https://doi.org/10.17221/152/2017-VETMED

Multiple myeloma associated with IgA lambda gammopathy and multiple myeloma oncogene 1 in a Yorkshire terrier

S. Kim[1], E. Son[2], S. Lee[2], S. Lee[2], H. Kim[1], M. Kang[1], C. Lee[1], H. Park[1]*

[1]Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea

[2]Jeil animal hospital, Seoul, Republic of Korea

*Corresponding author: parkhee@konkuk.ac.kr

ABSTRACT: An eight-year-old spayed female Yorkshire terrier was presented with a one-month history of conspicuous weight-bearing lameness in the right hindlimb, mild anorexia, intermittent vomiting and marked polydipsia and polyuria. Radiographs revealed circular radiolucent foci of variable size in the skeleton. Haematological and serum biochemistry examination revealed mild leucopoenia with severe neutropaenia, mild non-regenerative anaemia, moderate thrombocytopoenia, moderate hyperglobulinaemia, mild hypoalbuminaemia, mild azotaemia and moderate hypercalcaemia. Quantification of serum immunoglobulins revealed elevated IgA and IgG. Serum protein electrophoresis showed a broad appearance with a β-region spike. Plasma cells accounted for 7.6% of the cells in the bone marrow. Serum immunofixation electrophoresis (IFE) revealed IgA lambda gammopathy. Immunohistochemistry in the bone marrow was diffusely positive for multiple myeloma oncogene 1 (MUM-1) and CD20. To our knowledge, this is first case report of multiple myeloma associated with IgA lambda gammopathy confirmed via IFE and immunohistochemical expression of MUM-1 in a dog.

Keywords: dog; skeletal disease; pain; immunofixation electrophoresis; immunohistochemistry

List of abbreviations

A/G = albumin/globulin, **APTT** = activated partial thromboplastin time, **BUN** = blood urea nitrogen, **CREA** = creatinine, **Hb** = haemoglobin, **HCT** = haematocrit, **IFE** = immunofixation electrophoresis, **IHC** = immunohistochemical, **MCV** = mean corpuscular volume, **MM** = multiple myeloma, **MUM1** = multiple myeloma oncogene 1, **PT** = prothrombin time, **RBC** = red blood cells, **SPE** = serum protein electrophoresis, **WBC** = white blood cells

Multiple myeloma (MM) is characterised by the production of immunoglobulins by malignant plasma cells in the bone marrow (Sternberg et al. 2009; Flory and Rassnick 2010). MM is a systemic disease, and has been reported in humans, dogs, cats and horses (Pusterla et al. 2004; Patel et al. 2005; Flory and Rassnick 2010). It is a rare neoplasm in dogs and cats and MM accounts for only < 1% of malignant canine tumours (Giraudel et al. 2002). The clinical signs associated with canine MM include intermittent vomiting, anorexia, weight loss, lethargy and signs of skeletal disease (lameness, typical radiology, moth-eaten lesions). Most commonly affected are older dogs with a mean age of eight to 12 years at detection, and no breed or sex predilections exist (Flory and Rassnick 2010). In both humans (Boyle et al. 2014) and dogs (Sternberg et al. 2009), immunoglobulins generally include five types of heavy chains (IgG, IgA, IgM, IgD, and IgE) and two types of light chains (kappa and lambda). Each plasma cell produces only one type of heavy chain and one type of light chain. As in typical

Supported by the Bio and Medical Technology Development Program of the NRF funded by the Korean government, MSIP (Project No. 2016M3A9B6903437).

https://doi.org/10.17221/152/2017-VETMED

MM there is a clonal neoplastic population of plasma cells producing just one type of immunoglobulin; the distinction between heavy and light chains is highly relevant for the diagnosis and for monitoring the effects of therapy of MM (Andrea and Kenneth 2010; Katzmann et al. 2015). Commonly increased immunoglobulins in canine MM can be evaluated using serum protein electrophoresis (SPE) to detect the presence of a paraprotein (monoclonal protein) band (Jania and Andraszek 2016). The IgA lambda gammopathy described here was also validated using serum immunofixation electrophoresis (IFE); the use of this technique, as well as the confirmed expression of multiple myeloma oncogene 1 (MUM-1), are novel in the dog.

