1 Overview of Diagnosis, Categorization, Grading, and Staging

Identification of the process

The practicing veterinarian is sometimes presented with a patient who has a focal swelling. Good communication with the client, using mutually understood terminology, is important to avoid misunderstandings as a patient plan is developed. Tumor is a word of Latin derivation, meaning a swelling or protuberance—a "mass." This word is often misunderstood by the client as "cancer," but technically it includes masses formed by inflammatory infiltrates, controlled proliferations of hyperplastic cells, and uncontrolled proliferations of neoplastic cells (cancer). An astute clinician understands that the word tumor encompasses all of these processes. Failure to consider this wider definition can result in bias that causes an inaccurate or incomplete diagnosis.

Inflammation can be a result of tissue necrosis due to traumatic, chemical, thermal, or ischemic injury, or it can be due to primary or secondary infectious agents. The presence of neutrophils is a signal to be on the lookout for infectious organisms.

Hyperplastic proliferations of cells have a recognizable structure, may perform their usual physiological function, do not invade other tissues, and eventually undergo senescence and programmed cell death (apoptosis).

Neoplastic proliferations may contain cells of varied structure, may be functional or nonfunctional, may invade local tissues, may cause local tissue necrosis by their increasing bulk, tend to replicate in a disorderly manner, and have the ability to escape typical programmed cell death.

Within the realm of neoplasia or "cancer," there are the categories of benign and malignant. At a simplistic level, benign means good and malignant means bad. From a clinician's point of view, a benign cancer is one that stays in place and does not invade beyond the boundaries of the basement membranes or capsule, and a malignant cancer causes harm by invading locally and spreading to distant sites. It can be difficult to categorize a tumor based solely on a few cells collected by fine needle aspiration (FNA) or a small section of tissue collected by punch or needle biopsy. Hyperplastic lesions may become focally neoplastic,[1,2] benign tumors can undergo transformation to malignant neoplasia,[3] aggressive tumors can have large areas of necrosis and dense inflammation containing few tumor cells, and occasionally benign and malignant proliferations of multiple cell lines can arise simultaneously and in proximity.[4,5] So how is a tumor identified as malignant? The following list is an indication of the challenge of defining a malignant tumor:

When a mass is judged to be neoplastic, the following criteria can help determine if the tumor is malignant:

1 Has the normal architecture been lost in any area?
2 Is there nuclear and cellular pleomorphism such as nuclear hyperchromia, anisokaryosis, or multiple nuclei?
3 Is the mitotic count (MC) increased beyond what could be expected with a hyperplastic response or benign neoplasm?
4 Is there cellular necrosis that is not related to trauma?
5 Are tumor cells infiltrating into adjacent normal tissue?
6 Have tumor cells lost their normal orientation with the basement membrane (in epithelial tumors) and started to form irregular and disorganized clumps of cells?
7 Is there lymphatic invasion?
8 Is there lymph node invasion?

If points 7 or 8 are present, the tumor is considered malignant. If 1–6 are present, the likelihood of malignancy increases proportionately with an increased number of criteria (adapted from Zappulli et al.[6])

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Some tumors (anal sac apocrine carcinoma, pheochromocytoma, parathyroid tumor) can cause clinical illness and death without ever leaving the site of origin by releasing substances that disrupt normal metabolic functions. These tumor types are usually labeled malignant only when found at sites distant from the point of origin. The term "malignant" can be subjective in certain circumstances and should be used judiciously.

The path to successful treatment of the patient with a tumor begins with recognition of the lesion on a gross level, usually by the caretaker, sometimes by a groomer, or often during a physical exam by a veterinarian. A persistent and enlarging mass is the most commonly recognized lesion but a waxing and waning mass can also be a sign of a tumor such as a mast cell tumor (MCT). Some tumors, such as cutaneous lymphoma, can present initially as ulcerated sites, crusts, or multiple plaques. The next step is the assignment of the pathological process into a category of inflammation, hyperplasia, or neoplasia, or some combination of these categories. This can be accomplished at the point of care, usually with minimal fuss and expense, by aspirating the lesion with a needle and examining the individual cells. In the following chapters, this will be called FNA. With some tumors, especially papillomas, impression or scraping of the lesion can yield diagnostic cells, but generally this is not the ideal method of collection, as surface contamination can make evaluation difficult. All cytological samples presented here are stained with Wright–Giemsa (W-G) stain unless otherwise indicated. Figure 1.1 is a flow chart showing that the first step is to decide if the lesion is inflammatory, hyperplastic or neoplastic. If the lesion is neoplastic, the next step is to decide if it is benign or malignant. For example, the process can be named (e.g. a pyogranuloma versus lymphoid follicular hyperplasia versus follicular lymphoma), and if it is a neoplastic lymphoma, mitotic activity can be used to determine a likely behavior pattern (benign versus malignant).

Figure 1.2 shows an apocrine gland adenoma FNA. This cluster of small epithelial cells is suggestive of a proliferation of basaloid epithelial cells or the ductular epithelium of an apocrine gland. The cell nuclei are small and regular but present in numbers greater than expected from a normal or hyperplastic structure. There is scant inflammation, as shown by the neutrophil in this field, suggesting a benign tumor rather than a hyperplastic response to chronic inflammation.

When FNA of a mass reveals a population of proliferating cells, indicating the lesion is not merely an influx of inflammatory cells that can be relieved by medical means, biopsy with histopathological evaluation can show the architectural arrangement of the cells, allowing a more definitive diagnosis. This is the critical test that allows hyperplastic

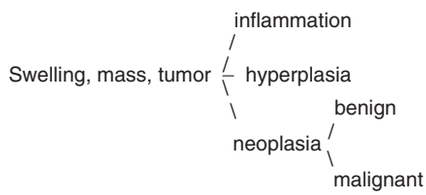

Figure 1.1 Terminology used to categorize a swelling, mass or tumor.

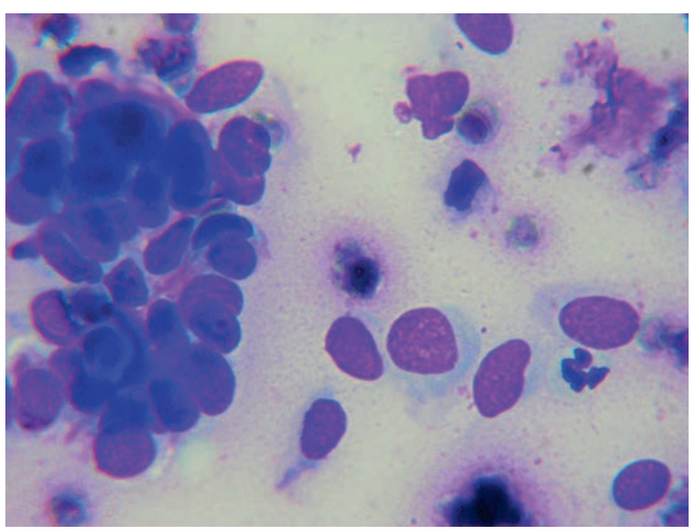

Figure 1.2 Apocrine gland adenoma fine needle aspiration. 50×.

growth to be distinguished from neoplastic growth based on how the cells are structurally arranged. FNA cannot evaluate architectural arrangement accurately because the cells are usually stripped of their association by the process of aspiration. The decision to take an incisional biopsy that removes a portion of the mass, or an excisional biopsy that removes all of the mass, should be based on factors such as the tumor type suggested by the FNA, the size of the lesion, the location of the lesion, the stage of the disease, and other parameters such as the overall health of the patient and wishes of the owner. Ultimately, the decision rests on the clinical judgment of the surgeon. All biopsies shown in this chapter are stained with hematoxylin and eosin (H&E), unless otherwise indicated.

In Figure 1.3, the biopsy shows the architecture of the gland aspirated (see Figure 1.2). There are double rows of small epithelial cells proliferating in a manner that does not invade the adjacent stroma, indicating that this is a benign apocrine gland tumor referred to as an apocrine ductular adenoma.

FNA can sometimes identify cells that are so clearly abnormal, either by morphology or cell density, that neoplasia can be diagnosed on a presumptive basis. A cytological diagnosis of a potential tumor type is important to help the surgeon make appropriate margin decisions. The surgeon will want wider margins for a potentially aggressive spindle cell tumor than for a probably benign histiocytoma, and tumors that tend to be poorly circumscribed, such as MCTs, will also require wider margins.

Figure 1.4 shows a transitional cell carcinoma FNA. An adult female mixed-breed dog was presented for hematuria and dysuria. A tentative diagnosis of cystitis was made based on clinical signs, and cystocentesis was performed to

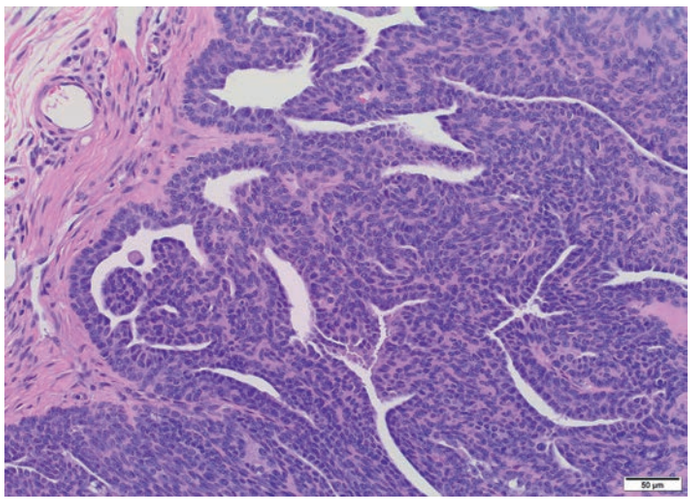

Figure 1.3 Apocrine gland ductular adenoma biopsy. 20×.

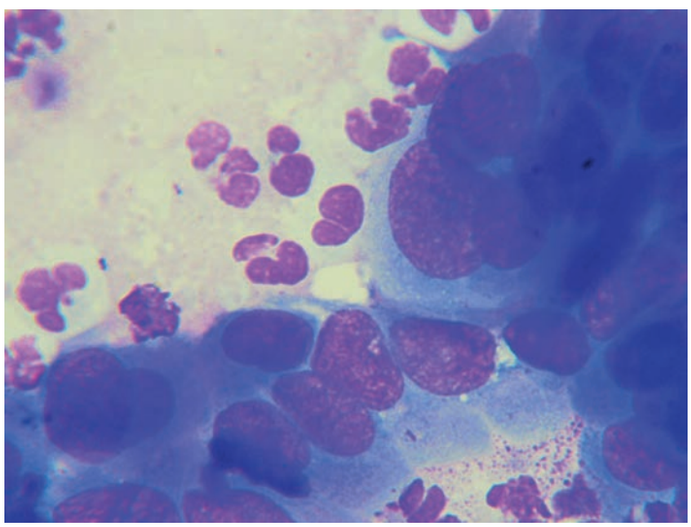

Figure 1.4 Transitional cell carcinoma FNA. 50×.

collect urine for routine urinalysis and sedimentation. Current practice is to catheterize or collect a free catch if there is any radiographic or ultrasound suggestion of neoplasia, so that there is no seeding of any potential tumor during cystocentesis. Cytological exam, in this case, revealed many clusters of large epithelioid cells with marked anisokaryosis (variation in nuclear size) and basophilic cytoplasm. There were scattered neutrophils, erythrocytes, and cellular debris. No infectious agents were seen. A preliminary diagnosis of neoplasia, probable transitional cell carcinoma, was made. This cytological diagnosis can be supported by evaluating the urine sample for genetic mutation of the BRAF gene, providing a significant benefit to the patient, as a treatment for infectious cystitis, based just on clinical signs, would not be curative and could delay the true diagnosis.

When a biopsy identifies the process as hyperplastic, and the margins are free of abnormal cells, it can be assumed that the lesion is cured. This does not preclude additional lesions arising adjacent to the removed mass.[4]

When a biopsy identifies the process as neoplastic, the tumor can be categorized into type and grade based on published guidelines derived from scores of biopsies and the statistical analysis of their behavior.

The cytological exam provides preliminary but not comprehensive categorization as architecture is not retained and therefore biopsy is considered the definitive test. The purpose of this atlas is to aid with the match of characteristics of cells obtained by FNA to characteristics of cells obtained by biopsy, and thereby hasten the decision process, a goal that is advantageous to the patient, the client and the veterinary clinician. The reader will be spared a detailed description of the original research that forms the basis for statistical analysis of the data and establishment of tumor classification and grading protocols but be assured that there has been an astonishing amount of research recently, and I am sure that the body of literature available will continue to expand at a rate difficult to assimilate. Inquiring minds are encouraged to review the documents in the journals and books listed in the reference section as these form the basis for the advancing knowledge base.

Identification of tumor types

The root categories of tumor types are derived from tissue of origin. Epithelial-origin tumors are designated as epithelioma or adenoma (benign) and carcinoma (malignant). Mesenchymal origin tumors are typically designated as an -oma prefixed by the tissue of origin (if benign) or -sarcoma prefixed by the tissue of origin (if malignant). Examples include (benign) fibroma and (malignant) fibrosarcoma. Discrete cells lacking cell-to-cell adhesion and originating from the specialized tissue and circulating cells of the immune system, such as lymphocytes, plasma cells, histiocytes, mast cells, and a transplantable chimeric neoplasm called a transmissible venereal tumor (TVT), are designated as round cell tumors, often -omas, prefixed by the tissue of origin (and sometimes indicated as benign or malignant). An example is benign plasmacytoma and malignant plasmacytoma or myeloma. There is a separate category for melanoma, which can have epithelioid, spindloid, and round cell characteristics within the same tumor (both benign and malignant tumors). An example is melanocytoma and malignant melanoma. It is important to remember that the labeling of a tumor as benign does not indicate that it will never behave in a malignant fashion in the future because tumors can evolve into a more aggressive population, with time, as mutations occur during periods of growth or post-exposure to disruptors (chemotherapy, radiation, chronic inflammation). It is also possible that a tumor labeled malignant will follow a benign course of behavior.

Grading

Grading, which is essentially formalized risk assessment, is performed by the pathologist using parameters that can only be evaluated by biopsy, including MC per 10 high-power fields (HPF) (40×), the presence of a recognizable pattern of growth, presence and amount of necrosis, and invasion into adjacent normal tissue. Advances in the field of oncology have necessitated a more stringent approach to the parameters of the grading systems in use and the need for the development of a flexible system that can update as the clinical database expands. Every month brings additional published studies that refine the grading systems, expand the number of cases evaluated, and define the outcomes and survival statistics. There are also new parameters evolving as immunohistochemical techniques and molecular markers are developed. Clinicians are aware that oncology protocols are a "moving target" and staying abreast of recent developments requires monitoring key journals such as *Veterinary Pathology*, participation in clinical board sites such as Veterinary Information Network, consultation with practicing Oncologists, and awareness of special research efforts at regional Veterinary Teaching Hospitals.

Mitotic activity is an important part of most grading systems. MC is most recently defined by the pathology community as the number of mitotic figures per 10 fields (mitotic figures/10 HPF) using a 40× lens and a 10× eyepiece, resulting in an area of 0.237 mm^2, with the number of fields examined being specified in the description.[7–11] Mitotic index (MI) is a controversial term that is sometimes used as the number of mitotic figures in 10 fields (mitotic figures/10 HPF), especially in older literature, but is more recently defined in the literature[7] as the number of cells undergoing mitosis divided by the number of cells not undergoing mitosis. This discrepancy can be confusing and one is advised to determine how the term is defined when it is used. MC is affected by the size of the field and so in future published studies, the field area should be defined as 0.237 mm^2 rather than, or in conjunction with, the less well-defined term HPF. To complicate matters further, both MC and MI can vary depending on which areas of the tumor are examined. The presence of necrosis and dense inflammatory infiltrates can make the identification of mitotic figures difficult, and small biopsies with less than 10 fields in size can make the enumeration of the MC less robust. There is heterogeneity in the density of mitotic figures and there is also pleomorphism in the shape of the mitotic spindle based on the phase of the cell cycle.[12] The rapidity and quality of fixation are critical to the preservation of mitotically active cells. Mitotic figures are difficult to identify in poorly fixed or necrotic tissues due to nuclear pyknosis and autolysis. There is a journal report of delayed fixation of an incisional biopsy of hepatocellular tumor resulting in a much higher ex vivo mitotic rate as cells divided rapidly in hypoxic and deteriorating tissue, compared to a much lower rate in the immediately fixed lobectomy sample.[13] Suboptimal sample handling can adversely affect the identification of grades. In most protocols, the grade is not based just on mitotic activity but also on other aspects of the proliferating population such as the amount of necrosis (also subjective and based on the section examined) and pattern of growth in the tissue. This heterogeneity introduces some variation in the assessment of tumor grade in many protocols[14] and may have contributed to the proliferation of several grading systems for some tumors as pathologists attempt to find the best system (Table 1.1). Some tumors, such as melanoma, are strongly dependent on the assessment of MC as a prognostic indicator and a poorly preserved sample could artifactually result in an inaccurate grade and an adverse effect on patient treatment.

Grading systems can use quantifiable descriptive terms such as low, medium, and high grades or can assign a numerical label (Table 1.2), and grading systems can use an equation to score several critical features that add up to a sum assigned to a grade (Table 1.3). All of the systems used are designed to succinctly convey the probability that a tumor will be aggressive and likely to invade local or distant tissues. Grading also allows a pathologist to give an oncologist a specified set of details designed to help choose and monitor appropriate therapy. The general practitioner and the oncologist or internist may have different preferences for grading protocols or treatment plans, and may desire different sets of information, resulting in a report listing several grading protocols applicable to the tumor described. These compilations of data can be useful even in the face of periodic modification as the database grows and our diagnostic tools become more refined to include immunohistochemical markers, molecular diagnostic parameters such as tumor growth fraction, genetic analysis gene mutations, flow cytometry, and polymerase chain reaction (PCR) of antigen receptor site rearrangements (PARR). Open communication between clinicians and specialists will be necessary to keep them apprised of new developments. Some of the more commonly used grading systems are demonstrated in Tables 1.1, 1.2, and 1.3.

Table 1.1 Multiple grading systems for lymphoma.

Tumor	System	Features
Lymphoma	NCI WF	Pattern, biology, survival
	Kiel	Pattern, morphology, immunophenotype
	WHO	Pattern, morphology, immunophenotype

Table 1.2 Multiple grading systems for mast cell tumor.

Tumor	System, reference	Grade; features
MCT	Patnaik, 14	1; confined to dermis, 0 mitoses/HPF, uniform nuclei 2; invades subcutis, 0–1 mitoses/HPF, rare binucleate cells 3; invades deep tissues, >3 mitoses in some fields, pleomorphic nuclei
	MSU, 14	Low; rare mitoses, confined to dermis, uniform nuclei High; invasive, frequent mitoses, pleomorphic nuclei

Table 1.3 Grading systems based on points for sarcoma in canines and mammary gland tumor in canines.

Tumor	Reference	Features
Sarcoma	14	Differentiation 1; regular appearance 2; poorly differentiated 3; pleomorphic Mitoses/10 HPF 1; 0–9 2; 10–19 3; >19 Tumor necrosis 1; no necrosis 2; <50% necrosis 3; >50% necrosis Score 1; 3 or less 2; 4–5 3; 6 or more
Mammary tumor	14	Tubule formation: 1; >75% 2; 10–75% 3; <10% Nuclear form: 1; uniform 2; variation 3; pleomorphic Mitoses/10 HPF 1; 0–9/10 HPF 2; 10–19/10 HPF 3; 20+/10 HPF Score: 1; low, 3–5 2; moderate, 6–7 3; high, 8–9

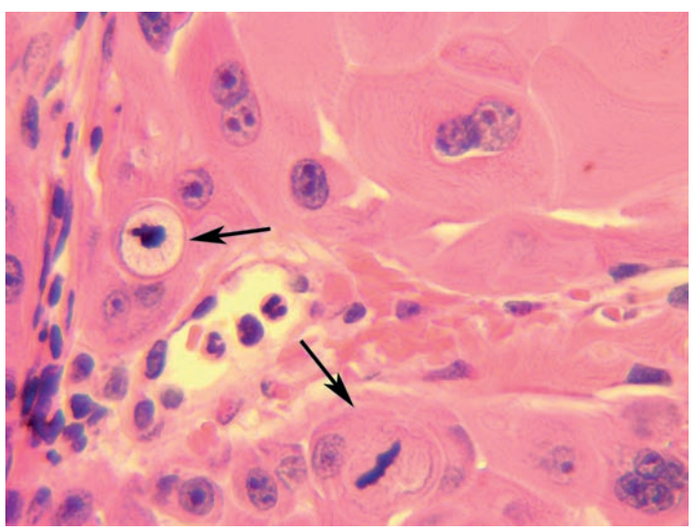

Figure 1.5 Squamous cell carcinoma biopsy. 50×.

Figure 1.5 shows a squamous cell carcinoma (SCC) biopsy with two mitotic figures. The cells in this biopsy are fairly well differentiated and recognizable as squamous epithelial cells and the mitotic figures (arrows) are clear. Evaluation of at least 10 HPF (40×) is recommended for assigning a grade. Fragmented or crushed tissue and biopsies smaller than 1.0 cm may have insufficient fields for proper evaluation of MC.

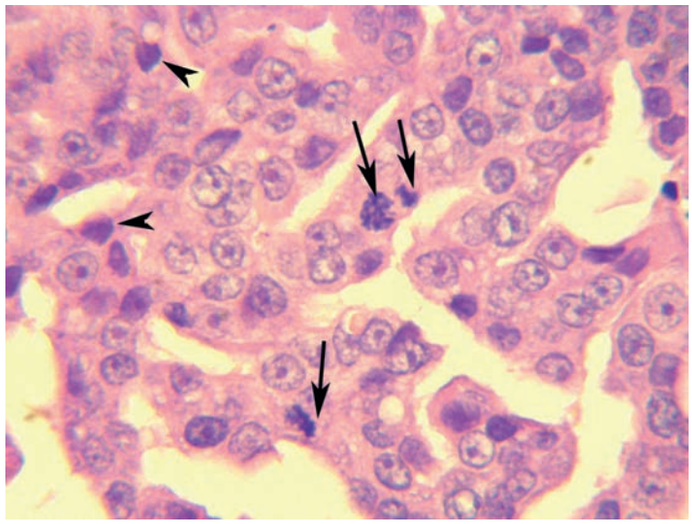

Figure 1.6 Apocrine adenocarcinoma biopsy. 50×.

Figure 1.6 shows an apocrine adenocarcinoma biopsy with three definitive (arrows) and two questionable (arrowheads) mitotic figures. Cells with vague and pyknotic nuclei can be difficult to assess for mitotic activity, a situation that introduces a source of discrepancy in the grading of some tumors. Necrosis and inflammation compound the problem by increasing the number of active and dying cells that are not necessarily tumor cells. Evaluation of 10 fields can be impossible if only tiny fragments are submitted for biopsy.

Staging

Staging is a clinical assessment that quantifies information such as the location of the tumor in the body, the size of the tumor, and whether there is local lymphatic or distant metastasis. The clinician performs the appropriate staging of a malignancy using FNA for cytological exam or biopsy, ultrasound or radiographs for documentation of lymphadenopathy or stromal pattern changes and thoracic radiographs and other imaging such as CT or MRI to look for evidence of metastasis. The pathologist can be of assistance if adjacent stroma or lymphatic tissue is submitted for analysis and lymphatic or vascular invasion can be documented on biopsy tissues.

Figure 1.7 shows lymphatic metastasis of mammary carcinoma. This biopsy of a mammary carcinoma revealed dilated lymphatics containing invasive carcinoma cells. This will only be seen if adjacent normal tissue containing lymphatics is included in the biopsy sample. Often the best area to look for compromised lymphatics is the superficial dermis, so it is helpful to submit the skin over the mass and at lateral margins, as well as deeper tissue.

Figure 1.8 shows lymph node metastasis of mammary carcinoma. This biopsy of a mammary tumor included a local lymph node that was found to have invasive carcinoma. This is where the pathologist can be helpful in the staging process.

There are some factors that can affect the use of grade and stage. Grade and stage are dynamic, not static, and each can progress independently to a different level. Grade and stage have elements of subjectivity; grade is based on a thin section of only a portion of the affected tissue so sampling error can occur, while stage can be affected by the quality and type of diagnostic procedures used (such as radiography versus magnetic resonance imaging [MRI]). If grade and stage are used to predict future behavior, the classification systems must have a sound basis and be applicable to the species and disease process to which they are applied. Histiocytic proliferations are infamous for their ability to regress or progress, spread to multiple sites, or completely disappear in dogs, yet they are, as currently recognized, always progressively more aggressive in cats.[15] It is important to recognize species and maybe even breed variability in behavior. MCTs in Siamese cats will sometimes spontaneously regress, but this would not be expected in Domestic Shorthair breeds (nor in dogs of any breed). Some tumors vary in behavior by site of origin and may have differing staging systems depending on location. Additionally, in some studies of melanocytic and mammary tumors, the presence of neoplastic cells in local lymphatics may not be correlated with survival time.[16, 17] The database is constantly expanding, and consultation with a specialist can provide the most current recommendations.

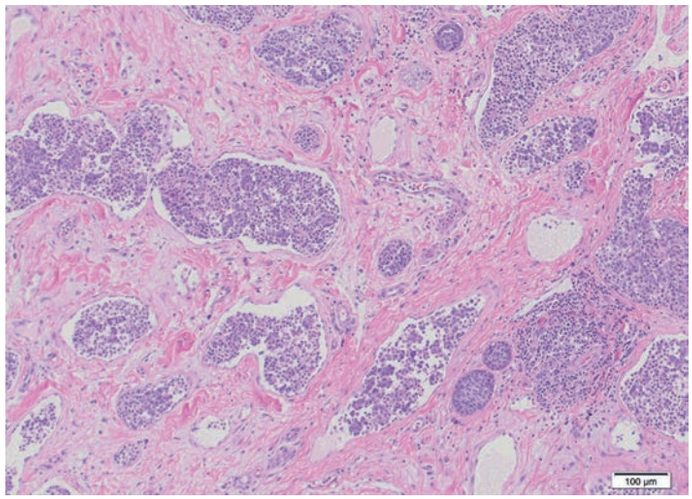

Figure 1.7 Mammary carcinoma lymphatic metastasis biopsy. 10×.

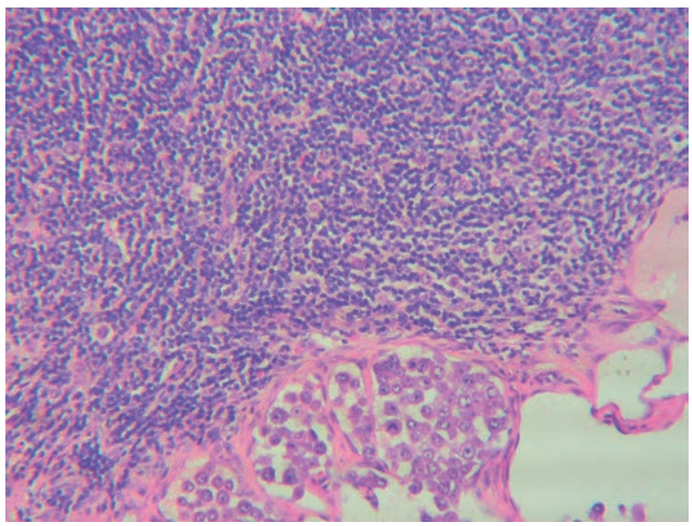

Figure 1.8 Mammary carcinoma lymph node metastasis biopsy. 20×.

Staging versus clinical behavior

A patient that presents with multiple masses can have a wide range of outcomes depending on the type of process causing the masses. A multifocal infectious or inflammatory disease might be expected to have a better prognosis than a widespread neoplastic disease. Aspiration of multiple masses is recommended as a first step because this will help determine if the masses are due to one process or multiple etiologies and can indicate infectious versus neoplastic etiology. This approach can yield the most favorable clinical outcome by treating the correct disease without delay. Staging will only be applicable after the disease process has been definitively diagnosed, regardless of how widespread the disease appears clinically.

Figure 1.9 shows a fungal granuloma. An adult male neutered cat presented with multiple skin masses, and aspiration of several masses revealed scattered histiocytes, small lymphocytes, macrophages, and *Cryptococcus neoformans*. The presence of a fungal agent indicates antifungal medical therapy would be appropriate, but immunosuppressive drugs or chemotherapy agents would be contraindicated and antibacterial therapy would be ineffective. The diagnosis made by FNA allows for timely therapy prior to biopsy or fungal serology in this case and demonstrates how a diagnosis must be made before staging is considered.

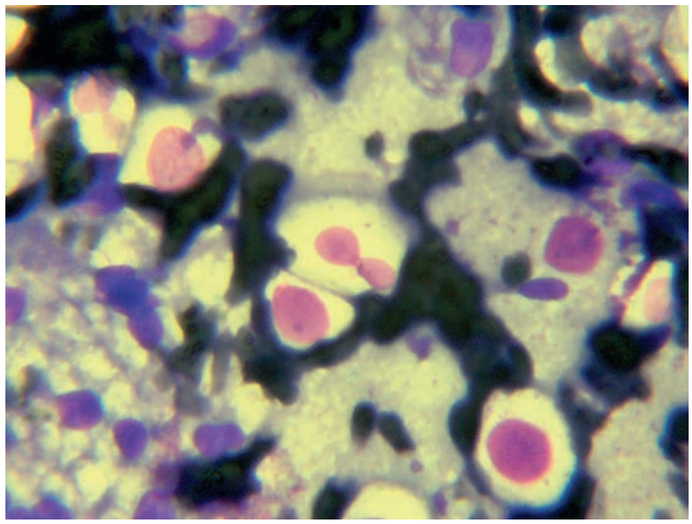

Figure 1.9 Fungal granuloma fine needle aspiration. 50×

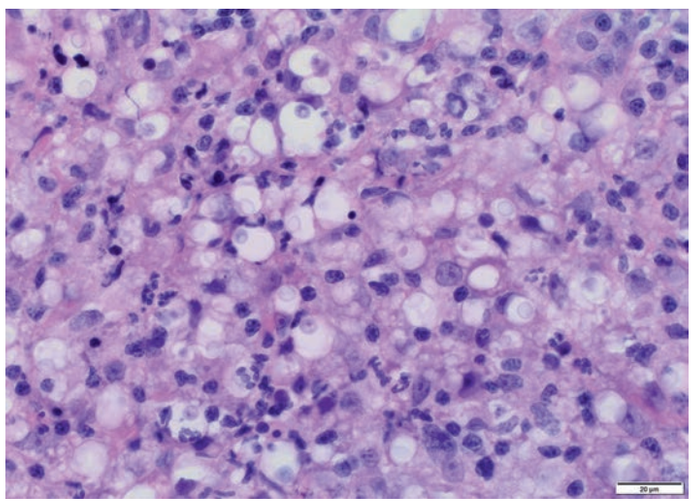

Figure 1.10 *Cryptococcus neoformans* biopsy. 40×.

Figure 1.10 shows a *C. neoformans* biopsy. This adult male neutered cat had multiple skin masses about the head and in the submandibular area, aspirates of which are illustrated in Figure 1.9. The nodules persisted despite therapy, and the biopsy revealed *Cryptococcus* organisms both in the skin nodules and lymph nodes. This disease may progress even with appropriate medical therapy, and this neurotropic organism can infiltrate the tissues of the nasal cavity, spreading to local lymph nodes and eventually invading the brain. Testing for immunosuppressive virus infection would be prudent.

Figure 1.11 shows a canine histiocytoma. Aspirate of several cutaneous masses in this 2-year-old dog revealed many round cells with fine chromatin and pale cytoplasm as well as scattered small lymphocytes and neutrophils. The preliminary diagnosis was multifocal histiocytoma, and biopsy was recommended for a definitive diagnosis because the lesions were multiple and persistent. A biopsy is not usually indicated at first presentation of this tumor type if there is only one mass and it regresses in a timely manner.

Figure 1.12 shows a canine cutaneous histiocytoma. This biopsy revealed that recurrent skin masses in a young dog were composed of sheets of round cells with moderate pale cytoplasm, occasional mitotic figures, and rare invasion into the epithelial layer by solitary cells. This lesion is expected to regress with time as tumoral Langerhans cells develop a more mature phenotype.[18] If this mass fails to regress, additional slides could be cut from the same block of fixed tissue used for the H&E slide and evaluated with Giemsa stain to exclude poorly granular MCT. Immunohistochemical

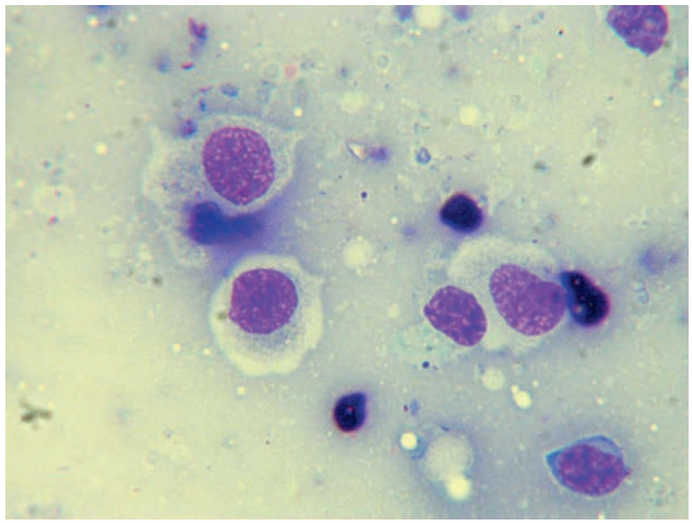

Figure 1.11 Canine histiocytoma fine needle aspiration. 50×.

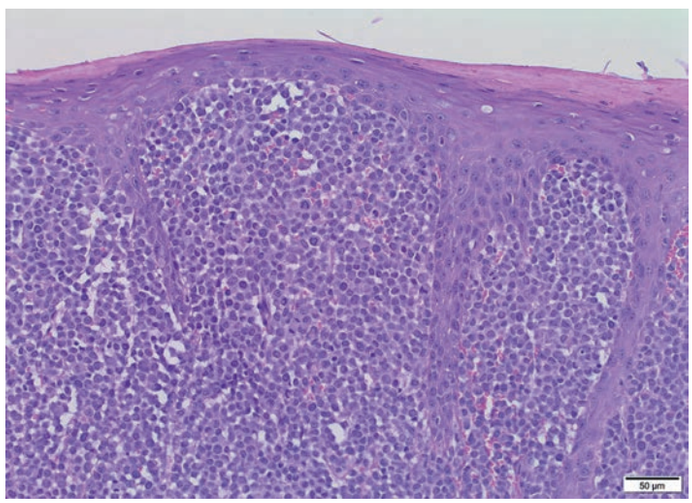

Figure 1.12 Canine cutaneous histiocytoma biopsy. 20×.

markers for T and B lymphocytes could also be applied to additional freshly cut slides to exclude cutaneous lymphoma. If there are concomitant genital masses, TVT should be considered. Additional immunohistochemical markers for histiocytes and other cell types are also available and are usually performed at research centers for best results. Consultation with laboratory personnel, the diagnostic pathologist who will be reading the tissue, and referral specialists such as oncologists or internists who may be working on the case will often yield the best plan for additional special stains in cases of persistent or progressive histiocytic tumors.

Figure 1.13 shows a cutaneous lymphoma collected by FNA. Aspirate of multiple skin masses on an adult, spayed female mixed-breed dog yielded a dense population of monomorphic round cells, about the size of the accompanying neutrophil, with round to occasionally cleaved nuclei, slightly clumped chromatin, and scant slightly basophilic cytoplasm. The preliminary diagnosis is cutaneous lymphoma.

Figure 1.14 shows a cutaneous lymphoma biopsy. Biopsy of several skin masses on the trunk of the adult dog in Figure 1.13 revealed variably dense infiltrates of fairly monomorphic medium-sized round cells that are invading the epidermis and forming microabscesses. This epithelial invasion is a hallmark of epitheliotrophic cutaneous T-cell lymphoma but on rare occasions scant epitheliotrophism can be seen with histiocytoma (personal experience, ARK). Unlike the histiocytoma in the previous figure, this disease will be progressive, and any therapy should be promptly initiated.

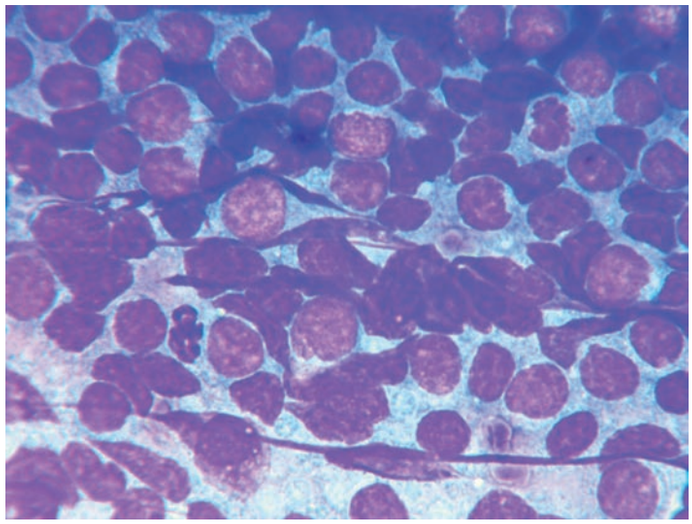

Figure 1.13 Cutaneous lymphoma fine needle aspiration. 50×.

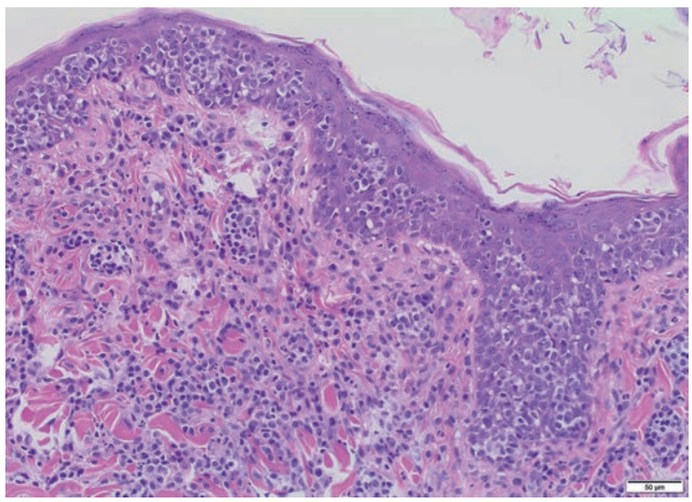

Figure 1.14 Cutaneous lymphoma biopsy. 20×.

Additional methods of qualifying lymphocytes and their expected behavior are sometimes available, and will be discussed in a later chapter. If this is an indolent T-cell lymphoma, the rate of progression may be slow in the early stages.[19, 20]

These three disease processes can have a somewhat similar clinical appearance but have distinguishing cytological and histological features that allow accurate diagnosis and therefore a more applicable prognosis and therapeutic regimen. Multiple skin masses and lymphadenopathy have different significance in these three diseases and therefore any staging or prognostic categorization must be done with a definitive diagnosis in hand.

Tumor classification by cell type: epithelial, mesenchymal, round cell and melanoma

Epithelial tumors

Epithelial neoplasms can arise as benign (epithelioma, adenoma) or malignant (carcinoma) tumors. Benign tumors may undergo transformation to malignant tumors.

Figure 1.15 shows an apocrine gland adenoma biopsy. This skin mass is composed of cystic spaces lined by single to double rows of well-differentiated apocrine epithelium, consisting of cells with small nuclei and scant cytoplasm that

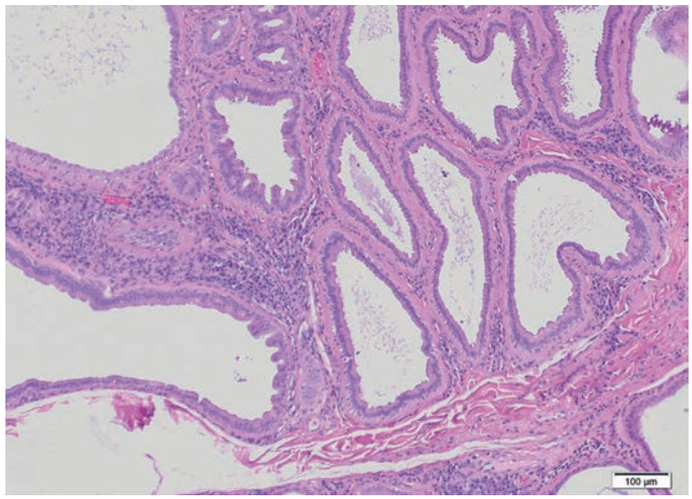

Figure 1.15 Apocrine gland adenoma biopsy. 10×.

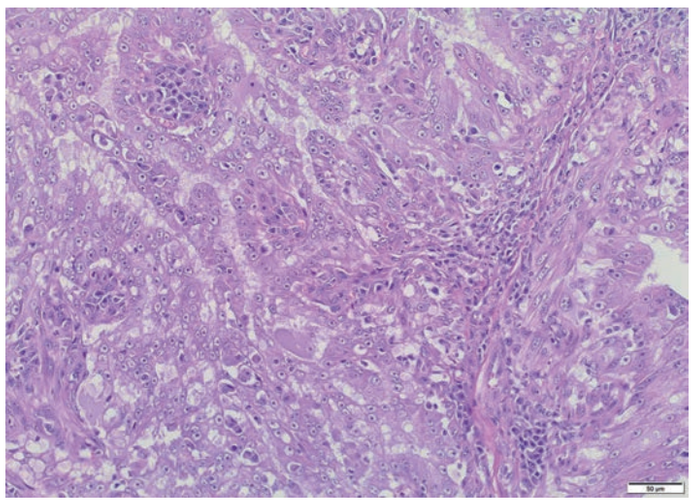

Figure 1.16 Apocrine gland carcinoma biopsy. 20×.

do not invade into underlying stroma. This tumor is benign, and complete excision with conservative margins of 0.2 cm should be curative.

Figure 1.16 shows an apocrine gland carcinoma biopsy. This invasive tumor can arise from an apocrine gland in the skin and rapidly invade adjacent stroma and lymphatic structures. There may be remnants of glands filled with neoplastic cells, or the cells may form sheets in the stroma with only a suggestion of the former glandular architecture. The nuclei are large with large nucleoli and vesiculated chromatin. There is cellular disorganization even in areas of retained glandular structure. Local lymph nodes and thoracic radiographs should be evaluated for evidence of metastasis. Complete wide excision is the minimal approach although clean margins can be difficult to obtain. It is recommended to evaluate local lymph nodes by biopsy or FNA and continued monitoring for regrowth or metastasis is advisable. Consultation with an oncologist could be helpful to determine the most efficacious approach to therapy.

Epithelial tumors can arise from the many structures of the haired skin, follicles, and associated glands. They are often benign or low grade but can undergo malignant transformation with time or can arise as de novo malignant tumor.

Figure 1.17 shows a trichoepithelioma biopsy. Adnexal tumors, especially cystic adnexal tumors, are common and are usually benign. They often grow around dilated follicles that fill with keratin. Evaluation of the epithelium lining the cyst

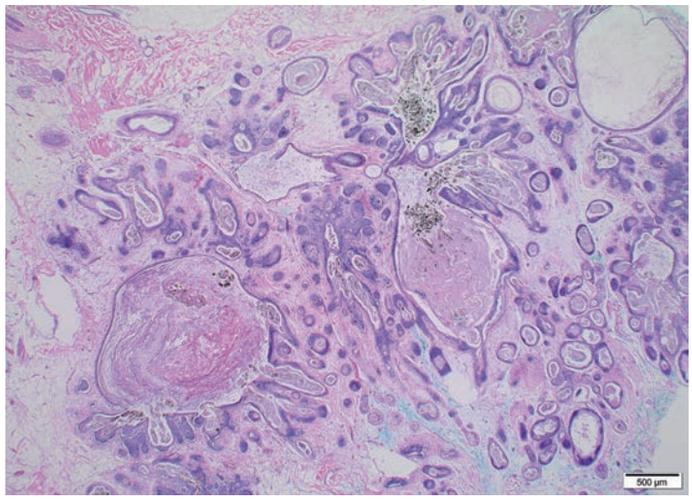

Figure 1.17 Trichoepithelioma biopsy. 10×.

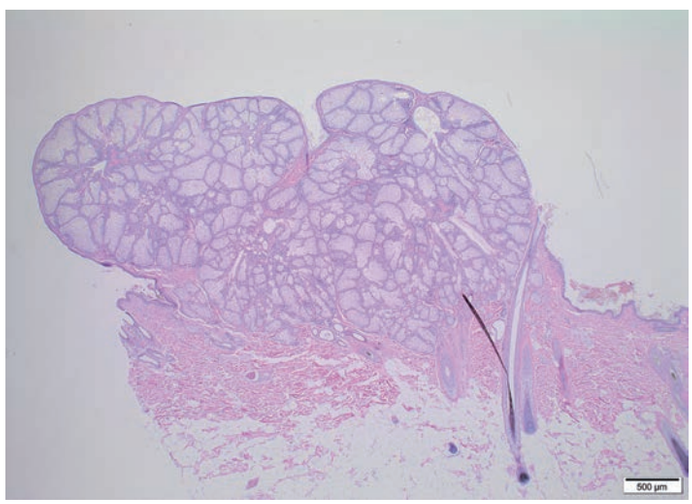

Figure 1.18 Sebaceous adenoma biopsy. 10×.

wall is necessary for diagnosis of the tumor type and is critical for accurate prognosis. Manual extrusion of the cyst contents with a biopsy of the keratin mass is unrewarding as there will be no cyst wall present in the sample. Rupture of the cyst prior to removal can result in severe cellulitis with an influx of pleomorphic phagocytic cells (macrophages and multinucleate giant cells) that can almost appear to be a neoplastic population. FNA is appropriate prior to removal to rule out other more aggressive tumor types such as MCT. Removal of this tumor with at least 0.2 cm of normal tissue at the margins is acceptable, and regrowth is not expected. Marginal excision ("shelling out") of the mass is not advisable because it may leave small amounts of tumor in the surgical bed to regrow or transform into a more aggressive form of this tumor.

