

CASE REPORT

Progression of cutaneous plasmacytoma to plasma cell leukemia in a dog

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Abstract: A 5-year-old male neutered Bernese Mountain Dog was presented for cutaneous plasmacytoma, which was treated by surgical excision. Four months later, the dog developed multiple skin masses, hyphema, pericardial and mild bicavitary effusions, myocardial masses, and marked plasmacytosis in the peripheral blood. Circulating plasma cells expressed CD34 and MHC class II by flow cytometry. Immunocytochemistry demonstrated that these cells were strongly positive for multiple myeloma oncogene 1/interferon regulatory factor 4 (MUM-1) and weakly to moderately positive for Pax5. The dog was hypoglobulinemic but had a monoclonal IgA gammopathy detected by serum immunofixation electrophoresis. The PCR analysis of antigen receptor gene rearrangements (PARR) by fragment analysis using GeneScan methodology revealed that plasmacytoid cells in the original cutaneous plasmacytoma and peripheral blood had an identical immunoglobulin heavy chain gene (IgH) rearrangement, indicating that both populations were derived from the same neoplastic clone. Canine cutaneous plasmacytoma rarely progresses to a malignant form and plasma cell leukemia is rarely diagnosed in the dog. This report describes a case of cutaneous plasmacytoma progressing to plasma cell leukemia with a rapid and aggressive clinical course. This report also highlights the utility of flow cytometry, immunocytochemistry, immunofixation electrophoresis, and PARR by fragment analysis using GeneScan methodology in the diagnosis of this hematopoietic neoplasm.

Case Presentation

A 5-year-old male neutered Bernese Mountain Dog was presented to the referring veterinarian for evaluation of 3 dermal masses of one-week duration. The dog was otherwise clinically healthy. The lesions were each raised, firm, erythematous, one-cm haired skin masses in the scapular and mid-cervical regions. Fine-needle aspirates of the 3 masses were performed and the cytologic pattern was comparable in all 3 sites. Cytology revealed the presence of a moderately heterogeneous population of round cells, arranged individually and in dense aggregates, which were 10–20 µm in diameter (Figure 1). Nuclei were round to rarely indented and often eccentrically placed, with moderately coarse chromatin and rarely small indistinct nucleoli. Cells had small to moderate amounts of basophilic

cytoplasm with discrete cell borders and frequently a perinuclear clear zone. There were moderate anisocytosis and anisokaryosis, rare binucleate cells, and small numbers of mitotic figures. There were small numbers of small mature lymphocytes in a blue amorphous background with minimal blood contamination and numerous ruptured cells. These results were consistent with a round cell tumor and most compatible with plasmacytoma or lymphoma with plasmacytoid features.

One week later, the dog was presented to a referral practice for the surgical excision of the 3 masses, although only one mass was detected on physical exam. A presurgery CBC was performed and hematology variables were unremarkable. The biochemistry profile revealed a mild elevation in creatinine (1.7 mg/dL, RI 0.5–1.5 mg/dL). Thoracic radiographs were

