Low-Dose Dexamethasone Suppression Testing for Hyperadrenocorticism

**PROFILE**

**Definition**
- Hyperadrenocorticism in dogs, also called canine Cushing’s syndrome (CCS), is a clinical syndrome with biochemical abnormalities resulting from excess cortisol production by the adrenal glands.\(^1,^2\)
- The prevalence of CCS in the general population of dogs in the United States is approximately 0.1\%.\(^3\)
- The initial index of suspicion results from history, clinical signs, and a minimum database; it should not be diagnosed without endocrine testing.\(^1,^3,^6\)
- The normal regulation of cortisol (Figure 1): Corticotropin-releasing hormone (CRH) from the hypothalamus stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH in turn stimulates the secretion of cortisol from the adrenal cortex. Cortisol exerts negative feedback on both ACTH and CRH secretion.

**Signalment**
- CCS affects middle-aged to older dogs. Approximately 75\% of dogs with pituitary-dependent hyperadrenocorticism (PDH) are older than 9 years (median age, 11.4 years), and 90\% of dogs with adrenal tumors are also older than 9 years (median age, 11.6 years).\(^1,^2,^7\)
- CCS occurs in all breeds, although certain breeds may be overrepresented (Table 1).

**Normal regulation of cortisol:** CRH from the hypothalamus stimulates the release of ACTH from the anterior pituitary. This causes release of cortisol from the adrenal cortex. Cortisol, in turn, exerts negative feedback on both the anterior pituitary and hypothalamus.

**Table 1. Breeds Commonly Affected by Hyperadrenocorticism\(^1,^2,^7\)**

<table>
<thead>
<tr>
<th>Pituitary-Dependent Breed</th>
<th>Adrenal Tumor Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian shepherd</td>
<td>Alaskan malamute</td>
</tr>
<tr>
<td>Beagle</td>
<td>Cocker spaniel</td>
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<tr>
<td>Dachshund</td>
<td>Dachshund</td>
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<tr>
<td>German shepherd</td>
<td>German shepherd</td>
</tr>
<tr>
<td>Labrador retriever</td>
<td>Labrador retriever</td>
</tr>
<tr>
<td>Poodle</td>
<td>Terriers</td>
</tr>
<tr>
<td>Terriers</td>
<td>Toy poodle</td>
</tr>
</tbody>
</table>

ACTH = adrenocorticotropic hormone; CCS = canine Cushing’s syndrome; CRH = corticotropin-releasing hormone; PDH = pituitary-dependent hyperadrenocorticism
In general, 75% of dogs with PDH weigh less than 20 kg, whereas 50% of dogs with adrenal tumors weigh more than 20 kg. Female dogs represent 55% to 60% of dogs with PDH and 60% to 65% of those with adrenal tumors.

Pathophysiology

Three causes of hyperadrenocorticism in dogs are PDH, adrenal tumor hyperadrenocorticism, and iatrogenic hyperadrenocorticism.

**PDH**

- **PDH** is most often caused by a pituitary adenoma (most often in the pars distalis) that secretes excessive ACTH, causing adrenocortical hyperplasia and excessive production of cortisol by the adrenal glands (Figure 2). Approximately 80% to 85% of dogs have naturally occurring CCS due to PDH.

**Adrenal tumor hyperadrenocorticism**

- Adrenal tumor hyperadrenocorticism is caused by a cortisol-secreting adrenocortical neoplasm (adenoma or carcinoma) that results in the atrophy of normal adrenal cells (Figure 3). Approximately 15% to 20% of cases of naturally occurring CCS can be attributed to adrenal tumors, and the tumors may be unilateral or bilateral.

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**Cortisol regulation in a dog with PDH:** Excessive ACTH is released by a pituitary adenoma causing adrenal gland hypertrophy and increased release of cortisol. This leads to ineffectual negative feedback of cortisol on the anterior pituitary and hypothalamus.

**Cortisol regulation in a dog with an adrenal tumor:** Excessive cortisol is secreted by a functional adrenocortical neoplasm that is not under the control of ACTH. Negative feedback of cortisol decreases the amount of ACTH released from the anterior pituitary and causes the nonneoplastic adrenal gland to atrophy and decrease its natural production of cortisol.
Iatrogenic hyperadrenocorticism is caused by excessive administration of glucocorticoids and results in atrophy of the adrenal cortices.

