

The Histopathology Laboratory in the Diagnosis of Neoplasia

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KEY WORDS

- histopathology
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- specimen handling
- prognosis
- terminology

SELECTION OF LABORATORY

Accuracy of histopathologic interpretation and turnaround time should be the main considerations in selecting a pathologist and laboratory for evaluation of specimens obtained via biopsy. Confidence in the diagnostic capabilities of the pathologist can be based on personal experience, references from other veterinary clinicians, knowledge of the training and experience of the pathologist, and certification of the pathologist by the American College of Veterinary Pathologists. As in every specialty, however, there are excellent pathologists who are not board certified. In any event, the pathologist should have had at least 3 years of supervised training in veterinary pathology. It is not professionally appropriate (nor legal in many states) for physician pathologists to interpret biopsies from domestic animal patients.

Convenience and cost are pragmatic considerations in selecting a laboratory. Many laboratories provide prepaid mailers, formalin containers, and phone-back service. These conveniences usually correlate with cost.

COLLECTION AND SUBMISSION OF SPECIMENS

Artifacts

The surgeon has an obligation to remove the tissue with as few artifacts as possible. Tissue damage due to crush and cautery is common. Crushing can be induced by digital compression or, more commonly, by forceps. Crushed tissue is often uninterpretable because of cell rupture and distortion. Cautery causes coagulation necrosis. Although it is sometimes possi-

ble to interpret overall tissue arrangement in cauterized tissue, evaluation of individual cellular morphology is hindered. Crush and cautery damage often is of little significance in large specimens but can render small biopsies (e.g., eyelid neoplasms) worthless.

Sampling

The removed, unfixed mass may be cut by the surgeon because of size, to obtain impression smears, or to observe tissue appearance. When selecting samples of large specimens to submit for histopathologic evaluation, consider any variation in tissue consistency, color, and texture and the junction of normal and abnormal tissue. For example, a large soft red area in a splenic hemangiosarcoma might contain only hemorrhage with no neoplastic cells, and the center of a large mammary carcinoma might be completely necrotic and uninterpretable histologically. In large masses it is always better to submit several smaller specimens from areas representing different consistencies or colors rather than one large specimen representing only one type of area. It is important to indicate this type of sampling in the request; otherwise, the pathologist may have difficulty distinguishing true surgical margins from sampling margins.

Margins

The surgical margin is often obvious on small specimens or those with capsules or skin attached. In large specimens from which the surgeon has submitted samples, however, the surgical margin frequently is not obvious. Surgical margins can be identified by suture, submission in separately marked containers, or markedly varying specimen sizes or shapes with accompanying explanations. Establishing margins and completeness of excision is usually of great interest in malignant neoplasms or locally aggressive nonmalignant lesions. Problems in evaluating margins can be divided into three categories:

- Laboratory error—Specimens must be “trimmed” to $\frac{1}{8}$ inch thickness and fit into porous cassettes for processing and embedding in paraffin (Figure 1). Trimming large specimens requires judgment and knowledge of orientation. Failure to include margins in the trimmed specimens likely will cause an error in margin assessment by the pathologist. Even if the trimmed specimen contains the appropriate margins, they possibly will not be present on the



Figure 1. Demonstration of correct placement of a trimmed specimen into a tissue cassette. The cassette then will be capped and placed in the tissue processor.

slide if they are slightly recessed from the cut surface of the trimmed specimen and if the histology technician does not section deeply enough into the paraffin block.

- **Clinician error**—In sampling a large specimen for submission to the laboratory, you (or your technician) might not include the margin with normal tissue. That is, the neoplastic tissue extends to the margin of the specimen you submitted but does not truly reflect the surgical margins. A different problem can arise in any size lesion that “shells out,” as such lesions can leave thin, grossly inapparent rims of neoplastic tissue in the patient (e.g., as is seen with hemangiopericytoma). While it is understandable that the surgeon is reluctant to excise more tissue than is necessary, it is highly likely that “shelled out” lesions have no normal tissue surrounding them and, not only might the pathologist have difficulty in establishing the completeness of excision but also might be unable to distinguish benign from malignant lesions. This latter issue can be a problem in mammary neoplasia. In complex (epithelial and myoepithelial) mammary neoplasia common in the bitch, it is not possible to distinguish between benign and malignant lesions without examining the infiltrative nature of the growth. This evaluation requires examination of the junction of the lesion with normal tissue.
- **Nobody’s error**—It is not always physically possible to find the surgical margin in small pedunculated specimens attached by narrow stalks. After fixation, these samples arrive at the laboratory looking like small prunes, and often, even after careful in-

spection, it is not possible to locate the attachment stalk. The histologic section of these specimens usually appears as an irregular sphere with no site of attachment visible in the plane of section. Often this is of no consequence, for such growth patterns usually indicate benign exophytic neoplasms; occasionally, however, melanomas of the oral cavity (all of which have malignant potential) grow in such a manner, and neoplastic cells can extend down the narrow stalk.

