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HYPERGAMMAGLOBULINEMIA AND MYELOMA IN FIVE TIGERS (*PANTHERA TIGRIS*): CLINICOPATHOLOGICAL FINDINGS

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Abstract: Five adult tigers (*Panthera tigris*) presented with a range of clinical signs, including paresis (2/5), lameness (2/5), ataxia (3/5), anorexia (5/5), and lethargy (5/5). Each tiger demonstrated elevated plasma globulin levels (7.8–14.8 g/dl; [reference interval 2–5.1 g/dl]) on routine biochemistry, confirmed as a monoclonal gammopathy using protein electrophoresis. Serum gammaglobulin concentration ranged from 5 to 7.5 g/dl, or 45.1–63.4% of total protein concentration. Azotemia was present in three tigers. Diagnostics and management varied with the presenting signs but included magnetic resonance imaging, radiography, chemotherapy, supportive care, and euthanasia. In each case, necropsy revealed a neoplastic plasma cell proliferation in the bone marrow and one or more extramedullary sites. Lytic lesions in the thoraco-lumbar spine were found in three animals, and one lesion was associated with spinal cord compression. Splenomegaly was present in 4/5 cases. Histopathology confirmed a plasma cell neoplasm in each case, and immunohistochemistry staining with multiple myeloma oncogene 1 (MUM1) was positive in each case. CD20 staining was performed in two cases and was positive in one. CD3 staining was performed in the same two cases, and was negative in each. Based on the clinical, gross, microscopic, and immunohistochemical findings, myeloma was diagnosed in all five tigers.

Key words: Hypergammaglobulinemia, myeloma, neoplasia, *Panthera tigris*, tiger.

INTRODUCTION

Monoclonal gammopathies result from the proliferation of a single clone of B-lymphocytes.³ It is an infrequent finding in domestic animals, primarily found in association with plasma cell neoplasia, including lymphoma, myeloma, chronic lymphocytic leukemia, and myeloma-related diseases (MRD) in cats.^{3,11,12} Less commonly, monoclonal gammopathies are a feature of chronic inflammatory conditions.³ Malignant plasma cell neoplasms are rare neoplastic conditions in both dogs and cats, accounting for <1% of malignant canine tumors and between 0.0012 and 0.0025% of malignant feline tumors.⁷ Differentiation of plasma cell origin from other round cells is achieved using an immunohistochemical stain with multiple myeloma oncogene 1 (MUM-1), a phenotypic marker used for characterization of B-cell lymphoma histogenesis.^{2,9} The immunohistochemical stain, CD20, is an alternative B-cell marker, which offers less reliability for myeloma diagnosis in dogs.⁹ Hypercalcemia, azotemia, cytopenias, and Bence-Jones proteinuria are all factors associated with a poor prognosis.¹² Treatment of myeloma has not been curative in any

species, and most animals survive less than 2 yr after diagnosis, despite chemotherapy.¹²

Myeloma has previously been reported in some nondomestic felid species, including a lion (*Panthera leo*), jaguar (*Panthera onca*), and snow leopard (*Uncia uncia*).^{4,8,11} Each of these cases demonstrated hyperglobulinemia and osteolytic lesions, although MUM1 was not used for an immunohistochemical confirmation of the cell type.

This case series compares the clinical, serum biochemical, hematologic, pathologic, and immunohistochemical findings in one male and four female captive tigers (*Panthera tigris*), where each presented with severe hypergammaglobulinemia.

CASE REPORTS

Over a period of 5 yr, five unrelated tigers were euthanized or died with myeloma diagnosed as the primary cause. The animals were housed at either a zoological facility in Tennessee (cases 1, 2, 3, and 5), or in the western United States (case 4). Each of the tigers were sedated for examination based on an estimated body weight, with target dosages of 0.02 mg/kg medetomidine intramuscularly (IM) (Wildlife Pharmaceuticals, Windsor, CO 80550, USA), and 0.2 mg/kg midazolam (NovaPlus, Hospira, Lake Forest, IL 60045, USA) IM, followed by 3 mg/kg ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA 50501, USA) IM. Hyperglobulinemia was detected on a routine biochemistry for all

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tigers. Serum protein electrophoresis was performed in all cases, at the University of Tennessee clinical pathology laboratory and urine protein electrophoresis was performed at the same laboratory in cases 1, 2, 3, and 5. Universally, a monoclonal gammopathy was characterized by a tall, narrow-based peak in the gamma globulin region, and was consistent with the presence of Bence-Jones proteinuria and/or a paraproteinemia. Necropsy was performed within 24 hr of death in each case.