Signs
- Common historical and physical abnormalities include varied combinations of polydipsia, polyuria, polyphagia, lethargy, muscle weakness, heat intolerance, panting, bilateral symmetrical alopecia, acne, calcinosis cutis, cutaneous hyperpigmentation, thin and bruised skin, anestrus, testicular atrophy, hepatomegaly, abdominal enlargement, facial paralysis, and exophthalmos.
- Complete blood count abnormalities may include a "stress leukogram," which is characterized by an absolute mature neutrophilia, lymphopenia, and +/- eosinopenia. 1
- Serum biochemistry abnormalities may include increased alkaline phosphatase and alanine transaminase enzyme activity. Cholesterol and glucose concentrations and bile acids may also be increased. Blood urea nitrogen and phosphate concentrations may be decreased. 1
- Urinalysis often reveals decreased specific gravity and evidence of urinary tract infection. 1

The low-dose dexamethasone suppression test (LDDST) can be used as a screening test for CCS. It measures the suppressive effect of dexamethasone on cortisol levels over time.

Methods
- Cortisol in plasma or serum is measured for the LDDST via a variety of assays including fluorometric, radioimmunoassay (most common and considered the gold standard), 9 enzyme-linked immunosorbent assay, chemiluminescence, and high-performance liquid chromatography.
- Blood is collected for a basal cortisol level prior to an intravenous injection of dexamethasone sodium phosphate or dexamethasone in polyethylene glycol (0.01 or 0.015 mg/kg body weight). A blood sample is collected again 8 hours after the dexamethasone injection. A 4-hour post-injection cortisol level may be used as a discriminating test (interpretation of results is discussed later in this article). 1,2,10
- Dexamethasone is used because it is a potent suppressor of ACTH secretion from the pituitary gland (and CRH from the hypothalamus) and does not interfere with the cortisol assay. 9
- Serum or heparinized plasma may be used (laboratory dependent).

Sensitivity & Specificity
- The sensitivity of the LDDST ranges from 85% to 100% depending on the study. This indicates the test's ability to identify true positives (patients that have CCS). 2,11,12
- The specificity of the LDDST ranges from 44% to 73% depending on the study. This indicates the test's ability to identify true negatives (patients that do not have CCS). 2,11,12

Interpretation of Results
- Dexamethasone given to a normal dog will cause inhibition of pituitary secretion of ACTH and therefore decrease cortisol secretion from the adrenal glands for 24 to 48 hours. 13
- Within 2 to 3 hours after administration of dexamethasone, plasma cortisol levels in dogs with a normal response will decrease to less than 1.4 mcg/dL (reference value dependent on the laboratory) and persist longer than 8 hours.
- Results should be evaluated using the laboratory's established reference intervals.
- Results are interpreted in light of signalment, history, physical examination findings, and hematologic and serum biochemistry abnormalities.
- Phenobarbital treatment does not appear to affect results of LDDST. 14
- A dog with no history, clinical signs, or serum biochemistry abnormalities and a normal LDDST is considered negative for CCS.
- A dog with history, clinical signs, and minimum database abnormalities consistent with CCS and increased cortisol levels after the 8-hour LDDST is considered to have CCS.
- Eight-hour plasma cortisol levels greater than 1.4 mcg/dL are consistent with a diagnosis of hyperadrenocorticism.
PDH vs Adrenal Tumors

