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ADRENAL FUNCTION TESTS AND THEIR CLINICAL USE IN DOGS

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Hyperadrenocorticism is a common canine endocrinopathy. Hyperadrenocorticism refers to the unregulated overproduction of cortisol by the adrenal cortex and may be primary (i.e. caused by abnormalities of the adrenal gland) or secondary (i.e. caused by pituitary overproduction of ACTH). Primary and secondary hyperadrenocorticism can also be referred to as adrenal-dependent and pituitary-dependent, respectively. Numerous strategies for diagnosing canine hyperadrenocorticism have been described in the veterinary literature. This talk will discuss the clinical application of commonly used diagnostic tests in the diagnosis of canine hyperadrenocorticism.

Diagnostic Testing Concepts

<table>
<thead>
<tr>
<th>Dz (+)</th>
<th>Dz (-)</th>
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<tr>
<td>Test (+)</td>
<td>a b</td>
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<tr>
<td>Test (-)</td>
<td>c d</td>
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Sensitivity = a / (a+c) = true positive
Specificity = d / (b+d) = true negative

The populations used to determine the sensitivity and specificity must be considered when evaluating the usefulness of adrenal function tests.

Pretest probability – All diagnostic tests will perform better when applied to a properly selected patient population. The pretest probability of disease is related to the prevalence of the disease among the dogs being tested. Since we usually do not know whether the dog being tested actually has the disease, another measure of a given test’s effectiveness is the post-test probability that any obtained result is correct. Another name for the posttest probability is the test’s “predictive value”. It is important to remember that, like the pretest probability, the posttest probability is heavily influenced by disease prevalence. (An example will follow)

Positive predictive value (+PPV) = a / (a+b) = proportion of diseased dogs with (+) test.
Negative predictive value (-PPV) = c / (c+d) proportion of non-diseased dogs with (-) tests.

(+): Predictive value increases as prevalence increases but decreases as prevalence decreases
(-): Predictive value decreases as prevalence increases but increases as prevalence decreases

Prevalence = (a+c) / (a+b+c+d)
Accuracy = (a+d) / (a+b+c+d)

Pretest probabilities relevant to HAC:

PDH - 85%
Adrenal tumor – 15% (50:50 probability of malignancy)
Are these probabilities valid?

- These prevalence estimates are at least 15 years old.
- The population was probably dogs with Cushing’s syndrome.
- The use of diagnostic US was in its infancy.

Testing for Hyperadrenocorticism

Patient selection – Identify dogs on the basis of historical, clinical, and laboratory findings. An appropriate clinical work-up increases the likelihood that the patient to be tested actually has the disease (i.e. increases the pre-test probability of disease).

Screening Tests

Urine cortisol:creatinine ratio

ACTH stimulation test

Low dose dexamethasone suppression test

Combination of ACTH stim test + LDDS test

Baseline cortisol concentration – Not recommended.

Measurement of glucocorticoid-induced isoform of ALP – Not recommended.

Differentiating tests

High dose dexamethasone test

Endogenous ACTH+ adrenal US

Diagnostic imaging

SCREENING TESTS

URINE CORTISOL:CREATININE RATIO

Rationale – Dogs with hyperadrenocorticism have elevated urine cortisol levels relative to normal dogs. Measurement of urine cortisol (indexed to urine creatine) is technically easy, is non-invasive, and can be a reasonable screening test when used appropriately.

Sensitivity: 75 – 100%

Specificity: LOW (20-25%); two studies reported >75%

Methodological differences between various studies can dramatically alter the diagnostic performance of the test. For example, the cut-off point used to distinguish between HAC and non-HAC significantly affects the test’s sensitivity and specificity. As a rule of thumb, as the cut-off value is lowered, sensitivity increases and specificity falls and vice versa when the cut-off value is increased.

Clinical use - In general, the UCCR test has a high sensitivity, so a normal UCCR value virtually eliminates the possibility of HAC. That is, the test can rule out HAC. However, the sensitivity is not 100% (and was as low as 75% in one study), so a normal UCCR in the face of a high suspicion for HAC should be followed up using another screening test. The specificity is poor overall, so a positive test result must be confirmed using a screening test with greater specificity.

Example 1. Use of a high cut-off value can enhance test specificity.


