Multicentric leiomyosarcoma in a young male horse

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Leiomyosarcoma is a rare malignant tumor of smooth muscle origin.
Tumors usually develop in the reproductive and alimentary tracts, which contain abundant smooth muscle.
Immunohistochemical staining of biopsy specimens is useful in diagnosis of leiomyosarcoma.

A 2-year-old Thoroughbred colt was referred to the George D. Widener Hospital, New Bolton Center, University of Pennsylvania, for an examination of the upper portion of the respiratory tract. The colt had a history of multiple firm, raised nodules of the mandible and maxilla since it was 6 months old. As a yearling, the colt had difficulty chewing hay normally but was able to maintain weight by eating a complete commercial feed as a soft mash.

The colt was previously evaluated as a yearling at a private referral hospital to determine the cause of the facial nodules and inability to properly masticate. At that time, the horse had multiple firm, nonpainful raised nodules distributed randomly throughout the entire skull. The swellings ranged in size from 5 mm to 2 cm in diameter. The colt was bright and alert; rectal temperature and heart and respiratory rates were within reference limits. Thoracic auscultation revealed no abnormal lung sounds in either hemithorax. Radiographs of the skull were obtained, and the masses appeared to be mineralized circular lesions. The occlusive surfaces of the premolar and molar teeth were not level. Results of a CBC and serum biochemical profile and fibrinogen concentration were within reference ranges. The serum total calcium concentration was 10.7% (reference range, 0 to 5.53%), and the calcium fractional excretion was low (1.39%; reference limit, 1.49 ± 1.58%).

As a yearling, an endoscopic examination of the nasal passages and larynx was performed, which revealed narrowing of the nasopharynx presumably secondary to the increased bone proliferation throughout the skull. The guttural pouches were normal, and the trachea was not evaluated.

Dynamic pharyngeal collapse was also observed on deep inspiration. Biopsy specimens of the right facial bone and right ramus of the mandible were obtained and submitted for histologic examination; a diagnosis of fibrosarcoma with bony proliferation was made on the basis of those findings. The colt was returned to the farm, fed the slurry diet, and began race training as planned by the owner. During exercise, the colt made a loud upper airway noise and would appear agitated with a markedly high respiratory rate and notable effort, compared with that observed at rest. Because of these exercise-related abnormalities, the colt (2 years old) was referred to New Bolton Center for additional diagnostic testing that included endoscopy during exercise on a high-speed treadmill.

On physical examination, the colt was bright and alert and in excellent body condition. The rectal temperature was 38°C (100.5°F), heart rate was 36 beats/min, and respiratory rate was 16 breaths/min. There were multiple swellings along the facial bones and both rami of the mandible; these swellings were firm and elicited no signs of pain on palpation. The colt had mild exophthalmia and epiphora bilaterally (more prominent in the left eye than in the right), which were considered secondary to the facial deformity caused by the nodules. Results of fundic examinations of both eyes were unremarkable. Airflow was detected through both nares, and a low-pitched upper respiratory noise was audible at rest. Thoracic auscultation revealed normal bronchovesicular sounds bilaterally with the referred upper airway noise. There was a bony protuberance present along the right fourth metatarsal bone (split bone); no signs of pain were elicited on palpation of the protuberance, which resembled a quiescent split bone injury. No other abnormalities were detected on physical examination.

At New Bolton Center, the colt had an excellent appetite, although it appeared to chew hay for a prolonged period, often quidding, and had difficulty moving the hay bolus into position to swallow. On the farm, the colt had been fed hay cubes and a complete pelleted feed; because the owner had noted that the colt could chew and swallow these preparations more easily than other feedstuffs.

Radiographs of the skull were obtained and revealed multifocal, primarily circular, mineral opacities involving all bones of the skull and mandible. Extensive bony proliferation was present in the premaxilla and ventral aspect of the mandible (Fig 1). The tooth roots were not clearly apparent, and there was destruction of both cortical and medullary bone (most remarkably in the rostral mandible). Multiple retained caps of the upper and lower premolars were present, and the right upper fourth premolar was cranially and...
axially malpositioned. Additional radiographs were obtained of the right and left metatarsi, left radius, right tibia, cervical portion of the vertebral column, and thorax. There were multiple mineral opacities (≤ 3 mm in diameter) within the medullary portion of the long bones and also within the vertebral bodies from C2 through C5. Thoracic radiography revealed a diffusely distributed mild interstitial pattern throughout the lung fields, which was considered clinically unimportant. On the basis of these radiographic abnormalities, differential diagnoses included fungal lesions, primary bone tumors (eg, fibrosarcoma, osteosarcoma, chondrosarcoma, or hemangiosarcoma), and metastatic bone disease.

