

## **Hypercortisolism I: Diagnostic Tips & Tricks**

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“Canine Cushing’s syndrome” (CCS) or hypercortisolism (HAC), refers to the clinical signs associated with the presence of excess glucocorticoids in the body, either from exogenous administration or endogenous production. There are three major forms of CCS. Iatrogenic CCS is caused by the exogenous administration of glucocorticoids. “Cushing’s disease,” or pituitary-dependent hypercortisolism (PDH), refers to excess cortisol production due to a pituitary tumor. Hypercortisolism can also be caused by an adrenal tumor (AT). Rarely, CCS is caused by ectopic secretion of ACTH or food-dependent, due to ectopic production of GIP receptors in the adrenal cortex.

Historically, the disease has been referred to as hyperadrenocorticism. Recently, however, Project ALIVE (<https://www.esve.org/alive/search.aspx>) has recommended the use of “Cushing’s syndrome,” instead of hyperadrenocorticism, to describe the clinical syndrome. Since the clinical signs of CCS are due to excess cortisol, CCS caused by a pituitary tumor is referred to as pituitary-dependent hypercortisolism, or PDH. This author attempts to follow ALIVE guidelines, but is not always successful.

Approximately 85% of all naturally occurring cases of CCS are pituitary-dependent, although numbers may be higher in some countries, with up to 94% of dogs having PDH in Australia (Langner, 2025). The remaining 15% are caused by adrenal tumors. Of dogs with PDH, about 85% are caused by adenomas arising from the anterior lobe; 15% are caused by adenomas in the intermediate lobe. The majority of dogs with PDH have microadenomas that are not visible to the naked eye. Other dogs have macroadenomas that can be seen with CT or MRI. In addition to CCS, these tumors can cause neurologic signs, but typically not until >10 mm in diameter (~15%).

Dogs with PDH secrete a much larger amount of ACTH than normal dogs. This excess stimulation from ACTH then causes over-secretion of cortisol from BOTH adrenal glands and results in bilateral adrenomegaly.

Dogs with AT usually secrete excessive cortisol from only one adrenal gland. Half of the adrenocortical tumors are adenomas, and the other half are adenocarcinomas. Since the hypothalamus and pituitary gland detect high levels of cortisol in the blood, CRH and ACTH secretion are dramatically decreased. Since the contralateral adrenal gland requires stimulation from ACTH to synthesize cortisol, cortisol secretion from that adrenal gland decreases, and the gland becomes atrophic.

Dogs with iatrogenic hyperadrenocorticism have very low levels of ACTH in their blood due to inhibition from the exogenous glucocorticoid. Chronic ACTH deficiency causes bilateral atrophy of the adrenal glands. As long as the dog is receiving glucocorticoids, it has clinical signs of hyperadrenocorticism, but the adrenal glands are atrophied. If chronic steroid administration is discontinued abruptly, pituitary

ACTH production will resume, but the atrophied adrenal cortices may be unable to produce sufficient cortisol in a stressful situation. This results in iatrogenic hypoadrenocorticism.

## **CLINICAL SIGNS AND CLINICOPATHOLOGIC AND RADIOGRAPHIC ABNORMALITIES**

The classic signs of CCS are panting, polyphagia, polydipsia, and polyuria. They also may have recurring skin infections or urinary tract infections. In hindsight, many dogs have also been less active, less likely to play, and less interactive for months preceding diagnosis. Muscle wasting, particularly leading to hindlimb weakness in larger dogs, may also occur.

Testing for CCS is rarely, if ever, indicated in patients that do not have clinical signs. **The vast majority of dogs with CCS do not have clinical signs of illness, such as vomiting, anorexia, or diarrhea.** The exception is dogs with pituitary macroadenomas, or metastatic adenocarcinomas. Unless either of these is suspected, clinically ill dogs should generally not be tested for hyperadrenocorticism. Non-adrenal illness interferes with endocrine testing, and treatment for hyperadrenocorticism is rarely recommended in dogs that are clinically ill.

Physical examination of patients with CCS often reveals the characteristic pot-bellied appearance. Dermatologic changes such as alopecia (or slow hair re-growth), thin skin, pigmentation, comedones, and dermatitis may be present.

Clinicopathologic abnormalities frequently found in dogs with naturally-occurring CCS include: increased ALP, ALT, cholesterol, and glucose (mild unless diabetic—5%); “stress” leukogram (neutrophilia, monocytosis, and lymphopenia); and isosthenuria (USG <1.020). Increased ALP and cholesterol are so common in these patients that it is very unlikely that a patient has CCS if they do not have at least one of these findings.

