Hypercalcemia and high serum parathyroid hormone-related protein concentration in a horse with multiple myeloma

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- In horses, absolute hypercalcemia is a rare condition that has been associated with renal disease, vitamin D toxicosis, hyperparathyroidism, and malignancy.
- Quantification of serum parathyroid hormone, ionized calcium, and parathyroid hormone-related protein (PTHrP) concentrations is useful in differentiating the causes of hypercalcemia.
- Concurrent demonstration of high serum PTHrP concentration and hyperglobulinemia in a horse with hypercalcemia should lead to a suspicion of multiple myeloma.
- If serum protein electrophoresis cannot definitively identify a paraprotein, quantification of specific immunoglobulin classes by radial immunodiffusion may be useful in confirming a diagnosis of multiple myeloma.

A 13-year-old Morgan gelding was examined because of mild weight loss and delayed shedding of the winter hair coat. The referring veterinarian had not found any physical abnormalities and submitted samples for a CBC and serum biochemical profile. Abnormalities included hyperproteinemia (12.6 g/dL; reference range, 5.4 to 7.8 g/dL), hyperglobulinemia (9.9 g/dL; reference range, 2.2 to 4.4 g/dL), and hypercalcemia (total calcium, 16.8 mg/dL; reference range, 10.0 to 13.5 mg/dL). The horse was referred to the Veterinary Teaching Hospital at the University of Georgia for further evaluation.

At the time of initial examination at the University of Georgia 2 months later, the owner reported that there had been no further changes in the horse's weight and that its appetite was excellent and its attitude, energy level, and performance were normal. On physical examination, the horse appeared bright and alert and was in good to excellent body condition (body weight, 426 kg [937 lb]). Rectal temperature, heart rate, and respiratory rate were within reference limits. Auscultation with a rebreathing bag did not elicit distress or a cough, and normal bronchovesicular sounds were heard. Transrectal palpation did not reveal any clinically important abnormalities.

A CBC and serum biochemical profile were performed. Abnormalities included hyperproteinemia (10.9 g/dL; reference range, 5.6 to 7.7 g/dL), hyperglobulinemia (8.5 g/dL; reference range, 2.1 to 5.4 g/dL), and hypercalcemia (total calcium, 16.2 mg/dL [reference range, 10.8 to 12.8 mg/dL]; ionized calcium, 2.56 mmol/L [reference range, 1.58 to 1.90 mmol/L]). Serum albumin concentration (2.4 g/dL; reference range, 2.3 to 3.5 g/dL) was normal. A urinary catheter was inserted, and a urine sample was obtained for urinalysis and determination of fractional excretion of electrolytes. Results of the urinalysis were unremarkable, but fractional urinary excretions of sodium (1.3%), potassium (84.5%), chloride (3.4%), and phosphorus (10.9%) were greater than previously reported reference values.[1]

Abdominocentesis was performed. Results of analysis of abdominal fluid were normal, except that protein concentration (3.1 g/dL; reference range, < 2 g/dL) was slightly high. Results of abdominal ultrasonography[a] were normal other than a slight increase in the echogenicity of the renal cortices, compared with echogenicity of the spleen and liver. Occasional minute hyperechoic foci that did not result in acoustic shadowing were seen throughout the renal parenchyma bilaterally.

Samples were submitted for serum protein electrophoresis and quantification of serum **parathyroid hormone (PTH)** and **parathyroid hormone-related protein (PTHrP)** concentrations; PTH and PTHrP concentrations were determined by a commercial laboratory[b] by means of radioimmunoassay.[2] Serum protein electrophoresis revealed polyclonal gammopathy (**Figure 1**). The PTH concentration was within reference limits (1.3 pmol/L; reference range, 0.25 to 2.0 pmol/L); the PTHrP concentration was less than the limit of detection.

