Pathology in Practice

In collaboration with the American College of Veterinary Pathologists

Figure 1—Images of the enlarged right forelimb of a 10-month-old 16.8-kg sexually intact female Boxer examined because of the physical abnormalities and severe lameness in the limb before (A) and after (B) limb amputation.

History

A 10-month-old 16.8-kg sexually intact female Boxer was presented because of a misshapen left forelimb with a large mass on the distal aspect and extreme lameness in the limb. The animal had a history of lethargy, anorexia, and decreased drinking. The forelimb was irregularly enlarged by the mass that was described as fast growing and painful.

Clinson Lui, BS; David Forster, DVM; Ana Alcaraz, DVM, PhD, DACVP*

College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA

*Corresponding author: Dr. Alcaraz (aalcaraz@westernu.edu)

<https://doi.org/10.2460/javma.19.07.0317>

Clinical and Gross Findings

Initial physical examination revealed that the animal was in thin body condition with no asymmetry except for the right antebrachium. During physical examination, the dog showed signs of lameness and pain. The right thoracic limb showed a firm enlargement affecting almost the complete length of the ulna and radius and muscle atrophy. On palpation, the mass was firm, smooth, and affected almost the complete length of the antebrachium, with marked edema in the paw. The skin was not freely moveable over the mass, and the mass did not preclude movement in the carpus or elbow joints.

Radiography (not shown) revealed a mass with complete lysis of radius and ulna primarily on the distal third with a possible pathological fracture line

along the proximal remnant of the radius. No metastasis was present on thoracic radiographs. The mass was 37 cm in length, 19 cm in width, and 11 cm in circumference. Distal to the mass, the paw exhibited severe swelling with pitting edema. The skin covering the mass had patchy alopecia and was taut but intact (**Figure 1**).

A CBC revealed mild leukocytosis (21.1×10^3 WBCs/ μL ; reference interval, 6×10^3 to 17.0×10^3 WBCs/ μL) characterized by moderate neutrophilia (18.36×10^3 neutrophils/ μL ; reference interval, 3.5×10^3 to 12×10^3 neutrophils/ μL) as well as mild normocytic anemia (5.34×10^6 RBCs/ μL ; reference interval, 5.5×10^6 to 8.5×10^6 RBCs/ μL) and mild thrombocytopenia (149×10^3 platelets/ μL ; reference interval, 200×10^3 to 500×10^3 platelets/ μL). Serum biochemical analyses revealed high alkaline phosphatase (ALP) activity (518 U/L; reference interval, 23 to 212 U/L).

The decision to surgically amputate the right forelimb including the scapula was recommended. The surgery was performed 2 weeks after presentation. The entire right forelimb and regional lymph nodes were submitted for pathology examination (**Figure 1**). The dog's behavior improved after surgery, and it was no longer anorexic or lethargic and appeared to have more energy.

The mass affecting the right antebrachium measured 37.5 cm in length, 19 cm in width, and 11 cm in circumference and did not affect the carpus or elbow joints. On cut section, the mass had a firm consistency and was easily sliced but gritty. The cross sections of the mass showed a solid multinodular mass with white firm areas mixed with soft red areas and occasional focal gritty areas, with no evidence of normal cortical bone or muscle. The skin covering the mass was intact with no ulcerations present. There was subcutaneous edema present but did not affect the mass. Two superficial cervical lymph nodes were enlarged (approximately 6×4 cm each on cut surface).

Formulate differential diagnoses, then continue reading.

Histopathologic Findings

Representative sections of the mass and lymph nodes were submitted for routine histopathology after formalin fixation with neutral-buffered 10% formalin. Just beneath the skin, the normal architecture of any preexistent tissue including bone and muscle of the affected area of the forelimb was effaced with neoplastic cells that expanded to the adjacent connective tissue. The neoplastic cells were arranged in solid irregular streams intermixed with variable amounts of disorganized acellular homogenous smooth pale eosinophilic material compatible with osteoid matrix (**Figure 2**). There were multifocal small coalescing areas of hemorrhage and necrosis scattered throughout within the neoplasm. (**Figure 3**)

Neoplastic cells were small to large ($< 20 \mu\text{m}$) polygonal to spindle with eosinophilic moderately sparse cytoplasm and indistinct cell borders. Nuclei

Figure 2—Photomicrograph of a section of the periphery of the mass described in **Figure 1**. Neoplastic cells are arranged in solid sheets intermixed with variable amounts of osteoid matrix. H&E stain; bar = 500 μm .

