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Case report

MULTIPLE MYELOMA WITH SKIN AND RENAL INVOLVEMENT AND *ANAPLASMA PHAGOCYTOPHILUM* CO-INFECTION IN A DOG

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An 11-year-old male Cocker Spaniel was presented with acute onset of apathy, polyuria and polydipsia. Blood examination showed hypoalbuminemia and hyperglobulinemia, while electrophoresis revealed monoclonal gammopathy. Subsequent tests showed glycosuria, proteinuria, a positive serology test for *Anaplasma spp.* and ultrasonographical changes of the liver and spleen. Urine electrophoresis confirmed the presence of Bence-Jones protein. The dog's condition deteriorated, and the dog had developed skin lesions on the neck and body. The owner decided to euthanize the dog and agreed to bone marrow and skin biopsy. Bone marrow cytology revealed an increased number of plasma cells and several cellular atypia. Histopathology of the skin lesions showed a round cell tumor of lymphoid or plasmacytoid origin. Subsequent immunohistology supported the diagnosis of multiple myeloma cutaneous involvement.

This case report describes unusual features observed in a dog with multiple myeloma.

Keywords: anaplasmosis; glycosuria; monoclonal gammopathy; plasma cell; skin metastasis

INTRODUCTION

Multiple myeloma (MM) is an uncommon neoplasm in dogs [1,2]. It is characterized by a proliferation of malignant plasma cells, which usually results in an excessive production of immunoglobulin [1-3]. Its prevalence is 1% of all neoplastic cases in dogs [1,4-7]. Commonly, it develops in older dogs (on average 8 to 9-years old) and sex predisposition is not observed [3,8,9]. Clinical signs result from hyperglobulinemia or neoplastic infiltration in the organs and bones [8,10]. Medical history usually reports

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lethargy, weakness, lameness, bleeding diathesis, polyuria/polydipsia, ophthalmologic problems or neurologic deficits [6,8-12].

The etiology of this disease is unknown. Environmental carcinogens exposure, chronic antigenic stimulation, and viral and genetic involvement are considered to be possible predisposing factors [3,8,10]. Rarely, there are reports of cutaneous plasmacytoma progression to multiple myeloma [3,13,14]. However, a study by Boostrom et al. (2017) did not report any MM development from 21 cutaneous plasmacytoma cases [15].

Diagnostics of MM involve peripheral blood and bone marrow examination and diagnostic imaging. Hypercalcemia, hypoalbuminemia, anemia, leucopoenia and thrombocytopenia are often seen in patients with MM, but the most common laboratory finding is hyperglobulinemia with inversion of the albumin to globulin ratio [2,3,8,12]. This is caused by increased production of immunoglobulins (Ig) by the neoplastic clones of plasma cells [1,2]. Subsequent electrophoresis reveals a monoclonal component [2]. Multiple myeloma is the main cause of monoclonal gammopathy [1]. Differential diagnoses for monoclonal gammopathy involve lymphopoietic tumors, amyloidosis, chronic pyoderma, ehrlichiosis, leishmaniasis or MGUS (monoclonal gammopathy of undetermined significance) [1-4,8,10,16]. Accumulation of paraprotein can result in coagulopathy, hyperviscosity syndrome and glomerulopathy [2,8]. For example, bleeding was observed in 42.3% of patients with MM [6].

Osteolysis is often observed, and it is caused by cytokines produced by neoplastic plasma cells, which support survival of osteoclasts, and their activity [2,3,8,10]. This can lead to pain of the affected bones, lameness or pathological fractures [2,8,13]. Osteolysis is present in 50% cases of MM [1,3,9], while the vertebrae can be affected in 25% of dogs with MM [13]. Some patients could suffer from general osteopenia without any focal lesions [13].

The key diagnostic method is bone marrow examination, which assesses the amount of plasma cells in this organ [8].