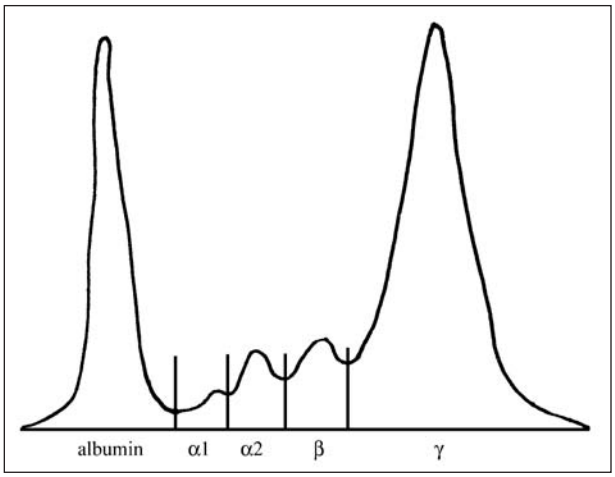

Figure 1—Densitometric tracing of results of serum protein electrophoresis performed on a 13-year-old Morgan gelding with a history of mild weight loss, hyperproteinemia, and hypercalcemia. Concentrations of albumin, α_1-globulin, α_2-globulin, and β-globulin were within reference limits, but γ-globulin concentration was high (6.3 g/dL; reference range, 0.6 to 1.9 g/dL). The symmetrical increase in the γ-globulin region was consistent with polyclonal gammopathy.

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At this time, the major problems that had been identified were a history of mild weight loss, polyclonal gammopathy, and hypercalcemia. The horse was discharged from the hospital because the owner elected to not pursue further diagnostic testing at that time.

Seven months later, the owner reported that the horse was again losing weight. The referring veterinarian submitted serum for quantification of ionized calcium, PTH, and PTHrP concentrations. Persistent ionized hypercalcemia (1.97 mmol/L) with a normal PTH concentration (1.6 pmol/L) was again confirmed. However, at this time, the PTHrP concentration was high (2.8 pmol/L; reference range, < 1 pmol/L). The normal serum PTH concentration in conjunction with the high serum PTHrP concentration provided presumptive evidence for a diagnosis of hypercalcemia secondary to malignancy.

Approximately 10 months after the owner's initial report of weight loss, the horse became more somnolent; weight loss progressed even though the horse had a good appetite. The owner reported that the horse had developed signs of mild colic and returned the horse to the University of Georgia Veterinary Teaching Hospital. Body weight was 399 kg (878 lb), representing a loss of 27 kg (59 lb) from 8 months previously. Voided urine was submitted for urinalysis, and blood was submitted for a CBC, serum biochemical profile, electrophoresis, radial immunodiffusion for quantification of immunoglobulin concentrations,[c] and bioassay for determination of serum tumor necrosis factor activity.[3] Results of the urinalysis were normal. Hematologic abnormalities included mature neutrophilia (15.5×10^6 neutrophils/µL; reference range, 2.9 to 8.5×10^6 neutrophils/µL). The only serum biochemical abnormalities were hyperglobulinemia (11.1 g/dL) and hypercalcemia (total calcium, 17.7 mg/dL). Tumor necrosis factor activity was not detected in serum. Serum protein electrophoresis revealed a sharp-edged peak in the β-γ-globulin region, suggesting either monoclonal or biclonal production of immunoglobulin (**Figure 2**). Radial immunodiffusion revealed a marked increase in serum IgA concentration (60,800 mg/dL; reference range, 153 ± 86 mg/dL). Serum IgG concentration was normal (1,500 mg/dL; reference range, 1,334 ± 350 mg/dL), and serum IgG (T) concentration (270 mg/dL; reference range, 821 ± 301 mg/dL) and serum IgM concentration (80 mg/dL; reference range, 120 ± 31 mg/dL) were low. Results of serum electrophoresis and radial immunodiffusion were consistent with a diagnosis of multiple myeloma with production of IgA paraprotein. Forty-eight hours after readmission, the horse was euthanatized at the owner's request.

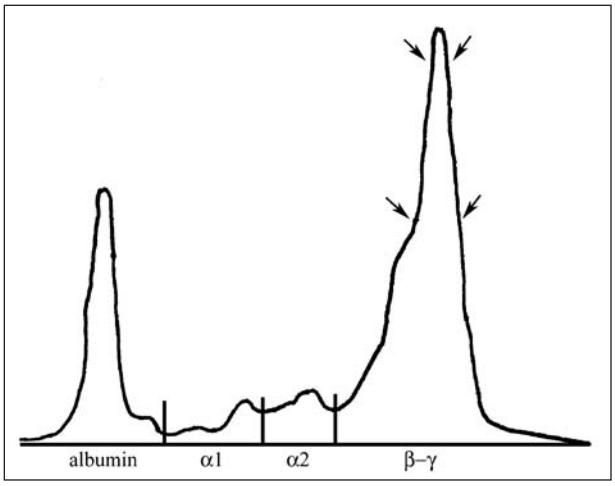

Figure 2—Densitometric tracing of results of serum protein electrophoresis performed 8 months later on the horse in Figure 1. The sharp-edged peak (arrows) in the β-γ-globulin region was consistent with monoclonal or biclonal production of immunoglobulin.

