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Diagnosis of Spontaneous Canine Hyperadrenocorticism: 2012 ACVIM Consensus Statement (Small Animal)

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This report offers a consensus opinion on the diagnosis of spontaneous canine hyperadrenocorticism. The possibility that a patient has hyperadrenocorticism is based on the history and physical examination. Endocrine tests should be performed only when clinical signs consistent with HAC are present. None of the biochemical screening or differentiating tests for hyperadrenocorticism are perfect. Imaging can also play a role. Awareness of hyperadrenocorticism has heightened over time. Thus, case presentation is more subtle. Due to the changes in manifestations as well as test technology the Panel believes that reference ranges should be reestablished. The role of cortisol precursors and sex hormones in causing a syndrome of occult hyperadrenocorticism remains unclear.

Key words: Adrenal cortex; Cushing's syndrome; Dog; Pituitary.

Clinical Presentation: Indications For Diagnostic Testing

The possibility that a patient has hyperadrenocorticism (HAC) is based on the history and physical examination. Endocrine tests should be performed only when clinical signs consistent with HAC are present. The Panel believes that because of heightened awareness of HAC, dogs are currently evaluated at much earlier stages of disease development. Consequently, clinical manifestations are more subtle, and the prevalence of clinical signs and physical examination find-

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Abbreviations:

ACTH	adrenocorticotrophic hormone
ALP	alkaline phosphatase
AT	adrenal tumor
cACTH	canine ACTH
CBC	complete blood count
CT	computed tomography
ELISA	enzyme-linked immunosorbent assay
EQUAS	External Quality Assurance Specimens
HAC	hyperadrenocorticism
HDDST	high-dose dexamethasone suppression test
HPAA	hypothalamic/pituitary/adrenal axis
IRMA	immunoradiometric assay
LDDST	low-dose dexamethasone suppression test
MRI	magnetic resonance imaging
PDH	pituitary-dependent hyperadrenocorticism
RIA	radioimmunoassay
UCCR	urinary corticoid : creatinine ratio

ings in individual dogs is less than that published several decades ago.

The primary indication for pursuing a diagnosis of HAC is the presence of one or more of the common clinical signs and physical examination findings (Table 1).^{1–10} If only 1 clinical sign is present, it is usually polyuria and polydipsia, or alopecia and skin changes suggestive of an endocrine disease.¹¹ Cases seen by dermatologists may have a different constellation of findings than those seen by internists. Failure

Table 1. Clinical manifestations of canine HAC.^{1–11,111–113} Categorization of frequency is based on identification at the time of initial presentation.

Common	Less Common	Uncommon
Polydipsia	Lethargy	Thromboembolism
Polyuria	Hyperpigmentation	Ligament rupture
Polyphagia	Comedones	Facial nerve palsy
Panting	Thin skin	Pseudomyotonia
Abdominal distention	Poor hair regrowth	Testicular atrophy
Endocrine alopecia	Urine leakage	Persistent anestrus
Hepatomegaly	Insulin-resistant	
Muscle weakness	diabetes mellitus	
Systemic hypertension		

HAC, hyperadrenocorticism.

Table 2. Common laboratory abnormalities in dogs with HAC.^{1–11,111,113}

CBC	Serum Biochemistry Panel	Urinalysis
Neutrophilic leukocytosis	Increased alkaline phosphatase	Specific gravity ≤ 1.018 – 1.020
Lymphopenia	Increased alanine aminotransferase	Proteinuria
Eosinopenia	Hypercholesterolemia	Indicators of urinary tract infection
Thrombocytosis	Hypertriglyceridemia	
Mild erythrocytosis	Hyperglycemia	

HAC, hyperadrenocorticism; CBC, complete blood count.

to identify multiple indicators for HAC does not rule out the disease. However, the more abnormalities identified, the stronger the indication to pursue testing. Less common clinical signs and physical examination findings add further support for diagnostic testing.

Less common clinical presentations of HAC include anestrus and testicular atrophy; ligament laxity that may lead to tearing and lameness¹²; facial palsy; and pseudomyotonia.^{13,14} Severe polyuria, urinary tract infection or both may lead to urine leaking, especially when the dog is asleep, and owner-perceived urinary incontinence. Hypercoagulability may result in spontaneous thromboembolism, typically involving pulmonary vessels and causing acute respiratory distress.^{15,16} Cortisol-induced insulin resistance may promote diabetes mellitus and impair exogenous insulin response.^{17,18} If less common clinical presentations are identified first, a thorough review of the history, physical examination findings, and routine laboratory test results often provides additional evidence for the disease. Failure to identify abnormalities listed in Tables 1 and 2 is a major negative indicator for the presence of HAC.

Clinical manifestations may develop secondary to mass-occupying effects of a pituitary or adrenal tumor (AT). A large pituitary tumor may cause neurologic

signs (pituitary macrotumor syndrome), including inappetence, anorexia, stupor, circling, aimless wandering, pacing, ataxia, and behavioral alterations. Although pituitary macrotumor syndrome develops in 10–25% of dogs months to years after HAC diagnosis, some have pituitary macrotumor syndrome, albeit subtle, at initial presentation. Documenting a large pituitary mass on computed tomography (CT) or magnetic resonance imaging (MRI) during the evaluation of neurologic signs supports testing for HAC. Adrenocortical carcinomas may invade the phrenicoabdominal vein, caudal vena cava, or both, causing retroperitoneal hemorrhage, blood-loss anemia and abdominal pain, or incite formation of a tumor thrombus that leads to ascites or rear limb paresis.^{19,20}

Testing for HAC is recommended after unexpected identification of an adrenal mass on imaging performed for another problem such as vomiting. A review of the history, physical examination findings, and results of routine blood and urine tests will usually, but not always, provide evidence for HAC, if present, and prompt additional testing. Because the presence of an AT dictates perioperative management, testing for HAC should be recommended before adrenalectomy.