Results of a CBC and serum biochemical analyses were unremarkable. Serum total calcium concentration was 11.99 mg/mL (reference range, 10.7 to 13.4 mg/mL). Serum phosphorus concentration ranged from 4.31 to 5.55 mg/mL (reference range, 1.9 to 5.4 mg/mL). Free-catch samples of voided urine were obtained during a 24-hour period to determine the fractional clearance values for calcium and phosphorus. The phosphorus fractional clearance was within reference range (0.2 to 0.4%; reference range, 0 to 0.5%). However, to properly determine the calcium urinary clearance, the entire contents of the bladder must be collected to obtain both the calcium carbonate crystal-poor and crystal-rich fractions; it was not possible, therefore, to calculate the calcium fractional clearance, because urine was not obtained in this manner. Serum calcitonin concentration was normal (16 pg/mL; reference range, 1 to 40 pg/mL). Serum ionized calcium concentration was 1.86 mmol/L (reference range, 1.58 to 1.90 mmol/L). Serum concentration of parathyroid hormone was 3.1 pmol/L (reference range, 0.25 to 2.0 pmol/L). The combination of these findings (moderately high serum concentration of parathyroid hormone and normal serum ionized calcium concentration) was considered inconclusive for a diagnosis of secondary nutritional hyperparathyroidism.

Endoscopic evaluation of nasal passages and larynx was performed to assess the effect of the bony abnormalities on the anatomy and function of the upper portion of the respiratory tract. The right nasal passage was narrowed to such an extent that the endoscope could not be passed through the right ventral meatus. There appeared to be thickening of the dorsal and ventral turbinates near the nasal septum. The left nasal passage was also narrow, although not as notably as the right nasal passage. The pharynx was narrow, but its resting function was normal. Because of the severe narrowing of the nasal passages detected during this endoscopic examination, a further evaluation of respiratory function during exercise on the high-speed treadmill was deemed unnecessary.

Biopsy specimens were obtained from the left mandible and right frontal bone during a standing procedure. The horse was sedated with xylazine (0.4 mg/kg [0.18 mg/lb], IV), and 3 mL of mepivacaine was injected SC over a swelling in the left mandible and right frontal bone. A 1.5-cm skin incision was made, and several samples of bone were obtained from each site with a Jamshidi biopsy needle. Skin edges of the incision were apposed with 2-0 nylon suture in a cruciate pattern, and the horse received a single dose of phenylbutazone (4.0 mg/kg [1.8 mg/lb], IV).

Histologically, the biopsy specimens consisted of hypercellular masses composed of interlacing bundles of spindle-shaped cells, interspersed with variable-sized foci of woven bone that frequently blended smoothly into the interlacing bundles. Aggregations of hemosiderin-laden macrophages and a few scattered neutrophils were occasionally seen. The spindle-shaped cells were characterized by scant wispy eosinophilic cytoplasm and an oval to elongated, vesicular to finely stippled nucleus that generally did not contain a nucleolus. Cell borders were indistinct and mitotic figures were rare (Fig 2). A section of woven bone and a small fragment of lamellar bone were also present in each of 2 sections of biopsy tissue examined histologically.

Sections of biopsy tissue were stained immunohistochemically by applying the appropriate primary anti-
body followed by 1 of 2 detection systems: a dextran polymer and conjugated secondary antibody with horseradish peroxidase or a labeled streptavidin-biotin system with horseradish peroxidase. The immunohistochemical stains used included anti-S100, antivimentin, clone IA4 anti-α smooth muscle actin, antidesmin, and anti-glial fibrillary acidic protein (GFAP) antibodies. In humans, uptake of anti-S100 antibody is associated with the presence of S100 protein in the cells of the nervous system and cells of melanocytic origin, regardless of their location in the body. Positive staining is identified by nucleocytoplasmic labeling of the cells containing S100 protein. In addition to identifying nervous system-related tissues, this antibody reacts with chondrocytes and adipocytes and may be used as an indicator of gastrointestinal tract stromal tumors, such as leiomyomas and neurolemmonomas in humans.