At necropsy, the carcass was in good condition with little depot fat. The walls of the distal fourth of the esophagus, the ileum, and the terminal portion of the rectum were severely thickened circumferentially. The surfaces of both kidneys had numerous dark-red, raised nodules ranging from 0.5 to 1.5 cm in diameter and extending into the cortex. Similar nodules were seen deep within the cortices. The marrow of multiple sternebrae contained white, poorly circumscribed nodules ranging from 0.5 to 1.5 cm in diameter.

Histologic examination of the kidney revealed that the infiltrative nodules were composed of diffuse sheets of neoplastic plasmacytoid cells (**Figure 3**). The neoplastic cells were round with mild anisocytosis and contained round, often eccentric, hyperchromatic nuclei. A rim of well-defined dark-blue cytoplasm, often with a prominent Golgi complex, surrounded the nucleus in these cells. Anisokaryosis was moderate, and occasional binucleate cells were observed. Mitotic figures were uncommon (< 1 per 40× field). Similar neoplastic plasmacytoid cells infiltrated bone marrow, many lymph nodes, and liver. In bone marrow, islands of neoplastic cells were found surrounded by normal hematopoietic cells. Neoplastic cells effaced the normal architecture of the lymph nodes and were found within the capsule, but did not infiltrate perinodal fat. Nonencapsulated nodules of neoplastic cells were present in the liver. There was severe hypertrophy of the smooth muscle of the esophagus, pylorus, ileum, and rectum.

Morphologic features of the neoplastic cells were suggestive of a plasma cell origin; immunohistochem-

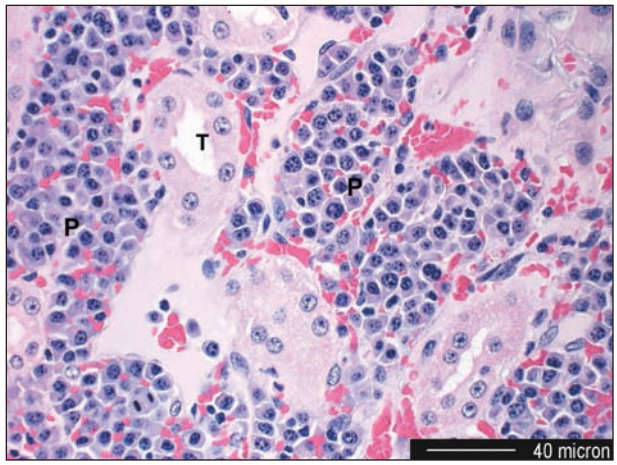

Figure 3—Photomicrograph of a section of kidney from a horse with multiple myeloma. Sheets of neoplastic cells that have features consistent with plasma cells (P) surround remnant renal tubules (T). H&E stain; bar = 40 µm.

istry was used to establish a final diagnosis. Tissues were incubated with a variety of primary antibodies, including sheep anti-horse IgA, IgM, and IgG[d] (all at a 1:400 dilution); rabbit anti-CD3[e] (at a 1:50 dilution); mouse anti-CD79α[e] (at a 1:30 dilution); mouse anti-BLA.36[f] (at a 1:50 dilution); and rabbit anti-PTHrP[g] (at a 1:500 dilution). Secondary biotinylated antibodies appropriate for the primary antibody were then applied, and the immunologic reaction was visualized with an avidin-biotin complex system with 3,3'-diaminobenzidine as the chromagen.[h] Antigen retrieval methods that were used included addition of 1mM EDTA (pH, 8) with microwaving (IgA), protease 2[i] (IgM), and citra[f] with steam (BLA.36, CD79α, and CD3). Neoplastic cells were immunopositive for IgA but immunonegative for CD3, CD79α, BLA.36, IgG, and IgM (**Figure 4**).

Immunohistochemistry was also used to establish that the neoplastic cells were producing PTHrP, as scattered neoplastic cells were immunopositive for PTHrP (**Figure 5**). The final diagnoses were multiple myeloma involving bone marrow, kidney, liver, and lymph nodes and multifocal smooth muscle hypertrophy of the alimentary tract. Neoplastic plasma cells secreted IgA and PTHrP, causing hypergammaglobulinemia and hypercalcemia.

