Cancer Cytology Interactive Case Series

Douglas H. Thamm, VMD, DACVIM (Oncology) Colorado State University

Fort Collins, CO

While cytology does not give the practitioner the same amount of information as histopathology does, it can provide important information that is rapidly available, inexpensive, and minimally invasive. Cytology can provide important information that can change how subsequent treatment and diagnostics take place. For example, the surgical approach to a subcutaneous mass may be altered depending on whether the mass is a lipoma or a mast cell tumor. Similarly, additional staging tests may be indicated prior to surgery for a malignant mass (e.g. thoracic radiographs, evaluation of the regional lymph node) that would not be necessary for a benign mass. Similarly, intra-operative cytology can sometimes be utilized to make rapid decisions about treatment when necessary. Even if a cytologic diagnosis cannot be made in-house, one critical contribution the practitioner can make is to evaluate the quality of a sample prior to its submission to an outside lab. In the event that a sample is inadequate, additional samples can be obtained while the patient is still in the clinic.

Obtaining the sample

There are a number of different ways that cytologic samples can be obtained. These can include needle aspiration, impression smears, scrapings, swabs, and evaluation of cell pellets from fluids.

For needle aspiration, the author usually recommends between 20 and 25 gauge, 1-inch needles. A 22-gauge needle is sufficient for most masses. Smaller needles can be used for masses where blood contamination occurs or where it is expected (e.g. thyroid), and larger needles for masses that exfoliate poorly. Two techniques have been described for aspiration: the "bare needle" or "stab" technique and the "syringe technique". The "bare needle" technique works well for most masses, and is preferred for masses where blood contamination is encountered. For the "bare needle" technique, the mass is immobilized, the needle is introduced, and then rapidly introduced into different parts of the mass in a pecking fashion. Two situations in which the "syringe" technique may yield better results are for tumors that do not exfoliate well (dry needle) and for very small tumors, where there is not room to peck the needle into the mass.

In contrast to its utility in dermatologic disease, impression smears are rarely useful for external lesions when neoplasia is suspected, as surface contamination with bacteria, inflammatory cells and debris often obscures the underlying disease process. In the author's practice, impression smears are primary used to evaluate incisional biopsy samples after they are obtained, either to obtain preliminary information on the possible diagnosis or to confirm the adequacy of the sample.

In the author's practice, scrapings of superficial lesions or swabs of cavities (e.g. ears, nasal cavity, vaginal vault) are also rarely useful when neoplasia is expected, due to the risk of surface contamination.

Smears of cell pellets can often be a useful way to concentrate the cellular component of a fluid such as effusion or urine. The sample is centrifuged, the supernatant is poured off, and then the pellet can be rolled onto a glass slide using a cotton-tipped applicator. The author often finds this technique useful for evaluating urine samples when transitional cell carcinoma is suspected.

Preparing the sample

For aspirates or fluids, there are 2 different techniques that can be utilized for making the smear: These include the pull-apart technique (horizontal or vertical) and the blood smear technique. A combination of the 2 techniques can sometimes be used on the same slide. The goal is to create at least one area on the slides that contain a monolayer of cells of relatively high density. It is critical when preparing cytology slides that the slides not be exposed to formalin fumes. This is most important when shipping slides to an outside lab. Air-dried cytology samples and formalin containers should never be shipped together.

Common problems

- Not enough cells: This can occur with tumors that do not exfoliate well (e.g. sarcomas), in very small lesions, in lesions with necrotic centers, or if sampling has been too timid (needle too small, inadequate vacuum).
- Blood contamination: This is encountered with excessive or prolonged aspiration in some tumors, and in highly vascular lesions (thyroid, haemangiosarcoma). Failure to blot impression smears can also lead to excessive blood contamination.
- Broken cells: This can occur if too much pressure is applied when using the pull-apart technique, if impression smears are smeared horizontally across the slide, and in tissues where the cells are excessively fragile (lymphoma would be the most common example).
- Too thick: This can occur with soft lesions that exfoliate very well (lymphoma), if too large a needle is used, or if a sample is not spread sufficiently on the slide.

Microscopic evaluation of samples

The author uses a 4-step diagnostic algorithm when evaluating suspected cancer cytology samples. The benefit of this algorithm is that it is not necessary to answer all 4 questions in order to obtain meaningful information about a case. The questions to answer, in order, are:

- 1. Is the sample adequate?
 - If not, re-sample.
- 2. What general class of disease does the sample fall into?

Normal/hyperplastic Inflammatory/infectious Neoplastic

3. If neoplastic, what is the tissue of origin?

Discrete/round cell Epithelial

Mesenchymal

4. Does the lesion appear benign or malignant?

Discrete or round-cell tumors include lymphoma, mast cell tumor, histiocytic tumors, transmissible venereal tumor, and plasma cell tumors. Poorly differentiated melanomas and sarcomas can sometimes have a discrete cell appearance. As the name implies, round cell tumors generally have a round shape, exfoliate abundantly, and the cells tend to remain discrete rather than exfoliating in sheets, clumps or clusters.

Epithelial tumors (adenomas, carcinomas) also generally exfoliate well, and are much more likely to form sheets or clumps. Furthermore, in certain epithelial tumors, acinar or glandular structures may be observed. The individual cells are typically round to polygonal.

Mesenchymal tumors classically do not exfoliate as well (although there are exceptions to this), and typically are individual rather than clumped or clustered. Many have a spindloid or fusiform shape, although poorly differentiated cells can sometimes be fairly round.

The classic cytologic criteria of malignancy include: Anisocytosis / anisokaryosis (variation in the size and shape of the cells and nuclei, respectively), macrokaryosis (large nuclei), increased nuclear:cytoplasmic ratio, multinucleation (can be normal in certain cell types), frequent or abnormal mitoses, and multiple or large nucleoli.

Step 1: Adequate YES NO sample? Step 2: NORMAL General INFLAMMATORY class? NEOPLASTIC Step 3: DISCRETE Tissue of **EPITHELIAL** Origin? MESENCHYMAL Step 4: BENIGN B9 vs MALIGNANT Malignant?

A schematic describing this 4-step algorithm is included below

The second hour of this session will apply this 4-step diagnostic algorithm to a series of clinical oncology cases.

References

Ghisleni G, Roccabianca P, Ceruti R, et al (2006). Correlation between fine-needle aspiration cytology and histopathology in the evaluation of cutaneous and subcutaneous masses from dogs and cats. Vet Clin Pathol Vol. 35: 24-30.

Langenbach A, McManus PM, Hendrick MJ, et al (2001). Sensitivity and specificity of methods of assessing the regional lymph nodes for evidence of metastasis in dogs and cats with solid tumors. J Am Vet Med Assoc Vol. 218: 1424-1428.

Meyer DJ. The acquisition and management of cytology specimens. In: Raskin RE, Meyer DJ (eds.). Atlas of Canine and Feline Cytology. Philadelphia, PA USA: Saunders, p. 1.

Rogers KS. Collection of specimens for cytology. In: Bonagura JD, Twedt DC (eds). Kirk's Current Veterinary Therapy XIV. St. Louis, MO USA: Saunders Elsevier, 2009, pp. 301-305.

Thrall MA. Diagnostic cytology in clinical oncology. In: Withrow SJ, Vail DM (eds.). Small Animal Clinical Oncology, 4th Ed. St. Louis, MO USA: Saunders Elsevier, 2007, pp. 112-133.