veterinary practice. Effusions are the abnormal or increased accumulation of this fluid in any of the body cavities that are lined by mesothelial cells. These include the thoracic, pericardial, and abdominal cavities. These body fluids accumulate as a result of one or more of a number of disease conditions, including but not limited to trauma, neoplasia, cardiovascular compromise, metabolic disorders (hypoalbuminemia), and infectious/inflammatory diseases.

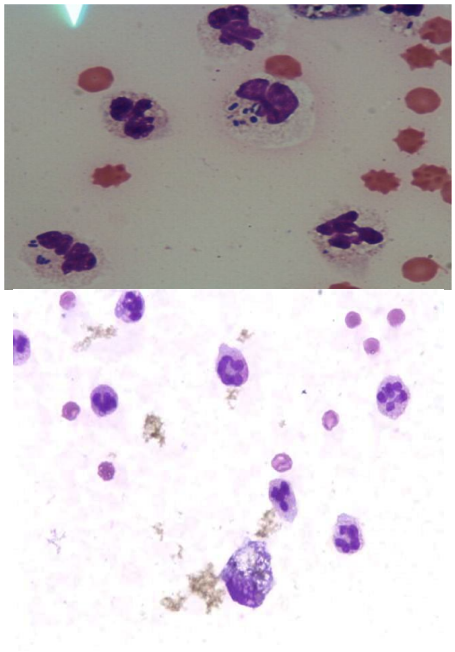
Aspiration biopsy needles

Design		Gauges
Chiba	25° bevel	22, 23
Turner	45° bevel	16, 18, 20, 22
Madayag	90° bevel	22
Greene	90° bevel	22, 23
Westcott	Slotter	20
Franseen	Trephine	18, 20, 22

Cells and structures seen in effusions

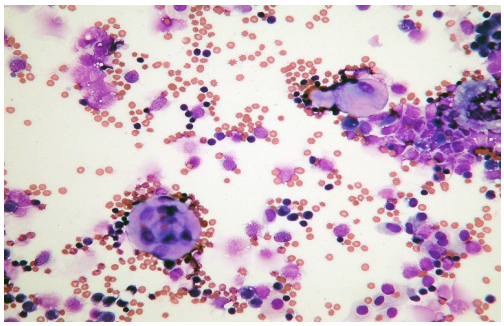
Neutrophils

Neutrophils are present to some degree in most effusions and tend to predominate in effusions associated with inflammation. Cytologically ,there are two types of neutrophils: degenerate and nondegenerate. (a) Degenerate neutrophils are neutrophils that have undergone hydropic degeneration. This is a morphological change that occurs in tissue or effusions because bacterial toxins alter cell membrane permeability. This allows water to diffuse into the cell and through the nuclear pores, causing the nucleus to swell, fill more of the cytoplasm, and stain homogeneously eosinophilic. This swollen, loose, homogenous eosinophilic nuclear chromatin pattern characterizes the degenerate neutrophils. (b) Nondegenerate neutrophils are neutrophils that have tightly clumped, hypersegmented, basophilic nuclear chromatin. These aged neutrophils are often seen phagocytized by macrophages (cytophagia). The presence of nondegenerate neutrophils suggests that the fluid is not septic, however bacteria that are not strong toxin producers, such as *Actinomyces* spp., may be associated with nondegenerate neutrophils.



Mesothelial / Macrophage – Type Cells

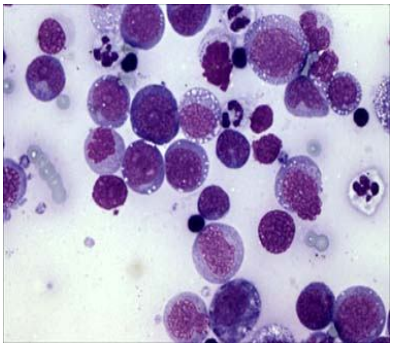
Mesothelial cells line the pleural, peritoneal, and pericardial cavities as well as visceral surfaces and are present in variable numbers in most effusions. They are large cells that may be present singly or in clusters. They generally contain a single round to oval nucleus but may be multinucleated. Their cytoplasm is slightly basophilic and may contain phagocytic debris, because activated mesothelial cells may become phagocytic.