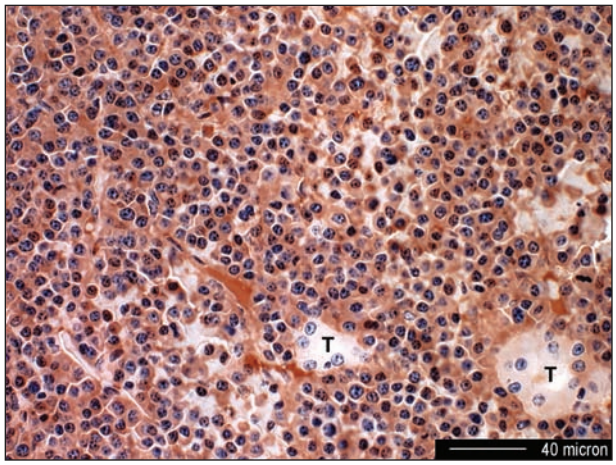

Figure 4—Photomicrograph of a section of kidney from the horse in Figure 3 following immunohistochemical staining for IgA. Virtually all the neoplastic plasma cells have diffuse cytoplasmic staining for IgA, whereas epithelium lining remnant renal tubules (T) does not stain. Hematoxylin counterstain; bar = 40 μm.

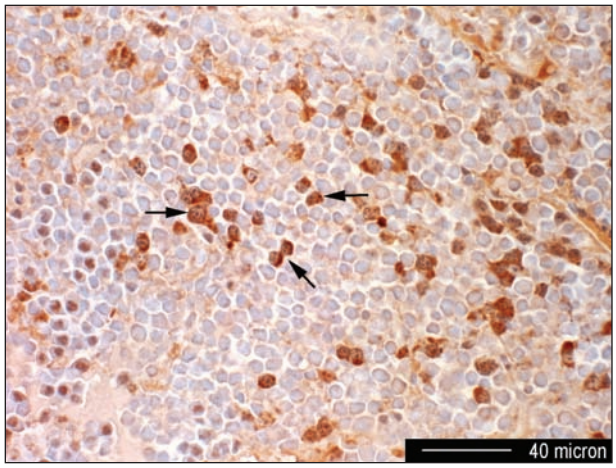

Figure 5—Photomicrograph of bone marrow from the horse in Figure 3 following immunohistochemical staining for parathyroid hormone-related protein (PTHrP). Scattered neoplastic cells stain intensively for PTHrP. Bar = 40 μm.

Multiple myeloma is a malignancy of plasma cells distributed in multiple sites that results in uncontrolled production of immunoglobulin (paraprotein) from a single plasma cell clone. It is an exceptionally rare disease in horses, with only 10 cases reported in the veterinary literature.[4] The most commonly reported reason for initial examination of horses with multiple myeloma is weight loss,[4] as was the case for the horse described in the present report.

In a previous report,[4] all 7 horses with multiple myeloma in which serum protein electrophoresis was performed had evidence of a paraprotein in the α-, β-, or β-γ-globulin region. In contrast, the initial serum protein electrophoresis performed on the horse described in the present report revealed polyclonal gammopathy. Polyclonal gammopathy is commonly associated with chronic inflammation or infection, chronic liver disease, neoplasia, and other conditions that cause nonspecific antigenic stimulation and activation of large numbers of B-cell clones, with synthesis of antibodies from all immunoglobulin classes. Later in the course of disease in this horse, when synthesis of a single immunoglobulin class from the malignant plasma cell clone presumably exceeded production of other immunoglobulins, serum protein electrophoresis revealed a sharp-edged band in the β-γ-globulin region consistent with monoclonal gammopathy. Further characterization of the immunoglobulin fractions by means of radial immunodiffusion identified a marked increase in serum IgA concentration, supporting the presumptive antemortem diagnosis of multiple myeloma with IgA as the monoclonal paraprotein. Subsequent postmortem immunohistochemical staining confirmed that neoplastic plasma cells were expressing IgA. Identification of IgA paraprotein is unique because all horses with multiple myeloma in a previous report[4] had IgG paraprotein.

The unusual occurrence of hypercalcemia in this horse greatly facilitated the initial diagnostic course. Causes of hypercalcemia in horses include acute and chronic renal failure, primary hyperparathyroidism, vitamin D toxicosis, and malignancy.[5-9] The normal serum creatinine and serum PTH concentrations were key findings that ruled out renal failure and hyperparathyroidism as causes of the hypercalcemia at the time of initial examination. There was no historical suggestion of vitamin D ingestion; thus, malignancy remained as the principal differential diagnosis for the hypercalcemia. Hypercalcemia of malignancy has been reported in association with several neoplastic diseases in horses, including lymphoma, squamous cell carcinoma, adrenocortical carcinoma, and gastric carcinoma.[10-14] When hypercalcemia of malignancy is suspected, a complete physical examination and diagnostic testing for the presence of cancer are recommended.[15] In the horse described in the present report, a thorough physical examination, hematologic and serum biochemical testing, analysis of peritoneal fluid, and abdominal ultrasonography were not helpful in identi-

fying a neoplastic disease. Detection of hypercalcemia in this horse at the time of initial examination was intriguing because almost a third of human patients with multiple myeloma have hypercalcemia.[4] Because the first serum protein electrophoresis did not identify a paraprotein, serum PTHrP concentration was quantified to confirm the diagnosis of hypercalcemia of malignancy.

