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CASE REPORT
Myeloma-related disorder with leukaemic progression in a cat

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A 10-year-old American Shorthair cat with nasal discharge, anorexia, and weight loss was found to have pancytopenia and hyperproteinaemia. Bone marrow aspiration revealed atypical plasma cells that totalled 50% of the nucleated bone marrow cells. The number of atypical plasma cells progressively increased in the peripheral blood during the observation period of 64 days. The cat did not respond to treatments with melphalan, chlorambucil, and prednisolone, and died 71 days after the initial presentation. Clinical, cytological, histopathological, and immunohistochemical findings in this case supported the diagnosis of myeloma-related disorder (MRD) with leukaemic progression.

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Historical diagnostic criteria for multiple myeloma (MM) in veterinary medicine require at least two of the following: paraproteinaemia/monoclonal gammopathy, radiographic evidence of osteolytic bone lesions, Bence-Jones proteinuria, and increase in the number of neoplastic plasma cells to more than 5% of the nucleated bone marrow cells.[1,2] However, MM and advanced extramedullary plasmacytoma cannot always be distinguished by these criteria; therefore, the diagnostic criteria for MM in cats remain controversial.[3–5] Recently, Mellor et al proposed an all-encompassing term 'myeloma-related disorders (MRD)' for neoplasms of plasma cell origin in cats, and indicated that cats with MRD commonly had extramedullary involvement at the initial clinical presentation[6] while human patients with the MM did not.[7,8] In the present report, we describe a feline case of MRD showing a progressive increase in the number of atypical plasma cells in the peripheral blood.

A 10-year-old neutered male American Shorthair cat was referred to the Veterinary Medical Center of the University of Tokyo with a 2-week history of nasal discharge, anorexia, and weight loss. On physical examination, the body weight was 6.4 kg (body condition score: 2/5), and visible mucous membranes were slightly pale. A complete blood cell count measured with a multiple automated haematology analyser (pocH-100iV diff; Sysmex, Hyogo, Japan) revealed non-regenerative anaemia and thrombocytopenia (Table 1). On a peripheral blood smear, plasmacytosis (2% of blood leukocytes) and mild rouleaux were noted. The blood chemical profile measured with an automated biochemical analyser (Dri-Chem 7000 V; FUJIFILM, Tokyo, Japan) indicated hyperproteinaemia, increases in alanine aminotransaminase and alkaline phosphatase activity, and increased blood urea nitrogen (Table 1). Serum free T_4 level measured by an automatic immunoassay analyser (IMX system; Abbott Diagnostics, North Chicago, IL) was below the reference interval at 0.88 ng/dl; (reference interval[9] 1–2.5 ng/dl) which supports the effects of other illness on thyroid levels. Tests for feline leukaemia virus antigen and feline immunodeficiency virus antibody were both negative (Snap FeLV/FIV Combo Test: Idexx, Westbrook, ME). A test for serum antibody directed to feline coronavirus was also negative (Marupi Lifetec, Osaka, Japan).

Thoracic and abdominal radiographs revealed cardiomegaly, hepatomegaly, and splenomegaly.

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Table 1. Haematological values and blood chemistry profiles.

Blood examination	Day 1	Day 17	Day 29	Day 36	Day 52	Day 64	Reference ranges*
Red blood cells ($\times 10^6$/μl)	3.92	5.76	4.82	4.20	5.77	3.67	NA
Haemoglobin (g/dl)	5.1	7.6	6.4	5.8	8.3	5.3	8.0–15.0
Packed cell volume (%)	17.0	23.0	18.0	17.0	23.0	17.0	30.0–45.0
White blood cells (μl)	3600	2300	2400	3800	6400	7800	5000–19,000
Neutrophils, band (μl)	0	40	140	190	NT	470	0–300
Neutrophils, segmented (μl)	2060	1310	1370	1630	NT	3,820	2000–12,000
Lymphocytes (μl)	1110	140	190	490	NT	80	1000–7000
Monocytes (μl)	220	0	50	110	NT	390	0–1000
Eosinophils (μl)	140	70	50	0	NT	0	0–1000
Basophilis (μl)	0	0	0	0	NT	0	NA
Atypical plasma cells (μl)	70	740	580	1330	NT	2,340	NA
Atypical plasma cells (%)	2	32	24	35	NT	30	NA
Platelets ($\times 10^3$/μl)	11	81	25	13	34	11	200–700
Blood urea nitrogen (mg/dl)	37.8	NT	NT	NT	284.5	169.0	10.0–30.0
Creatinine (mg/dl)	1.6	NT	NT	NT	9.9	7.4	0.8–2.0
Alkaline phosphatase (U/l)	381	NT	NT	NT	NT	356	<200
Alanine aminotransferase (U/l)	247	NT	NT	NT	NT	37	<80
Total protein (g/dl)	10.8	10.8	8.4	8.2	9.8	8.0	5.5–7.9
Albumin (g/dl)	3.5	NT	3.2	NT	3.5	NT	2.1–3.4
Ca (mg/dl)	11.4	NT	NT	NT	8.2	NT	8.0–12.0