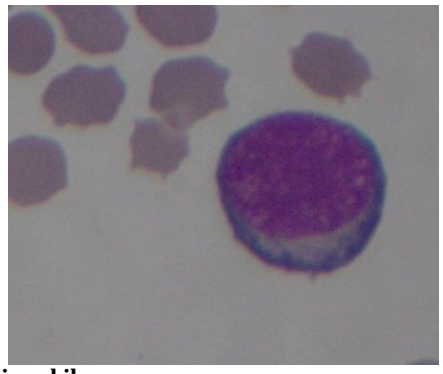
Lymphocytes

Lymphocytes are present in many effusions and may be the predominant cell type in chylous and lymphomatous effusions and sometimes in inflammatory effusions.

1. Chylous effusions primarily consist of small lymphocytes containing small amount of clear to blue cytoplasm, an oval to bean shaped nucleus, clumpy nuclear chromatin and no visible nucleoli. These cells are typically smaller than neutrophils.
2. Lymphomatous effusions primarily consist of lymphoblasts.Lymphoblasts are immature lymphocytes. They contain a light blue nucleolus which may be surrounded by a ring of chromatin. There are small vacuoles in the cytoplasm. These cells are not found in normal circulating blood and should be recorded if found in a differential smear.

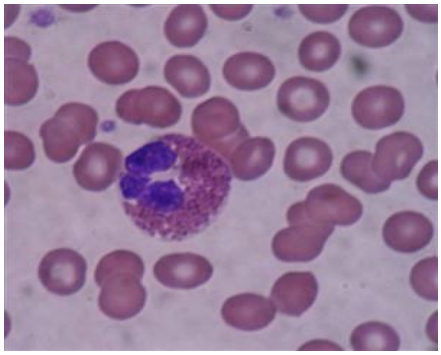


3. Reactive lymphocytes may be seen in inflammatory effusions. Reactive lymphocytes are also called plasma cells, are large lymphocytes with an egg shaped nucleus tending to locate at one end of the cells. They are about the same size as monocytes. The cytoplasm of a reactive lymphocyte stains a deeper blue and the chromatin is clumpedand may appear as spokes of a wheel. These cells are produced in response to an antigenic stimulus.



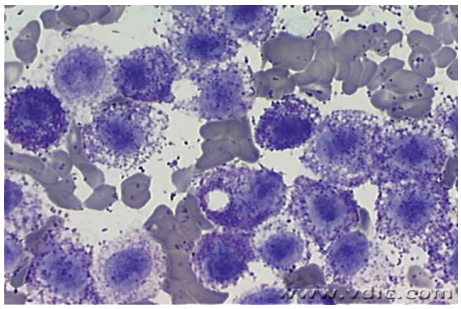
Eosinophils

Eosinophils may be present in effusions and are readily recognized by their rod- shaped, (in cats) or variably sized round (in dogs), orange granules.



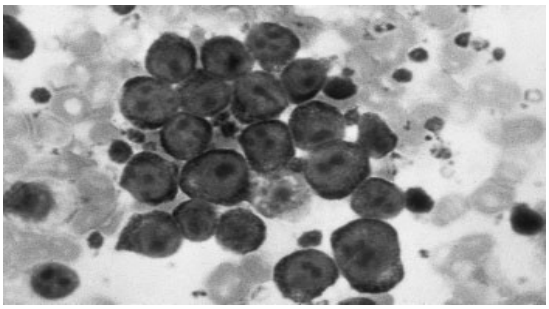
Mast cells

Mast cells are readily identified by their purple granules. Mast cell tumors within body cavities may be associated with effusions and frequently exfoliate large numbers of mast cells into the effusion.