Results of a complete blood count (CBC), biochemistry panel, urinalysis, urine protein : creatinine ratio, and blood pressure measurement may further support HAC (Table 2). No abnormality listed in Table 2 is pathognomonic for HAC. Laboratory test results and a blood pressure measurement must be interpreted within the context of the history and physical examination findings. An absence of common abnormalities noted in Table 1 should strongly decrease the suspicion of HAC. Conversely, failure to identify abnormalities listed in Table 2 does not, by itself, rule out HAC. If measured, bile acid concentrations may be mildly increased. A cause and effect relationship between HAC and formation of gall bladder mucoceles has yet to be clarified. Identification of bilateral adrenomegaly or an AT on abdominal ultrasound examination provides additional evidence to pursue the diagnosis of HAC in dogs with common abnormalities listed in Table 1. However, the presence of ultrasonographically normal-sized adrenal glands does not rule out HAC.

Ideally, testing for HAC should be avoided if serious illness exists. Many illnesses affect results of HAC screening tests.^{21,22} Testing for HAC is not mandatory at the time suspicion arises. Postponing testing until concurrent illness is resolved or controlled is recommended, but the concurrent illness must be considered.

In summary, indicators for performing diagnostic tests for HAC are:

- Compatible history and physical examination findings. The greater the number of findings, the stronger the suspicion. Biochemical panel, CBC, urinalysis, and urine protein : creatinine ratio

results and blood pressure measurement by themselves are not indications to test.

- A pituitary macrotumor.
- A diabetic dog with persistently poor response to high dosages of insulin not attributed to another cause, including owner issues.
- An adrenal mass.
- Persistent hypertension. (The Panel did not reach consensus on this point. Some would not test if hypertension was the only abnormality present.)

Screening Tests

No test has 100% diagnostic accuracy. Positive and negative predictive values are dependent upon disease prevalence. In a population appropriately screened so that disease prevalence is high, all diagnostic tests will be more accurate.

Diagnosis of HAC depends on demonstration of either: (1) increased cortisol production or (2) decreased sensitivity of the hypothalamic-pituitary-adrenal axis (HPAA) to negative glucocorticoid feedback. Measurement of a single basal cortisol concentration has no diagnostic value. Pulsatile adrenocorticotrophic hormone (ACTH) secretion results in variable cortisol concentrations^{23,24} which may at times be within the reference range. Dogs with nonadrenal illness (NAI) can have increased baseline cortisol concentrations.^{22,25}

The tests used most often include the low-dose dexamethasone suppression test (LDDST), urinary corticoid : creatinine ratio (UCCR), and ACTH stimulation test. Because all were introduced into veterinary medicine in the 1970s and 1980s, the Panel believes current reference ranges and cut-off values should be re-evaluated. First, measured cortisol concentrations differ among assays. Thus, values generally cannot be used interchangeably. Second, methods and assays have changed over previous decades, but new reference ranges were not usually generated. Third, studies from which reference ranges were derived had various shortcomings, namely comparison of dogs with HAC to healthy dogs rather than those suspected of having HAC; inclusion of groups with small numbers of dogs; and, use of controls with NAI that were not suspected of having HAC. Furthermore, trials to evaluate screening tests were performed in referral settings with a high disease prevalence, but the tests often are now used in primary care settings with a low disease prevalence. Fourth, the incidence of mild cases of HAC has appeared to increase over time, possibly because of heightened awareness and earlier patient presentation. Milder cases will have a lower degree of cortisol hypersecretion, and cut-off values previously established may not apply.

Any screening test may be negative in a patient with HAC. If a test is negative but suspicion for HAC remains, another test should be performed. If more than 1 test is negative, the possibility that the patient does not have HAC must be considered. Alternatively, the patient may have mild HAC and the tests have not yet become positive. It may be worthwhile to retest in 3–6 months if clinical signs progress.

Technical Aspects

Cortisol Assays. In serum or plasma, total cortisol (bound and free) is measured; in urine and saliva, only free cortisol is measured. Various techniques are available (eg, RIA, ELISA, chemiluminescence). To the Panel's knowledge, data regarding in-house cortisol measurements have not been published in the peer-reviewed literature; therefore, such methods were not considered.

Circulating cortisol concentrations differ depending on the assay. The EQUAS program run by Michigan State University provides data comparing measurements among laboratories. Consistent differences are reported. For example, cortisol measured by Immulite is higher than that measured by RIA (Dr R. Nachreiner, personal communication). Differences exist among laboratories using the same methodology. From the EQUAS XXXV report (July 2010), 27 laboratories using the Immulite assay found cortisol concentrations from 3.7 to 7.2 µg/dL (101–199 nmol/L) in the same sample. Eleven laboratories used the same RIA; cortisol concentrations in the same sample ranged from 3.0 to 5.0 µg/dL (83–137 nmol/L).

Tube Type, Sample Type, Time of Centrifugation, and Stability. Cortisol concentrations were the same whether measured on samples stored in glass or plastic,²⁶ serum or plasma,^{26,27} and centrifuged 10 minutes or 40 hours after blood collection.²⁷ Cortisol is stable in plasma and urine at 4 and 25°C for 5 days, but decreases in serum at 4, 25, and 37°C (compared to –20°C).²⁶ However, to ensure adequate sample integrity, the Panel recommends that after centrifugation samples either be refrigerated for up to 24 hours or frozen for longer at –20°C. Urine can be stored at 4°C for up to 4 days or at –20°C for >5 days. Samples should be sent to the laboratory overnight; sample type will not matter and no special packaging is needed.

Cross-Reactivity. Because of assay-dependent cross-reactivity among various steroids (prednisolone, prednisone, methylprednisolone, fludrocortisone, cortisone, hydrocortisone), the Panel recommends a 24 hour interval between the last steroid administration and cortisol measurement. However, the 24 hour time period will not eliminate the risk of adrenal suppression secondary to glucocorticoid administration.

Influence of Hemolysis and Lipemia. The effect of lipemia and hemolysis may differ among assays. The Panel recommends contacting the individual laboratory for information related to the assay used.

Hypothalamic-Pituitary-Adrenal Axis and Drugs. Many drugs affect human HPAA activity.²⁷ A number of these drugs are not used in veterinary medicine, but metoclopramide, clonidine, buprenorphine, codeine, clomipramine, ceruletide, and desmopressin are used in veterinary medicine. Except for desmopressin,²⁸ studies are lacking in veterinary medicine.

Exogenous progestins²⁹ and glucocorticoids can suppress the HPAA. The duration of suppression reflects duration of use, dose, administration route, form of

synthetic steroid (short- or long-acting), and individual sensitivity and cannot be predicted.³⁰

Conclusions

- No particular assay is recommended.
- Cortisol concentrations vary by assay and among laboratories using the same method. Reference ranges and cut-off values must be established by each laboratory; therefore, the Panel does not recommend specific reference ranges and cut-off values.
- Samples for cortisol measurement should be centrifuged within 1 hour after collection, immediately refrigerated or frozen for longer storage, and shipped overnight to a reference laboratory.

Low-Dose Dexamethasone Suppression Test

Test Principles. The LDDST can demonstrate decreased HPAA sensitivity to negative glucocorticoid feedback, 1 of the 2 characteristics of HAC diagnosis. Additionally, dexamethasone may be metabolized quicker in dogs with HAC than in healthy dogs.³¹ Resistance to dexamethasone suppression is not “all or nothing” but a continuum; slight resistance may be present in early or mild cases and more severe resistance may be present in advanced cases of HAC.³²

The LDDST as a Screening Test. A diagnosis of HAC is determined by the cortisol concentration 8 hours after dexamethasone administration. In human medicine, because patients with mild HAC may have greater sensitivity to dexamethasone suppression, cut-off values have decreased over time.³³ As stated above, the Panel suggests that updated cut-off values be established by each laboratory. However, no cut-off correctly identifies all patients with HAC.³⁴ In veterinary medicine, the reported sensitivity and specificity of the LDDST range from 85 to 100% and from 44 to 73%, respectively.^{6,21,22,35-43}

An “inverse” pattern, in which the cortisol concentration 8 hours after dexamethasone was below the cut-off value, but the cortisol concentration 4 hours post-dexamethasone was increased was described in 5 dogs with PDH.⁴¹ Because this pattern is highly suspicious for HAC, further testing should be pursued.

Dexamethasone Form, Dosage, and Time of Testing. In the 1st LDDST study, the best separation between healthy dogs and dogs with HAC was achieved by cortisol concentrations 8 hours after 0.01 mg/kg dexamethasone IV.³⁷ Intravenous dosages of 0.01 mg/kg dexamethasone sodium phosphate and 0.015 mg/kg dexamethasone polyethylene glycol yielded similar cortisol concentrations in dogs with HAC after 2, 4, 6, and 8 hours.³⁹ When comparing dexamethasone in the polyethylene glycol or sodium phosphate form, no differences were detected after 0.01 and 0.1 mg/kg dosages.⁴⁴ Dexamethasone sodium phosphate dosage should be calculated based on the active compound. According to Plumb’s Veterinary

Drug Handbook (7th ed), 1.3 mg dexamethasone sodium phosphate is equivalent to 1 mg dexamethasone.

Effect of Timing and Feeding. Dogs do not exhibit a circadian cortisol secretion.²³ Therefore, the Panel assumes that time of day does not affect LDDST results. The effect of feeding on LDDST results is unknown. The Panel recommends not feeding during the test. Fasting before testing is not necessary unless lipemia affects results of the cortisol assay used.

Influence of Drugs. Dexamethasone is metabolized primarily by cytochrome P450 3A4. Agents that increase the enzyme’s activity accelerate dexamethasone clearance and could cause false positive results. In humans, such agents include carbamazepine, phenytoin, rifampicin, barbiturates, and St. John’s wort. In veterinary medicine, only phenobarbital has been studied. Available evidence suggests no effect of phenobarbital on LDDST results, although occasionally phenobarbital-treated dogs may not show suppression.⁴⁵⁻⁴⁷

Conclusions

- The Panel considers the LDDST the screening test of choice unless iatrogenic HAC is suspected.
- The LDDST should be performed using 0.01–0.015 mg/kg dexamethasone sodium phosphate or polyethylene glycol IV; calculate dose using the parent compound and not the salt.
- The LDDST can be started any time of the day; avoid feeding during the test.
- Obtain blood samples before and 4 and 8 hours after dexamethasone administration.
- The cortisol concentration 8 hours after dexamethasone administration is used to diagnose HAC. It is the clinical experience of the Panel that in normal dogs cortisol concentrations 4 and 8 hours after 0.01 mg/kg dexamethasone are below or very close to the detection limit of current assays. New cut-off values should be established.
- An “inverse pattern” should prompt further testing for HAC.
- Because clinical signs and biochemical abnormalities in dogs on phenobarbital may be similar to those in dogs with HAC, confirmation of HAC in phenobarbital-treated dogs is challenging. If clinical and laboratory abnormalities persist after switching to another anticonvulsant (substantiating the suspicion of HAC), an LDDST then may be performed. If discontinuation of phenobarbital is impossible, LDDST results should be interpreted cautiously and further diagnostic testing considered.

ACTH Stimulation Test

Test Principles and Diagnostic Accuracy. The ACTH stimulation test assesses adrenocortical reserve and is the gold standard for diagnosis of iatrogenic HAC.

Because of its low sensitivity, its diagnostic usefulness as a screening test for spontaneous HAC is inferior to the LDDST.

The sensitivity of the ACTH stimulation test for all forms of spontaneous canine HAC ranges between 57 and 95%. It has been determined that for dogs with HAC because of an AT, sensitivity is 57–63%; for dogs with PDH it is 80–83%. Specificity ranges between 59 and 93%.^{6,21,36,43,48–51}

Form, Dosage, and Route of ACTH. Synthetic polypeptides containing the biologically active first 24 amino acids of ACTH are available, eg, Cortrosyn (cosyntropin) or Synacthen (tetracosactrin). The potency of the preparations has not been compared. Recently, Cosyntropin Injection was introduced for IV use only. No differences in cortisol concentrations were found in response to 250 µg Cortrosyn IM or Cosyntropin Injection IV in 18 healthy dogs.⁵² In some countries, compounded ACTH preparations are available. In 1 study, cortisol concentrations 60 minutes after administration of compounded ACTH (2.2 U/kg IM) were no different than after Cortrosyn (5 µg/kg IV).⁵³

No difference in mean peak cortisol concentration was detected when comparing IV and IM administration of 250 µg Cortrosyn in healthy dogs⁵⁴; IV and IM administration of 5 µg/kg Cortrosyn in healthy dogs and dogs with HAC⁵⁵; or IV administration of 250 µg/dog and 5 µg/kg Cortrosyn in dogs with HAC.^{56,57} When comparing various cosyntropin dosages (10, 5, 1, 0.5, 0.1, 0.05, 0.01 µg/kg) on cortisol concentrations in healthy dogs^{56–58}, the lowest dosage that stimulated maximal cortisol secretion was 0.5 µg/kg IV.⁵⁸ Depot tetracosactide (250 µg/kg IM) and cosyntropin (5 µg/kg IV) produced similar cortisol responses at 60 minutes after administration in healthy dogs.⁵⁹ However, neither cosyntropin dosages below 5 µg/kg nor tetracosactide depot have been assessed in dogs with HAC.

Sample Timing. After administration of Cortrosyn at 5 µg/kg or 250 µg/dog IV or IM, peak cortisol secretion occurs at 60–90 minutes.^{53–57} After 5 µg/kg IV, no difference was detected between 60- and 90-minute cortisol concentrations.^{53,55,56} Using 4 compounded products (2.2 U/kg IM) in healthy dogs, cortisol concentrations at 60 minutes were similar to each other as well as to concentrations after Cortrosyn (5 µg/kg IV). However, at later times cortisol concentrations varied considerably.⁵³

Effect of Timing and Feeding. Dogs do not exhibit circadian cortisol secretion.²³ Similar to the LDDST, the Panel assumes that time of day does not affect test results. Fasting before testing is not necessary unless lipemia affects results of the cortisol assay used.

Cosyntropin Storage. Cosyntropin can be reconstituted and frozen in aliquots at –20°C in plastic syringes for 6 months.⁶⁰ Whether Synacthen can be frozen has not been investigated; according to the manufacturer, it should be stored at 2–8°C.

Influence of Drugs. In people, cortisol response to ACTH may be decreased by serotonin receptor agon-

ists, progestagens, ketoconazole, and fluconazole and may be enhanced by propranolol.²⁷ In veterinary medicine, the ability of glucocorticoids of any form,³⁰ progestagens²⁹ and ketoconazole⁶¹ to suppress cortisol secretion is known. No effect on the ACTH stimulation test was documented overall or individually in healthy dogs treated with phenobarbital for 8⁶² (n = 12) or 29 weeks⁴⁶ (n = 12) or in epileptic dogs treated for 1 year⁴⁵ (n = 5) or >2 years⁶² (n = 5).

Conclusions

- The ACTH stimulation test is the gold standard for diagnosis of iatrogenic HAC. It is of less use for the diagnosis of spontaneous HAC.
- The ACTH stimulation test can be performed at any time of day.
- The effect of feeding on ACTH stimulation test results is unknown. The Panel recommends not feeding during the test.
- Because of greater purity and quality control, only use of synthetic ACTH is recommended and utilization of compounded ACTH is discouraged.
- Cortrosyn, Cosyntropin Injection, and Synacthen can be used interchangeably.
- Perform the test using 5 µg/kg of the preferred compound with blood samples drawn before and 60 minutes after administration. The Panel prefers IV administration.
- Depot tetracosactide needs to be given IM, but the Panel does not recommend its use until it has been tested in dogs with HAC.
- Progestagens, glucocorticoids, and ketoconazole suppress the HPA axis and decrease responses to ACTH. Phenobarbital does not appear to affect results.

Combined Dexamethasone Suppression/ACTH Stimulation Test

The combined test merges an ACTH stimulation test for screening with a high-dose dexamethasone suppression test for differentiating. As the diagnosis of HAC is based on ACTH stimulation test results, the combination test has a lower sensitivity than the LDDST.

Urinary Corticoid : Creatinine Ratio

Test Principles and Diagnostic Accuracy. The UCCR provides an integrated reflection of corticoid production, adjusting for fluctuations in blood concentrations.

Determination of basal UCCRs can be performed in tandem with a high-dose dexamethasone suppression test (see below). The combination has the advantage of potentially demonstrating both increased cortisol production and decreased sensitivity to glucocorticoid feedback.

When a single, random urine sample is collected in veterinary hospitals, the reported sensitivity and specificity of the UCCR for diagnosis of HAC ranges from

75–100%^{21,63–66} and 20–25%, respectively.^{21,63,64} However, using the protocol below, in dogs with physical and biochemical changes consistent with HAC, the sensitivity of finding 2 basal UCCRs above the cut-off level was 99% (95% confidence interval [CI], 94–100%) and the specificity was 77% (95% CI, 64–87%).⁴² In some dogs, considerable day-to-day variation exists in the UCCR. In mild cases, a UCCR may be just within the reference range 1 day and increased another day.

Protocol. To avoid the influence of stress,⁶⁷ urine for UCCR should be collected at home at least 2 days after a visit to a veterinary clinic. Although a UCCR sample can be collected at any time of day,⁶⁸ morning urine may be preferred because it usually represents several hours of urine production.

Influence of Drugs and Concurrent Disease. Glucocorticoids and other drugs that suppress cortisol secretion, such as progestagens,²⁹ can decrease a UCCR by suppressing endogenous cortisol secretion. Phenobarbital treatment does not affect UCCR.⁴⁷ Nonadrenal disease may cause endogenous stress and increased cortisol secretion. Therefore, high UCCRs in dogs without a high degree of clinical suspicion of HAC should be interpreted cautiously.

Conclusions

- The UCCR is a sensitive test to detect cortisol hypersecretion.
- To avoid false-positive results, urine should be collected at home minimally 2 days after a visit to a veterinary clinic.

Differentiating Tests

It is important to differentiate PDH and AT because treatment and prognosis differ. Spontaneous HAC from ectopic ACTH secretion⁶⁹ and food-stimulated cortisol secretion⁶⁹ are rare. Biochemical tests (canine ACTH or cACTH, LDDST, HDDST, dexamethasone suppression of the UCCR) can distinguish PDH and

AT, but no test is 100% accurate. Differentiating tests should not be done unless a positive result has been obtained on a screening test.

Endogenous ACTH Concentration

Test Principles. Canine ACTH is secreted from the pituitary gland in an episodic, pulsatile fashion in healthy dogs and those with PDH.^{23,71} A circadian rhythm has not been convincingly demonstrated, although 1 study reported higher plasma cACTH concentrations in late afternoon than in the morning.⁷² Concentrations of cACTH do not differ between healthy dogs and those with PDH, and its measurement is not useful to screen for HAC.⁷³ Measurement of cACTH is the most accurate stand-alone biochemical test for differentiating PDH from AT.

cACTH Assays. Immunoradiometric assay (IRMA) and chemiluminescent assays have been validated for cACTH measurement.^{74–77} Measured cACTH concentrations are lower using chemiluminescent technology than RIA.⁷⁶

The accuracy for differentiation of PDH from AT depends upon analytical sensitivity and the working range of the assay (Table 3). The most common problem with the cACTH assay is poor sensitivity. Some dogs with PDH have cACTH concentrations at or below the sensitivity of the assay, particularly with the Immulite 1000 analyzer. The largest study of cACTH in dogs with HAC used a 2-site solid-phase chemiluminescent immunometric assay (Immulite ACTH kit and Immulite 2000 analyzer) and showed excellent discrimination between PDH and AT.⁷⁵ No dogs with PDH had undetectable cACTH concentrations, likely because of the analytical sensitivity (5 pg/mL), but the range of cACTH concentrations for dogs with PDH was 6–1,250 pg/mL, with many dogs falling close to the lower end of the range. Thus, less sensitive assay systems (eg, Immulite 1000) would likely have poorer discrimination. Intra-assay and interassay variability (increased at lower cACTH concentrations), pulsatile

Table 3. Results of cACTH assays for dogs with HAC (last 10 years with currently available assays only).

Study	Assay	PDH	AT	Number Incorrect
Zeugswetter ⁷⁷	Immulite 1000	49 dogs <10–101 pg/mL	10 dogs <10 pg/mL	9/59
Rodriguez Pineiro ⁷⁵	Immulite 2000	91 dogs 6–1,250 pg/mL	18 dogs <5 pg/mL	0/109
Castillo ⁷²	Nichols IRMA	5 dogs 40–135 pg/mL	NA	NA
Scott-Moncrieff ⁷⁶	Immulite ACTH	11 dogs <10–50 pg/mL	4 dogs <10 pg/mL	4/15 (Immulite)
	Nichols IRMA	9–99 pg/mL	<10 pg/mL	3/15 (IRMA)
Gould ¹¹⁴	Nichols IRMA	21 dogs 28–1,132 pg/mL 1 dog <5 pg/mL	6 dogs <5 pg/ml 1 dog 76 pg/mL	2/29

ACTH, adrenocorticotrophic hormone; cACTH, canine ACTH; HAC, hyperadrenocorticism; IRMA, immunoradiometric assay; PDH, pituitary-dependent hyperadrenocorticism; AT, adrenal tumor.

ACTH secretion, and inappropriate sample handling allowing ACTH degradation increase the likelihood of a falsely low value in dogs with PDH.

Timing of Sample Collection. No clear evidence exists that the specific time of sample collection affects results or discriminatory power of the test.

Sample Handling. Plasma proteases degrade cACTH rapidly if samples are not cooled appropriately. Blood should be collected into chilled, silicon-coated glass or plastic tubes containing EDTA, centrifuged within 15 minutes (ideally in a cooled centrifuge), and the plasma transferred to plastic tubes and frozen immediately.^{74,76,78} Samples must stay frozen until analysis; if a courier is used for quick transport to a reference laboratory, ice may be sufficient. If samples are shipped, they should be sent overnight packed in dry ice.

Addition of the protease inhibitor aprotinin (Trasylol) prevents ACTH degradation by plasma proteases.⁷⁴ With the Immulite assay, aprotinin introduces an artifactual decrease⁷⁶ and is not recommended.

Discordant Test Results. Discordance between cACTH concentration and results of other differentiating tests sometimes occurs. Episodic cACTH secretion, poor assay sensitivity, and sample degradation are potential explanations. Stress and the presence of multiple adrenal disorders (ie, cortisol-secreting AT or PDH with pheochromocytoma; cortisol-secreting AT and PDH) also may influence ACTH concentrations. Ectopic ACTH secretion and food-stimulated cortisol secretion could also cause discordance.^{69,70}

Conclusions

- cACTH measurement is the most accurate stand-alone biochemical differentiating test.
- Reference ranges vary by technique; each laboratory must establish its own reference ranges.
- Sensitivity is a concern with some assays.
- Proper sample handling is critical.

Dexamethasone Suppression Testing

Test Principles. In normal dogs, dexamethasone administration causes rapid and prolonged suppression of cortisol secretion. In patients with an AT, dexamethasone at any dosage does not suppress cortisol secretion. In dogs with PDH, ACTH secretion is not appropriately suppressed by administration of a low dose of dexamethasone (0.01 mg/kg), but in 75% of dogs with PDH, cortisol concentrations decrease after administration of 0.1 mg/kg dexamethasone used in the high-dose dexamethasone suppression test. The other 25% of dogs with PDH do not demonstrate suppression even after receiving higher dexamethasone dosages.³⁵ In dogs with PDH that do not suppress, a large pituitary tumor is more likely.^{32,79}

Dexamethasone Form, Dosage, and Time of Testing. The HDDST should be performed as the LDDST except that the dosage of dexamethasone is 0.1 mg/kg IV. The free alcohol form should be avoided.

LDDST and HDDST as Differentiating Tests. The largest study evaluating both suppression tests included 181 dogs with PDH and 35 with AT.³⁵ Procedures used to classify dogs were fairly rigorous; however, some dogs with mitotane-responsive AT may have been included in the PDH group. The criteria proposed for identification of dogs with PDH using an LDDST were a 4-hour postdexamethasone cortisol concentration below the laboratory cut-off or <50% of the basal cortisol concentration or an 8-hour cortisol concentration <50% of the basal cortisol concentration, but greater than the laboratory cut-off. The criteria for suppression on the HDDST were a 4- or 8-hour cortisol concentration or both below the laboratory cut-off or <50% of the basal cortisol concentration. Approximately 75% of dogs with PDH met at least 1 criterion for suppression on either the LDDST or HDDST. Of dogs with PDH, 12% did not suppress on an LDDST but did on the HDDST. Dexamethasone resistance (ie, no criteria were met) occurred in all dogs with AT and the remainder of the dogs with PDH. The criteria proposed in this study still are well accepted, although no follow-up studies have been performed for confirmation. In 41 dogs with AT in another study, 28 LDDST and 30 HDDST were performed.⁶ No suppression was seen on any test.

Based on clinical experience, the Panel agrees that suppression in response to dexamethasone supports a diagnosis of PDH, and a dog with dexamethasone resistance can have either AT or PDH. However, cut-off values need to be re-evaluated.

Dexamethasone Suppression with UCCR. Decreased blood cortisol concentration after dexamethasone administration is reflected in decreased UCCR. After collection of a morning urine sample on 2 consecutive days at home, 3 doses of dexamethasone (0.1 mg/kg) are administered PO at 6- to 8-hour intervals, and a 3rd urine sample is collected the next morning. A decrease in the 3rd UCCR to <50% of the mean of the basal values is consistent with PDH.⁸⁰ Lack of suppression does not confirm AT. In 160 dogs with HAC (49 AT and 111 PDH), the UCCR in 72% of dogs with PDH suppressed to <50% of the basal UCCR.⁸¹ The other 28% of dogs with PDH were dexamethasone-resistant. In dogs with AT, the maximum suppression was 44% of the baseline sample.

Discordant Test Results. Discordance between results of suppression tests and other differentiating tests may occur for the same reasons as for cACTH measurement. Changes in dexamethasone metabolism also may influence results of suppression tests.^{31,82}

Conclusions

- Dexamethasone suppression can help distinguish PDH from AT. If suppression occurs, the patient likely has PDH. However, cut-off values should be reevaluated.

- Lack of suppression after dexamethasone administration on either the LDDST or HDDST does not confirm an AT because approximately 25% of dogs with PDH fail to suppress.
- Suppression to < 50% of baseline on an LDDST (using criteria outlined above) in a dog with HAC confirms the disease as pituitary-dependent.
- If there is no suppression on an LDDST, measurement of cACTH or abdominal ultrasound is recommended. If these tests are not available, the HDDST is an alternative but it will only provide differentiation in approximately 12% additional PDH cases.
- Results of either the LDDST or HDDST cannot be considered 100% absolute.

Diagnostic Imaging

Diagnosis of HAC cannot be performed solely with imaging (ultrasonography, CT, MRI) and must rely on hormone tests. Moreover, finding normal adrenal glands on imaging studies does not rule out HAC.

Radiography. Abdominal distension, good contrast because of abdominal fat deposition, hepatomegaly, and bladder distension may be seen as well as mineralization of the bronchi and pulmonary interstitium⁸² and of dermal and subcutaneous tissues in areas predisposed to calcinosis cutis. A small liver makes HAC unlikely.⁸³ An AT may be visualized either because of mass effect or tumoral calcification.

Ultrasonographic Imaging. Adrenal gland width is the most informative parameter. Because the long axis of an adrenal gland often is misaligned with either the medial or dorsal plane of the body, cross-sectional images may lead to oblique views and miscalculation of glandular dimensions. Breed and body size-related differences also must be considered.

Ultrasonography can estimate AT size and possibly vascular or local soft tissue invasion.^{85,86} Symmetrical, normal sized, or enlarged adrenal glands are found in dogs with PDH,⁸⁷ but mild asymmetry also may occur.^{88,89} Moderate asymmetry, contralateral adrenocortical atrophy (adrenal width <4 to 5 mm), destruction of normal tissue architecture, or some combination of these is consistent with an AT. Distinguishing macronodular hyperplasia from AT can be difficult with ultrasonography. Although most AT are unilateral, bilateral tumors may occur.^{86,90,91}

When an AT has been confirmed, certain findings suggest malignancy. Possible metastases may be identified by thoracic radiography and abdominal ultrasonography. Metastasis can be confirmed by ultrasound-guided biopsy. Adrenal gland width >4 cm is highly correlated with malignancy. Invasion into the vena cava or adjacent tissues can be detected by ultrasonography, but CT⁹² and MRI are more sensitive techniques to identify vascular invasion and detect metastases. Therefore, abdominal ultrasonography ideally should be followed by CT or MRI before adrenalectomy. Differentiating benign from malignant AT

often is difficult, even with histopathological examination. No dog should be sent to surgery for adrenalectomy without confirmation of the presence of an AT (and atrophy of contralateral adrenal gland) by abdominal ultrasound examination, CT, MRI, or some combination of these.

Pituitary Imaging. Pituitary imaging provides valuable information regarding treatment options and prognosis. Pituitary lesions range from small nests of hyperplastic cells to large tumors.⁵ The absence of neurological abnormalities does not exclude a pituitary macrotumor (ie, a tumor that is either >1 cm diameter, extends above the sella turcica, or has a pituitary/brain ratio of >0.31).^{32,92}

Because pituitary lesions may be quite small, contrast-enhanced CT and MRI may identify a normalized pituitary gland in dogs with PDH.^{32,88,94-96} The blood supply of the posterior pituitary gland is direct (arterial), whereas that of the anterior pituitary gland is mainly indirect via the pituitary portal system; dynamic contrast-enhanced CT takes advantage of this difference. In a dog with a normal pituitary gland, after IV administration of contrast medium, the posterior pituitary gland can be identified first. This phase is called the "pituitary flush," and its absence indicates atrophy of the posterior pituitary gland because of compression by a pituitary tumor. Displacement or distortion of the flush can be used to identify and localize anterior pituitary microtumors.⁹⁷ Dorsal displacement and decreased signal intensity of the posterior lobe on T1-weighted MRI also indicates the presence of a microtumor.⁹⁸

The Panel does not recommend a specific pituitary imaging technique; choice reflects availability and the type of information sought. Over time, some pituitary tumors become macrotumors. Because radiation therapy or hypophysectomy is required for their treatment and both are more effective with smaller tumors and in the absence of neurological abnormalities, the Panel recommends that pituitary imaging be considered for all dogs at the time of PDH diagnosis. If clinical features suggest a pituitary macrotumor, confirmation requires imaging. Imaging also is essential for treatment planning before either hypophysectomy or pituitary irradiation.

A cortisol-secreting AT and pituitary tumor may occur simultaneously.⁹⁹ Thus, 2 Panel members advise pituitary imaging in dogs with AT. All Panel members recommend pituitary imaging when discordant results of previous tests exist (eg, an AT is visualized but cACTH concentration is not low, the contralateral adrenal gland is not atrophied, [>4 to 5 mm], or part of the affected adrenal gland appears normal).

Conclusions

- Diagnostic images should be carefully interpreted and always in conjunction with hormonal studies.
- No dog should undergo adrenalectomy without confirmation of the presence of an AT (and atro-

phy of contralateral adrenal gland) by abdominal imaging.

- Metastases, vena caval invasion by tumor mass, adrenal width >4 cm, or some combination of these findings strongly suggests malignancy.
- Pituitary imaging is recommended in all cases of PDH and considered essential in some.

Measurement of Cortisol Precursors and Adrenal Sex Hormones

The syndrome of atypical or occult HAC is defined as “a syndrome in which a dog appears to have HAC based on history, physical examination, and clinicopathologic findings, but the LDDST, UCCR, and ACTH stimulation test fall into currently accepted reference ranges.” The Panel prefers the term “occult” over atypical, but also notes that in the human literature, occult HAC refers to individuals not showing typical signs of HAC, ie, those with subclinical or inapparent disease. Because the term is known, the Panel chose to continue to refer to the syndrome as “occult HAC.”

Current theory, which possibly is incorrect, is that “occult HAC” is because of the abnormal adrenocortical secretion of sex hormones. The Panel does not believe that sex hormones cause “occult HAC.” Readers are referred elsewhere¹⁰⁰ for a discussion of the evidence for or against the theory.