Figure 1.18 shows a sebaceous adenoma biopsy. Proliferations of sebaceous glands are the fifth most common skin tumor in dogs and the eighth most common in cats.[21] They are characterized by proliferations of well-differentiated variably vacuolated sebaceous epithelial cells confined by a reserve cell lining of smaller basaloid epithelium, often around cystic and debris-laden ducts and follicles. Nomenclature based on the architectural arrangement of the cells includes sebaceous gland hyperplasia consisting of enlarging sebaceous gland epithelium rimmed by a single layer of basaloid epithelium extending from the follicle to ducts to the lobule, sebaceous adenoma consisting of proliferating sebaceous gland epithelium rimmed by a single layer of basaloid epithelium extending in a disorderly pattern from the duct and sebaceous epithelioma consisting of proliferating basaloid reserve cells around occasional foci of sebaceous gland epithelium that do not always clearly extend from a duct. These are listed from least aggressive to most aggressive in Table 1.4. Complete excision with conservative margins of at least 0.2 cm of normal tissue is usually curative.

Table 1.4 Types of proliferations of sebaceous glands and the prognosis for each.[21]

Category	Prognosis
Sebaceous hyperplasia	Benign
Sebaceous adenoma	Low grade
Sebaceous epithelioma	Low to medium grade
Sebaceous carcinoma	High grade, invasive

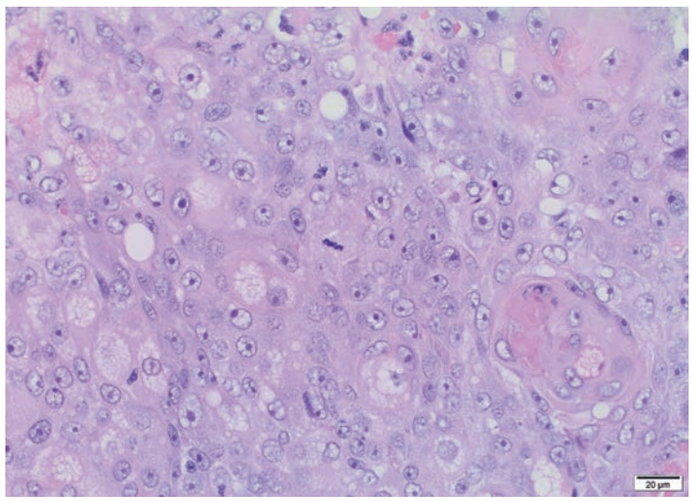

Figure 1.19 Sebaceous carcinoma biopsy. 40×

Figure 1.19 shows a sebaceous carcinoma biopsy. Sebaceous carcinoma is the malignant counterpart of benign sebaceous gland tumor and consists of poorly circumscribed proliferations of the sebaceous gland and reserve basaloid cells that are not associated with ducts and invade into normal stroma. There is nuclear atypia with occasionally vesiculated chromatin, prominent nucleoli, and variably dense to vacuolated cytoplasm. A presurgical FNA finding of pleomorphic nuclei can be the hint that wide margins are necessary to achieve complete excision and allow enough marginal normal tissue to evaluate the adjacent lymphatics for invasion. Upon receiving this diagnosis, staging and oncology consultation would be prudent due to the potential for aggressive behavior.

Figure 1.20 shows a biopsy of an SCC in situ. Carcinoma in situ (Bowen's disease) is seen mostly in cats and is a complex progression of preneoplastic to early neoplastic proliferations of squamous epithelial cells that are confined to the epidermis and do not cross the basement membrane into the subepidermis. The epithelial cells can be pigmented with the migration of melanophages and clumps of pigment into the underlying superficial dermis. This lesion can be promoted by solar exposure (actinic keratosis) or papillomavirus infection and can be multiple. If not completely excised, it can progress to invasive carcinoma.

Figure 1.21 shows a biopsy of an SCC. SCC is a disorganized proliferation of variably keratinizing squamous epithelial cells that cross the basement membrane and invade into underlying stroma. They can be well differentiated, forming nodules of keratinizing cells (keratin pearls), or they can be anaplastic and form sheets of pleomorphic epithelioid cells. There is usually nuclear atypia with large nuclei, vesiculated chromatin, prominent, usually single, nucleoli and variable cytoplasmic keratinization, which is often asynchronous to nuclear maturation (dyskeratosis). This tumor type will readily invade stroma and local lymphatics, metastasizing to local lymph nodes and further. FNA can reveal these atypical morphological features, leading to a preliminary diagnosis of carcinoma. This can allow for referral to a specialist or, if referral is declined, can indicate a need for staging with an evaluation of local lymph nodes and thoracic radiographs, as well as wide surgical excision. Excision without additional therapy often is only palliative.

Epithelial tumors of the mucous membranes (mouth, conjunctiva, vagina, urinary bladder, intestine) can exhibit a rapid growth rate with early stromal invasion and may metastasize to lymph nodes readily. Notable exceptions are epulis and papilloma, which are usually benign, but can become locally invasive with time.

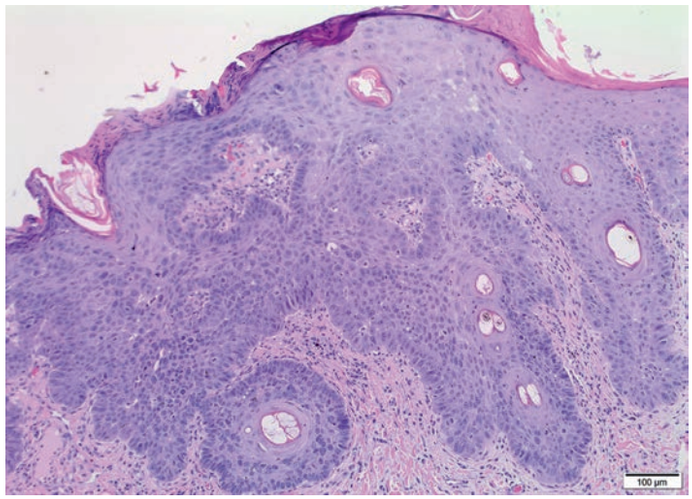

Figure 1.20 Carcinoma in situ biopsy. 10×.

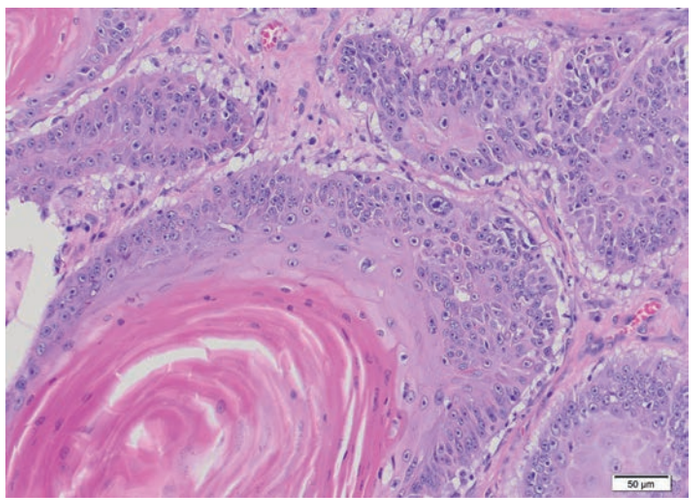

Figure 1.21 Squamous cell carcinoma biopsy. 20×.

Figure 1.22 shows a canine epulis biopsy. This pink, firm gingival mass from a dog reveals anastomosing chains of epithelial cells extending from the epithelium into a proliferative fibrous stroma without a visible breach of the interface basement membrane that would indicate stromal invasion. Complete excision of this low-grade biphasic lesion is often curative, but multiple foci may be present and additional masses may arise.

Figure 1.23 shows a canine ossifying acanthomatous ameloblastoma (ossifying acanthomatous epulis) biopsy. This pink, firm gingival mass from a dog reveals a more proliferative epithelial component with occasional production of bone. It is a low-grade tumor, although if not excised aggressively there is high potential for recurrence and removal by an oral surgeon is recommended. There is also a high potential for invasion into and destruction of the underlying bone, with the potential for transformation to a more aggressive tumor type such as SCC or osteosarcoma. Radiographs prior to surgery would be helpful to define the margins. Metastasis is not expected unless there is a transformation to a more aggressive tumor type.

Figure 1.24 shows a canine oral papilloma biopsy. This oral mass was one of many excised from the mouth of a dog. The thickened, hyperkeratotic epithelium is thrown into folds over a fibrous stroma, and there is no stromal invasion. There may be viral inclusions and cytopathic changes in the epithelial cells. This lesion is often the result of papillomavirus infection, it may be multiple, and additional lesions may arise. In an older dog with recurrent tumors, check for immunosuppression and consider the possibility of oral nonviral papillary squamous carcinoma. This tumor type will appear as a papilloma superficially but will have an invasive deep component and will behave as a carcinoma.[22]

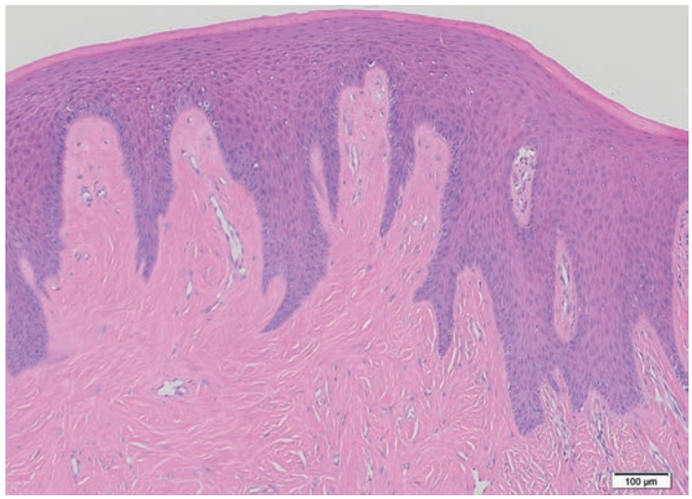

Figure 1.22 Canine epulis (peripheral odontogenic fibroma) biopsy. 10×.

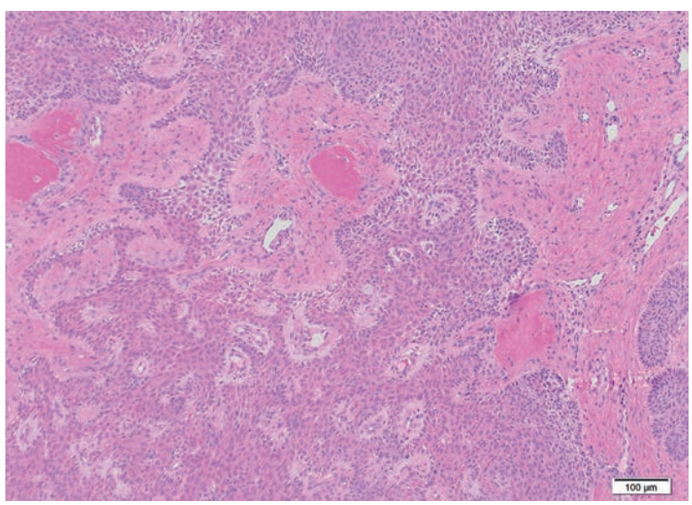

Figure 1.23 Canine ossifying acanthomatous epulis (ossifying acanthomatous ameloblastoma) biopsy. 10×.

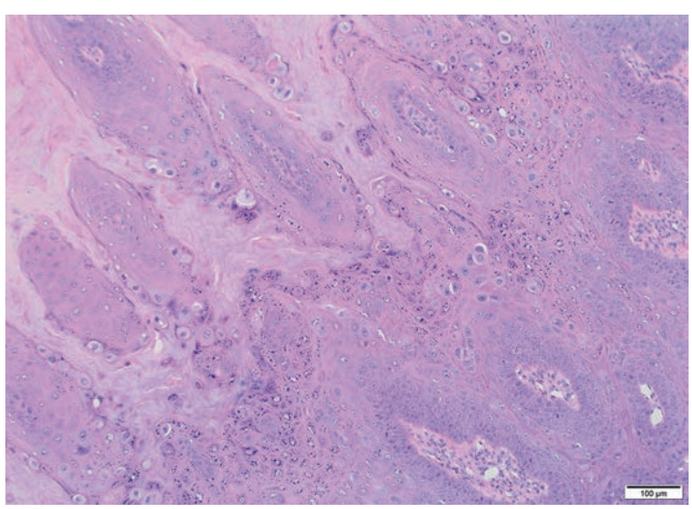

Figure 1.24 Canine oral papilloma biopsy. 20×.

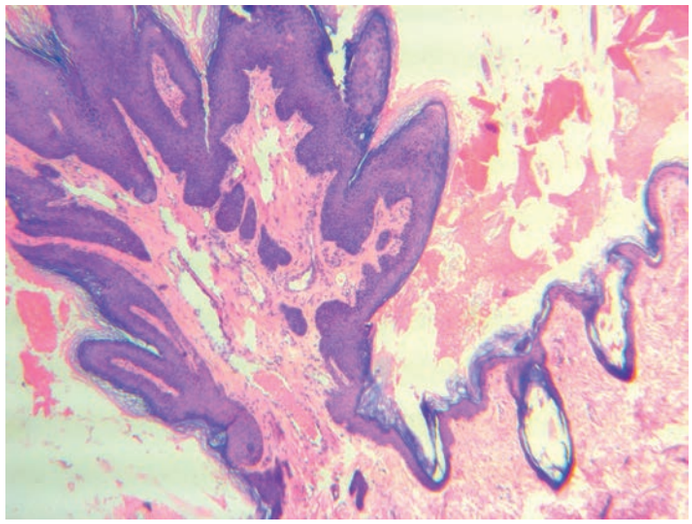

Figure 1.25 Canine conjunctival fibropapilloma biopsy. 10×.

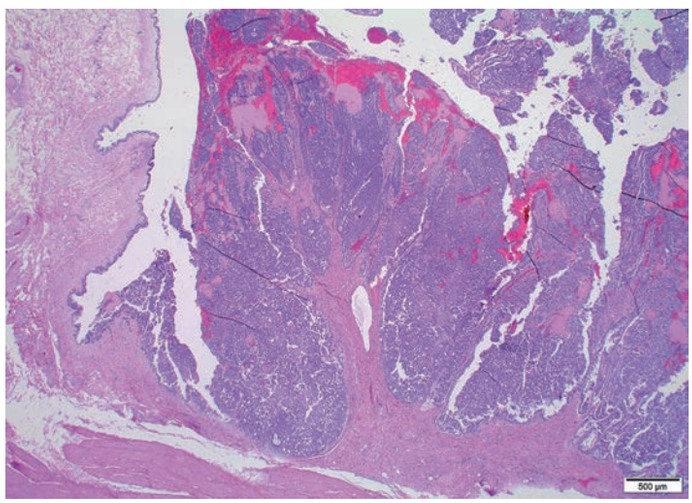

Figure 1.26 Canine urinary bladder transitional cell carcinoma biopsy. 10×.

Figure 1.25 shows a canine conjunctival fibropapilloma biopsy. This tumor may be associated with trauma, and it tends to arise at the mucocutaneous junction of the eyelid. The cytopathic effects present in viral papillomas are not seen. Thickened and mildly dysplastic hyperkeratotic epithelium is thrown into folds over a loose fibrous stroma without stromal invasion. There is no significant hyperkeratosis. Viral genetic material is absent, and viral antigens are not demonstrated. These lesions are often hyperpigmented and can be grossly similar to melanoma, thus the recommendation for removal when small.[23]

Figure 1.26 shows a canine urinary bladder transitional cell carcinoma biopsy. Biopsy evaluation of this bladder mass revealed a disorganized proliferation of pleomorphic epithelial cells with invasion into the underlying stroma. This tumor tends to metastasize to local lymph nodes and distant sites, but medical therapy can be palliative for a significant length of time with minimal complications in some dogs, and consultation with an oncologist for the most applicable and current therapy is suggested.

Figure 1.27 shows a biopsy of an SCC of the nose. This disorganized proliferation of pleomorphic squamous epithelial cells exhibits nuclear atypia and stromal invasion. The central keratinization of some nests of cells is the identifying feature of this lesion.

Figure 1.28 shows a canine SCC toe biopsy. This SCC (arrow) in the toe of an adult dog has invaded into the third phalangeal bone causing disassociation of the joint cartilage (arrowhead). This suggests a high-grade tumor, and

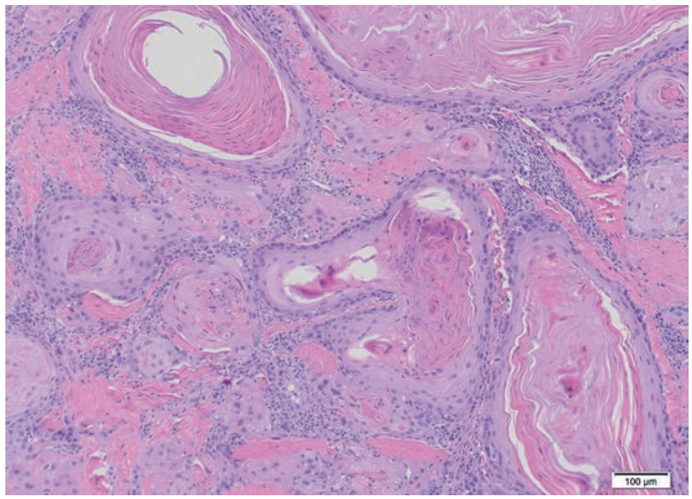

Figure 1.27 Squamous cell carcinoma of the nose biopsy. 20×.

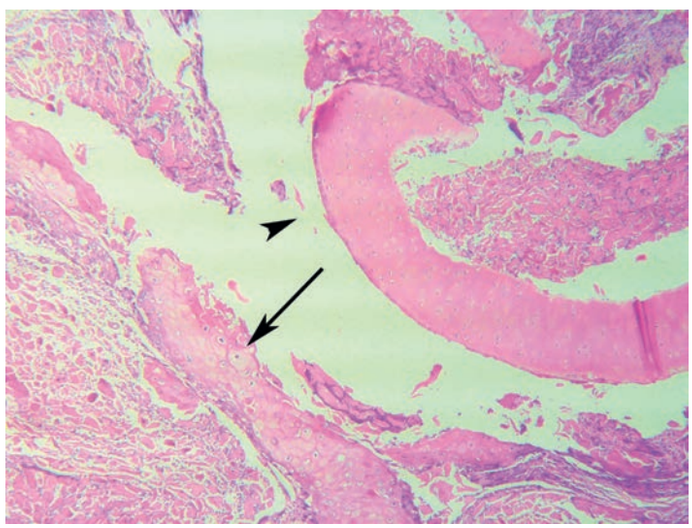

Figure 1.28 Canine squamous cell carcinoma toe biopsy. 2.5×.

evaluation of local lymph nodes with thoracic radiographs would be part of the minimal database. Excision of the entire digit would remove this painful joint, which is unlikely to return to normal function, and may be necessary to achieve wide margins of normal tissue and provide an assessment of bone and joint invasion.

Squamous epithelial tumors are often assessed by a combination of mitotic rate and invasion of stroma and lymphatics, and assigned a grade. Feline oral SCC mitotic rates, however, appear not to be predictive of behavior, but stage, and especially the presence of bone invasion, is predictive.[24, 25] Radiographs, therefore, may have high prognostic significance in feline oral SCC.

Figure 1.29 shows a feline SCC mouth biopsy. This SCC from the mouth of an adult cat is forming a keratin pearl at the left margin and is clearly invasive into the stroma of the submucosa. The scant eosinophilic material around the foci of tumor may be osteoid or bone, suggesting that this tumor is invading the underlying bone. Mitotic figures are not seen in this field, but a high-grade tumor is suspected due to the bone invasion.

Tumors arising from epithelial cells of internal organs are often not discovered until a late stage. In one survey, 76% of feline lung carcinomas had metastasized by the time of discovery.[26]

Figure 1.30 shows a lung bronchoalveolar carcinoma biopsy. The proliferation of atypical and disorganized bronchial lining epithelium can replace large areas of pulmonary parenchyma before clinical signs of respiratory distress are observed.

Table 1.5 describes a grading protocol for biopsy evaluation of canine pulmonary carcinoma.

Table 1.6 describes a grading protocol for biopsy evaluation of feline pulmonary carcinoma.

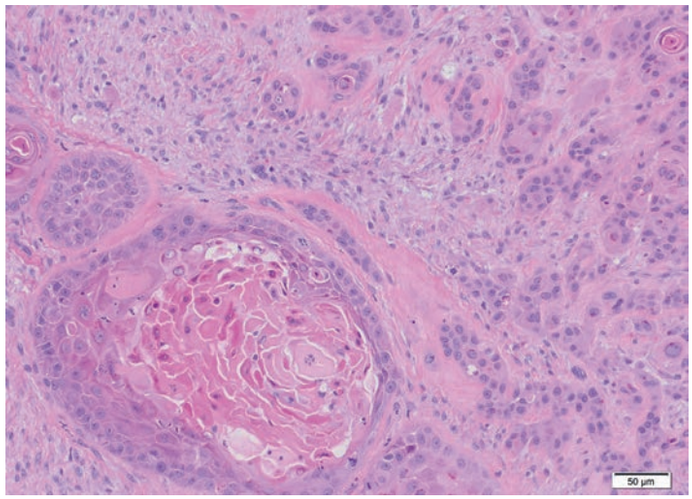

Figure 1.29 Feline squamous cell carcinoma mouth biopsy. 10×.

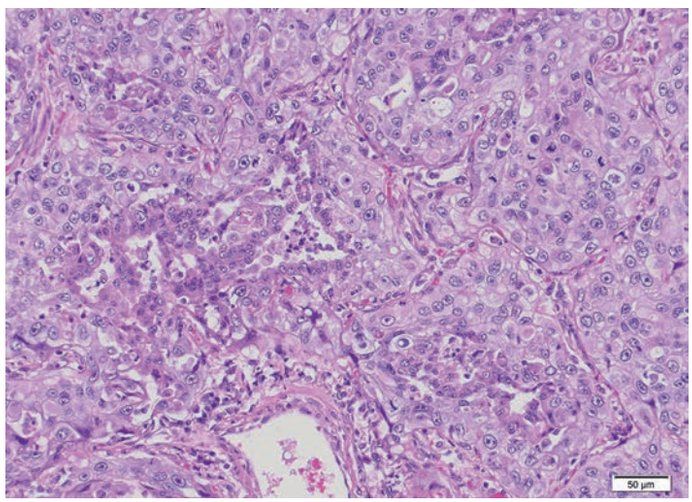

Figure 1.30 Lung bronchoalveolar carcinoma biopsy. 20×.

Feline pulmonary carcinomas are not common. They usually present clinically as either a solitary nodule, a large nodule with several smaller nodules, or coalescing nodules. They are classified into three grades according to their degree of differentiation, with high-grade tumors being the more common presentation and having a poor prognosis and short survival time. Retrospective studies confirm that clinical prognosis is satisfactorily predicted by these grading systems.[28] Metastasis to one or multiple digits is an occurrence referred to as feline lung-digit syndrome.

