Glucocorticoid-deficient hypoadrenocorticism secondary to intravascular lymphoma in the adrenal glands of a dog

ME Buckley, a* PS Chapman a and A Walsh b

Case report
A 2-year-old neutered male German Shepherd dog was presented with weakness, poor appetite and weight loss. Glucocorticoid-deficient hypoadrenocorticism was diagnosed with undetectable pre- and post-ACTH cortisol concentrations but normal sodium and potassium concentrations. Despite appropriate supplementation with glucocorticoids, the patient’s weakness progressed and neurological deficits developed. The patient was euthanased. Histopathological analysis of multiple organs, including the adrenal glands, showed an accumulation of neoplastic lymphocytes within blood vessels, consistent with a diagnosis of intravascular lymphoma. Histologically, in both adrenal glands, the architecture of the zona fasciculata and reticularis was disrupted by blood vessels congested with a neoplastic population of T-lymphocytes; the zona glomerulosa remained intact.

Conclusion
This is the first report of intravascular lymphoma causing glucocorticoid-deficient hypoadrenocorticism in a dog.

Keywords
adrenal glands; Addison’s disease; dogs; neoplasia

Abbreviations
ACTH, adrenocorticotropic hormone; CBC, complete blood count


Canine hypoadrenocorticism most commonly results from immune-mediated destruction of the canine adrenal glands. 1 However, rarely in humans and dogs other diseases of the adrenal glands, including neoplasia, granulomatous inflammation and infectious disease, can also cause hypoadrenocorticism. 2,3 Most dogs with hypoadrenocorticism are deficient in mineralocorticoids and glucocorticoids, though a subset of dogs may lack the typical electrolyte changes of hyperkalaemia and hyponatraemia. 1 This syndrome has commonly been referred to as atypical hypoadrenocorticism.

Intravascular lymphoma is a rare malignant neoplasm that occurs when lymphocytes proliferate predominantly within capillaries. 4,5 Capillary lumina become occluded by neoplastic cells, resulting in tissue ischaemia and a range of clinical abnormalities depending upon the affected organ. It is not uncommon for affected humans to have adrenal enlargement associated with adrenal insufficiency. 6 The authors believe that the case described here is unique as the first reported case of canine hypoadrenocorticism secondary to intravascular lymphoma and the first reported case of glucocorticoid-deficient hypoadrenocorticism secondary to any adrenal neoplasia.

Case report
A 30-kg neutered male German Shepherd Dog, estimated to be approximately 2 years old, was presented to the Veterinary Specialty and Emergency Center with a history of weakness, anorexia and weight loss. The dog had been transported from North Carolina by a rescue organisation 3 months previously and his history prior to that was unknown. Three weeks prior to presentation, the dog had been evaluated by the referring primary care veterinarian for the same signs and the diagnostics had been performed. A complete blood count (CBC) showed a mild leucopenia and moderate thrombocytopenia. A serum biochemistry profile showed mild hypoglycaemia, hypoalbuminaemia and azotaemia; serum sodium and potassium concentrations were within reference ranges (full results are shown in Table 1A). An in-house ELISA (Snap 4Dx, IDEXX) was positive for antibodies against Ehrlichia canis. Thoracic radiographs showed no significant abnormalities. Treatment was initiated with doxycycline at 10 mg/kg/day (300 mg once daily). An adrenocorticotropic hormone (ACTH) stimulation test had been performed, presumably because of the patient’s non-specific clinical signs, lack of a stress leucogram and the biochemical abnormalities, and showed pre- and post-cortisol levels <19.3 nmol/L before and 2 h after the intramuscular injection of 66 units of a compounded ACTH gel (Wedgewood Pharmacy). Treatment with prednisone was initiated at 15 mg total dose per day (0.5 mg/kg/day).

Two weeks after starting medical treatment, the clinical signs of weakness, lethargy and inappetence persisted. A serum biochemistry profile showed a mild persistent increase in the blood urea nitrogen concentration and mild increases in serum liver enzyme activities. A repeat CBC showed a worsening of the leucopenia, a new finding of neutropenia and normocytic, normochromic anaemia (Table 1B). A PCR panel (Fastpanel PCR Canine Tick Borne Panel Profile, Antech Diagnostics, FL, USA) did not amplify any DNA from Anaplasma phagocytophilum, Anaplasma platys, Babesia spp., Bartonella henselae, Bartonella vinsonii, Ehrlichia spp., Mycoplasma hemocanis/hematoparvum, Neorickettsia risticii or Rickettsia rickettsii. An immunofluorescent antibody titre for E. canis was negative at <1: 80.

