WHAT IS YOUR DIAGNOSIS?

A 9 year old, female neuter, English Springer Spaniel was presented to the R(D)SVS Internal Medicine Service for investigation with a three-week history of lethargy, pyrexia, polydipsia/polyuria and mild generalised lymphadenomegaly. An improvement in demeanour was seen with meloxicam and antibiotics (enrofloxacin then amoxycillin/clavulanate) but the pyrexia, polydipsia/polyuria and lymphadenomegaly were unchanged.

On clinical examination, the dog was bright, alert and responsive. The pulse rate was 100 beats/minute, the respiratory rate was 30 breaths per minute and the rectal temperature was 39.6°C. Moderate generalised lymphadenopathy was noted. There were widespread cutaneous plaques and subcutaneous nodules present. Abdominal palpation and rectal examination were unremarkable.

A blood sample was taken for routine haematology and serum biochemistry (results below). A urine sample was obtained by cystocentesis for full analysis and culture.

Haematology

Parameter	Result	Reference Range
RBC (x 10^{12}/l)	5.94	5.5-8.5
PCV (%)	40.0	39-55
Hb (g/dl)	13.7	12-18
MCV (fl)	67	60-77
MCHC (%)	34.3	32-36
TWBC (x 10^{9}/l)	9.0	6-15
Neutrophils (x 10^{9}/l)	7.29	3.6-12
Lymphocytes (x 10^{9}/l)	1.26	0.7-4.8
Monocytes (x 10^{9}/l)	0.36	0-1.5
Eosinophils (x 10^{9}/l)	0.09	0-1
Basophils (x 10^{9}/l)	0	0-0.2
Platelets (x 10^{9}/l)	471	200-500

Serum biochemistry

Parameter	Result	Reference Range
Total protein (g/l)	66.7	58-73
Albumin (g/l)	29.3	26-35
Globulin (g/l)	37.4*	18-37
Bile acids (µmol/l)	4.5	0-7
Total bilirubin (µmol/l)	3.1	0-6.8
Urea (mmol/l)	5.8	1.7-7.4
Creatinine (µmol/l)	106	40-132
Glucose (mmol/l)	6.2*	3-5
AP (iu/l)	60	20-60
ALT (iu/l)	26	21-102
Sodium (mmol/l)	148	139-154
Chloride (mmol/l)	111	99-115
Potassium (mmol/l)	5.1	3.6-5.6
Inorganic phosphate (mmol/l)	1.62	0.9-2.0
Total calcium (mmol/l)	3.12*	2.0-3.0

1) *What are your differential diagnoses for the hypercalcaemia?*

2) *What further investigations would you like to perform?*

1) Differential diagnoses for hypercalcaemia include (using the mnemonic HARD IONS G)
Hyperparathyroidism
Addison's disease
Renal failure
Vitamin **D** toxicosis e.g. from rodenticides, house plants or psoriasis creams
Idiopathic (this is mainly a feline condition)
Osteolytic e.g. osteomyelitis
Neoplasia e.g. lymphoma, anal sac adenocarcinoma
Spurious i.e. it is important to rule out lab error before starting exhaustive investigation
Granulomatous disease

2) The first step in the investigation of hypercalcaemia would be to demonstrate that the hypercalcaemia is a true finding by ideally demonstrating that the ionised calcium is elevated, or at least documenting that the total hypercalcaemia is a persistent finding. Ionised calcium is the active form and constitutes 50% of the total calcium with the remainder being either protein bound (40%) or bound to anions (10%). In this case the ionised calcium was 1.61 mmol/l (reference range 1.15-1.5) and could potentially explain the polydipsia/polyuria state in this case.