Figure 1.31 shows a biopsy of a pancreatic carcinoma metastatic to the liver. Ductular carcinoma in this adult female French Bulldog arose in the pancreas, metastasizing to adjacent organs including the liver as shown in this histological section. There is both stromal (arrowheads) and lymphatic (arrows) invasion in this section. The patient presented with vague clinical symptoms, mostly anorexia, and a gastrointestinal foreign body was suspected. A pancreatic mass that had already spread to multiple other internal organs was found on exploratory laparotomy, and a biopsy was performed postmortem.

Masses in internal organs may shed neoplastic cells into body cavity effusions. FNA of effusions is a rapid way to obtain a preliminary diagnosis. FNA of the mass can be performed prior to surgery if evaluation of an effusion is not diagnostic.

Figure 1.32 shows an intestinal adenocarcinoma FNA. An adult cat presented with abdominal effusion. FNA revealed clusters of epithelioid cells with marked anisokaryosis, prominent nucleoli, and basophilic cytoplasm that appear to contain fluid or mucin. When clusters of cells such as this are found free in the abdominal fluid, additional workup such as radiography or ultrasound is indicated to search for a mass that could be biopsied.

Table 1.5 Grading system of canine pulmonary carcinomas.[27]

Overall differentiation	Score
Well-differentiated (orderly arrangement of neoplastic cells to other cells, matrix, and basement membrane)	1
Moderately differentiated (contains areas with orderly cellular arrangement and areas with loss of cell-to-cell or cell-to-matrix organization)	2
Poorly differentiated (loss of neoplastic cell orientation to other cells and loss of polarity to the matrix or basement membrane)	3
Nuclear pleomorphism	Score
Mild (overall uniform nuclei with minimal anisocytosis and anisokaryosis)	1
Moderate (nuclei vary but with less than a 2-fold difference in size)	2
Severe (nuclei with a greater than 2-fold difference in size and many irregular shapes)	3
Mitotic count (in 10 HPFs)	Score
1–10	1
11–20	2
21–30	3
≥31	4
Nucleolar size	Score
Small (difficult to identify)	0.5
Medium (identifiable but not prominent)	1
Large (prominent and at least a third of the size of the nucleus)	1.5
Tumor necrosis	Score
None	0
1% to 20%	1
21% to 50%	2
>50%	3
Tumor fibrosis	Score
None	0
1% to 20%	0.5
21% to 50%	1
>50%	1.5
Demarcation	Score
Well-demarcated (sharp border between tumor and normal tissue but no capsule)	1
Moderately demarcated (areas of tumor tissue protrude into adjacent tissue)	2
Invasive (many areas of tumor tissue and separate tumor cells protrude into adjacent tissue; borders not distinguishable)	3
Histological grade	Total score
Grade 1 (low grade)	≤8.5
Grade 2 (medium grade)	9.0–14.0
Grade 3 (high grade)	≥14.5

Abbreviation: HPF, high-power field.
Area of view is not specified.

Table 1.6 Grading system of feline pulmonary carcinomas.[29]

	Histological features
Grade 1 (low grade) (well differentiated)	Monomorphic cellular and architectural phenotype Little to no epithelial stratification or solid growth patterns Distinct borders with little to no invasion
Grade 2 (medium grade) (moderately differentiated)	Less pleomorphism than poorly differentiated tumor No vascular invasion or intrapulmonary metastasis
Grade 3 (high grade) (poorly differentiated)	Disorganized, infiltrative growth of pleomorphic cells Vascular invasion and/or intrapulmonary metastasis

Figure 1.33 shows a biopsy of an intestinal mass in an adult cat that revealed invasion by glands from the lamina propria into the submucosa and muscular layers. These glands were lined by epithelial cells that were sometimes more than a single-cell layer thick, with large pleomorphic nuclei exhibiting vesiculated chromatin with prominent nucleoli, and basophilic cytoplasm with large vacuoles. There was an invasion into the local lymphatics by neoplastic rafts of cells with a similar morphological appearance to the cells in Figure 1.32.

Mammary tumors can be composed of proliferating tubules and glands (simple), proliferating tubuloglandular epithelium and myoepithelial cells (complex or biphasic), and proliferations of tubuloglandular epithelium and

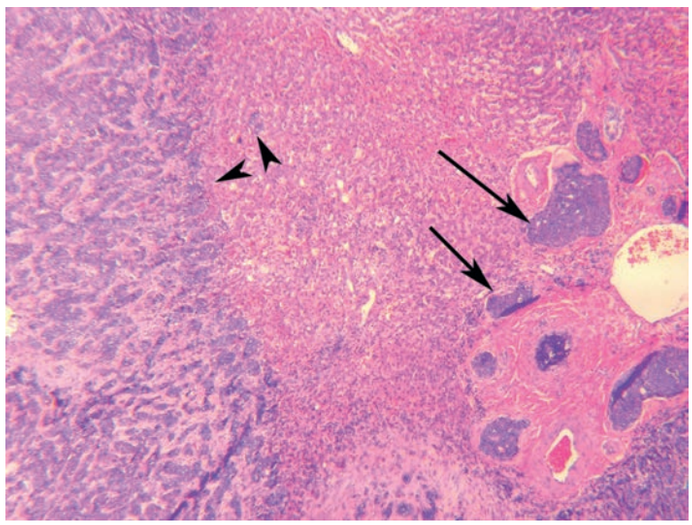

Figure 1.31 Pancreatic carcinoma metastatic to liver biopsy. 2.5×.

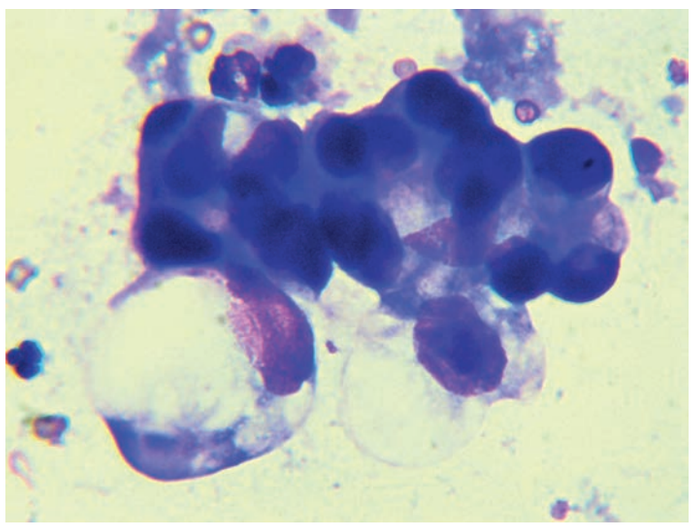

Figure 1.32 Intestinal adenocarcinoma in abdominal fluid fine needle aspiration. 50×.

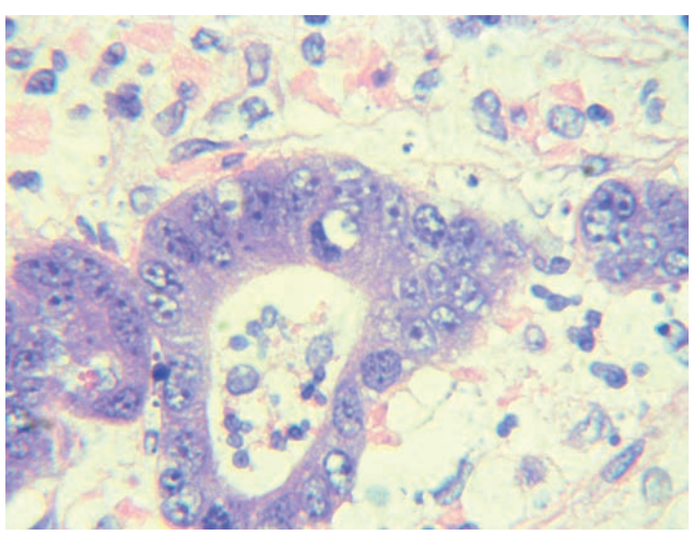

Figure 1.33 Intestinal adenocarcinoma biopsy. 40×

myoepithelium with the formation of cartilage and/or bone (mixed or triphasic). Simple epithelial tumors are graded using type, nuclear pleomorphism, and MC, and this grade correlates with the risk of invasion (see Tables 1.7 and 1.8). Most canine mammary tumors are epithelial and myoepithelial (complex or mixed), and the grading system devised for simple mammary tumors is not applicable to complex or mixed tumors.[30] In dogs, ovariohysterectomy (OHE) status and tumor grade, age, tumor stage, tumor subtype, and lymphatic metastasis were correlated with recurrence, metastasis, and survival time in one study and not related in another.[31, 32] Atypical ductal hyperplasia or epitheliosis in dogs is associated with malignant neoplastic transformation, as is stromal invasion.[33–35] Mixed mammary tumors, the most common type of tumor found in dogs, tend to be less aggressive unless they are carcinosarcomas.[36] Spindle cell tumors can arise in canine mammary glands, should be identified as to the cell type of origin using immunohistochemistry, and are graded using the canine soft tissue sarcoma (STS) grading protocol.[37] Fifty-three percent of feline mammary carcinomas had metastasized by the time of discovery.[38] Mixed mammary tumors (triphasic tumors) are not

Table 1.7 Grading system for canine (Pena) and feline mammary carcinomas (NHG, Mills)[14]

Histological features			
Tubule formation			Score
Comprises a majority of the tumor (>75%)			1
Present to a moderate degree (10–75%)			2
Little or none present (<10%)			3
Nuclear pleomorphism			Score
Small regular uniform nuclei			1
Moderate increase in size, vesiculation, and variability			2
Vesicular nuclei with marked variation in size and shape			3
MC (in 10 HPFs)[a]			Score
(Pena)	**(NHG)**	**(Mills)**	
DOG 0–9	CAT 0–8 mitoses	CAT (0–50)	1
DOG 10–18	CAT 9–16 mitoses	CAT (51–70)	2
DOG >20	CAT ≥17 mitoses	CAT (≥71)	3
Histological grade			Total score
Grade 1 (low grade)			3–5
Grade 2 (medium grade)			6–7
Grade 3 (high grade)			8–9

Abbreviations: HPF, high-power field; MC, mitotic count.
[a] MC is assessed at the periphery or in the most mitotically active parts of the tumor. Recently proposed modifications to the mitotic cutoffs are reported in parentheses (Mills et al). Area of view = 2.37 mm^2.

Table 1.8 Grading system of feline mammary carcinomas (Mills).[14]

Lymphovascular invasion	Score
Absent	0
Present	1
Nuclear shape[a]	Score
≤5% abnormal	0
>5% abnormal	1
MC (in 10 HPFs)[b]	Score
≤62	0
>62	1
Histological grade	Total score
Grade 1 (low grade)	0
Grade 2 (medium grade)	1
Grade 3 (high grade)	2–3

Abbreviations: HPF, high-power field; MC, mitotic count.
[a] Abnormal nuclear form includes any deviation from smooth nuclear contour or round/oval nuclear shape, such as clefting, angularity, corrugation, or amoeboid morphology assessed at high power in the least differentiated and/or most invasive portions of the tumor. The number of nuclei exhibiting the abnormal nuclear form is estimated and expressed as a percentage of the total number of nuclei within any given field.
[b] MC is assessed at the periphery or in the most mitotically active parts of the tumor. Area of view = 2.37 mm^2.

seen in cats but biphasic tumors are reported.[39, 40] With regards to the collection technique, it is difficult to evaluate stromal and lymphatic invasion if the tumor extends to all margins on the biopsy tissue submitted because one cannot look for invasion into normal adjacent stroma or lymphatics if neither is present. It is not advisable to "shell out" mammary tumors as the tissue tends to separate at the tumor interface yielding an inadequate margin.

Mammary chains are exposed to systemic factors known to promote mammary neoplasia, and it is not unusual for dogs and cats to develop tumors in multiple glands, and multiple tumors of the same or different type arising from different lobules in the same gland. In the mammary gland, hyperplastic lesions and benign tumors can develop into malignant tumors if not removed early or if incompletely removed.[41] An established grading system, the Pena system, considers the architectural arrangement, nuclear morphology, and the MC, totaling the score of the components into a category of low, moderate, or high. Tumors with epithelial and myoepithelial components, found commonly in dogs, are judged by the epithelial component alone unless the stromal component is also malignant in which case the grading system is applied to both populations. Mammary tumors in cats are predominantly epithelial and tend to be predominantly carcinomas with a high potential for lymphatic spread, and the high and low grades are statistically correlated with survivability.[42] There is not a well-established cytological grading system for canine or feline tumors, although increased nuclear pleomorphism is at least an indication for complete excision, and evolving protocols for the cat show a correlation with MC and histological grade.[43]

Figure 1.34 shows a biopsy of a mammary adenoma on a middle-aged spayed female dog. It reveals a proliferation of tubuloepithelial cells in dilated ducts and glands. There is no stromal invasion seen, and adjacent lymphatics are not significantly dilated. Excision of this mass is indicated because benign tumors can eventually become invasive. Grossly recognizable normal tissue at the lateral margins and a tissue plane at the deep margin would be the minimal margin width warranted if pre-biopsy FNA does not reveal pleomorphic cells suggestive of a more aggressive tumor type. It is not advisable to "shell out" mammary tumors even if the FNA looks benign.

Figure 1.35 shows a biopsy of a mammary complex adenoma on a middle-aged spayed female dog and reveals a proliferation of tubuloepithelial cells in dilated ducts and glands with proliferation of associated myoepithelium. This is a complex tumor, and grading for simple tumors is not applicable. Complex tumors are usually low grade, but complete excision in a timely fashion is recommended because this tumor may undergo malignant transformation with time. Conservative margins of grossly normal tissue at the lateral and deep margins are indicated if there is no FNA evidence of cellular pleomorphism.

Figure 1.36 shows a complex mammary adenoma biopsy with focal transformation to ductular carcinoma in an adjacent lobule. The larger mass in the lower right quadrant (arrow) is a complex, low-grade mammary tumor, which is not invading into surrounding stroma. In the upper center of the slide, there is a proliferation of ducts/alveoli (arrowhead) exhibiting focal atypia characterized by filling of the ducts with moderately pleomorphic epithelial cells that appear to have lost the normal arrangement of basally located nuclei that define the normal duct. This demonstrates, at the light microscope level, the concept of how plump, presumably hyperplastic cells might undergo loss of normal regulatory control, filling the ducts with disorganized epithelial cells (resulting in a diagnosis of epitheliosis, previously referred to as carcinoma in situ[34]) that eventually escape into the surrounding stroma.

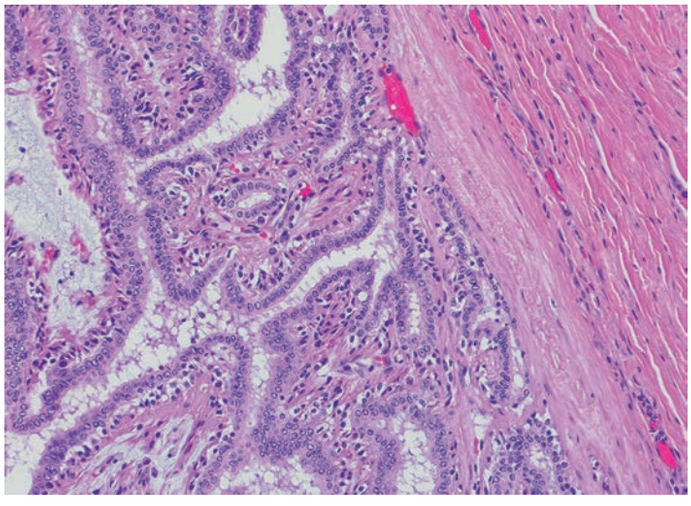

Figure 1.34 Canine mammary adenoma biopsy. 20×.

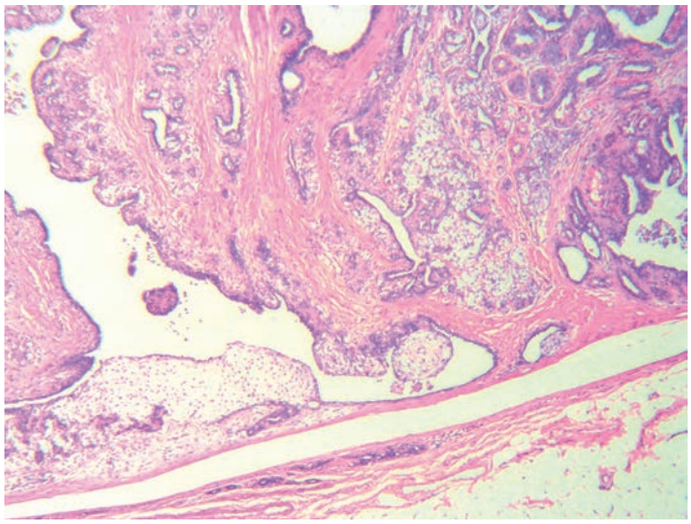

Figure 1.35 Canine mammary complex adenoma biopsy. 10×.

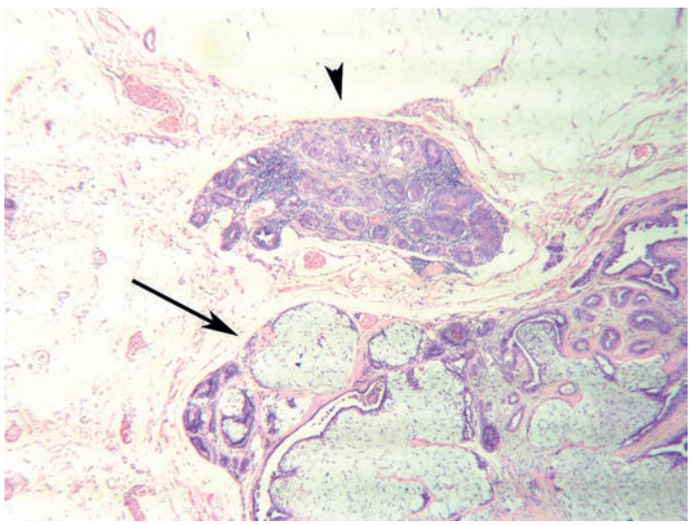

Figure 1.36 Complex mammary adenoma with focal intraductal carcinoma biopsy. 10×.

Figure 1.37 shows a closer view of the developing carcinoma in Figure 1.36. There is an irregular proliferation of cells filling rather than lining the tubules and exhibiting larger than normal nuclei with open chromatin, a probable mitotic figure, and abundant more deeply basophilic cytoplasm than the cells in the adjacent tubules. There appears to be early peritubular fibrosis and a peritubular inflammatory infiltrate of small lymphocytes and plasma cells. A more aggressive mammary tumor appears to be arising within this lobule. There is a recognizable inflammatory infiltrate of small lymphocytes and plasma cells associated with this lesion.

Figure 1.38 shows a biopsy of a mixed mammary tumor on a middle-aged sexually intact female mixed-breed dog. It reveals a proliferation of tubuloepithelial cells in dilated ducts and glands with the proliferation of associated myoepithelium, which is undergoing focal osseous and cartilaginous metaplasia. This tumor is low grade, but early excision is recommended because there is potential for malignant transformation with time. Conservative margins are indicated if there is no FNA evidence of cellular pleomorphism.

Figure 1.39 shows a canine invasive scirrhous carcinoma biopsy. This diffusely invasive mammary tumor in an adult female mixed-breed dog exhibits nuclear pleomorphism, frequent mitotic figures, and loss of normal architecture. This type of tumor has a high potential for invasion into lymphatics and progression to distant sites. FNA of this type of tumor may yield pleomorphic cells that would confirm the need for thoracic radiographs prior to surgery. Complete

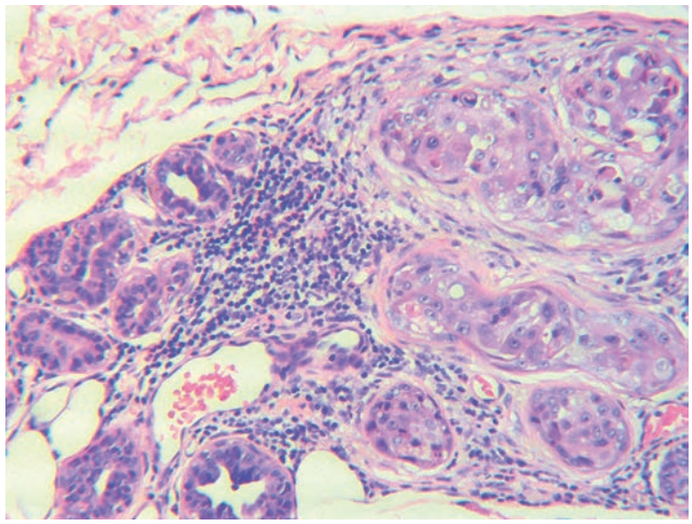

Figure 1.37 Mammary intraductular carcinoma. 20×.

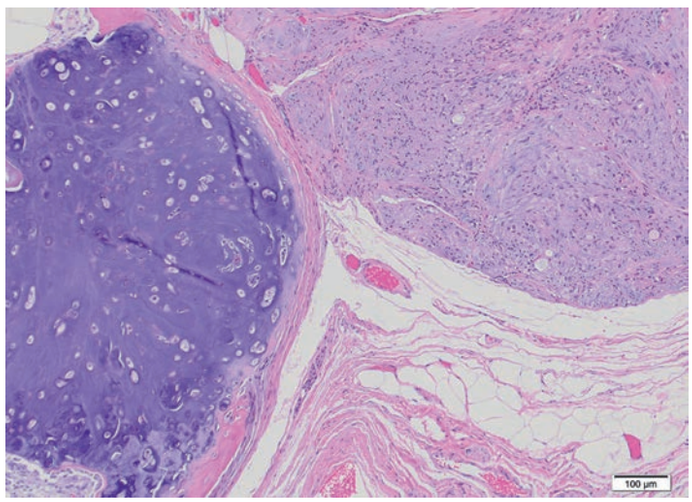

Figure 1.38 Canine mixed mammary tumor biopsy. 10×.

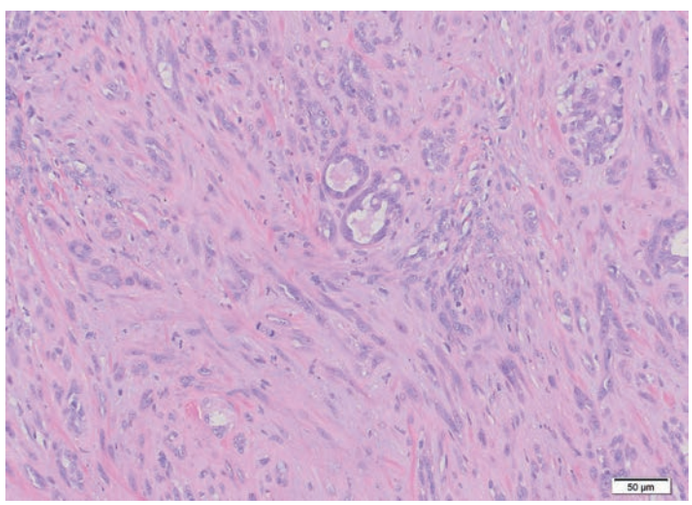

Figure 1.39 Canine invasive scirrhous carcinoma biopsy. 10×.

excision with wide margins would allow a search for lymphatic invasion, and submission of local lymph nodes could be helpful for staging if there is lymphadenopathy.