The previous recommendation to perform a urine culture in every newly diagnosed dog with CCS is currently controversial. The incidence of bacteriuria may be lower than previously reported, treatment of subclinical bacteriuria does not consistently resolve infection (and may result in more resistant bacteria), and the 2019 ISCAID guidelines do not recommend treatment of subclinical bacteriuria.

Radiographs in dogs with CCS usually reveal hepatomegaly. An adrenal tumor may be seen, if present. Half of all adrenal tumors are mineralized. Mineralization does NOT predict whether a tumor is an adenoma or adenocarcinoma. Thoracic radiographs may reveal metastasis from a tumor. Abdominal ultrasound may reveal bilaterally enlarged adrenal glands (PDH) or one large adrenal gland (AT) and a very small contralateral gland. Metastasis may also be found with ultrasound (most frequently in the liver).

## DEFINITIVE DIAGNOSIS

Diagnosis of CCS requires compatible clinical signs, clinicopathologic abnormalities, and specific endocrine tests. Specific tests for the diagnosis of CCS can be divided into SCREENING and DIFFERENTIATING tests. Screening tests, which help to identify patients with CCS, include the ACTH-stimulation test, low dose dexamethasone suppression test (LDDS), and urine cortisol:creatinine ratio (UCCR). Differentiating tests determine whether a patient with CCS has PDH or an AT. These include the LDDS, HDDS, endogenous ACTH, abdominal ultrasound, and the MRI and CT.

All of the screening and differentiating tests have advantages and disadvantages. However, the LDDS is preferred for most cases seen in general practice because it is very sensitive and can also be used for differentiation.

### Screening Tests

While the UCCR is a very sensitive test (up to 99%), it is not very specific and may be positive in patients with other disease. This test is often useful to rule-out CCS. A negative test almost always rules-out the diagnosis of CCS. However, a positive test result requires confirmation with another screening test. The UCCR is most useful in patients in which there is a low index of suspicion for the disease.

**Cut-off values provided by the laboratory receiving the diagnostic samples should be used for interpretation of all diagnostics. The values used below are guidelines only.**

The LDDS is a very useful test. It is very sensitive (~85-95%), and more specific than the UCCR (but less so than the ACTH stimulation test). Briefly, a serum pre-sample (0 hr) is collected from the patient immediately prior to administration of 0.01 mg/kg dexamethasone, IV. Another sample is drawn at 4 hrs and another sample at 8 hours. Note that if dexamethasone sodium phosphate is used, even though the bottle lists the concentration as 4 mg/mL, only 3 mg/mL is actually dexamethasone.

For DIAGNOSIS, not differentiation, of CCS, the clinician need only look at the 0 hr and the 8 hr samples. Remember—the dog must first be diagnosed with CCS before its etiology is determined. The 4-hr sample is not used for diagnosis. Although reference values vary with the laboratory, a dog is considered to have a normal response if the 8 hr cortisol sample is <1.4 µg/dL (<40 nmol/L, **or the cut-off provided by your laboratory**). A cortisol concentration greater than 1.4 µg/dL (>40 nmol/L, **or the cut-off provided by your laboratory**) is consistent with the diagnosis of hypercortisolism.

The ACTH stimulation test is the least sensitive, but most specific, of the three tests. Sensitivities as low as 60% have been reported. The test is less likely than the LDDS or UCCR to have a false positive result. The recommended protocol requires the collection of a pre-ACTH serum sample, prior to administration of 5 µg/kg (up to 250 µg) of synthetic cosyntropin, IV or IM. A post-serum sample is then collected 1 hour later. Remember to use your laboratory's cut-off values. In the absence of laboratory guidelines, cortisol values of <17 µg/dL (468 nmol/L) are NOT diagnostic for CCS; values from 17-22

µg/dL (468-605 nmol/L) are in the grey zone; and values >22 µg/dL (>605 nmol/L) are consistent with the diagnosis of CCS. Given the lower sensitivity, a cortisol value below the laboratory's cut-off does not rule out the diagnosis of CCS in a patient with compatible clinical signs.

### **Differentiation Tests**

After diagnosing a patient with CCS with one of the above methods, the next step is to determine whether the CCS is PDH or caused by an AT. This information is optimal for proper treatment of the disease. It is NOT possible to completely rule out a pituitary tumor without imaging of the abdomen and/or brain. However, it IS possible to rule it in.

