Abstract

Cytologic diagnosis by fine needle aspiration and other sampling techniques is a common and effective technique. Minimal equipment is needed. A diagnosis is available within an hour or less. Most veterinarians can learn to make many cytologic diagnoses within weeks. Cytologic diagnosis is a complement to other diagnostic methods such as ultrasound and histopathology. No test is 100% sensitive and 100% specific and one must understand the limitations of cytology as well as the advantages. Cytology is effective in neoplasia diagnosis but is usually not as specific as histopathology. Thus one should consider a cytologic diagnosis as tentative until a histologic diagnosis is made, with certain exceptions, especially lymphoma and hematopoietic neoplasia. Veterinarians can overestimate or underestimate the accuracy and precision of cytology as a diagnostic test. Cytologic diagnosis of infections is often more specific than histopathology because examination is more often with oil immersion lens of individual cells and organisms instead of relatively thick histologic sections examined with low magnification. One makes a cytologic diagnosis of inflammation and infection more often than neoplasia and those diagnoses are easier.

Recommended Reading


Author's references

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Representative Sample

The key to a good cytologic diagnosis is a good sample. The sample must come from a representative region of the lesion, organ or tissue being evaluated. There must be enough information on the smears to allow a conclusion. The cytologist should know what the clinical question is. That question may be as simple as “aspirate of a skin tumor” to a copy of the patient's journal with a hepatic aspirate and the specific question "Does this dog have a copper storage disease of the liver?". A cytologic report should begin with a description of the sample provided. That description is meant to indicate to the reader how much confidence one should have in the conclusion based on the number and quality of the cells and other material available in the sample for evaluation. The submitter of the sample should not be offended by these comments. One expects samples to vary in their cellularity and appearance, because they are taken from sites that vary in their composition.

A representative area of a lesion means that the cells obtained should lead to a diagnosis of the primary problem. A common error is to take a swab or impression smear from an ulcerated, infected, necrotic surface or even normal surface over a neoplasm in hopes of obtaining a diagnosis of a primary problem that is deeper. It is often easier to take a swab from a surface than to aspirate a neoplasm with viable cells but those samples are often not diagnostic. The cytologic conclusion of such a sample is likely to be inflammation and infection based on abundance of leukocytes and bacteria. The primary diagnosis could be a neoplasm under that ulcerated surface. A specific example is bronchial brushing of bronchi even those bronchioles near a lung tumor. The samples often have only hyperplastic epithelial cells, mucus or exudate, while an ultrasound guided fine needle aspirate of a lung mass usually gives the correct diagnosis of a neoplasm. Fluid cytology of fluids near neoplasms in the pericardial sac or abdomen often have only pathologic bleeding and or reactive mesothelial cells, so the absence of neoplastic cells in a sample does not mean the animal did not have neoplasia.

How many cells with good morphology are needed for a correct diagnosis? One study on mammary tumors evaluated only samples with over 200 epithelial cells with good morphology. A reasonably confident diagnosis can be made with much fewer cells in given situations. If an aspirate comes from the site where a mast cell tumor was removed earlier, a group of 5-10 immature mast cells on recheck can indicate regrowth.

Cells with good morphology are usually found in smears with a thin, monolayer area, which can be stained well. Thick smears have many cells but they may be too darkly stained and covered by other cells to identify cell features or even what the cell's identity is. Lymphoid cells, cells from endocrine tumors and cells from necrotic areas are very fragile and are easily lysed. Lysed cells and partially lysed (swollen cells) should not be used for a diagnosis. Partially swollen cells may have larger nuclei and nucleoli and thus look immature (for example like lymphoblasts in a lymph node aspirate) and cause an incorrect diagnosis of a malignant neoplasm.

Technique

A common technique is fine needle aspiration of tumors and organs. Fine needle means 23 G (0.6 mm) or smaller needle. This avoids tissue plugs more common in large needles. One should insert the needle "in and back" through a mass from one edge and repeat in another direction through the lesion after redirecting the needle. Do not slice and cut sideways through tissue causing tissue damage like cutting through tissue with a knife. If the lesion is likely vascular (e.g., liver) one can simply pack the needle with cells and not use any vacuum.
Expel a medium sized drop of fluid on a glass slide and slowly and gently streak it out as a blood smear. Drops that are too small (spray artifact) caused by spraying fluid out of the syringe, dry faster than one can streak them out in thin smears. Cells in these thick drops are too dark to evaluate well. Drops that are too large may cause smears that are too long and run off the end of the glass slide before a thin area is obtained. Some automated staining machines do not stain the last 5-10 mm of a glass slide, so the smear should end before that region.

Fluid cytology is a topic for itself, but the cell count and protein concentration are very useful in interpretation of fluids from body cavities (peritoneal, thorax, joint, CSF). Very thick fluids may require a "squash prep" to make a thin smear. Very cell poor fluids (CSF, BAL) require a cytocentrifuge to obtain consistently good smears with good morphology.

