

Note: To convert cortisol reported in nmol/L to mcg/dl, divide the concentration in nmol/L by 27.59.

Diagnosis

Serum alkaline phosphatase (ALP) measurement: Measurement of serum ALP activity has been used as a screening test for HAC. However, the best way to use measurement of ALP activity may be as a means to rule out the diagnosis of HAC. The sensitivity of this laboratory test is high, as ALP is above normal in 90% of dogs with HAC.^{1,2} However, the specificity is very low. Scottish terriers are now recognized as a breed that has higher serum ALP activity than do other breeds.³ (Of note, vacuolar hepatopathy is not specific for HAC either.⁴)

The corticosteroid-induced isoenzyme (CAP) has also been assessed and suggested to be a good screening test for HAC⁵; however specificity remains low. As with the total ALP activity, CAP activity is elevated in the vast majority of dogs with HAC or that have received exogenous corticosteroids. However, elevated CAP activity, either as absolute levels or as a percentage of total, occurs in dogs without exposure to exogenous corticosteroids or HAC. In general, CAP appears in a large number of samples when ALP is high for any reason.⁶ Dogs with, for example, non-hepatic neoplasia, liver disease, pyometra, pneumonia, congestive heart failure, and pancreatitis can have a CAP activity accounting for at least 50% of the total.⁷ More important, dogs more likely to be screened for HAC, e.g., those with hypothyroidism or diabetes or those given exogenous glucocorticoids or phenobarbital, can have elevated SAP levels with >50% CAP activity.^{8,9} Overall, the sensitivity of elevated CAP activity for glucocorticoid exposure (i.e., HAC or glucocorticoid therapy) is approximately 95%, but the specificity may be as low as 18%.^{10,11} In one study, the predictive value of a positive test and of a negative test was 50% and 80%, respectively; in other words, a dog with an increased CAP activity had a 50% chance of truly being exposed to glucocorticoids, while a dog with normal CAP activity had a 20% chance of glucocorticoid exposure.¹⁰

Occult hyperadrenocorticism: A syndrome termed “occult” HAC has recently been coined and refers to dogs that have clinical signs suggestive of HAC but normal ACTH stimulation test and/or LDDST results. Measurement of 17-hydroxyprogesterone (17OHP) has been advocated for diagnosis of “occult” HAC and is available through some commercial laboratories. The protocol requires ACTH stimulation testing with measurement of serum 17OHP concentration pre- and post-ACTH.

The first report of clinical signs thought to be due to sex hormone elevation described diffuse bilaterally symmetrical alopecia and hyperpigmentation in 7 Pomeranians.¹² Classic HAC was ruled out. Numerous sex hormones were measured pre- and post-ACTH; several abnormalities were noted and hypothesized to be due to a partial deficiency of 21-hydroxylase, an enzyme needed for cortisol synthesis.¹² In humans with 21-hydroxylase deficiency, cortisol is not synthesized, and cortisol precursors, most notably 17OHP and androgens, accumulate.¹³

More recently, a study of 23 dogs with clinical and laboratory findings suggestive of HAC were reported. Of the 23 dogs, 11 had an elevated cortisol response to ACTH. Of 10 dogs with a normal ACTH response, 6 had a positive LDDST. All 23 had an elevated 17OHP response to ACTH.¹⁴ The conclusion of the study was that serum 17OHP concentration post-ACTH stimulation is elevated in dogs with classic as well as occult HAC and that measurement of serum 17OHP concentration is a marker of adrenal dysfunction.

However, I believe inadequate substantiation exists that elevated serum sex hormones are clinically significant in PDH (adrenal tumors may be different). In humans, evidence suggests that elevated serum 17OHP does *not* cause clinical signs. Clinically silent 17OHP-secreting adrenal tumors occur.^{15,16} Massive elevations in serum 17OHP occur with 21-hydroxylase deficiency, yet clinically affected patients show signs either of aldosterone deficiency or androgen excess.^{13,17} Clinical signs of HAC do not occur despite 17OHP concentrations ranging from 3,000 to 40,000 ng/dl (reference range 20–600).¹⁷ Last, a “cryptic” syndrome of 21-hydroxylase deficiency exists in which affected people lack 21-hydroxylase and have hormonal abnormalities but no clinical signs. The factors that impose the phenotypic variability on the genotypic abnormality are unknown,¹⁷ but abnormal sex hormone elevations by themselves are not sufficient to cause clinical disease.