Figure 3—Photomicrograph of the forelimb mass described in **Figure 1** showing polygonal neoplastic cells with eosinophilic cytoplasm and indistinct cell borders. The osteoblasts and osteoclast are surrounding variable amounts of osteoid matrix. H&E stain; bar = 100 μm .

were round, with finely stippled chromatin, and with occasionally 1 to 2 prominent nucleoli and moderate degree of anisocytosis and anisokaryosis. There were areas with moderate numbers of multinucleated giant cells, with opposing variable sized areas of irregular osteoid matrix, interpreted as osteoclasts. Mitotic rates averaged 13 mitotic figures in 10 hpfs.

In the lymph node tissue, no substantial lesions were found. The subscapular and medullary sinuses were markedly expanded with edema and hemorrhage. There were numerous large, foamy macrophages within the medullary sinuses. There was also marked lymphoid hyperplasia with numerous prominent follicles that contained germinal centers. All the findings in the lymph node sections were consistent with reactive lymph node hyperplasia. There was no evidence of metastasis.

Morphologic Diagnosis and Case Summary

Right forelimb mass: antebrachial osteosarcoma (OSA).

Case summary: appendicular OSA with pathological fracture and bone lysis in a young dog.

Comments

Osteosarcoma is a malignant mesenchymal tumor of primitive bone cells that produce osteoid. Histologic appearance of OSAs varies markedly but a common feature is the presence of malignant osteoblasts. The tumor matrix may also contain variable quantities of cartilage, collagen, and tumor osteoid. Malignant osteoblasts may be spindle shaped and resemble fibroblasts or be rounded cells with basophilic cytoplasm and eccentric hyperchromatic nuclei. They can be present individually or as clusters of osteoid.¹ Mitotic figures are common and the amount of osteoid is variable. Multinucleated giant cells with features of osteoclasts are often present in OSA along with coagulation necrosis and hemorrhage. Osteosarcoma can be subclassified into 6 different categories depending on its histologic features: 1) poorly differentiated OSA, tumor cells vary from small cells resembling bone marrow stroma and presence of unequivocal tumor osteoid; these present with highly aggressive forming lytic bone lesions and pathological fractures; 2) osteoblastic OSAs are recognized by the presence of anaplastic osteoblasts throughout the tumor; the tumors have hyperchromatic eccentric nuclei and basophilic cytoplasm; 3) chondroblastic OSAs have malignant mesenchymal cells directly producing osteoid and chondroid matrix; 4) fibroblastic OSAs have a cell population of spindle cells similar to fibrosarcoma along with osteoid and bone formation by tumor cells; 5) telangiectatic OSA is an aggressive osteolytic tumor consisting of a mixture of solid areas and blood-filled spaces that resemble hemangiosarcoma but has spicules of osteoid among pleomorphic mesenchymal cells; and 6) giant-cell OSAs have giant cells that predominate. Histologically, OSA can sometimes be confused with a callus repair, and one must look for maturation within the callus as a differentiating feature in the cell population.¹ Osteosarcoma presents with more atypia compared to a normal callus.

Osteosarcoma is the most common primary bone tumor in dogs.² It accounts for 80% of their bone tumors. Osteosarcoma has been reported in cats, horses, cows, guinea pigs, birds, fish, and lizards.³ Etiology is associated with somatic copy-number alterations (SCNA) in cancer genes. At the level of point mutation, TP 53 is most affected with most mutations occurring in the DNA-binding domain or high frequency of SCNAs.³ Additionally, *RB1* gene mutations are speculated to correspond with OSA. However, a more recent study showed that mutations at CDKN2A/B are more specifically correlated with OSA

in dogs.³ This study found that 78% of canine samples of OSA had mutations at CDKN2A/B and only 6% had mutations at the *RB1* locus.³ These are tumor suppressor genes that, if altered, could lead to OSA. There have been many conflicting studies on whether there is a sex predisposition or reproductive status (sexually intact versus spayed or neutered) predisposition for OSA.⁴ The results of studies are inconsistent, and many confounding variables are often listed.⁴ Osteosarcoma has a bimodal age distribution with 80% of cases occurring in animals over 7 years old. There is a second peak where 6% to 8% of dogs affected by OSA are under 3 years of age.⁴ Median age of dogs at diagnosis with OSA is of 7.5 years, and older dogs that are taller and heavier have an increased risk of developing OSA. There is also an increased incidence of OSA in dogs between the ages of 6 and 24 months old.^{4,5} However, research on OSA in young dogs is limited.

