The Armed Forces Institute of Pathology
Department of Veterinary Pathology
Wednesday Slide Conference
2009-2010
Conference 22
28 April 2010

Conference Moderator:
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CASE I: AFIP 3147900.

Signalment: 17-year-old chestnut Hanoverian mare (*Equus caballus*).

History: This mare presented approximately seven years prior to euthanasia with lameness which progressed to swelling over the splint bones of the left forelimb. A bone scan showed intramedullary sclerosis of the left distal humerus occupying approximately one third of the bone and a suspected lesion of the glenoid cavity of the left scapula. The mare was treated with non-steroidal anti-inflammatory drugs (NSAIDs) and stall rest. No follow-up examination was recorded with reference to the lesions in the humerus and scapula.

Nearly two years prior to euthanasia, the mare presented with an approximately 10 cm diameter, rounded, boney tumor on the caudal aspect of the left mandible. The mandible was thickened in the horizontal plane, with a craterous depression immediately caudal to the mass. Radiographs revealed an "altered trabecular pattern of bone" extending dorsally into the mandibular cheek tooth roots.

Previous clinical history included a left-sided ethmoid hematoma diagnosed approximately eight years prior to euthanasia, which was treated with serial surgeries and formalin injection. Following complete recovery, the mare later developed a chronic, low-volume, bilateral, mucoid nasal discharge due to a mucocele, which resolved in response to 10% formalin injection. No further mucoid discharge or endoscopic changes to the ethmoid turbinates were observed following treatment. The reason for eventual euthanasia was not specified by the case contributor.

Gross Pathology: Necropsy examination was performed by the attending clinician, who reported boney masses in the left mandible and left scapula; the masses effaced pre-existing bone and contained coalescing pockets of soft, white material. The clinican's working diagnosis included osteosarcoma and fibrosarcoma.

Histopathologic Description: Bone, mandible (slide 6) and scapula (slide 7): Extending to the margins of the submitted tissue, expanding the medulla, separating and surrounding regularly-spaced, irregularly-shaped trabeculae of woven bone, and invading and effacing overlying cortical bone is an unencapsulated, infiltrative, well-demarcated moderately cellular neoplasm composed of spindle to stellate mesenchymal cells arranged in haphazard streams on a moderately edematous fibromyxomatous matrix. Trabeculae are lined by a single layer of plump, hypertrophied, active osteoblasts, and frequently, one or more osteoclasts within Howship's lacunae (osteoclastic osteolysis). Proliferating mesenchymal cells have indistinct cell borders, scant eosinophilic cytoplasm, and one oval to elongate nucleus with finely-stippled chromatin and 1-2 generally indistinct nucleoli. Mitoses are rare (less than 1 per ten 40X HPF). Scattered throughout the connective tissue are moderate numbers of osteoclasts, few aggregates of hematopoietic precursors (marrow elements), few perivascular foci of lymphocytes and plasma cells, and rare hemosiderin-laden macrophages. Multifocally, the spindle cells invade and replace the overlying woven or compact cortical bone, which also undergoes osteoclastic osteolysis. In some slides (particularly slide 7), there are aggregates of lymphocytes and plasma cells in the overlying fascia, and attached skeletal muscle myocytes exhibit sarcoplasmic pallor, swelling, vacuolation, with formation of contraction bands (degeneration) or are shrunken, angulated, fragmented, and have hypereosinophilic sarcoplasm with loss of cross striations and pyknosis (necrosis).

Contributor's Morphologic Diagnosis: Bone, mandible and scapula: Ossifying fibroma.

Contributor's Comment: The lesions in the submitted section of mandible (slide 6) and scapula (slide 7) are essentially identical. Ossifying fibroma is a benign, but invasive, lytic, and expansile fibro-osseous proliferation that is most commonly reported in the mandible in horses less than one year of age, where it is classified as equine juvenile mandibular ossifying fibroma. The lesion is less frequently seen in cats, dogs, and sheep, has been reported in greater kudus, a llama, a goat, and a rabbit, and has not been reported to undergo malignant transformation or metastasize.(reviewed in 4,6,8)

This case is unusual in that the affected mare was much older than is typical, and scapular involvement is uncharacteristic of this tumor, which classically presents as a well-demarcated, expansile, non-painful mass protruding from the rostral mandible.(4,6,8) However, ossifying fibroma has been reported in sites other than the mandible, including the maxilla, and the os penis of a dog.(5) Like the most commonly affected bones of the skull, the proximal scapula develops by intramembranous ossification, so its involvement in this case is not completely unexpected. Moreover, the lesion has been reported in tubular bones in humans and cats.(2)

AFIP Diagnosis: Bone, mandible and scapula: Ossifying fibroma.

