Canine pituitary dependent hyperadrenocorticism (PDH), also known as Cushing’s disease, is a common endocrine disorder in older dogs. This disorder is caused by a pituitary adenoma (PA) that secretes inappropriate amounts of adrenocorticotropic hormone (ACTH), which results in bilateral adrenal hyperplasia and disorderly and excessive production of cortisol by the adrenal gland.

Read Part 1 of this series—Comparative Epidemiology & Etiology in Dogs & Humans (November/December 2015)—at tvpjournal.com. This article briefly described the diagnosis of PDH, including clinical signs, common laboratory findings, and atypical presentations of canine PDH.

BASELINE DIAGNOSTICS

In all patients being screened for PDH, the following initial diagnostics should be performed prior to endocrine diagnostics:

- Thorough history, including prior treatment with systemic or topical glucocorticoids
- Physical examination (Table 1)
- Complete routine database, including complete blood count, serum biochemical profile, urinalysis, urine culture, and blood pressure (Table 2).

These diagnostics are important as the clinical signs of PDH are rarely pathognomonic, and patients are generally older and may have comorbidities that affect endocrine function tests (Table 3) and impact therapeutic options and prognosis.

ENDOCRINE DIAGNOSTICS

Specific endocrine tests and imaging modalities are available to both diagnose PDH (Table 3) and distinguish between the various causes of hyperadrenocorticism (HAC). No single test is perfect and, if the initial screening test is negative and high clinical suspicion of PDH exists, additional tests should be performed to determine a definitive diagnosis.

TABLE 2. Common Laboratory Findings in Dogs with PDH

<table>
<thead>
<tr>
<th>HEMATOLOGIC ABNORMALITIES</th>
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<tr>
<td>“Stress” leukogram:</td>
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<tr>
<td>» Neutrophilic leukocytosis</td>
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<tr>
<td>» Lymphopenia</td>
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<tr>
<td>» Eosinopenia</td>
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<tr>
<td>» Mild thrombocytosis</td>
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<td>» Mild erythrocytosis</td>
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<table>
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<tr>
<th>SERUM BIOCHEMICAL ABNORMALITIES</th>
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<tr>
<td>Increased serum alkaline phosphatase</td>
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<tr>
<td>Milder increase in alanine aminotransferase</td>
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<tr>
<td>Hypercholesterolemia</td>
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<tr>
<td>Hypertriglyceridemia</td>
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<td>Hyperglycemia</td>
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<tr>
<th>URINALYSIS ABNORMALITIES</th>
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<tr>
<td>Decreased urine specific gravity &lt; 1.018</td>
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<tr>
<td>Proteinuria</td>
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<td>Urinary tract infection (even in absence of pyuria and bacteriuria)</td>
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Several endocrine screening tests are available to arrive at a diagnosis of HAC, including:
• Low-dose dexamethasone suppression (LDDS)
• ACTH stimulation
• Urine cortisol to creatinine ratio (UCCR).

Additional tests may be required to differentiate PDH from other causes of hypercortisolemia, such as endogenous ACTH measurement and advanced imaging.

### INITIAL ENDOCRINE SCREENING

#### Low-Dose Dexamethasone Suppression

LDDS demonstrates decreased pituitary sensitivity to negative feedback from glucocorticoids, via mechanisms discussed in the first article in this series.

**Indications.** Most consider LDDS the screening test of choice for HAC unless the patient history suggests iatrogenic HAC, in which case ACTH stimulation is preferred.

**Sensitivity/Specificity.** In veterinary medicine, the reported sensitivity and specificity of the LDDS test range from 85% to 100% and from 44% to 73%, respectively.²⁻¹⁰

**Test Results.** Blood samples are obtained (1) before, (2) 4 hours after, and (3) 8 hours after dexamethasone administration (0.01 mg/kg IV). Diagnosis of HAC is based on lack of suppression of cortisol concentration 8 hours after dexamethasone administration.

### ACTH Stimulation

ACTH stimulation assesses adrenocortical reserve. Due to greater purity and quality control, use of synthetic ACTH is recommended. Use of compounded ACTH is discouraged, especially when monitoring patients on adrenolytic agents or adrenal enzyme blockers (see Synthetic Versus Compounded ACTH, page 38).

**Indications.** ACTH stimulation is the gold standard for diagnosis of iatrogenic HAC and spontaneous Addison’s disease (hypoadrenocorticism). Diagnosis of HAC is based on finding an elevated cortisol concentration (post ACTH administration) based on the reference range established by the laboratory.

