Histovet
Surgical Pathology

Choosing the Right Biopsy for the Job

Over the last five years, dramatic changes in imaging techniques and biopsy instrumentation have been nothing short of a revolution in veterinary medicine. Whereas formerly histologic evaluation was limited to samples obtained by surgical excision, today you have the additional options of ultrasound-guided fine needle aspiration, ultrasound-guided trucut biopsies, or visually-guided samples obtained via laparoscopy or endoscopy. It is easy to find descriptions of these techniques, but it is not at all easy to find any discussion about what technique is most appropriate for the disease that you suspect.

The principle is a simple one: cytology is appropriate when you suspect a disease in which the tissue architecture is not important, and in which the lesion is more-or-less diffuse within the tissue. Tissue core biopsies (most often, trucut biopsies) retrieve only tiny histologic samples. They are appropriate when you anticipate the histologic changes will be widespread within the tissue, and when you anticipate the need for at least some appreciation of architectural arrangement of the diseased tissue. Finally, larger traditional incisional or excisional biopsies are required when you anticipate the need for a broad geographic overview of the tissue organisation, to appreciate things like tumor invasion or the relative amounts of normal and abnormal tissue that may be critical to prognosis.

Cytology is fine for situations in which the change is likely to be diffuse within the tissue, and in which the critical diagnostic information is likely to be gained from examination of individual cell morphology or the detection of infectious agents, without regard for tissue architecture. These criteria apply to diseases like *feline hepatic lipidosis*, in which the fatty change affects virtually every hepatocyte. The enlarged kidney of a cat with suspected *renal lymphoma* would be another excellent candidate since the diagnosis is made by seeing large numbers of monotypic lymphocytes without regard to their architectural arrangement or their relationship to pre-existent renal tubules. *Cholangiohepatitis*, on the other hand, has only multifocal accumulations of leukocytes that may be missed with fine needle aspiration, and the diagnosis requires not only the detection of the leukocytes but also the precise anatomic location in which they accumulate. The same limitation applies to aspiration of *inflammatory skin disease* since in almost all cases the diagnosis requires not only an appreciation of what leukocytes are present but exactly where they are in relationship to the hair follicles, adnexal glands, and epidermis. A disease like *cirrhosis*, in which there may be no leukocytes and in which the diagnosis requires the appreciation of fairly coarse architectural reorganisation, is a terrible candidate for cytologic diagnosis.

Trucut biopsies are appropriate when you need at least some appreciation of tissue architecture and when the architectural changes are likely to occur in a fashion that would be captured in small biopsies. In contrast to cytology aspirates, these samples will allow us to appreciate the location of leukocytes relative to the pre-existent tissue architecture and they also allow us to appreciate how the individual cells (leukocytes or tumor cells) are behaving with respect to the pre-existent architecture and to one another.

This ability to evaluate tissue architecture becomes particularly critical in the diagnosis of malignancy since destruction of pre-existent architecture is often a critical requirement to distinguish neoplasia from inflammation.

Invasion by atypical cells through normal tissue barriers is an observation of great prognostic importance, easily appreciated in well-selected trucut biopsies but never with aspiration cytology. The greatest limitation of trucut biopsies is that they do not allow appreciation of "coarse" architectural change. They allow only a keyhole view of a tumor that may or may not have tissue invasion in that specific location. They also may not allow appreciation of the variability in the degree of histologic aggressiveness within a neoplasm.

In situations other than neoplasia, the same strengths and weaknesses apply. Trucut kidney biopsies, for example, are excellent for distinguishing *glomerulonephritis* from *amyloidosis* because, in both diseases, one expects virtually every glomerular tuft to be affected (the average trucut biopsy captures 4-8 glomeruli). On the other hand, a disease like *pyelonephritis* typically creates random radial corticomedullary streaks of inflammation, and the lesion may be missed with a trucut sample. Even if captured in the sample, the pathologist will not know whether the lesion reflects 10 percent or 90 percent of the kidney! Since the prognosis is directly correlated with the extent of the lesion, this is a critical weakness in the utility of renal trucut biopsies. Unless you have remarkable skill in guiding such trucut biopsy needles via ultrasound, trucut biopsies are usually not suitable when you anticipate the lesions will be random and multifocal (like metastatic neoplasia, lesions of bacteremia, etc.).