Conference Comment: During the conference, participants discussed the differential diagnosis for this case in detail. Without the benefit of knowing the clinical history and radiographic findings, most conference participants diagnosed fibrous osteodystrophy (FOD), a fairly common metabolic bone disease caused by persistent primary or secondary hyperparathyoridism. Primary hyperparathyroidism results from a functional parathyroid gland adenoma, and is far less common than secondary hyperparathyroidism in all domestic species. Secondary hyperparathyroidism can result from renal disease or a nutritional deficiency or imbalance (i.e. simple dietary calcium deficiency, dietary phosphorus excess, or vitamin D deficiency). Nutritional secondary hyperparathyroidism is most common in horses, a species exquisitely sensitive to the effects of high dietary phosphorus. Horses fed a diet containing a calcium to phosphorus ratio of 1:3 or wider are highly susceptible to FOD; this most commonly occurs in horses fed a diet consisting primarily of grain, giving rise to the colloquial term "bran disease" for the condition. The disease is also reported in horses grazing pastures high in oxalate, even when calcium and phosphorus are normal. Disease prevalence is highest in young growing horses, and the characteristic clinical presentation is bilateral swelling of the mandibles and maxillae, prompting another colloquial term, "big head." Serum PTH concentrations and urinary fractional clearance of phosphorus are increased in affected horses, and are more useful for antemortem diagnosis than are serum calcium and phosphate concentrations.(7)

Microscopically, FOD is characterized by the triad of increased osteoclastic bone resorption, fibroplasia, and increased osteoblastic activity with the formation of trabeculae of woven bone; all three features are also seen in normal bone remodeling and certain stages of fracture repair, and are present in this case as well.(7) Therefore, the diagnosis requires correlation with the clinical and radiographic findings, and examination of sections of bone distant from sites of bone remodeling or fracture repair. The numerous osteoclasts present in this case compelled some participants to discount ossifying fibroma entirely in favor of a diagnosis of FOD; however, the conference moderator cautioned participants to remember that any mesenchymal cell can secrete RANK-L, resulting in osteoclast differentiation and activation. In this case, fibrous osteodystrophy can be excluded only in light of the clinical history and radiographic findings.

The foci of inflammation within marrow spaces seen in this case are unusual, and are not typical of FOD or ossifying fibroma, urging some participants to consider osteomyelitis in the differential diagnosis. While inflammation in the marrow spaces and reactive new bone growth may result from chronic, productive osteomyelitis, the conference moderator and participants noted several features that argue against osteomyelitis in this case: 1) inflammation in osteomyelitis would be expected to occur in larger pockets, whereas in this case the foci of inflammatory cells are small and generally confined to perivascular areas; 2) the reactive trabecular bone in osteomyelitis would generally exhibit a distinct orientation around pockets of inflammation, whereas in the examined slides the new bone is arranged haphazardly; and 3) productive osteomyelitis generally yields reactive periosteal new bone growth that is oriented perpendicular to the cortex initially (though it may orient parallel to the cortex after remodeling), and would not typically produce the degree of cortical osteoclasis seen in this case.

Microscopically, ossifying fibroma is often described as intermediate between fibrous dysplasia and osteoma, two other benign bone-forming tumors considered by conference attendees and discussed during the conference. Fibrous dysplasia has been ascribed to a mesenchymal developmental abnormality, hamartomatous anomaly, or neoplastic process. While relatively common in humans, it has been only rarely reported in the horse, including once in the

accessory carpal bone causing sudden-onset lameness,(2) and once in the nasal cavity causing epistaxis.(3) Fibrous dysplasia closely resembles ossifying fibroma in all respects except one: while spicules of bone in ossifying fibroma are lined by a single layer of plump osteoblasts which apparently arise from the proliferating mesenchymal cells that separate them, spicules of bone in fibrous dysplasia form directly from mesenchymal cells and are not lined by osteoblasts.(2-4,6-8) At the other end of the spectrum, osteomas are composed primarily of dense trabeculae of woven bone that, as in ossifying fibroma, are lined by a layer of plump osteoblasts; however, osteomas generally project from the surface of the bone, and their bony trabeculae are more separated by smaller spaces with more loosely-arranged mesenchymal tissue than is present in ossifying fibroma.(4,6,8)

Because reports of fibrous dysplasia, ossifying fibroma, and osteoma in horses remain sparse, differences in prognosis among these benign fibro-osseous proliferations are not yet apparent, and the more practically important duty of the veterinary histopathologist is distinction between these proliferative lesions and malignant neoplasia. Affirming this, the submitting clinician's working diagnosis, based on the gross and radiographic findings, included osteosarcoma and fibrosarcoma. Osteosarcoma, while less common than ossifying fibroma in horses, is occasionally reported in the bones of the face and jaw and shares with ossifying fibroma the tendency to replace alveolar and cortical bone, causing tooth loosening and increased susceptibility to pathological fracture. Osteosarcoma is excluded in this case by the presence of regularly-spaced, irregularly-shaped spicules of well-differentiated woven bone, a feature characteristic of ossifying fibroma but not osteosarcoma. Moreover, the spicules of bone are lined by plump osteoblasts that are clearly distinct from the proliferating mesenchymal cells that separate the bone trabeculae. In contrast to the usually overtly malignant neoplastic cells of osteosarcoma, the proliferating mesenchymal cells in this case are microscopically bland, with a low mitotic rate, minimal anisocytosis and anisokaryosis, and absence of osteoid.(4,5,7,8) Similarly, fibrosarcoma is excluded based on the bland microscopic features of the proliferating mesenchymal cells and the gradual transition between the proliferative cell population and adjacent tissue.