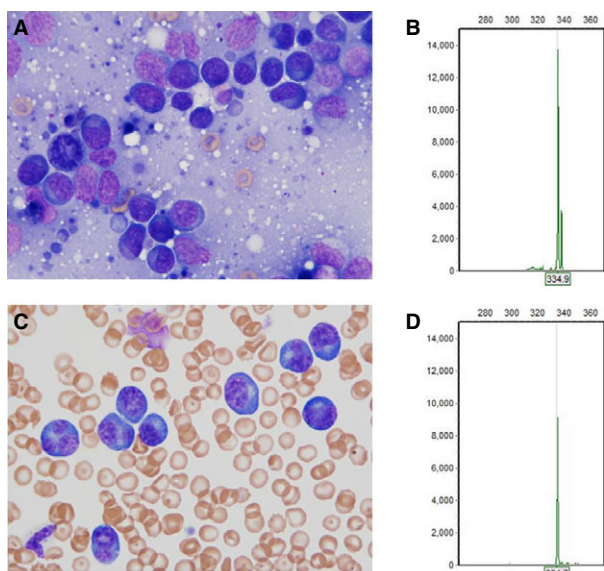


Figure 1. Cytologic pattern of a cutaneous mass and blood smear from a dog. Wright–Giemsa, $\times 100$ objective (**A** and **C**) and PCR for antigen receptor rearrangements (PARR) results (**B** and **D**) of a cutaneous plasmacytoma (**A**) and peripheral blood (**C**) in a dog with multiple plasmacytomas followed by plasma cell leukemia. (**A**) Direct smear of a fine-needle aspirate from a cutaneous mass in a dog. There are individualized discrete cells with plasmacytoid morphology, moderate anisocytosis and anisokaryosis, and mitotic figures. (**B**) Immunoglobulin heavy chain gene (IgH) amplification of DNA from the cutaneous mass. (**C**) Blood smear from a dog with circulating plasmacytoid cells. (**D**) IgH amplification of DNA from the blood. Results demonstrate a clonal IgH rearrangement in both populations. Amplicon sizes are identical by fragment analysis using GeneScan methodology, indicating that the plasmacytoid cells in both sites are derived from the same neoplastic clone.

unremarkable and an abdominal ultrasonographic examination revealed a prominent lymph node in the right middle abdomen, which was considered most consistent with a reactive process given the ultrasonographic appearance. Lymph node aspiration was not performed. The cutaneous mass was completely excised and histology revealed a densely cellular, multinodular mass extending from the superficial dermis into the subcutis (Figure 2). The cells were arranged in pseudopackets within a fine fibrovascular stroma. The cells were intermediate-sized and round, with often distinct cell borders and moderate to abundant eosinophilic cytoplasm. Nuclei were round with coarsely granular chromatin and rare single distinct nucleoli. Anisocytosis and anisokaryosis were mild with rare binucleate, multinucleate, and karyomegalic cells. Mitotic figures were frequent with 1–3 mitoses per HPF. Scattered throughout were small numbers of small mature lymphocytes and apoptotic cells. Neoplastic cells stained strongly positive for multiple myeloma oncogene 1/interferon regulatory factor 4

(MUM-1) (monoclonal rabbit anti-human MUM-1, BC5 clone; Biocare Medical, Concord, CA, USA) and approximately half of the cells had moderate to strong diffuse cytoplasmic staining for CD79a (monoclonal mouse anti-human CD79a, HM47/a9 clone; Biocare Medical) (Figure 2). There were few scattered small mature lymphocytes, which stained positive for CD3 (polyclonal rabbit anti-mouse CD3; Dako North America Inc., Carpinteria, CA, USA), dispersed throughout the mass. These results were considered consistent with plasmacytoma.

Four months following surgical excision of the cutaneous plasmacytoma, the dog represented with multiple large skin masses and hyphema in one eye. He had a history of a possible cough and intermittent diarrhea, but was otherwise bright and alert with a normal appetite. Thoracic radiographs, abdominal ultrasonographic examination, and echocardiogram were performed revealing pericardial effusion, mild pleural and peritoneal effusion, pulmonary masses, 2–3 focal areas of hypoechoic myocardium in the ventricular wall interpreted as most consistent with neoplasia, and a large left cranial abdominal mass. The pericardial effusion had large numbers of plasmacytoid cells with moderate anisocytosis and anisokaryosis, small numbers of small well-differentiated lymphocytes, neutrophils and macrophages, and moderate numbers of reactive mesothelial cells consistent with discrete cell neoplasia (data not shown). A CBC performed using the Advia 120 automated hematology analyzer (Siemens, Erlangen, Germany) was characterized by a marked leukocytosis ($87,900/\mu\text{L}$, RI 4000–15,500/ μL) due to marked plasmacytosis ($73,836/\mu\text{L}$) and mild thrombocytopenia ($134 \times 10^3/\mu\text{L}$, RI $170\text{--}400 \times 10^3/\mu\text{L}$). There was a normal HCT (42%, RI 36–60%) with mild metarubricytosis (2 nRBC/100 WBC) with no cytologic evidence of increased polychromasia. A reticulocyte count was not performed. A biochemical profile was performed on the AU680 Chemistry System (Beckman Coulter, Brea, CA, USA). Biochemical abnormalities included mild hypoproteinemia (4.9 g/dL, RI 5.0–7.4 g/dL), mildly increased ALT activity (148 U/L, RI 12–118 U/L), and mildly increased creatinine concentration (1.9 mg/dL, RI 0.5–1.6 mg/dL). Circulating plasmacytoid cells were 10–20 μm in diameter with a round to lobular nucleus, coarsely clumped chromatin, rarely 1–2 large round faint nucleoli, and moderate to abundant deeply basophilic cytoplasm with perinuclear clearing cytologically similar to the cells seen in the pericardial fluid (Figure 1).

