Diagnostic Testing for Hyperadrenocorticism

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Hyperadrenocorticism is associated with excessive production or administration of glucocorticoids and is one of the most commonly diagnosed endocrinopathies in the dog. Hyperadrenocorticism is rare in the cat.

ENDOCRINE SCREENING TESTS

A presumptive diagnosis of hyperadrenocorticism can be made from clinical signs, physical examination, routine laboratory tests and diagnostic imaging findings, but the diagnosis must be confirmed by hormonal assay. A single resting or basal plasma or serum cortisol determination is of very limited diagnostic value because of the overlap in cortisol concentrations obtained from normal and abnormal disease states. Plasma or serum cortisol values are only useful after dynamic manipulation with ACTH or dexamethasone. The most commonly used screening tests are the ACTH stimulation test or the low-dose dexamethasone suppression test, however, the urinary corticoid: creatinine has also proved a useful screening test. None of these tests are perfect and all are capable of giving false negative and false positive test results.

If a dog with clinical signs compatible with hyperadrenocorticism produces a negative result with one screening test, an alternative screening test should be used. False positive results can be obtained in dogs suffering from non-adrenal disease (Kaplan et al., 1995). Thus, a definitive diagnosis of hyperadrenocorticism should never be made purely
on the basis of results of one or more of these screening tests, especially in dogs without classic signs of hyperadrenocorticism or in dogs with known non-adrenal disease.

The relative merits of each test and interpretation are discussed below. The author's preference is to use the ACTH stimulation test as the first screening test and the low-dose dexamethasone suppression test if the ACTH stimulation test gives a normal result in a dog with clinical signs suspicious of hyperadrenocorticism.

**ACTH stimulation test**

**Advantages**

The ACTH stimulation test is the best screening test for distinguishing spontaneous from iatrogenic hyperadrenocorticism. In spontaneous hyperadrenocorticism, the ACTH stimulation test reliably identifies more than 50 per cent of dogs with adrenal-dependent hyperadrenocorticism and about 85 per cent of dogs with pituitary-dependent hyperadrenocorticism.

It is a simple and quick test to perform and documents excessive production of glucocorticoids by the adrenal cortex in cases of hyperadrenocorticism. The information gained is also useful in providing baseline information for monitoring mitotane therapy, although different criteria are used to interpret cortisol results during treatment.

**Disadvantages**

The ACTH stimulation test does not reliably differentiate adrenal-dependent from pituitary-dependent hyperadrenocorticism. A diagnosis of hyperadrenocorticism should not by excluded on the basis of a normal ACTH response if the clinical signs are compatible with the disease. Occasionally, an animal under chronic stress may develop some degree of adrenal hyperplasia, which produces an abnormal ACTH response. This may be seen for example with diabetes...
mellitus or pyometra and a normal cortisol response to ACTH stimulation will be obtained after treatment of the underlying disease in these cases.

Interpretation

It is essential to use absolute values for pre- and post- ACTH plasma cortisol concentrations rather than a ratio or percentage increase in post-ACTH cortisol concentration over the basal concentration. In normal dogs, pre ACTH cortisol concentrations are usually between 20 and 250 nmol/l with post ACTH cortisol concentrations between 200 to 450 nmol/l. Regardless of the pre-ACTH cortisol value, a diagnosis of hyperadrenocorticism can be confirmed by demonstrating a post-ACTH cortisol concentration greater than 600 nmol/l in dogs with clinical signs compatible the disease.

**Low-dose Dexamethasone Suppression Test**

**Advantages**

The low-dose dexamethasone suppression test is more reliable than the ACTH stimulation test in confirming hyperadrenocorticism, since the results are diagnostic in all adrenal-dependent cases and in 90 to 95 per cent of dogs with pituitary-dependent hyperadrenocorticism.

**Disadvantages**

The low-dose dexamethasone suppression test is not as accurate as the ACTH stimulation test for the detection of iatrogenic hyperadrenocorticism. The test is also affected by more variables than the ACTH stimulation test, takes 8 hours to complete and does not provide pre-treatment information that may used in monitoring the effects of mitotane therapy. The low-dose dexamethasone suppression test does not reliably differentiate pituitary-dependent from adrenal-dependent hyperadrenocorticism.