Finally, though not associated with the degree of cortical lysis present in this case, idiopathic enostosis-like lesions have been reported in the long bones of horses(1) and should be considered when a proliferation of woven bone is encountered in a diagnostic setting. An example from the right distal humerus of a Swedish Warmblood riding horse was reviewed in WSC 2008-2009, Conference 21, case IV.

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References:

1. Bassage LH, Ross MW: Enostosis-like lesions in the long bones of 10 horses: scintigraphic and radiographic features. Equine Vet J **30**:35-42, 1998
2. Jones NY, Patterson-Kane JC: Fibrous dysplasia in the accessory carpal bones of a horse. Equine Vet J **36:**93-95, 2004
3. Livesey MA, Keane DP, Sarmiento J: Epistaxis in a standardbred weanling caused by fibrous dysplasia. Equine Vet J **16:**144-146, 1984
4. Miller MA, Towle HAM, Heng HG, Greenberg CB, Pool RR: Mandibular ossifying fibroma in a dog. Vet Pathol **45:**203-206, 2008
5. Mirkovic TK, Shmon CL, Allen AL: Urinary obstruction secondary to an ossifying fibroma of the os penis in a dog. J Am Anim Hosp Assoc **40:**152-156, 2004
6. Speltz MC, Pool RR, Hayden DW: Pathology in practice: ossifying fibroma of the right mandible. J Am Vet Med Assoc **235:**1283-1285, 2009
7. Thompson K: Bones and joints. *In:* Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 82-88, 110-124. Elsevier Saunders, Philadelphia, PA, 2007
8. Thompson KG, Pool RR: Tumors of bones. *In:* Tumors in Domestic Animals, ed. Meuten DJ, 4th ed., pp. 248-255, Iowa State Univ. Press, Ames, IA, 2002

CASE II: 04-2259 (AFIP 2988004).

Signalment: 6-month-old, male, domestic shorthair cat (*Felis catus*).

History: This cat presented with lameness in right rear and left front legs. Radiographs revealed multiple old and new fractures in several bones. The animal was maintained on a diet of Hill's Science Diet® for 1.5 months and then became lame again with a new fracture.

Gross Pathology: All bones are brittle and fracture easily. Bone callus is present on several ribs, the left distal femur and the left distal ulna. The right distal tibia has a fresh oblique fracture.

Histopathologic Description: The radius and ulna are submitted. Both have a normal growth plate. The primary spongiosa lack osteoid and are mostly cartilage. Cartilage is retained in the secondary spongiosa and the metaphyseal bone. No osteoclast activity is seen. The radius has a healing fracture with a mixture of cartilage, bone and fibrous tissue.

Contributor's Morphologic Diagnosis: Osteogenesis imperfecta, probable.

Contributor's Comment: The bones in this cat are failing to produce osteoid. The growth plate is normal and no remodeling of bone by osteoclasts is seen. These features support a bone formation problem as the most likely disease. Osteogenesis imperfecta is the most likely cause for the changes in this bone. Confirmation of the disease as the cause would require examination of the teeth, which was not done in this case. The histologic appearance does not support other metabolic bone diseases as causes, including rickets and osteodystrophy fibrosa. Osteodystrophy fibrosa is characterized by bone resorption and fibrosis. Rickets has thick, abnormal growth plates and widened osteoid seams in the primary spongiosa.

Osteogenesis imperfecta is an inherited disease caused by a genetic defect of the COL1A1 or COL1A2 genes, which encode the procollagen molecules of collagen type 1. This results in quantitative and qualitative abnormalities in type 1 collagen. Bone and teeth are affected, but other type 1 collagen-rich tissues, such as skin, are not affected. Osteogenesis imperfecta has been reported in dogs, cats, cattle, sheep, mice and tigers. Defects in the COL1A1 and COL1A2 genes have been found in the Golden retriever dog and mice.

AFIP Diagnosis: 1. Bone, distal radius and ulna: Osteopenia, epiphyseal, focally extensive, marked, with metaphyseal reactive woven bone formation (fracture callus), synoviocyte hyperplasia, and pannus.
2. Bone, proximal humerus: Osteosclerosis, epiphyseal and metaphyseal, focally extensive, marked, with growth retardation lattices, mild trabecular woven bone formation, mild marrow fibrosis and myxomatous metaplasia, and focally extensive periosteal granulation tissue.