There are four diagnostic criteria for MM diagnosis establishing [1,2,4,7]:

– Markedly increased numbers of plasma cells in the bone marrow – over 20% [8]
– Monoclonal gammopathy
– Radiographic evidence of osteolysis
– Light chain proteinuria (Bence Jones protein).

Multiple myeloma is diagnosed if two or more criteria are fulfilled [1,2].

Dogs suffering from MM have a median survival time of 1.5 years [4,6]. Negative prognostic factors involve the presence of osteolytic lesions, hypercalcemia, Bence Jones proteinuria, and lack of bleeding diathesis [1,4,5,6,17].

Our case report describes a patient suffering from multiple myeloma with unusual features, such as the development of cutaneous lesions and glycosuria. These clinical signs are not commonly described in MM patients.

CASE PRESENTATION

The patient was an 11-year-old male Cocker Spaniel. The owner noticed he was apathetic, had polyuria and polydipsia, and was shaking while resting. No jerky treats were administered. Clinical examination revealed a mild hypotrichosis and an enlarged left cervical superficial lymph node. Other findings were unremarkable.

Hematology (ProCyte Dx Hematology Analyzer; ®IDEXX Laboratories, Inc, Hoofddorp, Netherlands) showed a mild non-regenerative anemia and monocytosis, while biochemistry (Cobas c111; ®Roche Diagnostics, Basel, Switzerland) revealed a low albumin level (20.1 g/l) and an increased amount of total protein (73.7 g/l). Total calcium level was within reference range; however, chloride and sodium levels were decreased. The glucose level was within the reference range as well. Total T4 was in the normal reference range (RIA analysis, RIA Laboratorium, Kosice, Slovakia) (26.5 nmol/l). Complete blood results are shown in Table 1.

Table 1. Overview of hematology and biochemistry results

Hematology	Result	Units	Ref. range
RBC	**4.89*10^{12}**	/l	5.65-8.87*10^{12}
HCT	**30.7**	%	37.3-61.7
HGB	**10.5**	g/dl	13.1-20.5
Retic	41.1	K/µl	10.0-110.0
WBC	12.4*10^{9}	/l	5.05-16.76*10^{9}
Neu	7.71*10^{9}	/l	2.95-11.64*10^{9}
Lym	1.94*10^{9}	/l	1.05-5.10*10^{9}
Mono	**2.61*10^{9}**	/l	0.16-1.12*10^{9}
Eos	0.13*10^{9}	/l	0.06-1.23*10^{9}
Baso	0.01*10^{9}	/l	0.00-0.10*10^{9}
PLT	263	K/µl	148-484
Biochemistry			
ALT	0.4	µkat/l	0-0.949
ALP	0.74	µkat/l	0-1.24
Crea	75.6	µmol/l	46-88
Urea	5.44	mmol/l	3.97-8.05
TP	73.7	g/l	47-74
Alb	**20.1**	g/l	26-41
Glu	4.69	mmol/l	3.6-5.8
Chol	7	mmol/l	3.3-7.4
TG	0.48	mmol/l	0.3-1.26
P	1.17	mmol/l	0.90-1.91
Ca	2.47	mmol/l	2.05-2.86
Cl	**109.5**	mmol/l	110-130
Na	**141.3**	mmol/l	143-151
K	4.33	mmol/l	3.5-5.1
Total T4	26.5	nmol/l	13-51

Ultrasound examination (ALOKA ProSound alpha 6; ®Hitachi Aloka Medical LTD, Tokyo, Japan) showed heteroechogenic parenchyma of the liver with mild hepatomegaly. One hypoechogenic nodulus in the spleen and a small amount of sediment in the urinary bladder were also present.

Urine examination showed decreased specific gravity (1.020), and dipstick analysis (Dekaphan Leuco diagnostic strips) showed pH 7 and a presence of erythrocytes or haemoglobin (2+), proteins (1+) and glucose (4+). Ketonuria was negative. The urine sediment was unremarkable. There were no erythrocytes or leucocytes present. UPC (protein to creatinine ratio) was increased (7.42) (Measured on Cobas c111; ®Roche Diagnostics, Basel, Switzerland).