Flow cytometry was performed on the peripheral blood as previously described¹ and analyzed with Kaluza software (Beckman Coulter Inc., Fullerton, CA,

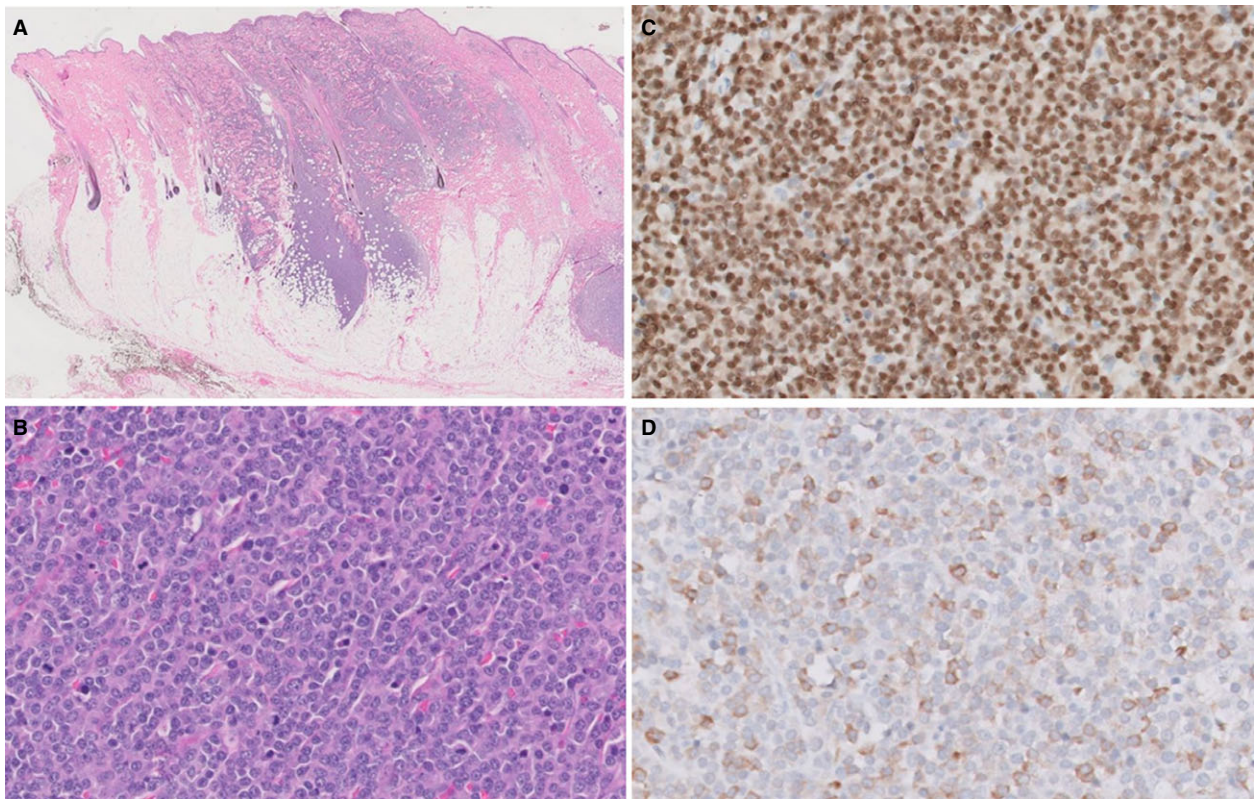


Figure 2. Histologic and immunohistochemical findings of a cutaneous plasmacytoma in a dog (**A** and **B**) H&E, (**C** and **D**) 3,3'-Diaminobenzidine tetrahydrochloride chromogen, hematoxylin counterstain. (**A**) The mass extends to the superficial dermis and has locally infiltrative borders into the subcutaneous adipose tissue. $\times 2$ objective. (**B**) Higher magnification view of neoplastic round cells with prominent mitotic activity and plasmacytoid features. $\times 40$ objective. (**C**) Immunohistochemistry for multiple myeloma oncogene 1/interferon regulatory factor 4 (MUM-1), demonstrating strong positive nuclear immunoreactivity for MUM-1 (plasma cell marker). $\times 40$ objective. (**D**) Immunohistochemistry for CD79a, demonstrating moderate to strong cytoplasmic positive immunoreactivity for CD79a (B cell marker) in a subset of neoplastic cells. $\times 40$ objective.

USA). Approximately 64,000 cells/ μL , which represented 73% of the cells present, had an aberrant phenotype, expressing CD34 and MHC class II. The cells did not express CD21 or CD45 (Figure 3). A clonal immunoglobulin heavy chain gene (IgH) rearrangement was detected in the peripheral blood by PCR analysis of antigen receptor gene rearrangements (PARR), as previously described,² with analysis by capillary electrophoresis and GeneMarker software (Soft