On initial presentation to the Center, the dog was subjectively lethargic but ambulatory with a normal gait. There was diffuse, mild muscle wasting with a body condition score of 3/9. There was mild
Alanine aminotransferase (10–100 IU/L) 3 16 47 (40–155) 4.04
Potassium (3.5–5.8 mmol/L) 2.02 1.02 3.01 (2.06–10.6) 2.71
Red blood cell (5.5–8.5 × 10^12/L) 5.57 3.92 2.96 (4.8–9.3) 2.11
Haematocrit (37–55%) 41.4 28.9 23.4 (17.6
Haemoglobin (120–180 g/L) 142 95 75 (121–203) 67
MCV (60.0–77.0 fl) 74.1 73.7 79 (58–79) 83.6
MCHC (300–375 g/L) 342 328 321 (300–380) 378
Platelets (175–500 × 10^9/L) 104 221 54 (170–400) 140
Reticulocyte count (10.0–110.0 × 10^9/L) 41.8 38.3 88 (264.8

Table 1. Clinicopathological abnormalities at various time points

<table>
<thead>
<tr>
<th>Analyte (reference range)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (4.1–7.9 mmol/L)</td>
<td>2.6</td>
<td>5.4</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Albumin (23–40 g/L)</td>
<td>22</td>
<td>25</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>BUN (2.5–9.6 mmol/L)</td>
<td>13.6</td>
<td>10.4</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Creatinine (44–159 μmol/L)</td>
<td>106</td>
<td>88</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Phosphorus (0.81–2.20 mmol/L)</td>
<td>2.29</td>
<td>1.94</td>
<td>1.81</td>
<td></td>
</tr>
<tr>
<td>Sodium (144–160 mmol/L)</td>
<td>153</td>
<td>154</td>
<td>147</td>
<td>151</td>
</tr>
<tr>
<td>Potassium (3.5–5.8 mmol/L)</td>
<td>4.4</td>
<td>5</td>
<td>5.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Alanine aminotransferase (10–100 IU/L)</td>
<td>95</td>
<td>166</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>γ-glutamyltransferase (0.0–7 IU/L)</td>
<td>3</td>
<td>16</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total white blood count (5.5–16.9 × 10^9/L)</td>
<td>3.64</td>
<td>2.09</td>
<td>4.7 (40–155) 4.04</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (2.0–12.0 × 10^9/L)</td>
<td>2.02</td>
<td>1.02</td>
<td>3.01 (2.06–10.6) 2.71</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (0.5–4.0 × 10^9/L)</td>
<td>0.63</td>
<td>0.38</td>
<td>1.08 (0.69–4.5) 0.43</td>
<td></td>
</tr>
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Reference ranges listed on the left unless otherwise noted.
A, initial evaluation by primary care veterinarian; B, 2 weeks after initial presentation; C, 3 days after date B and initial presentation to authors’ practice; D, 5 days after date C, approximately 3 weeks after initial presentation to primary care veterinarian.
BUN, blood urea nitrogen; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume.

discomfort on flexion of the neck and on palpation of the cervical spine. A CBC showed a marginally regenerative anaemia, a normal leukogram and thrombocytopenia (Table 1C).

An abdominal ultrasound examination showed no significant changes. Both adrenal glands were at the upper limits of normal size (maximum diameter of the cranial pole of the left adrenal gland 6.5 mm in the transverse plane; right adrenal gland 7.7 mm) with a normal shape and architecture.7,8 Lateral radiographs of the cervical, thoracic, lumbar and sacral spine documented a slightly narrowed C5–C6 disc space but no other significant abnormalities. Cytological analysis of a bone marrow aspirate from the right proximal humerus showed erythroid and megakaryocytic hyperplasia with evidence of left shifting and a myeloid to erythroid ratio of 1 : 3. No infectious agents or neoplastic cells were seen. The dose of prednisone was reduced to a physiological 5mg once daily (0.16 mg/kg/day).

The dog was re-presented 2 days later after becoming progressively weak. A full neurological examination revealed weak withdrawal reflexes, delayed postural reactions and an intermittent horizontal nystagmus with the slow phase to the left. A CBC and chemistry panel at that time showed progressive worsening of the anaemia, a mild elevation in alanine aminotransferase and a mild thrombocytopenia (Table 1D). Over the following 12 h, the dog became progressively dull and weak and was euthanased. A necropsy was performed, which revealed a mildly enlarged liver. The lungs, cardiac structures, spleen, stomach, intestines, adrenal glands, kidneys and spinal cord all appeared grossly normal.

Histopathological examination of multiple tissues included adrenal glands, lung, liver, spinal cord, skeletal muscle and sciatic nerve. Throughout all sections examined, small and medium-sized blood vessels contained large, round neoplastic cells (nuclei 2–3-fold greater than the diameter of a single erythrocyte) with distinct cell borders, scant to moderate granular eosinophilic cytoplasm and round to oval nuclei with coarsely stippled chromatin and 1–3 nucleoli. The neoplastic cells markedly dilated the blood vessels of the adrenal glands, resulting in disruption and loss of the zona fasciculata and zona reticularis (Figure 1). The remaining cortical cells in these zones were moderately vacuolated (Figure 2). The zona glomerulosa in both adrenal glands retained normal architecture. Immunohistochemistry was performed on paraffin-embedded adrenal gland tissue for the following markers: CD3, CD79a, CD18 and Pax5. Positive immunoreactivity was noted for CD3 (T-lymphocyte marker) and CD18 (pan-leucocyte/histiocyte marker) stains; neoplastic cells were negative for the B-cell markers, CD79a and Pax5 (Figure 3), confirming a diagnosis of T-cell intravascular lymphoma.