Margins are present 360 degrees around the specimen in any plane examined. It is not possible or even necessary for all these to be examined. Problems arise with trimming specimens that contain highly irregular masses with small grossly inapparent projections. A good example is the subcutaneous fibrosarcoma of the cat. It is possible for this mass to appear to be completely excised by a relatively wide margin in one plane of section while a long nonpalpable projection of the neoplasm extending to the specimen margin could be present in another plane.

Fixation

Specimens should be $\frac{1}{4}$ inch in one dimension to allow adequate penetration of formalin. Fixed tissues are trimmed by the pathologist or technician to fit into tissue cassettes, which hold the specimen during processing (Figure 1). Specimens in the cassettes are approximately $\frac{1}{8}$ inch in one dimension to allow penetration of the alcohols, xylene, and, eventually, liquid paraffin. Thinly shaved specimens are often difficult to embed and section. Specimens can contract and curl after fixation. Something that appeared to be an even surface on the cut fresh tissue might be very irregular after fixation. This is of no consequence in tissues $\frac{1}{4}$ inch thick because the fixed tissue can be trimmed further to a flat surface. It is physically impossible to trim thinly shaved fixed specimens. The histotechnician therefore might be unable to obtain a complete cross-section because the tissue curves into and away from the plane of the microtome knife (Figure 2).

Specimens should be fixed in 10 times their volume of clean formalin. Excessively bloody specimens can be rinsed in saline or “dirty” formalin kept for this purpose. Small specimens can be sent immediately to the laboratory. Specimens that have been fixed for 24 to 48 hours can be sent to the laboratory in only enough formalin necessary to keep them moist (e.g., wrapped in formalin-soaked gauze or paper towel). This technique allows you to avoid shipping large volumes of formalin.

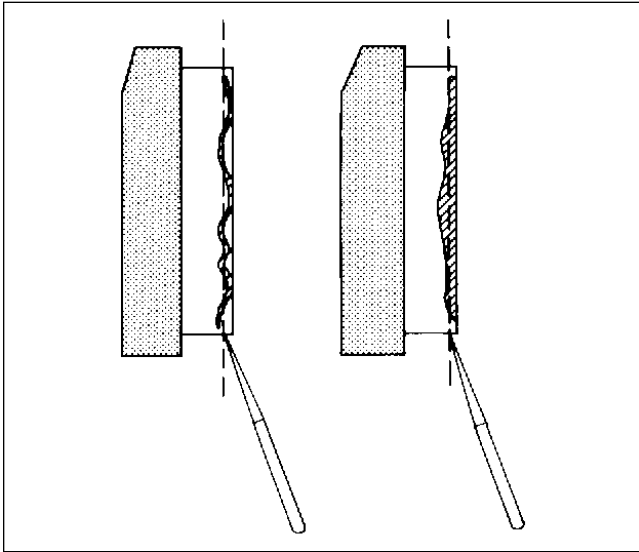


Figure 2. Diagram demonstrating consequence of microtome sectioning a specimen that is too thin (*left*) compared with an appropriately thick specimen (*right*).

Retaining and Discarding Specimens

As insurance against loss of specimens in transit to the laboratory, portions of specimens larger than $\frac{1}{4}$ inch can be retained in formalin and discarded after receipt of a histopathologic diagnosis that is compatible with the clinical findings. Never discard a surgically removed mass. Decisions whether grossly diagnostic lesions such as cysts, lipomas, or abscesses should be sent for histopathologic evaluation should be made in consultation with the owner. Often owners will request that such a lesion be submitted to reassure them that it is benign. If owners are reluctant or unable to afford histopathologic evaluation, consider letting them (or you) retain the sample in formalin in case there is a change of mind or circumstance. Specimens can be stored in formalin for years.

Signalment, History, and Description of Lesion

It is surprising how often the basic signalment can be helpful in certain circumstances. Breed information can sometimes enable the pathologist to be more definitive in classifying and offering prognosis in unusual lesions (e.g., anaplastic histiocytic malignancies in flat-coated retrievers). Be especially diligent for prompts such as age of the dog and duration of lesion when evaluating features of malignancy in complex (epithelial and myoepithelial) neoplasms in the mammary gland of the bitch. There can be very small focal transitions from benignancy to malignancy in older bitches with long-standing neoplasms of this type. Whether a dog is intact or castrated or spayed might

be helpful in distinguishing a lymphosarcoma from a transmissible venereal tumor in the genital region.

