Light-chain monoclonal gammopathy and cast nephropathy in a horse with multiple myeloma

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CASE DESCRIPTION

A 27-year-old Dutch Warmblood mare was referred to the University of Zurich’s Clinic for Equine Internal Medicine because of a history of lethargy, reluctance to move, weight loss, persistent hyperproteinemia, and recurrent episodes of mild lameness.

CLINICAL FINDINGS

Hematologic evaluation revealed anemia (RBC concentration, 3.84 \( \times 10^n \) cells/µL; reference range, 6.2 \( \times 10^n \) to 9.0 \( \times 10^n \) g/kg [0.68 \( \times 10^n \) to 1.12 \( \times 10^n \) g/lb], PO, q 24 h) was initiated. Results of a CBC and plasma biochemical analysis indicated severe hyperproteinemia (total protein concentration, 11.5 g/dL; reference range, 5.7 to 7.0 g/dL) and anemia (5.1 \( \times 10^n \) RBCs/µL; reference range, 6.2 \( \times 10^n \) RBCs/µL to 9.0 \( \times 10^n \) RBCs/µL). On recheck examination 2 months later, the clinical signs had not improved. The horse’s plasma ACTH concentration was within the reference range (25.5 pg/mL), but hyperproteinemia (plasma total protein concentration, 13.1 g/dL) and anemia (4.3 \( \times 10^n \) RBCs/µL) were persistent.

ABBREVIATIONS

SPEP  Serum protein electrophoresis
UPEP  Urine protein electrophoresis

CLINICAL RELEVANCE

Multiple myeloma is rarely reported in horses. A monoclonal peak on serum protein electrophoresis should raise the suspicion of neoplasia, specifically multiple myeloma. The findings for this patient confirmed the importance of considering neoplasia in horses with nonspecific clinical signs. (J Am Vet Med Assoc 2018;253:1177–1183)
ulin concentration, 9.2 g/dL; reference range, 2.6 to 4.0 g/dL) and moderate hypoalbuminemia (albumin concentration, 2 g/dL; reference range, 2.5 to 3.4 g/dL) was present on serum biochemical analysis, with the remainder of the results within respective reference ranges. The hyperglobulinemia was further evaluated by SPEP\(^b\) which revealed monoclonal gammopathy with a pronounced narrow peak in the $\gamma$ region ($\alpha_1$ globulin concentration, 0.32 g/dL; reference range, 0.28 to 0.76 g/dL); $\alpha_2$ globulin concentration, 0.71 g/dL; reference range, 0.69 to 1.31 g/dL); $\beta$ globulin concentration, 1.05 g/dL; reference range, 0.68 to 1.64 g/dL); and $\gamma$ globulin concentration, 6.85 g/dL; reference range, 2.32 to 3.46 g/dL) and confirmed hypoalbuminemia (albumin concentration, 2.0 g/dL; reference range, 2.32 to 3.46 g/dL; albumin-to-globulin ratio, 0.23 [reference range, 0.57 to 1.08]; Figure 1). Evaluation of a urine sample obtained by catheterization revealed isosthenuria (urine specific gravity, 1.014; reference range, 1.025 to 1.040) with no abnormalities evident on dipstick testing of the sample.

A previously described multiplex analytic approach\(^c\) was used to further characterize the monoclonal globulin (also known as myeloma [M] protein or paraprotein) detected by SPEP.\(^c\) This method enables characterization of the IgA, IgG subclasses (IgG1, IgG3, IgG4, IgG5, IgG6, and IgG7), IgE, and IgM.\(^2\) All measured immunoglobulin concentrations either were within the expected ranges or were low; therefore, the presence of $\kappa$ and $\lambda$ light chains was evaluated by immunofixation at the authors’ institution. Serum and urine samples were incubated on a polyacrylamide gel with a polyclonal rabbit anti–human $\kappa$ light-chain antibody\(^d\) and a polyclonal rabbit anti–human $\lambda$ light-chain antibody at 1:2,000 dilutions in PBS solution. Both antibodies had cross-reactivity with the respective equine immunoglobulins in previous sample testing in the authors’ facility. Additionally, serum and urine from a healthy horse were tested as reference samples. A goat anti-rabbit IgG peroxidase-conjugated antibody was used as the secondary antibody. A positive result for the $\kappa$ light chain at approximately 25 kDa was visualized; the result for $\lambda$ light-chain testing was negative. In contrast, samples from the healthy horse had a physiologic marked positive result for $\lambda$ light chain and a very slight positive result for $\kappa$ light-chain presence.\(^3\) Results for the patient were considered indicative of abnormal monoclonal proliferation.