Figure 1.40 shows a biopsy from a young sexually intact female cat and reveals tubulolalveolar structures lined by a single layer of plump epithelium, which is then surrounded by laminar layers of myoepithelium. This is fibroepithelial hyperplasia, a benign response to hormonal stimulation and should regress upon removal of the hormonal stimulus.

Figure 1.41 shows a feline ductular carcinoma biopsy. This aggressive and high-grade tumor in an adult spayed female cat retained a somewhat duct-like appearance, but there is a loss of normal lobular architecture and invasion into a fibrotic stroma. This type of tumor in the cat tends to invade lymphatics early. When taking a biopsy for initial diagnosis, wide margins are recommended, so adjacent lymphatics can be searched for invasive tumor. Small biopsy samples may fail to demonstrate the invasive nature of the tumor.

Figure 1.42 shows a biopsy of feline invasive ductular carcinoma in the lymphatic structure. There are clusters of invasive ductular carcinoma in this lymphatic vessel. The wide margins of the tissue submitted, from the case in Figure 1.41, allowed the demonstration of numerous dilated lymphatics containing invasive carcinoma.

Figure 1.43 shows a local lymph node that was also submitted with the tissue from Figure 1.41. There was an invasion into the lymph node by neoplastic ductular epithelial cells.

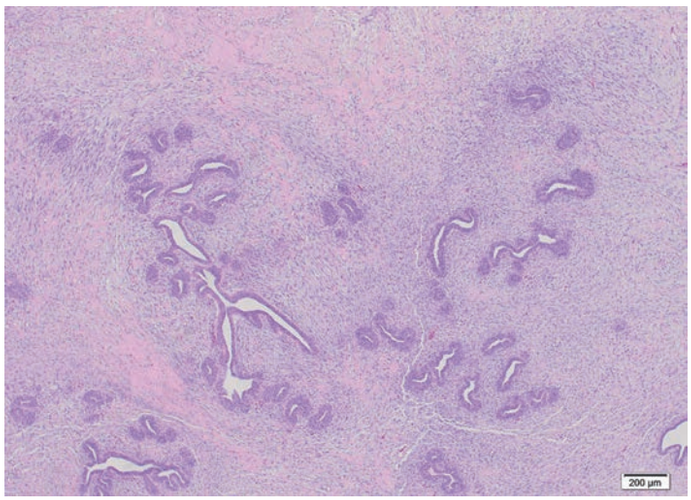

Figure 1.40 Feline mammary fibroepithelial hyperplasia biopsy. 10×.

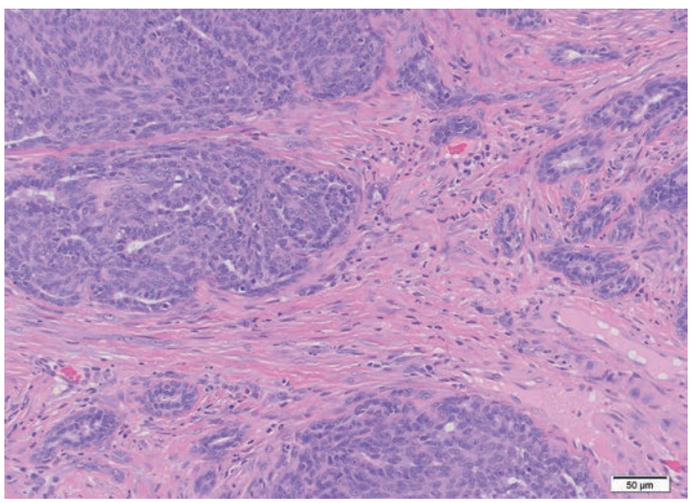

Figure 1.41 Feline ductular carcinoma biopsy. 20×.

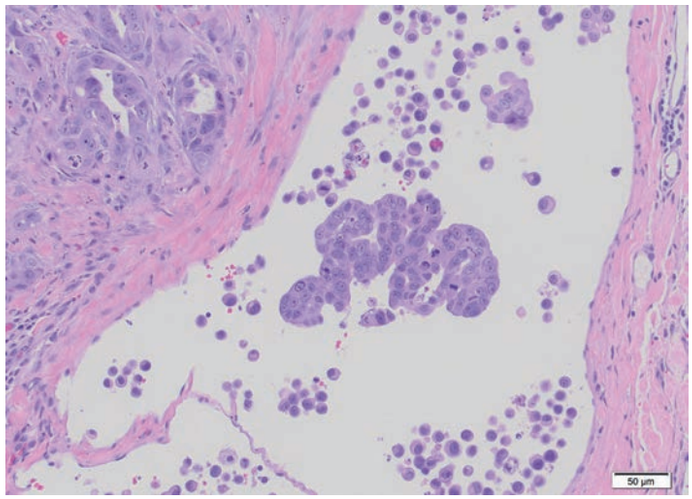

Figure 1.42 Feline invasive ductular carcinoma in lymphatic structure biopsy. 20×.

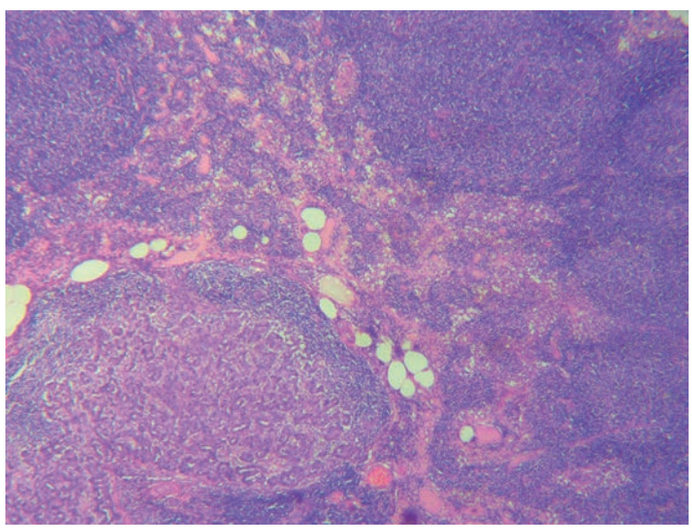

Figure 1.43 Feline invasive ductular carcinoma metastasis to local lymph node. 10×.

Mesenchymal tumors

Neoplasia of the stromal/spindle/mesenchymal cells of the body, also known as sarcoma when the proliferation is invasive, tends to be poorly circumscribed, as these cells are the framework of tissues and do not rest on a basement membrane. The human term STS encompasses many tissues, including smooth and striated muscle, adipose tissue, synovial tissue, and synovial linings, but in veterinary medicine the term STS and related histological grading system is, at this time, more limited to tumors of fibrocytes, nerve sheaths, and perivascular wall tissue often referred to as spindle cell tumors due to their spindloid appearance. These tumors usually grow first by local extension and then can metastasize to distant sites later in the course of the disease by hematogenous and lymphatic pathways. MC and size of clean margins measured in millimeters or centimeters are predictive of behavior, although more recent studies indicate that the status of surgical margins (clean versus not clean and size of the margin) is the main prognostic factor.[44] Complete removal of these tumors can be difficult to impossible depending on the location, and gross assessment of margins can be deceiving due to pseudo-encapsulation and extension of fibrils along fascial planes. Histological evaluation of surgical margins can be confounded by poorly delineated margins that are cauterized, torn, ripped and folded, or cut into. If the sample is large and requires an incision to allow formalin into the mass, it is best to make any cuts from a non-margin site such as the overlying skin.

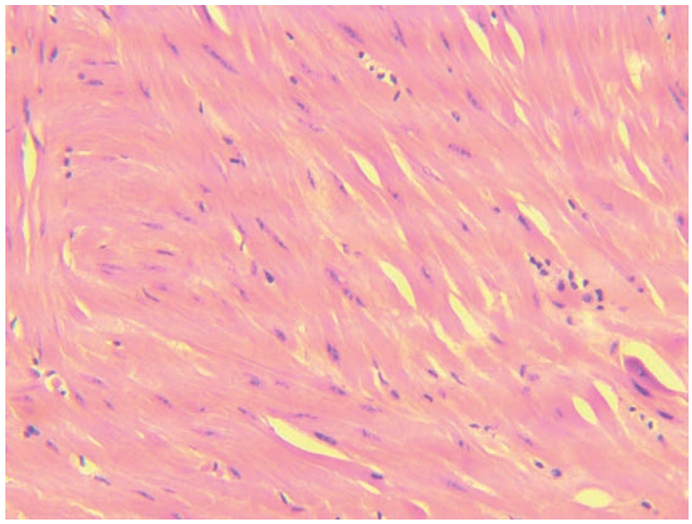

Figure 1.44 Spindle cell tumor grade 1 biopsy. 10×.

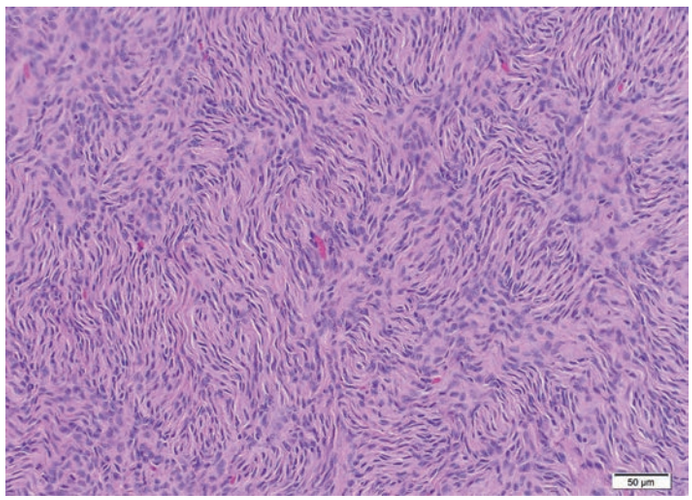

Figure 1.45 Spindle cell tumor grade 2 biopsy. 10×.

Figure 1.44 shows a spindle cell tumor grade 1 biopsy. This biopsy from the skin of an adult dog reveals anastomosing bundles of cells that are typical of smooth muscle cells. Mitotic figures are 0–1/HPF with an MC of 1/10 HPF. There is no necrosis. This tumor would have a score of 1 + 1 + 1 = 3 and is a grade 1 tumor (Table 1.3).

Figure 1.45 shows a biopsy from the flank of an adult mixed-breed dog is a disorganized proliferation of plump spindle cells of suspected nerve origin. There are 0–3 mitotic figures/HPF with an MC of 10/10 HPF. There are a few areas of necrosis, estimated at about 10% of the sample examined. This tumor would have a score of 2 + 2 + 2 = 6 and is a grade 2 tumor (Table 1.3).

Figure 1.46 shows a biopsy from the thigh of an adult beagle and is a disorganized proliferation of pleomorphic spindloid to epithelioid cells of indeterminate origin. There is moderate to marked anisokaryosis with prominent multiple nucleoli. There are 0–6 mitotic figures/HPF with an MC of 21/10 HPF. There is greater than 50% necrosis in the sections examined. This tumor would have a score of 3 + 3 + 3 = 9 and is a grade 3 tumor (Table 1.3).

Figure 1.47 shows a grade 2 spindle cell tumor. It was submitted with the history that the excision appeared complete because there was normal tissue in the marginal tissue beyond the excised capsule. It is very important to note that spindle cell tumors do not form a capsule, and the bands that appear to be capsules are actually tumors.

Figure 1.48 shows a grade 2 spindle cell tumor that has less dense tissue at the margin, and this was assumed to be normal tissue based on the gross appearance. The black ink indicates the surgical margin. Tumor extends to the inked

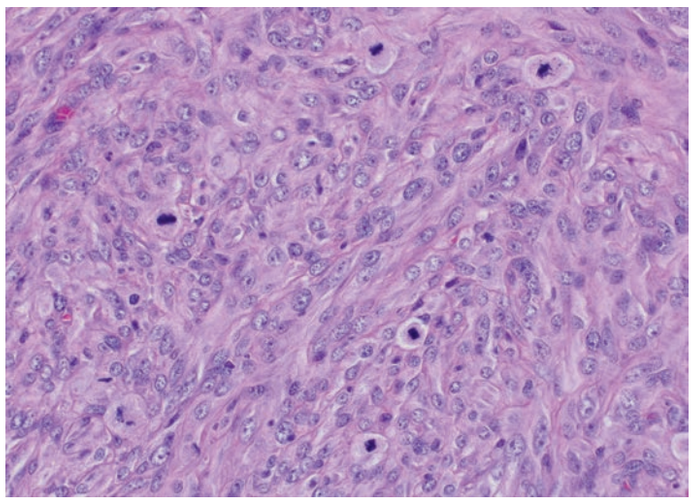

Figure 1.46 Spindle cell tumor grade 3 biopsy. 20×.

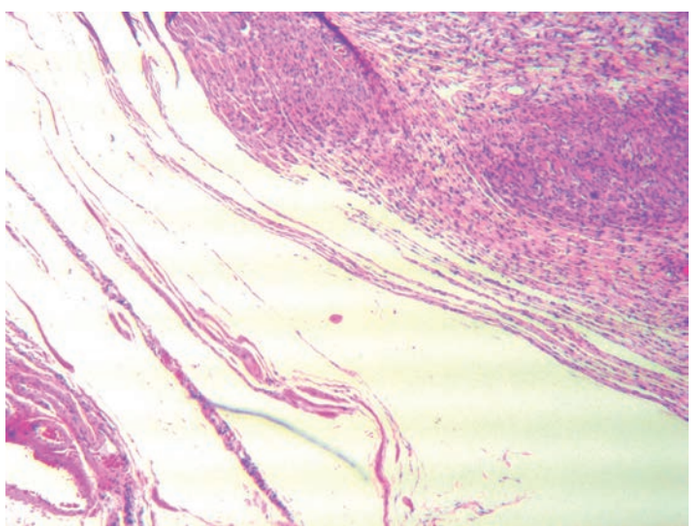

Figure 1.47 Spindle cell tumor biopsy. 2.5×.

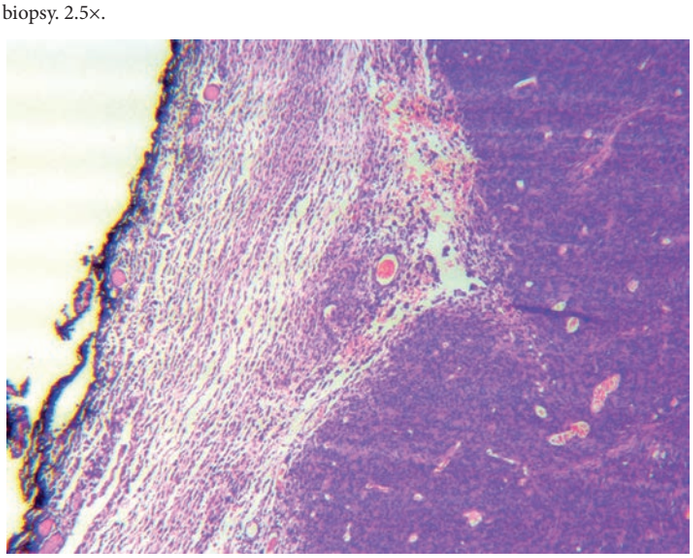

Figure 1.48 Spindle cell tumor biopsy. 2.5×.

margin. Surgical removal of spindle cell tumors is fraught with peril. Referral for presurgical imaging and removal by a specialist should be offered because this tumor often extends widely microscopically. If referral is declined, any surgical removal should be performed with the knowledge that the tumor is likely to extend beyond the gross bulk of the mass.

Since many stromal or spindle cells have a similar appearance on routine histology, immunohistochemical stains may be necessary to identify the cell type (fibroblasts, pericytes, myopericytes, smooth muscle, myofibroblasts, Schwann cells, perineural cells) for optimum prognostic significance and treatment choices. Immunohistochemistry may be most useful when referral for chemotherapy is a treatment option or for prognostic information. This test is most satisfactory when performed on frozen tissue sections, but formalin-fixed tissues are accepted and processed by many laboratories. Consultation with diagnostic lab personnel prior to submission is suggested for the most current information regarding test availability, submission requirements, and pricing. Chemotherapy is used mostly for control of growth as there are none specific or highly effective for spindle cells at this time. If chemotherapy will not be utilized and immunohistochemistry is not elected, then wide excision, with or without radiation therapy, is a standard therapy recommendation due to a similar biological behavior for many STS.[45] There is not a useful grading protocol for cytological specimens of spindle cell tumor at this time, and the prognostic success of grading proposals for visceral and oral spindle cell tumors is variable also.

Figure 1.49 shows a grade 2 spindle cell tumor in a dog. The tumor exhibits a storiform pattern with occasional swirls and palisading nuclei when stained with routine H&E stain. This pattern is suggestive of neural tissue, and the tumor is presumed to be a peripheral nerve sheath tumor, but immunohistochemistry would be necessary for a more definitive diagnosis of the tumor cell type.

Figure 1.50 shows a grade 2 spindle cell tumor that is forming swirls and nests around vascular spaces, suggesting a peripheral vascular wall myocyte origin. Immunohistochemistry could be performed for more definitive identification of the tumor cell type.

Stromal/spindle cell tumors in cats can be aggressive no matter what the mitotic rate, with a recurrence rate of 14% for peripheral nerve sheath tumors diagnosed as benign and 31% for peripheral nerve sheath tumors diagnosed as malignant, based on one study.[46]

Figure 1.51 shows a biopsy of a spindle cell tumor from an adult cat. It reveals many haphazardly arranged spindle cells and occasional large epithelioid cells with pleomorphic and lobulated nuclei, prominent nucleoli, and abundant cytoplasm. This cellular pleomorphism is the hallmark of many feline STS, especially those associated with an injection site, and is a useful feature when evaluating a tumor using FNA, as the finding of even rare pleomorphic cells on FNA is an indication for immediate aggressive therapy. A definitive grading system with predictive value has not been widely accepted.

Cutaneous hemangiosarcoma in dogs has a grading protocol that is based on a combination of mitotic rate and how deep the tumor extends, thereby estimating the potential for invasive behavior. Staging must take into account the presence of invasion beyond the local site. For example, stage I is confined to the dermis, stage II extends into subcutis

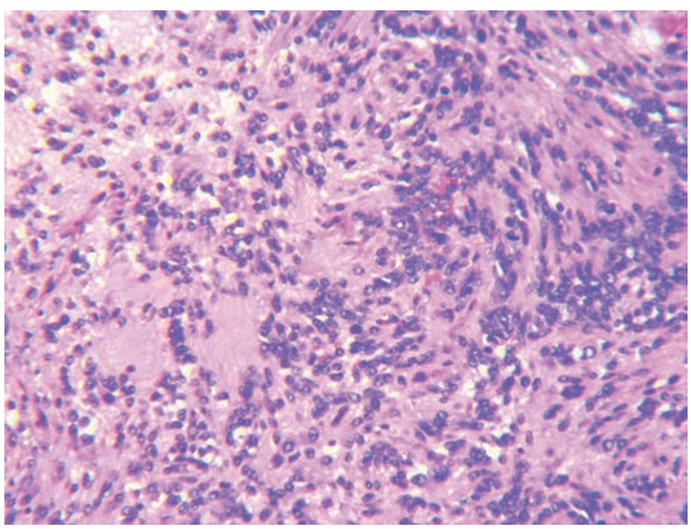

Figure 1.49 Spindle cell tumor biopsy. 10×.

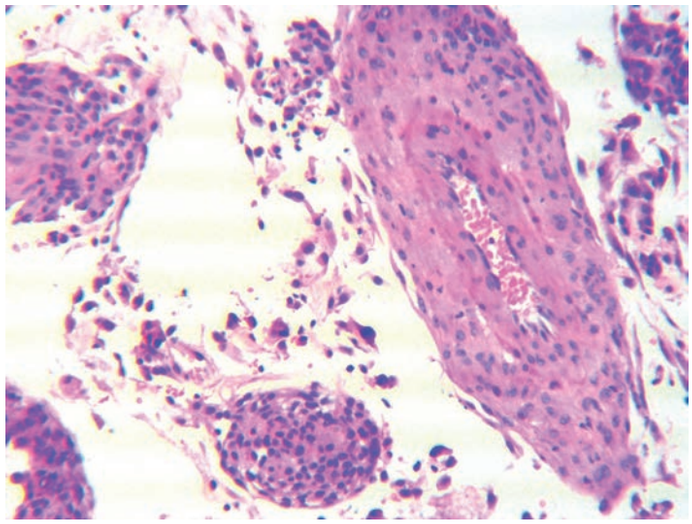

Figure 1.50 Spindle cell tumor biopsy. 10×.

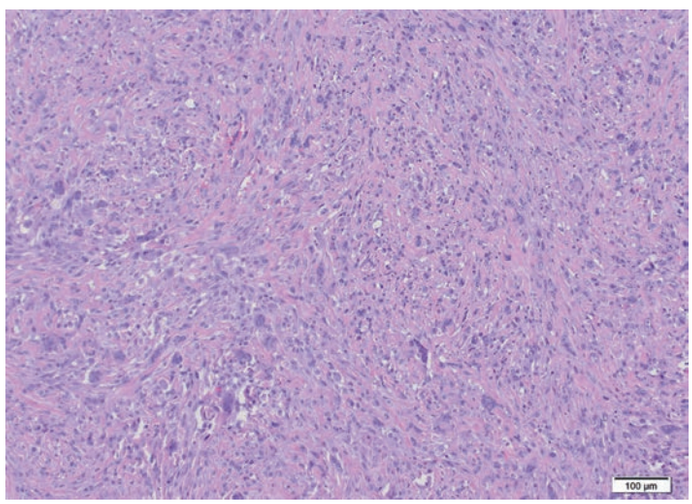

Figure 1.51 Feline soft tissue sarcoma. 10×.

and may exhibit regional lymph node involvement, and stage III invades structures such as muscle and involves distant metastasis.[47] Staging, while providing superior prognostic information, requires clinical information that cannot be determined from a single skin biopsy. Behavior in cats is unpredictable, ranging from locally invasive to metastatic, and attempts to grade may be misleading. Visceral hemangiosarcoma is quick to metastasize, and the prognosis for long-term survival ranges from guarded with moderate to high probability of distant metastasis in nonruptured tumors, to poor with a high probability of distant and local metastasis in ruptured tumors.[47] Splenic hemangiosarcoma grading systems have not been well established to correlate with survival and therefore are not in common use.[48] This lack of correlation may be because the large size and heterogeneity of the spleen combined with the tendency of splenic vascular tumors to be buried within abundant hemorrhage and hematomas can make gross identification of the actual tumor difficult.[49] Canine hemangiosarcoma can originate in the right heart so cardiac imaging should be considered prior to splenectomy. Benign hemangiomas can undergo malignant transformation to hemangiosarcoma, so evaluation of all excised lesions suspected of being vascular in origin is very important in order to confirm complete excision.

Figure 1.52 is a cutaneous hemangiosarcoma in an adult dog and consists of a somewhat circumscribed mass composed of vascular channels lined by pleomorphic endothelium. There were 0–2 mitotic figures/HPF. It was confined to the dermis and was considered to be low grade due to the relatively low mitotic rate and minimal local invasion.