Excisional or incisional surgical biopsies should be the gold standard against which all other biopsy techniques are measured. However, there are specific instances in which this most invasive of all biopsy techniques does not offer any substantial advantage over cytology or trucut biopsy, and a few cases in which it may even be less accurate. Examples of the last category are not frequent; the one that comes to mind is *gastrointestinal biopsy*. One might imagine that full thickness biopsies would always be preferred, but in fact full thickness samples are limited in the number one can take, and by the fact that they are virtually always taken without knowing anything about the mucosa hidden by what usually is a normal tunica muscularis. Endoscopy, while limited to the most anterior and posterior regions of the gastrointestinal tract, has the advantage of direct visualization of suspected mucosal lesions, and the ability to harvest very large numbers of samples in a relatively short interval. For diseases that are notoriously patchy like eosinophilic gastroenterocolitis, endoscopic biopsy offers a substantial advantage.
There are several important circumstances in which the optimal result comes from using a combination of excisional biopsy and cytology, each bringing its different "talents" to the microscope. There will be many times when *poorly melanotic melanoma*, for example, is more easily confirmed with cytology than with histology. *Anaplastic mast cell tumor* will almost always have at least a few metachromatic granules visible with cytology, but trying to find those granules in a histology sample may be virtually impossible. *Bone marrow* is best evaluated by a combination of histology core and cytology. The histology core sample is superior for assessing overall cellularity and architectural changes, while the cytology/aspiration offers a more accurate

assessment of what cell types are present. Cytology will often allow detection and identification of *infectious agents* with greater accuracy than histopathology.
Listed below are some "rules of thumb" about the relative reliability of cytology, trucut/endoscopic biopsy, and excisional biopsies for the diagnosis of some of the most familiar diseases that you will attempt to confirm via cytology or histology. I warn you that all internists or oncologists may not share my opinions . . . but of course they usually do not have to interpret the samples!

Tissue/Disease	Cytology	Core/Pinch	Excision	Comment
Bone				Core samples are technically difficult; results vary with experience of surgeon.
Neoplasia	+	+++	++++	Additional Information
Gastrointestinal				
Inflammation	-	+++	++	Endoscopy is superior for gastric and colonic mucosal disease, but will not detect disease in submucosa or muscularis.
Mucosal neoplasia	-	+++	++	
Mural neoplasia	+	-	++++	Additional information
Lymphangiectasia	-	++	++++	
Joint				
Arthritis	+++	-	N.D.[1]	Histopathology is indicated only if neoplasia is suspected.
Capsule thickening	-	++	+++	
Kidney				
Glomerulopathy	-	++++	N.D.	With careful assessment of serum biochemistry and urinalysis, biopsy is rarely indicated and (except for glomerular disease) almost impossible to interpret.
Diffuse nephrosis	-	+++	N.D.	
Pyeolnephritis	+	++	N.D.	Additional Information
End stage kidney	-	-	N.D.	
Liver				
Lipidosis	++	+++	++++	The correct diagnosis of lipidosis and steroid hepatopathy requires assessment of lesion extent; these are probably over-diagnosed with cytology alone.
Steroid hepatopathy	+	++	++++	
Other metabolic	+	++	+++	

Hepatitis	+	++	++++	
Cirrhosis	-	+	+++	
Lymphoma	+++	++++	++++	
Other neoplasia	+	++	++++	
Lung				Tissue biopsy is rarely done. Safety concerns outweigh all other considerations.
Focal densities	+	++ ++	++++	
Diffuse density	+		++++	Additional Information
Mammary Gland				Mammary cytology is notoriously inaccurate and cannot establish prognosis for neoplasia.
Neoplasia	- to +	++	++++	
Hyperplasia	-	++	++++	Additional Information
Mastitis	+	++	++++	
Skin				Cytology is useful for detection of agents and (with care) diagnosing pemphigus foliaceus, Malassezia and mites.
Inflammation	+	++++	++++	
Neoplasia	++	+++	++++	Additional Information
Alopecia	-	++++	++++	
Spleen				Splenic aspiration is useful only for diffuse round cell malignancies (lymphoma, mast cell tumor, and plasmacytoma).
Splenomegaly	+	++	++++	
Nodules	+	++	++++	
Urinary bladder				Cytology is moderately useful for diagnosing TCC in urinary sediment.
Mural thickening	+	+++	++++	
Cystitis	++	++	++++	Additional Information

[1] N.D.: not usually done as a diagnostic procedure

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Choosing the Right Biopsy for the Job: Bone

The histologic, and even the cytologic, diagnosis is straightforward but the problem is capturing the tumor cells which often lie deep within the lesion, surrounded by a protective shell of reactive new bone. If the tumor is extremely osteolytic, you may be able to get a sample with ordinary fine needle aspiration or surgical incisional biopsy, but those examples are infrequent. The biopsy instrument of choice is a bone core biopsy (Jamshidi needle is a common type). Ideally, you should measure the radiograph to predetermine how deeply you have to go in order to capture the osteolytic center of the lesion, which is where the diagnostic cells are going to be.

Choosing the Right Biopsy for the Job: Gastrointestinal

The problem with gastrointestinal biopsies of inflammatory disease is not in capturing the samples, but in interpreting them. The normal cellularity of the stomach, small intestine or colon of dogs and cats has never been defined, which seems like a fairly fundamental problem when dealing with so-called inflammatory bowel disease in which the very definition of the disease is "too many" leukocytes. There is virtually no agreement among pathologists as to how many leukocytes qualifies as too many, and what proportion is allowed to be eosinophils before it becomes abnormal. Distinguishing severe inflammatory bowel disease from early malignant lymphoma with shallow endoscopic biopsies can be difficult or impossible. Full thickness samples allow observation of submucosal or even transmural infiltration by the lymphocytes in malignant lymphoma, and thus a much more certain diagnosis. The shortcoming applies only to early disease. With full-fledged lymphoma, even endoscopic samples should allow a definitive diagnosis.