Case description

An eight-year-old, spayed female, Yorkshire terrier was presented with a one-month history of conspicuous weight-bearing lameness in the right hindlimb, mild anorexia, intermittent vomiting and marked polydipsia and polyuria. On clinical examination, the dog was underweight (body condition score 2/5, body weight 1.9 kg) and had pale mucous membranes and a mild elevated body temperature (39.6 °C). Generalised pain was noted on palpation and manipulation of the rear limbs. Neurological examination revealed proprioceptive deficits in the rear limbs.

Haematological examination showed mild leucopoenia with severe neutropaenia, mild normocytic normochromic non-regenerative anaemia (reticulocytes < 0.5%) with moderate thrombocytopaenia (Table 1). Results of serum biochemistry revealed mild hyperproteinaemia, mild hypoalbuminaemia, increased serum urea concentration, mildly increased creatinine (CREA), and moderate hypercalcaemia (Table 1). Urinalysis revealed isosthenuria (specific gravity 1.018, reference interval (RI), 1.015–1.045) with moderate proteinuria (1g/l, RI, negative). However, proteinuria for Bence Jones protein was not evident (IDEXX Laboratories, Inc., USA). Urine bacterial culture was negative. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were prolonged and D-dimer was within the normal reference range (Table 1). Results of SPE revealed an increase in β-globulin with normal α1, α2, and γ-globulin and low albumin (Table 2). SPE analysis showed a monoclonal spike migrating in the β region (Figure 1A, Antech Diagnostics, Inc., USA). Quantitative immunoglobulin tests revealed normal serum IgM and high IgA and IgG (Table 2). Serum IFE suggested the presence of monoclonal gammopathy with an IgA lambda type (Figure 1B). An infection was ruled out based on negative results of serum antigen to *Dirofilaria immitis*, antibodies to *Borrelia burgdorferi*, *Anaplasma phagocytophilum*,

Table 1. Abnormalities in complete blood count, serum chemistry and the coagulation test in the present case

Variables		Results	Reference interval
Complete blood count	WBC (× 10^9/l)	3	6.0–17.0
	neutrophil (× 10^9/l)	1.7	4.0–12.6
	lymphocyte (× 10^9/l)	1.1	0.8–5.1
	monocyte (× 10^9/l)	0.2	0.0–1.8
	HCT (Proportion of 1.0)	0.33	0.39–0.58
	RBC (10^{12}/l)	4.6	5.50–8.60
	Hb (g/l)	115	110–190
	MCV (fl)	71.9	62.0–72.0
	platelets (10^9/l)	53	120–480
Serum chemistry	serum urea concentrations (mmol/l)	19.35	3.28–10.42
	creatinine (umol/l)	132.6	35.36–123.76
	total protein (g/l)	79	50–72
	albumin (g/l)	22	26–33
	globulin (g/l)	57	28–42
	A/G ratio	0.38	0.8–1.7
	total calcium (mmol/l)	4.025	2.33–3.035
	phosphorus (mmol/l)	1.938	0.61–1.62
Coagulation tests	PT (s)	10.7	5.8–7.9
	APTT (s)	20.8	13.1–17.4
	D-dimer	0.1–0.3	0.3

A/G = albumin/globulin, APTT = activated partial thromboplastin time, Hb = haemoglobin, HCT = haematocrit, MCV = mean corpuscular volume, PT = prothrombin time, RBC = red blood cells, WBC = white blood cells

Table 2. Results of serum protein (SP) electrophoresis and quantitative immunoglobulin (QI) tests in the present case

