Cytology and biopsy of neoplastic lesions

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Cytology and histopathology are complimentary procedures with a compromise of possibly obtaining less information on cytology but having the advantage of being less intrusive. Histopathology evaluates architecture which allows for more detailed information on invasive potential and evaluation of margins but at the expense of the requirement for anaesthesia and higher costs and effects on the animal.

Limiting factors of cytology include the experience of the pathologist, but more importantly the quality of the sample.

A detailed history, signalment, site of aspiration or biopsy is fundamental when submitting cytology or histological preparations.

"Failure to provide complete and accurate information limits the ability of the pathologist to make clinical correlations from the histomorphologic findings and could even lead to misdiagnosis". Quote by John Cullen. NCSU College of Veterinary Medicine.

Sample collection for cytology

There are three basic techniques for sampling solid lesions:

- fine needle aspiration - with or without suction - methods of choice
- impression smears
- scrapings/swabs

Sampling techniques

Fine needle aspiration biopsy (FNA / FNB) with suction
Using 21-23G needles with a 5-10ml syringe is recommended. During aspiration, application of negative pressure should not exceed a few seconds, as this will cause disruption of blood vessels, and blood contamination.

Non aspiration technique for FNA/ FNB
Some authors consider this technique to be superior to other aspiration techniques. A syringe can be primed with approximately 1ml of air before sampling. The needle is then attached, so that the syringe can be used as a handle to house the needle without applying negative pressure.

Impression smears or Scrapings from surfaces
This technique is useful for ulcers, skin lesions or the cut surfaces of masses. The surface material often contains only necrotic cell debris, neutrophils, macrophages, squames and contaminating bacteria. Smears can be made from biopsy specimens after the blood has been blotted off the cut surface with a gauze swab.

General tips that may enhance the ability to obtain a cytological diagnosis

1. If the mass is large, aspiration of different areas may help alleviate inadequate sampling of the representative portion of the mass.
2. Surface sampling generally yields inflammation/necrosis and/or secondary infection.
3. Smears should be made quickly as slow slide making technique may cause clotting and /or deterioration and lysis of cells, with obvious loss of cellular detail.
4. Spraying material onto the slide with a shotgun effect is contraindicated as material tends to dry in thick blobs on the slide.
5. A starfish preparation (smearing cells with the needle) has been described - the cells are generally intact but are usually too thick for

evaluation.
6. If multiple masses are aspirated, fresh syringes and needles should be used for each mass, and smears labelled appropriately (on the same side of the slide that contains the material).
7. Submission of a number of appropriately labelled slides (4-6) is recommended to ensure adequate material is examined. This is imperative if sedation or anaesthesia is required in fractious animals.
8. Label slides clearly with a pencil on the frosted end with animals' name and sample site. Ballpoint pen or permanent markers dissolve in the fixative, wash off or become smudged during the fixation process (alcohol fixation with alcohol based stains, e.g. Diff-Quik®)
9. Strips of bandage stuck around slides and labelled is unacceptable practice.
10. Some stains may not stain all cells. For example Diff-Quik® does not reliably stain mast cells (especially in cats) and leukocyte granules in some other conditions such as mucopolysaccharidosis.
11. Double fixation of smears (up to two minutes) will enhance staining of mast cells with Diff-Quik® in cats.
12. Smears submitted to the lab with formalin fixed tissue for histopathology should not be packed in the same container, as formalin contamination (including formalin fumes) prevent proper staining with all Rowmanowsky stains (includes Diff-Quik®).
13. Refrigeration of any smears (cytology or blood smears) should be avoided to prevent condensation on the slide.

Histopathological samples – some tips and path peeves

1. Describe all lesions – describing one mass in the history and then squashing six into the pottle is unsatisfactory.
2. Ice cream containers with large amounts of cellotape or bandaging are inadequate and present a hazard to staff and couriers.
3. Samples should be placed into 10% buffered formalin at a 1:10 ratio in a wide necked sturdy plastic container. The lid should have an "O" ring seal.
4. Glass is not recommended. Samples should also be double/triple bagged – in accordance with courier regulations.
5. Avoid incising into the deep margin.
6. Surgical lamps can dry tissue, and thus immediate immersion in fixative is ideal.

Large samples such as whole limbs should be refrigerated and sent on ice, but should not be frozen. In cases of a large tumours, parallel incisions can be made (bread loafing) but this should not affect margins or tissue orientation. If very large, e.g. spleens, mammary strips, the sample can be prefixed in a large bucket then sent drained and triple bagged, or a smaller sample taken (which would preclude evaluation of margins).

Very small samples (endoscopic or pinch) should be placed in screen containers - preferably not on sponge or cardboard as these are prone to artefact. Multiple samples are recommended. Crush artefacts and/or forceps marks in small tissues can completely ruin the specimen. Small samples may result in non-diagnostic samples and one should be prepared (and make sure owner is prepared) that repeat biopsy may be necessary to get larger biopsies.

Wedge biopsies are preferable to needle biopsies as a study showed discordant diagnosis for about 50% of cases. A single core from a biopsy needle only represents less than $1x10^{-5}$ of the total liver of a 35kg dog.

Luminal organs should be flushed through with formalin.

Thin flat samples such as bladder or stomach should be placed into a tissue cassette with a foam pad to prevent curling. They can also be loosely stitched to pre-soaked cardboard. Needles are not to be used to pin samples to cardboard.