Diagnostic tests for the above differentials include:

Hyperparathyroidism: parathyroid hormone (PTH) assay, parathyroid ultrasound (one large parathyroid gland greater than 4mm in diameter)
Addison's disease: biochemistry and haematology findings (hyperkalaemia, hyponatraemia, azotaemia, eosinophilia), ACTH stimulation test
Renal disease: azotaemia in conjunction with an isosthenuric or mildly hypersthenuric urine (1.008-1.029) Note: in renal disease, often there is a total hypercalcaemia but ionised calcium is normal (although can be low, high or normal).
Vitamin D toxicosis: history of ingestion, increased vitamin D metabolite concentrations.
Osteolytic: history of pain or lameness, radiography
Neoplastic: biopsy, increased parathyroid hormone related peptide (PTHrp), palpation of an anal sac mass.
Granulomatous disease: biopsy, increased serum concentrations of 1,25 OH vitamin D

In this case, PTH, PTHrp the vitamin D metabolities, 25-OH-vitamin D and 1,25-OH-vitamin D, were measured. The results are below.

PTH (pg/ml)	28	10-60
PTH-rp (pmol/l)	0.9	<1.0
25-OH-vitamin D (ng/ml)	16.5	19.3-44
1,25-OH-vitamin D (pg/ml)	109	16-40

3) *Given these results, together with the clinical presentation, what is the most likely diagnosis?*

In this patient, the lymph node enlargement and hypercalcaemia, taken together, were most likely due to lymphoma or granulomatous disease. PTH was not increased making primary hyperparathyroidism unlikely. PTHrp did not support of diagnosis of malignancy but does not rule it out (Mellanby et al 2006a). Biopsy of the lymph nodes, skin plaques and subcutaneous nodules were consistent with pyogranulomatous lymphadenitis, dermatitis and panniculitis (Figures 1 and 2). . Samples were submitted for histopathology and culture (bacterial, fungal and mycobacterial) and special stains (Gram, Grocott and Zeihl-Neilson) were also performed, all of which were negative. To further investigate the possible infectious aetiologies of the pyogranulomatous inflammation, serology for *Toxoplasma* and *Neospora* and PCR blood tests for *Leishmania, Bartonella, Ehrlichia, Borrelia* and *Rickettsia* were submitted, all of which were negative.

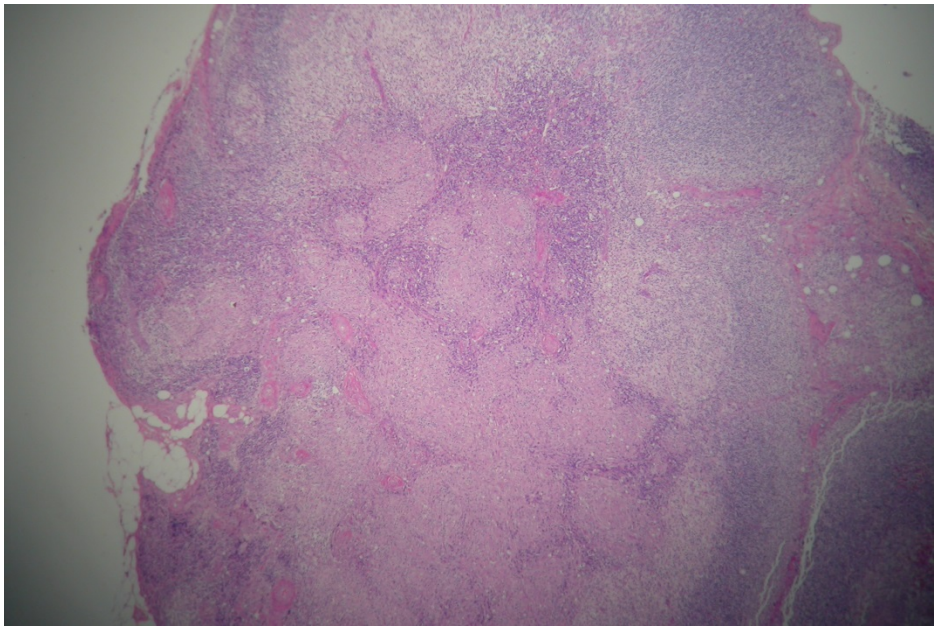

Figure 1. Popliteal lymph node histopathology demonstrating effacement of normal nodal architecture by pyogranulomatous inflammation.

