DIAGNOSIS OF CANINE HYPERADRENOCORTICISM: A CASE-BASED APPROACH
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Case 1: Signalment: 10 yr old, CM, Miniature poodle; History: Presented for teeth cleaning; PE: Severe dental tartar and moderate gingivitis; Labs: leukocytosis with neutrophilia, lymphopenia, monocytosis and eosinopenia; ALP 578 IU/L (35-280); urine specific gravity (USG) 1.015.

Hyperadrenocorticism (HAC) was suspected due to the elevated ALP. The owners were not sure if the dog was drinking more. A USG of 1.015 could be consistent with pu/pd. Which test to use to diagnose HAC, a low-dose dexamethasone suppression test (LDDST) or ACTH stimulation test (AST) depends on the situation. I make the following recommendations: 1. If a dog has no known non-adrenal illness (NAI) and moderate to severe clinical signs of HAC, do the LDDST. 2. If clinical signs are mild or only laboratory changes are present (e.g. increased ALP), do the AST. 3. If NAI is present, if the dog has received any form of exogenous glucocorticoid including topicals or phenobarbital, do the AST.

Since none of the tests for HAC are perfect, one question to ask is what would be worse, a false-negative or a false-positive result? In this case, I would say a false positive would be worse because it could Sentence the dog to unnecessary lifelong therapy. When the only problem noted is a high ALP, I would rather miss the diagnosis now (and revisit the idea later if signs progress) than falsely diagnose HAC. In general, the LDDST has a chance of a false negative of about 5% whereas with the AST it is about 20%. The flipside, however, is that the LDDST, in dogs with NAI, has as high as a 55% chance of giving a false positive. For the AST the chance of a false positive is about 15%. Therefore, for this case, I’d go with the AST.

The cost of Cortrosyn (cosyntropin) has increased dramatically so an alternative to use for an AST is commonly sought. No good substitute exists. The best option to reduce the cost of the test is to use a low dose (5 mcg/kg IV) of cosyntropin with blood samples drawn before and 1-hr post injection. Reconstituted Cortrosyn can be stored refrigerated in plastic vials for up to 4 mths and frozen for 6 mths. If freezing Cortrosyn, do so in smaller aliquots as the effect of thawing and refreezing is unknown. Compounded ACTH may be used with caution. We performed a study in normal dogs in which we gave Cortrosyn (5 mcg/kg IV) and 4 different compounded forms of ACTH (all manufacturers recommended a dose of 1 U/lb IM with blood samples drawn before injection and 2-hr post). Administration of all forms of compounded ACTH increased serum cortisol concentrations to a similar degree as did the Cortrosyn when values were compared at 60 min following injection. However, serum cortisol concentration had returned to baseline by 120 min when using 2 of the compounded forms. Thus, due to variability in duration of response, I recommend that when using compounded ACTH products samples should be collected before administration and at 1 and 2 hrs following injection.

CASE SUMMARY: On an AST, cortisol pre-ACTH was 224 nmol/L (reference range 10-160 nmol/L; to convert to mcg/dl divide cortisol in nmol/L by 27.6) and post-ACTH was 468 nmol/L (220-560 nmol/L). A urine protein/creatinine ratio (UPC) and blood pressure were measured as about 66% of HAC dogs have proteinuria and/or hypertension. Both were normal.

This could be a good case to measure a urine cortisol:creatinine ratio (UCCR). The UCCR is best used to rule out a diagnosis of HAC. Almost all dogs with HAC have an elevated UCCR, but the majority of dogs with an elevated UCCR do not have HAC. Thus, if the ratio is normal there is little chance the dog has HAC, but if the ratio is high, another screening test,
i.e. LDDST or AST, must be done to confirm the diagnosis. The UCCR is best measured on a urine collected at home. If in this dog the UCCR was normal, I would rule out HAC.

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. 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. In humans with 21-hydroxylase deficiency, cortisol is not synthesized and cortisol precursors, most notably 17OHP and androgens, accumulate.

More recently, a study of 23 dogs with clinical and laboratory findings suggestive of HAC was 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. 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. Massive elevations in serum 17OHP occur with 21-hydroxylase deficiency, yet clinically affected patients show signs either of aldosterone deficiency or androgen excess. Clinical signs of HAC do not occur despite 17OHP concentrations ranging from 3,000-40,000 ng/dl (reference range 20-600). Lastly, 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, 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. 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. 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”. Lastly, the specificity of the test may be as low as 70%, i.e. the chance of a false positive result is 30%. In one study of 35 dogs with neoplasia who did not have adrenal disease, 30% had an elevated serum 17OHP concentration post-ACTH stimulation. 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 normal ACTH 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. 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. More importantly, 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.

Two mechanisms have been proposed for progesterone’s ability to cause signs of glucocorticoid excess. Progestins, synthetic forms of progesterone, may either bind glucocorticoid receptors or may displace cortisol from its binding protein thereby elevating serum free cortisol concentration. Indeed, progestins can suppress endogenous ACTH secretion and cause adrenal atrophy, an action suggestive of glucocorticoid activity. 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. Similarly, during diestrus when serum progesterone concentrations are highest, adrenal secretion of cortisol in response to ACTH is greatest. Thus, directly equating activity of progesterone with that of progestins is inappropriate.

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 non-specifically by non-adrenal disease, production of all hormones increases proportionately.