The LDDS is most frequently used because of its ability to screen and differentiate. After the CCS has been diagnosed with an 8 hr sample >1.4 µg/dL (>40 nmol/L), several criteria for differentiation can be evaluated. The LDDS is consistent with PDH if: the 4 hr cortisol sample is <1.4 µg/dL (<40 nmol/L); the 4 hr cortisol sample is <1/2 baseline; or the 8 hr cortisol sample is <1/2 baseline. It is important to realize that some patients with PDH do not suppress at either time point. In fact, over half of the dogs that don't suppress actually have PDH!

The HDDS is very similar to the LDDS and has a slightly increased chance of picking up a dog with PDH. The protocol is the same as the LDDS, except that the dexamethasone dose is 0.1 mg/kg, IV. The HDDS is consistent with PDH if: the 4 hr OR 8 hr cortisol sample is <1.4 µg/dL (<40 nmol/L); or the 4 hr OR 8 hr cortisol sample is <1/2 baseline. Again, all dogs with PDH do NOT suppress with the HDDS. Approximately 50% of dogs that do not suppress have PDH.

Although clinically useful, the endogenous ACTH concentration is more difficult to obtain due to the lability of the hormone. A blood sample must be drawn into a plastic EDTA tube and centrifuged immediately. The plasma should be separated and frozen immediately, until reaching the diagnostic lab. Intuitively, high ACTH levels are consistent with PDH, and very low levels are consistent with ADH. Unfortunately, since ACTH is secreted episodically by the pituitary, and depending on the sensitivity of the assay used, it is possible for a dog with PDH to have a low ACTH value. So, although a high value is diagnostic for PDH, a low value does NOT rule it out, unless a highly sensitive assay is used.

Imaging studies are often helpful in the differentiation of PDH from AT. An abdominal ultrasound that reveals bilaterally enlarged adrenal glands is consistent with a diagnosis of PDH. If there is one very large adrenal gland and a very small contralateral gland, the dog has an AT.

MRI and CT can help diagnose PDH and give an indication of how large the tumor is. Tumors greater than 10 mm may cause neurologic signs (dullness, altered mental status, anorexia, etc.). Brain imaging will differentiate a macroadenoma from a microadenoma, and help identify patients that may benefit from radiation therapy. The indications for an MRI or CT are: 1. Evaluation of a neurologic dog with CCS; 2. Discriminating PDH from AT (if macroadenoma is present); 3. Identification of patients for radiation therapy. If PDH has been diagnosed by other methods and the dog does not have neurologic

signs, imaging of the brain is only indicated if the owner is willing to pursue radiation therapy if a large macroadenoma is present.

## **IATROGENIC CCS**

A dog with iatrogenic CCS generally shows clinical signs of CCS while receiving long-term glucocorticoids. However, if the steroid is suddenly discontinued, the atrophied adrenal glands will be unable to respond to stress, and clinical signs of hypoadrenocorticism may occur due to cortisol deficiency. Since exogenous glucocorticoids suppress ACTH secretion and may cause adrenal atrophy, an ACTH stimulation test is used to identify a patient with iatrogenic CCS. The test is performed as described above, except that the corticotrophin is given IV. A dog with iatrogenic CCS should have a flat-line response, generally with both baseline and 1 hr post-stimulation values of  $<1 \mu\text{g/dL}$  ( $<28 \text{ nmol/L}$ ).

It is important to note that even short courses of glucocorticoids may inhibit the adrenal response to ACTH for up to a month or more. These patients often have decreased, but not baseline, responses (post  $\sim 2 \mu\text{g/dL}$  –  $5 \mu\text{g/dL}$ ,  $55\text{--}140 \text{ nmol/L}$ ). Usually, careful questioning of the owner will reveal steroid administration, which may include topical steroids. Additionally, some steroids, such as prednisone, can interfere with the cortisol assay and falsely elevate the value. Thus, there should be at least a 24 hr “washout” period after administration of short-acting steroids, and longer for longer-acting steroids.

## **HOW I MONITOR DOGS ON TRILOSTANE**

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Trilostane is an enzyme inhibitor that decreases the production of cortisol and, to a lesser extent, aldosterone and other steroids. Based on its mechanism of action, it should not cause adrenal necrosis in the same way as mitotane, but trilostane can still lead to an Addisonian crisis due to hypocortisolemia. Additionally, trilostane has been reported to cause idiosyncratic adrenal necrosis in dogs (resulting in both glucocorticoid and mineralocorticoid deficiency), although rare. Survival times for PDH patients treated with trilostane and mitotane are similar (approximately two years) (Barker EN 2005). However, in my experience, if a veterinarian has little experience using either mitotane or trilostane, trilostane is easier to learn to use.