Genetics, State College, PA, USA; amended protocol described in Appendix S1). An IgH rearrangement of identical size by fragment analysis using GeneScan methodology was identified in archived cytology slides from the primary cutaneous plasmacytoma, indicating that the initial mass and the circulating cells were derived from the same clone (Figure 1). Treatment of the dog was initiated with melphalan, prednisone, metronidazole, and cefpodoxime.

Approximately one month later, and 6 days after completion of melphalan treatment, the dog was

presented for blindness and bilateral hyphema. The retinas could not be evaluated due to the hyphema. The skin lesions noted one month prior had almost completely resolved during melphalan treatment. The dog was bright and alert with no other abnormalities detected on physical exam. A CBC showed progression of disease with a marked leukocytosis (134,200/ μL , RI 4500–15,000/ μL), marked plasmacytosis (124,800/ μL), and mild monocytosis (1300/ μL , RI 200–1000/ μL). There were a moderate nonregenerative, normocytic, normochromic anemia (HCT 31%, RI 40–55%) and a moderate thrombocytopenia (85,000/ μL , RI 200–500 $\times 10^3$ / μL) with no platelet clumps detected. Biochemical abnormalities included moderately elevated AST activity (139 U/L, RI 16–55 U/L), mild hypoproteinemia (5.3 g/dL, RI 5.5–7.5 g/dL) and hypoglobulinemia (1.8 g/dL, RI 2.4–4.0 g/dL), mildly elevated creatinine concentration (2.0 mg/dL, 0.5–1.5 mg/dL), and mild hypocholesterolemia (127 mg/dL, 131–345 mg/dL). Flow cytometry of the peripheral