Neoplastic cells

Neoplastic cells may be observed in effusions with many different types of neoplasia. Identification of the neoplastic cells depends upon the viewers ability to recognize the cell type and signs of malignancy. Fine needle aspirate of lymph node from dog with multicentric lymphoma. Cytology reveals a population of large, neoplastic lymphoblasts.



Classification of effusions in dogs and cats

	Total protein	Cells per milliliter	Cell types	Special features
1. Transudate	<2.5 g/dl	<1000	Mononuclear	Low cellularity
2. Modified transudate	2.5–5.0 g/dl	1000–8000	Mononuclear	Cell type varies with etiology
3. Nonseptic exudate	>3.0 g/dl	>3000	Neutrophils	Neutrophils Nondegenerate
4. Septic exudate	>3.0 g/dl	>3000	Neutrophils	Degenerate neutrophils
5.Hemorrhagic	>3.0 g/dl	Variable	Similar to blood	Erythrophagia or hemosiderin in macrophages
6. Neoplastic	>2.5 g/dl	Variable	Tumor cells	Neoplastic cell population identified

Transudates

Pure transudates most frequently form as a result of hypoproteinemia from either increased loss or decreased production of albumin (Forrester *et al.*, 1988). Albumin maintains the plasma colloidal osmotic pressure within the vascular system, preventing leakage of fluid into and promoting reabsorption of fluid from the extravascular compartments, such as the body cavities. Transudates resulting from hypoalbuminemia alone usually require plasma albumin concentrations to be less than or equal to 1.0 g/dl . If hypertension is also present, however, as is sometimes seen associated with liver diseases transudates may accumulate when albumin concentrations are greater than 1.0 g/dl (Cowell *et al*.,1989).

Clinical conditions that result in pure transudate formation

Dog with Ascites (fluid accumulation in abdomen)

Modified transudates

Occur as a result of fluid leakage from lymphatics carrying high protein lymph or blood vessels. Such leakage is caused by increases in hydrostatic pressure or permeability. Both of these conditions allow high protein ultrafiltrate fluid to pass in to the cavity. Neither of these conditions results in chemotactants in the cavity ; therefore large numbers of inflammatory cells do not migrate into the fluid. Hence, high-protien (2.5–5.0 g/dl) ,low to

moderate cellularity (1000–8000 cells/ml) fluid develops. The TNCC of the modified transudate overlaps that of the transudate. Modified transudates vary in color from amber to white to red and are frequently slightly turbid to turbid. Nondegenerate neutrophils, mesothelial/macrophage cell types, small lymphocytes, or neoplastic cells may predominate, depending on the cause of the effusion. In general, they are caused by conditions that produce an increase in vascular hydrostatic pressure or permeability within capillaries or lymphatics.

Conditions resulting in modified transudates include congestive heart failure, lung atelectasis, diaphragmatic hernia, acute organ torsion, partial or complete obstruction to the cranial vena cava (thoracic effusion), caudal vena cava (abdominal effusion), or any disease resulting in intrahepatic portal hypertension.