Case 1

A 16-yr-old female tiger was presented for a 2-wk history of anorexia, lethargy, progressive hind limb paresis and ataxia. The plasma globulin concentration was elevated at 7.8 g/dl (reference interval 2–5.1 g/dl)¹⁰ and azotemia was present (blood urea nitrogen [BUN] 109 mg/dl [reference interval 15.3–52 mg/dl],¹⁰ plasma creatinine [Cre] concentration 4.3 mg/dl [reference interval 0.5–3.9 mg/dl]).¹⁰ Gammaglobulin concentrations were measured in serum (5 g/dl, 45.1% of total serum protein) and urine (0.9 g/dl, 38.3% of total urinary protein). Previous biochemistry from 2 mo prior to presentation showed similar azotemia (BUN 100 mg/dl, Cre 4.3 mg/dl) and hyperglobulinemia (6.5 mg/dl). Radiographic and high field magnetic resonance imaging (MRI) evaluations of the thoraco-lumbar spine were performed. Radiographically, there was widening of the spinal canal at the level of L3, with a concave appearance to the dorsal aspect of the vertebral body (Fig. 1). MRI examination revealed T2 hyperintense lesions within the vertebral bodies of L2, L3, L4, and equivocally in L1. These were T1 isointense to the spinal cord and strongly enhanced after contrast administration. There was extension of the vertebral body change at the level of L2–L3 into the spinal canal, resulting in an extradural compression and dorsal deviation of the spinal cord parenchyma (Fig. 2). Treatment was initiated with palliative radiation therapy and 6 mg melphalan orally (PO) (Diamondback Drugs, Scottsdale, AZ 85251, USA) three times weekly. Nevertheless, clinical deterioration was rapid, and euthanasia was performed 5 days later. Bloodwork performed at this time showed an increase in both globulin (8.6 g/dl) and BUN concentration (156 mg/dl), while Cre concentration had remained at 4.2 mg/dl.

Necropsy findings included an 85 × 5 × 15-mm, mottled red and tan, soft tissue mass arising in the ventral spinal canal spanning L2 to L4 and dorsally compressing the L3 spinal cord. The

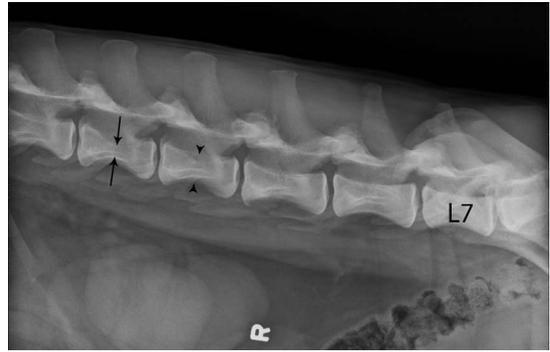


Figure 1. Lateral radiographs of the lumbar spine, case 1. Vertebral body of L3 (black arrows) is narrower than adjacent vertebra (black arrowheads), resulting in a widened spinal canal.

neoplasm was composed of sheets of round cells with coarsely stippled chromatin, moderate amphophilic cytoplasm, and occasional perinuclear cytoplasmic pallor, consistent with plasma cells. Within the overlying spinal cord was a focal area of cavitation with white matter vacuolation and gliosis. Neoplastic plasma cells were also found disrupting the renal cortex and around hepatic portal structures. Neoplastic cells within the vertebral mass were MUM1 positive.

Case 2

A 17-yr-old intact female tiger presented with a 1-wk history of anorexia. Routine hematology and biochemistry identified hyperglobulinemia (13.6 g/dl), anemia (hematocrit [HCT] 20.5% [reference interval 24–52.5%]),¹⁰ and azotemia (BUN 102 mg/dl, Cre 5.8 mg/dl). Serum protein electrophoresis showed a gammaglobulin concentration of 7.5 g/dl (47.6%). Gamma globulin concentration comprised 38.1% of the total urinary protein. Survey radiographs demonstrated well-circumscribed lucencies within the spinous process of T13 and the vertebral pedicles of L2. Treatment with 120 mg prednisolone PO (West-ward Pharmaceuticals, Eatontown, NJ 07724, USA) once a day (SID) and 6 mg melphalan PO (Diamondback Drugs) three times weekly was started. Ataxia was reported approximately 6 wk later. Repeat bloodwork revealed increased plasma globulin concentration of 14.8 g/dl, and worsening renal values (BUN 118 mg/dl, Cre 5.8 mg/dl). Euthanasia was performed based on the deteriorating clinical condition.