Conference Comment: In the distal radius and ulna, conference participants noted several changes in addition to those described by the contributor, including proteoglycan loss in the articular cartilage with empty chondrocyte lacunae (chondrocyte necrosis), synoviocyte hyperplasia, and proliferation of a layer of synoviocytes covering the articular cartilage (pannus). The relationship of the additional observed changes to the underlying condition in this kitten is unclear, but conference participants speculated that the radial fracture may have accounted for both the osteopenia in the radial epiphysis (i.e. due to disuse) and secondary degenerative lesions in the joint (i.e. due to instability). Joint laxity is a characteristic feature of osteogenesis imperfecta (OI), along with bone and tooth fragility and blue sclerae, and therefore may also have contributed to secondary joint lesions in this animal.

Additionally, many conference participants received a section of bone interpreted as proximal humerus instead of the distal radius and ulna described by the contributor. In contrast to the epiphyseal osteopenia noted in the radius, the primary lesion in the proximal humerus is osteosclerosis attributed to the persistence of primary spongiosa with increased cross connections between cartilage spicules resulting from decreased osteoclasis (growth retardation lattices). Throughout the metaphysis, there are scattered trabeculae of woven bone, and within the metaphyseal bone marrow there is mild fibrosis and/or myxomatous metaplasia. In the adjacent periosteum, there is focally extensive granulation tissue.

Osteogenesis imperfecta, though well-described in humans, remains a rare diagnosis in animals. As mentioned by the contributor, most cases of OI stem from mutations in COL1A1 and/or COL1A2 genes that encode the $\alpha 1$ and $\alpha 2$ chains of type I collagen, respectively. Collagen in bone, dentin, ligaments, tendons, and the sclera is primarily type I, resulting in the characteristic confinement of lesions to these anatomic locations.(1-6) While type I collagen also predominates in the skin, OI usually does not produce cutaneous lesions,(6) although skin fragility has been reported in affected newborn New Zealand Romney lambs.(1)

Noteworthy, OI represents a heterogenous group of diseases, and defects in glycosaminoglycan and proteoglycan metabolism(2) or deficient osteonectin synthesis(6) may also account for some cases. This observation, combined with evidence that a wide variety of mutations in COL1A1 and/or COL1A2 have been demonstrated in humans with

OI, explains the substantial phenotypic variation seen in the disease.(1) The conference moderator emphasized this point and noted that because mutations that produce OI can cause qualitative and/or quantitative collagen defects, the bone fragility that results is not necessarily related to bone mass, and thus a uniform set of pathognomonic microscopic findings cannot be relied upon for the diagnosis of OI. As a result, the definitive diagnosis of OI based solely on the tissues provided for histologic examination in this case is not possible, although the clinical history in combination with the gross and microscopic findings is certainly suggestive of the disease. As described by the contributor, the growth plates in this case are microscopically normal, an expected finding in OI since the collagen found in cartilage is primarily type II.(6)

The osteosclerotic lesions in the proximal humeral metaphysis and epiphysis led some participants to consider osteopetrosis in the differential diagnosis, and the conference moderator noted that osteopetrosis, like OI, may manifest as brittle bones prone to fracture. Osteopetrosis in cats has been associated with feline leukemia virus (FeLV) infection and chronic vitamin D toxicosis.(5) However, osteopetrosis classically results in a more severe failure of bone modeling than is present in this case. Additionally, although trabecular bone in OI is often described as microscopically normal or osteopenic, some cases, particularly those seen in lethal OI with skin fragility in New Zealand Romney lambs, result in persistent trabeculae of calcified cartilage that extend into long bone diaphyses and fill marrow cavities, accompanied by a paucity of osteoclasts, similar to osteopetrosis.(1,5)

Of note, some of the earliest cases of "OI" in dogs and cats were later shown to represent misdiagnoses of fibrous osteodystrophy (FOD) due to nutritional secondary hyperparathyroidism, with apparent familial susceptibility actually derived from exposure to common nutritional deficiencies.(2) The paucity of osteoclasts in this case argues against a diagnosis of FOD. Other considerations briefly discussed during conference included osteopenia secondary to protein calorie malnutrition and lack of osteoclasts due to inconsistent exposure to such anti-osteoclastic agents as lead or bisphosphonates. In addition, the lesion in the distal metaphyses of the radius and ulna is somewhat reminiscent of the scorbutic lattice of vitamin C deficiency (scurvy). However, conference participants uniformly agreed that there is more osteoid deposition on the primary spongiosa than would be expected in scurvy. Furthermore, the growth retardation lattice noted in the proximal humeral metaphysis is not expected in scurvy, and this case lacks other classic findings of scurvy, such as microfractures or infractions with associated hemorrhage and fibrin.(5,6)

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References:

1. Arthur DG, Thompson KG, Swarbrick P: Lethal osteogenesis imperfecta and skin fragility in newborn New Zealand Romney lambs. NZ Vet J **40:**112-116, 1992
2. Denholm LJ, Cole WG: Heritable bone fragility, joint laxity and dysplastic dentin in Fresian calves: a bovine syndrome of osteogenesis imperfecta. Aust Vet J **60:**9-17, 1983
3. Leeb F, Peters M, Brugmann M, Fehr M, Hewicker-Trautwein M: Osteogenesis imperfecta in two litters of dachshunds. Vet Pathol **40:**530-539, 2003

4.Seeliger F, Leeb T, Peters M, Brugmann M, Fehr M, Hewicker-Trautwein M: Osteogenesis imperfecta in two litters of Dachshunds. Vet Pathol **40**:530-539, 2003

5. Thompson K: Bones and joints. *In:* Jubb, Kennedy, and Palmer's Pathology of Domestic Animals, ed. Maxie MG, 5th ed., vol. 1, pp. 33-38. Saunders Elsevier, Philadelphia, PA, 2007

6.Weisbrode SE: Bones and joints. *In:* Pathologic Basis of Veterinary Disease, eds. McGavin MD, Zachary JF, 4th ed., pp. 1065-1066. Mosby Inc., St. Louis, MO, 2007

CASE III: 0801631 (AFIP 3122074).

Signalment: Mouse (*Mus musculus*), heterozygous F1 p53(+/-) transgenic (C3H/HeNTac female inbred x C57BL/6-Trp53tml Brd het N12 male inbred F_1), approximately 45 weeks of age, both sexes affected.

History: Several untreated and treated mice developed hindlimb paresis or paralysis and were euthanized.

Gross Pathology: In some mice there were masses involving the vertebral column (size ranged from only 1-2 mm raised above the rest of the vertebra to >1 cm masses attached to the vertebra); however, in some cases gross lesions were not noted.

Histopathologic Description: The vertebral body is expanded by numerous disorganized trabeculae of eosinophilic to amphophilic matrix (osteoid) which largely fills the marrow cavity, extends into the spinal canal, and extends slightly out from the external (periosteal) surface. Osteoblasts (tumor cells) are oriented at random with respect to the bone surfaces. They are plump and irregular-shaped, except where they are piled up into sheets extending into the spinal canal or in layers extending out from the periosteum, where they are more spindle-shaped. Cell boundaries are indistinct. The cytoplasm is faintly eosinophilic and often vacuolated. Nuclei are large and round, and occasionally there is a single prominent nucleolus. Mitotic figures are uncommon (1-2 per 0.238 mm^2 high power field). The spinal cord within the canal is misshapen; numerous vacuoles are present within the white matter (grey matter is rarely present in the sections provided). Where osteoid abuts muscle cells they occasionally are smaller than normal, with amphophilic to basophilic cytoplasm and centrally-located nuclei.

Contributor's Morphologic Diagnosis: Vertebral osteosarcoma with leukomalacia of the spinal cord and lumbar muscle degeneration and regeneration.

Contributor's Comment: Although it seems obvious that a mass need be only small in this location to cause clinical signs, osteosarcomas are not something one generally thinks of as a cause of paresis/paralysis when gross lesions are absent. The smaller tumors in these cases remind us that such a clinical observation requires very close examination of the spinal column, and in some cases tumors may best be detected by microscopic examination of the relevant (i.e. thoracic to lumbar) sections of spinal column.

This is a typical osteoblastic osteosarcoma with abundant osteoid production making diagnosis fairly simple. Osteosarcomas in mice can be osteoplastic, chondroblastic, osteoclastic, anaplastic, osteoblastic, fibroblastic, telangiectatic (vascular), or compound.(3) The National Toxicology Program records an overall incidence of osteosarcomas in 9-month-old p53 transgenic mice of 2.0% in males and 7.3% in females.(2) In the study in question, the maxilla was the most common site of osteosarcomas, with the vertebrae second most common; osteosarcomas were also recorded in the femur, tibia, and rib. In a mouse model of osteosarcoma development, wherein one or both p53 alleles were inactivated with or without inactivation of one or both Rb alleles, the most common sites of osteosarcoma development were the jaw (mandible followed by maxilla), followed by the hind leg/hip, and ribs and vertebrae.(6) Metastases were present (largely in the lungs and liver) in 9% of mice, and rarely in those animals presenting with tumors on the jaws (presumably because they had a significantly decreased lifespan due to the primary tumor). It was noted in that study also that many tumors were microscopic.(6)

The incidences of several tumors, predominantly osteosarcomas and soft tissue sarcomas, are increased in p53 transgenic mice.(1) This observation, together with the observations that a high percentage of osteogenic sarcomas have rearrangements or deletions of the p53 gene and that bone tumors occur as part of a syndrome of germline mutations of p53 (the Li-Fraumeni family syndrome), supports the hypothesis that inactivation of p53 is an important step in development of bone tumors.(1)

AFIP Diagnosis: Bone, vertebral arch: Osteosarcoma, osteoblastic and fibroblastic, with spinal cord compression atrophy.

