DIAGNOSING CANINE HYPERADRENOCORTICISM: WHAT IS THE ROLE OF SEX HORMONE PROFILES
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INTRODUCTION
Approximately 80 to 85% of cases of spontaneous canine hyperadrenocorticism (HAC) are due to pituitary dependent hyperadrenocorticism (PDH), with the remainder due to an adrenocortical tumor (AT). Cortisol is the most common secretory product of the adrenal gland in HAC, although excessive secretion of other adrenal hormones has also been documented.

SIGNALMENT
The median age for HAC in dogs is 11 years. There is a slight increased predisposition for females (55-65%). Larger breeds may be affected more frequently with AT than smaller breeds.

CLINICAL SIGNS OF HYPERADRENOCORTICISM
Common clinical signs include polydipsia, polyuria, polyphagia, abdominal enlargement, hepatomegaly, cutaneous changes, muscle weakness, decreased exercise tolerance, excessive panting, and hypertension. Less common clinical signs include, lethargy, insulin resistant diabetes mellitus, and incontinence; while rarely thromboembolism, ligament rupture, and pseudomyotonia are recognized. Neurologic abnormalities observed in pituitary macrotumor syndrome include behavior changes, inappetance, stupor, aimless wandering, circling, pacing, and ataxia.

LABORATORY CHANGES IN HYPERADRENOCORTICISM
Common laboratory abnormalities include neutrophilic leukocytosis, eosinopenia, lymphopenia, thrombocytosis and polycythemia. Changes on the biochemistry panel include increased alkaline phosphatase (ALP), milder increases in alanine aminotransferase (ALT) activity, hypercholesterolemia, hyperglycemia, and hypertriglyceridemia. Urinalysis may reveal low urine specific gravity, glucosuria, proteinuria or evidence of urinary tract infection. Dogs with HAC also have increased risk for calcium oxalate urolithiasis.

DIAGNOSIS OF HYPERADRENOCORTICISM
Diagnosis of Cushing’s syndrome is made by consideration of historical findings, physical examination, review of a laboratory minimum data base (CBC, serum biochemical profile, urinalysis) and performance of specific endocrine function tests. Common clinical tests for HAC include the ACTH stimulation test, low dose dexamethasone suppression (LDDS) test, and urine cortisol: creatinine ratio. Measurement of a baseline cortisol has no value for diagnosis. Testing for HAC should be performed in dogs with consistent clinical signs of HAC, clinical signs of a pituitary macrotumor, dogs with poorly controlled diabetes mellitus and suspected insulin resistance, dogs with an adrenal mass, and dogs with persistent hypertension for which no other underlying cause can be identified. Testing should not be initiated based on laboratory abnormalities alone. It is important to remember that systemic illness can interfere with testing and result in false positive test results; testing for HAC should be postponed until the patient is clinically well.

WHICH TEST SHOULD BE PERFORMED FIRST?
The cortisol:creatinine ratio is an excellent screening test for spontaneous HAC. If clinical signs of HAC are present and there is no history of exogenous corticosteroid administration, the LDDS is the most appropriate confirmatory test. If this test is abnormal or borderline the ACTH stimulation test may be used to confirm or support the diagnosis and obtain a baseline for monitoring response to treatment. If HAC is not confirmed by the LDDS test, an ACTH stimulation test may be performed if clinical suspicion for the disease is high. Testing should be repeated in 1-3 months if testing is negative but no other cause of the clinical signs is identified. In dogs with obvious clinical signs of HAC and persistent normal cortisol testing, measurement of a sex hormone profile may be considered (see below). In animals with suspected iatrogenic HAC, the ACTH stimulation test is the test of choice.

URINE CORTISOL: CREATININE RATIO: The UCCR provides an integrated reflection of cortisol production, thereby adjusting for fluctuations in blood cortisol concentrations. The reported sensitivity of the UCCR ranges from 75%-100% when the sample is randomly collected in the hospital while the reported specificity is 20-25%. In dogs
with clinical signs of HAC If two samples are collected at home at least 2 days after a veterinary visit, the UCCR performs better; predictive value of two basal UCCRs above the cut-off level is 0.88 and that of a negative test result is 0.98. Because of concerns about the specificity of the UCCR, a positive result should be confirmed by a second test. A UCCR well within the reference range makes a diagnosis of HAC unlikely. Reference ranges for UCCR vary between laboratories.

**LOW-DOSE DEXAMETHASONE SUPPRESSION TEST:** The LDDS demonstrates decreased sensitivity to glucocorticoid feedback in dogs with HAC. The LDDS relies on the fact that the administration of exogenous glucocorticoids should suppress the production of ACTH from the normal pituitary and therefore the production of cortisol from the normal adrenal. This suppression persists in normal dogs for 16-24 hours. Since dexamethasone is not detected by the cortisol assay, cortisol suppression can be measured after administration of exogenous dexamethasone. In dogs with pituitary dependent HAC the pituitary gland is less sensitive to glucocorticoid feedback while adrenal tumors function independently of ACTH, and in addition dexamethasone is metabolized more quickly in dogs with HAC. For these reasons in dogs with either form of HAC no suppression occurs 8 hours after low dose dexamethasone administration. The LDDS test has a sensitivity of 85–100% and a specificity of 44-73%. The LDDS should not be used before iatrogenic HAC has been excluded. The LDDS test requires a blood sample for the measurement of a baseline cortisol, followed by the administration of dexamethasone (dexamethasone sodium phosphate or dexamethasone in polyethylene glycol) IV at a dose of 0.01 mg/kg. The patient is then left undisturbed in a cage and a second blood sample is collected 8 hours later. In normal dogs the second sample will show suppression of the cortisol concentration typically to less than 1.0 μg/ml. Dogs with HAC do not demonstrate this suppression. Additional information may be obtained by measuring a cortisol concentration 4 hours after dexamethasone administration. If suppression occurs at 4 hours but "escape" occurs at 8 hours this is diagnostic for PDH and further differentiation testing is unnecessary.

**ACTH STIMULATION TEST:** The ACTH stimulation test relies on the assumption that hyperplastic or neoplastic adrenals have abnormally large reserves of cortisol and therefore hyper-respond to maximal stimulation by ACTH. Dogs with iatrogenic HAC will have a suppressed response to ACTH. The recommended protocol is collection of a baseline sample, administration of synthetic ACTH (cortrosyn, cosyntropin, tetracosactrin) at a dose of 5 μg/kg IV. A second sample is collected one hour later. In order to interpret the ACTH stimulation test, reference ranges must be established for each laboratory. The sensitivity of the ACTH stimulation test ranges between 57-83% while specificity is between 86 – 93%. Sensitivity is only 57-63% in dogs with functional adrenal tumors. The ACTH stimulation test does not distinguish between ADH and PDH but is useful in distinguishing between iatrogenic and spontaneous HAC. Because of the low sensitivity of this test, the ACTH stimulation test is not the first test of choice for spontaneous HAC. The ACTH stimulation test can be performed at any time of day and use of compounded ACTH is discouraged. Cosyntropin/cortrosyn can be reconstituted and frozen (e.g. in aliquots) at -20°C in plastic syringes for 6 months; whether Synacthen can be frozen has not been investigated.

**DIFFERENTIATION OF PDH FROM ADRENAL TUMORS**
Endocrine function tests such as the high and low dose dexamethasone suppression tests, endogenous ACTH concentration and diagnostic imaging are used to differentiate PDH from AT. Suppression of a basal cortisol concentration by more than 50% 4 or 8 hours after administration of either a low or high dose of dexamethasone is diagnostic for PDH. Lack of suppression however, is not diagnostic for ADH and additional testing is required. If greater than 50% suppression is seen at 4 or 8 hours on a LDDS, a HDDS test is unnecessary. Measurement of canine ACTH concentration is also useful in differentiating PDH form AT. In dogs with AT ACTH concentration should be low, whereas in dogs with PDH the concentration is normal or high. Reference ranges for endogenous ACTH vary between laboratories and assays. Because of episodic secretion of ACTH in PDH, the ACTH concentration in PDH varies from low to high. / If the ACTH concentration is above the laboratory cut-off PDH is confirmed but a low ACTH concentration does not confirm an adrenal tumor and should never be used alone to confirm a diagnosis of adrenal tumor.

