Cushing syndrome is an uncommon endocrine disorder with incidence of 2-3 cases per million of population per year in most countries. Its characteristic features result from the prolonged exposure of tissue to corticosteroids. The first step in its diagnosis rests on clinical suspicion followed by scrupulous history taking and careful physical examination. While lists of symptoms and signs may be found in many textbooks, most are common in patients without the diagnosis. The most distinctive and specific signs of Cushing syndrome (CS) include purple striae, proximal myopathy, easy bruising with thinned skin, facial plethora and unexplained osteoporosis. Many features such as central obesity, glucose intolerance or hypertension overlap with the metabolic syndrome. Depression, psychosis, menstrual irregularities, hirsutism, peripheral edema and fatigue are not unique to CS, and overlap with polycystic ovary syndrome and other diagnoses.

The most common cause of CS is the iatrogenic use of corticosteroids in different preparations: oral, topical, inhaled, intra-articular, nasal or in the form of eye drops. All should be suspected, and as they are usually related to potent non-cortisol preparations the circulating levels of cortisol will be low. When this is excluded, an endogenous source of hypercortisolism should be sought.

Hypercortisolism without the clinical features of CS occurs in pregnancy, anorexia nervosa, physical stress, strenuous exercise and with increased cortisol binding globulin (CBG) level (such as in pregnancy or on exogenous estrogens). Therefore, every test measuring serum total cortisol should be performed after stopping oral exogenous estrogens, oral contraceptive pills, hormone replacement therapy 4 to 6 weeks beforehand, although transdermal preparations may not cause these changes.

Adrenocorticotrophin (ACTH)-dependent CS/Cushing disease (CD) is most common and accounts for 80% to 85% of causes of CS, of which pituitary adenomas comprise 80% to 90% and the remaining 10% to 20% are secondary to ectopic ACTH secretion. In women, CD is 10-fold more prevalent than ectopic CS, while in men the ratio is 3:1. ACTH-independent CS is caused by an adrenal adenoma, carcinoma or (very rarely) by bilateral adrenal hyperplasia.
invited review

Biochemical confirmation of Cushing syndrome

According to the guidelines of the Endocrine Society, the recommended first line tests for confirming hypercortisolism include midnight salivary cortisol (2 tests required), the 1 mg overnight dexamethasone suppression test or the low-dose dexamethasone suppression test (LDDST), or a 24-hour urinary free cortisol (UFC) (at least 3 tests). However, in our opinion the UFC is only of value when the diagnosis is usually obvious and the disorder severe; in mild cases it is of very little discriminant value.

In the presence of suggestive clinical features and positive tests, it is recommended to refer the patient to an endocrinologist with experience in the diagnosis of the condition. If biochemical screening is negative and a high index of clinical suspicion remains, it is possible that there is cyclical CS. It is important to remain open to the diagnosis and repeat the investigations at a time when it appears to be clinically active.

Overnight dexamethasone suppression test

As higher than physiological doses of exogenous steroids should suppress ACTH and cortisol production in healthy individuals, dexamethasone is used in the diagnostic assessment of CS. In the overnight dexamethasone suppression test (ONDST), the patient takes 1 mg of dexamethasone between 23:00 and 00:00 (midnight), and serum cortisol is measured between 08:00 and 09:00 the following morning. If it is suppressed below 50 nmol/L, CS is extremely unlikely (at that point in time, thus not excluding cyclical cortisol secretion).

The diagnostic sensitivity of this test is >95%, but its specificity is rather low at 70% to 80%. However, the clinician should be aware that decreased absorption of dexamethasone, or medications which increase dexamethasone metabolism such as phenytoin, carbamazepine, pioglitazone, rifampicin or phenobarbitone, would give false positive results. The opposite applies to liver enzymes inhibitors (cimetidine, ciprofloxacin, fluoxetine or itraconazole). Measuring the dexamethasone level along with the cortisol level could be useful in cases suspected of malabsorption although the assay is expensive and may not be available, and normative values are required. Another option suggested is the administration of the dexamethasone intravenously at 5 mcg/kg/h over 5 hours, and measuring cortisol 4 hours after stopping the infusion, when the same cut-off value of less than 50 nmol/L has been used to exclude CS.

Low-dose dexamethasone suppression test

During a LDDST, 0.5mg of dexamethasone is administered orally every 6 hours for 48 hours from 09:00. The serum cortisol at 09:00 is measured at 24 and 48 hours. A cut-off value of 50 nmol/L is used to exclude CS. The LDDST is more cumbersome than the overnight test, but combines a similar sensitivity of 95% with a much higher specificity of 97% to 100%.