The amino acid sequence of PTHrP is 70% homologous to that of PTH, and the actions of PTHrP are therefore similar to those of PTH.[16] With identification of PTHrP and its gene in 1987, it has since become clear that essentially every cell in the body makes PTHrP under normal conditions.[16,17] It has a broad range of physiologic functions, including stimulation of bone resorption, vasorelaxation, and cell proliferation; regulation of placental calcium transport, organogenesis, parturition, lactation, and vascular smooth muscle proliferation; and development of the skeletal system.[16] Despite its wide distribution in the body, PTHrP is normally present in minute amounts (< 1 pmol/L) in the circulation[16,18] and high serum PTHrP concentrations have been found in conjunction only with pathologic diseases, principally malignancy.[18] Parathyroid hormone-related protein may be synthesized by normal cells activated by the presence of a malignancy or by neoplastic cells. With many neoplastic diseases, serum PTHrP concentration is directly correlated with serum calcium concentration.[18] However, with some malignant conditions, there is no direct correlation between serum PTHrP and calcium concentrations, and in these instances, hypercalcemia may develop secondary to direct bone destruction resulting from tumor invasion or may be a result of the actions of other hormones or cytokines, transforming growth factor-α, lymphotoxin, tumor necrosis factor, interleukin-1α, and 1,25 dihydroxyvitamin D.[19] The high serum PTHrP concentration in the horse described in the present report led to a presumptive diagnosis of hypercalcemia of malignancy, despite the fact that an underlying neoplasm could not be definitely identified antemortem. Rarely, hypercalcemia with a concurrent increase in serum PTHrP concentration occurs when there is no detectable neoplastic disease.[20,21] However, in this horse, the unusual combination of high serum IgA concentration and high serum PTHrP concentration strongly supported an antemortem diagnosis of multiple myeloma.

The clinical importance of the multifocal smooth muscle hypertrophy of the alimentary tract seen postmortem is unclear because this finding has not been reported previously in horses with multiple myeloma.[4] Idiopathic muscular hypertrophy of the distal esophagus and distal ileum has been reported as an incidental postmortem finding in horses; however, distal ileal hypertrophy may also lead to intestinal obstruction.[22] It seems plausible that clinical signs of mild abdominal pain that developed late in the course of the illness were caused by partial luminal obstruction secondary to the smooth muscle hypertrophy. Skeletal muscle hypertrophy has been reported rarely in people with multiple myeloma[23] and has been proposed to be attributable to the effects of PTHrP. Parathyroid hormone-related protein is an important growth factor for vascular smooth muscle,[24] and it is interesting to speculate that increased production of PTHrP may have been responsible for some of the smooth muscle hypertrophy in this horse.

As demonstrated in this horse, quantification of serum PTH, ionized calcium, and PTHrP concentrations is useful in distinguishing primary hyperparathyroidism, secondary hyperparathyroidism, and hypercalcemia of malignancy in horses. This case was unique in that serum protein electrophoresis was not initially helpful in securing a diagnosis of multiple myeloma and the paraprotein was IgA. Quantification of individual immunoglobulin classes by means of radial immunodiffusion was useful in the diagnosis of multiple myeloma in this horse and should be included in the diagnostic plan when multiple myeloma is suspected and serum protein electrophoresis does not reveal a monoclonal spike.

[a]ATL 3000, Philips, Atlanta, Ga.
[b]Animal Health Diagnostic Laboratory, Lansing, Mich.
[c]Immunopanel, VMRD Inc, Pullman, Wash.
[d]Anti-horse IgA, IgM, and IgG, Bethyl Laboratories Inc, Montgomery, Tex.
[e]Anti-CD3 and anti-CD79α, Dako, Carpinteria, Calif.
[f]Anti-BLA.36, BioGenex, San Ramon, Calif.
[g]Anti-PTHrP, Oncogene Sciences Ab-2, Cambridge, Mass.
[h]Avidin-biotin peroxidase complex, 3,3'-diaminobenzidine, Vector Laboratories, Burlingame, Calif.
[i]Protease 2, Ventana Medical Systems Inc, Tucson, Ariz.

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