It can be argued that the more information you provide to the pathologist, the more you bias the histopathologic diagnosis, and to some extent this might be true! In some cases no history or description is needed because the histopathologic appearance is definitive and the prognosis would be similar independent of description, history, and even signalment. Nevertheless, such information should be provided to ensure better treatment of the patient in case the lesion is not definitive. Because it is not possible to predict which cases might not be definitive, a guideline would be that the amount of clinical and descriptive information should correlate directly with the complexity of the case and with your concern that the sample might not reflect the clinical problem and should correlate inversely with how small the sample is compared to the size of the mass. If there is a clearly well-demarcated, completely excised skin lump in an otherwise healthy dog and the entire specimen is being submitted, there is not much that needs to be told. In this type of case, the signalment and location and the statement “otherwise clinically normal animal” are likely all that is needed. Information that guides the pathologist in suggesting differential diagnoses or additional tests in nondefinitive biopsies is most valuable.

For example: A diagnosis of reactive periosteal bone formation is made on a small bone biopsy sample. Only cortical and periosteal bone are present in the specimen. The history states “radiographic bone lesion on distal radius.” The sample is consistent with the history and with trauma, and the pathologist can justifiably conclude that there is no evidence of neoplasia or inflammation *in the sample* and therefore would not recommend any additional sampling. However, if the history stated that the bone lesion was both lytic and productive, the pathologist should comment that there is no microscopic explanation in the biopsy specimen for the lysis that was described. The pathologist should suggest that a deeper biopsy is indicated because even though there is no evidence of neoplasia or inflammation *in the sample*, the description of the lesion suggests there is neoplasia or inflammation *in the animal*.

Correlation of Histopathologic and Clinical Diagnoses

It is necessary to distinguish between conflicts of bias and conflicts of fact. A lytic, aggressive lesion in a long bone with a histopathologic diagnosis of suppurative osteomyelitis might be a *conflict of bias* with

the clinical and radiographic diagnosis of osteosarcoma, but these findings are in fact consistent with a histopathologic diagnosis of osteomyelitis. In this case it is appropriate to accept the histopathologic diagnosis as correct unless you had evidence that there might be marked secondary suppuration in an osteosarcoma.

An invasive and osteolytic soft tissue mass in the gingiva with a histopathologic diagnosis of squamous papilloma is a *conflict of facts* with a clinical diagnosis of an aggressive gingival lesion (malignancy or inflammation). It would *not* be appropriate to accept the histopathologic diagnosis in this case. Conflicts of fact require consultation between clinician and pathologist. The problem could be an incorrect diagnosis by the pathologist, a nonrepresentative biopsy, or an inexplicable discrepancy between histologic appearance and biologic behavior.

BASIS OF PROGNOSIS

Historical

The single most important factor in determining the prognosis of a neoplasm is experience with clinically and histopathologically similar neoplasms. Predictions of biologic behavior for malignant lesions is known only in terms of averages; a malignant diagnosis on a canine mammary gland does not always predict malignant behavior.¹

Margins

For many neoplasms, unless a margin is available for evaluation of invasive potential, it is difficult to distinguish between benignancy and malignancy (e.g., canine mammary complex adenoma versus complex carcinoma).² Histopathologic evaluation of the surgical margins is critical for prognosis of recurrence.

Differentiation and Grading

The correlation between biologic behavior and histopathologic degree of differentiation is poor for many neoplasms in veterinary medicine. Some neoplasms can have prominent anaplastic features yet are benign—the common leading example of this is the canine histiocytoma. Others can be very well differentiated and yet have relentlessly malignant behavior—an extreme example of this is the very well differentiated fibrosarcoma in the oral cavity of dogs.³ In some cases it is not possible to predict biologic behavior based on the histopathologic appearance. Examples: 7% of dogs with grade I mast cell tumors die of this lesion,⁴ 10% of benign-appearing cutaneous melanomas in dogs behave malignantly,⁵ and only 15% of even the most anaplastic (based on mitotic index) cu-

taneous and subcutaneous fibrous connective tissue sarcomas in dogs metastasize.⁶

While recognizing the limitations of predicting biologic behavior of a specific neoplasm for the individual patient, clinically useful histopathologic grading has been recommended for mast cell tumors,⁴ cutaneous melanomas,⁵ and mammary neoplasia⁷ in dogs.

Vascular Invasion

Vascular invasion is considered to be a significant histopathologic finding and should be so indicated on the pathology report. It is not always possible for pathologists to determine whether tumor cells are within a lymphatic, capillary, or venule, and thus they sometimes use the noncommittal term *vascular invasion*. As a general rule, nonhematopoietic sarcomas metastasize via the blood vascular system and carcinomas metastasize via the lymphatic system.