Exudates

Exudates are the result of leakage of fluid from abnormal or altered vasculature. This generally occurs because of an inflammatory process or chemotactic stimuli within the body cavity . The inflammatory process increases serosal and vascular permeability, resulting in a fluid with elevated protein and often a high TNCC. Exudates are further classified as septic or nonseptic depending on whether or not infectious agents are identified in the fluid. Classification as a septic exudate would indicate that microorganisms have been identified microscopically or by culture techniques. Nonseptic exudates result from noninfectious causes of inflammation in the body cavity, including conditions that cause long-standing modified transudates. Exudates may vary in color from white to amber to pink, but they are usually turbid. The protein content is usually high (>3 g/dL), and the cell counts are typically higher than 3000 cells per milliliter. The exception to this is uroperitoneum, which has a low cell count and protein because of the accumulation of urine in the body cavity. The numeric parameters of exudates overlap with those of modified transudates; however, in exudates, the neutrophil is generally the predominant cell population, indicating the presence of inflammation. The neutrophils are often accompanied by variable numbers of other inflammatory cells, including macrophages, lymphocytes, eosinophils, and mesothelial cells. The morphologic appearance of the neutrophils may give an indication as to whether the exudate is septic or not. Specific degenerative changes in neutrophils suggest the presence of sepsis. Degenerative changes are nuclear changes that indicate cell death. Some degenerative changes, such as pyknosis, are the result of slow cell death in a relatively nontoxic environment . Pyknotic cells are more typically observed in nonseptic exudates, and pyknosis alone should not alert the clinician to the presence of sepsis. Karyolysis and karyorrhexis are degenerative changes that indicate a more rapid cell death in a more toxic environment . Karyolytic cells have a pale swollen nucleus similar to the appearance of cells lysed during sample preparation. Cells lysed during sample preparation have a lysed cytoplasmic membrane, however, unlike karyolytic cells, where the cytoplasmic membrane is still intact. The presence of karyolysis and karyorrhexis warrants strong consideration of sepsis, a thorough examination of the fluid for the presence of intracellular or extracellular microorganisms, and microbial culture .Although most exudates contain a predominant population of neutrophils, some inflammatory fluids may, in addition to neutrophils, contain a significant (10%) eosinophil component. These exudates may be specifically termed eosinophilic effusions . Eosinophilic effusions are infrequently seen in veterinary medicine. In one study, approximately half of these effusions were associated with neoplasms, including lymphoma, systemic mastocytosis,and hemangiosarcoma (HSA). Other causes of eosinophilic effusions include allergic hypersensitivity conditions, parasitic diseases (eg, heartworm disease, intestinal parasites), pneumothorax, lung lobe torsion, intestinal lymphangiectasia, and lymphomatoid granulomatosis.

Nonseptic exudates

A number of clinical conditions, such as feline infectious peritonitis (FIP), foreign objects or material in the body cavity, pancreatitis, steatitis, bile or urine leakage, neoplasms, torsion of internal organs (eg, lung lobes, liver lobes, spleen), inflamed internal organs, or walled-off abscesses, may result in a nonseptic exudate.. Other nonseptic exudates have general features of elevated protein and TNCC with a predominant population ofnondegenerate neutrophils with lower numbers of hypersegmented neutrophils and pyknotic cells. Specific conditions resulting in nonseptic exudates include the following.

Uroperitoneum, Bile peritonitis, Feline infectious peritonitis (FIP.

Septic exudates

The identification of phagocytized intracellular organisms, usually bacteria, distinguishes a septic exudate from a nonseptic one. The absence of microscopically identifiable bacteria does not always rule out sepsis, however, and further investigation, such as culture, may be warranted when exudates are identified, particularly if degenerative changes are seen in the neutrophils. The predominant cell type in most septic exudates is the neutrophil. Many of these cells are degenerate as evidenced by nuclear karyolysis

(swollen pale nucleus) or karyorrhexis (nuclear fragmentation). Karyolytic neutrophils in an effusion warrant suspicion of sepsis, but definitive identification relies on the presence of intracellular organism. Extracellular bacteria may also be observed, but care must be taken to ensure that these organisms are not contaminants, normal flora, or present in used staining solutions. Numerous bacterial types have been associated with septic exudates in the dog and cat. Most septic exudates, particularly in the cat, involve anaerobes or facultative anaerobe. In general, identification of bacterial species and appropriate microbial therapy should be determined by culture techniques. The exception may be Actinomyces and Nocardia, which appear microscopically as characteristic long, filamentous, beaded rods along with the presence of ‘‘sulfur granules,’’ which are microcolonies of bacteria .Many septic exudates occur by introducing organisms into the body cavity via traumatic puncture wounds; bite wounds; perforation of the intestinal tract; migrating foreign bodies; ruptured pulmonary, hepatic, or prostatic abscesses; pyometra; pneumonia; or pleuritis. Most septic exudates involve bacterial sepsis; however, infections with Mycoplasma, rickettsial agents, fungal agents, and parasites may occur less frequently.

