Cytology of Neoplasms that Occur on the Limbs
Rick Alleman, DVM, PhD, DABVP, DACVP

I. Introduction

The purpose of this material is to provide information that may be useful in the identification of tumors associated with various structures on the limbs. A brief explanation of the cytologic appearance of specific tumors and their biological behavior is provided.

II. Tumors of Bone and Cartilage

Any tissues present within bone can undergo neoplastic transformation; however, most tumors will arise from the mesenchymal precursors of osseous and cartilaginous tissues. Although less common, tumors may also arise from vascular or connective tissue cells in bone. Most bone and cartilage tumors of the limbs are malignant. Benign osteomas and chondromas occur predominantly on the skull and flat bones.

A. Osteosarcoma

1. Osteosarcoma is the most common malignant bone tumor of the limbs. They frequently occur in giant and large breed dogs, but may also be seen in small dogs, cats, and many other species.

2. Site predilection: In large dogs they have a site predilection for the metaphyseal regions of the proximal humerus, distal radius and ulnar, and proximal or distal tibia and femur. In small dogs (< 15 kg) less than ¼ of the osteosarcomas occur on the appendicular skeleton with the femur being the most common site, not the distal radius. In small dogs, most occur on the axial skeleton, skull, ribs, vertebrae or pelvis.

3. Although they are bony neoplasms, tissue aspirates from osteosarcomas are usually easily obtained using a standard 20 gauge or 22 gauge needle and syringe. If dense cortical bone is still present around the lesion a bone marrow aspirate needle may be used to penetrate the cortical bone, and the stylet can then be removed once the needle is embedded in the mass.

4. Cytologic appearance: Aspirates contain moderate to large numbers of individualized, oval to spindle shaped cells (malignant osteoblasts). These cells contain moderate amounts of deeply basophilic cytoplasm with discrete cytoplasmic borders, giving some osteosarcomas more of a round cell appearance. Nuclei are round to oval and often eccentrically located in the cytoplasm (flag cells). There is marked anisokaryosis (variation in nuclear size) and nuclei contain clumped chromatin with prominent, multiple, pleomorphic nucleoli. Multinucleation is a distinguishing feature, along with the presence of amorphous pink material (osteoid) that can be seen both extracellularly and as granules within the cytoplasm of neoplastic cells.

5. Biological behavior: In the canine species, appendicular osteosarcomas are very aggressive, and hematogenous spread to the lungs occurs early in the disease process.
Cures are rarely obtained. Although some may survive up to a year, median survival time with amputation alone is 2 to 4 months. Amputation in combination with cisplatin chemotherapy has a reported one year survival time of 40%. Rare reports of spontaneous remission of appendicular osteosarcoma has been reported in dogs (JAVMA, 219:614-618, 2001)

However, osteosarcomas located on the mandible and axial skeleton may have a better prognosis than those seen on the ribs or appendicular skeleton. In the feline species, osteosarcomas are less aggressive and typically metastasize late in the course of the disease. Reported median survival time following surgical removal is 4 to 5 years.

B. Chondrosarcoma:

1. *Chondrosarcomas* are malignant tumors characterized by the formation of cartilage. They occur more frequently in large, but not giant breed dogs. They have been reported rarely in cats.

2. Site predilection: These tumors more commonly occur on the flat bones with 69% occurring on the head (nose and mandible), ribs, or pelvis. Metaphyseal regions of long bones may also be affected.

3. Cytologic appearance: Cytologically chondrosarcomas are similar in appearance to osteosarcomas with a predominant population of individually arranged, oval to spindle shaped cells. These cells are sometimes embedded in a pink, amorphous matrix material (chondroid). Chondroid does not usually stain as dark as osteoid and more resembles a mucus-like material. Multinucleated giant cells may also be observed, but not as frequently as in osteosarcomas.

4. Biological behavior: Biologically, they appear to grow more slowly than osteosarcomas, and metastasis usually occurs late in the disease. In dogs, the metastatic rate is approximately 20%. The lung is the primary site of metastasis, particularly with rib tumors. In cats, too few reports are available to define the behavior, but pulmonary metastasis has been reported. Aggressive surgical resection is the treatment of choice and may be curative, however, survival data is limited.

C. Fibrosarcomas:

1. *Fibrosarcomas* are malignant neoplasms of fibroblasts. They most commonly occur in the skin and subcutis of the extremities and trunk, and in the oral cavity. However, they may less frequently arise from stromal elements in the medullary cavity of long bones, causing severe bony lysis. Soft tissue fibrosarcomas are commonly seen in cats. The high incidence in this species has been associated with tumor growth at sites of previous inoculations, and as the results of infections with feline sarcoma virus.

2. Cytologic appearance: Preparations contain low to moderate numbers of individually arranged, spindle-shaped cells. Nuclei are oval to pleomorphic and anisokaryosis is usually present. Nucleoli are frequently prominent and multiple. Nuclear chromatin may be coarse.
3. **Biological behavior**: These tumors are usually locally invasive but are typically slow to metastasize. Because they are slow to metastasize, amputation is the treatment of choice.