Interpretation
Interpretation of the results of a low-dose dexamethasone suppression test must be based on the laboratory’s normal range for the dose and preparation of dexamethasone administered. If the dose of dexamethasone fails to adequately suppress circulating cortisol concentrations in a dog with compatible clinical signs, a diagnosis of hyperadrenocorticism is confirmed. While basal and 8-hour post-dexamethasone samples are most important for interpretation of the test, one or more samples taken at intermediate times (for example, 2, 4, or 6 hours) during the test period may also prove helpful. If a plasma cortisol concentration determined two to six hours after dexamethasone injection is suppressed normally or near-normally (to below 40 mmol/l), while the 8-hour sample shows escape from cortisol suppression, then a diagnosis of pituitary-dependent hyperadrenocorticism is indicated (Peterson, 1984).

**Urinary corticoid: creatinine ratio**

Evaluation of urinary corticoid: creatinine ratio rather than the more laborious 24-hour urinary corticoid excretion has been shown to be a simple and valuable screening test (Rijnberk et al., 1988).

Cortisol and its metabolites are excreted in urine. By measuring urine cortisol in the morning sample, the concentration will reflect cortisol release over a period over several hours, thereby adjusting for fluctuations in plasma cortisol concentrations. The concentration of cortisol in urine increases with increased plasma concentrations. Relating the urine cortisol concentration to urine creatinine concentration provides a correction for any differences in urine concentration.

Urine is collected in the morning for cortisol and creatinine estimations. It is preferable for the dog to be at home for this test so that the dog is subjected to as little stress as possible otherwise abnormal cortisol concentrations will be found in the urine. The urine corticoid: creatinine ratio is determined by dividing the urine cortisol concentration (in µmol/l) by the urine creatinine concentration (in µmol/l).
Interpretation

Reference ratio for normal dogs is less than 10 x 10^-6 (Stolp et al., 1983). The urine cortisol: creatinine ratio is increased above the normal (> 10 x 10^-6) in dogs with hyperadrenocorticism. However the ratio is also increased in many dogs with non-adrenal illness (Smiley and Peterson 1993). Therefore while this simple test appears highly sensitive in detecting hyperadrenocorticism in dogs, it is not that specific. The test does provide a good screening test for hyperadrenocorticism and values in the normal range make a diagnosis of hyperadrenocorticism highly unlikely. The urine cortisol: creatinine ratio does not reliably differentiate pituitary-dependent from adrenal-dependent hyperadrenocorticism unless the ratio exceeds 100 x 10^-6, when it becomes very likely that the dog is suffering from pituitary-dependent hyperadrenocorticism (Galac et al., 1997). The test is of little value, however, in monitoring the response to mitotane therapy in dogs with hyperadrenocorticism (Guptill et al., 1997).

Serum 17-hydroxyprogesterone

The measurement of precursors of cortisol, particularly 17-hydroxyprogesterone (OHP), has been shown to be of value in dogs and cats with clinical signs consistent with hyperadrenocorticism, supporting haematological and biochemical findings, yet are negative on the ACTH stimulation test and/or low-dose suppression test (Ristic et al., 2002). The serum OHP concentration increases excessively following ACTH stimulation in animals with hyperadrenocorticism, whether pituitary- or adrenal-dependent. The test is usually performed on samples taken for a routine ACTH stimulation test, when the cortisol concentrations have not produced a definitive diagnosis. OHP concentrations are affected by non-adrenal illness and therefore it is important that there is an adequate index of suspicion of hyperadrenocorticism before requesting this test.

Endocrine Tests To Differentiate the Cause of Hyperadrenocorticism
The ability to differentiate between pituitary- and adrenal-dependent hyperadrenocorticism can have important implications in providing the most effective method of management for the disease. An accurate test is therefore required to differentiate pituitary from adrenal causes of hyperadrenocorticism. The high-dose dexamethasone suppression test was the most commonly used test for differentiating the cause of hyperadrenocorticism, but its accuracy has recently been brought into question. Canine ACTH assays are now readily available and the determination of the plasma ACTH concentration has been shown to provide reliable discrimination between pituitary and adrenal causes of hyperadrenocorticism. Diagnostic imaging techniques, particularly abdominal ultrasonography, have also proved sensitive in distinguishing dogs with pituitary-dependent hyperadrenocorticism from dogs with adrenocortical tumours. Recognition of metastatic lesions with radiography and/or ultrasonography, however, is the only method that can distinguish dogs with adenomas from dogs with carcinomas (Reusch and Feldman, 1991).