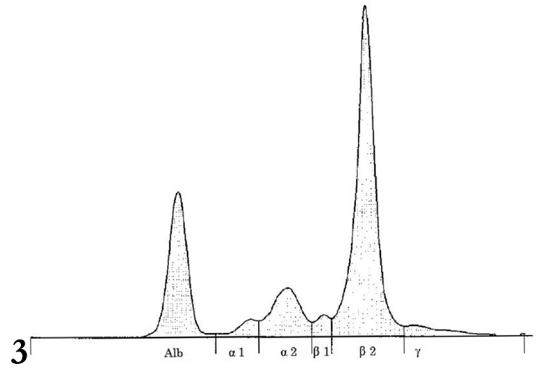
SNAP 4Dx Plus Test (®IDEXX Laboratories, Inc. Hoofddorp, Netherlands), a screening test for vector-borne diseases, was performed and resulted in a positive finding of *Anaplasma spp*. The dog was treated with doxycycline at a dose of 10 mg/kg once daily for 28 days. Subsequent serology of the two serum samples taken 4 weeks apart showed that the antibody titer for *Anaplasma phagocytophilum* decreased markedly. The antibody titer before treatment was 11.55 TE, while the titer after 4 weeks of treatment was 0.27 TE (negative is less than 8 TE) (ELISA technique, LABOKLIN GmbH & Co. KG; Bad Kissingen, Germany). PCR examination for *Anaplasma spp*. in the first sample was performed post mortem with a negative result (PCR method, laboratorium SAV; Košice, Slovakia).

The patient's condition deteriorated and several skin lesions developed on the face and the left side of the body (Figure 1, 2). The skin lesions were comprised of multiple cutaneous nodules with their size being in between 0.5 cm to 2 cm, which had a firm consistency and red coloration. The left cervical superficial lymph node and both popliteal lymph nodes were enlarged. Other lymph nodes, including axillary lymph nodes, were normal. Subsequent blood examination showed increased total proteins (80.5 g/l) while the albumin level was decreased (20.4 g/l). Serum electrophoresis (diagnostic kits Hydragel 7 Proteine, Sebia Corporate, Lisses, Evry Cedex, France) showed a monoclonal peak in the beta region (Figure 3). Based on these findings, the



Figure 1 and 2. Skin lesions on the head and the neck.
Author: MVDr. Hana Turna

urine sample was examined by electrophoresis (performed at LABOKLIN GmbH & Co. KG; Bad Kissingen, Germany). This examination found a prominent band in the gamma globulin region, which indicated the presence of Bence Jones proteinuria (Figure 4).



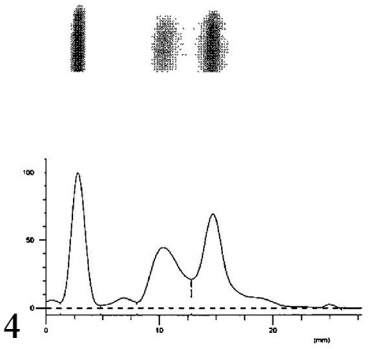


Figure 3. Serum electrophoresis – monoclonal gammopathy in a beta-globulin region.
Author: MVDr. Csilla Tothova, PhD.

Figure 4. Urine electrophoresis - prominent band in gamma globulin region.
Source: LABOKLIN GmbH & Co. KG; Bad Kissingen, Germany

Subsequently, X-rays of the long bones and vertebrae were conducted; however, there were no signs of osteolysis.

Diagnosis of multiple myeloma was proposed and a bone marrow examination was recommended. The owner declined further diagnostic tests and decided upon euthanasia of the patient. Biopsies of bone marrow and skin lesions were performed with the owner's consent immediately after euthanasia. Samples were sent to a specialized laboratory (LABOKLIN GmbH & Co. KG; Bad Kissingen, Germany). Bone marrow cytology (Diff-Quik-staining) showed an increased number of non-plasmatic plasma cells (20%), and several cells showed cellular atypia, such as mild anisokaryosis, prominent nuclei and presence of binucleated cells. In addition, PCR of bone marrow for *Leishmania infantum* was performed and reported a negative result (LABOKLIN GmbH & Co. KG; Bad Kissingen, Germany).

