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SELECTING THE MOST APPROPRIATE TEST FOR HYPERADRENOCORTICISM

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Canine hyperadrenocorticism is notoriously difficult to diagnose. Many of the clinical features and clinicopathological abnormalities are shared by a variety of other disorders and of the numerous adrenal function tests recommended, no true gold standard exists. There is a wide variation in veterinary input, invasiveness, time and cost between the tests and each differs significantly with regard to diagnostic sensitivity, specificity and efficiency. Some of the tests are capable of differentiating the site of the lesion (pituitary versus adrenal) while others may be useful in monitoring the response to therapy. Interpretation of test results in individual patients requires knowledge of the characteristics of each test. However, whatever test is chosen, the diagnostic performance is significantly enhanced by increasing the prevalence of the disease in the population under test.

Selecting appropriate cases

Hyperadrenocorticism is a disease of middle-aged to older dogs of either sex. Small breed dogs appear predisposed to pituitary-dependent hyperadrenocorticism (PDH) while the likelihood of functional adrenal tumours (AT) increases in larger dogs (>20 kg). Numerous clinical signs are possible but most commonly include polyuria/polydipsia, polyphagia, abdominal distension and a variety of dermatological abnormalities. Pathognomonic features such as calcinos is cutis, are rare, and as likely in iatrogenic as in naturally occurring hyperadrenocorticism. Routine clinicopathological abnormalities include mild erythrocytosis, a stress leucogram, dilute urine, increased liver enzymes (with a disproportionate increase in alkaline phosphatase), hypercholesterolaemia, hypertriglyceridaemia and increased bile acids. Although these abnormalities are not specific for hyperadrenocorticism, almost all cushingoid dogs have at least one abnormality. At UCD, the measurement of alanine aminotransferase concentration is the most diagnostically efficient single routine clinicopathological parameter for hyperadrenocorticism but is more useful in ruling out hyperadrenocorticism than diagnosing the disorder.

Adrenocorticotropic hormone response test

The adenocorticotropic hormone (ACTH) response test is generally used to screen for the presence of hyperadrenocorticism. It can distinguish between iatrogenic and spontaneous hyperadrenocorticism but does not reliably distinguish between PDH and functional AT. It is useful in the monitoring of adrenocortico lytic therapy.

It is a simple and quick test easily performed in the practice environment. The standard protocol is measurement of circulating cortisol concentration before and 1-hour after a single intramuscular or intravenous injection of synthetic ACTH (tetracosactrin, cosyntropin) at 250 µg/dog, although doses as low as 1.0 µg/kg can be used. Dogs with hyperadrenocorticism theoretically have an exaggerated response to ACTH. The absolute post-ACTH cortisol concentration is most frequently used to assess the response during an ACTH stimulation test. Although values above the reference range are frequently cited as abnormal, most clinicians use a value that significantly exceeds this
range, often between 600 and 650 nmol/l. Approximately 80 % of dogs with PDH have an exaggerated cortisol response to ACTH while only approximately 60 % of dogs with AT have such results. Despite the low sensitivity of this test, an advantage is that it is highly specific (approximately 0.9). The likelihood of an abnormal result in a non-cushionoid dog generally increases the more severely or chronically ill the animal is. Occasionally, dogs with AT have a subnormal cortisol response to exogenous ACTH.

In conclusion, an abnormal cortisol response in a dog with suspicious clinical and clinicopathological features is supportive of hyperadrenocorticism but provides no information on the site of the lesion. Although abnormal results can occur particularly in an animal known to have concurrent non-adrenal illness (e.g. unstable diabetes mellitus), a more common diagnostic dilemma is finding a reference range (or rarely subnormal) cortisol response in a highly suspicious case. Decreasing the post ACTH cortisol cut-off point (e.g. to approximately 500 nmol/l) for hyperadrenocorticism improves sensitivity with minimal effect on specificity and helps improve the diagnostic performance of this test. Alternatively, a more sensitive test should be considered in these suspicious cases.

**Low-dose dexamethasone suppression test**

In healthy dogs, glucocorticoids exert negative feedback inhibition on ACTH secretion. A low-dose of dexamethasone (0.01 – 0.015 mg/kg) administered intravenously to healthy dogs, causes inhibition of ACTH secretion and reduced plasma cortisol concentrations within 2 to 3 hours lasting up to 8 hours. In hyperadrenocorticism, the 8-hour cortisol value is not sufficiently suppressed and remains above approximately 30 to 40 nmol/l. The low-dose dexamethasone suppression test is considered an extremely sensitive diagnostic test. In approximately 95 % of dogs with PDH and up to 100 % of dogs with AT, cortisol suppression is inadequate. However, some dogs with hyperadrenocorticism have suppressed values at 8 hours and suppressed or elevated (inverse results) values at 3 or 4 hours. Unfortunately, false-positive test results frequently occur in dogs with non-adrenal disease and the specificity of the test has been reported to be as low as 0.44. In general, the more severe the non-adrenal illness, the more likely that cortisol suppression will not occur. The high negative predictive value of this test means that hyperadrenocorticism is unlikely if cortisol suppression occurs, but in some individual cases this does hold true.