Choosing the Right Biopsy for the Job: Kidney

Many kidney biopsies are taken just because we have the technology to do so, without really understanding the profound limitations of trucut biopsies in establishing prognosis. Sequential clinical chemistry and urinalysis will usually tell you all you need to know to establish therapy and prognosis. My advice: consider very carefully what exact piece of information you would like to learn, and then contact your pathologist to see if kidney biopsy has a reasonable chance of providing that information!

Choosing the Right Biopsy for the Job: Lung

We desperately need a more useful technique for sampling lung. Transtracheal wash is almost useless, and BAL is only slightly better when it comes to providing any type of useful information about pathogenesis, therapy or prognosis. Percutaneous trucut biopsies have proven extremely useful in investigating equine lung disease, but everyone is very nervous about the risk of pneumothorax when using that technique in dogs or cats. It is so rarely done that I do not know if anyone has documented the actual risk.

At least in my experience (meaning my own cases and those of colleagues), our ability to accurately capture and interpret fine needle aspiration samples of lung masses is poor. The sensitivity of the

technique is low, and lung tissue frequently undergoes dysplastic change that is essentially indistinguishable from epithelial malignancy. Beware of any diagnosis of adenocarcinoma based upon lung aspiration cytology.

Choosing the Right Biopsy for the Job: Mammary Gland

In my opinion, cytology has virtually no place in the investigation of mammary enlargement in dogs or cats. Cytology cannot determine prognosis since it cannot reliably distinguish behaviorally benign from behaviorally malignant mammary neoplasia in either species. Many tumors have a lot of inflammation within them, and mammary gland with inflammatory disease will have dysplastic changes easily mistaken for neoplasia. There is too much regional variation in the presence or absence of predictors of malignancy to justify the use of trucut biopsies. When it comes to mammary masses, full excisional biopsies are still the rule . . . for good reason.

There may be some very special circumstances in which cytology or a trucut sample is justifiable. For example, is the enlarged inguinal nodule adjacent to a mammary tumor another tumor, a reactive lymph node, or lymph node with metastasis? Is a mammary lump in a dog with multiple mast cell tumors another mast cell tumor, or is it an independent mammary tumor? Is the lump recurring along my suture line some kind of postoperative inflammation, or is it a recurring tumor?

Choosing the Right Biopsy for the Job: Skin

The current standard biopsy technique for the investigation of skin disease is a series of 3-5 mm skin punch biopsies. Experts can take one or two samples from the perfect primary lesion; most of us would be better advised to take at least a single sample from every macroscopic variant we can identify! There is no golden rule about whether you are better to take the center of the lesion, the periphery, or adjacent normal skin since it varies from disease to disease. Taking at least one biopsy from normal skin is an excellent idea. And, by the way: if you are going to carefully describe each lesion in terms of character and location, then do not waste that effort by throwing all of the samples into the same bottle! If you think it is important that the pathologist know which biopsy comes from which exact location, then they must be placed in separate bottles or identified by colored suture or some other foolproof method. Samples never, ever stay adherent to tongue depressors!

. . . and remember: skin biopsy should not be the diagnostic message of last resort after you have exhausted every drug in the pharmacy! As in all other pathology, the diagnostic specificity of skin lesions is greatest early in the disease, and falls progressively over time. If your first course of therapy based on clinical criteria has had no effect, then THAT is the time for biopsy.

Choosing the Right Biopsy for the Job: Urinary Bladder

Thickened urinary bladders are usually the result of interstitial cystitis rather than neoplasia. The histologic appearance is epithelial ulceration and osmotic mural edema without any significant lymphocytic infiltration. The lesion is qualitatively indistinguishable from urinary epithelial ulceration caused by urinary calculi. Multifocal ulceration gives rise to really impressive multifocal

edema or its sequel of papillary reparative epithelial hyperplasia, both of which can easily be mistaken for neoplasia. The microscope is magical!
Transitional cell carcinoma can be diagnosed on direct or (better) sedimented samples of urine, but many cytologists will be cautious in making the diagnosis if there is any evidence of concurrent inflammatory disease. Transitional epithelium is notorious for its tendency to become dysplastic (hyperchromasia, anisokaryosis, binucleation) in the face of ongoing irritation of any type, and so the risk of over-diagnosing transitional cell carcinoma is substantial. There are a number of techniques using catheter tips and other devices to perform "traumatic flushes" of the urethra that will dislodge chunks of tissue large enough for histologic assessment. Anything that will get even tiny histology samples is greatly preferred over cytology, and the diagnosis can usually be made with a high degree of confidence.

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