Because urine dipstick testing is insensitive to $\lambda$ and $\kappa$ light-chain detection,\(^4\) UPEP was performed to test for possible excretion of the paraprotein. Proteinuria was present with 1 pronounced narrow peak evident on UPEP. Immunofixation of a urine sample was performed as previously described to test for the presence of the $\lambda$ light chain. A positive result was obtained with a band at 25 kDa, confirming Bence-Jones proteinuria.\(^3\)

Ultrasonographic examination of the abdomen was performed; the spleen was identified immediately adjacent to the left body wall, extending from the flank to the right side of the ventral midline, and subjectively appeared enlarged. Multiple hypoechoic nodules measuring up to 2.8 cm in diameter were detected throughout the parenchyma of the subjectively enlarged spleen (Figure 2). Examination of remaining abdominal structures did not reveal any abnormalities. The horse was sedated by admin-

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**Figure 1**—Graph depicting SPEP results for a 27-year-old Dutch Warmblood mare that was referred to a veterinary teaching hospital because of a history of lethargy, reluctance to move, weight loss, persistent hyperproteinemia, and recurrent episodes of lameness. There is a distinct, narrow monoclonal peak in the $\gamma$ globulin region and hypoalbuminemia. The albumin-to-globulin ratio was 0.23.

**Figure 2**—Representative ultrasonographic images of the spleen of the same horse as in Figure 1. Multiple hypoechoic nodules (arrows) measuring up to 2.8 cm were detected throughout the parenchyma of the subjectively enlarged spleen; the largest nodule found (A) and several smaller nodules (B) are shown.
istration of xylazine (0.5 mg/kg [0.23 mg/lb], IV). Ultrasound-guided fine-needle aspiration of splenic nodules was performed, and cytologic examination of Wright-Giemsa-stained smears revealed moderate plasma cell hyperplasia with signs of dysplasia, consistent with an extramedullary plasmacytoma. Results of ultrasonographic examination of the thorax were unremarkable, and a mild bronchial pattern was observed on thoracic radiographs.

A diagnosis of multiple myeloma was suspected, and a bone marrow biopsy sample was obtained from the sternum by use of a Jamshidi bone marrow biopsy needle. The horse was sedated with detomidine (10 µg/kg, [4.5 µg/lb], IV) and butorphanol (10 µg/kg, IV) for this procedure. The sample was submitted to the Institute of Veterinary Pathology at the author's institution for histologic examination (Figure 3). The bone marrow was densely cellular with normal resident hematopoietic cell lines comprising approximately 70% and nonresident round cells in variably sized groups comprising approximately 30% of the cell population. The latter cells had eccentric, clockface, round nuclei and signs of atypia, including anisokaryosis, anisocytosis, and rare binucleation. Combined immunohistochemical staining for κ and λ light chains confirmed that these cells were of plasma cell origin and demonstrated clustering of the cells in groups. Taken together, the results of clinical and laboratory analyses confirmed a diagnosis of multiple myeloma.

Owing to the horse's age and poor prognosis, treatment was declined by the owner. Recommendation for palliative care included medical pain relief as necessary and general supportive care such as feeding a high-energy diet to counteract progressive weight loss. The owner was advised not to exercise the horse but monitor its general condition daily.