An additional value in performing a low dose dexamethasone suppression test is its ability to distinguish between PDH and AT in up to 60 % of cases when circulating cortisol concentrations are measured at 3 or 4 and at 8 hours. Criteria that indicate a diagnosis of PDH include a decrease of circulating cortisol concentration to less than a laboratory reference value at 3 or 4 hours, less than 50 % of the baseline value at 3 or 4 hours, or less than 50 % of the baseline value at 8 hours. However, PDH cannot be ruled out if suppression does not occur.

**Urinary cortisol:creatinine ratio**

The determination of the cortisol (corticoid):creatinine ratio (UCCR) in urine samples taken in the morning can be used in the investigation of hyperadrenocorticism in dogs. It is the least stressful of all the diagnostic tests as owners can obtain samples in the home environment. It is also extremely sensitive with a reported range of 0.75 to 1.0. However, it lacks specificity with values as low as 0.21 especially if the animal is stressed or concurrent moderate to severe non-adrenal illness is present. Overall, its high negative predictive value suggests that hyperadrenocorticism is unlikely if the UCCR is within the
reference range but that further investigation for hyperadrenocorticism is warranted if it is elevated. Extremely elevated UCCR values occur almost exclusively in PDH. Suppression to greater than 50% of baseline following oral administration of three doses of dexamethasone (0.1 mg/kg) is similarly consistent with PDH. Overall, it is considered an inappropriate test for accurate monitoring of adrenocorticolytic therapy.

**Measurement of 17α-hydroxyprogesterone concentrations**

Within the adrenal gland, 17-α-hydroxylase catalyses the conversion of pregnenolone to 17α-hydroxyprogesterone (17-OHP). It is ACTH responsive and 17-OHP is further metabolised by 21-hydroxylase and 11-β-hydroxylase to produce 11-deoxycortisol and cortisol, respectively.

Its measurement has also proven useful in the investigation of dogs with AT that have a subnormal cortisol response to exogenous ACTH. In these individual (and rare cases), the adrenal gland presumably retains the ability to respond to ACTH but the cortisol production pathway is not intact.

Recent research has suggested that measurement of 17-OHP is also useful particularly in dogs with clinical and clinicopathological signs suggestive of hyperadrenocorticism that do not exhibit classical results using traditional tests, although this is unusual. However, because of the overlap in test results between healthy and sick dogs and those with hyperadrenocorticism, it cannot be advocated as a routine screening test for hyperadrenocorticism. A variety of other sex hormones can potentially be measured during an ACTH response test but the interindividual variation in results limits their value in investigating hyperadrenocorticism.

**Plasma adrenocorticotropic hormone concentration**

Measurement of circulating ACTH concentration is an excellent test to discriminate between PDH and AT but has no role to play in the diagnosis of the condition. Dogs with PDH release large amounts of ACTH but dogs with AT have reduced ACTH output. In dogs, inappropriately elevated plasma ACTH concentrations are consistent with PDH while low values are consistent with AT. Meticulous sample handling procedures (cold collection and immediate freezing) are necessary to avoid degradation and falsely low values. Aprotinin has a profound preservative effect upon canine plasma ACTH and it may be possible to submit unfrozen plasma samples to which aprotinin has been added.

**Diagnostic imaging**

Ultrasonography is the most widely available useful imaging method for assessing dogs with hyperadrenocorticism. However, like endogenous ACTH measurement, abdominal ultrasonography is most valuable in distinguishing PDH from functional AT. As a screening test it has limitations because of the overlap in adrenal gland size between healthy and sick dogs and those with PDH and because unilateral adrenal gland enlargement may represent incidental non-functioning adrenal tumours or those capable of producing other hormones. Co-existing PDH and AT has been described in a few cases of hyperadrenocorticism. In such cases, the results of the adrenal function tests and endogenous ACTH measurement are at variance with the ultrasonographic appearance of the adrenal glands.
Conclusions

Whilst hyperadrenocorticism is undoubtedly difficult to diagnosis, selecting appropriate cases significantly increases the diagnostic performance. It serves to minimise inappropriately diagnosing hyperadrenocorticism in dogs with non-adrenal illness and to have confidence to consider more diagnostic tests in animals highly suspicious of the disorder but with one negative adrenal function test result.

If finances allow, the selection of a highly specific test with one of high sensitivity maximises diagnostic performance. In our clinic, the ACTH response test and low dose dexamethasone suppression test are recommended in all suspicious patients. The simultaneous measurement of 17-OHP during the ACTH response test is not considered routinely but is reserved for investigation of animals with suspicious clinical signs and a subnormal response to ACTH administration. Measurement of endogenous ACTH concentration and diagnostic imaging are most useful in distinguishing between PDH and AT when a diagnosis of hyperadrenocorticism has been confirmed.

References


