

Pitfalls in the Investigation of Cushing's Syndrome



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Cushing's syndrome is classically associated with a number of clinical findings, including central obesity, a "moon face" appearance, and muscle wasting, resulting from hypercortisolism. There is a 5:1 female preponderance. Problems are encountered not only with diagnosis, but also in determining the etiology. Many pitfalls exist at each level of investigation.

Several disorders have clinical and laboratory features overlapping with those of Cushing's syndrome. These include obesity, polycystic ovarian syndrome (PCOS), and the metabolic syndrome. There are also several situations where the hypothalamic-pituitary-adrenal axis is hyperactive, and in these situations patients can have features and laboratory findings of Cushing's syndrome. These include chronic alcoholism, anxiety, and depression, and this can be classified as "pseudo-Cushing's syndrome." Few symptoms are specific to Cushing's; although, skin atrophy, violaceous striae, and muscle weakness, all features of a catabolic state, are not seen in PCOS or the metabolic syndrome.¹ Episodic Cushing's, where hypercortisol states occur in a relapsing-remitting

pattern, further complicates diagnosis, and this condition is thought to be more common than previously assumed.²

Establishing a Diagnosis of Cushing's Syndrome

Urinary Free Cortisol

The most important initial step is to establish the diagnosis of Cushing's syndrome. An effective starting point is to collect three urine collections measuring urinary free cortisol (UFC) and creatinine.

If all three urinary free cortisol values are greater than three times the upper limit of normal, Cushing's syndrome is very likely. Pitfalls in this situation include false positives due to pseudo-Cushing's syndrome or drug interactions interfering with the assay.

If all three urinary free cortisol measurements are below the upper limit of normal, Cushing's syndrome is very unlikely. However, episodic Cushing's may give a false negative result if the urines are collected during a quiescent phase.

The use of inhaled corticosteroids or steroid creams can cause adrenal suppression, resulting in decreased cortisol secretion and low urinary cortisol. The presence of abnormal glucocorticoid receptors can result in glucocorticoid hypersensitivity, resulting in manifestations of cortisol excess with normal cortisol levels.

In the final scenario, urinary free cortisols may be above the upper limit of normal but less than three times normal. In this case, Cushing's syndrome is a possibility, and further diagnostic testing is required, because obesity, pseudo-Cushing's, the metabolic syndrome, and PCOS can also give such values.

Dexamethasone Suppression Testing

A further diagnostic test to establish the diagnosis of Cushing's syndrome is a 1 mg overnight dexamethasone suppression test. A decrease in serum cortisol the following morning to less than 50 nmol/l tends to rule out Cushing's syndrome. Pitfalls for this test include possible misinterpretation due to increased corticosteroid binding globulin (CBG), such as in those on the birth control pill or in early pregnancy. Increased hepatic dexamethasone metabolism resulting from medications, such as antiepileptic medication, decreases serum dexamethasone so that it does not suppress cortisol secretion. Episodic Cushing's can also give UFC levels in any range. This test has a high sensitivity and specificity but it is less than 100%.³

A two day dexamethasone suppression test, giving 0.5 mg every 6 hours, can also be performed. In normal subjects, the UFC should suppress to less than 37 nmol per 24 hours, and the serum cortisol on the morning after the last dose of dexamethasone should be less than 50 nmol/l. However, some patients with mild Cushing's

syndrome will suppress, and some patients with pseudo-Cushing's will not suppress.

A corticotropin-releasing hormone (CRH) injection (100 ug) can be given after a 48-hour low dose dexamethasone test. A serum cortisol after 15 min of > 35nmol/l suggests Cushing's, and a cortisol of < 35nmol/l suggests pseudo-Cushing's. This test was initially thought to be useful for distinguishing pseudo-Cushing's from true Cushing's but recent results have shown that it does not add much diagnostic value beyond the simpler dexamethasone suppression test.⁴

Late night Cortisol measurement

The diurnal variation in cortisol levels that is a part of normal physiology is often lost in Cushing's syndrome, and this feature is used for two diagnostic tests. The first is sleeping serum midnight cortisol measurement. This is effective in differentiating pseudo and true Cushing's.⁵ In Cushing's syndrome, the value should be > 50 nmol/l, while in pseudo-Cushing's syndrome it should be < 50 nmol/l. Unfortunately, this test is impractical for use in Canada, because it requires overnight hospital admission and a blood test at a very specific time with the patient asleep or having just recently awakened.

The second test measures late night salivary cortisol.⁶ Cortisol in saliva is unbound and, thus, unaffected by CBG levels. A high salivary cortisol at midnight is highly specific for Cushing's syndrome. Unfortunately, this test has not been standardized, but it makes it possible to make serial measurements at home, and it can be used to diagnose and differentiate episodic Cushing's. This test is not widely available currently due to the handling requirements of the specimen, but it should become more accessible in the near future.⁷

ACTH Dependant vs. ACTH Independent Causes

Once the diagnosis of Cushing's syndrome has been established, the next step is to determine whether the cause is adrenocorticotrophic hormone (ACTH) dependent or independent. Serum ACTH measurements, along with serum cortisol, are taken on several morning samples. A pitfall of ACTH measurement is the need for a cold tube and refrigerated centrifuge. This may not be available in some sites where blood is taken, so it is important to check with the lab before the patient is sent.