Two weeks after the referral examination and workup, the horse was euthanized because of repeated episodes of lethargy, anorexia, and signs of colic. Complete necropsy was performed at the Institute of Veterinary Pathology at the authors' institution. Radiography of the long bones was performed to evaluate for the presence of osteolytic processes. Results of ultrasonographic examination of the thorax were unremarkable, and a mild bronchial pattern was observed on thoracic radiographs.

The bone marrow consisted macroscopically of adipose tissue, with gross evidence of hematopoiesis limited to the proximal aspects of the femurs. There was no alteration of cortical thickness. Subjectively, the spleen appeared moderately enlarged with multifocal to coalescing pale foci of 2- to 10-mm diameter on the cut surface. Several melanomas were also present, including dermal perianal and perivulvar nodules and infiltrative masses in the masticatory, pectoral, subscapularis, and dorsal lumbar muscles.

Figure 3—Photomicrographs of a bone marrow sample from the same horse as in Figure 1. A—The thinner ellipse indicates a region in which hematopoietic precursor cells are dominant, whereas the thicker ellipse indicates a region that primarily includes nonresident neoplastic cells (gold-brown pigment indicates hemosiderin within macrophages). H&E stain; bar = 100 µm. B—Immunohistochemical analysis for immunoglobulin κ and λ light chains reveals strong positive staining (brown) of the neoplastic cell population. Immunohistochemical stain; bar = 50 µm.
Proximal tubular epithelial cells often had granular intracytoplasmic staining for κ light chains, reflecting uptake of this product from the filtrate. Mild thickening of tubular and glomerular capillary basement membranes stained with periodic acid–Schiff reagent was interpreted as age related. These findings suggested a diagnosis of cast nephropathy. The results of macroscopic and histologic examinations confirmed a diagnosis of multiple myeloma with production of κ light chains and cast nephropathy.

Discussion

The horse of this report had nonspecific clinical signs such as lethargy, reluctance to move, intermittent mild lameness, and weight loss over a 5-month period. In people with multiple myeloma, typical clinical signs include bone pain, fatigue, generalized weakness, and weight loss. Signs of chronic or acute skeletal pain are most commonly reported in dogs at the time of multiple myeloma diagnosis. In a previous report of a horse with nonsecretory myeloma, chronic lameness was the only clinical sign on initial examination. The term myeloma bone disease is used in human medicine to describe the pathophysiology of increased osteoclastic activity with suppressed osteoblastic activity leading to bone lesions causing pain, reluctance to move, and fractures. The observed nonspecific lameness and reluctance to move in the horse of this report might have been explained by bone pain caused by lytic lesions; however, radiographs of the long bones did not show any evidence of lytic lesions, and although bone marrow was completely replaced by plasma cells in some areas, macroscopic bone lesions were not found on necropsy. Further diagnostic tests were not performed in this case, but scintigraphy has been used to identify bone disease associated with myeloma and other types of cancer in horses. In our geriatric patient, a musculoskeletal condition unrelated to the primary disease, such as chronic degenerative joint disease, was considered the likely cause for the observed lameness.