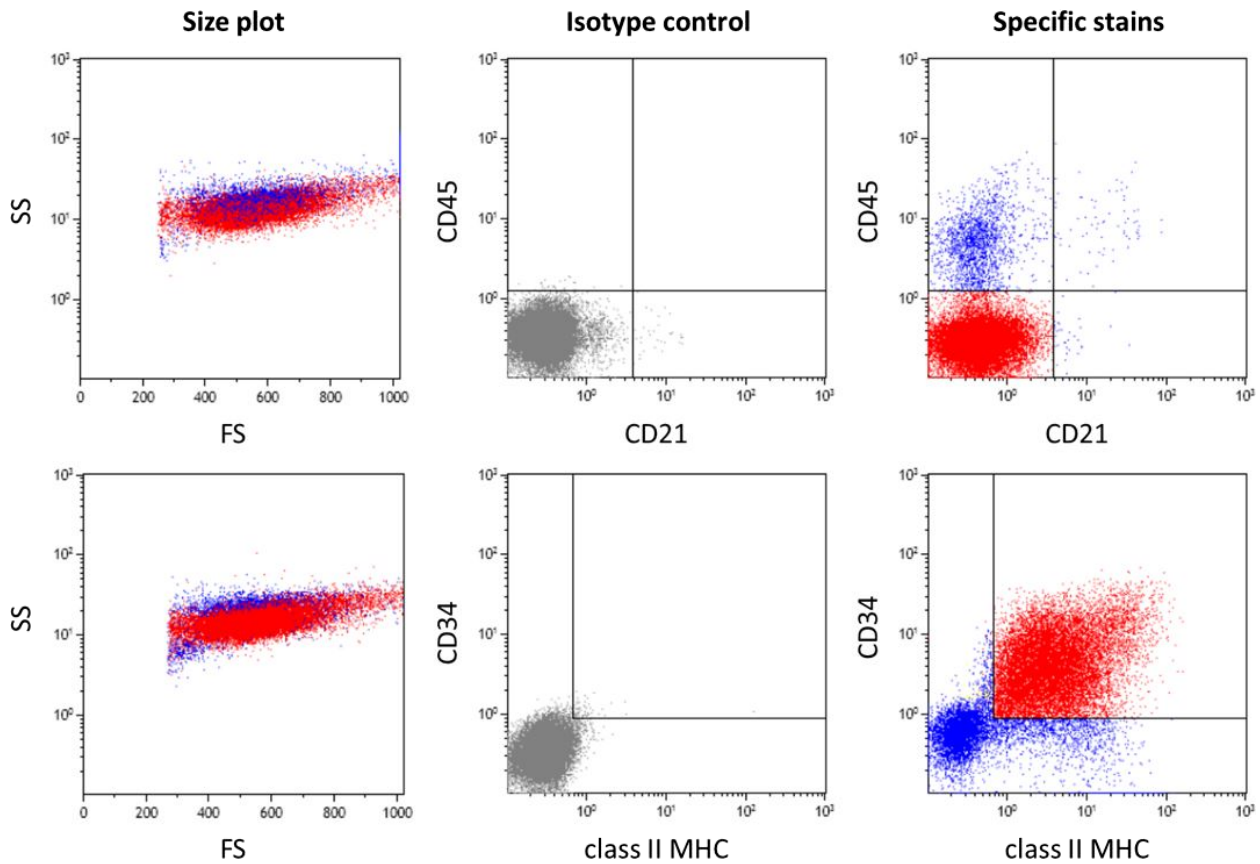


Figure 3. Flow cytometric immunophenotyping of neoplastic plasmacytoid cells in the peripheral blood of a dog. Size plots on the left with forward scatter (FS) on the horizontal axis and side scatter (SS) on the vertical axis show normal peripheral blood leukocytes, including neutrophils, monocytes, and small lymphocytes in blue, and neoplastic plasma cells in red. Fluorescence dot plots on the right side show that neoplastic cells (red) do not express CD45 or CD21 (top right plot) and do express CD34 and MHC class II (bottom right plot). The middle 2 panels show the dot plots of the isotype controls.

blood revealed 97,000 cells/ μ L with the same aberrant phenotype seen in the first blood sample, which represented 72% of the cells present. Circulating plasmacytoid cells had the same morphology as seen one month earlier. These cells had strong diffuse nuclear staining for MUM-1 (monoclonal mouse anti-human MUM-1, MUM1p clone; Dako North America Inc.), weak to moderate stippled nuclear staining for Pax5 (monoclonal mouse anti-human Pax5, DAK-Pax5 clone; Dako North America Inc.), and negative staining for CD3 (monoclonal mouse anti-human CD3, LN10 clone; Leica Biosystems Newcastle Ltd., Newcastle Upon Tyne, UK) (Figure 4). Serum was submitted for protein electrophoresis (SPE), immunofixation electrophoresis (IFE), and immunoglobulin quantification, as previously described³ (details provided in Appendix S2). SPE and IFE were performed using an agarose gel electrophoresis system according to the manufacturer’s instructions (HYDRASYS Agarose Gel Electrophoresis System; Sebia Inc., Norcross, GA,

USA). For IFE, sheep anti-dog IgG and goat anti-dog IgA, IgM, and lambda light chain antisera were used to characterize the paraprotein (Bethyl Laboratories Inc., Montgomery, TX, USA). Immunoglobulin fraction quantification was performed on serum using canine IgA, IgG, and IgM radial immunodiffusion test kits according to the manufacturer’s instructions (Triple J Farms, Kent Laboratories, Bellingham, WA, USA). The serum protein electrophoresis alone did not clearly reveal a monoclonal gammopathy, whereas immunofixation electrophoresis demonstrated a restricted band in the IgA lane (Figure 5). Immunoglobulin quantification revealed a moderate elevation in IgA (588 mg/dL, RI 40–160 mg/dL) and decreased quantities of IgG (427 mg/dL, RI 1000–2000 mg/dL) and IgM (22 mg/dL, RI 100–200 mg/dL). These results indicate an IgA monoclonal gammopathy despite the decrease in total globulins. The dog died at home the following day and a postmortem examination was not performed.