4. **Vaccine-associated fibrosarcomas**: Increased incidence of these tumors coincided with the introduction of mandatory rabies vaccinations and the FeLV vaccine. Aluminum hydroxide or aluminum phosphate adjuvants are implicated in the pathogenesis. The estimated incidence is about 1 in 5,000. The tumors are best managed with aggressive surgical resection. Recommendations are to vaccinate with RV and FeLV on the rear limbs in order to allow amputation should tumors occur. Post-operative radiation may be considered for microscopic residual tumor or unclean margins, but reports thus far indicate only moderate response. Chemotherapy with mitoxantrone or doxorubicin has been ineffective. Acemannan, a chemical synthesized from the aloe vera plant, may have some benefit by causing a lymphocytic infiltrate, edema, and tumor necrosis. However, more controlled studies are needed to assess the effectiveness. Unless limb amputation is an option, most cats develop local tumor recurrence and are euthanized. Metastasis occurs in 11% of reported cases and survival rates vary, but 2 year survival is generally considered poor (<20%). Survival rates may be higher in tumors located on the limbs where amputation is possible (JAVMA 216:58-62, 2001).

D. **Hemangiosarcoma**

1. **Hemangiosarcomas** of bone arise from the vascular endothelium and represent about 2% to 3% of all primary bone tumors. However, these neoplasms are more commonly associated with the heart (right atrium), the spleen, the liver or the subcutis.

2. **Site predilection**: In osseous hemangiosarcomas the ribs and the proximal humerus are the sites most commonly involved, and Great Dane, Boxer, and German Shepherd breeds appear to be over represented.

3. **Cytologic appearance**: The aspirates are usually bloody and often show evidence of intralesional hemorrhage with erythropagia or hemosiderin laden macrophages. Tumor cells exfoliate poorly and are often absent from aspirated material. When present they have an appearance typical of a malignant, mesenchymal tumor. Low numbers of individually arranged, spindle-shaped cells are seen. The cells are usually large and plump with wispy, pale, veil-like cytoplasm that frequently contains small, punctate, clear, cytoplasmic vacuoles. Nuclei are large and pleomorphic and display many features of malignancy.

4. **Biological behavior**: As with hemangiosarcomas in other areas of the body, wide and early metastasis is likely. It may be difficult to tell if the bony neoplasm is the primary tumor or metastatic site. If there are no metastatic lesions, amputation may be helpful. Combination chemotherapy (VAC protocol) may increase survival time.

III. **Limb Tumors of Soft Tissue Origin**
A. **Synovial cell sarcoma:** As with bone tumors, neoplasms of the joint may arise from any number of different tissue types. Primary joint tumors occur most commonly in middle-aged, large breed dogs, at large, appendicular joints (often stifle). Most of these lesions in the dog and cat are malignant, and arise from synovioblastic mesenchymal cells; histiocytic cells are connective tissue. Synovial cell sarcoma (SCS) is reported to be the most common primary joint neoplasm. However, a recent study of 35 synovial tumors employing immunohistochemistry seems to suggest otherwise. The authors recommend that immunohistochemistry is necessary to make definitive diagnosis of and appropriate prognosis for synovial tumors. We will discuss the synovial cell sarcoma as it is currently understood, but references to other tumors of the joint are made in the section on “Evaluation of Synovial Fluid (Heather Wamsley)”. It currently appears that Rottweilers have a predisposition to development of histiocytic tumors (histiocytic sarcoma) in the joint. These lesions are very aggressive with rapid metastasis (91%), usually by the time of diagnosis.

1. **Site predilection:** These are infrequently diagnosed tumors, usually involving the stifle, but may also occur on the elbow, or shoulder.

2. **Cytologic appearance:** Cytologically, synovial cell sarcomas contain individually arranged, oval shaped cells with eccentrically located nuclei. Moderate to marked anaplastic features are usually observed. Similar to osteosarcomas and chondrosarcomas, a second population of spindle-shaped cells is also seen. Abundant, pale, amorphous, pink, granular material (mucin) is frequently observed in the background. There are usually sufficient nuclear criteria for malignancy to make a diagnosis of sarcoma (anisokaryosis, pleomorphism, prominent multiple nucleoli). The location of the lesion, along with the mucinous background material, suggest the joint as the tissue of origin.

3. **Biological behavior:** The biological behavior of these tumors is variable, but in general they are locally very invasive, causing bony lysis and frequent recurrence if surgical excision is not complete. Metastasis is said to occur in about 25% of the cases.

B. **Hemangiopericytoma:**

1. **Hemangiopericytomas** are mesenchymal neoplasms of undetermined origin. Some investigators believe the tumor arises from pericytes that line the blood vessels, hence the name. Recent immunohistochemical characterization (vimentin and actin positive) supports this hypothesis. Thus far, the dog is the only domestic animal in which this neoplasm has been reported, however, these tumors are fairly common in the canine species.