In dogs as well, the relationship between elevated serum sex hormone concentrations and disease is unclear. First, of 6 sex hormones assessed in the alopecic Pomeranians, only serum 17OHP post-ACTH stimulation was

significantly different between affected and unaffected dogs. When affected males and females were assessed separately, the males did not have an elevated serum 17OHP.¹² In 28 dogs diagnosed with Alopecia X, treatment with melatonin led to partial or complete hair regrowth in 64% despite no change in serum sex hormone concentrations.¹⁸ Furthermore, in 276 dogs with Alopecia X, including 63 Pomeranians, 73% had at least one basal or post-ACTH sex hormone concentration greater than the normal range. However, despite the preponderance of elevations in sex hormone concentrations, no consistent sex hormone abnormalities were identified, and it was concluded that it is more appropriate to refer to this syndrome as “alopecia associated with follicular arrest rather than equating it with an adrenal hormone imbalance.”¹⁹ Last, the specificity of the test may be as low as 70%, i.e., the chance of a false positive result is 30%.^{20, 21} In one study of 35 dogs with neoplasia who did not have adrenal disease, 30% had an elevated serum 17OHP concentration post-ACTH stimulation.²⁰ **Thus, dogs without adrenal disease clearly can have elevated sex hormone concentrations as they do cortisol concentrations, and sex hormones may be more likely to be falsely elevated by NAI as compared to cortisol.**

Second, problems exist with the study that attributed occult HAC to elevated 17OHP concentration. Classifying all 23 dogs as having occult HAC was inappropriate, as 17 had a standard stimulation test or LDDST consistent with HAC and were not occult. Three dogs had a normal ACTH stimulation test and low plasma cortisol throughout an LDDST, results not unusual in dogs with adrenal tumors. Only 3 dogs were diagnosed with pituitary-dependent HAC despite having both a normal ACTH stimulation and LDDST.¹⁴ This suggests that occult HAC may account for only a small percentage of HAC cases. In 64 dogs documented to have HAC, no dog was negative on both the standard tests, even calling into question the likelihood that occult HAC exists.²² More important, follow-up in 2 of the dogs refutes the idea that 17OHP could cause the supposed syndrome. When treated with trilostane, an inhibitor of cortisol synthesis, these dogs improved despite an increase in serum 17OHP concentrations.¹⁴

Two mechanisms have been proposed for progesterone’s ability to cause signs of glucocorticoid excess. Progestins, synthetic forms of progesterone, may either bind glucocorticoid receptors²³ or may displace cortisol from its binding protein, thereby elevating serum free cortisol concentration.²⁴ Indeed, progestins can suppress endogenous ACTH secretion and cause adrenal atrophy, an action suggestive of glucocorticoid activity.²⁵⁻²⁷ Accordingly, progesterone may do the same. Examination of Pomeranians with Alopecia X, however, refutes the likelihood of either mechanism occurring. If elevated serum 17OHP concentration as seen in those dogs is sufficient to cause clinical disease due to glucocorticoid actions of 17OHP, endogenous ACTH concentration should be suppressed due to negative feedback at the pituitary. To the contrary, Pomeranians with elevated serum 17OHP concentrations had higher plasma ACTH concentrations than healthy dogs.¹² Similarly, during diestrus when serum progesterone concentrations are highest, adrenal secretion of cortisol in response to ACTH is greatest.²⁸ Thus, directly equating activity of progesterone with that of progestins is inappropriate.²⁹⁻³¹

In cases of pituitary-dependent occult HAC, how or why normal adrenocortical tissue should have altered steroid synthesis is unknown. As such, how likely activation of the pituitary-adrenal axis from NAI would be to also cause a shift toward synthesis and secretion of sex hormones is unknown. In a study we performed, linear regression analysis found significant correlation between post-ACTH serum cortisol, 17OHP, and corticosterone concentrations both in dogs with neoplasia and those suspected of having HAC, **suggesting that as adrenal function is increased either by adrenal disease or nonspecifically by non-adrenal disease, production of all hormones increases proportionately.**²⁰