Compared UCCR in dogs with HAC and ill dogs without HAC. The calculated sensitivity of the UCCR test was 100%, the specificity was 85%. The cut-off value used to distinguish HAC and non-HAC was high.
Specificity was high because the UCCR is dogs with HAC above the cut-off had. In this case, sensitivity was also high probably because, the control group (Dogs without HAC) had only mild illnesses – i.e. less likely to have a significant stress response which could increase UCCR and interfere with the test’s discriminatory value.

Example 2. Characteristics of the patients tested are important when considering test performance.


The UCCR was compared for normal dogs, dogs with HAC, dogs suspected of having HAC, and dogs with severe non-adrenal illness. Normal and suspect HAC dogs had lower UCCR than HAC dogs, but there was no difference between the UCCR from dogs with HAC and ill dogs without HAC. The calculated sensitivity was 92% and 97% for normal and HAC dogs but 92% and 21% for HAC and ill dogs. This study’s use of a low cut-off value (especially a value higher than the mean value for normal dogs) increased sensitivity but lowered specificity because of the influence of factors (e.g. illness) that significantly enhance cortisol production produces a large overlap in UCCR values and a poor ability to discriminate between HAC and ill dogs.

LOW DOSE DEXAMETHASONE SUPPRESSION TEST

Rationale – The normal hypothalamic-pituitary-adrenal axis is exquisitely sensitive to feedback inhibition by cortisol. This test uses a low dose of glucocorticoid to test the responsiveness of the HPA. Dexamethasone should suppress cortisol secretion in normal dogs while cortisol secretion will not be suppressed in dogs with HAC

Sensitivity: Individual studies report 85 – 100%. Overall sensitivity is about 95% (combined data from various studies).

Specificity: ~ 70% (as low as 44% in one study)

Study population is important again and, like the UCCR, specificity falls when the test is performed on dogs affected with severe non-adrenal illness. Sampling time can also influence sensitivity and specificity; the above values were based on 8-hr post DEX results.

Clinical Use – The LDDS test does not distinguish the etiology of HPA activation in dogs with HAC. Recent studies have clearly shown that inappropriate use of the LDDS test will result in inaccurate results, especially when animals with moderate to severe non-adrenal illnesses are tested. It is not the test’s sensitivity but its specificity that plagues the interpretation of the LDDS test. Even so, specificity with the LDDS test is generally higher than UCCR even when testing ill dogs because it is the responsiveness of the pituitary-adrenal axis that is tested, unlike the UCCR, which is simply related to cortisol production. Pituitary responsiveness should remain under most circumstances. However, chronic, non-adrenal illness appears to be associated with refractory pituitary responsiveness as evidenced by failure to reduce ACTH release in response to DEX.

Example 3. Illustration of the effect of prevalence on predictive values.


The sensitivity of the LDDS test was 96% and the specificity was around 75% in this study. Normal dogs were not evaluated, but the study population included dogs with non-adrenal illness. Data from this study can be used to illustrate the importance of testing only dogs that are likely to have HAC. The prevalence of HAC in this study was about 50%, which is very high relative to the general population of dogs but may adequately represent the population of dogs typically tested for HAC (in other words, when we decided to test there is a 50:50 chance that the dog has HAC). The predictive value of a positive or negative test (i.e. how likely is the result to be correct) is greatly affected by disease prevalence. Thus, in the study population, the positive predictive value of the LDDS is estimated at 79% and the negative predictive value is estimated to be 6%. In real-life terms, this means that a positive test will be correct only 79% of the time (21% of dogs with a positive test will in fact not have HAC) and that a negative test will be correct only 6% of the time (94% of dogs that test negative for HAC will in fact actually have the disease). Compare these values to a population with a prevalence of 1% (which may reflect the general population of dogs), given the same test sensitivity and specificity. The positive predictive value is
ACTH STIMULATION TEST

Rationale: ACTH is the major physiological stimulator of the adrenal cortex. This test uses stimulation with exogenous ACTH to get an estimate of functional adrenal mass. The amount of cortisol released in response to maximal ACTH stimulation will parallel the adrenal mass and will be greater in dogs with HAC when compared to dogs without HAC. Clinically, the ACTH STIM test suffers from some of the same drawbacks as the LDDS test, such as an inability to distinguish dogs with HAC from those with severe non-adrenal illness.