Larger dog breeds develop OSA at a younger age than dogs of smaller breeds.⁵ Specific breeds, like Boxers, Great Danes, and German Shepherd Dogs, are at increased risk of OSA, compared with small breed dogs.^{2,4,6} Dogs weighing > 20 kg account for 90% of the cases. In dogs, OSA affects mostly the appendicular skeleton, with forelimbs being affected twice as often as hind limbs and 75% of the cases having the metaphysis as the most commonly affected location.^{2,6} The preferential sites are on the distal radius, proximal humerus, proximal tibia, and distal femur. The distal radius and ulna area is the most common location, accounting for 40% of all cases. Osteosarcoma is locally aggressive and causes lysis, production of bone, soft tissue swelling, and possibly pathological fractures.^{2,5} However, OSA does not usually cross a joint surface. Metastasis is extremely common and spreads through the hematogenous route. Up to 90% of all animals found with osteosarcoma have metastatic disease found on necropsy, most often affecting the lungs.¹

High serum ALP activity was seen in our patient, and this occurs in association with osteoblastic activity and bone remodeling. Elevated ALP (actual cutoffs vary depending on the laboratory) is considered a poor prognostic indicator for dogs with appendicular OSA.^{2,5,7} Preoperative elevation of ALP is associated with shorter disease-free interval and survival time. Dogs on average had a median survival time of 76 to 120 days.⁵

Dogs can be used as models for human OSA because dogs morphologically and biologically similar.⁸ Fibrosarcomatous subtypes have favorable prognoses in both animals and humans.⁸ However, age and site predilections are different. In most cases, older dogs and a smaller number of younger dogs are usually affected by osteosarcoma, whereas in humans, it is adolescents and a smaller numbers of older people who are mostly affected by the disease.^{4,8} The forelimb is mostly affected in dogs, whereas the knee is the target location in humans.⁴ There are still enough similarities, so human therapeutic options may become a therapeutic model for canine osteosarcoma.

In our case, the OSA followed the traditional presentation and common location that affected the distal portion of the radius and ulna. Because the dog was a Boxer and 10 months of age, it is also one of the more susceptible breeds and fits in the described bimodal age distribution. Two months after surgery, the dog became extremely lethargic and anorexic and had no interest in anything around it. The dog was taken to the emergency room, at which point, detection of metastases was reported but not described. The owner elected euthanasia without postmortem examination.

References

1. Thompson K. Bones and joints. In: Maxie MG, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. Vol. 1. Elsevier Saunders; 2008:112–118.
2. North S, Banks T. *Introduction to Small Animal Oncology*. Elsevier, Saunders; 2009:209–220.
3. Sakthikumar S, Elvers I, Kim J, et al. SETD2 is recurrently mutated in whole-exome sequenced canine osteosarcoma. *Cancer Res*. 2018;78(13):3421–3431.
4. Makielski KM, Mills LJ, Sarver AL, et al. Risk factors for development of canine and human osteosarcoma: a comparative review. *Vet Sci*. 2019;6(2):1–19.
5. Withrow S, Vail VM, Page RL. *Small Animal Clinical Oncology*. 5th ed. Elsevier, Saunders; 2013:435–448.
6. Hoenerhoff MJ, Kiupel M, Rosenstern D, Pool RR. Multipotential osteosarcoma with various mesenchymal differentiations in a young dog. *Vet Pathol*. 2004;41(3):264–268.
7. Kuntz CA, Dernell WS, Powers BE, Withrow S. Extraskelatal osteosarcomas in dogs: 14 cases. *J Am Anim Hosp Assoc*. 1998;34(1):26–30.
8. Misdorp W. Animal model of human disease-skeletal osteosarcoma. *Am J Pathol*. 1980;98(1):285–288.