Unfortunately, the ability of chronic NAI to affect sex hormone testing has not received critical appraisal, as has the standard ACTH stimulation test. Besides 17OHP, the endocrine lab at Tennessee will also measure cortisol, estradiol, progesterone, testosterone, and androstenedione pre- and post-ACTH. **However, the clinical significance of this test has not been determined.** Two studies have determined that 30% of dogs with NAI but without HAC have elevated 17OHP concentrations post-ACTH.^{20, 21} **The likelihood of elevations in any of the other sex hormones measured for diagnosis of occult HAC has not been evaluated at all.**

Routine pituitary imaging: Treatment for a pituitary macroadenoma, i.e., an adenoma > 1 cm diameter, requires radiation therapy for local control of the tumor. Survival post-radiation depends on tumor size and the presence of neurological signs before treatment—the smaller the tumor and the milder the neurological signs (or absent), the better a dog will respond to therapy and the longer the survival.³² In 24 dogs with neurological signs secondary to a pituitary tumor and treated with radiation therapy, 5 dogs worsened during therapy, 5 dogs had stable disease, and 14 dogs improved. After irradiation, a stable period was observed in 20 dogs, with 10 achieving complete remission

and 10 partial remission. Time to improvement of neurological signs was 9–35 days after beginning treatment.³² In dogs with PDH followed over 1 year, 6 dogs (46%) had tumor growth. Of 13 with masses visible on MRI, 4 (36%) developed neurological signs within 1 year.^{33,34} In response, a question has been raised whether all dogs with PDH should have pituitary imaging to determine if a tumor is visible and if preemptive treatment should be considered. The endocrinology text by Feldman and Nelson makes the following recommendations (for dogs with no neurological signs at the time of diagnosis): All dogs with PDH should have CT or MRI performed at the time of diagnosis. If no mass is seen, the dog should be treated medically with no follow-up imaging required. If a mass 3–7 mm is seen, medical treatment should be administered and imaging repeated in 12–18 months. If the mass is >8 mm diameter, radiation therapy should be pursued. Medical therapy can be added if clinical signs continue for greater than 3–6 months or if they recur.³⁵ No benefit has been shown to irradiation of tumors smaller than 8 mm.

Treatment

To treat or not to treat: An “urban legend” exists that survival is the same whether or not a dog with HAC is treated. Honestly, that has never been evaluated to my knowledge. It may be true for some dogs, but I do not think all. It also is a quality of life issue for both owner and dog.

I do believe that not all dogs with positive tests for HAC need to be treated, however, and that the decision should be made on a case-by-case basis. In deciding when to treat, I look at the dog, quality of life, the owner, and clinical signs. None of the drugs are cheap, and neither mitotane nor trilostane are benign, so treatment is not to be taken lightly. If the only clinical sign truly is something like elevated ALP, I don’t treat. If the issue is only cosmetic (poor hair), I also don’t usually treat. If a dog is mildly pu/pd and an owner can live with it, I don’t treat. But if a dog is getting the owner up in the middle of the night all the time to be let out, I do treat. I do go back and review with the owner questions that might relate to clinical signs, e.g., if the dog has stopped jumping on furniture (a sign of possible muscle weakness). I also look for evidence of clinical signs the owner might not note, e.g., look at urine s.g. to see if there is evidence of pu/pd. I also look for proteinuria (do a UPC) and hypertension—either or both of these are present in the majority of HAC dogs, and both can damage the body. So if either or both are present, I’m more aggressive about treating. Having said all that, sometimes there are clinical signs an owner doesn’t notice or has attributed to old age until the HAC is treated—e.g., not playing—and when the HAC is treated, activity increases.

Trilostane: Trilostane inhibits an adrenal enzyme, 3 β -hydroxysteroid dehydrogenase, thereby suppressing production of progesterone and its end products, including cortisol and aldosterone. A few studies have been published regarding the use of trilostane in a total of 119 dogs with pituitary-dependent hyperadrenocorticism (PDH).³⁶⁻³⁸ Since little is known about how trilostane should be used and monitored for greatest efficacy, the 3 studies are hard to compare, as treatment goals, doses, and monitoring protocols were different.