Neoplastic cells in the biopsy specimens from the horse of this report reacted positively with anti-S100 stain, as they did with anti-vimentin stain. Anti-vimentin immunopositivity may be used in humans to identify tissues of mesenchymal origin, such as lymphoid cells, endothelial cells, fibroblasts, and smooth muscle cells. Anti-vimentin staining is also used as an indicator that improper or prolonged tissue fixation has not resulted in inaccurate immunohistochemical staining properties of the tissue. The vimentin epitope is extremely sensitive to over-fixation with a variety of tissue fixatives; failure to identify the presence of mesenchymal cells, such as fibroblasts in the stroma of a specimen (regardless of the histogenesis of the tumor), indicates the tissue is not appropriate for immunohistochemical staining.

Cellular actin proteins may be identified by antibodies, such as anti-α smooth muscle actin. There are 6 different isoforms of these contractile proteins; γ and α isoforms are commonly found in tissues containing a homogenous population of myocytes and less frequently in nonhomogenous tissues, in which myocytes and myofibroblasts or myoepithelial cells may contain the γ and α isoforms. Clone IA4 anti-α smooth muscle actin reacts with myocytes, myofibroblasts, and myoepithelial cells. Anti-α smooth muscle actin immunopositive staining is identified as a diffuse, dark-brown coloration of the cytoplasm of the neoplastic cells; such immunopositivity was observed in the neoplastic cells of the biopsy specimens from the horse of this report. Positive staining with anti-α smooth muscle actin antibody suggests that the neoplastic cells are smooth muscle in origin. In the biopsy specimens from the horse of this report, neoplastic cells stained faintly with clone D33 anti-desmin stain. Desmin, a cytoplasmic intermediate filament protein, is frequently used to identify cells of myogenic origin. In humans, both striated and smooth muscle cells will have positive immunoreactivity. The clone D33 anti-desmin antibody does not cross-react with other intermediate filament proteins and thus has tissue specificity; it does not, however, have species specificity. In our laboratory, the D33 clone has demonstrated good immunoreactivity with equine muscle cells (data not shown); therefore, we cannot definitively determine why the neoplastic cells reacted only faintly with the anti-desmin antibody. Perhaps the number of desmin epitopes present in some of the neoplastic cells was below the level of immunohistochemical detection afforded by this particular clone and detection system. However, it was interesting to note that the vascular smooth muscle in the biopsy specimens reacted more intensely than the neoplastic cells with both the anti-desmin and anti-α smooth muscle actin antibodies.

The epitope for GFAP is present in human astrocytes, ependymal cells, and some Schwann cells, Kupffer cells, and chondrocytes. Myogenic cells do not react with anti-GFAP antibody, and the neoplastic cells in the biopsy specimens from the horse of this report were immunonegative to this immunohistochemical stain.

On the basis of the histopathologic features and immunohistochemical staining properties of the biopsy specimens from the Thoroughbred, the diagnosis of leiomyosarcoma with extensive metaplastic bone formation was made. Other plausible causes of the lesions and associated clinical signs in the colt included fibrosarcoma (as diagnosed initially from histologic findings) and nutritional secondary hyperparathyroidism, causing osteodystrophia fibrosa. Osteodystrophia fibrosa induced by nutritional secondary hyperparathyroidism was considered on the basis of signalment and historical evidence of abnormal phosphorous fractional excretion. Approximately 1 year prior to evaluation at New Bolton Center, urinalysis conducted at another hospital revealed that the fractional excretion of phosphorus was markedly high and the calcium fractional excretion was slightly low, but plasma concentrations of calcium and phosphorus were within reference ranges. Those laboratory values supported a diagnosis of nutritional secondary hyperparathyroidism. However, results of tests conducted when the horse was evaluated at New Bolton Center indicated that phosphorus fractional excretion in urine and plasma calcium and phosphorus concentrations were all within reference ranges. Although the parathyroid hormone concentration was mildly high (3.1 pmol/L), this abnormality alone is inconclusive for a diagnosis of nutritional secondary hyperparathyroidism. Additionally, the lesions observed radiographically were inconsistent with a diagnosis of osteodystrophia fibrosa.