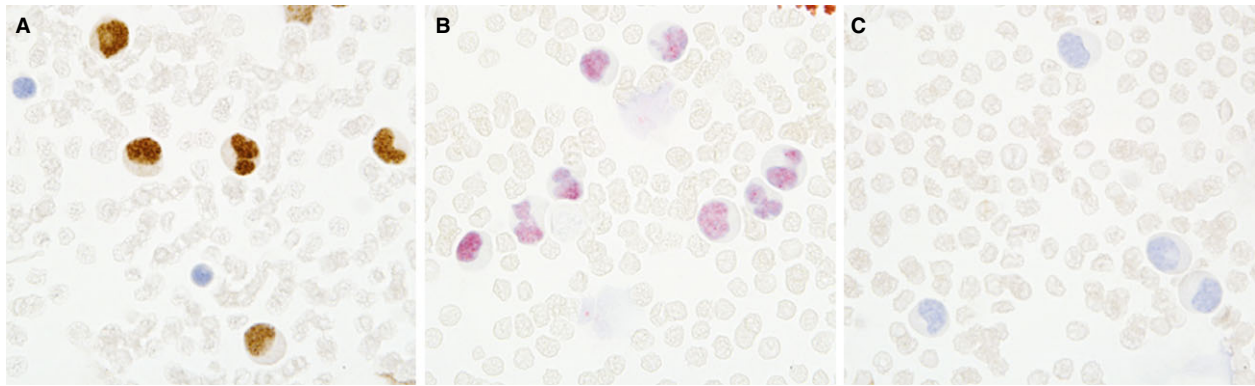


Figure 4. Immunocytochemical findings of neoplastic plasmacytoid cells in the peripheral blood of a dog. Hematoxylin counterstain, $\times 100$ (A and C) 3,3'-Diaminobenzidine tetrahydrochloride chromogen, (B) Fast Red chromogen. (A) Immunocytochemistry for MUM-1, (B) immunocytochemistry for Pax5, and (C) immunocytochemistry for CD3. Neoplastic cells have strong diffuse nuclear staining for MUM-1, weak to moderate stippled nuclear staining for Pax5, and negative staining for CD3.

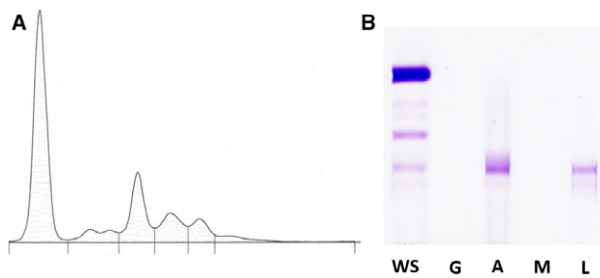


Figure 5. Serum protein electrophoresis (A) and immunofixation electrophoresis (B) of whole serum from a dog with multiple cutaneous plasmacytomas followed by plasma cell leukemia. (A) The peak to the left represents albumin and the small peak in the center likely represents acute phase proteins. There is no evidence of a monoclonal gammopathy. (B) There is a restricted band in the IgA (A) lane and light chain (L) lane. The IgG and IgM lanes do not have appreciable bands. WS indicates anti-whole serum; G, anti-canine IgG heavy chain; A, anti-canine IgA heavy chain; M, anti-canine IgM heavy chain; L, anti-canine light chain.

Discussion

Myeloma-related disorders (MRD) in dogs arise from plasma cells or immunoglobulin secreting B lymphocyte precursor cells that have undergone neoplastic transformation. The MRD classification includes a number of syndromes, including multiple myeloma, cutaneous and noncutaneous extramedullary plasmacytoma, solitary osseous plasmacytoma, IgM macroglobulinemia, and immunoglobulin secreting lymphomas and leukemias.⁴ Extramedullary plasmacytomas in dogs are most commonly found in the skin, oral cavity and alimentary tract, although they have rarely been described at other sites.^{4,5} Cutaneous plasmacytomas are typically benign and curable with surgical excision, yet there are rare case

reports describing progression from cutaneous plasmacytoma to multiple myeloma in dogs and people.^{6–8} These dogs with more aggressive disease had multiple cutaneous plasmacytomas most compatible with the anaplastic histologic subtype. The case described here also had multiple cutaneous plasmacytomas, although morphology was more compatible with the mature histologic subtype.⁹ Clinically, this dog experienced a progression from multiple cutaneous plasmacytomas to plasma cell leukemia over the course of 4 months. Plasma cell leukemias are very rarely seen in veterinary medicine and, as yet, poorly defined.

In human medicine, plasma cell leukemia is a rare aggressive variant of myeloma.^{10,11} Plasma cell leukemias are defined as having $> 20\%$ plasma cells in the peripheral blood, with a total cell count exceeding 2×10^9 plasma cells/L. Primary plasma cell leukemias occur in patients without previous evidence of myeloma, whereas secondary plasma cell leukemias occur in advanced multiple myeloma patients who develop a leukemic phase of their disease. It is important to differentiate human patients with primary and secondary plasma cell leukemias, because they have a different clinical course. Primary plasma cell leukemia patients tend to have a lower prevalence of osteolytic lesions and bone pain, and a higher prevalence of lymphadenopathy, liver and spleen involvement, pulmonary findings, pleural effusion, neurologic deficits, and extramedullary soft tissue plasmacytomas.^{10,11} There is more extensive bone marrow involvement and a higher prevalence of anemia and thrombocytopenia in patients with primary plasma cell leukemia than in those with secondary plasma cell leukemia, and ultimately a more aggressive disease course and

shorter survival period compared to patients with secondary plasma cell leukemia.

It is difficult to classify this canine case as primary or secondary plasma cell leukemia. The clinical features are most similar to primary plasma cell leukemia in people. This dog did not have osteolytic lesions on thoracic radiographs or detectable bone pain, did have pleural effusion and extramedullary plasmacytomas, and was presumed to have myocardial, pulmonary, and lymph node involvement because the imaging changes occurred during disease progression. However, tumor involvement in these latter 3 sites was not confirmed by cytology or histopathology. Bone marrow was also not examined in this case, although the degree of plasmacytosis in the peripheral blood and presence of anemia and thrombocytopenia suggest extensive myelophthisis. The disease progression was rapid and aggressive, as seen with human cases of primary plasma cell leukemia. However, the clonally identical cutaneous plasmacytomas preceded overt evidence of bone marrow involvement, and this finding suggests the possibility that the plasma cell leukemia may have been secondary.

The serum creatinine was only mildly elevated and did not increase significantly during the course of disease. As a urinalysis was not performed, it is difficult to assess possible renal disease. The activities of ALT and AST were mildly to moderately elevated at different time points. As CK activity was normal at the time of AST elevation, and was not measured at the time ALT was increased, the changes in ALT and AST suggest mild hepatocellular injury, possibly secondary to tumor infiltration in the hepatic parenchyma, drug therapy, or circulatory disturbance associated with hyperviscosity syndrome. The echocardiogram suggested neoplasia within the myocardium, so cardiac muscle injury may have contributed to AST elevation as well. The mild decrease in cholesterol may be attributed to multiple myeloma, as hypocholesterolemia has been reported in cats¹² and people with multiple myeloma, and is thought to be due to increased utilization of cholesterol by malignant plasma cells.¹³

The immunophenotype of the plasma cells described in this case has not been previously reported. In people, normal plasma cells lack expression of pan-B cell antigens CD20 and CD22, show heterogeneous expression of CD19, CD45, and CD56, and express high levels of CD38 and CD138. Myeloma plasma cells may have multiple combinations of aberrant expression patterns, including downregulation of CD38 and CD45 expression, absence of CD19 expression, gain of CD20 or CD117 expression, and high expression of CD56.^{14,15} Antibodies to most of these proteins are not

available in veterinary medicine, and the immunophenotype of normal or neoplastic canine plasma cells has not been reported in the literature. Typically, using our standard flow cytometry antibody panel, normal plasma cells have low to absent CD21 antigen expression, high expression of CD45 antigen and MHC class II, and no expression of CD34 antigen (unpublished observations, EDR/PRA/ACA 1/14/2016). In this case, the plasma cells expressed MHC class II, but not the other proteins commonly expressed on plasma cells (low CD21 and CD45). There is evidence of CD34-expressing multiple myeloma stem cells in the human literature.^{16–18} In this case, CD34 expression likely represents aberrant expression of the antigen on neoplastic cells rather than true stem cell origin given the cytologic morphology of these cells. The majority of plasma cells in the peripheral blood of the dog reported here had a well-differentiated morphology, though many were polylobated and pleomorphic. Low numbers of cells had an immature phenotype with more dispersed chromatin, a higher nuclear/cytoplasmic ratio, and prominent nucleoli. This pattern of CD34 expression on nonstem cell-derived lymphoproliferative disorders is consistent with what has been reported in a subset of canine B-cell lymphomas.¹⁹

Immunocytochemical staining of peripheral blood smears for MUM-1, Pax5, and CD3 was useful in confirming plasma cell origin as immunohistologic reactivity for MUM-1 is highly specific for canine plasma cell tumors.²⁰ Pax5 is exclusively expressed in B cells from the pro-B lymphocyte stage to the mature B-cell stage and its expression must be downregulated for differentiation of B cells into plasma cells, therefore normal plasma cells are negative for Pax5 expression. Neoplastic myeloma plasma cells are typically negative for Pax5, but rarely will maintain Pax5 expression in people.²¹ In this case, Pax5 expression appeared downregulated, given the faint staining pattern, but was present in the majority of circulating neoplastic plasma cells. As CD3 is a T-cell specific marker, it is expected to be negative on plasma cells.

This case also demonstrates the utility of PARR using fragment analysis with GeneScan methodology, which allows precise size identification of the IgH rearrangement to single base resolution. This technology is useful in determining whether 2 hematopoietic tumors are derived from the same clone. Immunoglobulin H rearrangements are expected in MRD in dogs and are used to diagnose and monitor myeloma patients in human medicine. In this case, the amplified rearranged IgH products were of identical size in the initial cutaneous and subsequent peripheral blood plasma cell populations, confirming that the plasma

cell leukemia and original cutaneous plasmacytoma arose from the same clone.

Monoclonal IgA gammopathies have been previously described in canine patients with B-cell neoplasia and normal serum globulin levels.³ However, this finding has not been previously described in dogs with this degree of hypoglobulinemia. This case report demonstrates the value of assessing serum proteins in the face of a normal or low total globulin concentration and highlights the increased sensitivity of IFE over conventional SPE for detecting monoclonal bands. Further evidence suggesting the significance of this increased IgA is the clinical finding of hyphema and blindness, likely attributable to hyperviscosity syndrome. Hyperviscosity syndrome is a common sequela in multiple myeloma and is likely due to the quantity, abnormal polymerization, and abnormal shape of the paraprotein.²² Although it seems counterintuitive that a dog with hypoglobulinemia could develop hyperviscosity syndrome, it is possible that the high level of IgA, combined with abnormal shape or polymerization of the paraprotein and hyperleukocytosis caused hyperviscosity syndrome.

This case report describes the rapid and aggressive progression of myeloma-related disease in a dog, from multiple cutaneous plasmacytomas to plasma-cell leukemia. This case report also presents a unique flow cytometric phenotype for neoplastic plasma cells and highlights the utility of additional diagnostics, including PARR by fragment analysis using GeneScan methodology, IFE, and immunocytochemistry for MUM-1, Pax5, and CD3, in diagnosis of this hematopoietic neoplasm.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Methods for PCR analysis of antigen receptor gene rearrangements (PARR).

Appendix S2. Methods for serum protein electrophoresis (SPE), immunofixation electrophoresis (IFE), and immunoglobulin fraction quantification.