**High-dose dexamethasone suppression test**

This test is indicated in those cases where the diagnosis of hyperadrenocorticism has been established by a screening test, but the differentiation of adrenal-dependent and pituitary-dependent hyperadrenocorticism has not been determined. The high dose of dexamethasone inhibits pituitary ACTH secretion through negative feedback in pituitary-dependent hyperadrenocorticism thus suppressing of cortisol concentrations. Adrenocortical tumours are autonomous and thus cortisol is not suppressed. However, it has been shown that approximately 20 to 30 per cent of pituitary-dependent cases will not suppress with this test making it poor discriminatory test. The high-dose dexamethasone suppression test does not differentiate adrenocortical adenomas from adrenocortical carcinomas.

**Plasma endogenous ACTH concentration**
Stringent and meticulous sample handling is crucial since ACTH activity in the plasma will reduce rapidly resulting in falsely low values and incorrect interpretation. The endogenous ACTH assay used must be validated for use in dogs. Measurement of basal endogenous ACTH concentrations is of no value in the diagnosis of hyperadrenocorticism because of the episodic secretion of ACTH in the normal dog and the overlapping values with those dogs with hyperadrenocorticism. Interpretation-Endogenous ACTH concentrations in normal dogs range from 13 to 46 pg/ml. Dogs with adrenal tumours have very low endogenous ACTH concentrations (< 5 pg/ml) whereas cases with pituitary-dependant hyperadrenocorticism tend to have high-normal to high concentrations (> 28 pg/ml) (Gould et al., 2001).

**Diagnostic imaging**

Abdominal radiography, abdominal ultrasonography, and abdominal and cranial computed tomography (CT) and magnetic resonance imaging (MRI) can be used to differentiate pituitary-dependant hyperadrenocorticism and adrenal-dependent hyperadrenocorticism. Adrenal ultrasonography is an accurate means of differentiating between pituitary and adrenal causes of hyperadrenocorticism. Finding both adrenal glands have a similar size and are of normal shape suggests pituitary-dependent hyperadrenocorticism, whereas a mass in the adrenal area suggests adrenal-dependent hyperadrenocorticism in a dog with confirmed hyperadrenocorticism.

CT and MRI have proved useful in the diagnosis of adrenal tumours, adrenal hyperplasia and large pituitary tumours, but both techniques are expensive to perform and are not widely available (Voorhout et al., 1988; Bertoy et al., 1995; Duesberg et al., 1995). In a study comparing abdominal survey radiography with CT for the detection of adrenocortical tumours, CT accurately localised all tumours whereas abdominal radiography only accurately localised 55 per cent of the cases (Voorhout et al., 1990). This was due to the fact that tumours less than 20 mm in diameter could not be seen on abdominal radiographs. CT can
also identify invasion of the caudal vena cava by the tumour and adhesions between the adrenal gland and the caudal vena cava.

Although no comparative studies have been carried out in dogs, magnetic resonance imaging has been found to be superior to CT in detecting ACTH-secreting tumours of the pituitary gland in human beings. MRI is extremely sensitive and can detect pituitary tumours as small as 3 mm in dogs and cats (Bertoy et al., 1995). Large pituitary tumours (up to 12 mm in diameter) have been shown to be present without causing neurological signs, whereas in another study pituitary masses ranging in size from 8 to 24 mm were associated with neurological signs (Duesberg et al., 1995). In those cases with neurological signs, MRI or CT examination of the brain is essential for accurate planning if pituitary irradiation is to be considered.

Diagnostic imaging can also be used to distinguish between benign and malignant tumours of the adrenal cortex. Malignant tumours may invade the caudal vena cava and spread to the liver and lungs.