Sensitivity: 73 – 95% in dogs with PDH and AT. Sensitivity is about 80%, if all studies are combined (looked at PDH + AT or PDH or AT alone). If dogs with PDH and AT are examined separately, the sensitivity is 87% and 61%, respectively.

Clinical Use: There may be several explanations why the sensitivity of this test is less than might be anticipated. First, a minimum degree of adrenal hyperplasia may be needed before the ACTH stim test is abnormal. Second, the presence of symptoms may not always correlate to sufficient adrenal mass to make the ACTH stim test abnormal. Dogs with adrenal tumors may have reduced adrenal mass (atrophy of non-neoplastic gland) or reduced ACTH responsiveness. The ACTH stimulation test cannot be used to distinguish between PDH and AT because results from dogs with these diseases have significant overlap.

COMBINATION DEXAMETHASONE SUPPRESSION/ACTH STIMULATION TEST

Sensitivity varies from 76 – 93%. The use of the test and its interpretation is somewhat controversial. The sensitivity of the test will vary depending on whether the interpretation encompasses the results from both tests versus each test separately. Sensitivity will be low when a positive result is only assigned when there is suppression by DEX and hyperresponsiveness to ACTH. However, sensitivity will be increased if the ACTH portion of the test is considered alone, with the same interpretation as the stand-alone test.

DIFFERENTIATION TESTS

HIGH DOSE DEXAMETHASONE SUPPRESSION TEST

Rationale: The adrenal response (cortisol production) to high doses of glucocorticoids can, in theory, distinguish pituitary-dependent HAC from adrenal-dependent HAC. The basis for the test is that the HPA axis, while abnormal, can still function. The rationale is that high levels of dexamethasone will suppress overproduction of ACTH in pituitary dependent disease and in turn suppress adrenal cortisol production but that dexamethasone will be unable to affect cortisol production when autonomous adrenal hyperfunction (e.g. from an adrenal tumor) is present. A condition characterized low circulating endogenous ACTH.

Clinical Use: The purpose of the HDDS test is to discriminate pituitary-dependent from adrenal-dependent hyperadrenocorticism, not to distinguish normal dogs from dogs with HAC. Thus, when you perform a HDDS test you have already made a diagnosis of HAC and a screening test (preferably the LDDS test or ACTH stimulation test) should have been positive prior to the HDDS test. However, sensitivity for diagnosis of PDH may be as low as 70%. This can be due to the site of the pituitary tumor–pars intermedia tumors are not be subject to cortisol feedback – or the tumor may lack the receptors or cellular pathways needed for feedback mechanism. Estimates of the specificity of this test are not available, but are likely to be high. Only rare AT will suppress in response to the HDDS test and even then, suppression is not complete.

ENDOGENOUS ACTH

Rationale: This test should have clear advantages over the others. Cortisol secretion is driven by persistently elevated ACTH in dogs with pituitary-dependent HAC. In contrast, adrenal hypersecretion of cortisol results in suppressed ACTH production in dogs with adrenal dependent HAC. Thus, ACTH measurement should be an ideal test to distinguish these forms of the disease. The endogenous ACTH level should be high in dogs with pituitary dependent HAC and low in dogs with adrenal-dependent HAC.

Clinical Use: Measurement of endogenous ACTH has limited value as a screening test because some dogs with PDH have ACTH values in the reference range. However, measuring the endogenous ACTH level is useful, for distinguishing between PDH and AT. Used as a differentiation test, the ACTH level correctly diagnosed PDH or AT in 82% of dogs tested (aggregate data from multiple studies). When dogs with non-diagnostic (i.e. inconclusive) tests were re-tested, a correct diagnosis could be made in 96% of dogs.

Example 4. Combining tests can enhance diagnostic usefulness

This prospective study employed a single determination of endogenous ACTH combined with adrenal US to distinguish PDH from AT in 29 dogs with HAC. The sensitivity of the combined tests was 100% and the sensitivity was 95% for distinguishing PDH and AT.

SUMMARY

• HAC screening tests have a high sensitivity but perform poorly if applied without discretion. The positive predictive value can be increased by testing only those dogs that have a high pretest probability of having HAC.

• Avoid testing ill dogs or dogs with chronic non-adrenal diseases if possible. HAC screening tests are often abnormal in this population of dogs due to non-specific activation of the hypothalamic-pituitary-adrenal axis.

• Tests used to distinguish PDH from AT will let you down every time if your patient selection for the screening process has not been appropriate.