Variables		Results	Reference interval
SP electrophoresis	albumin (g/l)	22.1 (28.2%)	26–33
	α1-globulin (g/l)	2.2 (2.8%)	2.0–5.0
	α2-globulin (g/l)	9.9 (12.5%)	3.0–11
	β-globulin (g/l)	40 (50.7%)	6.0–12
	γ-globulin (g/l)	4.6 (5.8%)	5.0–18
QI tests	IgA (g/l)	over 15	0.2–1.5
	IgM (g/l)	1.89	0.7–2.7
	IgG (g/l)	22	10–20

https://doi.org/10.17221/152/2017-VETMED

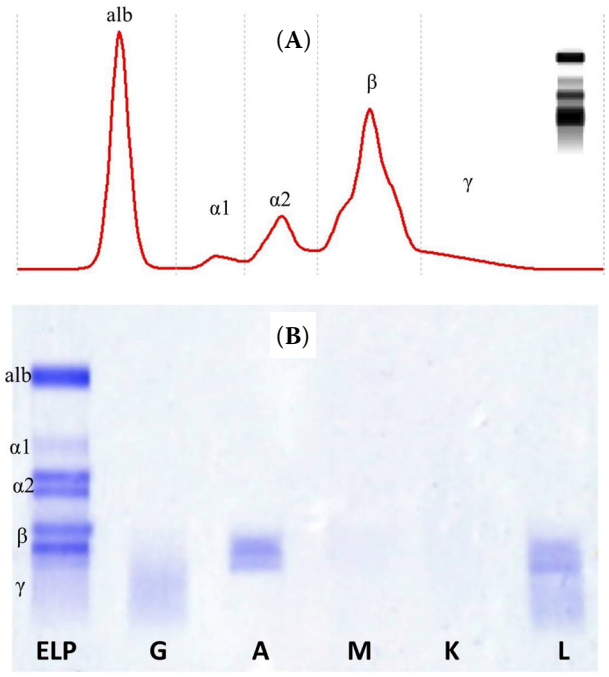

Figure 1. Schematic representation of serum protein electrophoresis (**A**) and immunofixation electrophoresis (**B**) results. (**A**) The monoclonal protein was identified as a monoclonal spike in the ß region. The broad appearance of the ß region was due to the superimposition of polyclonal immunoglobulins migrating with the monoclonal spike. (**B**) The subtype of the monoclonal protein was identified as bands (lanes A and L) on the immunofixation electrophoresis

alb = albumin; α1 = α1-globulin; α2 = α2-globulin; β = β-globulin; γ = γ-globulin; ELP = electrophoresis; G = IgG; A = IgA; M = IgM; K = kappa; L = lambda