NT = not tested, NA = not available.
*The reference ranges were based on '*Hematology*'[10] by Susan M Cotter for haematologic values and '*Laboratory profiles of small animal diseases*, 3rd edn'[9] for blood chemistry profiles.

Echocardiography revealed thickening of the interventricular septum and left ventricular free wall and dilatation of the left atrium. Radiographic examinations of extremities, vertebrae and costae revealed no osteolytic lesion. Abdominal ultrasonography demonstrated a mixed echoic mass with a diameter of 2.5 cm in the spleen as well as an ascites and dilated hepatic veins.

The cat was initially treated with prednisolone (Predonine; Shionogi, 0.8 mg/kg, PO, q12 h) and furosemide (Lasix; Sanofi-Aventis, 0.7 mg/kg, PO, q12 h) in addition to intravenous whole blood transfusions (40–60 ml) four times until day 16.

On day 17, atypical plasma cells, which contained a round or pleomorphic nucleus, moderately basophilic cytoplasm, and poorly defined perinuclear Golgi zone, were observed on the peripheral blood smear, accounting for 32% of the blood leukocytes (Fig 1A and Table 1). In order to stabilise the patient before general anaesthesia and bone marrow aspiration, a whole blood transfusion was carried out on days 17 and 18. The bone marrow aspirate obtained on day 18 revealed hypercellularity with proliferation of atypical plasma cells (50.4% of all nucleated cells) and a relative decrease in the numbers of erythroid and myeloid cells (Table 2, Fig 1C and D). The plasma cells in the bone marrow showed marked anisocytosis, marked anisokaryosis, and occasional bi-nucleation. These cells were similar in appearance to the plasmablasts described by Mellor et al[12] possessing an eccentric round to pleomorphic nucleus with a fine chromatin pattern, one to several nucleoli, and moderately basophilic cytoplasm containing fine azurophilic granules (Fig 1D). Serum protein electrophoresis revealed M-protein in the γ-globulin fraction which accounted for 34.8% (3.8 g/dl) of the total protein concentration (10.8 g/dl) (Fig 2). Bence-Jones protein was shown to be present in the urine by a heat precipitation method. From these findings, the cat was diagnosed to have MRD.

Chemotherapy with melphalan (Alkeran; GlaxoSmithKline, 2 mg/m^2, PO, q48 h) was started from day 21. Although the number of circulating plasma cells slightly decreased, there was a progression of pancytopenia; therefore, melphalan was discontinued. Thereafter, the cat was treated with prednisolone and recombinant human granulocyte-colony stimulating factor (Neutrogin; Chugai Pharmaceutical, 5 μg/kg, SC, on days 29 and 38) in addition to the intermittent whole blood transfusions (40–45 ml, nine times, from day 36 to day 65). An increase in the atypical plasma cells in the peripheral blood (1330/μl, 35% of blood leukocytes) (Table 1) was observed on day 36. On day 38, atypical plasma cells with villous cytoplasmic projections were observed in the peripheral blood (Fig 1B). On day 52, elevated blood urea nitrogen (284.5 mg/dl) and creatinine (9.9 mg/dl) concentrations were found, and the cat showed appendicular oedema. Chemotherapy with chlorambucil (Leukeran; GlaxoSmithKline, 3 mg/m^2, PO, q24 h) was started from day 58; however, an increase of large-sized atypical plasma cells was noted in the peripheral blood on