Conference Comment: The contributor provides a succinct overview of osteosarcoma in this transgenic mouse model, and readers may wish to review the conference proceedings for WSC 2009-2010, Conference 11, case III for a general discussion of osteosarcoma. Put simply, neoplasia results from the clonal expansion of a single precursor cell that has incurred nonlethal genetic damage. Specifically, four classes of normal regulatory genes comprise the primary targets of genetic damage: 1) growth-promoting proto-oncogenes, 2) growth-inhibiting tumor suppressor genes, 3) genes that regulate apoptosis, and 4) genes involved in DNA repair.(5) This case is the second neoplasm reviewed during this conference year from a mouse model with increased susceptibility to neoplasia; WSC 2009-2010, Conference 1, case II is a mandibular ameloblastic odontogenic tumor in a Tg.AC hemizygous mouse attributed to the expression of the *ras* oncogene, exemplifying the first of the classes of targets of genetic damage in carcinogenesis listed above. By contrast, neoplasia in the present case, as described by the contributor, is attributed to germline mutations of the *p53* gene, and thus illustrates the second of these classes of targets.

As indicated by its distinction as the "guardian of the genome" and "molecular policeman," *p53* is among the most influential and best characterized tumor suppressor genes known, and is the most common target for genetic damage in human neoplasia; *p53* mutations are identified in over 50% of human tumors. The *p53* gene encodes the p53 protein, a transcription factor whose role is essentially to prevent the propagation of cells with genetic damage, a

function it accomplishes via three mechanisms: 1) temporary cell cycle arrest (i.e. quiescence), 2) permanent cell cycle arrest (i.e. senescence), and/or 3) apoptosis. Cell cycle arrest conferred by p53 is largely mediated by p53-dependent transcription of the CDK inhibitor p21, which inhibits cyclin-CDK complexes and phosphorylation (i.e. inactivation) of the retinoblastoma (RB) protein; the inhibition prevents cell cycle progression from the late G_1 phase to the S phase (i.e. the "point of no return") at the G_1/S checkpoint, providing an opportunity for repair of DNA damage. Apoptosis, on the other hand, is induced by p53 in the face of irreversible DNA damage, and results from p53-directed transcription of such pro-apoptotic genes as *BAX* and *PUMA*.(5)

The contributor alluded to the germline *p53* mutation in this transgenic mouse as analogous to the Li-Fraumeni syndrome of humans, discussion of which is best preceded by a cursory review of the "two-hit" hypothesis of oncogenesis. This hypothesis applies to a number of genes, but best illustrated by the *RB* tumor suppressor gene. Basically, the "two-hit" hypothesis states that oncogenesis depends on the presence of two mutations (i.e. "hits") involving both alleles of a given tumor suppressor gene. As applied to the *RB* gene, retinoblastoma occurs only in the presence of two mutations – one in each allele. Children with familial retinoblastoma inherit one defective copy of the *RB* gene as the result of a germline mutation (i.e. the first "hit"), and the second (i.e. somatic) mutation occurs sporadically. In sporadic cases, a single retinoblast must suffer two separate somatic mutations in the *RB* gene – one in each allele – to result in retinoblastoma. Therefore, children who inherit a germline *RB* mutation are at increased risk for developing retinoblastoma, because only one additional "hit" is required to confer complete loss of RB function. A germline mutation in *p53* is the basis of the Li-Fraumeni syndrome, which is directly analogous to germline *RB* mutations in familial retinoblastoma. Affected humans with the Li-Fraumeni syndrome have a 25-fold higher chance of developing malignant neoplasia by age 50 than the general population.(5)

Wide histomorphological variation within a given tumor and between individual cases is typical of osteosarcoma, which may complicate tumor classification.(4) Accordingly, the neoplasm in this case exhibits substantial intratumoral histomorphologic variation, ranging from an expansile highly productive zone at the periphery, to an invasive lytic internal zone. Based upon this finding, the conference moderator encouraged participants to consider and discuss alternative diagnoses, including osteosarcoma arising in osteoma, or even a collision tumor. Close examination of the interface between the neoplasm and surrounding muscle is helpful in excluding these possibilities; in osteoma and reactive bone, as alluded to in case I above, a gradual transition between a dense fibrous layer and a well-differentiated layer of osteoblasts lining trabeculae is expected, whereas no such transition is present in this case and atypical ovoid neoplastic cells are present throughout the neoplasm.