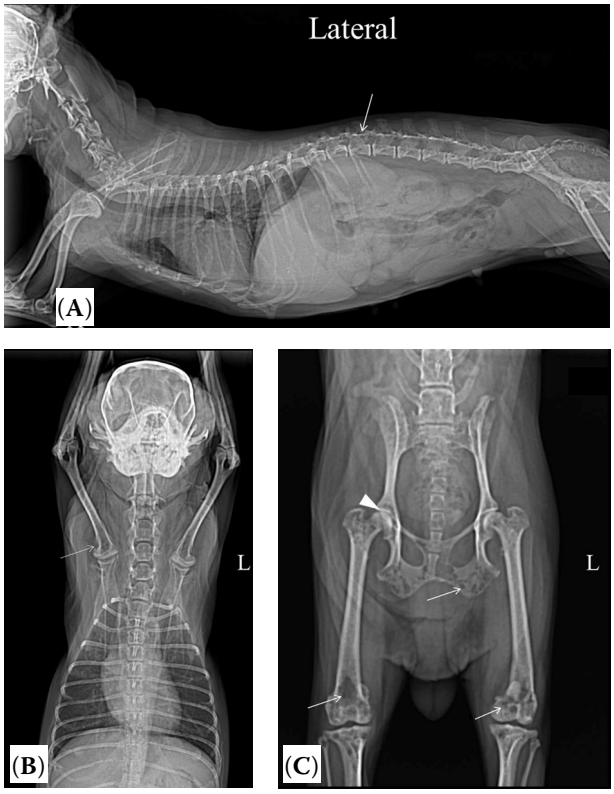

Figure 2. Lateral (**A**) and ventrodorsal (**B** and **C**) radiographs revealed multiple discrete radiolucent (punched-out) osteolytic lesions (arrows) of variable size affecting the skeleton (humerus, scapula, radius, ulna, vertebra, ilium, ischium, femora, tibia, fibula). Several small lucent foci were identified in regions of the proximal diaphysis, distal physis and epiphysis of the long bones and multiple spinous processes. (**C**) The right femoral head with a pathologic fracture (arrow-head)

Anaplasma platys, *Ehrlichia canis* and *Ehrlichia ewingii* (SNAP® 4Dx® Plus Test, IDEXX Laboratories, Inc.).

Imaging studies including radiographic examination of the skeleton revealed multiple discrete radiolucent foci (punched-out) of osteolytic lesions of variable size affecting multiple parts of the skeleton (humerus, scapula, radius, ulna, vertebra, ilium, ischium, femur, tibia, fibula), particularly the long bones (Figures 2A–2C). The right femoral head had a pathologic fracture (Figure 2C). Ultrasonographic examination revealed increased echogenicity of the cortex and corticomedullary junction and a hyperechoic band (medullary rim sign) in both kidneys and when compared to the spleen and liver parenchyma.

The cytology after bone marrow aspiration from the humerus showed hypocellularity (Figure 3). The myeloid : erythroid ratio was 1.90 : 1.00 (RI, 0.75–2.50 : 1.00). Plasma cells were characterised by an eccentric round nuclei, abundant deep blue cytoplasm and prominent, clear Golgi apparatuses (Figures 3A and 3B). Multinucleated plasma cells with anisokaryosis and round nuclei were arranged randomly (Figure 3C) and (Figure 3A and 3C) plasma cells with flame cell morphology, characterised by irregular and fragmented or rounded eosinophilic cytoplasmic projections were evident. Plasma cells accounted for 7.6% of the cells in the bone marrow. In a core bone marrow biopsy near the osteolytic lesion of the right ilium, bone marrow was replaced by an infiltrative, highly cellular neoplasm (IDEXX Laboratories, Inc., USA) (Figure 4A). The neoplasm was composed of round cells arranged in sheets supported by existing stroma. Neoplastic cells had distinct cell borders and amphophilic cytoplasm. Nuclei were round-to-oval with finely to coarsely stippled chromatin and one to two indistinct nucleoli. Mild anisocytosis and anisokaryosis were present (Figure 4A). We performed immunohistochemical (IHC) staining on bone marrow specimens

https://doi.org/10.17221/152/2017-VETMED

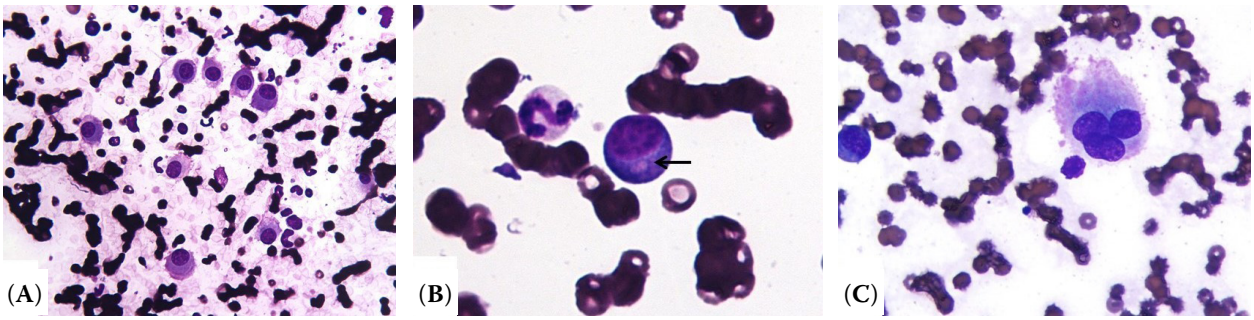

Figure 3. Cytology of the bone marrow aspirate taken from the humerus. (**A**) Plasma cells characterised by eccentric round nuclei, abundant deep blue cytoplasm, and a prominent clear Golgi apparatuses. (**B**) A plasma cell with a prominent clear Golgi apparatus (black arrow) and a neutrophil. (**C**) Multinucleated plasma cells with anisokaryosis and round nuclei arranged randomly, and (**A** and **C**) plasma cells with flame cell morphology, characterised by irregular and fragmented or rounded eosinophilic cytoplasmic projections. Rouleaux formation was visible on the bone marrow smear (**A**–**C**). Diff-quick stain, 200 × magnification (**A**), 630 × magnification (**B**), 400 × magnification (**C**)

from an osteolytic lesion for MUM1 (plasma cell marker), CD20 (B lymphocyte marker) and CD3 (T lymphocytes marker) (IDEXX Laboratories, Inc., USA). On IHC staining of a bone marrow biopsy from the radiolucent area of the ilium, approximately 70–80% of the neoplastic cells were positive for MUM-1 (Figure 4B) and approximately 5–10% were positive for CD20 (Figure 4C). However, neoplastic cells were diffusely negative for CD3 (Figure 4D). Therefore, the neoplasm could be diagnosed as multiple myeloma. Based on the above findings, this case was diagnosed as a MM IgA lambda gammopathy.

The dog was hospitalised and treated daily with oral melphalan (Alkeran, GlaxoWellcome, NC, 0.1 mg/kg, twice a day for ten days) and prednisolone (Solondo Tab.,