Cytologic Interpretation

What are the easiest diagnoses? These are ones that a veterinarian should not send to a cytologist and pay $25-50 for a diagnosis. Some skin masses are common and easy. Lipomas have many fat droplets on the unstained glass slides and few mature fat cells when stained. Most can have a diagnosis before streaking out the drop of fluid from the aspirate, but one should stain the smears to be sure they do not contain other cell types. Epidermal inclusion cysts have large numbers of mature squames and a little debris. Mast cell tumors have large numbers of mast cells (round cells with round nuclei and many granules) and perhaps many eosinophils. Malignant mast cell tumors may be confusing but well differentiated tumors are easily diagnosed. Abscesses have many neutrophils and often many bacteria. Other inflammatory skin lesions may have variable numbers of macrophages, lymphocytes and eosinophils. Bacteria, fungi or foreign bodies may be seen. When in doubt send it to a cytologist and then compare the cytologist's report with your initial impression. It is not hard to see where the border is between what you can diagnose and what you need to send out. One will also not have to pay for a cytology review when the smears have only blood and not enough cells for anyone to make a diagnosis. Fibrous hard lesions may not give up cells to an aspirate and if no cells are present in the aspirate, there is no reason to send the sample for diagnosis.

Inflammatory lesions should have moderate to large numbers of leukocytes. The type of leukocyte indicates the morphologic diagnosis (e.g., chronic pyogranulomatous inflammation). Based on the type of inflammation, site and clinical presentation, one looks for certain types of causes, usually bacteria. If a fungus is found it is first described. The size is judged against erythrocytes (6-7 um in diameter) or neutrophils (15 um). One uses various atlases (see reading list) to aid in identifying fungi and parasites.

Diagnosis of neoplastic lesions requires more experience and training. But most beginning cytologists can recognize very malignant neoplasms when the smears have large numbers of non-inflammatory cells that look very immature and variable. There are 2 main questions to answer with samples containing large numbers of one type of tissue (non-inflammatory cell) cell. One is "How malignant does it look?" The other is "What type of cell is this?".

Cytologic criteria of malignancy usually are based on increased variability and immaturity of the cells. Benign cells should be fairly uniform in size, shape and maturity. Increasing variation in size and shape of chromatin, nucleoli and nuclei indicate increasingly strong evidence of malignancy. The more immature a cell appears, the more likely it is to be malignant. Immaturity is indicated by larger size of nuclei and nucleoli and that the chromatin becomes finer (smaller granules) and more spread out with clear space between granules. Irregular chromatin patterns (e.g., parachromatin clearing) indicate malignancy. Nucleoli, which vary greatly in size, number and shape (sharp angular nucleoli, not round), indicate malignancy. Atypical mitotic figures (tripolar, lagging chromosomes) are strong indicators of malignancy when present.

Cell type is indicated by cell shape and any tendency to bind together. Epithelial cells (and mesothelial cells, endothelial cells and synovial cells) form surfaces so have tight cell:cell junctions that persist to some degree in a smear. Finding cells in pairs, rows, sheets or other
patterns suggest an epithelial cell origin. Mesenchymal cells often form spindle or stellate cells. One may see an intracellular matrix like collagen or osteoid to indicate a connective tissue (mesenchymal cell origin). A cytologic diagnosis that a mass is neoplastic, malignant and epithelial cell in origin is what should be expected. A cytologic diagnosis of "carcinoma" (type not indicated) from a lung mass is a very adequate diagnosis for deciding what to do next with the animal. Too many lectures and books give the impression that cytology should give specific diagnosis to neoplasms (e.g., malignant hemangiopericytoma). Specific typing of neoplasms should be left to histopathology.

Cytology smear from many types of tumors have some round cells that are individual (discrete) but there are some tumors classified as round cell tumors. These are lymphomas, mast cell tumors, transmissible venereal cell tumor and canine cutaneous histiocytoma. Some other malignant neoplasms such as amelanotic melanoma and anaplastic carcinoma may have mainly round cells. I have used the diagnosis "very malignant large round cell tumor" in cases that were not the traditional 4 round cell tumors. A diagnosis that a mass is a very malignant neoplasm is often quite sufficient for the veterinarian to give proper advice to the owner.

In summary, cytologic diagnosis is very useful to give a rapid diagnosis. One must understand how much confidence to put in that cytologic diagnosis, given the clinical presentation. That requires an understanding of what evidence was available and how much is needed for a confident diagnosis. That confidence varies with each case and situation. When in doubt talk to the cytologist if you did not look at the slides yourself. You may provide new information that allows the cytologist to come to a different conclusion or at least ranking of what diagnosis is most likely.

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