Severe hyperproteinemia characterized by hypoalbuminemia and hyperglobulinemia was the main clinicopathological finding in this case. Serum proteins mainly consist of albumin and a variety of globulins that are characterized and divided into fractions (α, β, and γ globulins) according to their electrophoretic mobility. Increased circulating protein concentrations can result from panhyperproteinemia or hyperglobulinemia. Panhyperproteinemia occurs with severe dehydration and is associated with an increased PCV. Hyperglobulinemia occurs with a variety of disorders and should be further investigated by SPEP. Increases in the α-globulin fraction represent an acute phase response that is associated with acute inflammatory disorders.
ized by an increase in the β-globulin fraction can occur with active liver disease. The γ-globulin fraction includes immunoglobulins. Polyclonal gammopathy is characterized by a broad increase throughout the α, β, and γ regions, whereas monoclonal gammopathy is reflected by a pattern with a single pronounced spike in 1 region. Gammapathy is among the most common abnormalities identified by SPEP in people and small animals. Of 140 dogs with abnormal SPEP results, the most frequent diagnoses obtained were infectious or inflammatory conditions (56.4%) and neoplastic disorders (15%). Similar results were obtained from cats, with 80 of 136 (58.8%) having diagnoses of infectious or inflammatory conditions and 14 of 136 (10.3%) having neoplastic disorders. Similar studies are lacking in horses, but gammopathies have been described in horses with neoplasia, hepatic disease, and abdominal abscesses. Monoclonal gammapathy, as observed in the horse of this report, is a very rare abnormality seen on SPEP. Of 3,925 SPEPs performed on canine samples, only 20 (0.5%) identified the presence of a monoclonal gammapathy. Monoclonal gammapathy is pathognomonic for a plasma cell dyscrasia, where 1 clone of an abnormal plasma cell or B cell produces a single immunoglobulin or immunoglobulin heavy or light chain. This protein is evident on SPEP as a monoclonal spike and referred to as myeloma protein, paraprotein, or monoclonal protein. The most common differential diagnosis for monoclonal gammapathy in dogs is multiple myeloma, and the monoclonal gammapathy identified in our patient strongly suggested the diagnosis of multiple myeloma. In general, hyperproteinemia merits further investigation, and a lymphoproliferative disorder, such as multiple myeloma, can be expected with a monoclonal gammapathy.

In human medicine, the diagnosis of multiple myeloma requires the fulfillment of criteria established by the International Myeloma Working Group. According to those criteria, clonal plasma cells must represent > 10% of the bone marrow population as determined by biopsy, or an extramedullary plasmacytoma must be diagnosed. In addition, ≥ 1 of the following related organ or tissue impairments must be present: increased circulating calcium concentration, renal insufficiency, anemia, and bone lesions (CRAB). Other organ damage resulting from conditions such as hyperviscosity or amyloidosis is considered nonspecific. In the current case, further investigations were performed to achieve a definite diagnosis. Results of bone marrow biopsy revealed that > 10% of the cell population consisted of plasma cells, and extramedullary plasmacytoma was found in the spleen. Additional tissue impairment was evidenced by anemia. Taken together, these results confirmed a diagnosis of multiple myeloma. Although this is a rare neoplastic condition in horses, the authors suggest the use of the aforementioned guidelines when multiple myeloma is suspected.

In human patients, multiple myeloma accounts for only 1 percent of neoplastic disorders, with an annual incidence of 0.004% to 0.005%. Multiple myelomas are also uncommon in dogs and cats, with only individual cases or small case series reported. In an electronic literature search, we identified only 21 cases of suspected plasma cell dyscrasias, including multiple myeloma, in horses since 1959. Monoclonal gammapathies were reported in association with chronic lymphocytic leukemia or solitary plasmacytoma or without a specific origin identified. Very few cases of multiple myeloma have been reported in this species. Most multiple myelomas produce a monoclonal protein. The plasma cells can produce either complete immunoglobulins or only the light-chain component. In people, IgG is the most frequently reported myeloma protein (52%), followed by IgA (21%), κ or λ light chain (16%), IgD (2%), and IgM (0.5%), with some having bicalon products (2%). In horses, IgA, IgG, and IgG(T) gammapathies associated with multiple myeloma have been reported. The myeloma protein in the present report was identified as κ light chain. Horses are a λ light chain–dominant species. It has been shown that the λ to κ ratio in healthy horses is largely dominated by the λ chains (ratio of 96%;4%). A dominance in κ light chains indicates a monoclonal proliferation. To our knowledge, this is the first report of a light-chain gammapathy in a horse. In addition, very few cases of myeloma involving light-chain gammapathy of dogs and cats have been described. The κ light-chain isotype is most commonly produced in human patients with this condition.