**Sensitivity/Specificity.** Sensitivity of ACTH stimulation for all forms of spontaneous canine HAC ranges from 57% to 95%. In dogs with PDH, sensitivity is 80% to 83%. Specificity ranges from 59% to 93%.¹¹⁻¹⁸

Due to its low sensitivity, it is inferior to LDDS as a screening test for spontaneous HAC. However, many clinicians still use ACTH stimulation because of its convenient study duration of 60 minutes and relative effectiveness in the clinical setting, especially when combined with abdominal ultrasound evaluation and interpreted in light of the history and physical examination.
Synthetic polypeptides, such as Cortrosyn (cosyntropin) or Synacthen (tetracosactrin), contain the biologically active first 24 amino acids of ACTH; however, their potencies have not been compared.

In several studies, after administration of cosyntropin (5 mcg/kg or 250 mcg/dog IV or IM), peak cortisol concentrations occurred at 60 to 90 minutes. After administration of 5 mcg/kg IV, no difference was detected between 60- and 90-minute cortisol concentrations.\(^{13,16}\)

Cosyntropin can be reconstituted, divided into aliquots in plastic syringes, and frozen at -20°C for 6 months.\(^{19}\) Whether tetracosactrin can be frozen has not been investigated; according to the manufacturer, store it at temperatures from 2°C to 8°C.

**Compounded ACTH products** have been evaluated in healthy dogs. Sixty minutes after administration, cortisol concentrations were similar among 4 compounded products (2.2 U/kg IM) and cosyntropin (5 mcg/kg IV). However, at later times, cortisol concentrations varied considerably with the compounded products.\(^{13}\)

**Urine Cortisol to Creatinine Ratio**

UCCR provides an integrated reflection of cortisol production, adjusting for fluctuations in blood concentrations. Determination of basal UCCRs can be performed alone or in tandem with other endocrine testing.

**Indications.** UCCR can be used as a screening test for hypercortisolemia, although a single positive result should not be overinterpreted. Adding oral dexamethasone suppression to UCCR testing has the advantage of potentially demonstrating both increased cortisol production and decreased sensitivity to glucocorticoid feedback.

**Sensitivity/Specificity.** When a single, random urine sample is collected in veterinary hospitals, reported sensitivity and specificity of UCCR for diagnosis of HAC ranges from 75% to 100% and 20% to 25%, respectively.\(^{20,21}\) In some dogs, there is considerable day-to-day variation in UCCR results.\(^{22}\)

However, in dogs with physical and biochemical changes consistent with HAC, when 2 basal UCCRs were above the cut-off level:\(^{22}\)

- Sensitivity was 99%; 95% confidence interval (94%–100%)
- Specificity was 77%; 95% confidence interval (64%–87%).

**DIFFERENTIATING PDH FROM ADH: LABORATORY ANALYSIS**

Given that PDH and adrenal dependent hyperadrenocorticism (ADH) are the most common forms of HAC, and that their treatment options and prognoses differ, it is important to recommend additional testing to determine the exact etiology of HAC. Several diagnostic modalities are commonly used to differentiate between PDH and ADH (Table 4):

- **Endogenous ACTH**
- Dexamethasone suppression
- Dexamethasone suppression with UCCR.

**Endogenous ACTH**

Canine ACTH is secreted from the pituitary gland in an episodic, pulsatile fashion in both healthy dogs and those with PDH.\(^{1}\) A circadian rhythm has not been convincingly demonstrated, although one study reported higher plasma concentrations of canine ACTH (cACTH) in late afternoon than in the morning.\(^{23}\)

**Indications.** Concentrations of cACTH do not differ between healthy dogs and those with PDH, and its measurement is not useful to screen for HAC. However, measurement of cACTH is the most accurate stand-alone biochemical test for differentiating PDH from an adrenocortical tumor (AT), but the sensitivity of the assay differs with methodology. Normal or elevated concentrations of cACTH are consistent with PDH, while suppressed values are consistent with ADH.

**Sensitivity/Specificity.** The most common problem with the cACTH assay is poor sensitivity. The largest study of cACTH in dogs with HAC used a 2-site solid-phase chemiluminescent immunometric assay (Immulite 2000 Immunoassay System for ACTH; healthcare.siemens.com) and showed excellent discrimination between PDH and AT.