Examination of the biopsy specimens obtained initially (ie, approx 1 year prior to the colt’s evaluation at New Bolton Center) indicated fibrosarcoma. Fibrosarcoma is a malignant tumor that arises from fibrous connective tissue and can be found within peripheral or central bone. Fibrosarcomas are considered rare in horses and may arise from the periosteum, fascia, or even soft tissue structures; these tumors cause bone destruction without bone production. Fibrosarcomas are considered to be invasive tumors that do not commonly metastasize but frequently recur if surgical resection is incomplete. Histologically, fibrosarcomas may be difficult to differentiate from leiomyosarcomas. It could be speculated that the primary clinical problem in the colt of this report was nutritional secondary hyperparathyroidism with subsequent metaplasia and malignant transformation, although this has not been reported.

Leiomyosarcoma is a rare malignant tumor origi-
ating from smooth muscle. These tumors usually develop in areas that contain abundant smooth muscle, such as the reproductive and alimentary tracts. They have also been detected in the retroperitoneal space, kidneys, skeletal muscle, and the head and neck region. Characterization of the clinical progression of leiomyosarcomas is difficult because of the low frequency with which these tumors have been identified; however, local destruction with areas of tumor necrosis and metastasis has been reported. In dogs, the duodenum, liver, and spleen have been reported to have a high incidence of metastasis, compared with other tumor locations. In the few reports of leiomyosarcoma in horses, the reproductive and gastrointestinal tracts were affected. Two reports involved middle-aged horses (age range, 12 to 14 years); of these, 1 horse had leiomyosarcoma of the esophagus, stomach, and liver, and the other had a pedunculated mass associated with the jejunum. Another report is of a pedunculated mass that protruded into the rectum of a 4-year-old Quarter Horse mare. Additionally, there is a report of testicular leiomyosarcoma in an 11-month-old Thoroughbred; in that colt, metastatic masses within the masseter muscle, caudal vena cava, cervical and popliteal lymph nodes, and diaphragm were detected. The neoplastic cells from those testicular (primary) and metastatic masses were evaluated by immunohistochemistry and found to be immunopositive for anti-smooth muscle actin.

Leiomyosarcoma in the oral cavity has been rarely reported in humans, and primary tumors in the maxilla or mandible are considered exceptionally rare. Involvement of the maxilla has been reported more frequently than involvement of the mandible; persons affected range in age from 15 to 85 years, and there appears to be male predilection. However, there is 1 report of a leiomyosarcoma in the mandible of a 7-year-old girl. The clinical manifestation of leiomyosarcoma of the maxilla is often nonspecific, involves a mass that may or may not be painful, has a smooth surface with irregular margins, closely adheres to the underlying mucosa and bone, and may be associated with loosening of the teeth. Physical examination and serum biochemical evaluations are reported to be unremarkable in most affected humans. Several years prior to the diagnosis of leiomyosarcoma, a small number of these patients had received radiation treatment for retinoblastoma. Diagnosis was made on the basis of findings of microscopic and immunohistochemical examination of a biopsy specimen of the mass; immunopositivity to anti-smooth muscle actin or anti-desmin antibodies was considered diagnostic for leiomyosarcoma. Because of the aggressive nature of these tumors, recurrence and metastasis to local lymph nodes and lungs is common. Surgical excision of the tumor is considered the most appropriate primary treatment, although radiation therapy is also used infrequently. The origin of leiomyosarcomas located in the head and neck region of humans is unknown. It has been speculated these tumors arise from vascular smooth muscle or undifferentiated mesenchymal cells.

To the authors' knowledge, leiomyosarcoma that affects portions of the musculoskeletal system has not been reported in horses. In the horse of this report, the origin of the tumor was unknown, but smooth muscle of the vasculature was suspected because of the involvement of multiple sites and lack of other anatomically appropriate sources of smooth muscle at the affected sites. Tumors arising from vascular smooth muscle are considered rare despite the large quantity of smooth muscle tissue found in the vasculature. At present, there are no species-specific antibodies available that would enable confirmation of the suspicion that vascular smooth muscle was the origin of the leiomyosarcoma in the colt of this report. At 6 years of age (approx 3 years since the last examination), the colt was being fed a complete feed slurry and free-choice grass. Difficulty chewing hay continued, but weight was maintained. The horse was unable to pursue an athletic career because of severe narrowing of the nasal passages secondary to the bony lesions. At this time, there had been no externally visible progression of the neoplastic process. However, the long-term prognosis for this colt was considered guarded because of the extensive and diffuse distribution of the masses throughout multiple bones.

References