Renal disease, a condition commonly seen in humans with light-chain multiple myeloma, was identified in the horse of the present report at necropsy. Renal tubular disease was suspected on the basis of urinalysis results, despite the fact that serum creatinine concentration was within the reference range. Inability to concentrate urine develops when at least two-thirds of nephron function is lost. This is classically referred to as renal insufficiency, even in the absence of azotemia. Dipstick analysis for protein yielded negative results because this test does not react to light chains. The UPEP results revealed proteinuria with substantial amounts of κ light-chain (Bence-Jones) proteins confirmed by immunofixation. Complete immunoglobulins or immunoglobulin heavy chains cannot pass the glomerular wall; this results in their deposition at this site, causing glomerulonephritis. In contrast, free light chains pass through the glomerular wall. In tubules, light chains bind to mucoproteins, leading to precipitation and formation of obstructive casts. Infiltration of light chains into the interstitium occurs secondary to tubular rupture, which causes inflammation. Myeloma cast nephropathy is the most common renal lesion associated with multiple myeloma and is considered one of the additional defining factors for the disease in people. Myeloma kidney
disease or light-chain cast nephropathy describes the disorder in which monoclonal light chains (Bence-Jones proteins) in the urine lead to acute or chronic renal failure.\textsuperscript{42} Histologically, the kidneys have characteristic features comprising tubular cast formation and tubular injury without evidence of glomerular disease. On immunofluorescence testing, casts stain intensely for either \( \kappa \) or \( \lambda \) light chain.\textsuperscript{40} Low-molecular-weight proteinuria and tubular casts have been described in a horse with multiple myeloma, but the monoclonal protein was not identified in that case.\textsuperscript{31}

A treatment regime comprising a single dose of cyclophosphamide followed by a course of melphalan was declined by the owner of the horse of this report. At present, multiple myeloma is considered an incurable disease, and treatment is directed toward remission or clinical improvement. The use of systemic cytotoxic drugs has been infrequently reported in equine patients, relative to information available in human and companion animal medicine.\textsuperscript{43}

For the patient of this report, SPEP and UPEP were useful tools for investigation of hyperproteinemia and diagnosis of multiple myeloma. The findings for this patient reaffirmed the importance of considering neoplasia in horses with nonspecific clinical signs and suggested that multiple myeloma should also be added to the list of differential diagnoses when there is unexplained renal insufficiency.

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The authors declare that there were no conflicts of interest.

**Footnotes**

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**References**

Effect of age on the pharmacokinetics and distribution of tulathromycin in interstitial and pulmonary epithelial lining fluid in healthy calves

Danielle A. Mzyk et al

OBJECTIVE
To compare the plasma pharmacokinetics of tulathromycin between 3-week-old (preweaned) and 6-month-old (weaned) calves and to characterize the distributions of tulathromycin into pulmonary epithelial lining fluid (PELF) and interstitial fluid (ISF) of preweaned and weaned calves following SC administration of a single dose (2.5 mg/kg).

ANIMALS
8 healthy 3-week-old and 8 healthy 6-month-old Holstein steers.

PROCEDURES
A jugular catheter and SC ultrafiltration probe were aseptically placed in the neck of each calf before tulathromycin administration. Blood, ISF, and bronchoalveolar lavage fluid samples were collected at predetermined times before and after tulathromycin administration for quantification of drug concentration. A urea dilution method was used to estimate tulathromycin concentration in PELF from that in bronchoalveolar lavage fluid. Tulathromycin–plasma protein binding was determined by in vitro methods. Plasma pharmacokinetics were determined by a 2-compartment model. Pharmacokinetic parameters and drug concentrations were compared between preweaned and weaned calves.

RESULTS
Clearance and volume of distribution per fraction of tulathromycin absorbed were significantly greater for weaned calves than preweaned calves. Tulathromycin–plasma protein binding was significantly greater for weaned calves than preweaned calves. Maximum PELF tulathromycin concentration was significantly greater than the maximum plasma and maximum ISF tulathromycin concentrations in both groups.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that age affected multiple pharmacokinetic parameters of tulathromycin, likely owing to physiologic changes as calves mature from preruminants to ruminants. Knowledge of these changes may be useful in the development of studies to evaluate potential dose adjustments during treatment of calves with respiratory tract disease. (Am J Vet Res 2018;79:1193–1203)