The likelihood of falsely low ACTH values in dogs with PDH is increased by:

- Intra-assay and interassay variability (increased at lower cACTH concentrations)
- Pulsatile ACTH secretion
- Inappropriate sample handling allowing ACTH degradation.\(^{23-28}\)

**Dexamethasone Suppression**

Dexamethasone administration in:

- **Normal dogs:** Causes rapid and prolonged suppression of cortisol secretion
- **Patients with an AT:** At any dosage does not suppress cortisol secretion
- **Dogs with PDH:**^\(^{2,29}\)
» Does not appropriately suppress ACTH secretion (therefore does not suppress cortisol) when a low dose (0.01 mg/kg) is administered
» In 75% of dogs with PDH, ACTH and cortisol concentrations decrease when a high dose (0.1 mg/kg) is administered
» In 25% of dogs with PDH, suppression of ACTH and cortisol does not occur even after administration of higher dosages; in these patients, a large pituitary tumor or a tumor developing from the pars intermedia is more likely.

A description of how to perform dexamethasone suppression tests is outlined in Table 4. The largest study evaluating both suppression tests—LDDS and high-dose dexamethasone suppression (HDDS)—included dogs with PDH (n = 181) and ATs (n = 35).2 With LDDS, criteria for identifying dogs with PDH included:
• 4-hour post LDDS cortisol concentrations below laboratory cutoff or < 50% of basal cortisol concentration
• 8-hour post LDDS cortisol concentrations < 50% of the basal cortisol concentration and greater than the laboratory cutoff.

With HDDS, criteria for cortisol suppression were a 4- and/or 8-hour cortisol concentration below the laboratory cutoff or < 50% of the basal cortisol concentration.

Evaluation of the pituitary-adrenal axis is indicated in the following circumstances:
» Patients with clinical signs and laboratory findings consistent with PDH and in which nonadrenal illness has been ruled out or is well controlled.
» Patients in which an adrenal/pituitary mass or bilateral adrenal hyperplasia has been discovered in conjunction with compatible clinical signs.
» Patients with an incidentally discovered adrenal mass, with adrenalectomy being considered.
» Diabetic dogs with insulin resistance.

Approximately 75% of dogs with PDH met at least one criterion for suppression on either LDDS (88%) or HDDS (12%).

Dexamethasone resistance (ie, no criteria were met) occurred in all dogs with AT and the remainder (25%) of the dogs with PDH. In another study of 41 dogs with ATs, 28 LDDS and 30 HDDS tests were performed, with no suppression seen on any test.

In dogs demonstrating lack of suppression with LDDS, use of endogenous ACTH rather than HDDS is recommended to differentiate PDH from ADH. Since suppression in response to dexamethasone supports a diagnosis of PDH, a dog with dexamethasone resistance can have either AT or PDH.

Evaluation of the Pituitary–Adrenal Axis

<table>
<thead>
<tr>
<th>TABLE 4. Diagnostic Tests That Differentiate Between PDH and ADH</th>
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<tbody>
<tr>
<td>TEST</td>
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<tr>
<td>Low-Dose Dexamethasone Suppressiona</td>
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<td>High-Dose Dexamethasone Suppressiona</td>
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<tr>
<td>Endogenous ACTH</td>
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a. LDDS and HDDS results cannot be considered 100% absolute; when imaging and endocrine test results conflict, the latter should be given preference.
b. Calculate dose using parent compound.
c. For HDDS, avoid the free alcohol form.
d. Addition of the protease inhibitor aprotinin prevents ACTH degradation by plasma proteases and greatly facilitates sample handling. Check with your laboratory regarding suitability because, with some assays (ie, Immulite), aprotinin introduces an artifactual decrease in results.
Dexamethasone Suppression with UCCR
Decreased blood cortisol concentration after dexamethasone administration is reflected in decreased UCCR.

After the patient's owner/handler collects a morning urine sample on 2 consecutive days, 3 doses of dexamethasone (0.1 mg/kg PO) are administered at 6 to 8 hour intervals, with a third urine sample collected the next morning.

Decrease in the third UCCR to < 50% of the mean cortisol basal values is consistent with PDH. Lack of suppression does not confirm AT.

In 160 dogs with HAC (49 with ATs, 111 with PDH), the UCCR in 72% of dogs with PDH suppressed to < 50% of the basal UCCR, while the other 28% of those with PDH were dexamethasone-resistant. In dogs with ATs, maximum suppression was 44% of the baseline sample.31

DIFFERENTIATING PDH FROM ADH: IMAGING
While imaging can be very helpful in differentiating PDH from ADH, it cannot be used to establish a diagnosis of HAC. Moreover, finding normal adrenal glands on imaging studies does not rule out HAC.