ACTH Independent Cushing's Syndrome

If ACTH levels are found to be suppressed, the probable source of the excess cortisol is the adrenal gland. This unregulated cortisol production can come from a solitary adrenal nodule, from bilateral adrenal macronodular hyperplasia, from a rare adrenal cause of cortisol excess, from micronodular pigmented adrenal hyperplasia, or from adrenocortical carcinoma.

An adrenal CT scan can assess the size of the adrenals and detect abnormal growth. Bilateral diffuse enlargement would be suggestive of ACTH stimulation, but micronodular pigmented hyperplasia can present with bilateral diffuse enlargement. Macronodular hyperplasia presents with bilateral adrenal enlargement with nodules of varying size, which autonomously secrete cortisol. Macronodular hyperplasia is unlikely to be malignant. Adrenal nodules of low density and less than 4 cm are rarely malignant. Nodules 6 cm or greater with a higher density have a high incidence of malignancy.

Adrenal vein catheter studies can be performed to assess the relative levels of cortisol secretion from both adrenals. A pitfall of adrenal vein catheter studies is that the adrenal veins are difficult to catheterize, and an expert radiologist is required to catheterize both adrenals so that simultaneous blood samples can be taken from each adrenal.

ACTH Dependent Cushing's Syndrome, Pituitary vs. Ectopic Etiology

When a patient is found to have both elevated cortisol and elevated ACTH, the Cushing's syndrome is ACTH dependant. The next step is to determine whether the etiology is pituitary or ectopic in origin.

Pituitary adenomas comprise at least eighty percent of ACTH dependent etiologies. They are associated with a progressive onset, moderate levels of ACTH, and high UFC levels.

An ectopic source of ACTH is suspected when ACTH levels are very high, UFC levels are very high, and serum potassium levels are low. The low potassium is due to the inability of the kidney to protect the mineralocorticoid receptors from the very high levels of cortisol. Some ectopic tumours secrete altered forms of ACTH, which are biologically active but do not react effectively with the antibodies used in the ACTH assay so the levels do not appear high on the assay. Ectopic tumours can also secrete a variety of other peptides, including gastrin, calcitonin, and glucagon. Approximately 50% of ectopic tumours are in the lung (either carcinoid or small cell lung tumours). Other sites include the thymus, pancreas, medullary thyroid, and adrenal gland.


The investigation of ACTH dependant Cushing's syndrome starts with a pituitary MRI with gadolinium enhancement. If an adenoma greater than 6 mm is found, this is most likely the source of the ACTH. ACTH secreting tumours can be small, and, since the incidence of nonfunctioning incidental adenomas in the general population is in excess of 10%, additional studies are required to determine if the adenoma is ACTH secreting.

A high dose dexamethasone suppression test is used to differentiate between a pituitary and an ectopic source. A single 8 mg dose is given at 11pm with cortisol measured the morning before and the morning after the dose. With pituitary adenomas, cortisol levels should fall by greater than 50%. This test is quite specific but only about 70% sensitive. A corticotrophin-releasing hormone (CRH) stimulation test with subsequent ACTH and cortisol measurements can be conducted on the premise that pituitary adenomas will respond to CRH, while ectopic tumours will not. Some pituitary tumours will not respond to CRH, and this test has not been standardized, as both ovine and human CRH are used and do not have the same half-life.

In the literature, the most specific test to differentiate between pituitary and ectopic sources is petrosal sinus blood sampling for ACTH. In this test, catheters are inserted into the femoral veins and directed into the petrosal sinuses draining blood from the pituitary. CRH is injected into a peripheral vein, and blood is then sampled from both the petrosal sinuses and the peripheral vein at the same time. A ratio of 3:1 petrosal versus peripheral ACTH indicates a pituitary source. Although this test is discussed in the literature as the best test for this purpose, in reality, there are a limited number of centres with the expertise and the facilities to perform this test. There are also some potential complications, so this test is reserved for cases where the diagnosis is uncertain, and it should only be done in centres with adequate experience.

If the source is determined to be ectopic, MRI and CT imaging of the chest and abdomen can be used to detect the source. In up to 20% of patients the source cannot be found and long-term follow-up is required.

Conclusion

In summary, 24-hour urinary free cortisol measurement, 1 mg dexamethasone suppression testing, and late night salivary cortisol measurement are the most efficient and practical initial diagnostic tools for Cushing's syndrome. One meta-analysis found these three tests to be similar and highly accurate.⁸ Further investigation involves determining ACTH dependent and independent etiologies, and if ACTH dependent, determining ectopic versus pituitary origins with further testing. In situations where there is a high clinical suspicion of Cushing's syndrome, but testing does not support the diagnosis, episodic cortisol secretion should be considered, and questioning regarding the use of glucocorticoid inhalers and skin creams should be reviewed. 

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