Midnight serum and salivary cortisol

A loss of the circadian rhythm of cortisol secretion is one of the most sensitive indicators of CS. Therefore, the midnight serum or salivary cortisol seems to be an attractive investigative choice. In our in-patient practice we use two midnight serum sleeping cortisol tests along with the LDDST to confirm hypercortisolism. A midnight serum sleeping cortisol has a high predictive value for diagnosing CS provided the patient is asleep, which is often difficult to achieve in a hospital environment, and the patient has been admitted for at least 48 hours. It is therefore of much greater use as a confirmatory or excluding test rather than as a screening procedure.

A late-night salivary cortisol (SC) correlates well with serum free cortisol; it is reproducible, stress-free and is particularly useful in diagnosing CS in children or when multiple tests are needed in cyclical CS. Saliva is collected by expectoration or using a salivette at home by the patient; activity seems not to be an important variable. Salivary cortisol is stable at room temperature for 7 days. The cut-off value depends on the assay used and suggested values for a radioimmunoassay (RIA) with expectoration range between 3.8-15 nmol/L, for an RIA with salivette 3.6-12 nmol/L, and 2.24.3 nmol/L for an enzyme-linked immunosorbent assay.

However, it is vital that the assay is calibrated for a given population and patient group. There was strong correlation reported between midnight serum cortisol and SC in patients with CS and in the normal population. The same was confirmed for UFC and SC in CS but not in the normal population and obese people. A meta-analysis by Carroll et al showed a pooled sensitivity of 92% and specificity of 96% for diagnosing CS.

The sensitivity increases further if two tests are combined; for example—night SC with the 1 mg DST, which has a sensitivity of 100% and a specificity of 95.5%. This approach seems to be very attractive for the pediatric population. SC may be particularly useful in early pregnancy or in women on oral contraceptive pills when this cannot be stopped. Using the SC as a diagnostic test is not recommended in mild CS, as is often the case in adrenal incidentalomas, when a combination of midnight serum cortisol and the 1 mg DST has been suggested.
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24-hour urinary free cortisol
Unbound cortisol accounts for 5% to 10% of total cortisol and is the physiologically active moiety. As it is unbound, it passes through the kidneys, but most of it is reabsorbed and thus only a small percentage excreted unchanged. In majority of assays used, the upper normal range of UFC ranges between 220 and 330 nmol per 24 hours. Up to 3 collections with normal results are required to exclude CS, and levels exceeding 4 times the upper normal range are highly suggestive of the condition. The sensitivity of UFC in the diagnosis of CS ranges between 95% and 100%, but it has low specificity (40%-50%) and even in CS one out of four collections could be within the normal range in 11% of patients.9

UFC measures unbound cortisol and therefore CBG levels do not affect it. On the other hand, it cannot be used in patients with chronic renal failure, and thus should be interpreted in the context of the creatinine clearance.16 A high fluid intake (>5L/24 hours) will increase UFC.17 Medications that falsely elevate UFC include carbamazepine, fenofibrate, carbenoxolone, some synthetic steroids and liquorice.3 As many of us increasingly see mild cases of CS, where the UFC is least helpful, we do not recommend its routine use.5

Differentiating Between Cushing Disease and Pseudo-Cushing Disease
Mild CS is often difficult to distinguish from pseudo-Cushing disease states such as alcoholism and severe depression, especially since depression could be a feature of CS itself. Hypercortisolism could be present as well in anxiety disorders, severe obesity and poorly controlled diabetes. Clinically, patients with pseudo-Cushing conditions, i.e., where there may be some clinical features of CS and some abnormal biochemistry, but where CS is not present, may be a difficult clinical problem. In patients with alcoholic pseudo-Cushing, the elevated cortisol level should return to the normal range after several days of alcohol abstinence, while the high levels of cortisol in severe depression (often with a maintained circadian rhythm) should improve with successful treatment of the depression. However, if treatment of the primary problem is not possible further investigations may be warranted. Pseudo-Cushing-related hypercortisolism often results in overlap in values with CS in terms of the 24h UFC, and may not suppress even on the LDDST.18 The corticotrophin-releasing hormone (CRH) stimulation test shows a blunted ACTH response in depression, but nevertheless overlaps with both CS and the ectopic ACTH syndrome.19

If initial biochemical evaluation does not differentiate between CS and pseudo-Cushing, it has been suggested that the combination of LDDST with the CRH test may be of some value. After 48 hours of dexamethasone 0.5 mg every 6 hours, 100 µg of CRH is administered intravenously and cortisol is measured after 15 minutes. In the study by Yanovski et al the values of cortisol above 38 nmol/L predicted CS with sensitivity and specificity of 100%.18 However, more recent studies have not supported the increased accuracy of this cumbersome test over a simple LDDST.

ACTH-Dependence
Once CS is confirmed, plasma ACTH should be measured. A sample for plasma ACTH needs to be spun immediately and frozen; otherwise the result could be falsely negative. At least two measurements should be performed: if ACTH is <5-10 ng/L CS is ACTH-independent; if it is more than 15-20 ng/L it is ACTH dependent and relevant images could be requested. ACTH levels between 5-15 ng/mL are less conclusive and we would recommend exploring the ACTH response to CRH if this occurs (see below).20

Appropriate Imaging in Cushing disease

Pituitary
After confirming the ACTH dependence of CS, an MRI of the pituitary gland should be performed. In the majority of patients, an ACTH-secreting pituitary microadenoma (<1 cm in maximal diameter) causes CD. An unenhanced scan detects a pituitary adenoma only in 50% of patients, but this improves to 50% to 60% on a gadolinium-enhanced scan, and may improve further with dynamic contrast scanning.21,22 However, since the mean diameter of these tumors is only 6 mm, and even less in children, many tumors may not be able to be imaged. It is also worth noting that pituitary lesions are found incidentally in 10% of the normal population.23 Typically, a pituitary microadenoma appears as hypodense to normal pituitary tissue, may cause pituitary stalk deviation, and in 95% of cases does not enhance after gadolinium administration. It may show mild enhancement in the remaining 5% of patients with pituitary adenomas, rendering the lesion isodense in comparison to the surrounding tissue.24 It has been reported that MRI of the pituitary gland is concordant with the intra-operative surgical findings in 75% to 98% of patients.9

For pituitary macroadenomas, which comprise about 5% of patients with CD, the planning of surgical treatment enabling cure, cavernous sinus involvement and the proximity of internal carotid arteries is very important. CT of the pituitary gland should be reserved
only for patients in whom MRI scanning is contraindicated: it is less accurate, identifying some 30% of tumors.

**Adrenals**

ACTH-independent CS may be related to an adrenal adenoma, adrenal carcinoma, bilateral multi-nodular adrenal hyperplasia or primary pigmented nodular adrenal dysplasia (PPNAD, which is associated with Carney complex). CT provides the best resolution for imaging of the adrenal gland, although MRI may provide additional functional information and avoids radiation. In the normal adrenal each limb measures on average of 5 mm, which is comparable to the thickness of the diaphragm, while the adrenal body measures around 1 cm. A heterogeneous adrenal mass bigger than 6 cm is highly suspicious of malignant behavior, which is more certain when vascular or capsular invasion and lymph node involvement are present. A study by Slattery et al analysing contrast wash-out on CT imaging of adrenal carcinomas revealed that all carcinomas behave like non-adenomas with delayed contrast clearance.

A benign cortisol secreting adrenal adenoma has similar features to non-functioning adenoma, with (usually) a high fat content shown by <10 Hounsfield units on CT imaging, or signal loss on out-of-phase sequences on MRI. In case of unilateral adrenal mass secreting cortisol, the contralateral adrenal gland should be atrophic, although this is hard to quantify. With bilateral ACTH-independent multi-nodular adrenal hyperplasia (AIMAH), the adrenals appear bulky with an irregular contour and/or with multiple nodules measuring 0.5-5.5 cm, which have the characteristics of lipid-rich masses. On MRI they are hypointense to liver on T1-weighted images and hyper- or isointense on T2. The adrenals may look normal or uni-/bilateral micronodular in PPNAD: the nodules are hypodense on T1 and T2-weighted images on MRI and are microscopically pigmented with an atrophy of surrounding cortex. This is generally a disease of the adolescent years.

**Ectopic lesions**

When there is suspicion of an ectopic source of ACTH, CT scanning of the chest with contrast should be arranged first, as about 50% of cases a bronchial carcinoid or small cell lung cancer is found. Identifying a bronchial carcinoid is most challenging, as it usually is a small lesion between 3-15 mm in diameter and difficult to distinguish from a granuloma or hamartoma. Very rarely, neuroendocrine tumors of the pancreas, intestine, thymus, medullary carcinoma of the thyroid, phaeochromocytoma or mesothelioma cause the ectopic ACTH secretion.

MRI scanning may help in visualizing small neuroendocrine tumors of the pancreas; Indium–labelled scintigraphy or fludeoxyglucose (18F) positron emission tomography (FDG PET) are not especially helpful in increasing the detection of neuroendocrine tumours over and above the CT and MRI findings. In 12% to 20% of ectopic ACTH-dependent CS no source of ACTH is found, and repeating imaging 6 to 12 months is recommended.