Overall, trilostane appears to be highly effective in suppressing cortisol secretion.³⁶⁻³⁸ In 3 studies pu/pd resolved over the first 6 months (mainly within the first 1–2 months) in 91%, while polyphagia resolved in 81%.³⁹ In 62% of dogs with dermatological abnormalities, there was marked improvement that took up to 3 months. In 8 dogs, signs were poorly controlled.³⁶ A second study looked at 11 dogs with PDH. All had dermatological problems, and 10 were pu/pd. Coat and skin condition returned to normal in 9 dogs within 6 months; in 1 dog improvement was noted after 1 year. All 10 had a decrease in water intake within 7 weeks, and 9 had complete resolution at a median of 11 weeks. Polyphagia decreased in 9 of 10; in 1 dog it took approximately 6 months. Of 4 dogs that had increased panting, improvement was noted in 4 and resolution in 2. By 6 months, 9 of 11 owners were pleased with the results.³⁷

The package insert now states to start administration of trilostane (2-5 mg/kg) once daily, but I recommend BID based on recent studies (below), trying for the lower end of the range. In most dogs, dosage adjustments, both up and down, will be required. Authors of one study noted that in most dogs there was an initial sensitivity to the drug, followed by a need for an increase in dose. After time, the dose required hit a plateau.³⁸ Interestingly, the final dose required for control has varied greatly between studies. Part of the discrepancy may relate to the differences in what was considered the ideal post-ACTH serum cortisol concentration. In one study the median final dose was 6.1 mg/kg³⁷, while another study found the therapeutic dose for most dogs is likely to be 16–19 mg/kg.³⁸ In any case, the point remains that each dog should be started on the recommended dose, and then the dose should be adjusted according to ACTH stimulation test results. Survival is at least as good as that achieved with mitotane therapy.³⁶⁻³⁸

Reported adverse effects for the most part are relatively mild, including lethargy and vomiting, but fatality has occurred.^{36,38} Although some studies found relatively low incidence of side effects, mild, self-limiting side effects such as diarrhea, vomiting, and lethargy were noted by 63% of owners in one study.³⁹ Trilostane can affect aldosterone secretion as well as cortisol, so an Addisonian crisis can occur.^{36,38} Excess adrenal suppression can occur at any time during therapy.^{36,38} One dog died despite appropriate treatment for hypoadrenocorticism, and the true cause of death remained undetermined.³⁶

As with mitotane, excess adrenal gland suppression can occur and warrants discontinuing medication (see below) and lowering of the dose. Although in theory the effects of trilostane as an enzyme inhibitor should be rapidly reversible (e.g., within a couple of days), suppression can last weeks to months. In a few cases, trilostane was discontinued when cortisol secretion was noted to be too low, and cortisol secretion remained low for 6 weeks to 4 months but eventually returned to pre-therapy levels.³⁸ In an additional case, signs of glucocorticoid and mineralocorticoid deficiency occurred in a dog being treated with trilostane, and bilateral adrenal necrosis was documented. The etiology of the necrosis was undetermined.⁴⁰ The hypoadrenocorticism lasted for at least 3 months,⁴⁰ but likely will be permanent for the life of the dog. How often acute iatrogenic hypoadrenocorticism will occur in dogs treated with trilostane is unknown, but adrenocortical necrosis happens more often than previously believed. In a recent study, histopathology was performed on the adrenal glands of 7 dogs receiving trilostane for treatment of PDH or an adrenal tumor. Necrosis was present in glands from 5 dogs, and it was severe in 2!⁴¹

A few questions still need to be answered. First, the optimal post-ACTH serum cortisol concentration should be determined. The different studies done so far had different target ranges. The goal for post-ACTH cortisol concentration in one study was to be below 250 nmol/L (9 µg/dl),³⁶ but the goal was 30–70 nmol/L (1–2.5 mcg/dl) in the second study³⁷ and 25–125 nmol/L (1–4.5 µg/dl) in the third.³⁸ The ideal timing of post-pill sampling also needs to be elucidated. Post-ACTH cortisol may vary with the interval between dosing and testing.⁴² Based on their experience, the authors of the third study recommended doing an ACTH stimulation test 3–8 hours after the last dose,³⁸ while another author specifically recommends testing at 4–6 hours post-pill.³⁹ Last, the appropriate starting dose and interval (daily or BID) need clarification. The authors of one study recommended starting with once-daily dosing,³⁸ while other authors recommended initiating therapy twice daily.⁴³ How long control must be maintained throughout the day needs to be elucidated. For example, is control for 12 hours adequate, or does it need to last 24 hours?

If minor side effects are seen, stop the drug for 3–5 days and then restart giving trilostane at a lower dose for 1 week before continuing with the initial dosing scheme. An ACTH stimulation test should be performed at 10–14 d, 30 d, and 90 days after being on a full dose of trilostane. The test should be performed 4–6 hrs post-pill. If the post-ACTH cortisol concentration is <20 nmol/L, stop the trilostane for 48–72 hours.³⁹ At this point, some recommend restarting trilostane at a lower dose,³⁹ but given the long-term suppression seen in some cases, I believe that, ideally, an ACTH stimulation test should be performed and trilostane should not be reinstated until cortisol secretion has recovered. If the post-ACTH cortisol is >200 nmol/L, increase the dose of trilostane. If the post-ACTH serum cortisol concentration obtained is 20–200 nmol/L but the clinical signs are continuing, then BID therapy should be used. The same dose that was given once daily should be given twice (e.g., if giving 30 mg once daily, then double it to 30 mg twice daily). Once the clinical condition of the dog and the dose have stabilized, an ACTH stimulation test should be performed every 3–6 months, and serum potassium concentration should be measured to check for hyperkalemia.

One fairly recent study assessed the use of trilostane BID in 44 dogs with PDH.⁴⁴ The initial dose of trilostane was 15 mg, PO BID for dogs <5 kg, 30 mg BID for dogs 5–20 kg, 60 mg a.m. and 30 mg p.m. for dogs 20–40 kg, and 60 mg BID for dogs >40 kg. At the first recheck (7 days later), the ACTH stimulation test was started 4–6 hours post-pill to assess the maximum effect of the drug. Good control was judged on the basis of clinical signs and a serum cortisol concentration pre- and post-ACTH of 30–110 nmol/L. The dose was adjusted by 25–50% increments. On further rechecks, the ACTH stimulation test was initiated 8–12 hours post-pill. Good control was believed to be a post-ACTH cortisol concentration of 30–250 nmol/L.

Mean initial dose of trilostane was 6.2 mg/kg (range 2.4–15.0) divided BID. The dose was not changed over the course of the study in 10 dogs, was increased in 19, was reduced in 5, and was both increased and reduced in 10. Over the course of the study, at all rechecks the mean dose was between 6 and 8 mg/kg divided BID, but the range was approximately 2–20. Adverse reactions were seen in 25% of cases related to low cortisol concentration. In 11%, trilostane therapy was discontinued due to prolonged suppression of serum cortisol concentration. In 4 dogs adrenal

function returned to normal and no further treatment was needed; one dog was treated as an Addisonian. Mean survival time was 930 days. Another more recent study from Davis also recommends giving trilostane BID.^{4, 5} Trilostane has been used to treat a few dogs with adrenal tumors.^{39, 46} Not enough information is available to ascertain whether the treatment protocol or efficacy varies if treating dogs with PDH versus those with an adrenal tumor. However, it should be remembered that trilostane is not cytotoxic as mitotane is. In other words, mitotane is truly a chemotherapeutic drug in this instance, killing primary neoplastic cells and, perhaps, metastatic cells as well. Trilostane simply would control tumoral secretion, not growth. In fact, in dogs with PDH treated with trilostane, the size of the adrenal glands increased.⁴⁷ Whether trilostane would be a better alternative to ketoconazole therapy to control the clinical signs of HAC pre-adrenalectomy remains to be determined.

A few disadvantages exist for using trilostane. The largest is availability. At the current time, trilostane is not approved for use in the United States. To obtain trilostane legally in the United States, a letter must be filed with the FDA. Second, the cost of trilostane will be 2–3 times that of mitotane depending on the size of the dog.³⁸ Since repeat ACTH stimulation testing is needed with mitotane or trilostane, the cost of repeat evaluation would be the same for either drug. Last, until the questions about required duration of action are answered, this author recommends use of mitotane in dogs with serious complications of HAC exist and breaks in control could be detrimental, e.g., in dogs with pulmonary thromboembolism.

References available upon request.