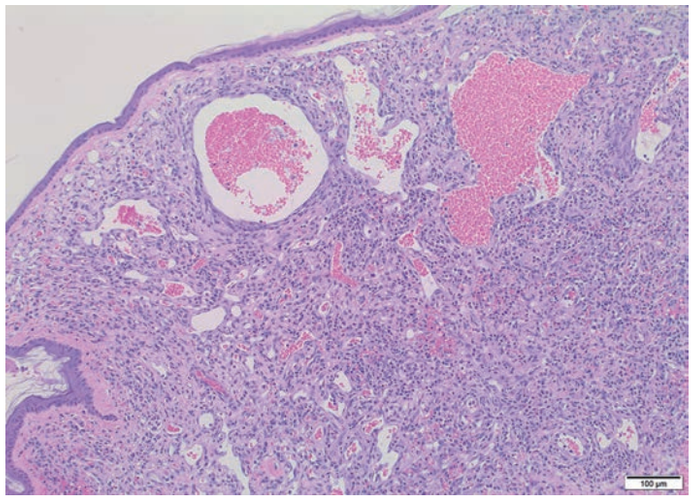

Figure 1.52 Canine cutaneous hemangiosarcoma. 10×.

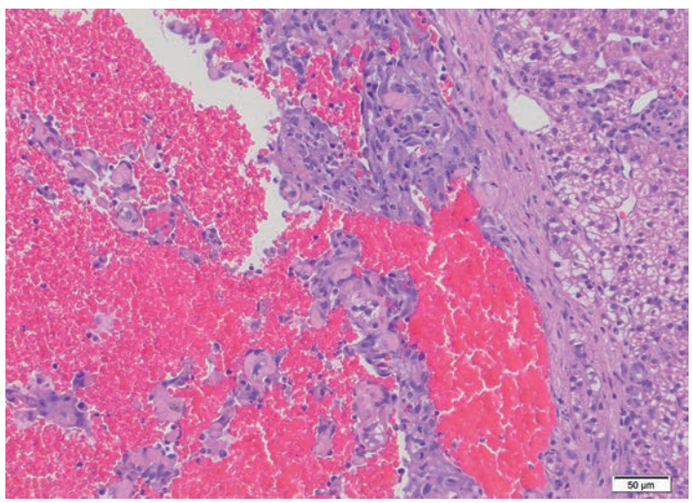

Figure 1.53 Canine hepatic hemangiosarcoma. 20×.

Figure 1.53 shows a hepatic hemangiosarcoma, diagnosed at necropsy in an adult male Weimaraner. This was likely a metastatic lesion as there was hemangiosarcoma in the spleen, and hemo-abdomen was observed upon opening the abdominal cavity. Preliminary diagnosis was made by identifying abdominal fluid on ultrasound and confirming abundant free blood by FNA.

Figure 1.54 shows a spleen with hemangiosarcoma. This aggressive vascular neoplasm often produces a necrotic or hemorrhagic area in the spleen, so collection of the biopsy sample at the margin of a necrotic or hemorrhagic area can yield the best results. Table 1.9 describes a grading system for biopsy evaluation of splenic hemangiosarcoma.

The most common bone tumor is canine osteosarcoma. Behavior (time until metastasis and survival time) appears to be correlated to the site of occurrence. Bone tumors of the head and jaw tend to metastasize less readily than bone tumors arising at other sites. Osteosarcoma of the scapula has a significantly greater hazard for death than appendicular sites. Every 100% increase in serum alkaline phosphatase (ALP) increases the hazard of death by 1.7, and tumor grade at this site is not predictive according to one report.[50] In a retrospective study of appendicular osteosarcoma, the number of mitotic figures/3HPF was more significantly associated with outcome than histological grade.[51] The primary treatment for this tumor is early complete excision with other treatments such as chemotherapy for palliation. Thoracic radiographs are suggested.

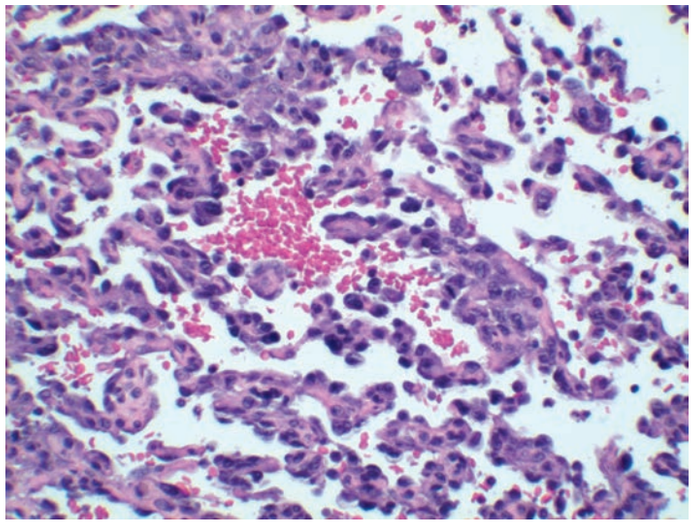

Figure 1.54 Canine splenic hemangiosarcoma. 20×.

Table 1.9 Grading system for splenic hemangiosarcoma.[48]

Differentiation	Score
Well differentiated: numerous irregular vascular channels predominate in all fields	1
Moderately differentiated: ≥50% of the tumor has well-defined vascular channels	2
Poorly differentiated: most of the tumor is solid sheets of spindle cells with few vascular channels	3
Nuclear pleomorphism	Score
No difference in nuclear size and shape	0
Minimal variation	1
Moderate variation (2× size difference)	2
Marked variation (>2× size difference)	3
Mitotic count (in 10 HPFs)[a]	Score
0–10	0
11–20	1
21–30	2
>30	3
Tumor necrosis	Score
No necrosis	0
<25%	1
25–50%	2
>50%	3
Histological grade	Total score
Grade 1 (low grade)	≤5
Grade 2 (medium grade)	6–9
Grade 3 (high grade)	10–12

Abbreviation: HPF, high-power field.
[a] Assessed in 10 contiguous HPFs in the region with the greatest mitotic activity, avoiding areas of hemorrhages and necrosis. Area of view is not specified.

Osteochondromatosis, a benign lesion, was reported to undergo malignant transformation to chondrosarcoma over a course of 20 months in one reported case.[52] Parosteal osteosarcoma of the skull is usually low grade initially, but can with time become aggressive, invading the skull.[53] Spontaneous regression of osteosarcoma has also been reported.[54] Due to the many variables associated with predicting the behavior of this tumor type, consultation with an oncologist would be prudent.

Figure 1.55 An adult mixed-breed dog presented with a firm mass on the left dorsal skull that on biopsy consisted of well-differentiated cartilage and bone with lacunae containing osteocytes and chondrocytes with small nuclei. This is typical of a benign or low-grade bone tumor such as osteochondroma. Metastasis is not expected, and complete excision may be curative.

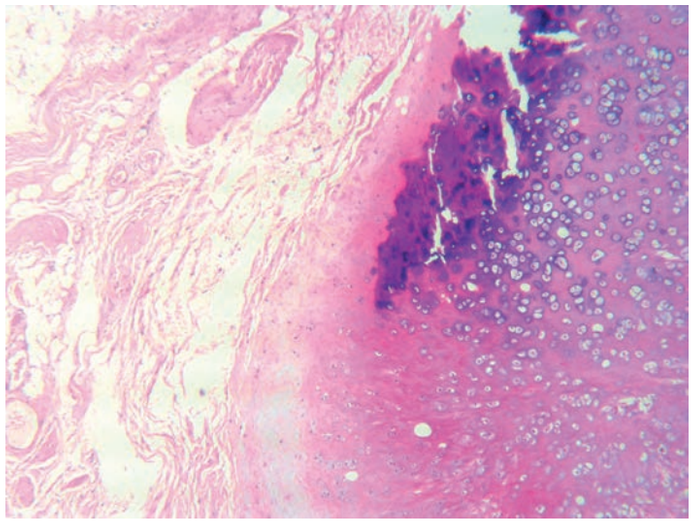

Figure 1.55 Osteochondroma biopsy. 2.5×.

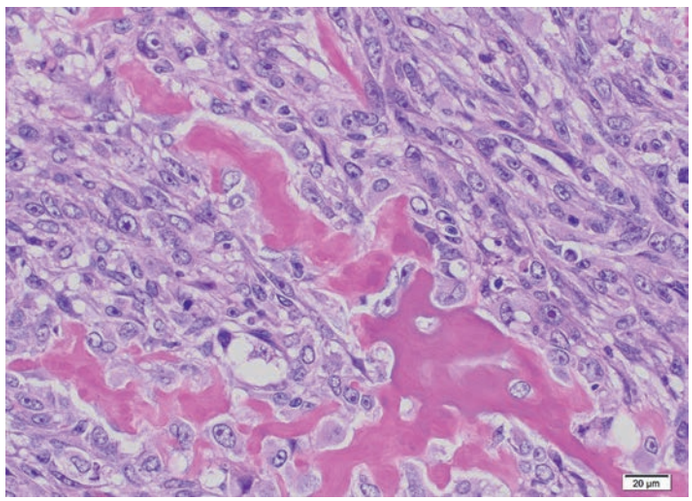

Figure 1.56 Osteosarcoma biopsy. 20×.

Figure 1.56 shows a biopsy of an adult German Shepherd Dog presented with a mass near the orbit. Biopsy reveals a haphazard proliferation of bone, cartilage, and occasional sheets of pleomorphic spindloid to epithelioid cells diagnosed as osteosarcoma. This tumor is moderately well differentiated, producing tumor bone, and at this site is unlikely to produce early metastasis. Euthanasia was elected due to the extreme deformation of the skull and ocular involvement. Two grading systems have been developed for canine osteosarcoma, and one has been modified for feline osteosarcoma, but there is a lack of reliable prognostic efficacy for either, possibly related to a strong factor of location-linked behavior.[14] A grading system that has recently been developed for canine multilobular tumor of bone has also shown promise but is of uncertain significance until additional studies are performed.[14]

Figure 1.57 shows a biopsy of an adult Mastiff presented with left front leg lameness. Radiographs revealed a lytic lesion of the left humerus, and the biopsy revealed a proliferation of pleomorphic epithelioid cells with rare multinucleated cells. Some cells appear to be nestled within scant eosinophilic material suggestive of osteoid, supporting a diagnosis of osteosarcoma. Definitive diagnosis of this tumor with evaluation of mitotic rate can be difficult if small samples from core biopsies are submitted, as this tumor can form in close association with necrotic bone, reactive bone, and periosteal hyperplasia, creating a heterogeneous pattern that can lead to sampling error. It can be a challenge to obtain a sample of adequate size and diagnostic quality without creating a site of instability.

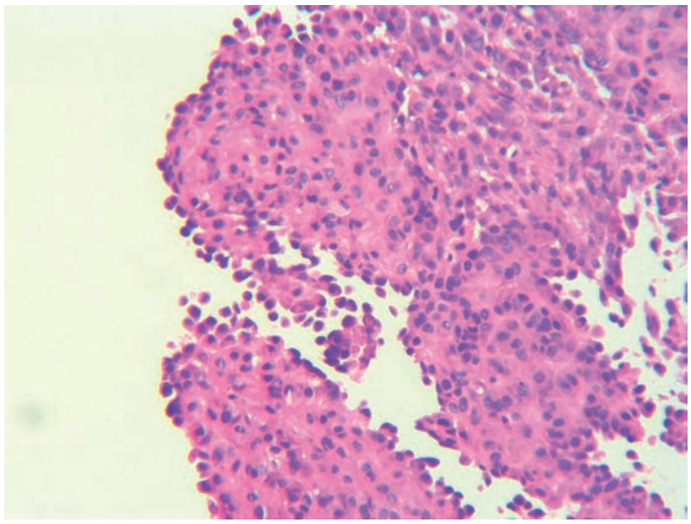

Figure 1.57 Osteosarcoma biopsy. 10×.

Round cell tumors

Mast cell tumor

Canine cutaneous MCTs have historically been graded with a three-part system historically referred to as the Patnaik system.[55] Grade 1 (low-grade tumors) have 0 mitotic figures/HPF, are well differentiated, and are confined to the subepidermis and superficial dermis. Grade 2 (medium-grade tumors) have 0–2 mitotic figures/HPF, rare binucleate cells, and are dermal to subcutaneous. Grade 3 (high-grade tumors) have three or more mitotic figures/HPF, are pleomorphic, and extend to subcutaneous or deeper tissues. There is generally good agreement between pathologists in the diagnosis of a grade 3 MCT but grade 1 and 2 are more often in dispute, which has clinical significance prognostically due to the generally benign nature of grade 1 MCT, the generally aggressive nature of grade 3 MCT, and the widely variable behavior of grade 2 MCT. This system is designed for cutaneous primary tumors and is not sufficiently validated, at this time, for primarily subcutaneous, mucosal, or visceral tumors. A recent study suggests all intranasal MCTs should be considered high grade.[56] It is important to remember that even grade 1 MCT can, at times, behave in an aggressive manner and it is likely that tumor types do not remain static over long periods of time, presumably having the potential to become more aggressive as they persist or recur.

A more recent system developed at Michigan State University by Kiupel et al. (which will be referred to as the two-tier scale) uses a two-part grading protocol, based predominantly on nuclear morphology, in which a high-grade tumor is diagnosed if there are >7 mitosis/10 HPF, 3 multinucleated cells in 10 HPF, or 3 bizarre nuclei in 10 HPF and a low-grade tumor is diagnosed if these conditions are not met.[57] MC is an important part of the grading process of both systems, with one study indicating an MC <5/10 HPF had a 70-month survival and MC> 5/10 HPF had a 2-month survival.[58] In a study comparing the two systems over a 5-year period, in the three-level (Patnaik) grading system there was 0% mortality due to tumors labeled grade 1 (low grade, 1 of 3), 23% mortality due to tumors labeled grade 2 (medium grade, 2 of 2), and 100% mortality in tumors labeled grade 3 (high grade, 3 of 3). In the two-tier grading system, there was 6% mortality due to tumors labeled low grade (1 of 2) and 71% mortality due to tumors labeled high grade (2 of 2), and the newer two-tier system was deemed to be more clinically predictive on a statistical basis.[59] A retrospective study of metastatic MCTs revealed that all primary tumors were high grade, mostly from the inguinal area, and metastatic lesions presented frequently as variably sized foci of mast cells in the lymph nodes, spleen, liver, skin, bone marrow, kidneys, heart, and lungs in decreasing order of occurrence.[60] Wide surgical excision with 1.5 cm lateral margins and 1 tissue plane at the deep margin is the general primary recommendation for treatment, with radiation and chemotherapeutic options available.

Figure 1.58 shows a canine cutaneous MCT grade 1 Patnaik scale. This cutaneous canine MCT is confined to the superficial dermis, mitotic figures are rare, and the mast cells appear well differentiated with small, monomorphic nuclei. This tumor is a grade 1 Patnaik scale tumor and a low-grade two-tier scale tumor (Table 1.2).

Figure 1.59 shows a canine cutaneous MCT grade 2 Patnaik scale. There are variably granulated mast cells with small but slightly pleomorphic nuclei, 0–1 mitotic figures/HPF with a mitotic rate of 2/10 HPF, and the tumor extends to the deep dermis. This tumor would be a grade 2 Patnaik scale and a low-grade two-tier scale (Table 1.2).

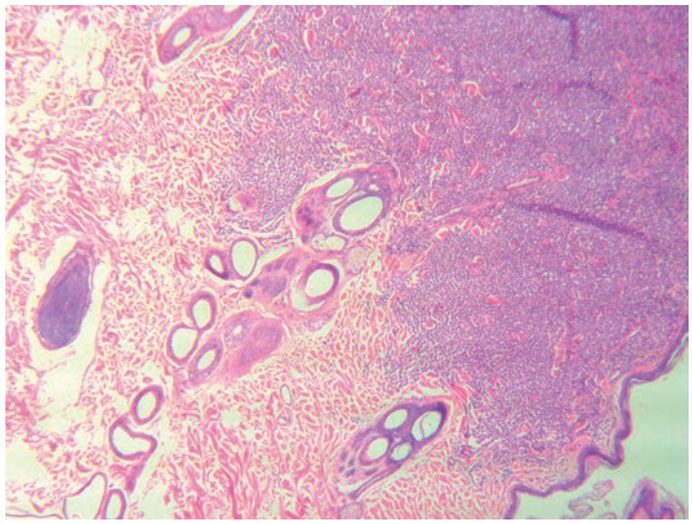

Figure 1.58 Canine cutaneous mast cell tumor grade 1 biopsy. 2.5×.

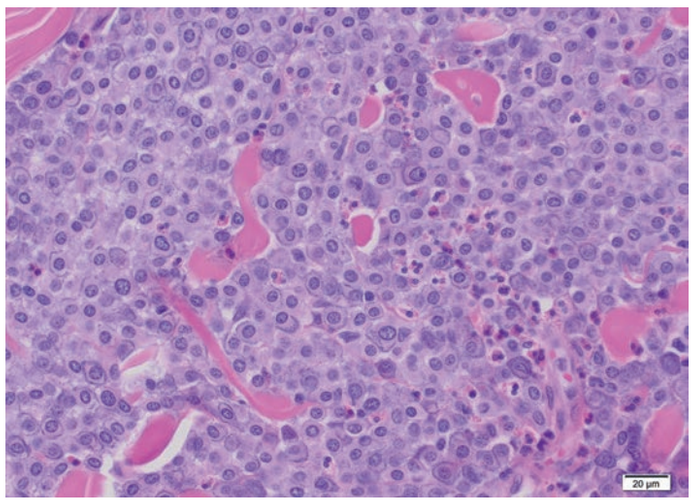

Figure 1.59 Canine cutaneous mast cell tumor grade 2 biopsy. 20×.

Figure 1.60 shows a canine cutaneous MCT grade 2 Patnaik scale. This grade 2 (Patnaik), low-grade (two-tier) cutaneous MCT has small, variably granulated mast cells with small nuclei with mild anisokaryosis and is confined to the dermis. There are areas of collagen necrosis with dense infiltrates of eosinophils. Identification of mitotic figures is difficult in these areas, and they should be avoided because the irregular appearance of eosinophil nuclei could cause a spurious elevation in the MC.

Figure 1.61 shows the aspirate of a mass from a 6-year-old spayed female Mastiff dog that revealed pleomorphic mast cells with moderate to marked anisokaryosis and frequent cells with multiple nucleoli, prominent nucleoli in some cells, and variable cytoplasmic granulation. Rare mitotic figures are seen. Note that most of the cells are larger than the neutrophil in the top middle right and the lymphocyte at the top middle left. There is a recently developed grading system for cytological evaluation of canine primary cutaneous MCT that appears to correlate well with histological grade and seems to have prognostic usefulness.[61] Table 1.10 describes a grading system for FNA and cytologic evaluation of canine mast cell tumors. This tumor would have a cytological grade suggestive of a high-grade tumor.

Figure 1.62 shows a canine cutaneous MCT grade 3 Patnaik scale. This biopsy of the aspirated mass in Figure 1.61 reveals a proliferation of moderately pleomorphic mast cells with identifiable cytoplasmic granules, and numerous large epithelioid cells without distinct cytoplasmic granules and with marked anisokaryosis and multiple nucleoli in an

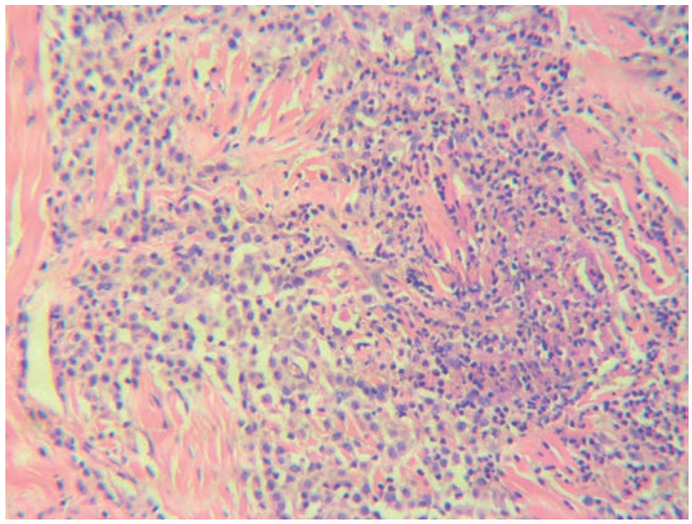

Figure 1.60 Canine cutaneous mast cell tumor grade 2 biopsy. 10×.

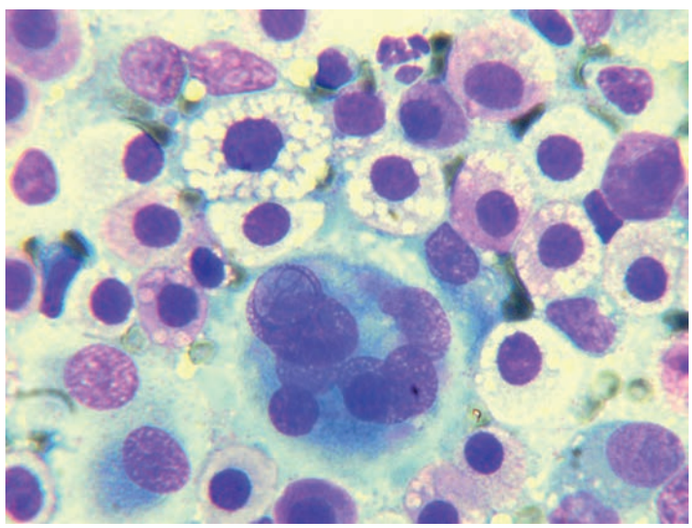

Figure 1.61 Canine mast cell tumor fine needle aspiration consistent with a cytological high-grade tumor. 50×.

Table 1.10 Cytological grading system of canine cutaneous mast cell tumor.[61]

High grade	Poorly granulated on modified Wright's stain *Or* two of the following: Presence of mitoses Anisokaryosis (defined as a variation of the nuclear size greater than 50%) Binucleation/multinucleation Nuclear pleomorphism
Low grade	If not classified as high grade

Obtained by evaluating only one smear of minimal cellularity of 100 intact cells per case.

edematous background. This tumor extended into subcutaneous tissue and to all margins. There were low numbers of mitotic figures, but the extensive invasion of deep tissues and extreme cellular pleomorphism suggested a diagnosis of grade 3 Patnaik scale, high-grade two-tier scale, MCT (Table 1.2). Giemsa stain was recommended for confirmation that the pleomorphic cells were mast cells.

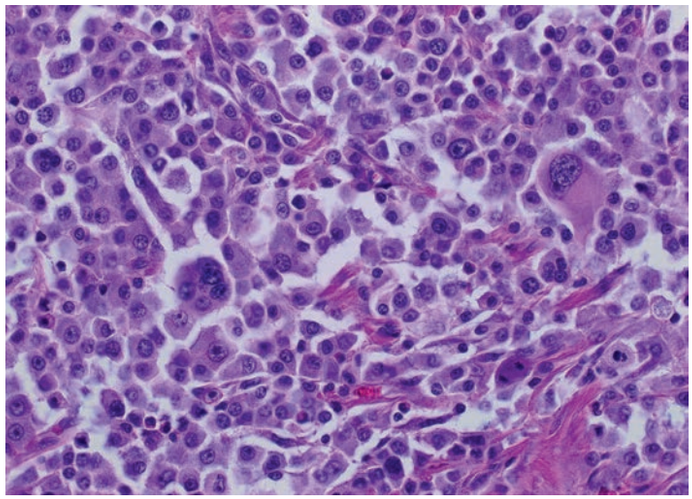

Figure 1.62 Canine cutaneous mast cell tumor grade 3. 20×.