- Dexamethasone will not suppress ACTH release from the anterior pituitary in dogs with PDH. Therefore, stimulated adrenal glands continue to release cortisol at increased concentrations. Dogs with PDH have an 8-hour cortisol level ≥ 1.4 mcg/dL.
- Dexamethasone will not suppress cortisol production in dogs with adrenal tumors because the tumor is not under the control of ACTH. The 8-hour (and 4-hour) post dexamethasone injection cortisol concentration will be ≥ 1.4 mcg/dL.
- Sixty-five percent of dogs with PDH meet at least one of the following criteria, which separate PDH from adrenal tumor hyperadrenocorticism:
  - The 4-hour cortisol concentration is < 1.4 mcg/dL.
  - The 4-hour cortisol concentration is < 50% of the basal cortisol concentration.
  - The 8-hour cortisol concentration is < 50% of the basal cortisol concentration.
  - Most dogs with PDH will have an 8-hour cortisol concentration > 1.4 mcg/dL.1
- Thirty-five percent of dogs with CCS have equivocal LDDST results that do not differentiate PDH from adrenal tumor hyperadrenocorticism, as defined by the following criteria (Table 2):
  - The 4-hour and 8-hour cortisol concentrations are ≥ the basal cortisol concentration.
  - The 4-hour and/or 8-hour cortisol concentrations are suppressed but still > 50% of basal concentration.
- A 4-hour postinjection blood sample of cortisol concentration may be useful in distinguishing PDH from adrenal tumor hyperadrenocorticism.
- If the 4-hour sample is not suggestive of PDH, then a discriminating test (such as measurement of endogenous ACTH concentration or a high-dose dexamethasone suppression test) may be useful.1,2

Inconclusive Results

If there is high clinical suspicion for CCS, dogs with inconclusive or normal results should be retested or have another screening test used (urinary cortisol:creatinine ratio). The duration of action of dexamethasone is over 48 hours, with a half-life of 119 to 136 minutes in the dog; therefore, retesting can be done in 1 to 2 weeks.15,16

Table 2. Low-Dose Dexamethasone Suppression Test Interpretation*

<table>
<thead>
<tr>
<th>Basal Cortisol</th>
<th>4-Hour Cortisol</th>
<th>8-Hour Cortisol</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 1.4 mcg/dL</td>
<td>&lt; 1.4 mcg/dL</td>
<td>Negative</td>
</tr>
<tr>
<td>Normal–Increased</td>
<td>&gt; 1.4 mcg/dL or &gt; 50% basal</td>
<td>&gt; 1.4 mcg/dL or &gt; 50% basal</td>
<td>PDH or adrenal tumor</td>
</tr>
<tr>
<td>Normal–Increased</td>
<td>&lt; 1.4 mcg/dL or &lt; 50% basal</td>
<td>&lt; 50% basal</td>
<td>PDH</td>
</tr>
<tr>
<td>Normal–Increased</td>
<td>&gt; 1.4 mcg/dL</td>
<td>&gt; 1.4 mcg/dL</td>
<td>Adrenal tumor</td>
</tr>
</tbody>
</table>

*An elevated 8-hour cortisol level is consistent with a diagnosis of hyperadrenocorticism if signalment, history, physical examination findings, and hematologic and serum biochemistry abnormalities fit the clinical presentation.
Test Limitations

- LDDST cannot distinguish iatrogenic hyperadrenocorticism from naturally occurring CCS.
- The 8-hour LDDST cannot differentiate PDH from adrenal tumor hyperadrenocorticism.
- Stress from confinement and handling, spontaneous fluctuations in cortisol, and nonadrenal diseases may interfere with results (false positives). Cortisol levels in these dogs may fail to suppress normally and therefore results of the LDDST may be misleading.
- In addition, the testing period is long (8 hours) and multiple blood samples are needed.

Additional Diagnostics

- Radiography of the abdomen may reveal hepatomegaly, distention of the urinary bladder, and adrenal masses.
- Radiography of the thorax may reveal mineralization of the airways or metastases.
- Radiographs may show osteopenia and calcinosis cutis.
- Ultrasonography of the abdomen may be helpful in discriminating PDH from adrenal tumor hyperadrenocorticism by the appearance of the adrenal glands in a dog already diagnosed with CCS.
- CT and MRI scans are being used to visualize the pituitary and adrenal glands in dogs.

Patient Monitoring

- LDDST should not be used to monitor patient response to treatment.
- Response to treatment is monitored using the ACTH stimulation test. Baseline ACTH stimulation testing is obtained prior to treatment and used to periodically evaluate response to treatment.

ACTH = adrenocorticotropic hormone; CCS = canine Cushing's syndrome; CT = computed tomography; LDDST = low-dose dexamethasone suppression test; MRI = magnetic resonance imaging; PDH = pituitary-dependent hyperadrenocorticism.