Assessment of margins

This is not possible with masses that have been cut in half, and only half submitted. It is recommended that margins are inked (within 30 minutes using surgical ink, applied with a swab or wooden applicator stick) or sutures can be placed, (specific numbers) prior to placing in formalin as this causes shrinking which may alter

margins. The submission should be accompanied with a written explanation for orientation.

Large masses that require bread-loafing should be inked first. This should be allowed to dry for 10 mins before placing in formalin.

Black, yellow or green are preferable to red or blue as these latter two colours may be difficult to identify on H&E sections.

Samples should also be taken from the tumour bed, appropriately labelled and submitted for examination.

Tips for biopsy of various tissues

Liver: Wedge biopsies are preferable over core biopsies as these are small and prone to crush artefact. They often contain samples of lung and muscle.
Lymph nodes: a full thickness wedge or whole node.
Skin: 4mm punch biopsies of skin. Skin masses should be completely excised rather than using skin punches or Tru Cuts, and should be submitted without post-surgical incision/s.
Gut: pinch biopsies of gut are often too small to demonstrate adequate architecture.
Spleen: Submission of splenic mass biopsies are preferred to submission of whole spleen.
Bone biopsy: Jamshidi needle biopsy used. Two radiographs (lateral and cranio-caudal) are evaluated. It is important to biopsy the centre of the lesion, which can be located using palpable anatomical landmarks. Biopsy of the periphery may result in sampling normal reactive bone surrounding the tumour.

Grading and staging of some of the more common tumours

Mast cell tumours

Histopathology is required for grading (low grade versus high grade). Grading is based on mitotic index, number of multinucleated cells (three or more nuclei), bizarre nuclei, or karyomegaly.

Staging includes CBC, Biochemistry, abdominal ultrasound, regional lymph node cytology.
Abdominal ultrasound and FNA of spleen or liver if enlarged; if nodal metastases or systemic signs present; or if known high grade tumour.

High grade tumours are associated with shorter time/potential for metastasis. Prognostic panel and proliferation markers for MCT can be done on paraffin blocks and is recommended especially for high grade tumours where tyrosine kinase inhibitors are to be used for therapy.

Prognostic panel markers for MCT (immunohistochemistry):
- c-KIT pattern (expression) staining (this has also been detected in internal aggressive tumours in cats)
- Ki-67 index - proliferation index
- AgNOR quantification - proliferation index
- Proliferation markers (PCR) - KIT Mutation detection

Refer to flowchart:
https://www.dcpah.msu.edu/Sections/Immunohistochemistry/WEBCD.IHC.REF.003.pdf
https://www.dcpah.msu.edu/Sections/Surgical_Pathology/

Malignant lymphoma

This is one of the most common tumours in dogs and cats. It is a systemic disease except for epitheliotropic lymphoma of the skin, e.g. usually originate in lymph nodes, spleen, and bone marrow, but can arise in other tissues. The most common forms of lymphoma in the dog are intermediate to high grade, multicentric (involve all nodes).

Staging provides useful diagnostic and prognostic information. Recommendations include:
Full CBC, chemistry (including ionised Ca), bone marrow evaluation three-view chest radiographs, abdominal

imaging/ultrasound.

Immunophenotype (immunocyto/histochemistry gives prognostic information for T (CD3) and B (CD20, 79a). The latter is most common and generally has a better prognosis.

Histopathology is indicated (if questionable cytology, solitary node, slowly growing nodes, desire for more detailed histology information, or low grade indolent tumours (which is a difficult diagnosis on cytology alone) and which may be reported as reactive, hyperplastic or as emerging lymphoma.) (In some cases, cytologic samples from indolent lymphomas are reported as consistent with intermediate to high grade lymphoma.)

Advanced imaging (CT/MRI if suspected CNS involvement).

Flow cytometry (liquid suspensions) and PCR for antigen receptor rearrangement (PARR [clonality] – liquid or cytology slides) look for abnormalities within lymphoid populations - homogenous lymphoid populations are more likely to be lymphoma or leukaemia.

The World Health Organization (WHO) classification of stages of lymphoma (based on degree of metastasis and invasiveness) are as follows:

- **Stage I: S**ingle lymph node or lymphoid tissue in single organ (excluding the bone marrow)
- **Stage II:** Regional lymphadenopathy (restricted to one side of diaphragm), with or without involvement of the tonsils.
- **Stage III:** Generalized lymphadenopathy
- **Stage IV:** Enlargement of the liver and spleen or hepatosplenomegaly (with or without lymphadenopathy)
- **Stage V:** Bone marrow, blood or involvement of other extranodal sites (eyes, CNS kidneys, skin, gastrointestinal tract, lungs, etc.

Stages are further classified to clinical substage a (no clinical signs) or b (with clinical signs/hypercalcaemia (usually T cell). This aids in prediction of remission duration and survival; dogs in substage b have shorter remission and survival times.

Less than 29% of dogs have indolent lymphoma (this varies depending on the study), which includes follicular lymphoma (all appeared to be B-cell derived), marginal zone lymphoma and T-zone (T-cell derived) lymphomas.

In dogs, negative prognostic indicators for lymphoma include stage IV or V disease, substage b disease, T-cell immunophenotype, presence of a mediastinal mass, anaemia and prolonged pre-treatment with corticosteroids.

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