Multiple cutaneous nodules in a dog
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Signalment:
‘Billy’, 6.5 years old neutered male black&white Cocker Spaniel.

History:
Billy presented to the University of Edinburgh’s Oncology Service for investigation of numerous, rapidly growing, cutaneous and subcutaneous nodules. The first nodule appeared six months earlier and in the 6 weeks before presentation multiple nodules had rapidly appeared and grown.

Physical examination:
On presentation Billy was bright, alert and responsive, with a body condition score of 6/9. Thoracic auscultation revealed a mild increase in bronchovesicular lung sounds. His left mandibular and right prescapular lymph nodes were enlarged and firm. He had multiple (>90), firm nodules to masses throughout his body; they were variable in size (0.2 – 6 cm) and location (cutaneous, subcutaneous and muscular). The only location were pain could be elicited was the right hip, that was painful at extension. The rest of the general physical examination was unremarkable.

Diagnostic procedures:
Haematology revealed mild anaemia (PCV 0.356 l/l, reference interval [RI] 0.39 - 0.55) and mild thrombocytopenia (platelets 116 x10⁹/l, RI 200 – 500). On examination of the blood smear, the anaemia appeared non-regenerative, and mild thrombocytopenia was confirmed; occasional large, round cells were noted (Figure 1).

The alterations observed in a comprehensive serum biochemistry profile included a mild increase in ALT (141 U/l, RI 21 – 102), moderate increase in ALP (259 U/l, RI 20 – 60), and a mild hyperproteinaemia (77.2 g/l, RI 58 – 73) due to hyperglobulinemia (45.2 g/l, RI 18 – 37). Triglycerides (2.0 mmol/l, RI 0.57 – 1.14) were also mildly increased. Other analytes (e.g. albumin, totCa, fCa) were within RI.

Fine needle aspirates from the cutaneous lesions and lymph nodes were performed and submitted for evaluation (Figures 2 – 4).

Thoracic radiographs revealed a bronchointerstitial pattern, tracheobronchial lymphadenomegaly, a solitary pulmonary nodule, and a soft tissue mass caudal to the right brachium. Pelvic radiographs revealed sublumbar lymphadenomegaly and a soft tissue mass cranial to the left femur (Figure 5).

Abdominal ultrasound revealed hepatomegaly with ill-defined hypoechoic regions up to 1cm in diameter. Throughout the splenic parenchyma there were extensive, partially coalescing, hyperechoic nodules and striations. Within the peritoneum there were numerous cystic and hypoechoic nodules up to 1.4cm in diameter.

Imaging findings were suggestive of disseminated neoplasia.

Questions:
1) Considering the clinical presentation, the diagnostic findings, and the cytology figures, what would be your main differential diagnoses?
2) What other tests would you suggest to perform?
**Figures:**

**Figure 1:** Blood smear, Modified Wright, 1000x magnification

**Figure 2:** FNA from a cutaneous nodule, May-Grünwald Giemsa, 500x magnification
Figure 3: FNA from a cutaneous nodule, May-Grünwald Giemsa, 500x magnification

Figure 4: FNA from the left submandibular lymph node, May-Grünwald Giemsa, 500x magnification
Figure 5:

Pelvic radiograph: a nodule is visible within the soft tissues cranial to the left femur (arrow).
Cytologic description:
The smears from the cutaneous lesions (Figures 2-3) had a moderate background of erythrocytes and were highly cellular. A population of large, atypical round cells predominated. Cells were 30 to 40 μm in diameter and had a moderate amount of basophilic cytoplasm that frequently contained low numbers of small, discrete, and colourless vacuoles. The nuclei were generally eccentrically located, and had irregular shapes ranging from rounded, to bean-shaped, to irregularly indented. The chromatin was finely stippled and one or multiple, round, variably-sized and -prominent nucleoli were seen. Mitotic figures were frequent, anisocytosis and anisokaryosis were moderate to marked; binucleation and multinucleation are present, and occasional macronuclei and satellite micronuclei were noted. The same population of large atypical cells was present in the lymph node aspirates (Figure 4), where also a moderate amount of pinkish to lavender-staining amorphous material (necrotic debris) and necrotic cells were seen. Rare, large, round cells with morphological features similar to what described in the cutaneous smears were also observed on blood smear evaluation (Figure 1).

Considering the cytological and haematological findings, the diagnosis was disseminated round cell neoplasia, and the main differential diagnoses were histiocytic sarcoma or plasma cell neoplasia. Lymphoma was considered another less likely differential.

Further testing:
To further investigate the hyperglobulinemia, serum protein electrophoresis (SPE) was performed. The trace showed a narrow peak in the β/γ region, highly suggestive of a monoclonal peak (Figure 6).

Fine needle aspirates were obtained from liver and spleen. Neoplastic cells were present in the smears from both organs. These cells had cytological features very similar to what described in the cutaneous lesion, but in the hepatic aspirates the cells frequently had bright pink staining of the peripheral cytoplasm (flame cell appearance) (Figure 7). The flame cell morphology was less frequent and less prominent in the splenic aspirates.

Bone marrow smears revealed hypercellular spicules (80 to 90%). Large neoplastic cells comprised 31.5% of the total nucleated cells, and the flame cell appearance was frequent and prominent. Erythroid and myeloid precursors were present to completion and showed orderly maturation; mild dysplastic changes were noted in the granulocytic precursors (giant bands and metamyelocytes) and megakaryocytic series (multiple individualised nuclei) (Figure 8).

A cutaneous nodule from the right flank was excised and submitted for histopathological evaluation. Infiltrating the dermis and subcutis there was a multilobular mass composed of sheets of round cells. These cells had a moderate amount of eosinophilic cytoplasm and round nuclei with fine to coarse chromatin and generally inconspicuous nucleoli. There was moderate cellular pleomorphism and scattered multinucleated cells were observed. The morphological findings were considered indicative of a plasma cell tumour.

A panel of immunohistochemical markers was applied on the cutaneous nodule sections. Approximately 75-85% of the neoplastic cells showed moderate to strong, positive, intranuclear staining for MUM-1, and scattered individual cells (less than 15%) showed weak to mild, positive membranous to cytoplasmic staining for CD79a. Approximately 85-95 % of the neoplastic cells exhibited moderate to strong membranous, positive staining for CD3. Neoplastic cells did not exhibit positive staining for CD18 nor PAX5.

Unstained spleen and bone marrow smears, and a cutaneous nodule section were stained with methyl green pyronin, a histochemical stain used to visualise RNA and DNA. Pyronin stains RNA in a bright pink-red colour, while methyl green stains DNA purple to bluish. Due to their high content of RNA in the cytoplasm, plasma cells normally exhibit marked cytoplasmic phyroninophilia (bright pink-red colour). Neoplastic cells in cytologic and histologic specimens demonstrated marked cytoplasmic phyroninophyilia (Figure 9).

Diagnosis:
Disseminated plasma cell tumour
Treatment and follow up:
Billy is currently undergoing chemotherapy treatment. His protocol consists of:
- A first administration of cyclophosphamide (250 mg/m² orally), followed by a second dose (200 mg/m² orally) after two weeks;
- Melphalan (7 mg/m², orally, once a day for 5 days, every 3 weeks), started the day after the second cyclophosphamide administration. At the time of writing, two 5-day cycles have been administered.
- Prednisolone (0.5 mg/kg, orally, once daily) started 5 days after the first cyclophosphamide dose; administration reduced to every other day 14 days after the first dose of melphalan.

Billy is responding very well to the treatment, and no significant chemotherapy side effects have been reported, apart from ongoing increased appetite (due to steroids), and an episode of grade I neutropenia (2.14 x10⁹/L, RI 3.6-12) after the first cyclophosphamide dose.

At the time of the second cyclophosphamide administration, six selected target cutaneous lesions showed a 45% reduction in size, and the globulin levels had decreased slightly (41.7g/l previously 45.2g/l, RI 18-37). At the time of the second melphalan cycle, most of the cutaneous lesions have completely disappeared and the globulin level further decreased to the normal range (30.7 g/l).

Discussion:
Disorders arising from plasma cells include conditions such as monoclonal gammopathy of unknown significance (MGUS), Waldenström's macroglobulinemia, solitary plasmacytoma (extramedullary and osseous), and multiple myeloma. [1]

Recently revised guidelines for diagnosing multiple myeloma in human medicine [2] require the presence of >10% clonal plasma cells in the bone marrow or a biopsy-proven bony or extramedullary plasmacytoma, in addition to one or more myeloma-defining events:

a. evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder (specifically hypercalcemia, renal insufficiency, anaemia, and bone lesions)

b. clonal bone marrow plasma cells >60%

c. involved:uninvolved free light chain ratio ≥100 (involved free light chain must be ≥100mg/L)

d. > 1 focal lesion on MRI studies (at least 5 mm in size)