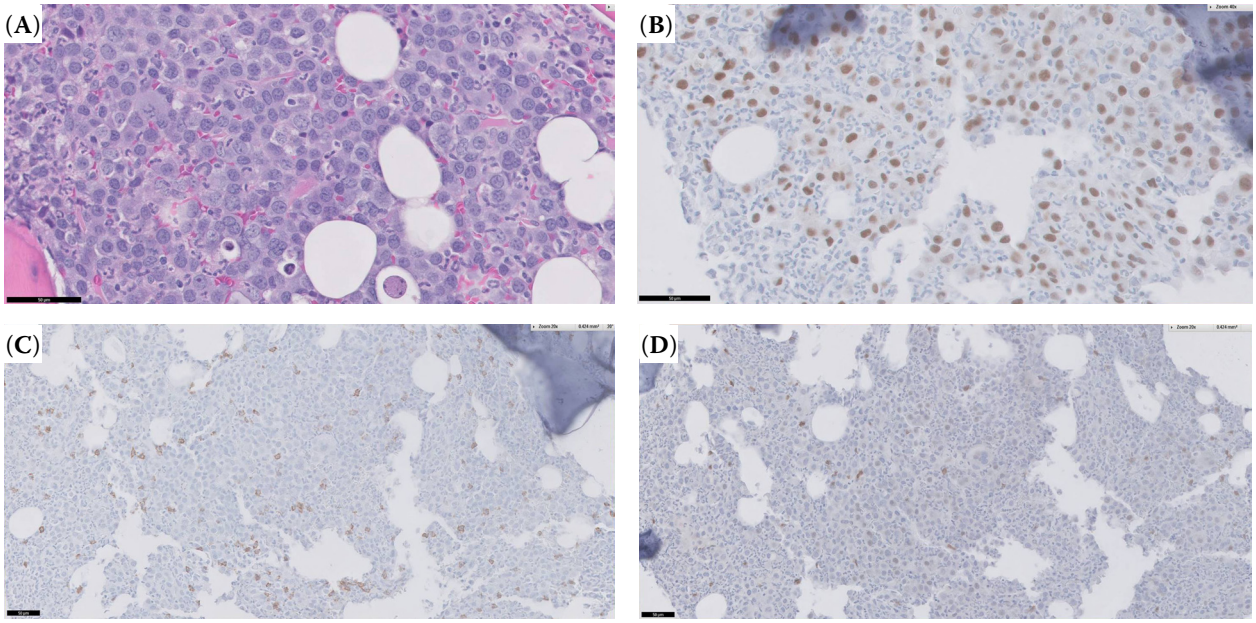

Figure 4. Histopathologic and immunohistochemical (IHC) characteristics of the bone marrow core biopsy from a lytic lesion in the left ilium. (**A**) The parenchyma is hypercellular (80%) with 20% adipose tissue. The neoplasm is composed of round cells arranged in sheets supported by existing stroma. Neoplastic cells have distinct cell borders and amphophilic cytoplasm. Nuclei are round-to-oval with finely to coarsely stippled chromatin and 1–2 indistinct nucleoli. Mild anisocytosis and anisokaryosis are present. H&E stain; bar = 50 µm. (**B**) IHC staining for multiple myeloma oncogene 1 (MUM-1), approximately 70–80% of the neoplastic cells are labelled (positive, brown colour). Bar = 50 µm. (**C**) IHC staining for CD20: Approximately 5–10% neoplastic cells are labelled (positive, brown colour). Bar = 50 µm. (**D**) IHC staining for CD3: neoplastic cells are diffusely negative. Bar = 50 µm

https://doi.org/10.17221/152/2017-VETMED

Yuhan, Republic of Korea, 0.5 mg/kg, twice a day for ten days). In addition, Tramadol HCl (Tridol Caps, Yuhan, Republic of Korea, 1 mg/kg, twice a day for seven days) was administered orally for pain relief. Saline diuresis was initiated (0.9% saline administered at 2.5 times the maintenance rate for 24 hours) for hypercalcaemia. After 72 hours, the serum calcium concentration had decreased to within the normal range. Amoxicillin-clavulanic acid (Amocla Tab, Kuhnil Pharm. 12.5 mg/kg, twice a day) was administered orally for ten days to prevent bacterial infections. During hospitalisation, the clinical signs improved. After ten days, the dog was discharged on a combination of melphalan (0.05 mg/kg, twice a day for four months) and prednisolone (0.5 mg/kg, every other day for 30 days). The dog tolerated treatment for 130 days without complications; however, it suffered a relapse with MM 153 days after the initial treatment. The owner declined further treatment due to financial concerns.

DISCUSSION AND CONCLUSIONS

The diagnostic criteria for MM in both humans (Rajkumar et al. 2014) and dogs (Flory and Rassnick 2010) has historically been based on the demonstration of at least two of the following: (1) monoclonal gammopathy or paraproteinaemia, (2) radiographic evidence of osteolytic bone lesions, (3) > 10% plasma cells in the bone marrow, and (4) Bence Jones proteinuria. This case was consistent with the criteria of MM, showing an lg spike on SPE and lytic bone lesions. In addition, MM-related findings included bone pain and abnormal features such as anaemia, hypercalcaemia, azotaemia, hypoalbuminaemia and demineralisation of the skeleton.

While a minimum of 10% bone marrow plasmacytosis is conventionally required to diagnose MM, the proportion of bone marrow plasma cells was 7.6% in this case. As histology was not diagnostic, we performed IHC staining of bone marrow specimens from an osteolytic lesion to confirm MM/neoplastic plasma cell proliferation (Brunnert and Altman 1991; Ramos-Vara et al. 2007). IHC results were diffusely positive for MUM-1 and CD20 and diffusely negative for CD3. MUM-1 has been identified as an oncogene in MM and is involved in lymphoid cell differentiation, particularly in the production of plasma cells. CD20 is expressed from the pre-B cell stage to the activated B cell stage. In human medicine (Sukpanichnant et al. 1994), 40% of 176 patients diagnosed with MM via histological and immunohistological techniques reportedly had less than 10% plasma cells in bone marrow aspirates. Compared to IHC methods, enumeration of plasma cells via routine staining of bone marrow aspirate smears may underestimate plasmacytosis, making the traditional 10% threshold problematic. These findings indicate the presence of plasma cell clonality in the bone marrow, and, therefore, a diagnosis of MM was made.

In this case, the broad appearance of the β region in the SPE cellulose acetate strip was difficult to interpret because of the superimposition of polyclonal immunoglobulins migrating with the monoclonal spike. Therefore, serum IFE was performed to identify the subtype of the monoclonal protein being produced by the myeloma cells. In both humans (Zamora-Ortiz et al. 2014) and dogs (Seelig et al. 2010), serum IFE is the gold standard for immunochemical characterisation and detection of M-proteins. Therefore, it is used as the main method for monitoring and diagnosing MM. In human medicine (Li et al. 2015), serum IFE was reported to be more sensitive than SPE and serum-free light chain analysis in the detection of unexpected protein bands and relapse in MM patients with autologous stem cell transplants. Therefore, the initial SPE should always be performed in combination with serum IFE to confirm monoclonality, as well as to determine the Ig heavy and light chain classes. As a result, IgA lambda monoclonal gammopathy was effectively determined by SPE and IFE.

Flame cells are characterised by abundant pink eosinophilic cytoplasm in plasma cells and are occasionally observed in reactive or neoplastic medical conditions in dogs and cats (Zinkl et al. 1983). To date, they have mainly been reported in association with IgA-producing plasma cells. Flame cells were identified in some plasma cells (Figures 4A and 4C). Therefore, the presence of flame cells and IgA in this case suggests that flame cells produce IgA.

This dog exhibited normocytic, normochromic cells and non-regenerative anaemia, leucopoenia with neutropaenia and concurrent thrombocytopenia. In such situations, pancytopaenia may be observed in conjunction with marked bone marrow infiltration of neoplastic cells (Sternberg et al. 2009). Hypercalcaemia in MM, such as in this case, is reportedly associated with tumour-induced osteolysis (Sternberg et al. 2009; Tripp et al. 2009), and hypercalcaemia and myeloma-related renal disease may have contributed to the observed polyuria and polydipsia. Renal insufficiency is most commonly associated with (Seelig et al. 2010): (1) excessive light chain production overwhelming the catabolic capacity of renal tubular cells, light chains forming complexes with proteins, resulting in tubular casts that can induce renal tubular obstruction and light chains that can induce inflammation and cytokine release, which may cause renal damage, (2)

https://doi.org/10.17221/152/2017-VETMED

hypercalcaemia leading to prerenal azotaemia secondary to antidiuretic hormone inhibition and eventual renal mineralisation (Sheafor et al. 1995), and (3) reduced renal perfusion due to hyperviscosity syndrome.

Although we here describe only one MM case with IgA gammopathy which could not be definitively diagnosed as MM because the ratio of plasma cells was less than 10%, the diagnosis was further confirmed by IFE and IHC analysis. The use of IFE and IHC analysis can help in diagnosing or ruling out MM.

REFERENCES

Boyle EM, Fouquet G, Guidez S, Bonnet S, Demarquette H, Dulery R, Herbaux C, Noel MP, Manier S, Schraen S, Onraed B, Faucompre J, Hennache B, Petillon MO, Mathiot C, Avet-Loiseau H, Facon TJ, Harding S, Moreau P, Leleu X (2014): IgA kappa/IgA lambda heavy/light chain assessment in the management of patients with IgA myeloma. Cancer 120, 3952–3957.

Brunnert SR, Altman NH (1991): Identification of immunoglobulin light chains in canine extramedullary plasmacytomas by thioflavine T and immunohistochemistry. Journal of Veterinary Diagnostic Investigation 3, 245–251.

Flory AB, Rassnick KM (2010): Multiple myeloma and plasma cell tumors. In: Cote E (ed.): Clinical Veterinary Advisor: Dogs and Cats. 2nd edn. Elsevier Health Sciences. 730–732.

Giraudel JM, Pages JP, Guelfi JF (2002): Monoclonal gammopathies in the dog: a retrospective study of 18 cases (1986–1999) and literature review. Journal of the American Animal Hospital Association 38, 135–147.

Jania B, Andraszek K (2016): Application of native agarose gel electrophoresis of serum proteins in veterinary diagnostics. Journal of Veterinary Research 60, 501–508.

Katzmann JA, Willrich MA, Kohlhagen MC, Kyle RA, Murray DL, Snyder MR, Rajkumar SV, Dispenzieri A (2015): Monitoring IgA multiple myeloma: immunoglobulin heavy/light chain assays. Clinical Chemistry 61, 360–367.

Li W, Zhou JZ, Chang HR, Dai LJ, Zhu ZL, Feng YF, Wu DP (2015): Comparison of SPE, IFE, and FLC in monitoring patients with multiple myeloma after autologous stem cell transplantation. Cell Biochemistry and Biophysics 73, 643–647.

Patel RT, Caceres A, French AF, McManus PM (2005): Multiple myeloma in 16 cats: a retrospective study. Veterinary Clinical Pathology 34, 341–352.

Pusterla N, Stacy BA, Vernau W, De Cock HE, Magdesian KG (2004): Immunoglobulin A monoclonal gammopathy in two horses with multiple myeloma. Veterinary Record 155, 19–23.

Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, Kumar S, Hillengass J, Kastritis E, Richardson P, Landgren O, Bruno Paiva B, Dispenzieri A, Weiss B, LeLeu X, Zweegman S, Lonial S, Rosinol L, Zamagni E, Jagannath S, Sezer O, Kristinsson SY, Caers J, Usmani SZ, Lahuerta JJ, Johnsen HE, Beksac M, Cavo M, Goldschmidt H, Terpos E, Kyle RA, Anderson KC, Durie BGM, Miguel JFS (2014): International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncology 15, e538–e548.

Ramos-Vara JA, Miller MA, Valli VEO (2007): Immunohistochemical detection of multiple myeloma 1/interferon regulatory factor 4 (MUM1/IRF-4) in canine plasmacytoma: comparison with CD79a and CD20. Veterinary Pathology 44, 875–884.

Seelig DM, Perry JA, Avery AC, Avery PR (2010): Monoclonal gammopathy without hyperglobulinemia in 2 dogs with IgA secretory neoplasms. Veterinary Clinical Pathology 39, 447–453.

Sheafor SE, Gamblin RM, Couto CG (1995): Hypercalcemia in two cats with multiple myeloma. Journal of the American Animal Hospital Association 32, 503–508.

Sternberg R, Wypij J, Barger AM (2009): Overview of multiple myeloma in dogs and cats. Veterinary Medicine 104, 468–476.

Sukpanichnant S, Cousar JB, Leelasiri A, Graber SE, Greer JP, Collins RD (1994): Diagnostic criteria and histologic grading in multiple myeloma: histologic and immunohistologic analysis of 176 cases with clinical correlation. Human Pathology 25, 308–318.

Tripp CD, Bryan JN, Wills TB (2009): Presumptive increase in protein bound serum calcium in a dog with multiple myeloma. Veterinary Clinical Pathology 38, 87–90.

Zamora-Ortiz G, Velazquez-Sanchez-de-Cima S, Hernandez-Reyes J, Martagon-Herrera NA, Ruiz-Arguelles A, Ruiz-Delgado GJ, Ruiz-Arguelles GJ (2014): Poor performance of the total kappa/lambda light chain quantification in the diagnosis and follow-up of patients with multiple myeloma. Revista de Investigacion Clinica 66, 314–318.

Zinkl JG, LeCouteur RA, Davis DC, Saunders GK (1983): "Flaming" plasma cells in a dog with IgA multiple myeloma. Veterinary Clinical Pathology 12, 15–19.

Received: November 30, 2017
Accepted after corrections: March 25, 2018