Two skin samples obtained by excisional biopsy were fixed by 4% paraformaldehyde solution and sent for histopathological evaluation. Histopathology (Hematoxylin and eosin staining, Giemsa staining; LABOKLIN GmbH & Co. KG; Bad Kissingen, Germany) of the skin lesions confirmed the presence of a cutaneous round cell tumor of lymphoid or plasmacytoid origin (Figure 5 and 6). Morphologic similarity of the samples suggested that these lesions were part of the same malignant process. However, the tumor was poorly differentiated.

Immunohistology of skin lesions was carried out (LABOKLIN GmbH & Co. KG; Bad Kissingen, Germany). The neoplastic round cells showed strong immuno-positive

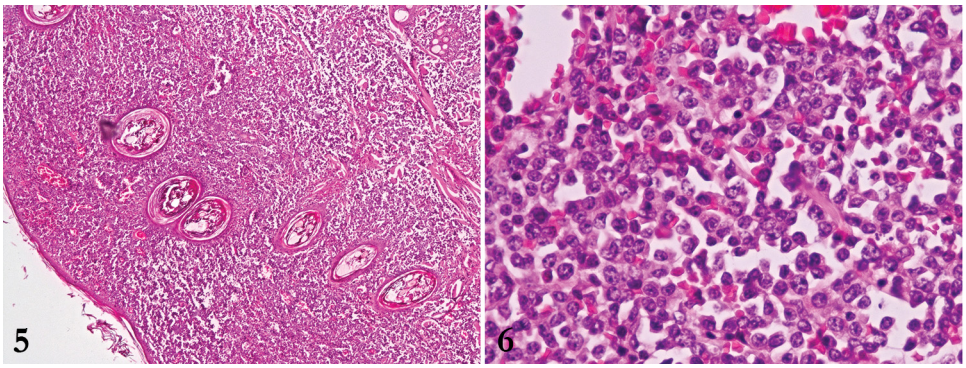


Figure 5. Histopathology of the skin lesion, H&E stain, magnification 4 x.
Author of the photo: Ines Hoffmann, MSc, LABOKLIN GmbH & Co. KG
Figure 6. Histopathology of the skin lesion, H&E stain, magnification 40 x.
Description: multifocal nodular expansion of the dermis by sheaths of round cells. The cells have distinct boundaries, moderate amounts of eosinophilic cytoplasm and round nuclei with one to three nucleoli. There are up to 3 mitoses per hpf.
Author of the photo and the description: Ines Hoffmann, MSc, LABOKLIN GmbH & Co. KG