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References:

1. Hung J, Anderson R: p53: functions, mutations and sarcomas. Acta Orthop Scand Suppl **273:**68-73, 1997
2. Kissling GE: Historical control rates for NTP Tg.AC p53 mice. National Toxicology Program (available at http://ntp.niehs.nih.gov/?objectid=522CD99F-F1F6-975E-74484178722ECB7C), 2008
3. Long PH, Leininger JR: Bones, joints, and synovia. *In:* Pathology of the Mouse, ed. Maronpot RR, pp. 665-671. Cache River Press, Vienna, IL, 1999
4. Slayter MV, Boosinger TR, Inskeep W, Pool RR, Dämmrich K, Larsen S: Histological Classification of Bone and Joint Tumors of Domestic Animals, 2nd series, vol. I, ed. Schulman FY, pp. 9-11. Armed Forces Institute of Pathology (in cooperation with the CL Davis DVM Foundation and The World Health Organization Collaborating Center for Comparative Oncology), Washington, DC, 1994
5. Stricker TP, Kumar V: Neoplasia. *In:* Robbins and Cotran Pathologic Basis of Disease, eds. Kumar V, Abbas AK, Fausto N, Aster JC, 8th ed., pp. 273-292. Saunders Elsevier, Philadelphia, PA, 2010
6. Walkley CR, Qudsi R, Sankaran VG, Perry JA, Gostissa M, Roth SI, Rodda SJ, Snay E, Dunning P, Fahey FH, Alt FW, McMahon AP, Orkin SH: Conditional mouse osteosarcoma, dependent on p53 loss and potentiated by loss of Rb, mimics the human disease. Genes Dev **22:**1662-1676, 2008

CASE IV: AFIP 08001 (AFIP 3148218).

Signalment: Rats (*Rattus norvegicus*) (Crl:CD®[SD]), 6 weeks old (± 1 week), males, 218-236 grams.

History: Rats received an aerosolized, inhaled, dry powder formulation of corticosteroid for 45 minutes, once daily for 14 days. Scheduled sacrifice was performed at the termination of the study. This study was conducted in an

Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility in accordance with the National Research Council (NRC) Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act.

Gross Pathology: None.

Histopathologic Description: Bone (stifle joint; tibia): In comparison to untreated control animals, there is a diffuse, mild to moderate thinning of the tibial physeal growth plate cartilage with retention of normal architecture, as well as elongation of the zone of the tibial primary spongiosa. Subjectively, the growth plate-to-spongiosa ratio varies from 1:2 to 1:6 (compared to approximately 1:1 in control animals). Within the region of the primary spongiosa there is minimal to mild thinning of trabecular bone, with widening of the intervening spaces and multifocal dilation of the intervening vasculature. There is a mild to moderate, diffuse hypocellularity of bone marrow hematopoietic elements with a corresponding increase in marrow lipocytes.

Contributor's Morphologic Diagnosis: Proximal tibia: Osteochondrodysplasia, mild to moderate, with minimal to mild trabecular osteopenia and moderate elongation of the primary spongiosa.

Contributor's Comment: These findings are attributed to systemic corticosteroid exposure following repeated inhalation at high doses. Other changes in these rats included mild decreases in lymphocyte counts, minimal increases in neutrophil counts, as well as slight hypertriglyceridemia and elevated gamma glutamyl transferase (GGT). In addition, other findings seen histologically included thymic lymphocyte depletion (increased apoptosis with tingible body macrophages), adrenocortical atrophy and depletion of peripheral lymphoid tissue (splenic white pulp and lymph nodes). These changes are all commonly recognized effects of exogenous systemic corticosteroid exposure.

Normal longitudinal bone growth occurs as a result of endochondral ossification, a highly coordinated process whereby the production of a cartilaginous scaffold at the growth plate is replaced by bone. Many factors have been identified as playing a role in this process; hormones such as estrogens, thyroid and growth hormone, leptin, sex hormones and glucocorticoids, as well as growth factors such as insulin-like growth factor, Indian hedgehog, PTH-rp, fibroblast growth factor, bone morphogenetic proteins, vascular endothelial growth factor (VEGF) and vitamin D have all been implicated.(2,6)

Glucocorticoids exert their effect via the ubiquitous glucocorticoid receptor or the less widely distributed mineralocorticoid receptor. The glucocorticoid receptor is a cytoplasmic receptor which, upon ligand binding, forms a multi-component complex, translocates to the nucleus and functions as a transcription factor, binding to promoter regions of glucocorticoid responsive genes. This activity may promote or repress transcription depending on the gene involved. As well, the glucocorticoid-bound receptor complex may exert an indirect effect, by interfering with the normal function of other transcription factors (nuclear factor-κB or smad-3 for example). The result is a myriad of effects, both desired and unwanted, across multiple systems.(4)

Glucocorticoids are generally considered inhibitory to endochondral ossification and have been associated with a number of adverse skeletal affects, including delayed or reduced growth and osteopenia. Glucocorticoids exert a direct effect on growth plate chondrocytes, which express the glucocorticoid receptor, by decreasing the rate of chondrocyte proliferation as well as increasing the rate of chondrocyte apoptosis at the zone of hypertrophy.(2) Glucocorticoids also interact with other mediators of bone growth. Insulin-like growth factor-1 (IGF-1) is considered a key mediator of bone growth, and its expression at the growth plate is determined by the relative influence of locally acting glucocorticoids, thyroid and growth hormones. In this regard, glucocorticoids have been shown to modulate both IGF-1 production and growth hormone receptor expression at the growth plate.(2) Other cell populations responsible for growth and remodeling are also impacted by glucorticoid exposure; osteoblast activity is typically inhibited, with decreased matrix/osteoid production whilst osteclastic activity is often increased, resulting in an uncoupling of the tightly controlled interplay between these two populations.(4)