Hemorrhagic effusions

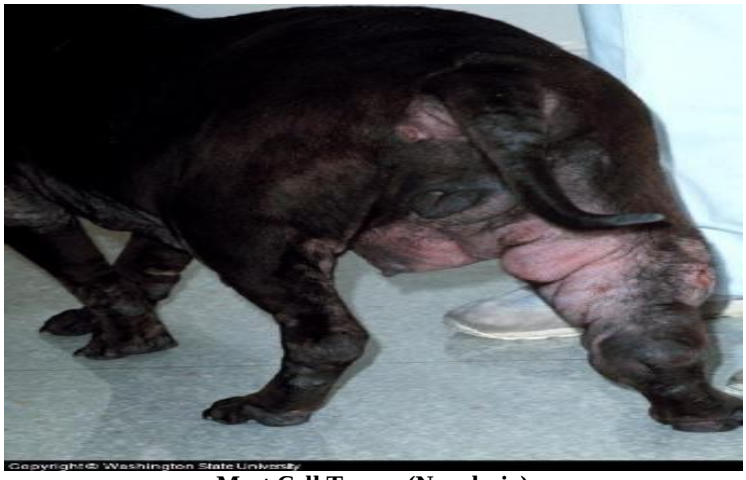
Hemorrhagic effusions can result from ruptured vessels or alterations in vascular endothelial integrity that is normally maintained by the interaction of platelets and various clotting factors. Hemorrhagic effusions grossly and microscopically contain a certain amount of blood, and the PCV of the fluid should be at least 10% to 25% of the peripheral blood. These fluids must be distinguished from the iatrogenic blood contamination that might occur during any sampling procedure. Several factors may help in distinguishing between these two processes, but peracute hemorrhage occurring less than 45 cinomas, adeno carcinomas and rarely sarcomas have been diagnosed by cytologic evaluation of effusions.

minutes after sampling may be impossible to distinguish from iatrogenic contamination. One distinguishing factor is that platelets are usually not seen in hemorrhagic effusions present for more than 1 hour before sampling. Similarly, because of the rapid mechanical defibrination that occurs after extravagation, blood that is the result of hemorrhage into a body cavity does not clot, even in a clot tube. Additionally, true hemorrhagic effusions eventually contain reactive macrophages with phagocytized erythrocytes or intracytoplasmic hemosiderin and/or hematoidin. Conversely, iatrogenic contamination with peripheral blood during sampling contains platelets and usually clots after collection. There are no specific numeric values that define a hemorrhagic effusion; however, hemorrhagic fluid with leukocyte counts significantly higher than that seen in the peripheral blood should be considered inflammatory as well.

Neoplastic effusions

Neoplasia is a common cause of effusions in dogs and cats. In one report, 57% of pericardial effusions and 11% of peritoneal and pleural effusions in the dog were the result of neoplasia. In the same study, neoplastic effusions accounted for 37% of the pleural effusions in cats. Neoplastic processes occurring within the body cavities may result in various types of fluid accumulations, including modified transudates, exudates, and hemorrhagic effusions. In one study involving more than 400 peritoneal and pleural effusions in dogs and cats, even in the hands of experienced cytopathologists, cytologic evaluation for the detection of tumors had a sensitivity of 64% and 61% in dogs and cats, respectively (Hirschberger *et al*. 1999). Most effusions caused by tumors not exfoliating neoplastic cells are in the modified transudate range. However, most effusions caused by tumors that are exfoliating cells into the cavity and are secondarily inflamed are in the exudates category. Lymphoma, mast cell tumor, mesothelioma, and various car

Hemangiosarcoma in a dog



Mast Cell Tumor (Neoplasia)

Complications of FNAC

Complications are few and seldom serious. The incidence of major complications reported is well below 1% and generally in the range of 0.05% (Frable,1989). Serious complications include: needle track seeding, pneumothorax in animals with axillary masses, transient acute swelling, hematomas, and histological alterations. More serious and sometimes life-threatening complications may occur with aspiration of deep organs. In the chest, these include massive hemorrhage, air embolism, and tamponade. Risk factors to be considered that may influence the development of complications following FNA are patient'sage, presence of underlying disease, and bleeding disorders. Also exerting influence on the rate of complications are location, size, and the depth of the mass; needle size; number of passes andlevel of experience of the aspirator.