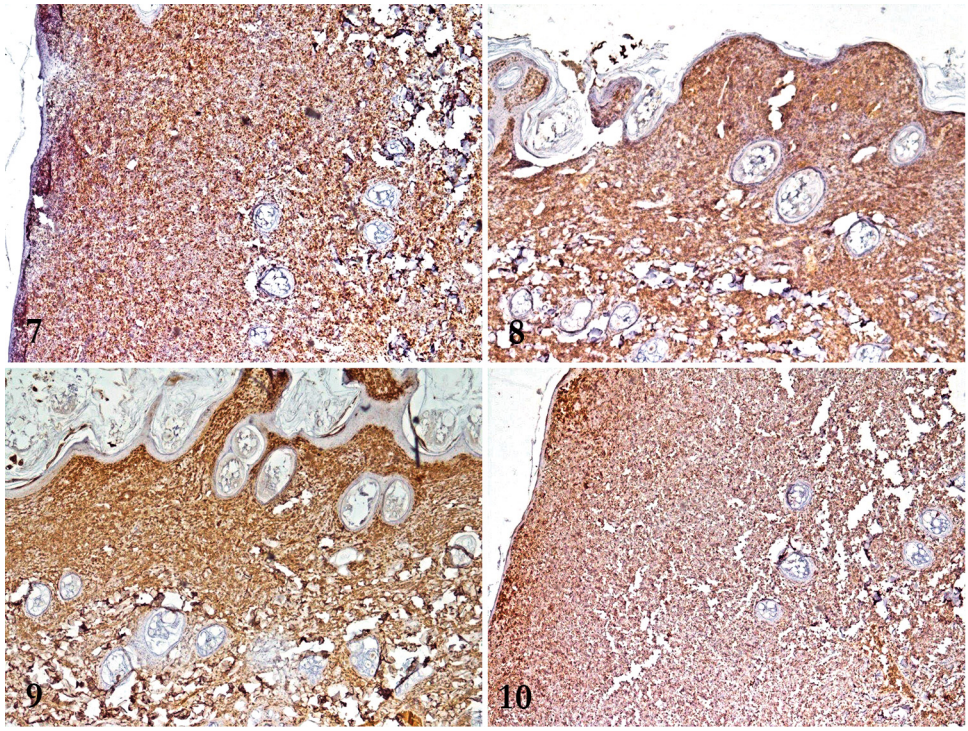


Figure 7. Immunohistology of skin lesions with strong immuno-positive staining for CD79a, magnification 4 x. Author of the photo: Ines Hoffmann, MSc, LABOKLIN GmbH & Co. KG
Figure 8. Immunohistology of skin lesions with strong immuno-positive staining for CD20, magnification 4 x. Author of the photo: Ines Hoffmann, MSc, LABOKLIN GmbH & Co. KG
Figure 9. Immunohistology of skin lesions with strong immuno-positive staining for IgG, magnification 4 x. Author of the photo: Ines Hoffmann, MSc, LABOKLIN GmbH & Co. KG
Figure 10. Immunohistology of skin lesions with strong immuno-positive staining for lambda light chains, magnification 4 x. Author of the photo: Ines Hoffmann, MSc, LABOKLIN GmbH & Co. KG

staining for CD79a (Figure 7), CD20 (Figure 8), IgG (Figure 9) and lambda light chains (Figure 10). The neoplastic cells did not express CD3. Valli et al. (2016) states that neoplastic cells in most canine cases of MM stain positively with CD79a while they are negative with CD3 [3]. Therefore, the immuno-positive staining supports a diagnosis of multiple myeloma cutaneous involvement.

DISCUSSION

This article describes uncommon clinical features in a dog with multiple myeloma. Cutaneous involvement with this neoplasm is seen rarely in humans [18], and there are only a few published cases involving multiple myeloma connected with skin lesions in a dog [19-21]. In humans, cutaneous involvement with MM is associated with a worse prognosis [18]. Most patients die within 12 months of the diagnosis, and autopsy often reveals extensive plasmacytic infiltration in several organs [18]. Previous case reports of MM with cutaneous involvement in dogs also reported a short survival time despite the administered therapy [19-21]. Mayer et al. (2008) proposed that cutaneous involvement with multiple myeloma can be a negative prognostic factor in canine patients as well as in humans, where it is a sign of advanced disease and a high tumor burden. [20] Presented case also reports significant deterioration of the patient condition in the time of skin lesion development.

Renal disease is common in multiple myeloma [8,10], renal insufficiency and proteinuria are observed most often, however, Fanconi syndrome can also be seen rarely [22]. Fanconi syndrome is caused by monoclonal light chains, which are excessively filtrated by the kidneys [22,23]. These light chains are usually composed of kappa light chains, which form crystals in the cytoplasm of proximal tubular epithelial cells [23]. Crystal formation induces cytotoxicity, which results in proximal tubular dysfunction and subsequently in Fanconi syndrome [23]. There are several reports of Fanconi syndrome connected with MM in humans [23-27], but there are no reports involving canine patients. Unfortunately, a test for aminoaciduria was not performed in this case, so Fanconi syndrome cannot be confirmed. However, glycosuria is one of the typical findings connected with this syndrome. Although renal histology was not performed, it is probable that the glycosuria in the patient was connected with multiple myeloma given the acute onset of polyuria and polydipsia and the presence of light chain (Bence Jones) proteinuria. To the author's knowledge, this is the first reported glycosuria concurrent with multiple myeloma in a dog.

Another interesting finding involves the concurrent anaplasmosis of the patient. A hypothesis by Kallick et al. (2015) proposed that Ehrlichia and Anaplasma infection could alter the immune system in humans and cause its dysfunction resulting in a wide variety of diseases such as leukemia, multiple myeloma, or myelodysplastic and autoimmune disorders [28]. In addition, Sukumaran et al. (2005) found that *Anaplasma phagocytophilum* infection alters neutrophil gene expression [29]. Some changes observed in their study were similar to the ones seen in patients with leukemia

[29]. This hypothesis is supported by a case report published by Geigy et al. (2013) that describes a dog with a history of multiple chronic vector-borne infections that subsequently developed multiple myeloma [30]. The patient had persistently increased titers of antibodies to *Ehrlichia canis* and *Anaplasma phagocytophilum* [30].

The patient in the presented case report had a negative PCR blood test for *Anaplasma spp*. but had an increased antibody titer that decreased after 4 weeks. Positive serologic results suggest a past or current infection; however, the negative PCR test from blood sample indicates a lack of active infection [31]. Therefore, these results could indicate previous infection with *Anaplasma phagocytophilum* in the patient. Based on previously mentioned hypotheses, this infection could be a predisposing factor for development of MM in the patient.

CONCLUSION

The presented case report shows uncommon clinical features connected with multiple myeloma in a dog. The skin lesions probably resulted from massive infiltration of neoplastic cells within the body, while excessive light chain production could lead to tubular dysfunction, which resulted in glycosuria. The presence of chronic *Anaplasma phagocytophilum* infection highlighted the necessity to search for pre-existing vector-borne infections, which can be predisposing factors in multiple myeloma patients.

Authors' contributions

HT was the attending physician of the dog and wrote the manuscript. CT carried out the electrophoresis. MK and AV performed biopsy of the bone marrow and the skin. MF carried out the diagnostic imagining (USG and X-ray). SG carried out urine analysis. MT helped with laboratory analysis interpretation and helped to draft the manuscript. MS helped with interpretation of the results and revised the manuscript critically.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

the owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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MULTIPLI MIJELOM SA PRISUTNIM PROMENAMA NA KOŽI I BUBREZIMA I KOINFEKCIJOM *ANAPLASMA PHAGOCYTOPHILUM* KOD PSA

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Prikazan je slučaj jedanaestogodišnjeg mužjaka koker španijela koji je doveden na pregled zbog nagle pojave apatije, poliurije i polidipsije. Pregledom krvi utvrđena je hipoalbuminemija i hiperglobulinemija, dok je elektroforeza pokazala monoklonsku gamapatiju. Naknadni testovi su pokazali glukozuriju, proteinuriju, pozitivan serološki test na *Anaplasma* spp. i ultrazvučne promene jetre i slezine. Elektroforeza urina potvrdila je prisustvo Bence-Jones proteina. Stanje psa se pogoršalo, a pas je imao lezije na koži na vratu i trupa. Vlasnik je odlučio da eutanazira psa i pristao je na biopsiju koštane srži i kože. Citološki pregled koštane srži otkrila je povećan broj plazma ćelija i prisustvo atipičnih ćelija. Histopatološka analiza promena na koži je pokazala okruglućelijski tumor limfoidnog ili plazmacitnog porekla. Naknadna imunohistohemijska ispitivanja su potvrdila dijagnozu multiplog mijeloma kože.

Ovaj prikaz slučaja opisuje neobične karakteristike uočene kod psa sa multiplim mijelomom.