Discussion

In this dog, the initial presenting signs were non-specific yet consistent with a diagnosis of glucocorticoid-deficient hypoadrenalism based on hypocortisolaemia in the face of ACTH stimulation testing and normal electrolyte levels. It has previously been assumed that so-called atypical hypoadrenalism is caused by glucocorticoid deficiency without mineralocorticoid deficiency. However, a recent
study found no significant difference in aldosterone concentrations between patients with and without electrolyte changes and questioned the term ‘atypical hypoadrenocorticism’. In this patient the aldosterone concentrations were not measured, but histopathology showed relative sparing of the zona glomerulosa. Although histopathology is not necessarily predictive of endocrine function, this does make aldosterone deficiency less likely.

The clinical response to glucocorticoid supplementation was incomplete, which is unexpected in a patient strictly with hypocortisolae-mia. It was initially suspected that the persistent clinical signs were from a vector-borne or fungal infection. However, given the final diagnosis, it is likely that many of the clinical signs were related to the underlying neoplastic disease and not directly to the endocrinopathy. The clinical signs associated with intravascular lymphoma are very variable and most cases are diagnosed at postmortem. Progressive neurological signs, as seen in this dog are a frequently described manifestation. As in this patient, most cases of intravascular lymphoma in veterinary patients have a T-cell phenotype, in contrast to humans in whom 90% of cases have a B-cell phenotype. Given the dearth of information on this disease in veterinary patients, it is unknown if this immunophenotypic difference influences the response to treatment or what the ideal treatment protocol might be. In one human study using a multi-agent anthracycline-based protocol, the mean survival was 13 months. The addition of the anti-CD20 antibody, rituximab, to such protocols shows promise of improving outcomes.

In addition to the lack of resolution of the clinical signs in the present case, the adrenal ultrasound findings were also inconsistent with a diagnosis of uncomplicated hypoadrenocorticism secondary to adrenal atrophy. In a recent study of dogs with hypoadrenocorticism, the measured thickness of the left adrenal gland was less than 3.2 mm in 28 of 29 dogs and the right adrenal gland less than 3.2 mm in 18 of 22 dogs. Neither adrenal gland was thicker than 4.5 mm in any dog. Based on these data, using abdominal ultrasound as a sole screening test would have excluded a diagnosis of hypoadrenocorticism in our patient. Occasional cases have been described of dogs with hypoadrenocorticism secondary to adrenal necrosis, neoplasia or abscessing inflammation and these cases had normal to large-sized adrenal glands. In humans, infections, including fungal disease and tuberculosis, have been described as causing hypoadrenocorticism with adrenomegaly, but no such cases have been definitively reported in dogs. In the present patient, the neoplastic infiltration documented on histopathology accounts for the unexpectedly normal size of the adrenal glands. This report demonstrates that a normal adrenal gland thickness on ultrasound examination cannot exclude a diagnosis of hypoadrenocorticism in dogs and infiltrative adrenal gland disease should be considered where this is the case.

Figure 1. Adrenal gland (H&E, ×40). Red bar indicates the zona fascicu-lata and zona reticularis infiltrated with neoplastic cells. Black bar indi-cates an intact zona glomerulosa.

Figure 2. Adrenal gland, zona reticularis (H&E, ×400). Arrow depicts vacuolation of a cortical cell. Two blood vessels distended with neoplastic cells are circled.

Figure 3. CD3 positive cells within the zona reticularis (immunoperoxi-dase, ×400).
Finally, leukopenia, lymphopenia and thrombocytopenia are also not expected in a patient with uncomplicated hypoadrenocorticism. By the time of our initial evaluation, the patient was also neutropenic and these cytopenias led to our further evaluation for vector-borne diseases or infiltrative bone marrow disease. The cytopenias were ultimately attributed to the intravascular lymphoma, although without evidence of neoplastic infiltration on the bone marrow aspirate sample, the mechanism for these cytopenias was not readily apparent. Various cytopenias have been reported in humans and dogs with intravascular lymphoma and are suspected to arise secondary to haemophagocytic syndrome, bone marrow infiltration or other undetermined mechanisms.

Although one retrospective study of 38 cases of bilateral metastatic neoplasia affecting the adrenal glands included one dog with lymphoma that was reported to have hypoadrenocorticism, no clinical details were provided of the endocrinopathy in that case. Only one previous case of hypoadrenocorticism secondary to bilateral adrenal neoplasia has been more completely described in the literature. The dog was diagnosed with glucocorticoid- and mineralocorticoid-deficient hypoadrenocorticism secondary to a bilateral anaplastic infiltrative adrenal neoplasm. We therefore believe that the case described here is the first reported case of glucocorticoid-deficient hypoadrenocorticism secondary to bilateral adrenal gland neoplasia and the first well-described case of intravascular lymphoma affecting the adrenal glands in a dog. The clinical signs of hypoadrenocorticism can be extremely variable and can mimic neoplastic diseases.Clinicians should consider the possibility of infiltrative adrenal gland disease such as intravascular lymphoma if the adrenal glands show normal thickness on ultrasound or the response to treatment for hypoadrenocorticism is poor.

References


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