Pancytopenia in a dog

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Signalment: 12 year old castrated male Miniature Pinscher Dog

History: This patient first presented to the Bailey Small Animal Teaching Hospital at Auburn University (AU-SATH) Emergency and Critical Care (ECC) service on 6/22/15 for evaluation of a two-month history of lethargy, weight-loss, and decreased appetite. Notable laboratory findings at that visit included mild, poorly-regenerative anemia (HCT-31.8%; absolute reticulocytes 94,000/µL), increased RDW (17.9%), and moderate to marked thrombocytopenia (38,000/µL; only rare platelet clumps noted on smear). Serum biochemistry abnormalities included hyperproteinemia (13.59 g/dL), hypoalbuminemia (1.3 g/dL), hyperglobulinemia (11.5 g/dL), and hypercalcemia (15.9 mg/dL). Radiographs revealed small radiolucent opacities of the dorsal spinous processes of T10 and L2 vertebrae. A bone marrow aspirate was obtained and a representative photomicrograph can be seen in Figure 1. The patient was discharged with the standard of care oral medications for the condition diagnosed.

Figure 1. Bone marrow aspirate (direct smear), Modified Wright stain, original magnification 40x.

Clinical Findings: The patient did well at home until about a week later, in the afternoon of 6/29/15, when the owners noticed trembling, hypersalivation, vomiting and diarrhea. The owners re-presented the patient to the AU-SATH ECC service. On physical exam, the patient was depressed, febrile (105°F, ~40.56°C), tachycardic (160 beats/min), and tachypneic (48 breaths/min). This patient was also hypotensive (systolic blood pressure ~60 mmHg). A small amount of free peritoneal fluid was noted on a brief abdominal ultrasound, and the effusion was submitted for analysis (see text below, and Figure 4 and Figure 5).

Laboratory Findings: A CBC was performed on an Advia® 120 hematology analyzer on 6/29/15 (Table 1). A representative peripheral blood photomicrograph from this feathered edge can be seen in Figure 2.
Table 1: CBC (Advia® 120 hematology analyzer) results (6/29/15)

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>UNITS</th>
<th>REFERENCE INTERVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT</td>
<td>22.9</td>
<td>%</td>
<td>38.7-59.2</td>
</tr>
<tr>
<td>RBC</td>
<td>3.67</td>
<td>x 10^9/L</td>
<td>6.02 - 8.64</td>
</tr>
<tr>
<td>Hgb</td>
<td>8.1</td>
<td>g/dL</td>
<td>13.1 - 20.1</td>
</tr>
<tr>
<td>MCV</td>
<td>62.4</td>
<td>fL</td>
<td>60.5-73.8</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.6</td>
<td>g/dL</td>
<td>32-37.9</td>
</tr>
<tr>
<td>RDW</td>
<td>21.4</td>
<td>%</td>
<td>11.2-14.4</td>
</tr>
<tr>
<td>Retic. (abs.)</td>
<td>90,700</td>
<td>cells/µL</td>
<td>0-60,000</td>
</tr>
<tr>
<td>nRBC</td>
<td>33</td>
<td>/100 WBC</td>
<td>0-10</td>
</tr>
<tr>
<td>WBC (corrected)</td>
<td>2,660</td>
<td>cells/µL</td>
<td>5,090-17,410</td>
</tr>
<tr>
<td>Seg. Neutrophils</td>
<td>931</td>
<td>cells/µL</td>
<td>2,600-10,400</td>
</tr>
<tr>
<td>Bands</td>
<td>1,197</td>
<td>cells/µL</td>
<td>0-300</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>186</td>
<td>cells/µL</td>
<td>390-6,730</td>
</tr>
<tr>
<td>Monocytes</td>
<td>346</td>
<td>cells/µL</td>
<td>160-1,160</td>
</tr>
<tr>
<td>Platelets</td>
<td>46,000</td>
<td>cells/µL</td>
<td>152,000-518,000</td>
</tr>
<tr>
<td>MPV</td>
<td>8.9</td>
<td>fL</td>
<td>8-14.6</td>
</tr>
</tbody>
</table>