Benefits of FNAC

- Cost effectiveness (simple and cheap)
- It has lower risk than surgical biopsy.
- It is readily repeatable and useful for multifocal lesions.
- Minimal physical and psychological discomfort for the patient.
- Rapid reporting and bedside diagnosis of neoplastic, hyperplastic, and inflammatory masses.
- Therapeutic procedure for the evacuation of cystic lesions.
- Permits the diagnosis of some benign conditions for which there is no need for surgery.
- It is a rapid means of confirmation of recurrence of previously treated malignancy without surgery (Vargas and Masood, 2003).

Limitations

- Sampling is scanty and histological architecture is lost thereby rendering impossible diagnosis based on histology.

- Inflammatory, metaplastic or degenerative lesions may mimic malignancy.
- Diagnosis is indefinite in some conditions such as follicular adenoma vs. carcinoma of the thyroid.
- Samples taken may not be representative of the lesion.
- Difficulty of cytological diagnosis in some conditions e.g. lymphomas (Orell,2003).

CONCLUSIONS

Fine needle aspiration is a relatively non-invasive, less painful and quicker method when compared to other methods of tissue sampling such as surgical biopsyand has a significantly shorter recovery time. It is a quicker method of diagnosis of diseases like cancer, giving more options for treatment, or that benign lumps are diagnosed without the need for surgery.

Fine needle aspiration biopsies do require some expertise to perform and interpret. To ensure that an accurate result is achieved, it is important that the general practitioner, radiologist, surgeon, pathologist or oncologist who performs procedure has experience in fine needle aspiration biopsy. FNA is being increasingly utilized in clinical diagnosis in order to avoid invasive investigations, surgical biopsies and costly exploratory surgeries (Diamantis *et al*.,2009). The technique allows cost-effective and efficient tissue sampling and has the potential to provide cells and fluid for molecular, genetic and proteomic analyses. These analyses may become very important in the future for diagnosis and prognosis of tumor disease for establishing the effectiveness of therapy.

REFERENCES

1. Cowell RL, Tyler RD and Meinkoth JH. Abdominal and Thoracic Fluid. In: Cowell RL, TylerRD (eds). Diagnostic cytology of the dog and cat. Goleta. Am Vet Publ. 1989; 151–166.
2. Diamantis A, Magiorkinis E and Koutselini H. Fine-needle aspiration (FNA) biopsy: historical aspects, *Folia Histochemica et Cytobiologica*. 2009;47(2):191-197.
3. Forrester SD, Troy GC and Fossum TW. Pathophysiology and diagnostic considerations.Compend Contin Educ Pract Vet. 1988; 10:121–137.
4. Frable WJ. Needle aspiration biopsy: past, present and future.Hum. Patho. 1989;20: 504-517.
5. Hirschberger J, DeNicola DB, Hermanns W. Sensitivity and specificity of cytologic evaluation in the diagnosis of neoplasia in body fluids from dogs and cats. Vet Clin Patho. 1999;28:142–146.
6. Orell SR. Pitfalls in fine needle aspiration cytology. Cytopathology. 2003;14:173-182.
7. Vargas HI and Masood S. Implementation of a minimally invasive breast biopsy program in countries with limited resources. Breast J.2003;9(2):81-85.
8. Webb AJ. History of Fine Needle Aspiration Livingstone. Philadelphia. 2003;237-255.