**ACTH-dependent disease: Cushing disease or an ectopic source?**

Patients with ectopic ACTH-dependant CS have significantly higher cortisol levels compared to CD. In a retrospective analysis of 245 patients with ACTH-dependent CS by Isidori et al, the mean (SD) baseline cortisol in CD was 629 (225) nmol/L, while in ectopic ACTH-dependent CS it was 1221 (864) nmol/L. In the LDDST, serum cortisol levels do not change significantly in ectopic CS but suppress by 30% in patients with CD, predicting CS secondary to ACTH secreting pituitary adenoma with a sensitivity of 82% and specificity of 79%. Almost 100% of patients with ectopic ACTH-dependent CS, present with hypokalemia, which is found only in about 10% of patients with CD. This is related to higher levels of cortisol, which saturate 11-β-hydroxysteroid dehydrogenase type 2. This enzyme is responsible for converting cortisol to cortisone and preventing its mineralocorticoid effect in the kidney.

**High dose dexamethasone suppression test**

In many endocrine centers, the high-dose dexamethasone suppression test (HDDST) follows the LDDST with 2 mg of dexamethasone every 6 hours for 48 hours, or with a single dose of 8 mg of dexamethasone: 80% of patients with pituitary-dependent CS suppress cortisol to less than 50% compared to the baseline level, but this is uncommon with an ectopic source. Interestingly, in study by Isidori et al there was no further significant suppression of cortisol from the 24 hour to 48 hour value on both LDDST and HDDST. However, as noted above, the suppression on the LDDST can be almost as valuable, and we have abandoned the use of the high-dose test in most instances.

**CRH stimulation test**

Corticotrophin-releasing hormone stimulates ACTH-
Secreting pituitary tumor cells as they usually express CRH-1 receptors, which stimulates cortisol release. This is rare in ectopic ACTH production. During the CRH test 100 µg or 1 µg/kg of ovine or human CRH is injected intravenously and ACTH and cortisol are measured at -15 and 0 minutes as a baseline then at 15, 30, 45, 60 and 90 minutes. The sensitivity of the test is similar with the use of ovine and human CRH. The absolute values are not helpful in differentiating CD from the ectopic CS: an increase of ACTH by 35% to 50% from the baseline level and cortisol by 20% is highly suggestive of a pituitary source of ACTH. However, in an analysis of several published series 7% to 14% of patients with histologically-proven CD failed to increase ACTH or cortisol in response to CRH.

**Bilateral inferior petrosal sinus sampling**

Bilateral inferior petrosal sinus sampling (BIPSS) re-
mains the gold standard diagnostic test for CD, and except for patients with pituitary macroadenoma we recommend its use in all patients with ACTH-dependent CS. The pituitary effluent drains via the cavernous sinus to the inferior petrosal sinuses; therefore, measuring a gradient of ACTH in the inferior petrosal sinuses (IPS) and peripheral blood, preferably with CRH stimulation, should confirm the diagnosis of CD as opposed to an ectopic source. This invasive technique requires a highly skilled interventional radiologist to achieve a high success rate, and therefore it should be performed only in specialised endocrine centers. In cyclical CS, it should be performed only during a period of hypercortisolemia. Venous catheters are placed in IPS bilaterally and the correct position is confirmed by venography or digital angiography; sampling of the cavernous sinuses is probably of no additional benefit, but measurement of prolactin with the ACTH may confirm catheter positioning. ACTH is measured simultaneously from bilateral IPS and a peripheral vein at 0, 3-5 and 8-10 minutes, ideally after stimulation with 100 µg of CRH intravenously (desmopressin is cheaper and has been used in some centers). An IPS to peripheral ACTH ratio of more than 2:1 on non-stimulated and more than 3:1 on CRH-stimulated sampling is consistent with a diagnosis of CD, with a combined sensitivity and specificity of 95% to 99%.35 Bilateral IPS sampling can also help with lateralization of a pituitary lesion only in around 72% of patients in adults, increasing to 83% with CRH stimulation.35 The predictive value of bilateral IPS sampling for lateralization is higher in children.36 A basal or stimulated ACTH gradient between IPS of more than 1.4 is suggestive of lateralization.34, 35 Venography at the time of sampling can help avoid false lateralization. This may relate to the shunting between both IPS.37 It should be noted, however, that not all authorities agree as to the value of this technique in lateralisation.

Conclusions and Management Algorithm
CS remains one of the most challenging and fascinating problems in clinical endocrinology, and it is very satisfying if correctly diagnosed and cured. Due to the complexity of investigations and the need for invasive procedures, it is of patient benefit to be referred early to a specialised endocrine center, when there is a high suspicion of CS. We recommend that outpatient midnight salivary cortisol or a variation on the dexamethasone suppression test be performed as a screening procedure, and then we admit the patient for a confirmatory midnight serum cortisol, a CRH test and imaging procedures (Figure 1). If the CRH and LDDST tests both suggest CD, and the MRI of the pituitary demonstrates a lesion, in some circumstances it might be appropriate to refer immediately for transsphenoidal surgery. However, we generally prefer to perform a bilateral IPS sampling study to increase our certainty.
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