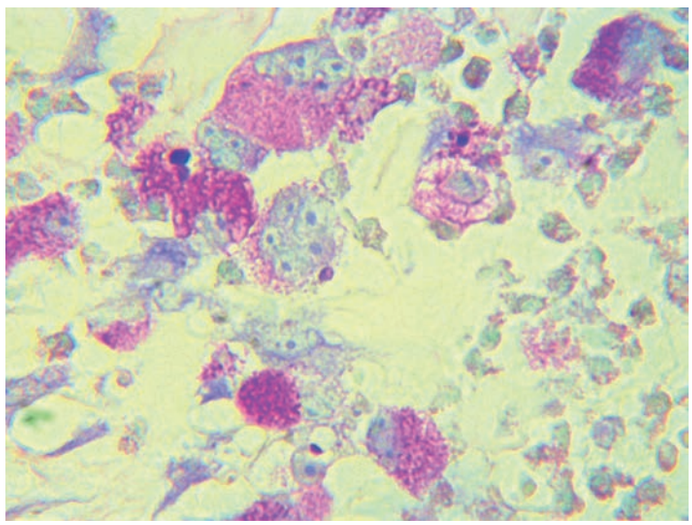

Figure 1.63 Canine cutaneous mast cell tumor grade 3 Giemsa stain. 50×.

Figure 1.63 shows a canine cutaneous MCT grade 3 Patnaik, Giemsa stain. Giemsa stain of the biopsy in Figure 1.61 reveals metachromatic granules in the giant epithelioid cells, revealing them to be anaplastic mast cells. This tumor is confirmed as a grade 3 Patnaik scale and high-grade two-tier scale MCT.

In the study that comparatively assessed tumors with both the two-part (low, high) and three-part (1–3) systems, all grade 1 cutaneous canine MCTs were diagnosed as low grade, all grade 3 MCTs were diagnosed as high grade, and 82% of grade 2 MCTs were diagnosed as low grade with 18% of grade 2 MCTs diagnosed as high grade. It should be noted that regarding the clinical relevance of this study, the mortality rate for two-tier low-grade MCT was 6%, and the mortality rate for two-tier high-grade MCT was 71%, while the mortality rate for Patnaik grade 1 was 0%, grade 2 was 23%, and grade 3 was 100%. Grading of MCT does not predict behavior with 100% accuracy, but both systems were significantly associated with prognosis, and there was greater concordance among pathologists when the two-tier system was used.[59]

Figure 1.64 shows a cutaneous MCT that was graded as a grade 3 Patnaik, high-grade two-tier, due to the many mitotic figures in a certain area of the tumor. There appear to be nine or more mitotic figures in this field.

Figure 1.65 shows the same cutaneous MCT seen in Figure 1.64. The mitotic rate is low in this field, and most of the irregular nuclei are eosinophils. This demonstrates one source of variability in grading, because some tumors have areas of significant variation in the density of mitotic figures.

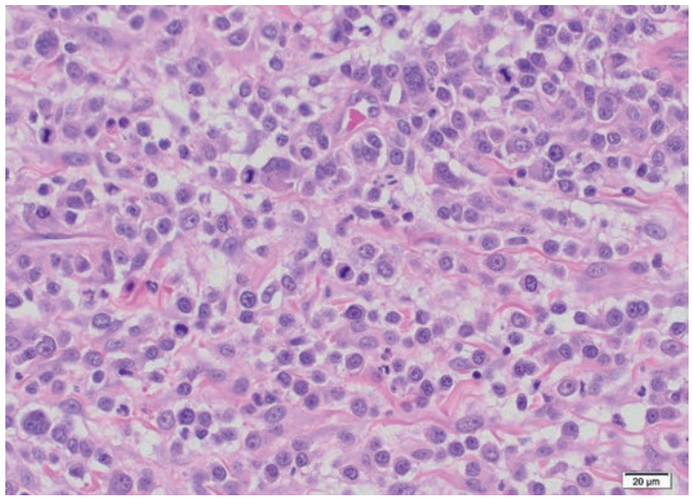

Figure 1.64 Canine cutaneous mast cell tumor high mitotic count biopsy. 20×.

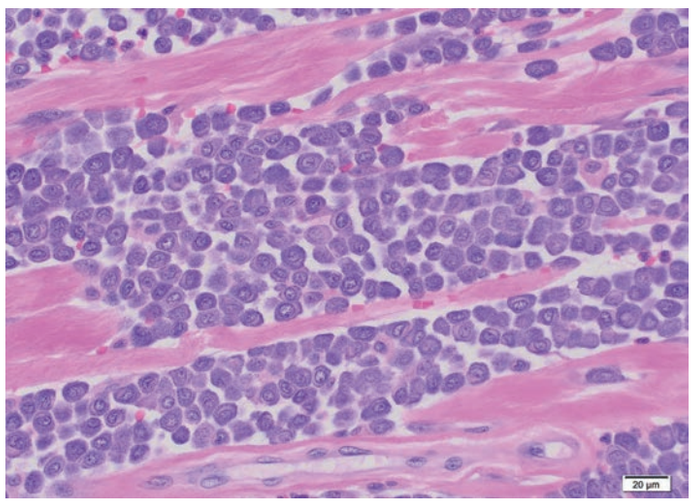

Figure 1.65 Canine mast cell tumor low mitotic count biopsy. 20×.

Canine MCTs with tyrosine kinase receptor (KIT) dysregulation may behave in a more aggressive fashion.[62] A more recent study reveals that of the prognostic factors including the clinical features of stage, breed, site, recurrence, grade, the immunohistochemical features including Ki-67 labeling index, KITr expression, evaluation of metalloproteinases and fibroblast activating protein, and the molecular features including c-KIT oncogene mutations, internal tandem duplications in exons 11, 8, and 9, the two variables that were independent predictors of overall survival were stage and tumor recurrence.[63]

Feline MCTs do not reveal a prognostic association with KIT expression, but higher mitotic rates are suggestive of more aggressive behavior.[64]

Figure 1.66 is a biopsy from an adult cat with a skin mass that revealed sheets of mast cells with rare mitotic figures. This would be considered a low-grade MCT. Complete excision of this type of lesion is often curative. A two-tier grading system has been proposed for histological evaluation of feline cutaneous MCT. High-grade tumors are associated with significantly reduced survival using this system, but in one author's experience (ARK) a low grade does not always preclude aggressive behavior.[65] Table 1.11 describes a grading system for biopsy evaluation of feline cutaneous mast cell tumors.

Figure 1.67 shows the splenic biopsy from an adult cat with a diffusely enlarged spleen. This is an indication for immediate diagnostic workup. The complete blood count (CBC) can be searched for circulating mast cells, and evaluation of abdominal fluid for mast cells is also an appropriate procedure prior to surgery. Mast cells are not normally

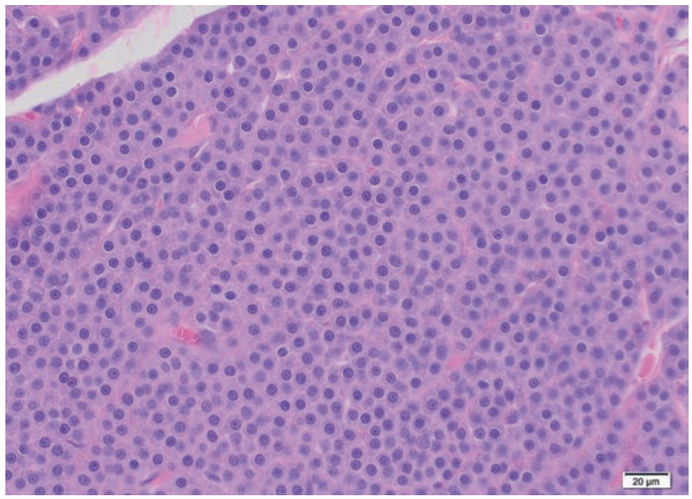

Figure 1.66 Feline cutaneous mast cell tumor biopsy. 20×.

Table 1.11 Grading system of feline cutaneous mast cell tumor.[65]

High grade	Mitotic count[a] >5/10 HPFs *And* at least two of the following: Diameter >1.5 cm Irregular nuclear shape (majority of cells) Nucleolar prominence/chromatin clusters (>50% of cells)
Low grade	If not classified as high grade

Abbreviation: HPF, high-power field.
[a] Assessed in 10 contiguous HPFs in the region with the greatest mitotic activity. Area of view = 2.37 mm^2.

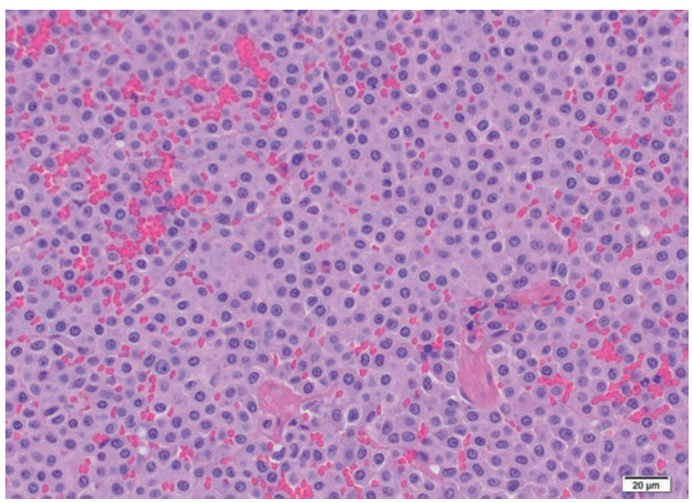

Figure 1.67 Feline splenic mast cell tumor biopsy. 20×.

seen in these fluids, and the finding of more than rare mast cells in the CBC or abdominal fluid is supportive of splenic MCT when there is splenomegaly. FNA of the spleen can be performed if these tests are not diagnostic. Also check for skin masses, palpate lymph nodes, and perform thoracic radiographs with abdominal ultrasound or radiographs. Splenectomy is the treatment of choice with reported remission times of 12–19 months. Degranulation of splenic MCT can lead to fatal hypotension so gentle handling is important.[66] Oncology consultation is suggested.

Table 1.12 Grading system of canine lymphoma.[67]

Histological grade	MC[a]
Grade 1 (low grade)	0–5/HPF
Grade 2 (medium grade)	6–10/HPF
Grade 3 (high grade)	>10/HPF

Abbreviations: MC, mitotic count; HPF, high-power field.
[a] Mitoses were counted in 10 fields at 400× and then the average was determined. Area of view is not specified.

Lymphoma

Lymphoma's histological grade is based on mitotic activity in a histological section of tissue. Table 1.12 describes a grading system for biopsy evaluation of lymphoma. There are numerous classifications or types of lymphoma, with both lymph node lymphoma and extranodal lymphomas that exhibit site-specific biological behavior. There is a subset of nodal and extranodal lymphomas called nodular lymphoma (marginal zone, mantle zone, follicular and T-zone lymphomas).[68, 69] These types of lymphoma tend to be indolent with a low MC and long survival times. Nodular (follicular, mantle zone, marginal zone, and T-zone) lymphoma can be difficult to differentiate from nodular hyperplasia with routine histopathology, so immunohistochemical stains or flow cytometry can be used to identify the phenotypic makeup of the population if PARR does not identify a diagnostic clonal population.[68–72] These additional diagnostic tools are discussed more thoroughly in Chapter 8. Use of these tests in one case followed a progression from a nodal marginal zone lymphoma to a multifocal diffuse large B-cell lymphoma, supporting the concept of progressive transformation of a neoplastic population from an indolent tumor type to a more aggressive tumor type, although of the same B-lymphocyte clone.[3] In another example, site-specific canine hepatosplenic T-cell lymphoma and hepatic T-cell lymphoma are poorly responsive to therapy, and they usually behave in an aggressive (clinically high grade) biological fashion.[73, 74] T-cell lymphoma at a number of sites including skin and liver can be indolent (clinically low grade), and quality of life can be maintained without aggressive therapy for long periods of time.[75] Regardless of the location or pattern of architecture, however, these lymphomas are made of cells that are divided into subtypes based on their molecular characteristics. Broadly dividing cell types into three groups results in group 1 consisting of low-grade T-cell lymphoma (T zone lymphoma), group 2 consisting of high-grade T-cell lymphoma (lymphoblastic T-cell lymphoma and peripheral T-cell lymphoma not otherwise specified), and B-cell lymphoma (marginal B-cell lymphoma, diffuse large B-cell lymphoma, and Burkitt lymphoma).[10, 76] A histological evaluation with immunophenotyping is required for definitive subtyping that can be reliably associated with survival.[10]

The most common presentation is a patient with large peripheral lymph nodes. This nodal lymphoma is graded based on MC with 0–5/HPF graded low, 6–10/HPF graded intermediate, and >10/HPF graded high.[10, 67] In one retrospective study, dogs with low-grade T-cell lymphoma had a median survival rate of 622 days, dogs with high-grade T-cell lymphoma had a median survival rate of 162 days, and dogs with B-cell centroblastic, the most common type, had a median survival rate of 127–221 days, across multiple treatment protocols.[10] Classification can be based on immunophenotype (T or B), maturity of cells, nuclear size based on comparison to erythrocytes (small is <1.5× the RBC, intermediate is 1.5–2.0× the size of the RBC, and large is >2× the size of the RBC), and growth pattern (diffuse or nodular). Subtyping is based on immunophenotyping for B and T cell lineage. This can be performed on biopsy samples by immunohistochemistry and on FNA samples using flow cytometry or PARR. Reactive hyperplasia may progress to low-grade or high-grade lymphoma.[3] In cases where definitive diagnosis of neoplasia is difficult due to a morphologically heterogeneous lymphocyte population as determined by FNA or biopsy, PARR can identify clonal populations that would support a diagnosis of neoplasia.[77] In cats, lymphoma prognosis is significantly affected by both retroviral infection and site of the neoplasm.[78]

Figure 1.68 shows a reactive lymph node biopsy. Enlarged lymph nodes can be a result of follicular hyperplasia due to antigenic stimulation. There should be multiple well-circumscribed cortical nodules composed of germinal centers of B-cell origin lined by a wall of more dense small lymphocytes of mostly T-cell origin. The medullary sinusoids should be well-defined and contain plasma cells and small lymphocytes. FNA will usually reveal a mixed population of small and large lymphocytes. Progression from reactive hyperplasia to neoplasia has been reported.[3]

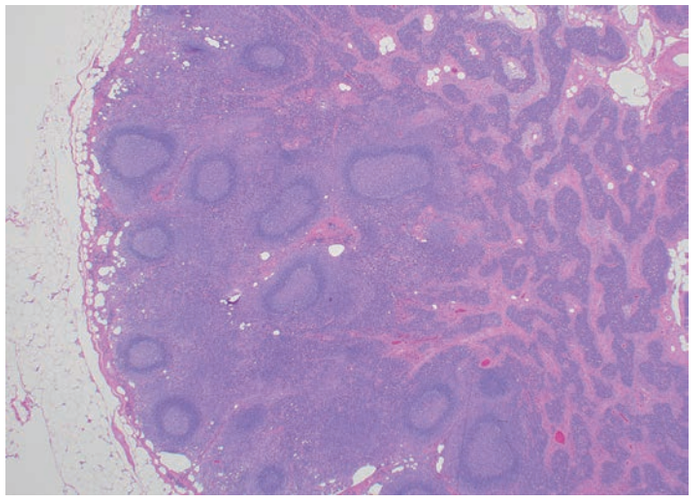

Figure 1.68 Reactive lymph node biopsy. 2.5×.

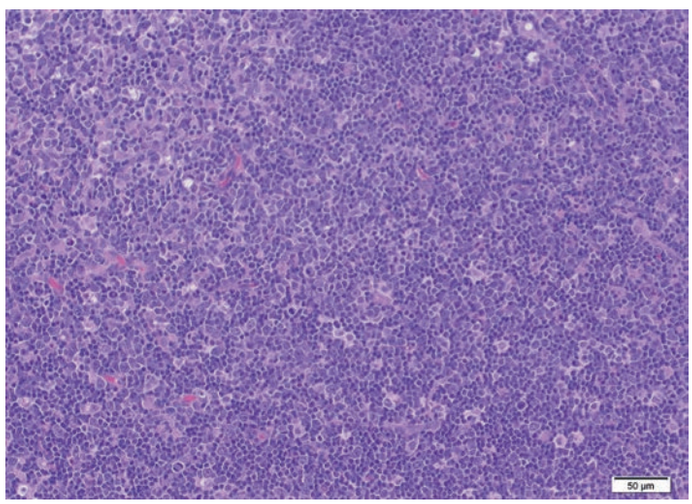

Figure 1.69 Lymph node lymphoma, low-grade biopsy. 10×.

Figure 1.69 shows a biopsy of a lymph node with lymphoma that reveals a loss of the normal follicular architecture with replacement of the follicles and sinusoids by sheets of monomorphic lymphocytes. This low-grade lymphoma exhibits few mitotic figures.

Figure 1.70 shows a biopsy of a high-grade lymphoma that reveals loss of architecture similar to Figure 1.69 but there will be many mitotic figures.

Figure 1.71 shows a cutaneous lymphoma. Cutaneous lymphoma can be seen in many skin locations from haired skin to mucosal surfaces. In epitheliotrophic cutaneous T-cell lymphoma, the most diagnostically helpful feature of this tumor is finding invasion into the epidermal layer by neoplastic round cells. Biopsy samples must have some intact epidermis to identify this trait.

Figure 1.72 shows a splenic malignant lymphoma biopsy. Histological evaluation of a diffusely enlarged spleen revealed sheets of T-cell lymphocytes. This tumor type is often aggressive and generally responds poorly to therapy, although low-grade, slow-growing types have been reported.[73, 74]

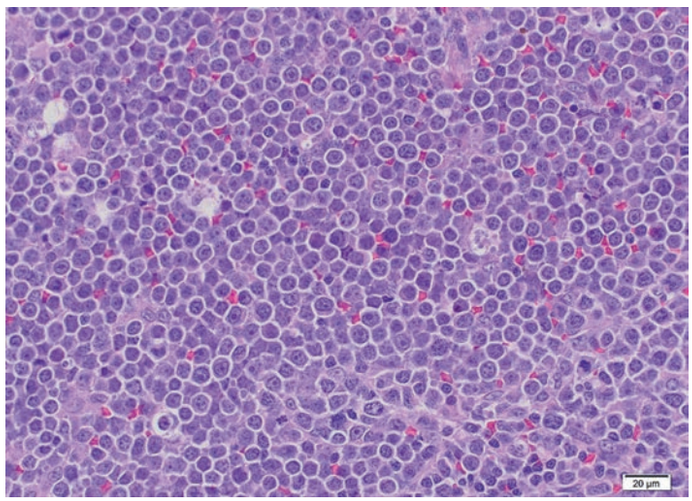

Figure 1.70 Lymph node lymphoma, high-grade biopsy. 20×.

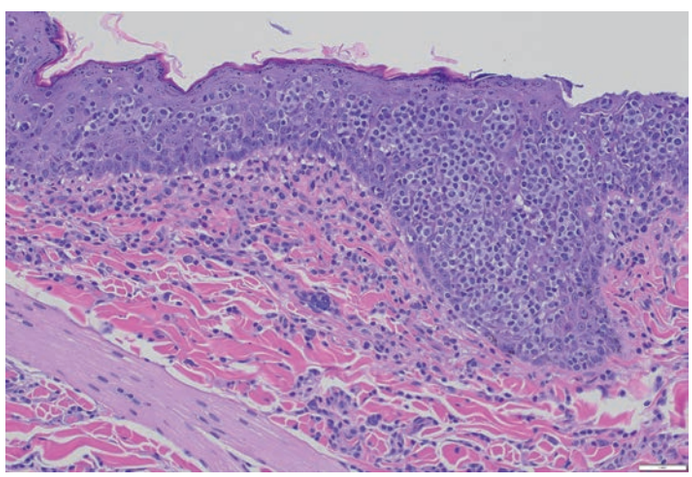

Figure 1.71 Cutaneous lymphoma biopsy. 10×.

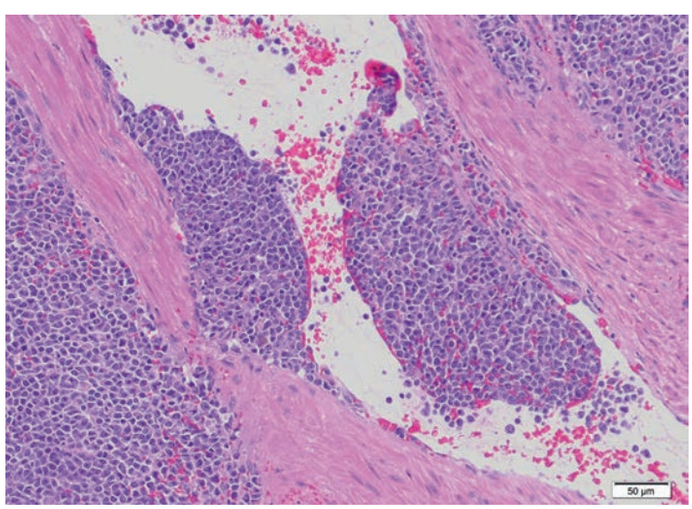

Figure 1.72 Splenic malignant lymphoma biopsy. 10×.

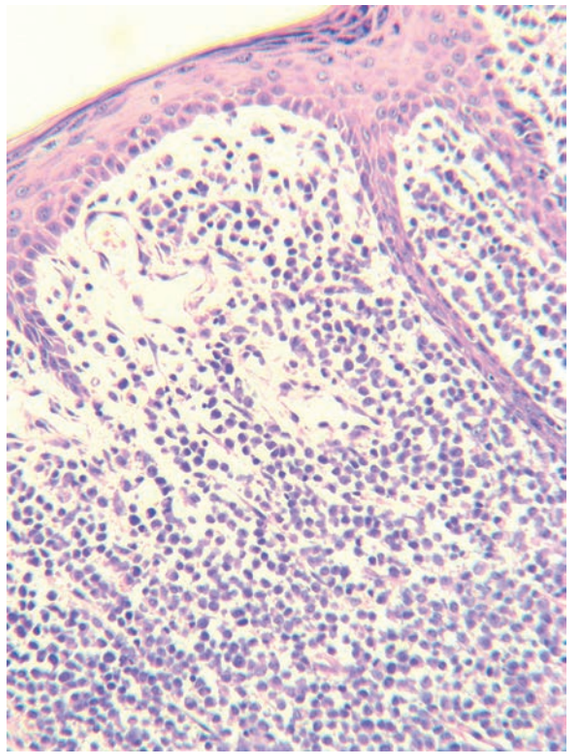

Figure 1.73 Canine cutaneous histiocytoma biopsy. 10×.