There is no induction phase involved with the administration of trilostane. Dogs are started on a 2-3 mg/kg total daily dose (SID or divided and given BID) and seen for a recheck 10-14 days later. The goals of treating dogs with trilostane are to improve clinical signs and quality of life and avoid side effects, including cortisol oversuppression. During rechecks, it is essential to assess the clinical signs of the patient based on the owners' assessment of how much the dog is drinking, urinating, and eating. Any lethargy, diarrhea, vomiting, or refusal to eat should also be noted. Although the ACTH stimulation test has been used to assess treatment efficacy for the past two decades, recent evidence suggests that a pre-pill cortisol concentration correlates with clinical signs, as well as an ACTH stimulation test. However, ACTH stimulation tests are still sometimes necessary, particularly if the patient is showing any signs of illness consistent with Addison's disease.

The pre-pill cortisol is measured from blood taken just before the morning pill is administered. If the patient is well-controlled and the pre-pill cortisol is  $>1.4\text{--}2\text{ }\mu\text{g/dL}$ , continuing the current dose is probably safe. However, if the value is  $<1.4\text{--}2\text{ }\mu\text{g/dL}$  or the dog shows any signs of illness, an ACTH stimulation test is indicated. If the patient is not clinically controlled (still polyuric, polydipsic, and/or polyphagic), and the pre-pill cortisol is  $>6.0\text{ }\mu\text{g/dL}$ , it is likely safe to increase the dose by 25-50% (or split to BID), and recheck in 2-4 weeks. If the pre-pill cortisol is  $<3.0\text{ }\mu\text{g/dL}$ , I recommend performing an ACTH stimulation test before increasing the dose.

An ACTH stimulation test can be used either instead of pre-pill cortisol monitoring or in addition to pre-pill cortisol monitoring. The test should be started 3-4 hours post-pill to assess cortisol levels at peak inhibition. Thus, the pill should be given in the morning if the dog is on SID dosing. A well-controlled dog's target post-stimulation cortisol concentration is  $2\text{--}6\text{ }\mu\text{g/dL}$ , but this MUST be interpreted in light of clinical signs. This range is flexible, depending on clinical response; a dog that has been on trilostane for six months and is doing well with a post-stimulation value of  $1.6\text{ }\mu\text{g/dL}$  may be acceptable, whereas a dog that has GI signs with a post-stimulation value of  $2.1\text{ }\mu\text{g/dL}$  may need his dose decreased. Similar holds true at the upper end of the range.

Notably, the effects of a given trilostane dose often increase even after the first two weeks of therapy. For example, if a dog is on 30 mg once daily and has a post-ACTH stimulation cortisol value of 9 ug/dL at 14 days, this may decrease to 5.5 ug/dL two weeks later, even if the dog is on the same dose. Thus, if the 14-day post-stim cortisol is <10 ug/dL following initiation of trilostane, I usually wait to increase the dose until after the next ACTH stimulation test two weeks later. Depending on those ACTH stim results, the trilostane dose may be increased or decreased by 20-50%.

Each time the trilostane dose is changed, a pre-pill cortisol and/or ACTH stimulation test should be run about 2-4 weeks later (2 weeks if the dose has been decreased due to over-suppression of cortisol). After the appropriate dose is determined, the dog should return for monitoring (assessment of clinical signs AND pre-pill cortisol +/- ACTH stimulation test) two to four weeks later, three months later, and then every 3-6 months. The trilostane dose may need to be increased, as patients seem to become more resistant to it with time. Additionally, adrenocortical necrosis is possible at any point during therapy. It MUST be stressed that owners still need to be cautioned that this drug can also lead to disastrous consequences if appropriate monitoring is not followed.

Dogs seem to get regulated more quickly on twice-daily dosing. Some dogs aren't as well-controlled on once-daily trilostane dosing, likely because the duration of efficacy of trilostane is variable, from 10-18 hrs. In these dogs, the dose may be divided and given BID. Dogs on the BID protocol generally need a lower total daily dose than those on SID dosing, and their clinical signs are often better controlled. Because of the increased efficacy of trilostane based on ACTH stimulation tests, some authors prefer to start patients on BID dosing (1-2 mg/kg BID).