**DIAGNOSTIC IMAGING**
Approximately 57% of adrenal tumors are identified on abdominal radiographs as a soft tissue mass or a mineralized opacity, compared to 72% with ultrasonography. Other radiographic findings may include hepatomegaly, osteopenia, dystrophic mineralization and distension of the urinary bladder. Thoracic radiographs
may reveal evidence of metastasis, pulmonary thromboembolism or mineralization. In dogs with PDH, ultrasound of the adrenal glands typically reveals bilaterally symmetrical enlargement of the adrenal glands with preservation of normal adrenal architecture. The glands may not be identical in size but the smaller adrenal gland in dogs with PDH typically has a dorso-ventral width greater than 5 mm. The size of the adrenal glands may be within the normal range in some dogs with PDH. In dogs with a functional adrenal tumor there is unilateral adrenal gland enlargement with abnormal adrenal gland architecture while the contralateral adrenal gland is small (<5mm in dorso-ventral width). Evidence of tumor thrombus within the vena cava is detected on ultrasound in 11% of dogs with adrenocortical tumors and evidence of distant metastasis to the liver, kidneys, and other abdominal organs may be present. Bilateral adrenal tumors and macronodular hyperplasia of the adrenal gland in dogs with PDH may complicate the interpretation of ultrasound findings. Computed tomography is also a useful tool for evaluating the adrenal glands, especially in dogs with adrenal tumors. MRI or CT of the brain is helpful in determining pituitary tumor size in dogs with PDH. Approximately 70% of dogs with PDH have a detectable pituitary tumor on CT or MRI.

**ATYPICAL OR OCCULT HYPERADRENOCORTICISM**

These terms are used to describe dogs in which clinical signs and response to treatment are consistent with a diagnosis of HAC, but the standard screening tests (ACTH stimulation test, low dose dexamethasone suppression test) results are within the reference range. The reasons for the clinical signs in these patients are currently unknown. It has been proposed that increased circulating concentrations of steroid adrenal hormones other than cortisol (e.g. progesterone, 17- hydroxyprogesterone, androstenedione, dehydroepiandrosterone) may be responsible for the clinical signs in occult HAC but this is controversial except in the few documented cases of sex hormone secreting adrenal tumors. Sex hormone secreting adrenocortical tumors can be identified because of the presence of an adrenal mass; in these dogs cortisol concentration after ACTH administration is typically suppressed below the reference range. Although dogs with apparent pituitary dependent occult HAC have been reported in the literature they are rare. Other potential reasons for normal screening test results in occult HAC patients may include individuals with increased sensitivity to glucocorticoids, inappropriately high reference ranges for cortisol in dogs with early of mild HAC, as well as rare forms of HAC such as food dependent HAC.

**SEX HORMONE PROFILES IN DOGS WITH HYPERADRENOCORTICISM**

Hormones that have been reported to be increased in the few documented cases of occult HAC include serum dehydroepiandrosterone, androstenedione, progesterone, and 17-hydroxyprogesterone. Concentration of 17-hydroxyprogesterone and progesterone in dogs with HAC has been evaluated more extensively than other sex hormones. Studies suggest that sensitivity and specificity of progesterone for diagnosis of HAC are 88% and 55% and for 17-hydroxyprogesterone are 91% and 59% respectively. Sex hormone testing should not be considered as first line testing for dogs with HAC. Measurement of sex hormone profiles should only be considered in dogs with clinical signs of HAC that have repeated normal LDDS and ACTH stimulation test results and no other cause for their clinical signs identified. One indication for testing that is well accepted is the presence of inappropriately low cortisol concentrations on HAC screening tests in dogs with no exposure to exogenous glucocorticoids (suggestive of sex hormone secreting AT).

**FURTHER READING**


Behrend EN; Kemppainen RJ; et al. Serum 17-α-hydroxyprogesterone and corticosterone concentrations in dogs with nonadrenal neoplasia and dogs with suspected hyperadrenocorticism. Journal American Veterinary Medical Association 2005;227:1762-1767.

Hill KE; Scott-Moncrieff JC; et al. Secretion of sex hormones in dogs with adrenal dysfunction. Journal American Veterinary Medical Association 2005;226:556-561