The diagnosis of standard HAC is never based solely on basal cortisol concentration. No evidence exists that measurement of basal serum sex hormone concentrations are any more reliable for diagnosis of adrenal dysfunction. Thus, the following discussion will focus on ACTH-stimulated concentrations, which are a measure of adrenal reserve.

Clinical Picture

Only 14 cases in the veterinary literature meet the definition.^{101–104} No specific phenotype for “occult HAC” is apparent.

Although sudden acquired retinal degeneration syndrome and hyperphosphatasemia in Scottish Terriers¹⁰⁵ have been linked with “occult HAC,” causative evidence is lacking. If only post-ACTH sex hormone concentrations were considered, no single sex hormone was increased in more than 62% of dogs with retinal degeneration, and no single hormone was consistently increased. Similarly, in Scottish Terriers with hyperphosphatasemia, no single hormone was consistently increased. Furthermore, more Scottish Terriers without hyperphosphatasemia had increased sex hormones than did those with increased enzyme activity. Correlation is not causation.

Indications for Diagnostic Testing. Testing for “occult HAC” should not be undertaken if no clinical indication for testing for classic HAC exists. If the clinical picture fits, the primary indication for measuring cortisol precursors and adrenal sex hormones is when a dog is tested for HAC with an ACTH stimulation test or LDDST and all cortisol concentrations, including basal, are below the

reference range. If administration of exogenous glucocorticoids of any form or of medications that alter cortisol synthesis (eg, ketoconazole) is ruled out, a sex hormone-secreting AT may be present. The ultrasonographic finding of an AT in such patients would further support the diagnosis, but the lack of visualizing an AT does not rule it out. Secretion of progesterone and 17- α -hydroxyprogesterone (17OHP) or other sex hormone or cortisol precursor^{103,106} may suppress pituitary ACTH secretion and cause atrophy of normal adrenocortical tissue. A cause and effect relationship between AT sex hormone secretion and clinical signs has been documented,^{103,104,107} whereas a causative relationship with PDH and sex hormones has not. Furthermore, AT cells can dedifferentiate, losing ability to synthesize enzymes in the hormone synthesis pathways. Thus, a sex hormone or cortisol precursor may be the end-product of hormone synthesis, not cortisol. If pituitary-dependent “occult HAC” exists, how or why adrenocortical tissue should have altered steroid synthesis is unexplained.

Therefore, if clinical signs are mild, the Panel recommends waiting and retesting for classical HAC when progression is noted. If clinical signs are moderate to severe, abdominal ultrasound examination should be performed. If the adrenal glands are normal, the differential diagnoses for the patient should be reconsidered. If bilateral adrenomegaly is present, pituitary CT or MRI should be considered to identify a pituitary tumor causing early HAC. Lastly, food-stimulated HAC should be considered as a diagnosis, as in these patients fasting cortisol concentration may be low.

Sex Hormone Testing. Measurement of serum sex hormone concentrations has been advocated for diagnosis of “occult HAC.” Use of a sex hormone panel has been proposed to increase sensitivity and specificity over measurement of a single hormone alone. Increased concentrations of any of the sex hormones are common, with increases in estradiol noted in approximately 40% of panels submitted to a reference laboratory.¹⁰⁸

On the other hand, dogs with NAI might have increased sex hormone concentrations compared to healthy dogs because of adaptation of adrenocortical function to the stresses of chronic illness. Dogs with chronic NAI had a 14%²¹ or 36%²² chance of having post-ACTH stimulation cortisol concentrations consistent with HAC. Dogs without adrenal disease also can have increased sex hormone concentrations, and sex hormones may be more likely to be falsely increased by NAI than cortisol. In 1 study, post-ACTH serum cortisol, 17OHP, and corticosterone concentrations were significantly correlated both in dogs with neoplasia and in those suspected of having HAC, suggesting that as adrenal function is increased either by adrenal disease or by NAI, production of all hormones increases proportionately.⁴⁸ Test specificity for 17OHP may be as low as 59–70%.^{48,51,109} The specificity of progesterone measurement in a single study was 55%.⁵¹ In 6 dogs with either pheochromocytoma or a nonfunctional AT, serum concentrations of androstenedione, progesterone, 17OHP, testosterone, estradiol, or some combination of these were increased.¹⁰⁷

Alternate Theories. The Panel recognizes cases that fulfill criteria for “occult HAC.” Three Panel members will test for “occult HAC” by measuring sex hormones in specific cases after all other differential diagnoses have been excluded.

A few explanations exist for the existence of such cases. First, as discussed above, the reference ranges and cut-off values for the LDDST need to be reestablished; the Panel believes they should be lower than they currently are, resulting in some dogs with “occult HAC” actually having typical HAC. If so, dogs with mild or early HAC that are “normal” on tests using current cut-off values may not be with revised (lower) values. Second, variable cortisol sensitivity exists in humans¹¹⁰ and may occur in dogs. Dogs with high sensitivity may show clinical signs of HAC at cortisol concentrations considered “normal” for the general population. Accordingly, the appropriate name for the syndrome may be “suspected HAC.” Third, dogs that meet the definition for “occult HAC” may have rare forms such as food-dependent HAC. Other explanations also may exist.

Conclusions

- Sex hormones have not been proven to cause “occult HAC.”
- In general, if the clinical picture does not fit testing for classic HAC, it does not fit testing for “occult HAC.”
- One indication for testing of “occult HAC” is inappropriately low cortisol concentrations on HAC screening tests.
- The specificity of adrenal sex hormone panel testing is low.
- Finding an AT does not mean HAC is present. Given the specificity of sex hormone testing, a sex hormone panel must be interpreted cautiously if clinical signs of HAC are lacking.

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