Necropsy identified a moderately enlarged, pale spleen; a 10 mm diameter, discreetly margined, red, soft focus within the spinous process of T13;

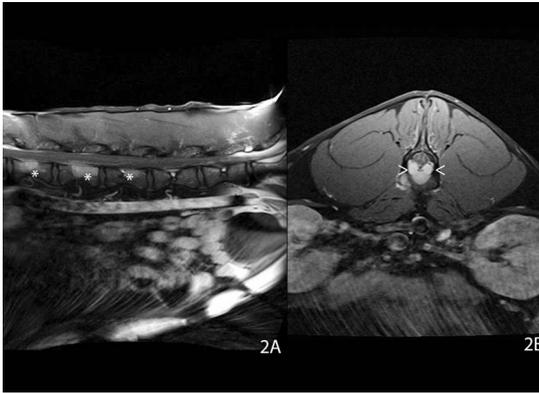


Figure 2. Postcontrast magnetic resonance imaging of the lumbar spine, case 1. **A.** Sagittal T1 postcontrast with fat suppression. Hyperintense lesions are seen within the vertebral bodies of L2, L3, and L4 (*). **B.** Transverse T1 postcontrast with fat suppression at the level of L3. The contrast enhancing lesion noted in the body of L3 (white arrowheads depicting lateral margins) is seen extending into the spinal canal, resulting in an extradural spinal cord compression and deviation.

and multiple pale tan, irregular, up to $5 \times 3 \times 3$ -cm soft, gray tan masses along the mesenteric border of the jejunum.

Histologically, the spleen was expanded by sheets of neoplastic plasmacytoid round cells, which were occasionally multinucleated or karyomegalic. Bone marrow from the femurs and humeri contained similar neoplastic cells. Within osteolytic lesions identified grossly and radiographically within T13 and L3, neoplastic cells were associated with punctate areas of bony loss. Jejunal mesenteric lymph nodes were expanded by a marked chronic granulomatous lymphadenitis. Neoplastic cells within the spleen were positive for CD20 and MUM1, and negative for CD3.

Case 3

An approximately 18-yr-old castrated male tiger presented with lethargy and left forelimb lameness of 7 days duration. Blood was collected, and demonstrated a plasma globulin concentration of 10.1 g/dl. Serum gammaglobulin concentration was 7.2 g/dl (61.2%). Urine, collected by catheterization, also showed a monoclonal gammopathy. Whole body radiographs and abdominal ultrasound indicated moderate osteoarthritis of the left elbow joint and splenic enlargement. An ultrasound-guided splenic fine-needle aspirate was consistent with plasma cell neoplasia. A bone marrow aspiration was unsuccessful because the

cytological preparation did not contain bone marrow particles. Treatment for presumptive myeloma with cyclophosphamide 53 mg three times weekly (Cytoxan, Diamondback Drugs) PO, 1600 mg fluconazole SID (Glenmark Pharmaceuticals, Mahwah, NJ 07652, USA) PO, and 140 mg prednisone SID (West-ward Pharmaceuticals) PO was initiated and the tiger improved clinically. Bloodwork performed at 5 wk and again at 6 mo revealed a plasma globulin concentration of 11.4 g/dl and 10.3 g/dl respectively. Renal values (BUN, Cre, and phosphorous) stayed within reference intervals over the course of the disease. The animal died 7 mo after the initial diagnosis.

Gross and histological examination confirmed the cause of death as a right atrioventricular thrombus. Neoplastic plasma cells were identified within a single discrete mass within the spleen and in the femoral bone marrow. No bony lesions were identified grossly. In the spleen, the neoplastic cells were positive for MUM1 and negative for CD20 and CD3.

Case 4

A 15-yr-old intact female tiger presented with anorexia and worsening lethargy of approximately 4 days duration. Examination under anesthesia demonstrated an enlarged, ruptured spleen, which was immediately removed via a standard midline approach. Blood collected at the time of surgery revealed an elevated plasma globulin concentration of 9.0 g/dl, azotemia (BUN 107 mg/dl, Cre 12 mg/dl). The serum monoclonal gammopathy concentration was 6.8 g/dl (63.4%). The tiger died during recovery from anesthesia.

Necropsy findings included a grossly enlarged, friable spleen with a 2-cm focal rupture and an associated hematoma. Neoplastic plasma cells were present throughout the spleen, liver, bone marrow, and, less extensively, the adrenal glands. Anesthesia-associated death was attributed to mild myocardial fibrosis and right-sided cardiac insufficiency, as well as extensive neoplastic infiltrate. In the spleen, the neoplastic cells were positive for MUM1.

Case 5

An 18-yr-old female tiger presented for lethargy and anorexia of approximately 1-wk duration. The plasma globulin concentration was 8.3 g/dl. Serum gammaglobulin concentration was 4.8 g/dl (45.6%), and a urinary monoclonal gammopathy was identified with 85% of urinary protein in the gamma fraction. Treatment was initiated with

regular fluid administration, 100 mg prednisone SID (West-ward Pharmaceuticals), and 15 mg mirtazapine PO SID (Aurobindo Pharmaceuticals LLC, Dayton, NJ 08810, USA). Variations in diet (offering rabbit and horse liver) were provided, in an attempt to improve appetite. This protocol showed intermittent success in appetite restoration. Ataxia, hindlimb paresis, and a left forelimb lameness developed 4 wk after initial presentation. Radiographs showed multifocal variably sized polyostotic lytic lesions within the left humerus, vertebrae, and ribs, which also had evidence of pathological fractures. Euthanasia was performed.

At necropsy, lesions were limited to the skeleton and included extensive, coalescing punctate regions of soft, dark red, bone loss associated with extensive bony remodeling. The vertebral body of L5 was most severely affected with obliteration of the vertebral body and extension of the neoplasm into the surrounding musculature (Fig. 3). The dorsal processes of multiple cervical and thoracic vertebrae had extensive central bone loss with peripheral bony remodeling and multiple ribs were fractured bilaterally with poorly formed bony calluses at the fracture sites. Nearly 60% of the bone marrow within the left and right humeri and femurs was replaced by tumor and the left humeral head was depressed and fractured into innumerable fragments. The spleen was grossly normal. Neoplastic plasma cells comprised nearly 50% of the bone marrow (Fig. 4) and were positive for MUM 1.

DISCUSSION

Antemortem diagnosis of myeloma is based on an accumulation of clinical findings, including radiographic or visual evidence of osteolytic lesions, a monoclonal gammopathy, the presence of Bence-Jones proteinuria, and bone marrow plasmacytosis.¹¹ The five cases included in this case series demonstrated an initial marked serum hypergammaglobulinemia, from 4 to 7.5 g/dl, or 45.1–63.4% of the total protein concentration, and this was the principal inclusion criterion for this study. The diagnosis of myeloma was supported by a monoclonal gammopathy in serum, and histologic confirmation of MUM1-positive proliferating malignant plasma cells. Three cases were further supported by lytic bone lesions, and four cases with a monoclonal urinary gammopathy, consistent with Bence-Jones proteinuria. The use of MUM1 has not been previously reported in nondomestic felids with suspected plasma cell neoplasia. CD20 was used in two

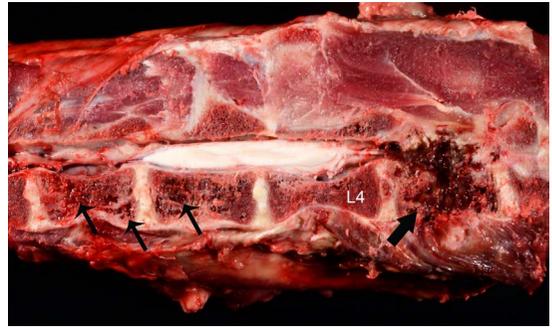


Figure 3. Image of the unfixed spinal column at the level of the L2 to L5 vertebrae, case 5. The L5 vertebral body is effaced by a poorly demarcated, highly lytic, soft red neoplasm (thick arrow). Smaller lytic foci are present within the L2 and L3 vertebrae (thin arrows).

cases and was positive in one, appearing to support the lower reliability in myeloma when compared with MUM1.⁹ Additionally, CD3 was used in two cases and was negative for T lymphocytes, as expected.