Angiogenesis is an important part of the endochondral ossification process. In piglets treated with short term prednisolone, there was a decrease in VEGF mRNA expression by hypertrophic chondrocytes, with diminished capillary ingrowth and loss of parallel organization in the metaphysis.(5) Interestingly, trabecular bone length in the primary spongiosa was also diminished, in contrast to the case presented here. Elongation of the primary spongiosa, as was seen in our case, appears not to be specifically referenced within the literature; it seems reasonable to hypothesize that the net outcome of glucorticoid exposure seen here results from the balance of relative dose or

exposure both systemically and at the growth plate, acting in concert at multiple levels in young, actively growing rats under the conditions of this study.

This case also demonstrates marrow hematopoietic hypocellularity with a corresponding increase in marrow lipocytes. This is also a recognized effect of glucocorticoid therapy and has been implicated in the pathogenesis of steroid-induced osteonecrosis. Rather than unmasking existent adipocytes due to loss of hematopoietic elements, a shift in differentiation of pluripotential marrow stem cells has been proposed to occur under the influence of glucocorticoids.(1)

AFIP Diagnosis: Bone, proximal tibia: Metaphyseal osteosclerosis, focally extensive, marked, with retention of unmodeled primary trabeculae.

Conference Comment: The slide submitted for this case prompted a brief discussion of the benefits and drawbacks of the sagittal and frontal planes of section. The preferred plane of section depends on which structures are of greatest interest in a given study, and must be carefully considered. Studies in which the cruciate ligaments, articular cartilage, and joint space are of primary interest are generally best supported by frontal sectioning of the stifle, whereas assessment of the growth plate of the distal femur, of particular interest in this case, is best accomplished by sagittal sectioning.

As in the section of proximal humerus discussed in case II of this conference, metaphyseal osteosclerosis in this case is the result of retention of unmodeled primary trabeculae. The contributor's other assessments, including thinning of the physeal cartilage, increased growth plate-to-spongiosa ratio, and bone marrow hypocellularity are substantiated by the microscopic images supplied from unaffected control animals; without the benefit of the control images for reference, conference participants were unable to confidently make the same interpretations. Regardless, the conference moderator emphasized that the presence of retained unmodeled primary trabeculae is far more reliable evidence of deficient osteoclasis than is the subjective determination that osteoclast numbers are reduced, particularly when a section from an age-matched control is not available for evaluation. As in case II, this finding prompts the consideration of other anti-osteoclast agents, including lead and bisphosphonates. Participants noted the apparent lack of similar lesions in the epiphysis, and speculated that this unexpected finding may have resulted from a different growth rate or plane of section.

The finding of osteosclerosis in this case is counterintuitive; due to their well-known catabolic properties, glucocorticoids would be expected to result in increased osteoclast lifespan and osteopenia. The contributor offers several plausible explanations for this irony. Conference participants discussed the important roles that dosage route and frequency may have on the net effect of a given drug, using as a corollary the paradox of parathyroid hormone. As alluded to in the discussion of fibrous osteodystrophy in case I of this conference, persistent hyperparathyroidism is known to increase bone resorption. Less obviously, parathyroid hormone (PTH) can also stimulate bone formation and increased bone mass, depending on the route and frequency of administration. Specifically, while continuous infusions of PTH cause bone resorption, intermittent injections of the same hormone cause increased bone formation and bone mineral density. This is the therapeutic basis for teriparatide (Forteo™), a recombinant drug composed of the first 34 amino acids of PTH, which exerts its main biologic effects and is clinically shown to decrease the risk of fractures and increase bone mineral density in postmenopausal women with osteoporosis when administered as a daily subcutaneous injection.(2)

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References:

1. Cui, Q, Wang GJ, Balain G: Steroid-induced adipogenesis in a pluripotential cell line from bone marrow. J Bone Joint Surg Am **79:**1054-1063, 1997
2. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH: Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med **344:**1434-1441, 2001
3. Nilsson O, Marino R, De Luca F, Phillip M, Baron J: Endocrine regulation of the growth plate. Horm Res **64:**157-165, 2005
4. Schäcke H, Döcke WD, Asadullah K: Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther **96:**23-43, 2002

5. Smink JJ, Bucholz IM, Hamers N, van Tilburg CM, Christis C, Sakkers RJ, de Meer K, van Bull-Offers SC, Koedam JA: Short-term glucocorticoid treatment of piglets causes changes in growth plate morphology and angiogenesis. Osteoarthritis Cartilage **12:**864-871, 2003
6. van der Eerden BC, Karperien M, Wit JM: Systemic and local regulation of the growth plate. Endocr Rev **24:**782-801, 2003