While vascular invasion is appropriately interpreted as an indication that metastatic disease is likely, remember that only 0.1% to 1% of circulating tumor cells successfully induce metastatic foci.⁸

Tumor cells in vessels can be artifacts. It is common to see neoplastic cells in large arteries and veins in biopsy specimens of canine testicles with seminomas. These are a consequence of physical manipulation of the testis during castration and are not reflections of true vascular invasion. This is supported by the low incidence of metastasis of canine seminomas.⁹

Size

Good correlation exists between survival time and size of feline mammary carcinomas. Cats with carcinomas 2 cm and smaller in diameter have median survival times of more than 3 years, whereas cats with carcinomas greater than 3 cm in diameter have median survival times of 6 months.¹⁰

Significance of Descriptive Modifiers

Many descriptive modifiers are of little clinical significance. Examples in the dog are cystic, papillary, or tubular simple mammary carcinomas,² nonproductive (osteolytic) osteosarcoma,¹¹ and epithelioid ocular melanoma.¹² For the most part, these descriptive modifiers are not known to correlate with any biologic behavior and are used by the pathologist to more completely convey what is present. However, the modifiers *simple* and *squamous* mammary carcinoma in the bitch have been correlated with more aggressive behavior than complex carcinomas.² Additionally, it has recently been shown that infiltrative papillary transitional cell carcinomas in bladders of dogs are less

likely to metastasize than are nonpapillary infiltrative carcinomas.¹³

Examples of modifiers with extreme clinical significance are parosteal osteosarcomas, which metastasize much more slowly than medullary and periosteal osteosarcomas,¹¹ and acanthomatous epulides, which are invasive and recur compared with fibrous or ossifying epulides.¹⁴

Location

Differences in biologic behavior can vary markedly with location. One of the most common examples is malignant melanoma in the dog. Oral malignant melanomas usually have a very poor prognosis compared with cutaneous malignant melanomas.⁵ The literature is not consistent about the behavior of digital cutaneous malignant melanomas in dogs compared with that of cutaneous malignant melanomas at other sites.^{5,15} Very well differentiated fibrosarcomas (difficult to differentiate from reactive fibrosis) in the maxilla and mandible of dogs can be extremely locally invasive and have a 20% chance of metastasis.³ This behavior would not be predicted from the microscopic appearance without knowing the location of the lesion.

An example of very slight variation in location affecting biologic behavior is the squamous cell carcinoma of the digit. A recent survey indicates a 1 year survival rate for dogs with squamous cell carcinoma of the digit arising in the subungual region, a rate that is significantly higher than for squamous cell carcinomas arising elsewhere in the digit!¹⁵

Confusing Terminology

Veterinary pathologists do not use a standard system for classification of neoplasms. The International Histological Classification of Tumors of Domestic Animals sponsored by the World Health Organization¹⁶ has not gained universal acceptance. This is most evident for canine mammary neoplasia. Some pathologists do not distinguish between benign mixed mammary tumors (epithelium with cartilage, bone, or fat) and complex adenomas (secretory epithelium and myoepithelium). In fact, with sufficient sampling it is possible to find cartilage, bone, or fat in some complex adenomas, emphasizing that the distinction between these two benign lesions might not be absolute. Complex mammary carcinomas (neoplastic secretory epithelium and myoepithelium, at least one of which is malignant) may be called *carcinomas* arising in a mixed tumor by pathologists not comfortable with the World Health Organization system. New editions of these guidelines are now being prepared, but there is

concern that history will repeat itself.

Because basal cell tumors often appear invasive and may have a high mitotic index, the classification *basal cell carcinoma* is preferred by some pathologists. Unless there is evidence of vascular invasion, however, basal cell tumors (carcinomas) rarely metastasize or recur after complete excision.¹⁷

Adenomatous hyperplasia is often used interchangeably with *adenoma*. This may be appropriate or inappropriate. Hyperthyroidism in cats is often associated with multinodular adenomatous hyperplasia of follicular cells. It has been suggested that these may coalesce to form thyroid follicular adenomas.¹⁸ It would be inappropriate, however, to classify the marked diffuse thyroid follicular cell hyperplasia of iodine deficiency as an adenoma.

THE FUTURE

Great advances have been made in molecular biology and the detection of neoplasia.¹⁹ Many publications that use immunohistochemical techniques to classify and categorize neoplasms are present in the veterinary literature. Studies using techniques such as flow cytometry,²⁰ in situ markers of cellular proliferation,²¹ and silver-stained nucleolar cluster size or number²² have been reported. Such studies are of major interest, but the main tools of the pathology laboratory in the diagnosis of neoplasia of domestic animals in the near future will remain unchanged from the present: The pathology laboratory will provide relatively inexpensive and effective routine tissue processing, sectioning, and staining with manual, qualitative evaluation and interpretation (i.e., morphologic diagnosis) by a trained professional.

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