In veterinary medicine, the conventional criteria for diagnosis of multiple myeloma involve demonstration of at least two [3] to three [4] of the following:

i. monoclonal gammopathy

ii. lytic bone lesions

iii. plasma cell infiltration in the bone marrow

iv. Bence-Jones proteinuria

Canine cutaneous plasmacytomas are usually benign tumours that carry a good prognosis; provided complete surgical margins are achieved, excision is considered curative. [1, 5] Cases of multiple cutaneous plasmacytomas are rare, [6] and they have been described also as part of a disseminated and aggressive myeloma process; [7, 8] a case of a subcutaneous solitary plasmacytoma progressing to multiple myeloma has been reported. [9]

In human medicine, cutaneous involvement in multiple myeloma is rare, generally appears at a late stage of the disease, and is associated with aggressive biological behaviour and short survival. [10] Extramedullary plasmacytomas are typically solitary tumours, most commonly found in the upper respiratory tract and they frequently have an indolent clinical course and only rarely progress to multiple myeloma. [11, 12] Usually the two neoplasms can be distinguished by clinical manifestation, since the histopathological appearance is overlapping.

Our case fulfils the criteria for diagnosis of multiple myeloma: the SPE trace was suggestive of monoclonal gammopathy, and neoplastic plasma cells were present in the marrow. It is uncertain whether the neoplasia originated extramedullary (skin or visceral organs) or from the bone marrow. Considering the progressively extensive cutaneous involvement, infiltration of spleen and liver, relatively milder involvement of the bone marrow, and absence of overt clinical signs, an extramedullary origin of the disease was speculated, although the lack of further investigations (such as haematology and biochemistry profiles, imaging or bone
marrow examination) at the time of presentation of the first nodule does not allow a definitive conclusion.

Presence of circulating plasma cells in multiple myeloma is reported in approximately 10% of dogs. [5] In humans, a recent study detected clonal circulating plasma cells via flow cytometry in 54% of multiple myeloma patients, and high numbers of circulating cells were associated with high-risk disease. [13]

Some morphological differences were seen between the cells in the cytology smears from the different sites. In particular, the presence of flame cells was noted mainly in liver and bone marrow smears. The peripheral cytoplasm of flame cells contains numerous cisterns of endoplasmic reticulum distended by immunoglobulins, which stain pink on MGG. The secretory obstruction destroys the cell margin, and cells shed fragments of immunoglobulin-laden cytoplasm through a process named clasmation. [14] The flame cell appearance is considered typical of plasma cells. In our case, the strong cytoplasmic pyroninophilia of the neoplastic cells was indicative of a high RNA content, due to the abundant rough endoplasmic reticulum, and supported a plasma cell origin of the neoplasia. [9, 15]

Most of the immunohistochemistry markers used in this case were consistent with a plasma cell origin. The cells showed positive staining for MUM-1, which is a transcription factor involved in lymphoid cell differentiation, and is considered specific for canine plasma cell tumours; [16] there was also weak positivity for CD79a, which is a surface marker present on both immature and mature lymphocytes, including plasma cells. [16] Lack of expression was expected for PAX5 (pan-B-cell marker, absent in plasma cells [12]), and CD18 (a pan leukocyte marker that is not constantly expressed in plasma cell tumours [7, 16]). The strong positivity for CD3 was unexpected in our case, as CD3 is a marker for T lymphocytes and T cell lymphomas. MUM-1 is expressed by activated T-lymphocytes and can be expressed in human T-cell lymphomas. [16] However, although very uncommon, reports of aberrant expression of CD3 in human multiple myelomas are reported. [17-19]

In our case, the majority of findings are supportive of a diagnosis of plasma cell neoplasia: cytological appearance of the cells (particularly the flame cell morphology), pyroninophilia, SPE trace suggestive of monoclonal gammopathy, histological features, and most of the immunohistochemical markers. We therefore considered our case a disseminated plasma cell tumour with aberrant CD3 expression; confirmation of B-lymphocyte lineage (e.g. with PCR assay for Antigen Receptor Rearrangement) would be required for a more definitive diagnosis. To our knowledge, aberrant expression of CD3 in plasma cell tumours has not yet been reported in veterinary medicine.

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Additional figures:

Figure 6: Agarose Gel Serum Protein Electrophoresis (IDEXX laboratories)

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Total protein: 76.30 g/l   A/G ratio: 0.69 (RI 0.60 – 1.50)

Figure 7: FNAs from liver, May-Grünwald Giemsa, 500x magnification. Prominent flame cell appearance of the neoplastic cells.
Figure 8: bone marrow smear, May-Grünwald Giemsa, 400x magnification. Presence of neoplastic flame cells (arrowheads), and giant band and metamyelocyte (arrows).

Figure 9: methyl green pyronin stain, spleen FNA (A, 500x magnification) and cutaneous nodule section (B, 200x magnification). Marked cytoplasmic phyroninophilia of the cells.

References: