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CASE REPORT

Companion or pet animals

Subdiagnostic Cushing's syndrome in a Labrador retriever diagnosed with progesterone-secreting adrenocortical neoplasia and late liver metastasis

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Abstract

A 5-year-old, neutered male Labrador retriever was presented for poor hair regrowth following clipping, lethargy, exercise intolerance, polyphagia, polydipsia, polyuria and heat-seeking behaviour. A bradyarrhythmia due to a second-degree atrioventricular block and poor cardiac contractility was found. On abdominal ultrasound, a left adrenal mass was detected, and a functional progesterone-secreting tumour was diagnosed and confirmed on histopathology and electron microscopy. After the initiation of inodilator drug therapy and adrenalectomy with a subsequent decline in progesterone concentrations, the clinical signs resolved. The dog was represented 2 years later with similar clinical signs. The dog's progesterone concentrations were again elevated, and a metastatic liver mass was detected on abdominal ultrasound examination and computed tomography scan. After liver lobectomy, the diagnosis was confirmed on histopathology, the clinical signs resolved, and progesterone concentrations normalised. This report describes the presentation of a dog with progesterone-secreting adrenocortical neoplasia and late metastasis.

BACKGROUND

Approximately 75% of primary adrenal tumours are of adrenocortical origin and may produce glucocorticoids, mineralocorticoids or sex hormones, depending on the cortical zone that is involved.[1,2] The remaining 25% are of neuroendocrine origin.[2] Sex hormone-secreting adrenocortical tumours, including those that produce androstenedione, progesterone, 17-hydroxyprogesterone, testosterone and oestradiol, have previously been described, but are rare in dogs.[3–5] A few studies have described clinical signs caused by progesterone-secreting tumours that are similar to what is seen in cases with hypercortisolaemia.[5–8] One explanation for this phenomenon is that excess cortisol precursors, such as progesterone, can displace cortisol from its binding proteins, leading to an excess of free cortisol even though the total serum cortisol concentration is decreased.[9,10] Measurement of sex hormone concentrations, particularly progesterone and 17-hydroxyprogesterone, is therefore warranted with certain case presentations.[11]

Dogs have previously been classified as having occult or atypical hyperadrenocorticism (AHAC) when a non-cortisol hormone with glucocorticoid activity, such as the abovementioned sex hormones, causes clinical signs consistent with hyperadrenocorticism in the absence of proven hypercortisolaemia.[12,13] Due to the controversy surrounding the classification of AHAC, the European Society of Veterinary Endocrinology (ESVE) has recently formulated criteria according to the Agreeing Language in Veterinary Endocrinology (ALIVE) project to redefine previously 'atypical/occult' cases to 'subdiagnostic Cushing's syndrome' (Figure 1).[14]

Adrenocorticotrophic hormone (ACTH)-independent subdiagnostic Cushing's syndrome is caused by the secretion of a non-cortisol hormone by an adrenal gland tumour.[14] About 50% of adrenocortical tumours are malignant and are characterised by local vascular invasion and metastasis to distant organs such as the liver, regional lymph nodes, spleen and lungs, necessitating pre-surgical staging.[15] This report describes the diverse clinical signs that may be seen in a dog with primary progesterone-secreting adrenocortical neoplasia. It also highlights the necessity of long-term monitoring of these cases due to the potential occurrence of late metastasis.

LEARNING POINTS/TAKE HOME MESSAGES

- Functional adrenal tumours may produce glucocorticoids, mineralocorticoids, sex hormones or catecholamines, depending on which area of the adrenal gland is affected.
- When clinical signs of Cushing's syndrome are present without concurrent hypercortisolaemia, adrenal sex hormone concentrations should be measured.
- Dogs that present with concurrent bradyarrhythmias should undergo diagnostic imaging to screen for intra-abdominal masses as part of their work-up.
- The recurrence of clinical signs after adrenalectomy should prompt further investigation to detect possible metastasis.

CASE PRESENTATION

A 5-year-old, 40 kg, neutered male Labrador retriever was referred by the hospital's primary care service for suspected hypothyroidism. The dog had been presented to them with a complaint of poor hair regrowth following clipping, lethargy, polyphagia and heat-seeking behaviour. Lumpectomy was performed 5 months before presentation to remove a cutaneous mass of unknown origin from the right lateral thigh area. Since then, the hair in this area has failed to regrow (Figure 2). The owner also commented that in the past few months, the dog showed exercise intolerance and preferred to lie in the sun. The dog was fed a good quality veterinary diet, and there was no history of changes in weight. Polyuria, polydipsia and polyphagia were suspected based on the history obtained from the owner. A physical examination was performed at the time of presentation and an irregular bradyarrhythmia with a heart rate of 54 beats per minute was auscultated. Alopecia and hyperpigmentation were noted over the right lateral thigh area. No other coat changes were present.

INVESTIGATIONS

A complete blood count was performed on the Siemens ADVIA 2120i, and a blood smear evaluation revealed no abnormalities (Table 1). Serum biochemistry was performed on the Cobas Integra 400 Plus Analyser (Roche) and the most significant findings included mild hypoproteinaemia due to moderate hypoglobulinaemia and mildly decreased creatinine (Table 2). *Ancylostoma caninum* eggs were detected on the faecal flotation, which was performed as part of the routine minimum database for dogs admitted to the internal medicine section. Urinalysis collected via free flow revealed a urine specific gravity of 1.022, with the urine dipstick and sediment analysis being unremarkable. Due to signs related to a decrease in metabolic rate (lethargy, exercise intolerance, heat-seeking behaviour) and dermatological changes, total thyroxine and thyroid stimulating hormone concentration was performed on the Siemens Immulite 2000 Immunoassay System Analyser. The results were within reference limits, making a diagnosis of hypothyroidism unlikely (Table 3). An electrocardiogram (ECG) revealed a second-degree (Mobitz type II) atrioventricular (AV) block. Non-invasive systolic, diastolic and mean arterial blood pressure measurements using high-definition oscillometry were 128, 69 and 76 mmHg, respectively (average of six readings). Echocardiography revealed poor cardiac contractility, with a fractional shortening percentage of 18.3% (reference interval 28%–45%) and mild tricuspid and mitral valve regurgitation. Cardiac troponin I performed on the Beckman Coulter UniCel Dxl 800, was mildly elevated (71.6 ng/L; reference interval 0–17.5 ng/L). Abdominal ultrasonography revealed left-sided adrenomegaly of 2.2 cm, with loss of normal architecture, cavitary lesions and heterogenous parenchyma (Figure 3). No sonographic evidence of abdominal metastasis or blood vessel invasion was found. The right adrenal gland could not be visualised. No evidence of pulmonary metastases or cardiomegaly was seen on three-view thoracic radiographs.

The case presentation and adrenomegaly prompted the investigation of a functional cortical or medullary adrenal tumour. An ACTH stimulation test using 5 μg/kg of synthetic ACTH (tetracosactid; Alfasigma) was administered intramuscularly. Cortisol and progesterone measurements were performed on the Siemens Immulite 2000 Immunoassay System Analyser. The pre-ACTH cortisol concentration was within reference limits, with a mild increase in the post-ACTH cortisol concentration (Table 3). With the same ACTH stimulation procedure, the pre- and post-ACTH serum

The criteria according to the ALIVE definition for the diagnosis of ACTH-independent subdiagnostic Cushing's syndrome:

1. Clinical signs consistent with Cushing's syndrome, and
2. results of dynamic testing of pituitary-adrenal function being normal or below normal, and
3. one of the following:
   i. abdominal ultrasound findings characteristic of a glucocorticoid-secreting adrenal tumour according to the ALIVE criteria for adrenal imaging, or
   ii. a suppressed endogenous ACTH concentration

FIGURE 1 Agreeing Language in Veterinary Endocrinology (ALIVE) criteria for the diagnosis of adrenocorticotrophic hormone (ACTH)-independent subdiagnostic Cushing's syndrome.

FIGURE 2 The Labrador retriever at its final pre-adrenalectomy health screening, showing poor hair regrowth over the area that was previously clipped.

TABLE 1 Haematology results at initial presentation (pre-operative), 2 years (representation pre-operative) and 3 years later.

Test	At presentation	2 years	3 years	Reference interval
Haemoglobin (g/L)	150	155	156	120–180
Red cell count ($\times10^{12}$/L)	6.59	6.63	6.72	5.5–8.5
Haematocrit (L/L)	0.44	0.46	0.46	0.37–0.55
Mean corpuscular volume (fL)	66.8	68.8	68.9	60–77
Mean corpuscular haemoglobin concentration (g/dL)	34.2	34.1	33.7	32–36
White cell count ($\times10^9$/L)	6.21	8.69	8.05	6–15
Segmented neutrophils ($\times10^9$/L)	4.22	6.69	5.39	3–11.5
Band neutrophils ($\times10^9$/L)	0.00	0.00	0.00	0–0.5
Lymphocytes ($\times10^9$/L)	1.43	1.13	1.85	1–4.8
Monocytes ($\times10^9$/L)	0.43	0.78	0.32	0.15–1.35
Eosinophils ($\times10^9$/L)	0.12	0.09	0.48	0.1–1.25
Basophils ($\times10^9$/L)	0.00	0.00	0.00	0–0.1
Platelet count ($\times10^9$/L)	261	358	318	200–500

TABLE 2 Serum biochemistry results at initial presentation (pre-operative), 2 years (representation pre-operative) and 3 years after initial presentation.

Test	At presentation	2 years	3 years	Reference interval
Total serum protein (g/L)	49.0	54.7	56.3	56–73
Albumin (g/L)	35.3	37.9	39.2	28–41
Globulin (g/L)	13.7	16.8	17.1	20–41
Alanine aminotransferase (U/L)	49.7	105.9	38.9	9–73
Alkaline phosphatase (U/L)	43.0	227	49.0	20–165
Urea (mmol/L)	6.20	3.90	3.80	2.3–8.9
Creatinine (μmol/L)	43.0	44.0	82.0	59–109
Sodium (mmol/L)	148	144	145	142–151
Potassium (mmol/L)	5.00	4.91	4.64	3.6–5.1
Ionised calcium (mmol/L)			1.33	1.1–1.4

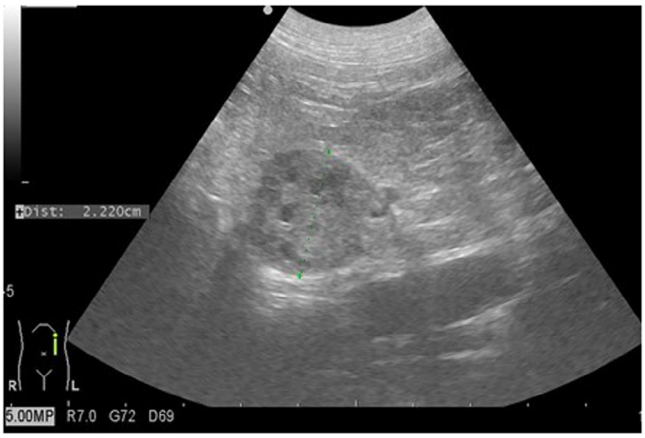

FIGURE 3 Ultrasound image of a 2.2 cm left adrenal gland showing loss of normal architecture, a heterogenous echogenicity, and multiple round hypoechoic structures.

progesterone concentrations were severely elevated (Table 3).[16] Urinary metanephrine and normetanephrine concentrations measured on the Sciex LC/MS/MS liquid chromatography system were not deemed to be consistent with a diagnosis of phaeochromocytoma (Table 3).[17] At the time of this case presentation, a validated assay for the measurement of endogenous ACTH (eACTH) was not available at the hospital's clinical pathology laboratory. The strict guidelines for sample handling could not be guaranteed while couriering the sample to a referral laboratory and eACTH was not measured.

Based on these results, a sex hormone-secreting tumour of the left adrenal gland was suspected. Adrenalectomy was therefore indicated in this case.

DIFFERENTIAL DIAGNOSIS

Without associated pruritis, the main differentials for failure of hair regrowth after clipping in this dog included post-clipping alopecia, endocrinopathies (hypothyroidism, hyperestrogenism, hypercortisolism), alopecia X and seasonal flank alopecia. The heat-seeking behaviour could have been due to a decrease in metabolic rate as is seen with hypothyroidism, muscle loss, cardiovascular disease or anaemia. In general, differentials for polyuria and polydipsia are categorised according to the conditions causing primary polydipsia and those causing primary polyuria. The combination of polydipsia, polyuria and polyphagia was, however, suggestive of endocrinopathy, such as hypercortisolism, diabetes mellitus or rarely (in dogs) hyperthyroidism. The differentials for exercise intolerance can broadly be divided into primary neuromuscular disorders and secondary disorders that impair the function of the neuromuscular system, such as cardiovascular and respiratory diseases, metabolic or endocrine disease and haematopoietic disorders. Differentials for the persistent hypoglobulinaemia seen in this dog included failure of passive transfer of immunoglobulins, acquired immunodeficiencies due to infections or neoplasia, idiopathic hypoglobulinaemia and less likely, inherited hypogammaglobulinaemia. Differential diagnoses for bradyarrhythmia and AV blocks are discussed in the text.

In this dog, an underlying endocrinopathy and cardiovascular disease were the most likely differentials for causing the

TABLE 3 Endocrine results at initial presentation (pre-operative), days 1 and 15 (post-operative), 9 months after surgery, 2 years (pre-operative) and 3 years after initial presentation.

Test	Presentation	Day 1 post-operative	Day 15 post-operative	9 months post-operative	2 years pre-operative	3 years	Reference interval
Total T4 (nmol/L)	16.9				12.3		13–41
Thyroid stimulating hormone (ng/mL)	0.054				0.34		0–0.45
Pre-ACTH cortisol (nmol/L)	111.0	<27.6		57.7	206	44.1	20–200
Post-ACTH cortisol (nmol/L)	259	<27.6		227	430	287	<450
Pre-ACTH progesterone (nmol/L)	7.85		<0.64	<0.64	3.07	<0.64	0.00–0.95[a]
Post-ACTH progesterone (nmol/L)	28.10		<0.64	4.13	19.0	2.84	0.95–6.00[a]
Metanephrine/creatinine (µmol/mol)	<32						BQL
Normetanephrine/creatinine (µmol/mol)	<87.3						BQL

Abbreviations: ACTH, adrenocorticotrophic hormone; BQL, below quantification limit for reference laboratory; T4, thyroxine.
[a]Reference interval from published sources.[16]

dog's clinical signs. The dog's hypoglobulinaemia was likely due to acquired immunodeficiency caused by its underlying disease or idiopathic disease.

TREATMENT

The dog's poor cardiac contractility and second-degree AV block were of anaesthetic concern, and treatment with 0.25 mg/kg pimobendan (Vetmedin; Boehringer Ingelheim) orally every 12 h and 1000 mg taurine (Taurine; Solal Ascendis Health) orally every 24 h was started. On follow-up echocardiography 5 weeks later, the cardiac contractility had markedly improved and the fractional shortening normalised (40.4%; reference interval 28%–45%). However, the signs of exercise intolerance and the second-degree AV block were still present.

For the adrenalectomy procedure, the dog was premedicated with 3 µg/kg fentanyl (Fentanyl; Pharma-Q) intravenously and a single dose of 0.01 mg/kg glycopyrrolate (Robinul; Aspen Pharmacare) intramuscularly. The glycopyrrolate was administered to ameliorate the dog's AV block. Diazepam (Pax; Aspen Pharmacare) 0.5 mg/kg intravenously and 4 mg/kg propofol (Propoven; Fresenius Kabi) intravenously were given as induction agents. Isoflurane (Isofor; Safeline Pharmaceuticals) was used for anaesthetic maintenance. The left adrenal gland was removed through a standard midline celiotomy. Early local invasion of the mass into the caudal vena cava was present, necessitating a partial caval venectomy. No further intra-operative complications were encountered.

Post-operatively the dog received intravenous constant rate infusions of lactated Ringer's solution (Ringer-Lactate solution; Fresenius Kabi) at 100 mL/h (one and a half times the daily maintenance requirements) and 3 µg/kg/h fentanyl. Pimobendan was continued as previously prescribed. The dog was ambulatory within 4 h after the procedure and received physical therapy to minimise the risk of thromboembolism formation. Blood pressure was measured every 2 h, and blood glucose with monitoring of vital parameters was performed every 3 h since hypotension and cortisol insufficiency are common complications.[18–20] Venous blood gas analyses to measure electrolyte concentrations, were performed daily on the Siemens RAPIDPoint 500. Continuous ECG performed due to the risk of post-operative cardiac arrhythmias,[19,20] revealed a mild sinus tachycardia with resolution of the previous AV block. The AV block did not recur post-operatively. Pre- and post-ACTH cortisol concentrations measured 12 h after the procedure were below the laboratory detection limit (Table 3). This finding coincided with a decline in habitus and appetite, tachypnoea, tachycardia and mild hypotension with systolic, diastolic and mean arterial blood pressures of 101, 54 and 71 mmHg, respectively. Sodium, potassium and glucose concentrations remained within reference limits despite these abnormalities. Plasma aldosterone was not measured. Adrenocortical insufficiency was suspected and 0.1 mg/kg dexamethasone (Kortico; Bayer) intravenously every 24 h, and 15 µg/kg fludrocortisone acetate (Florinef; Aspen Pharmacare) orally divided every 12 h, was started. When the dog started eating well 3 days post-operatively, the dexamethasone was switched to 0.5 mg/kg prednisolone (Lenisolone; Aspen Pharmacare) orally every 24 h.

OUTCOME AND FOLLOW-UP

Histopathological and electron microscopy (EM) findings of the adrenal gland were consistent with a diagnosis of an adrenocortical adenocarcinoma (see Supporting Information for full report). Histopathological evaluation revealed an expansile partially encapsulated, well-circumscribed, densely cellular mass, effacing the medulla and compressing the adjacent adrenal cortex against the capsule. The mass consisted of tightly to loosely packed trabeculae, cords and nests of neoplastic cells (Figure 4). The cells were polygonal to spindle shaped with distinct cell borders. The nuclei were round to rarely fusiform with finely stippled chromatin and small often inconspicuous single to multiple basophilic nucleoli. There were eight mitotic cells counted in 10 high-power fields (400×) and rare single cell necrosis. Only compressed atrophic adrenal medullar cells labelled positive with chromogranin A immunohistochemistry for neuroendocrine granules (Figure 5). The pathologist was concerned about the presence of a concurrent phaeochromocytoma and further evaluation using EM was performed. EM evaluation did not reveal any cells containing cytoplasmic membrane-bound epinephrine or norepinephrine granules with halos, which ruled out the possibility of a phaeochromocytoma (Figure 6).

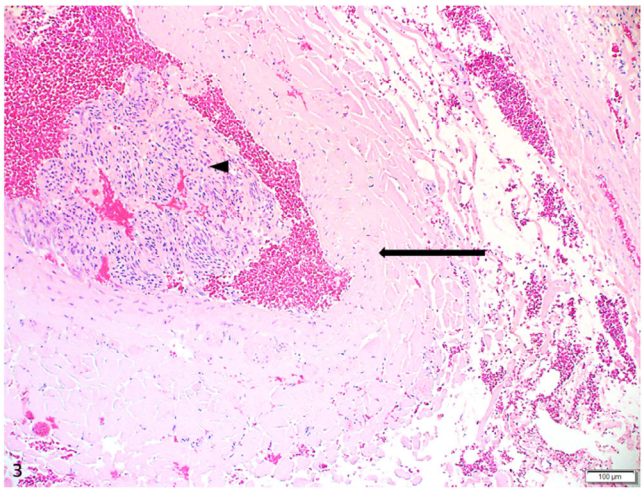

FIGURE 4 Adrenocortical adenocarcinoma showing intravascular invasion of an intracapsular arteriole (arrow) by neoplastic cells (arrowhead). H&E, haematoxylin and eosin.

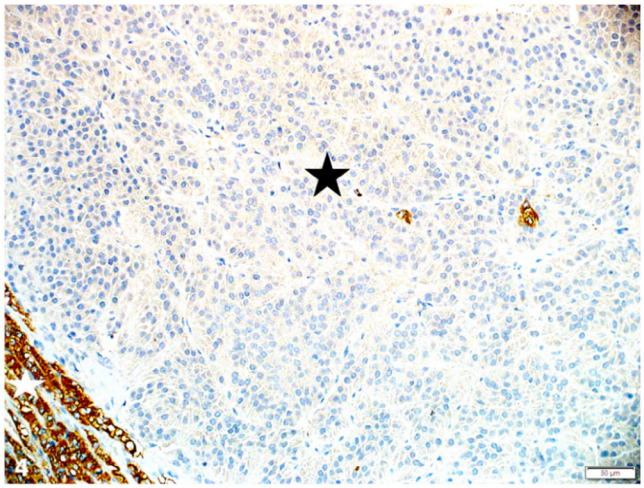

FIGURE 5 Adrenocortical adenocarcinoma showing no labelling of neoplastic adrenocortical mass (black star) with positive labelling of remnant adrenal gland medulla (white star). Chromogranin A immunohistochemical staining.

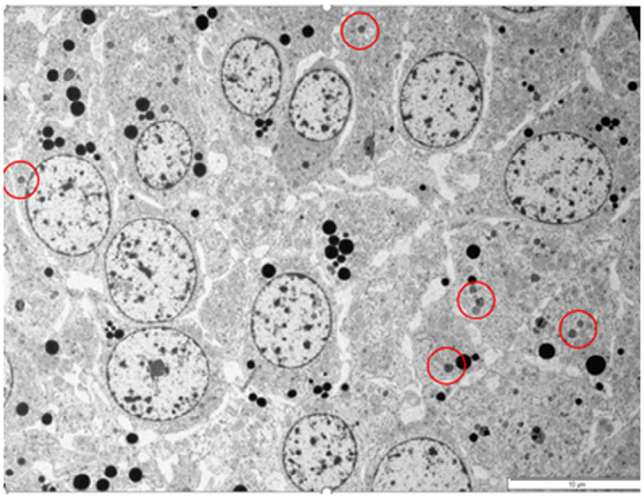

FIGURE 6 Transmission electron micrograph illustrating polygonal-shaped cells with large, round and centrally located nuclei and intracytoplasmic electron-dense inclusions. Note the absence of epinephrine or norepinephrine cytoplasmic granules and the presence of small melanin granules (circled red).

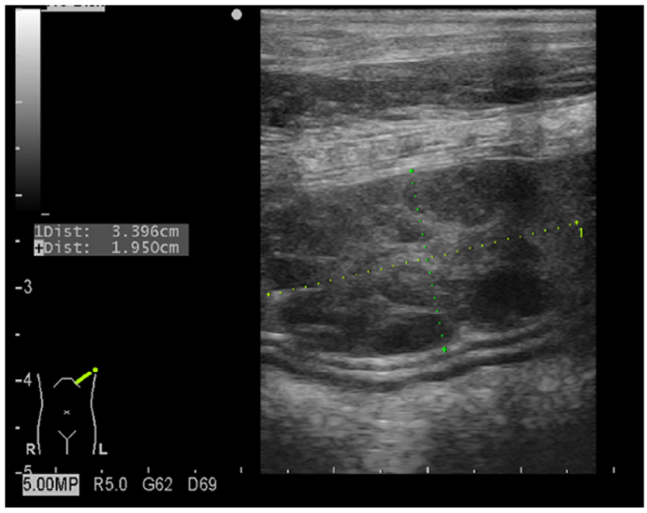

FIGURE 7 Intercostal view with the marker facing dorsally, showing a single, oval, well-marginated 3.3 cm × 1.9 cm mass with a mixed echogenicity in the left liver lobe.

At the follow-up visit 2 weeks after adrenalectomy, the dog's electrolytes were within reference intervals. The serum progesterone concentrations decreased to undetectable (Table 3). The dog's heart rate had normalised to 76 beats per minute with a return to normal sinus rhythm on ECG. Two months after surgery, the dog's echocardiogram still indicated normal contractility with a normal sinus rhythm on the ECG. Abdominal ultrasound revealed no evidence of metastases. The owner commented that although the alopecia had not improved and that they were unsure about the persistence of heat-seeking behaviour, the rest of the dog's presenting clinical signs had resolved. The fludrocortisone acetate was tapered and discontinued within 1 month after surgery. The prednisolone dosage was tapered and eventually discontinued after 3 months. Unfortunately, the owner decided to discontinue pimobendan 3 months after surgery.

On subsequent follow-up visits, the alopecia had resolved, no metastases were detected on abdominal ultrasound and the right adrenal gland could be visualised, measuring 6.4 mm in dorsoventral height. A normal sinus rhythm was present, but echocardiography showed decreased cardiac contractility without pimobendan, and treatment was reinstated. Pre- and post-ACTH progesterone and cortisol concentrations were within acceptable limits (Table 3).

The dog was represented with clinical signs similar to the initial presentation almost 2 years later. The owner commented that the dog was lethargic again, drinking more water, and had poor hair regrowth after his most recent grooming. The blood results are shown in Table 3. Abdominal ultrasound revealed a single, oval, well-marginated 3.4 cm × 2 cm mass with a mixed echogenicity in the left liver lobe (Figure 7). Efforts to obtain a diagnostic ultrasound-guided sample via fine-needle aspiration were unsuccessful. The right adrenal gland now measured 4.5 mm in dorsoventral height, which based on the dog's weight, was below the lower size limit.[21] A computed tomography (CT) scan revealed no abnormalities in the thoracic cavity, but the abdominal cavity revealed a bilobed, smoothly marginated hypodense oval lesion measuring 2.6 cm × 1.7 cm (dorsal portion) and 3.4 cm × 2.4 cm (ventral portion) in the caudo-lateral aspect of the left liver division (Figure 8). On post-contrast images, the dorsal part

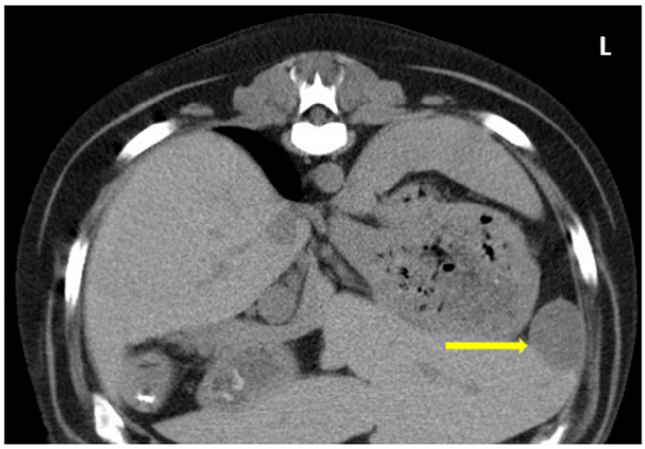

FIGURE 8 Pre-contrast transverse computed tomography image showing a smoothly marginated hypodense oval lesion in the caudo-lateral aspect of the left liver lobe division (yellow arrow).

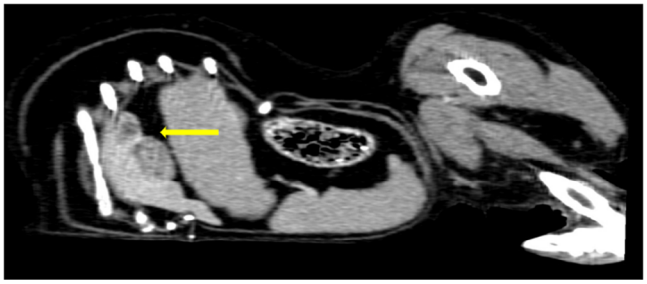

FIGURE 9 Post-contrast sagittal computed tomography image of a bilobed, smoothly marginated heterogenous oval lesion measuring 2.6 cm × 1.7 cm (dorsal portion) and 3.4 cm × 2.4 cm (ventral portion) in the caudo-lateral aspect of the left liver division (yellow arrow).

showed ring enhancement and a trabeculated appearance (Figure 9). Based on the similar clinical presentation and progesterone concentration results, it was suspected that the new liver mass could be a metastatic lesion and liver lobectomy was advised. Thromboelastography performed on the TEG 5000 Thromboelastograph Hemostasis System (Haemonetics Corporation) before the liver lobectomy revealed normocoagulable tracing. A celiotomy with a left middle liver lobectomy was performed. Post-operative monitoring was similar to what was previously described. Due to the dramatic changes in basal cortisol concentrations seen pre- and post-operatively at the initial surgery, it was of interest to see if the trend would be repeatable with the liver lobectomy in light of the suspicion of a metastatic lesion. Testing revealed a pre-operative cortisol concentration of 186 nmol/L and a 12-h post-operative concentration of less than 27 nmol/L (reference interval 20–200). Despite not performing an ACTH stimulation test to confirm post-operative adrenocortical insufficiency and guide treatment decisions, physiological doses of prednisolone were started and tapered over 4 weeks. Recovery from surgery was otherwise uneventful.

Histopathological evaluation of the liver mass performed by the same histopathologist revealed a population of neoplastic cells similar to those previously diagnosed with adrenocortical adenocarcinoma. Metastasis from the primary adrenal tumour was therefore suspected to be the most likely origin of the liver mass based on the similar clinical presentation, pre-operatively elevated progesterone concentrations and similar histopathological findings when the two tumours were compared.

The dog showed no recurrence of previous clinical signs at its last follow-up visit (3 years after the initial presentation). Echocardiography showed stable heart disease and ECG showed a normal respiratory sinus arrhythmia. Abdominal ultrasound showed no significant abnormalities. Routine blood work and progesterone and cortisol concentrations were within acceptable limits (Tables 1–3).

DISCUSSION

Based on the recent ALIVE project criteria,[14] ACTH-independent subdiagnostic Cushing's syndrome caused by a progesterone-secreting adrenocortical adenocarcinoma was diagnosed. Late metastasis to the liver was an unexpected, rare finding in this case, which to the author's knowledge, has not been reported thus far.

Subdiagnostic Cushing's syndrome was previously referred to as AHAC. The diagnosis of AHAC was controversial necessitating the formulation of criteria to categorise cases that present with signs typical of Cushing's syndrome but with non-definitive results of dynamic pituitary–adrenal function testing.[12,14] Subdiagnostic Cushing's syndrome is a clinical syndrome resulting from secretion of non-cortisol hormones such as progesterone, which have glucocorticoid effects, by adrenal tumours.[14] Progesterone-secreting tumours have been reported to result in clinical signs similar to hypercortisolism.[5–8,22] As was also seen in this dog, it has been reported that excess sex hormones are unlikely to cause clinicopathological findings that would typically be seen in dogs with hypercortisolism.[11] Similarly, in a recent report of two dogs with hyperprogesteronism, minimal clinicopathological changes were present.[5]

Primary adrenal tumours are rare, representing only 0.17%–0.76% of all tumours in dogs.[23] In a case series looking at functional adrenal tumours, 63% were carcinomas.[24] Indications for adrenalectomy are when a functional adrenocortical tumour is confirmed, the diameter of the mass is more than 4–6 cm, or when vascular invasion is present.[25,26] A functional tumour causing hypercortisolism was initially suspected due to the presence of consistent clinical signs and historical information. However, the results of dynamic functional testing of the pituitary–adrenal axis were not consistent with hypercortisolism. It should be kept in mind that only an ACTH stimulation test was performed in this case and not a low-dose dexamethasone suppression test (LDDST), which is a limitation of this report. Although the specificity of the ACTH stimulation test is higher, the LDDST is more sensitive than the ACTH stimulation test particularly in cases where an adrenocortical carcinoma is suspected (sensitivity: 85%–100% and 57%–63%, respectively).[27]

While testing of all sex hormones is recommended,[4,6] progesterone concentrations were measured first since it was the only canine sex hormone test commercially available. Not measuring the other sex hormones is a limitation of this report and would be interesting to note for future cases. Given the finding of hyperprogesteronaemia in the setting of an ACTH stimulation test and meeting the criteria to diagnose a functional adrenal tumour, it was determined that identification of other sex hormones would not be necessary for

successful clinical management of the dog. Initial pre-surgical staging revealed no evidence of spread and vascular invasion, which was only discovered during the surgical procedure. In a recent study of 302 dogs that underwent unilateral adrenalectomy, 15% had evidence of vascular invasion but it was not associated with survival to discharge as an independent risk factor.[28] In another study, six of 52 (12%) cases that underwent adrenalectomy were euthanased due to recurrence of clinical signs from metastases.[19] Three of the six cases were diagnosed with adrenocortical carcinoma.[19] Although the study did not specify when these dogs were represented with metastases, these findings would suggest that metastasis of this subset of tumours is rare. This finding is similar to that of another study, which included 52 cases, of which six died from diffuse metastases to the liver.[23] These dogs had a median survival time of 70 days, which is in contrast with the current case report, which documented only the recurrence of clinical signs almost 2 years after the initial adrenalectomy, coinciding with the detection of a new mass in the liver. In a recent report of two dogs with hyperprogesteronism due to adrenocortical neoplasia, no evidence of metastasis was found initially and at 10 and 18 months, respectively, after diagnosis.[5]

Twelve hours post-adrenalectomy, the dog developed signs of hypoadrenocorticism, which prompted temporary supplementation with glucocorticoids and mineralocorticoids. Due to its intrinsic glucocorticoid effect, prolonged elevation in progesterone concentrations has been reported to suppress eACTH concentrations with resultant functional suppression of the contralateral adrenal gland.[6,10,29] Individuals should therefore either receive intra-/post-operative glucocorticoid supplementation as a standard of care or be monitored intensively for the development of adrenocortical insufficiency. Endogenous ACTH is an accurate test for distinguishing between ACTH-dependent and ACTH-independent hypercortisolism in dogs.[27] The test is, however, very susceptible to pre-analytical error, which could result in inaccurate test interpretation.[30] The measurement of eACTH in this case would have been valuable in the context of the new ESVE ALIVE guidelines and is recommended for similar cases. In addition, the measurement of plasma aldosterone would further guide post-operative treatment. Even though the dog became hypotensive 12 h after adrenalectomy, it was more likely due to glucocorticoid rather than mineralocorticoid insufficiency based on normal electrolyte measurements at the time. Taking this into consideration and without further demonstrating aldosterone deficiency, supplementation with mineralocorticoids was likely unnecessary.

The dog also presented with suspected pre-clinical dilated cardiomyopathy (DCM) based on poor myocardial contractility seen on repeated echocardiographic scans. However, it could be argued that the dog already had clinical DCM if the exercise intolerance and lethargy at presentation were partly due to decreased myocardial function. Contractility improved after treatment with pimobendan which is the drug of choice for this condition.[31] Interestingly, despite this improvement, the dog's exercise intolerance only resolved after adrenalectomy. The dog also initially presented with bradyarrhythmia due to a second-degree AV block. Bradyarrhythmias can be physiological due to gastrointestinal or other intra-abdominal, respiratory, nervous system or ocular disorders causing increased vagal tone.[32,33] It can also be pathological due to myocardial cell injury, electrolyte disturbances or sinus node dysfunction.[32,33] An atropine response test was not performed in this case, but the dog had a positive response to the glycopyrrolate used as a premedicant. The AV block and resultant bradyarrhythmia resolved after adrenalectomy, prior to discharge. Based on the results of the cardiac troponin I, which was used as a marker of myocardial damage, the concentrations were not deemed elevated to a degree to be associated with significant cardiac disease.[34,35] Thus, it was suspected that the dog's bradyarrhythmia was physiological in origin due to the adrenal mass. Two previous case reports in dogs have described adrenal tumours (sex hormone-secreting and phaeochromocytoma) with concurrent bradyarrhythmia and second-degree AV blocks.[6,36] In the case with the sex hormone-secreting tumour, progesterone, 17-hydroxyprogesterone, androstenedione and oestradiol were increased.[6] Neither of these cases reported on the outcome of the bradyarrhythmias. While the dog's presenting clinical signs in the current case report were largely attributed to excessive progesterone concentrations, the bradyarrhythmia could also have contributed to the lethargy and exercise intolerance seen.

Subdiagnostic Cushing's syndrome should be considered in cases meeting the new ALIVE criteria. This report provides a glimpse at a practical approach to a novel topic in small animal endocrinology. It further highlights the importance of diagnostic imaging and testing pituitary–adrenal function, eACTH and sex hormone concentrations in dogs that present with signs of hypercortisolism and adrenomegaly. Due to the late occurrence of metastasis in this dog, owners should be instructed to monitor for the recurrence of clinical signs, which should prompt further investigation.

AUTHOR CONTRIBUTIONS

Primary clinician involved with the case and case report write-up: Anri Celliers. *Assistance with case management and case report editing*: Paolo Pazzi. *Reporting pathologists*: Nonkululeko Raseasala and Emily Mitchell. *Electron microscopy*: Antionette Lensink.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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IMAGE QUIZ

Referring to Figure 2, this is an ultrasound image of a 2.2 cm left adrenal gland showing loss of normal architecture, a heterogenous echogenicity and multiple round hypoechoic structures.

MULTIPLE CHOICE QUESTION

According to the ALIVE criteria for the diagnosis of ACTH-independent subdiagnostic Cushing's syndrome, which additional test should be performed if a dog presents with a history

and clinical signs suggestive of hypercortisolism but with a normal LDDST and ACTH stimulation test?

POSSIBLE ANSWERS TO MULTIPLE CHOICE QUESTION

A. Endogenous ACTH concentration
B. Urine normetanephrine:creatinine ratio
C. Plasma aldosterone concentration
D. Urine cortisol:creatinine ratio

CORRECT ANSWER

A. Endogenous ACTH concentration

Measurement of the endogenous ACTH concentration is necessary to distinguish ACTH-independent hypercortisolism from ACTH-dependent hypercortisolism. With ACTH-independent subdiagnostic Cushing's syndrome, endogenous ACTH is expected to be suppressed.