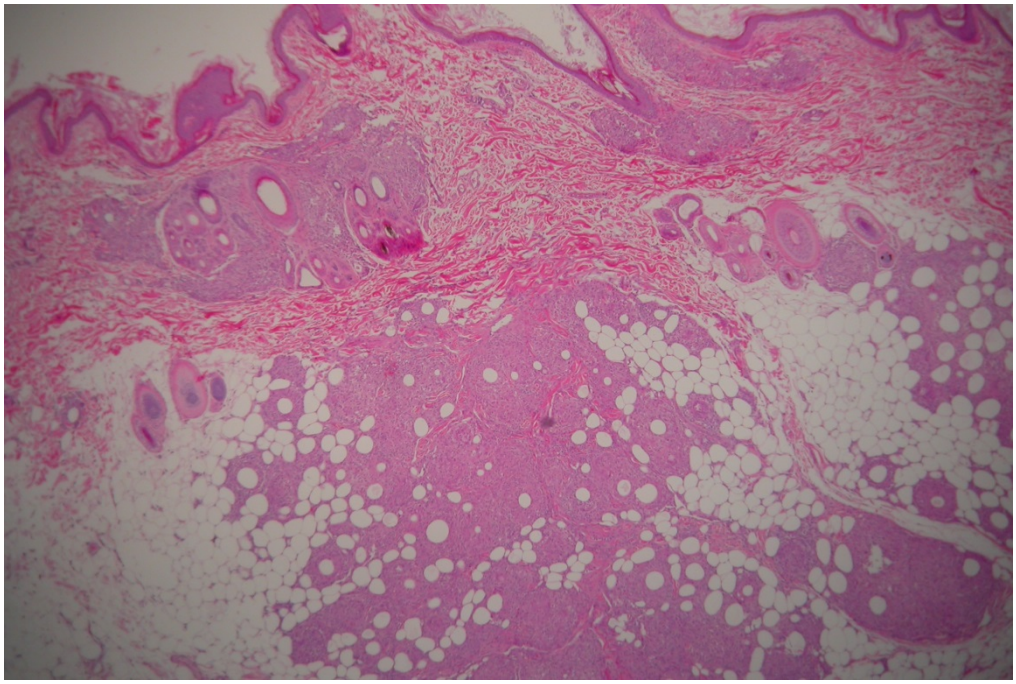

Figure 2: Note pyogranulomatous infiltration of dermis, epidermis and sub-dermal fat.

Management

Fluid therapy was commenced at 4 ml/kg/hr of normal saline to reduce the serum calcium concentrations during the investigation. Once a diagnosis was reached, prednisolone therapy was started at 1 mg/kg/day in divided doses. Lymphadenopathy and skin lesions resolved by day 40. After day 86, serum calcium results remained normal and prednisolone therapy was stopped on day 186. The animal was disease-free and calcium levels normal 1 year later.

Discussion

The clinical diagnosis in this case was of granulomatous inflammation with secondary hypercalcaemia. The hypercalcaemia was likely to be secondary to the increased serum 1,25-OH-vitamin D concentrations (Mellanby et al 2006b). Dysregulated production of 1,25-dihydroxyvitamin D by macrophages is a recognised cause of hypercalcaemia in granulomatous disease in humans. Granulomatous disease in dogs has been reported to occur secondary to various infectious agents; however, none were identified in this case. It has been reported that there appears to be a breed-related predisposition to sterile granulomatous lymphadenitis and pyogranulomatous dermatitis in English Springer spaniels. In this case, and previously reported cases, a positive response to immunosuppressive doses of glucocorticoids has been documented and may suggest an immune-mediated pathogenesis in this breed.

References

Mellanby, R.J., Craig, R., Evans, H., Herrtage, M.E. (2006a) Plasma parathyroid hormone related protein concentrations in dogs with calcium metabolism disorders. *Veterinary Record*. **159,** 833-838

Mellanby, R.J., Mellor, P.J., Villiers, E.J., Herrtage, M.E., Halsall, D, O'Rahilly, S., McNeil, P.E., Mee, A.P., Berry, J.L. (2006b) Hypercalcaemia associated with granulomatous lymphadenitis and elevated 1,25 dihydroxyvitamin D concentration in a dog. *Journal of Small Animal Practice*. **47,** 207-212