Hypergammaglobulinemia can be also caused by chronic inflammatory processes. It is possible that the chronic granulomatous lymphadenitis of the jejunal mesenteric lymph nodes contributed to the elevated plasma globulin concentration in case 2.^{3,11}

The frequency of myeloma increases with age in domestic cats, and usually presents with vague clinical signs, including weight loss, anorexia, and lethargy.^{11,12} The vague clinical signs observed in domestic animals and the increasing risk of disease with age appear to be consistent with those seen in tigers. In comparison with humans and dogs, osteolytic lesions are rare in domestic cats, and multiple extramedullary plasma cell neoplasms are more frequent.⁷ Osteolytic lesions may be more common in nondomestic felids than in domestic cats, as three tigers (cases 1, 2, and 5) had osteolytic lesions of the vertebrae and case 5 had extensive appendicular involvement. Osteolytic lesions were also noted in previous reports from nondomestic felids: in the pelvis of a lion and jaguar and the vertebrae of a snow leopard. These findings support the hypothesis that osteolytic lesions of the axial skeleton are a relatively common finding in myeloma in nondomestic felids.^{5,8,11} The use of diagnostic imaging, both radiographs and MRI, proved useful in demonstrating bone lesions and should be considered in clinical cases presenting with vague clinical signs, particularly when hypergammaglobulinemia is present.

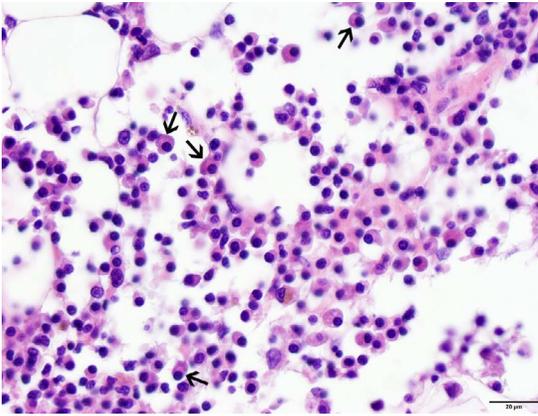


Figure 4. Plasma cell neoplasm within the L5 vertebrae, case 5. The bone marrow is replaced by sheets of neoplastic round cells with hyperchromatic nuclei and moderate amounts of eosinophilic cytoplasm with occasionally distinct perinuclear clearings (arrows). Hematoxylin and eosin (H&E).

Factors associated with poor prognosis include azotemia, which was present in three of the five tigers in this case series and Bence-Jones proteinuria, present in the four tigers that had the test performed. Poor prognosis is also associated with hypercalcemia, cytopenias, and leukemia, none of which were present in these cases.¹² Azotemia worsened over the course of disease in cases 1 and 2 but did not significantly change in case 3 despite surviving 7 mo after diagnosis. Renal impairment is a possible complication of myeloma, due to both toxic effects of the myeloma light chain proteins on proximal renal tubules and renal obstruction due to the accumulation of light chain proteins and subsequent formation of casts in distal tubules.¹ Although neoplastic cells were present in the kidney of case 1, other findings including tubular necrosis or protein casts were absent. In the affected cats, histologic changes of interstitial nephritis with fibrosis, typical of chronic renal disease in both domestic and nondomestic felids, suggests chronic renal disease likely contributed to the elevations in plasma BUN and Cre concentrations in these cases. Plasma globulin concentrations increased in each case where repeated bloodwork was available. Although it is difficult to assess the rate of this increase in relation to protein secreting neoplastic cells for use as a prognostic indicator,¹² it is interesting that the plasma globulin concentrations continued to increase irrespective of the treatment protocol used.

Treatment protocols and survival time for these cases was mixed, but the stage of disease at the

time of diagnosis was also variable. Case 3 survived 6 mo after initial diagnosis. Splenic plasmacytoma was initially diagnosed in this case via fine-needle aspirate antemortem; however, postmortem histopathology on the bone marrow was more consistent with a diagnosis of myeloma. A noted sequela of myeloma in humans is hypercoagulability, possibly due to the hyperglobulinemia.⁵ This may have been a factor in case 3, where the cause of death was a right atrioventricular thrombus. Case 4 died during recovery from anesthesia, following a splenectomy for a splenic rupture identified with ultrasound. This complication of myeloma in the spleen has not previously been reported in animals and is extremely rare in human cases.⁶ Cases 2 and 5 were euthanized after development of hind-limb neurological deficits, which occurred at 6 and 4 wk, respectively, from initial clinical signs of anorexia and lethargy. Case 1 was first presented after the development of neurological deficits, and the rapid deterioration led to euthanasia within 5 days. This high mortality rate is consistent with domestic species.¹²

Any adult tiger presenting with vague clinical signs of lethargy, anorexia, and/or peripheral neurologic deficits should be examined and blood work that includes a complete blood count, serum biochemistry profile, and urinalysis should be performed with particular attention to serum globulin concentrations. Histologic examination of tissue collected at necropsy should include the use of MUM1 immunohistochemical staining to distinguish this neoplastic disorder from mast cell tumors, lymphoma, or other round cell neoplasms.

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