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. The likelihood of elevations in any of the other sex hormones measured for diagnosis of occult HAC has not been evaluated at all.

**Case 2:** Signalment: 10 yr old, CM, Miniature poodle; History: Presented for teeth cleaning, increased water consumption; PE: Severe dental tartar and moderate gingivitis, bilateral partial alopecia, thin skin on ventral abdomen; Labs: leukocytosis with neutrophilia,
lymphopenia, monocyteosis and eosinopenia; ALP 578 IU/L (35-280); USG 1.005, urine protein 1+.; UPC = 3.2 ; systolic blood pressure 185 mm Hg.

Again, the question to ask is: Which is worse, a false-positive or a false-negative? This dog is an archetypal HAC case and, if the dog does have HAC, should be treated as soon as possible. Therefore, I judge that a false negative is worse. Since the LDDST has a smaller chance of a false negative, I would do that test first. In addition, the LDDST may also differentiate between PDH and an adrenal tumor (AT). Results on an LDDST consistent with PDH are: 1. Suppression of serum cortisol at 4 hrs post-dexamethasone (dex) but not at 8 hrs. 2. Lack of suppression of serum cortisol concentration but a decrease to <50% of baseline at 4 and/or 8 hrs post-dex. If a dog meets either or both of these criteria, PDH is present. If a dog does not meet the criteria, there is still at least a 50-50 chance the HAC is PDH or AT. Differentiation must be achieved by other means.

Since false positive and false negative results are possible with either the LDDST or AST, if there is any doubt about the accuracy of the results, perform the other test. This is not a case in which I would measure a UCCR. I highly suspect HAC. If the UCCR is elevated suggesting HAC, another test such as the LDDST or AST must be done. One study found the chance of a false negative on the UCCR to be as high as 25%. Given that, in a case where I strongly suspect HAC, even if the UCCR is normal, I’ll still do the LDDST or AST. In other words, in cases like this dog, whether the UCCR is normal or not, the next step would still be an AST or, preferably, the LDDST, so the UCCR would not be helpful.

**Case Summary:** On an LDDST, pre-dex cortisol 242 nmol/L (reference range 10-160 nmol/L), 4-hr post-dex cortisol 58 nmol/L (reference range <30 nmol/L), 8-hr post-dex cortisol 25 nmol/L (reference range <30 nmol/L).

In theory, the important post-dex sample for determining if HAC is present is at 8 hr. If that is abnormal, the results are consistent with HAC. In this case, the 8-hr sample is borderline. Also troublesome is the fact that the 4-hr was not adequately suppressed. Given a high suspicion of HAC in this dog, I would now do an AST. Even though overall the ACTH stim is more likely to give a false negative, dogs with HAC can have a false negative on the LDDST and a true positive on the AST. If, in this case, the ACTH stim is positive, I would treat based on the clinical signs (clinical judgment as to which test to believe). Based on the LDDST, if the dog has HAC, it is PDH and no differentiating tests need to be done.

**Case 3:** **Signalment:** 10 yr old, CM, Miniature poodle; **History:** Presented for teeth cleaning, pu/pd; **PE:** Severe dental tartar and moderate gingivitis, bilateral partial alopecia, thin skin; **Labs:** leukocytosis with neutrophilia, lymphopenia, monocyteosis and eosinopenia; ALP 578 IU/L (35-280); USG 1.005, urine protein 1+; Urine protein/creatinine ratio = 3.2 (<0.5); systolic blood pressure 185 mm Hg; LDDST: Pre-dex cortisol 242 nmol/L, 4-hr post-dex cortisol 186 nmol/L, 8-hr post-dex cortisol 186 nmol/L.

This dog has HAC based on the findings, but which type must now be determined. Treatment options and protocols and prognosis vary depending on the form present. The choices for differentiation include a high-dose dex suppression test (HDDST), measurement of endogenous ACTH (eACTH) concentration or abdominal ultrasound. On an HDDST, the criteria for determining if a dog has PDH are: 1. Suppression of serum cortisol at 4 and/or 8 hrs post-dex. 2. Lack of suppression of serum cortisol but a decrease to <50% of baseline at 4 and/or 8 hrs post-dex. **Lack of suppression in response to the high dose does NOT mean a dog has an AT.** For those animals that do not suppress on a HDDST, approximately 50% have
Therefore, the HDDST can never confirm the presence of an AT, and if no suppression is seen on an HDDST another differentiation test must be done. Also, if an LDDST was done for screening and failed to determine if the dog had PDH, the HDDST is also unlikely to give the differentiation. For measurement of eACTH, special handling is required, but only a single blood sample is required and the presence of an AT or PDH can be confirmed. Unfortunately, a “grey zone” exists; if the eACTH concentration falls in that range, it is impossible to determine if the dog has PDH or AT. Chance of getting a “grey zone” result is 18%. If eACTH measurement is repeated, the chance drops to 4%. Ultrasound can be very helpful depending on the skill of the ultrasonographer. If both adrenal glands are not visualized, however, it should not be assumed that the dog has an AT.

CASE SUMMARY: Since the LDDST did not also differentiate, the HDDST was unlikely to be helpful (see above), so an eACTH sample was submitted. The eACTH concentration was 76 pg/ml (eACTH >15 pg/ml consistent with PDH).

References available from author upon request.