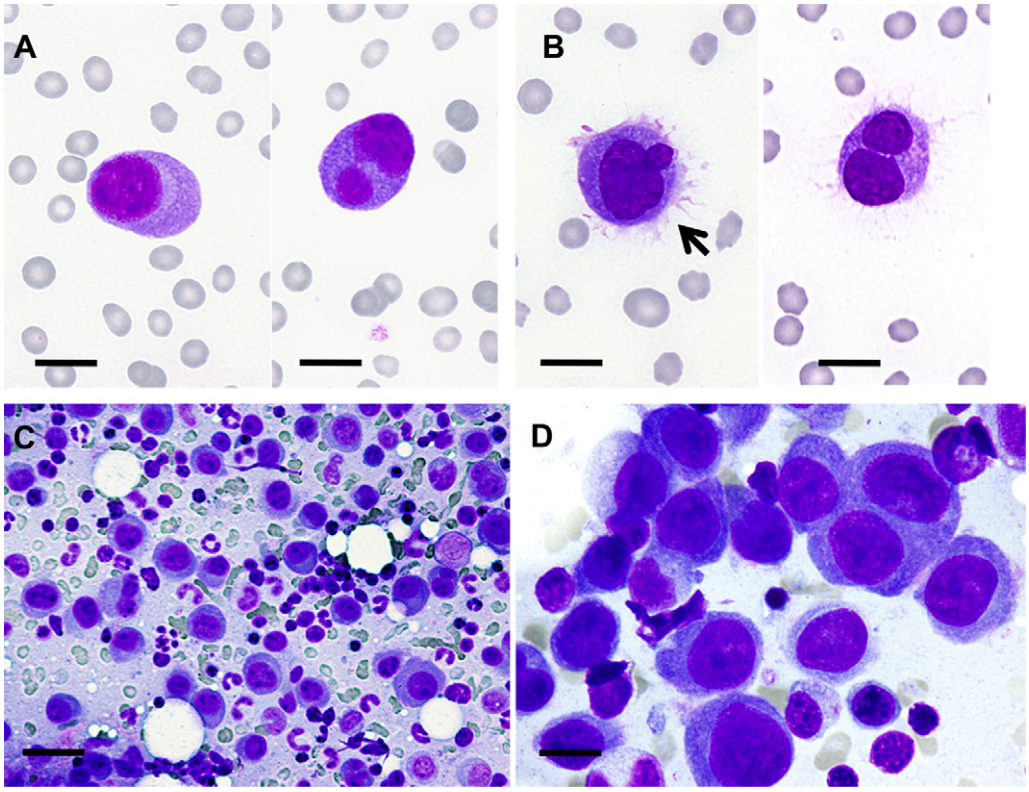

Fig 1. Cytological findings of the peripheral blood and bone marrow. (A) Peripheral blood smear on day 17: two atypical plasma cells are shown. (B) Peripheral blood smear on day 38: several plasma cells with villous cytoplasmic projections are observed (arrow). (C, D) Bone marrow smear on day 18: proliferation of atypical plasma cells. A–D: Wright–Giemsa stain. Bars = 10 μm (A, B, D), 50 μm (C).

Table 2. Myelograms.

Bone marrow cells	Day 18	Day 71	Reference ranges*
Proerythroblasts	0.4%	0%	1.00 ± 0.54%
Basophilic erythroblasts	1.4%	0.4%	4.02 ± 1.56%
Polychromatic erythroblasts	8.0%	14.7%	17.57 ± 4.48%
Orthochromatic erythroblasts	14.6%	1.2%	5.54 ± 3.15%
Myeloblasts	0.2%	0%	0.08 ± 0.16%
Promyelocytes	0.8%	0.4%	1.74 ± 1.04%
Neutrophilic myelocytes	1.4%	1.6%	4.31 ± 2.49%
Neutrophilic metamyelocytes	3.2%	0.4%	10.06 ± 3.20%
Neutrophils, band	6.8%	0.4%	14.40 ± 1.30%
Neutrophils, segmented	10.0%	1.2%	12.86 ± 4.85%
Eosinophils, band	0.2%	0%	0.49 ± 0.40%
Eosinophils, segmented	0.4%	0%	0.60 ± 0.20%
Monocytes	0.4%	0%	0.77 ± 0.51%
Lymphocytes	1.2%	0%	16.13 ± 2.92%
Atypical plasma cells	50.4%	79.0%	0.80 ± 0.60%
Megakaryocytes	0.6%	0.4%	NA
Myeloid/erythroid ratio	0.94	0.27	1.63 ± 0.35

NA = not available.
*The reference ranges were based on '*Schalm's veterinary hematology*, 5th edn'.[11]

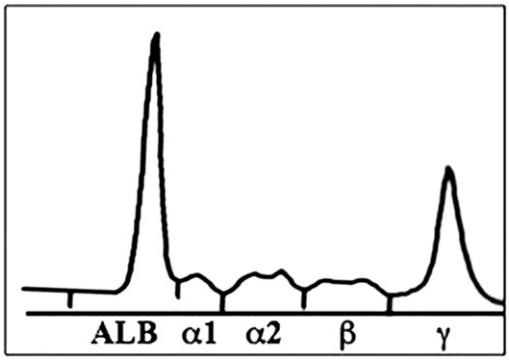

Fig 2. Serum protein electrophoretogram on day 18. The narrow spike is noted in the γ-globulin region. The total serum protein concentration was 10.8 g/dl. The concentrations of albumin (ALB), α_1, α_2, β and γ globulin were 4.1 g/dl (38.4%), 0.5 g/dl (4.9%), 1.2 g/dl (10.8%), 1.2 g/dl (11.1%) and 3.8 g/dl (34.8%), respectively.

day 64 (2340/μl, 30% of blood leukocytes) (Table 1). On day 66, in addition to the persistent thrombocytopenia, prolonged activated partial thromboplastin time (61.6 s, reference interval 21−45 s) and elevated concentration of fibrin/fibrinogen degradation product (5 μg/ml, reference interval <2.5 μg/ml) were noted, supporting the development of disseminated intravascular coagulation (DIC). The cat developed epistaxis and melaena, and died 71 days after the initial presentation.

An autopsy revealed generalised subcutaneous oedema with pleural effusion and ascites (approximately 500 ml). The lung was diffusely congested and oedematous. The spleen and liver were diffusely enlarged, and there were multifocal haemorrhagic foci on their surfaces. There was no mass lesion in the spleen. Lymph nodes were grossly unremarkable. An impression smear of the bone marrow showed marked proliferation of atypical plasma cells (79% of all nucleated cells) and decreases in the number of the normal erythroid, myeloid, and megakaryocytic cells (Table 2). On the impression smear of the spleen, a large number of atypical plasma cells were also noted.

Histopathological examinations of the bone marrow revealed diffuse proliferation of medium- to large-sized plasma cells surrounded by the remnant haematopoietic cells (Fig 3A). The plasma cells were similar in appearance to those previously described, and the majority had marked anisocytosis, marked anisokaryosis, and bi- or multi-nucleation. Mitotic figures were rare. In the spleen, the normal structures were almost completely replaced by a diffuse proliferation of plasma cells, accompanied by minimal extramedullary haematopoiesis (Fig 3B). The neoplastic foci were scattered throughout in the liver. The neoplastic plasma cells infiltrated into the hepatic central vein, sinusoid, and parenchyma, altering the normal architectures. The neoplastic plasma cells were also observed in the vessels of the visceral organs including heart, lung, kidney, intestine, pancreas, urinary bladder, and thyroid gland; however, there was no parenchymal infiltration of the neoplastic plasma cells in these organs. In both kidneys, glomerular mesangial cell proliferation and dilated medullary tubules containing proteinaceous casts were observed. The left ventricular wall of the heart was hypertrophic (thickness, 12 mm) and there was marked congestive oedema of the lung.

Immunohistochemistry for detection of IgG, IgM, IgA, and Ig light chain was performed in the bone marrow and kidney sections. To detect IgG, IgM and IgA, primary antibodies directed to feline IgG, IgM, and IgA (Bethyl Laboratories, Montgomery, TX), respectively, and labelled streptavidin biotinylated antibody method were employed. For detection of Ig light chain, a direct immunochemistry using horseradish peroxidase-conjugated anti-feline Ig light chain (κ and λ) (Bethyl Laboratories) was carried out. For the negative control, Tris-buffered saline was applied to the tissue sections instead of primary antibodies for immunohistochemistry. Most of the neoplastic cells in the bone marrow were negative for IgG, IgM, and IgA, but 5−10% of these neoplastic cells were positive for Ig light chain (Fig 3C). In the kidney, tubular casts and granules in the tubular epithelium were also positive for Ig light chain (Fig 3D).

Significant plasmacytosis in the peripheral blood is rare in feline MRD. In a report by Mellor et al,[6] a small number of circulating plasma cells were observed in only 1/24 cats with MRD. In a case report by Rahakrishnan et al,[13] there was progression from a cutaneous plasmacytoma to dissemination of neoplastic plasma cells in the marrow (73%) and peripheral blood with associated neutropenia and thrombocytopenia. This is in contrast to this case where anaemia, thrombocytopenia and atypical plasmacytosis were observed on initial presentation. The progressive marrow involvement by the proliferative plasma cells uncommonly results in leukaemia[4] but likely contributes to the cytopenias.

Median survival time in cats with MRD has been variously reported at 284 days[6] and at 6 months.[14] The cat in this report did not respond to the combined chemotherapy regime and survived only 71 days. This is consistent with a previous case report where the cat succumbed within 3.5 months and was also treated with multiple combined chemotherapy regimes. The classification scheme by Mellor et al[12] defines a high proportion of plasmablasts as consistent with poorly differentiated MRD with short survival times (median 14 days). In the case described in this study, most of the neoplastic plasma cells corresponded to plasmablasts, therefore, the cat could be diagnosed with poorly differentiated MRD according to the categorisation system.[12] Moreover, a feline case having MRD with significant plasmacytosis previously reported[13] was treated with prednisolone, melphalan, doxorubicin,

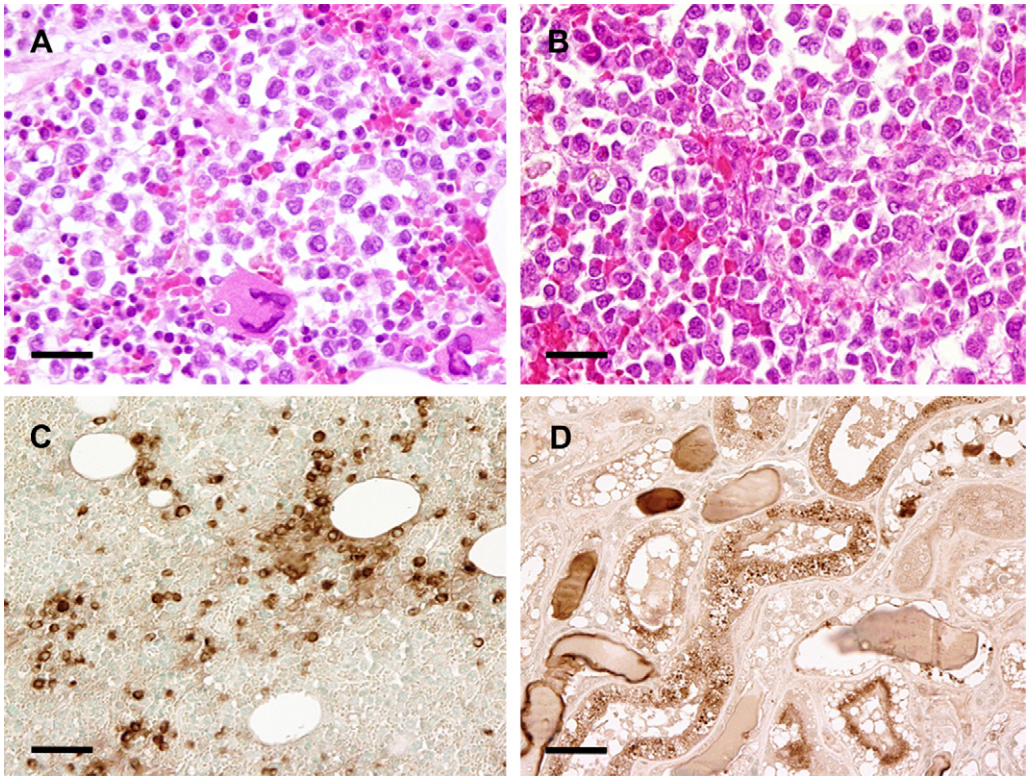

Fig 3. Histopathological findings of the bone marrow (A) and spleen (B) and immunohistochemical findings of the bone marrow (C) and kidney (D) obtained at autopsy. (A) Diffuse proliferation of neoplastic plasma cells surrounded by the cells of normal haematopoietic lineages are observed. (B) Normal splenic structures are substituted for the diffusely proliferated atypical plasma cells. (C) A proportion (5−10%) of the neoplastic cells are shown to be positive for Ig light chain in the cytoplasm. (D) Tubular casts and granules in tubular epithelia are shown to be positive for Ig light chain. A, B: Haematoxylin−eosin stain. C, D: Immunohistochemistry for feline Ig light chain (κ and λ), followed by methyl green counterstain. Bars = 25 μm (A, B), 50 μm (C, D).

L-asparaginase, and lomustine, but the cat was euthanased 3.5 months after confirming the leukaemic progression. Thereby, significant peripheral plasmacytosis may indicate a poor prognosis in feline MRD although studies on a large number of cases are warranted.

The changes of the echocardiography were consistent with those in hypertrophic cardiomyopathy; however, they might be associated with the long-term anaemia and blood hyperviscosity due to hyperglobulinaemia in the present cat. Appendicular oedema and pulmonary oedema detected during the observation period could be caused by the chronic heart failure.

Multiple blood transfusion, 14 in total, failed to maintain the PCV in this cat (Table 1). The replacement of marrow by the proliferating neoplastic population, blood loss due to the thrombocytopenia and reactions to the transfused blood elements may all have played a role in the persistent anaemia.

The present report details a case of feline MRD with leukaemic progression, appearing as a rare disease condition in cats. Further accumulation of the cases is needed in order to define its prognosis and therapeutic responses.

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