Radiography
Imaging results may include:
- Abdominal distension
- Good contrast due to abdominal fat deposition
- Hepatomegaly
- Bladder distension
- Mineralization of bronchi and pulmonary interstitium, and of dermal and subcutaneous tissues in areas predisposed to calcinosis cutis.

A small liver makes HAC unlikely. An AT may be visualized either due to mass effect or tumor calcification.32,33

Adrenal Gland Imaging
Adrenal gland width is the most informative parameter identified on ultrasonography (Figure 1). However, the following may affect correct measurement:
- Long axis of 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 differences
- Macronodular hyperplasia (a rare form of PDH) and some ATs can be difficult to differentiate.

Test Results. In dogs with PDH, ultrasonography reveals normal sized or enlarged adrenal glands that are typically symmetrical; however, mild asymmetry may occur. In dogs with ATs, ultrasonography reveals moderate asymmetry, contralateral adrenocortical atrophy (adrenal width < 4–5 mm), destruction of normal tissue architecture, or some combination of these findings.34,35

Ultrasonography can also estimate AT size and possibly vascular or local soft tissue invasion. Although most ATs are unilateral, bilateral tumors may occur and in these patients, endogenous ACTH concentrations should be determined.

Metastasis. When an AT has been confirmed, certain thoracic and ultrasonographic findings suggest malignancy, including:
- Adrenal gland width > 4 cm
- Invasion into the vena cava or adjacent tissues.

However, computed tomography (CT) (Figure 2) and magnetic resonance imaging (MRI) are more sensitive techniques to identify vascular invasion and detect metastases.36

Adrenalectomy should not be performed without confirming the presence of an AT (and atrophy of contralateral adrenal gland) by abdominal ultrasonography (Figure 3), CT, MRI, or some
combination of these. Metastasis can be confirmed by ultrasound-guided biopsy, but differentiating benign from malignant AT often is difficult, even with histopathologic examination.

**Pituitary Imaging**

Pituitary imaging provides valuable information regarding treatment options and prognosis. Pituitary lesions range from microscopic nests of hyperplastic cells to large tumors. Absence of neurologic abnormalities does not exclude pituitary macrotumors (ie, tumors that are > 1 cm in diameter, extend above the sella turcica, or have a pituitary:brain ratio of > 0.31).

**Indications.** Pituitary imaging should be considered for all dogs at time of PDH diagnosis, especially if hypophysectomy or radiation therapy is being considered. If clinical features suggest a pituitary macrotumor, confirmation requires imaging; clinical signs include:
- Disorientation and aimless wandering
- Severe polyuria/polydipsia
- Lethargy
- Hyporexia to anorexia
- Visual impairment
- Seizures (in some patients).

**Test Results.** Because pituitary lesions may be quite small, contrast-enhanced CT and MRI (Figure 4 and Figures 5–6, page 42) may identify normal sized pituitary glands in dogs with PDH. However, dynamic contrast-enhanced CT takes advantage of the pituitary gland’s blood supply:
- The posterior pituitary gland’s blood supply is direct (arterial)
- The anterior pituitary gland’s blood supply is mainly indirect via the pituitary portal system.

In dogs with normal pituitary glands, after IV administration of contrast medium, the posterior pituitary gland can be identified first—this phase is called the “pituitary flush.” Its absence indicates atrophy of the posterior pituitary gland due to compression by a pituitary tumor. Displacement or distortion of the flush can be used to identify and localize anterior pituitary microtumors.

Dorsal displacement and decreased signal intensity of the posterior lobe on T1-weighted MRI indicates the presence of a microtumor.

**IN SUMMARY**

Diagnosis of PDH requires incorporating information from the history, physical examination, and routine laboratory tests. Specific endocrine tests
and imaging modalities are available to diagnose HAC and distinguish between the various causes of hypercortisolism. No single test is perfect and, if the initial screening test is negative and high clinical suspicion of HAC exists, additional tests should be performed. Endocrine evaluation of patients with HAC and nonadrenal illness can be difficult, and it is important to eliminate or manage the concurrent illness before undertaking adrenal function tests.

ACTH = adrenocorticotrophic hormone; ADH = adrenal dependent hyperadrenocorticism; AT = adrenocortical tumor; cACTH = canine ACTH; CT = computed tomography; HAC = hyperadrenocorticism; HDDS = high-dose dexamethasone suppression; LDDS = low-dose dexamethasone suppression; MRI = magnetic resonance imaging; PA = pituitary adenoma; PDH = pituitary dependent hyperadrenocorticism; UCCR = urine cortisol to creatinine ratio

References