2. **Site predilection:** The most common site for the hemangiopericytoma is the extremities, particularly the lateral surface of the hind limbs. Tumors may present as a firm nodule or as a soft gelatinous swelling, mimicking a lipoma.

3. **Cytological appearance:** Cytologically, preparations of needle aspirates taken from hemangiopericytomas are usually very cellular. In fact, an extremely cellular aspirate from a mesenchymal tumor is a diagnostic feature of the hemangiopericytoma. Aspirates contain large numbers of individually arranged mesenchymal cells with very
thin, wispy cytoplasmic tails. Low numbers of small, punctate, clear, cytoplasmic vacuoles are frequently observed. Nuclei are round to oval, plump, and often contain one or two prominent nucleoli. Anisokaryosis is moderate to marked. Binucleation and sometimes small multinucleated cells (3 or 4 nuclei) are occasionally seen.

4. **Biological behavior:** These tumors are locally invasive and recurrence following local resection has been reported in 26% to 60% of the cases. Metastasis is rare (<5% of cases). Aggressive surgical excision is the treatment of choice. These tumors may become more infiltrative and aggressive with each surgical excision; therefore, the initial attempt at surgical removal provides the best opportunity for a cure.

C. **Plasmacytoma:**

1. Extramedullary plasmacytomas are lymphoid neoplasms with site predilections for the skin (76%), frequently occurring on the digits and foreleg (32%) as well as the oral cavity (28%), ears, and gastrointestinal tract. This tumor more commonly affects dogs; however, there are reported cases in the cat. In the dog, there may be a breed predilection in Cocker Spaniels (11.1%), Boxers (9.4%), and Airedale Terriers (7.7%).

2. **Cytologic appearance:** Plasmacytomas have the cytologic appearance of other round cell tumors with aspirates yielding moderate to large numbers of individually arranged, round to oval cells with discrete cytoplasmic borders. These cells contain variable amounts of deeply basophilic cytoplasm and sometimes have a characteristic "plasmacytoid" appearance with an eccentrically-located round nucleus. A perinuclear clear area "Golgi zone" is sometimes seen in the cytoplasm. There is marked anisokaryosis and binucleation and multinucleation is occasionally observed.

3. **Biological behavior:** In the dog, extramedullary plasmacytomas are usually considered benign and surgical removal is curative in most cases. Only rare cases of local tumor recurrence and metastasis have been reported. Even though the cytological appearance may be identical in either species, in the cat, the tumor may be more aggressive and become systemic with associated metastasis and monoclonal gammopathies. In either species, plasmacytomas can, on occasion be associated with local amyloid production, and rarely with amyloidosis or hypercalcemia.

D. **Melanoma:**

1. **Melanomas** may occur anywhere on the skin or in the oral cavity. However, in the dog, there is also a site predilection for the digits, particularly the nail-bed germinal epithelium. In this location, as well as in the oral cavity, melanomas are considered malignant, regardless of the cytologic differentiation. (see tumors of the oral cavity for cytologic appearance and biological behavior).

E. **Squamous cell carcinoma:** Squamous cell carcinomas are malignant tumors of squamous epithelium. They may be found in numerous locations, but have site predilections for the head (ears, lips, nose, and oral cavity) as well as the digits (nail-bed epithelium). Most nail bed SCCs occur in the dog.
1. **Cytologic appearance:** Cytologically, most tumors are composed of anaplastic appearing squamous cells seen occurring singularly and occasionally in small clumps. The cells are usually very pleomorphic with variable N:C ratio. They contain moderate amounts of lightly basophilic to aqua-blue cytoplasm. Some cells contain small, clear, punctate perinuclear vacuoles. "Tadpole" like cells with eccentrically located nuclei and single blunted cytoplasmic tails are occasionally seen. The nuclei are pleomorphic with coarse chromatin and prominent, multiple nucleoli. Well-differentiated SCCs may contain large numbers of fairly mature squamous epithelium.

**Note:** Subungual keratoacanthomas are lesions of the digit that are composed of well-differentiated, keratinized squamous epithelium. These lesions may invade bone, but are rare to metastasize. Surgical excision is usually curative.

2. **Biological behavior:** Most squamous cell carcinomas of the skin metastasize slowly, but those arising from nail-bed epithelium are highly aggressive and spread early to regional lymph nodes. The prognosis is guarded at best. Treatment consists of surgical excision of the affected digit or limb amputation if invasion is extensive. Metastatic lesions may often be present early in the disease. Cisplatin chemotherapy may be beneficial, but detailed studies are lacking.

IV. Conclusion

A. It is often important, sometimes critical, to be able to recognize the site predilections for certain neoplasms. This information can be used to help support a cytologic diagnosis.

B. In addition, once the diagnosis is made, it is important to recognize that location of the lesion may play a critical role in determining the biological behavior of the tumor, as well as therapeutic and prognostic information.