Histiocytoma

Categorization of histiocytic proliferations can be more complex than the other round cell tumors.[79, 80] There is not a grading protocol for histiocytic tumors. In the dog, histiocytic proliferations can be classified as reactive, nonneoplastic (reactive cutaneous histiocytosis, reactive systemic histiocytosis), they can be a benign neoplasm (cutaneous histiocytoma) or they can be a malignant neoplasm (localized histiocytic sarcoma, disseminated histiocytic sarcoma). Reactive histiocytosis both cutaneous and systemic, is usually a mix of activated interstitial dendritic cells and active lymphocytes. Histiocytoma is a benign cutaneous proliferation of Langerhans cells and is seen most often in young dogs. Cutaneous histiocytoma may spontaneously regress, but rare cases have been reported of multifocal lesions invading local lymph nodes prior to regressing. Histiocytic sarcoma is the malignant form of this tumor, usually arising from interstitial dendritic cells but sometimes Langerhans cells, and it can arise anywhere in the body, with a high likelihood of distant metastasis.[81] Canine histiocytic sarcoma arising in internal organs reportedly had a metastatic rate of 66% and a median survival time of 14.4–43.6 days, whereas tumors of the limbs had a metastatic rate of 28% and a median survival time of 125.6–164.4 days.[82] Hemophagocytic histiocytic sarcoma arises from macrophages.[79]

Benign cutaneous histiocytoma is not recognized to occur in the cat, at this time.[79] Feline progressive histiocytosis and feline pulmonary Langerhans cell histiocytosis are sometimes indolent but progressive and eventually debilitating, and histiocytic sarcoma and hemophagocytic histiocytic sarcoma can metastasize early and widely.[83, 84] The median survival in one study was 150 days for histiocytic sarcoma and 470 days for feline progressive histiocytosis and, as with other tumors in cats, the immunophenotype of the neoplastic population can be fluid.[85] The feline proliferations can be reactive or neoplastic, but both exhibit relentless progression.

Figure 1.73 shows a canine cutaneous histiocytoma biopsy. Canine cutaneous histiocytoma often presents as an ulcerated dome-shaped mass composed of sheets of histiocytes with fairly monomorphic round to reniform nuclei, scattered mitotic figures, bland chromatin, and moderate pale cytoplasm. They are often arranged in loose arrays extending up to the epidermis, sometimes with epidermal invasion. At the margins, there are often foci of small lymphocytes and plasma cells.

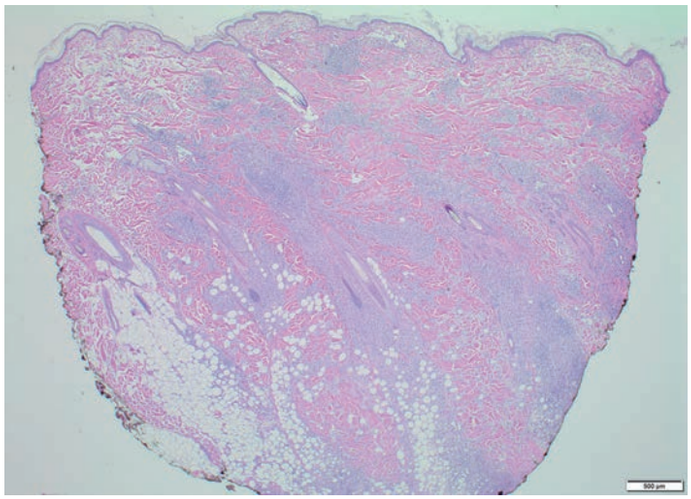

Figure 1.74 Canine cutaneous histiocytosis biopsy. 2.5×.

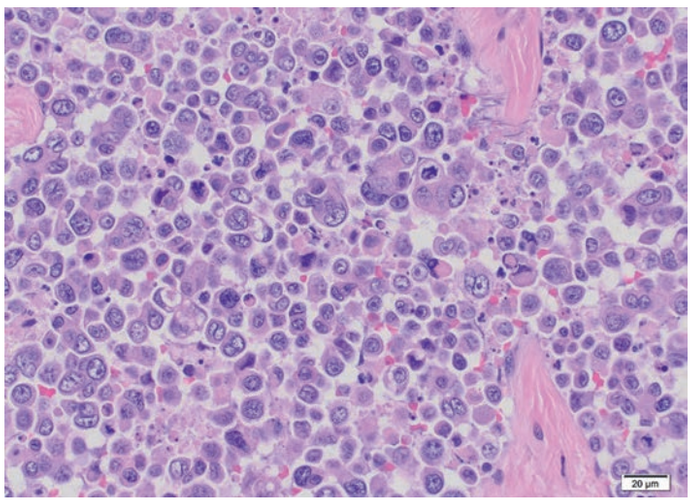

Figure 1.75 Histiocytic sarcoma biopsy. 20×.

Figure 1.74 shows a biopsy of canine cutaneous histiocytosis. A clinical history of multiple, sometimes waxing and waning, epithelioid cell infiltrates with moderately pleomorphic nuclei and abundant coarse cytoplasm is typical of histiocytosis. Immunohistochemistry would be necessary for a definitive diagnosis of the tumor type.

Figure 1.75 shows a histiocytic sarcoma biopsy. This adult male Schnauzer was presented for a subcutaneous mass on the thorax. Biopsy revealed areas of spindle cells with a storiform pattern interspersed with areas of epithelioid and multinucleate cells. There were 0–3 mitotic figures/HPF with 12 mitotic figures/10 HPF. The mass extended to surgical margins. The epithelioid and multinucleate cells are suggestive of histiocytic lineage, and a presumptive diagnosis of histiocytic sarcoma was made. Confirmation of the cell type with immunohistochemistry was advised. Thoracic and abdominal radiographic and ultrasound evaluation, complete laboratory evaluation, and examination of local lymph nodes are advised due to the tendency of this tumor type to spread widely. Additional imaging modalities could be helpful if thoracic radiographs are negative. Consultation with a specialist for the most current therapeutic protocol is suggested.

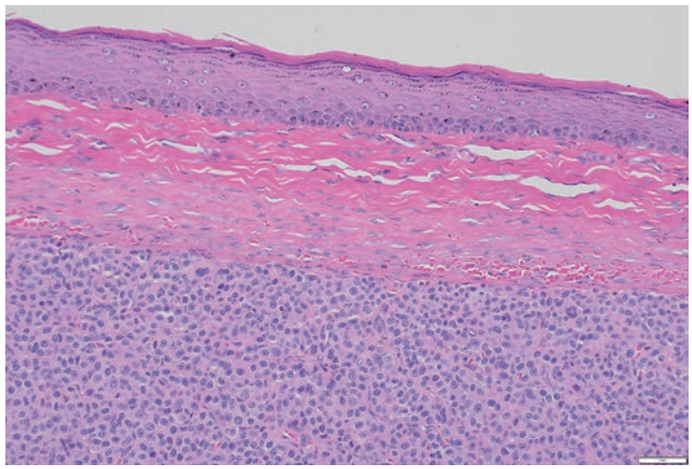

Figure 1.76 Plasma cell tumor biopsy. 10×.

Plasmacytoma

Plasma cell tumor is a common round cell tumor in the skin of dogs and is sometimes seen in cats. It is usually benign in spite of a pleomorphic appearance to the cells, but malignant forms can occur. Prognosis is best correlated to two factors: factor one being if there are tumor cells infiltrating bone and internal organs with associated hypercalcemia, and factor 2 being if there is production of serum or urine myeloma proteins with subsequent organ damage due to hyperviscosity.[86] The presence of either factor lowers the prognosis.

Figure 1.76 shows a plasma cell tumor biopsy. This dome-shaped hairless skin mass on an adult female Bulldog was biopsied and revealed many round cells with eccentric nuclei and numerous binucleate and trinucleate cells with occasional cells exhibiting large lobular nuclei with dense chromatin. This nuclear pleomorphism is a diagnostic feature of benign plasma cell tumor.

Melanoma

In the canine species, melanoma prognosis is heavily influenced by location and mitotic rate.[87] Because some of the criteria are subjective, several parameters should be considered when evaluating melanocytic tumors.[16] In all locations, distant metastasis, nuclear atypia, MC, degree of pigmentation and markers of proliferative rate (Ki67 index) will have a statistically significant prognostic ability. Stage of disease and lymphatic invasion is predictive in oral melanoma and junctional activity is predictive in lip and digital tumors. Oral and lip melanocytic neoplasms have a poorer prognosis with distant metastasis, lymphatic invasion, greater than 4 mitotic figures/10HPF, greater than 30% atypical nuclei, less than 50% pigmentation, and deep invasion with lysis of underlying bone. Cutaneous and digital melanocytic tumors have a poorer prognosis with distant metastasis, lymphatic invasion, greater than 3 mitotic figures/10HPF, greater than 20% nuclear atypia, scant pigment, and invasion beyond dermis. Tumor thickness in melanocytic tumors of the skin can be useful to distinguish melanocytoma from melanoma with 0.45 cm defined as the cutoff with 87%sensitivity, and the level of infiltration by tumor is associated with the outcome as well.[88] Canine melanomas can sometimes be managed with the melanoma vaccine if complete resection is possible. Melanomas located on mucosal surfaces that exhibit c-KIT receptor expression (a tyrosine kinase receptor), exhibit longer survival,[89] although this has not been shown to be useful as a treatment modality.[90] Immunofluorescence measurement of activated C-kinase (RACK1) was used to identify cell and nuclear size, which was helpful to distinguish between melanocytoma and melanoma.[91] Melanomas on haired skin tend to be more indolent and metastasize later in the course of the disease, but highly aggressive tumors can occur. Skin tumors with an MC of less than 3/10 HPF tend to be less aggressive and are often benign, and oral tumors with an MC of less than 4/10 HPF tend to be less aggressive, which means that complete excision with greater than 0.5 cm margins and no evidence of lymphatic or distant spread can result in a longer remission. Since this tumor can become more aggressive with time, large slow-growing tumors can develop areas of pleomorphism and high mitotic activity as they age, and in general the area of highest mitotic rate not adjacent to areas of ulceration should be chosen for evaluation. Occasionally the tumor is so pleomorphic and poorly pigmented that immunohistochemical stains are necessary for a definitive diagnosis of the tumor type, as this pluripotential cell type that arises from neuroectoderm can mimic all the other cell lines.[92] Junctional activity at the mucosal/submucosal interface is an important diagnostic indicator for this tumor so including tissue with intact overlying mucosa will often confirm the tumor type without the need for special stains.

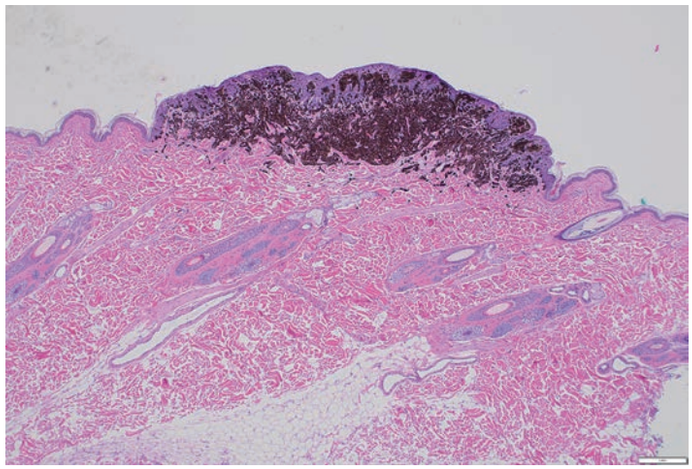

Figure 1.77 Well-differentiated dermal melanoma of canine skin biopsy. 10×.

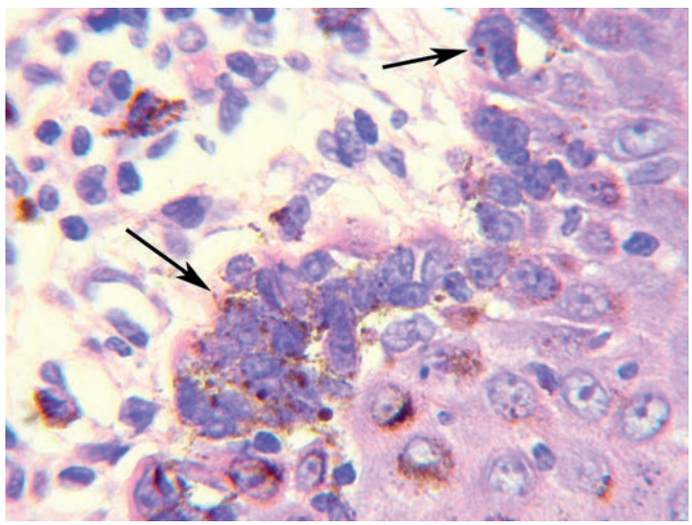

Figure 1.78 Melanoma biopsy showing junctional activity. 40×.

In the feline species, melanoma is uncommon, often amelanotic, and like the dog, the cells can be pleomorphic and mimic other tumors; therefore, immunohistochemistry may be necessary for a definitive diagnosis of the tumor type. Behavior is unpredictable but tends to be aggressive with a median survival time of 87 days and a longer survival time of greater than 100 days when treated aggressively.[93] A grading system has been proposed for tumors of the lips, nasal planum, and oral and nasal mucosa where tumors with greater than 4 mitotic figures/10HPF and/or intratumoral necrosis are considered high grade and tumors arising elsewhere are considered high grade if they exhibit both criteria.[94] In this study, the median survival was 90 days.

Figure 1.77 shows a well-differentiated tumor that exhibits deeply pigmented tumor cells in the subepidermal region. No mitotic figures are seen in the less well-pigmented areas. There is junctional activity with at least 0.2 cm of normal tissue at the margins, which suggests complete excision of this low-grade tumor although ideally at least 0.5 cm of normal tissue at the lateral margins and 1 fascial plane deep is the preferred minimal margin.[87]

Figure 1.78 shows a melanoma biopsy with junctional activity (arrows). Junctional activity (nested proliferations of the neoplastic melanocytes) is a distinguishing feature of the melanoma. It can only be evaluated if there is intact skin present, so submission of completely ulcerated lesions can delay definitive diagnosis in poorly differentiated tumors.

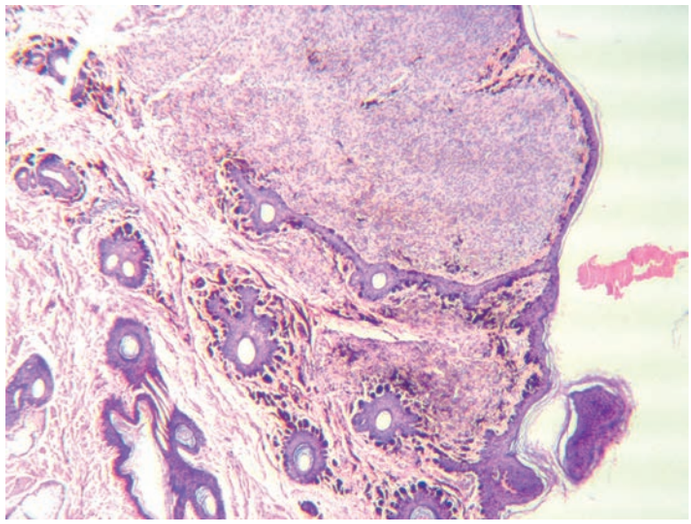

Figure 1.79 Biopsy of a well-differentiated cutaneous melanoma. 2.5×.

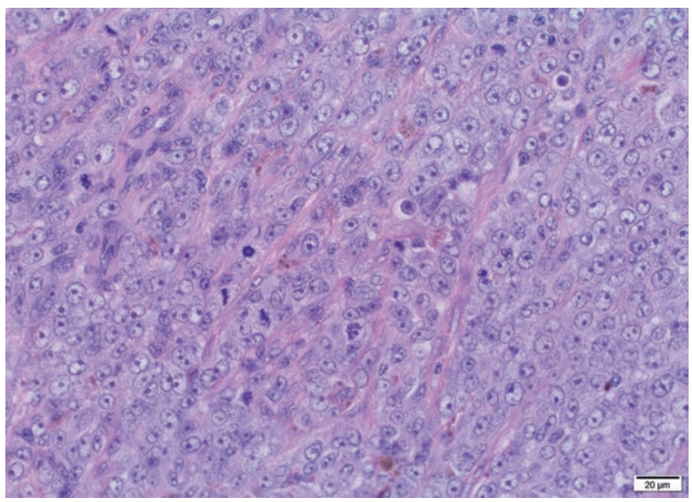

Figure 1.80 Biopsy of a poorly differentiated cutaneous melanoma. 20×.

Figure 1.79 shows a biopsy of cutaneous melanoma that is well differentiated. This variably pigmented proliferation of melanocytes demonstrates 0–1 mitotic figures/HPF with an MC of 2/10 HPF. There is junctional activity at the interface in some areas. This tumor is considered to be a well-differentiated tumor.

Figure 1.80 shows a biopsy of cutaneous melanoma that is poorly differentiated. This poorly pigmented proliferation of pleomorphic epithelioid to spindloid cells exhibits marked anisokaryosis and multilobular nuclei. Overall MC was 19/10HPF, although there are only scattered mitotic figures in this field. These cells are not clearly of melanocyte origin, and the presence of junctional activity at the epidermal interface is necessary for a definitive diagnosis without resorting to immunohistochemistry. Samples taken from ulcerated areas may not have any epithelium, resulting in an inability to look for junctional activity. This tumor type has a high probability for metastasis and/or regrowth so an accurate diagnosis is critical.

Figure 1.81 is an FNA of a pleomorphic, poorly differentiated melanoma that reveals a spindle cell with minimal melanin and an epithelioid cell with moderate melanin. This pleomorphism in a population is a hallmark of melanoma.

Figure 1.82 shows a biopsy of poorly differentiated melanoma that reveals a heterogeneous population of poorly pigmented spindloid melanocytes adjacent to a population of more deeply pigmented melanocytes in the same tumor, demonstrating the cellular pleomorphism seen in the aspirate in Figure 1.81 Biopsy at different sites can yield a very different appearing tumor.

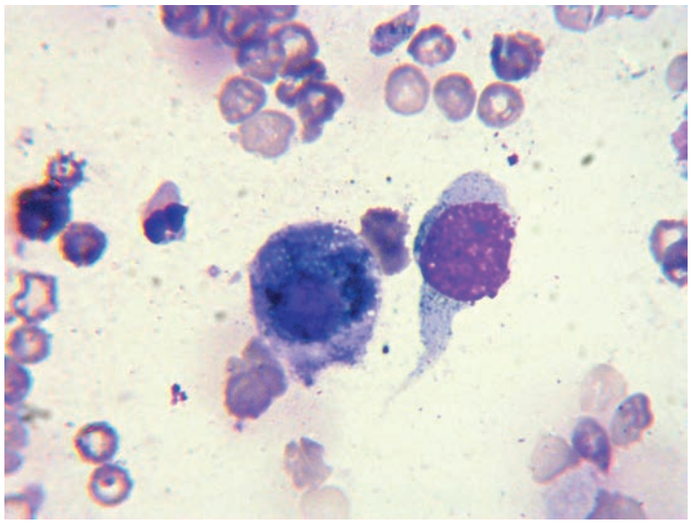

Figure 1.81 Cutaneous melanoma fine needle aspiration. 50×.

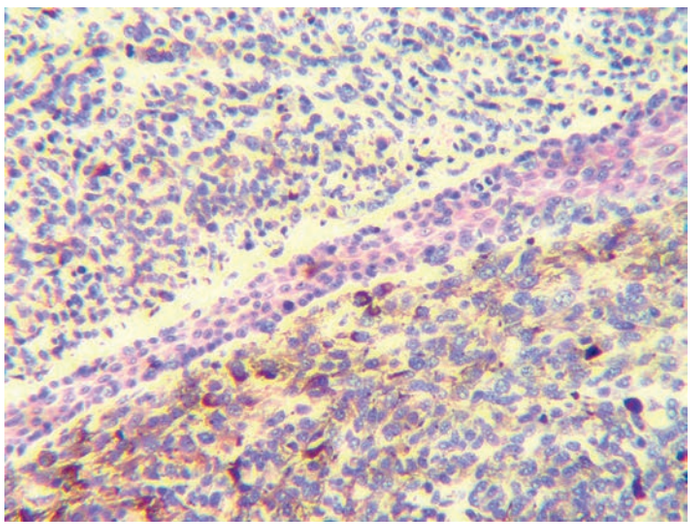

Figure 1.82 Cutaneous melanoma, poorly differentiated biopsy. 10×.

Conclusion

Some tumors are significantly influenced by location, others by mitotic rate, and others by multiple factors such as reproductive hormone receptors, hypoxia, and tyrosine kinase receptor expression (KIT).[95, 96] Benign tumors can grow large without becoming aggressive, can become malignant and metastasize, or sometimes can spontaneously disappear. Malignant tumors can contain populations of benign or reactive cells, confusing the diagnosis, and reactive populations can sometimes look malignant histologically. For the owner with limited funding, or the owner who declines referral, treatment by the general practitioner with antibiotics such as doxycycline for infectious diseases that cause immune dysfunction and chronic reactive lymphoid proliferations, cyclooxygenase inhibitors for epithelial tumors, tyrosine kinase inhibitors for MCTs and gastrointestinal stromal tumors (GIST) that exhibit receptor alterations, judicious use of immunosuppressive drugs for lymphoproliferative diseases, and excision with clean margins, may result in extension of lifespan with a good quality of life. Consultation with a specialist regarding current therapy recommendations may yield the most satisfactory results because this field is rapidly advancing and the published literature can experience some delay. For the owner with high expectations, testing beyond basic histopathology (immunohistochemistry, PARR,

c-KIT analysis for the specified (KIT) tyrosine kinase receptor mutation, silver staining of nucleolar organizer regions (Ag-NOR), and other developing procedures) is likely necessary to provide adequate information for prognosis and therapy.

A few key points regarding some common tumors can be useful to the general practitioner.

Epithelial tumors tend to be more aggressive when simple (one cell type such as a purely glandular tumor) as opposed to compound (e.g., epithelial and myoepithelial populations as in a mixed mammary tumor). The location can be predictive (SCC may be invasive earlier at mucosal sites than at haired skin sites). Invasion by epithelial tumors into adjacent stroma and lymphatics, and especially bone, is generally a bad prognostic indicator.

Histiocytic tumors in any location have a poor prognosis in cats, but in dogs the location is strongly correlated with behavior (cutaneous histiocytoma versus splenic histiocytic sarcoma).

Lymphoid neoplasia prognosis can be correlated to cell type. B-cell tumors tend to be rapidly expansive, in both dogs and cats, while T-cell tumors can be aggressive or indolent (slow growing) with the mitotic rate predictive of behavior in dogs.

MCT behavior correlates to grade, which is significantly influenced by mitotic rate and nuclear pleomorphism in dogs. In cats, there is no currently accepted grading protocol predictive of behavior, but site (skin versus internal organs) and mitotic rate can affect behavioral characteristics.

Melanoma morbidity and mortality are most affected by complete excision or other prompt therapy, and the mitotic rate and degree of nuclear pleomorphism are valuable prognostic indicators.

Sarcomas in dogs also can be best mitigated by early wide excision, and mitotic rate and cellular pleomorphism are strongly correlated to behavior. In cats, certain sarcomas, sometimes associated with tissue injury due to various causes, are highly aggressive and location is predictive in that the ability to completely amputate the affected area with wide margins can increase survival time.

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