Figure 2. Peripheral blood smear at the feathered edge, Modified Wright stain, original magnification 10x.  
Inset: Magnified view (100x) of one of the cells highlighted by the black box outlines.
Peritoneal effusion analysis (6/29/15):

- **Appearance**: red, turbid
- **Total protein**: 7.0 g/dL
- **Total nucleated cell concentration**: 28,530/µL
- **Total RBC concentration**: 100,000/µL
- **Cytologic findings**: representative photomicrographs are provided (Figure 3 and Figure 4).

![Figure 3. Peritoneal fluid (direct smear), Modified Wright stain, original magnification 40x.](image1)

![Figure 4. Peritoneal fluid (direct smear), Modified Wright stain, original magnification 100x.](image2)
Questions:
1. What is your presumptive diagnosis based on the tests completed on 6/22/15?
2. What medication(s) was this patient likely prescribed based on the initial diagnostics? Does this medication or medications have any potential adverse effects that may contribute to the morbidity noted on 6/29/15?
3. Discuss all possible mechanistic explanations for the cytopenias present in this patient on 6/29/15.
4. What is the significance, if any, of the leukogram and nRBCs on the CBC from 6/29/15?
5. How would you classify and interpret the peritoneal body cavity effusion? What mechanisms may have contributed to this effusion? Are these findings typical for your presumptive diagnosis from Question 1?

Interpretation of Images and Data:
- **Bone Marrow Aspirate (Figure 1):** The photomicrograph shows a highly cellular field of bone marrow comprised of 40-50% large discrete round cells with well-defined margins and a moderate amount of intensely basophilic cytoplasm, 1-2 perinuclear clear zones, round to irregularly round nuclei that are sometimes eccentric, with coarse chromatin and variably distinct nucleoli interpreted as neoplastic plasma cells. Anisocytosis and anisokaryosis of this population were moderate, and some binucleated plasma cells were noted (not pictured). Some of these plasma cells had “flame cell” morphology (not pictured). There were also a moderate number of late erythroid and myeloid precursors in the image shown.
- **CBC Hematology Data (Table 1):** There is pancytopenia characterized by moderate normocytic, normochromic, poorly regenerative anemia, moderate to marked thrombocytopenia (without evidence of megakaryocytic response based on MPV), and moderate to marked leukopenia characterized by severe neutropenia with a degenerative left shift and lymphopenia. There is a rubricytosis/normoblastemia that appears to be inappropriate based on the absence of a substantial regenerative response to anemia.
- **Blood Smear (Figure 2):** There was a paucity of nucleated cells on the blood smear, but low numbers of large round cells similar in appearance to those from the bone marrow (black boxes, inset) were rarely observed at the feathered edge and within the body of the smear. The interpretation was circulating neoplastic plasma cells.
- **Peritoneal Effusion (Fluid Analysis Data; Figure 3 and Figure 4):** Based on physical appearance and cell counts, this fluid appeared to be an exudate. Cytologic evaluation of direct and concentrated smears revealed about 50% of the cells to be neoplastic plasma cells similar in appearance to those from the bone marrow aspirate and blood (Figure 3), as well as ~50% neutrophils, both degenerate and non-degenerate, occasionally containing single to multiple rod bacteria (Figure 4). Rod bacteria were also seen free in the background. The interpretation was plasma cell neoplasia as well as septic purulent inflammation of unknown source.

**Diagnosis:** (1) Multiple myeloma with neoplastic cells in circulation and in the peritoneal effusion; (2) Peritoneal exudate: Septic purulent inflammation (unknown source); (3) Pancytopenia with degenerative left shift and inappropriate rubricytosis/normoblastemia.

**Outcome:** The patient remained unstable and hypotensive despite attempts at resuscitation with crystalloid fluid boluses and empirical antimicrobial therapy for sepsis with IV ampicillin sulbactam.
Acute Kidney Injury (AKI) was diagnosed based on the serum creatinine increasing from 0.9 mg/dL to 1.8 mg/dL within 24 hours (NOVA blood gas instrument). Based on the septic peritonitis of unknown source, exploratory surgery was recommended, but due to the grave prognosis, the owners elected humane euthanasia. However, the patient arrested before this could be performed. A necropsy was offered but declined by owners.

Answers to Questions:

1. The combination of marked hyperglobulinemia with hypercalcemia, multiple lytic bone lesions, and a bone marrow infiltrate of ~30% atypical plasma cells was diagnostic for multiple myeloma (MM). The marked hypercalcemia was attributed to one or more of the following: increased protein-bound fraction of calcium, increased production of PTH-rP, and/or osteolysis.

2. The patient was discharged with melphalan (0.14 mg/kg once daily orally) and prednisone (1 mg/kg once daily orally) to treat the multiple myeloma, as well as omeprazole (0.6 mg/kg once daily orally) and a 500 mg sucralfate slurry (three times daily orally) for gastroprotectant effects. Melphalan’s main adverse effect is bone marrow suppression, with delayed thrombocytopenia and/or neutropenia most common [7]. There is some evidence that smaller canine patients may be disproportionately at risk for severe myelosuppression, as demonstrated in a small phase I clinical trial where eighty-eight percent (88%) of dogs with neoplasia weighing less than 14 kg given appropriate doses of melphalan IV developed neutropenia <1,500/µL and/or thrombocytopenia <80,000/µL [5].

   Prednisone is often initiated along with melphalan based on apparent synergistic effects against MM cells. The dose used in this patient was in the range where anti-inflammatory but not immunosuppressive effects are expected. While unlikely based on the dose and duration of administration, prednisone may have increased the risk of a possible gastrointestinal ulcer or perforation in this patient.

3. The pancytopenia in this patient on 6/29/15 was likely due to multiple concurrent mechanisms. As the poorly regenerative anemia and moderate to marked thrombocytopenia were present at the initial visit on 6/22/15, the main explanation for the initial bicytopenia was myelophthisis due to infiltrative plasma cell neoplasia. The anemia may have also had a concurrent component due to anemia of chronic/inflammatory disease secondary to the MM. Supporting this latter hypothesis, the patient did have a hypoferremia at the time of original diagnosis (serum iron 58 µg/dL, RI: 76 – 229). Splenic sequestration was another possibility for thrombocytopenia, as this patient had splenomegaly on abdominal ultrasound. Finally, while a full coagulation panel was not performed, there was some evidence of possible consumptive coagulation and increased fibrinolysis based on increased fibrin-degradation products or FDPs (> 20 µg/mL, no RI) and decreased antithrombin (55%, RI >150).

   The marked neutropenia on 6/29/15 may have been due to myelosuppression from the administration of melphalan. However, other possibilities included accumulation of neutrophils within the septic peritoneal effusion and/or increased vascular margination secondary to sepsis. Similar mechanisms for anemia and thrombocytopenia were likely present at this time point, but given the clinical and cytologic evidence of sepsis, DIC and hemorrhage may have been further contributors those cytopenias on 6/29/15. The melphalan may have further contributed to decreased thrombopoiesis.
4. A “degenerative left-shift” (DLS) has multiple definitions, but one of the most commonly used is when the concentration of immature granulocytes is greater than the concentration of mature granulocytes; this phenomenon is often said to be a strong negative prognostic indicator. A recent case-control retrospective study of 319 hospitalized dogs with DLS (cases) and 918 hospitalized control dogs supported this assertion with a hazard ratio of 1.9 for in-hospital death or euthanasia among cases relative to controls [2].

Increased nRBCs in circulation are significant for two reasons. First, most automated hematology analyzer methodologies (including impedance and light scatter) count nRBCs as leukocytes, falsely increasing this concentration and requiring correction according to the formula $[\text{WBC}_{\text{corr}}] = 100 / (100 + \text{nRBC})$ [6]. This is particularly important in patients with low-normal or mildly decreased leukocyte concentrations, as small downward changes may be clinically meaningful. Second, the presence of nRBCs in circulation have been associated with mortality in a population of dogs with the systemic inflammatory response syndrome (SIRS), and the presence and magnitude of rubricytosis/normoblastemia has been associated with non-survivors in a population of dogs with heatstroke [1,4].

5. Based on the increased cell and protein concentration, this fluid would be classified as an exudate [6]. This unusual fluid appears to have components of both a neoplastic exudate and a septic inflammatory exudate. Involvement of MM in body cavity effusions is rare in domestic species, but was reported in one dog with a peritoneal effusion [8]. In that case the fluid cell concentration was low at 1,500/µL with a high protein concentration (7.9 g/dL); 25% of nucleated cells were large or giant atypical plasma cells that were often multinucleated (up to 4 nuclei). The patient in that report was euthanized without necropsy, and no association with prognosis could be made.

The source of the sepsis was unknown in this case due to a lack of follow-up imaging or necropsy. Possibilities considered most likely were gastrointestinal rupture (possibly secondary to MM infiltrating and weakening tissue or medications as described above) or translocation. Neutropenia may have been a predisposing risk factor for sepsis, but this alone should not have resulted in bacterial contamination of the peritoneal cavity.

**Discussion:** Multiple myeloma (MM) is a systemic clonal proliferation of neoplastic plasma cells that produce immunoglobulin [7]. MM is a relatively common neoplastic disorder of bone marrow: based on a large retrospective of 717 canine bone marrow specimens with myriad diagnoses including neoplastic and non-neoplastic disease, the incidence of MM was 3.5%, just behind lymphoma (4% of samples) [9]. Pathologic complications often arise from altered blood viscosity due to marked hyperglobulinemia (with excessive cardiac workload and secondary end-organ damage to the retina, brain, and kidneys), as well as increased susceptibility to infection, coagulation abnormalities, hypercalcemia (possibly contributing to kidney injury), and one or more cytopenias [7].

Diagnosis of MM in dogs is based on identification of a monoclonal gammopathy in serum/plasma, Bence-Jones proteinuria (immunoglobulin light chains in the urine), one or more lytic bone lesions, hypercalcemia, and increased bone marrow plasma cell concentration (>20%) [7]. Differentials for canine patients presenting with moderate to marked hyperglobulinemia include other immunoglobulin-secreting lymphoid malignancies, chronic infectious/inflammatory conditions (especially *Ehrlichia canis*, and *Leishmania* spp., where endemic), and monoclonal gammopathy of unknown significance [7]. Differentiating neoplastic from reactive disorders can be accomplished with serum or urine protein electrophoresis and/or polymerase chain reaction showing monoclonal antigen receptor rearrangement. In this patient’s case, a serum protein electrophoresis was submitted to the
Cornell University Animal Health Diagnostic Center and demonstrated a marked monoclonal spike in the gamma globulin region.

Alkylating chemotherapy drugs are the mainstay of MM treatment, most commonly melphalan (with or without prednisone), followed by less common choices including cyclophosphamide, chlorambucil, and lomustine (CCNU) [7]. The prognosis for MM with appropriate treatment is considered fair to good, with published median survival times of approximately 18 months [3]. However, the presence of marked hypercalcemia, Bence-Jones proteinuria, and numerous lytic bone lesions appear to have negative implications for treatment response [3]. Additionally, extensive abdominal organ involvement and presence of plasmablasts or atypical giant cell forms has been associated with worse prognosis in human patients with MM [8]. The presence of neoplastic MM cells in effusions has not been clearly associated with outcome in domestic species, but is rare based on only one previous report in the literature. In this patient, the cytopenias and presence of sepsis likely contributed to the very poor outcome. While the lack of surgical exploratory or necropsy precluded a definitive diagnosis for the etiology of sepsis, complications from extensive marrow involvement and drug toxicity likely lead to either GI rupture or translocation, ultimately leading to systemic infection and possibly DIC.

References:


