Cushing's syndrome

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Cushing's syndrome results from lengthy and inappropriate exposure to excessive glucocorticoids. Untreated, it has significant morbidity and mortality. The syndrome remains a challenge to diagnose and manage. Here, we review the current understanding of pathogenesis, clinical features, diagnostic, and differential diagnostic approaches. We provide diagnostic algorithms and recommendations for management.

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Cushing's syndrome results from lengthy and inappropriate exposure to excessive concentrations of circulating free glucocorticoids. When presentation is florid, diagnosis is usually straightforward. However, this diagnosis is increasingly being considered when the phenotype is subtle, and in common disorders such as type 2 diabetes and obesity. Only once the diagnosis of Cushing's syndrome is established can the underlying cause be searched for. This investigation is frequently a complex process needing all the skill of doctors, endocrinologists, chemical pathologists, radiologists, and surgeons.[1–3] We review advances in the understanding of the biology of Cushing's syndrome and discuss its diagnosis, differential diagnosis, and management. We focus on recent developments and highlight areas of controversy.

The most common cause of Cushing's syndrome is use of supraphysiological amounts of exogenous glucocorticoids, including topical or inhaled corticosteroids (iatrogenic Cushing's syndrome). Thus, adequate knowledge of an individual's medication history is essential for diagnosis. Rarely, patients might present with factitious Cushing's syndrome, with covert use of glucocorticoids, which can be a substantial diagnostic challenge, especially if hydrocortisone is being taken, since use of this substance will cause raised concentrations of circulating cortisol. Here, we will focus on endogenous Cushing's syndrome.

Epidemiology and prognosis

Patients with incompletely controlled Cushing's syndrome have a five-fold excess mortality, lending urgency to its ascertainment,[4] although this rate might not necessarily apply to patients with the subtle clinical and biochemical phenotype being increasingly diagnosed. Depending on the population studied, incidence of the disorder ranges from 0·7 to 2·4 per million population per year.[4–6] New data, however, suggest that Cushing's syndrome is more common than had previously been thought. In screening studies of obese patients with type 2 diabetes, especially those with poor blood glucose control and hypertension, the reported prevalence of Cushing's syndrome is between 2% and 5%.[7–9] In these studies, diagnosis of the disorder was not suspected on the basis of clinical features, but patients' metabolic control improved after intervention for their Cushing's syndrome. If confirmed in further large-scale prospective studies, these data suggest that more widespread screening for Cushing's syndrome in such patients is warranted, although researchers still need to prove that control of cortisol excess is more beneficial than attention to more specific abnormalities of metabolic and cardiovascular risk. The presentation and investigation of adrenal incidentalomas with sub-clinical Cushing's syndrome is beyond the scope of this Seminar.

Causes of Cushing's syndrome

Endogenous Cushing's syndrome is more common in women than men and is divided into corticotropin-dependent and corticotropin-independent causes (table 1). Overall, corticotropin-dependent causes account for about 80–85% of cases, and of these, 80% are due to pituitary adenomas (Cushing's disease), with the remaining 20% or so due to ectopic corticotropin syndrome.[10–12] Ectopic corticotropin secretion most usually takes place with small-cell carcinoma of the lung and bronchial carcinoid tumours, but might also arise with almost any endocrine tumour from many different organs (eg, phaeochromocytoma, pancreatic neuroendocrine tumours, gut carcinoids). Classically, when due to small-cell carcinoma of the lung, or widely metastatic cancer, ectopic corticotropin syndrome can have a rapid onset with severe features, although in some patients a paraneoplastic wasting syndrome can mask hypercortisolism, and hypokalaemia might be a clue to diagnosis. By contrast, the clinical phenotype (and some biochemical features) of carcinoid tumours can be indistinguishable from that of Cushing's disease.[10,11]

Corticotropin-independent Cushing's syndrome is due in most instances to a unilateral tumour: adrenal adenoma in 60% and adrenal carcinoma in 40% of cases. Very rare adrenal causes of Cushing's syndrome are corticotropin-independent macronodular adrenal hyperplasia, primary pigmented nodular adrenal disease (either as isolated

Search strategy and selection criteria

We searched MEDLINE from January, 2000, to October, 2005. We used search terms "Cushing's" OR "Cushing's" AND "Syndrome". We selected publications from this 5-year period, but our search also included other commonly referenced and highly regarded older publications known to us, and those that we judged appropriate. Several review articles or book chapters were included because they provided comprehensive overviews beyond the scope of this Seminar.

	Proportion	Female:male
Corticotropin-dependent		
Cushing's disease	70%	3·5:1·0
Ectopic corticotropin syndrome	10%	1:1
Unknown source of corticotropin*	5%	5:1
Corticotropin-independent		
Adrenal adenoma	10%	4:1
Adrenal carcinoma	5%	1:1
Macronodular hyperplasia	<2%	1:1
Primary pigmented nodular adrenal disease	<2%	1:1
McCune-Albright syndrome	<2%	1:1

*Patients might ultimately prove to have Cushing's disease.

Table 1: **Causes of Cushing's syndrome**

disease or as part of Carney complex), and McCune-Albright syndrome.[1,2,13]

Pathogenesis

Although Cushing's disease is the most common form of endogenous Cushing's syndrome, little is known about the underlying pathogenesis of these pituitary tumours.[14] In general, corticotrope tumours show especially low expression of the cyclin-dependent inhibitor p27,[15] overexpression of cyclin E,[16] and a high Ki67 expression indicative of high proliferative activity. Preponderance of reproductive-aged women might suggest a role of oestrogen, and there is a male predominance in prepubertal Cushing's disease.[17] Corticotrope tumours are usually only a few mm in diameter, on average 6 mm, and are larger than 1 cm (macroadenoma) in only 6% of cases.[18]

More is known about the synthesis and secretion of corticotropin.[19] Corticotrope tumours express the pro-opiomelanocortin gene (*POMC*), the peptide product of which is subsequently cleaved to corticotropin. By contrast with most microadenomas, such processing is relatively inefficient in corticotrope macroadenomas, which secrete large amounts of unprocessed *POMC*.[18,20,21] Some pituitary macroadenomas are silent corticotrope adenomas and can present with tumour mass effects (eg, optic chiasm compression) alone. Patients with an initial absence of features of Cushing's syndrome might progress to overt disease. These tumours can be diagnosed preoperatively, and followed up postoperatively, by measuring the amount of *POMC* in plasma.[22]

Tumours causing Cushing's disease are resistant to the effects of glucocorticoids, but *POMC* expression and corticotropin secretion are nevertheless partly reduced by high doses of dexamethasone in 80% of cases.[1,23] Recent data show loss of corticotropin receptor expression on corticotropes, enhanced inactivation of cortisol by 11β-hydroxysteroid dehydrogenase,[24,25] and reduced expression of bridging protein, which is associated with glucocorticoid feedback.[26] These data somewhat account for the resistance to glucocorticoids apparent in Cushing's disease. About 90% of tumours express the corticotropin-releasing hormone-1 receptor, as evidenced by the release of corticotropin in response to exogenously administered corticotropin-releasing hormone. Tumours also express the vasopressin-3 receptor and respond to vasopressin and desmopressin in vitro and in vivo.[27,28] In ectopic corticotropin syndrome, study of the human DMS-79 cell line—a small-cell lung cancer model—has shown that *POMC* is activated by transcription factors distinct from those in the pituitary (including E2F factors)[29,30] that are able to bind the promoter in an unmethylated state.[31] By contrast, carcinoid tumours, which have a more benign behaviour, show a molecular phenotype closer to that of pituitary corticotrope tumours.[32]

By contrast with the above, we know more about rare causes of adrenal Cushing's syndrome. Corticotropin-independent macronodular adrenal hyperplasia is characterised in many cases by aberrant expression of receptors in both adrenal glands that are not normally present (ectopic expression) or by amplified expression of receptors that are usually present (eutopic expression).[13] Cortisol secretion in these patients is mediated by functional membrane receptors for gastric inhibitory peptide (food-dependent Cushing's);[33–36] vasopressin;[37–40] catecholamines;[41,42] interleukin 1;[43] leptin;[44] lutenising hormone;[45] serotonin, or possibly by other unrecognised ligands.[13] In cases in which receptors are coupled to enhanced cyclic AMP, activation is thought to cause hyperplasia, frequently over many years.[33] Furthermore, the in-vitro responses of adrenal tissue obtained at surgery from these patients parallels the in-vivo response to peptides.[46] The fact that these receptors might be present in bilateral macronodular adrenal hyperplasia associated with subclinical Cushing's syndrome emphasises their potential causative role,[47] and further weight is given to this notion by the finding that expression of gastric inhibitory peptide is sufficient to induce adrenocortical growth.[48] Thus, such aberrant or excessive receptor expression seems to play an important pathological part. The causes of abnormal expression of these receptors are not known. Aberrant receptors also occur in unilateral adenomas but much less commonly than in corticotropin-independent macronodular adrenal hyperplasia.[49] Adrenal glands from patients with corticotropin-dependent disease also show expression of gastric inhibitory peptide receptors.[50] Activation of the corticotropin receptor pathway might be associated with aberrant expression of gastric inhibitory peptide receptors that eventually causes corticotropin-independent disease, and such aberrant expression could be merely an epiphenomenon of the hyperplastic drive. Finally, a constitutively active mutant corticotropin receptor has been identified in a patient with corticotropin-independent Cushing's syndrome.[51]

Primary pigmented nodular adrenal disease causes small nodules on the adrenal gland that might not be visualised on imaging. Diagnosis can be difficult to make,

because features might be mild and cyclic in nature. It can be sporadic or part of Carney complex (an autosomal dominant multiple neoplasia syndrome); most cases present in late childhood or in young adults.[52,53] Of the very rare forms of familial Cushing's syndrome, Carney complex is the most frequent and needs lifelong surveillance for potentially fatal complications, including cardiac myxomas. Germline mutations of the regulatory subunit R1A of protein kinase A (*PRKAR1A*) are present in about 45% of patients with Carney complex[54,55] and in sporadic primary pigmented nodular adrenal disease.[56] These patients with Carney complex and sporadic primary pigmented nodular adrenal disease show a paradoxical rise in cortisol secretion in response to dexamethasone associated with heightened expression of the glucocorticoid receptor.[57]

McCune-Albright syndrome is due to a postzygotic activating mutation in the *GNAS1* gene. The resulting tissue mosaicism produces a varied phenotype, and the disease can present in the first few weeks of life. These mutations lead to constitutive steroidogenesis in the affected adrenal nodules.[58] Mutations of *GNAS1* have also been seen in corticotropin-independent macronodular adrenal hyperplasia.[59]

With respect to adrenal cortical tumours, new data show a high rate of β catenin mutations, particularly in adenomas,[60] and rarely mutations of *PRKAR1A*.[61] Molecular changes that distinguish adrenal cortical carcinomas from adenomas are being increasingly recognised: in carcinomas, allele loss or loss of imprinting at the 11p15 locus is common.[62] This loss is associated with overexpression of insulin-like growth factor II and reduced expression of p57/KIP2 [62,63] an imbalance that favours cell growth. A specific germline mutation of *TP53* was associated with a high rate of adrenocortical carcinoma in Brazilian patients.[64,65]

	Proportion
Obesity or weight gain	95%*
Facial plethora	90%
Rounded face	90%
Decreased libido	90%
Thin skin	85%
Decrease linear growth in children	70–80%
Menstrual irregularity	80%
Hypertension	75%
Hirsutism	75%
Depression/emotional lability	70%
Easy bruising	65%
Glucose intolerance	60%
Weakness	60%
Osteopenia or fracture	50%
Nephrolithiasis	50%

*100% in children.[67]

Table 2: **Clinical features of Cushing's syndrome**[1,67,71,72]

Clinical features of Cushing's syndrome

Table 2 summarises clinical features of Cushing's syndrome. These are variably present in any given patient and can differ in a cyclic way, causing diagnostic difficulty. The diagnosis is being increasingly considered in patients with metabolic syndrome, who might have mild features of slow onset, and diagnosis can be a substantial diagnostic challenge. Signs that most reliably distinguish Cushing's syndrome from obesity are those of protein wasting—presence of thin skin in the young, easy bruising, and proximal weakness. In children, presenting features differ, with obesity and decreased linear growth especially evident.[66–70] Important data shows the difference in presentation between women and men, with purple striae, muscle atrophy, osteoporosis, and kidney stones more frequent in men.[71] Renal stones are present in about 50% of all patients,[72] but are usually not apparent clinically. Gonadal dysfunction is common in both sexes. Adverse effects of glucocorticoids on bone metabolism are shown by decreased bone-mineral density, although the exact incidence is not known[73] and it tends to return to normal over time after effective treatment.[74] Bone loss can be more severe in primary adrenal Cushing's syndrome than pituitary-dependent Cushing's syndrome.[75,76]

More than 70% of patients with Cushing's syndrome can present with psychiatric symptoms ranging from anxiety to frank psychosis; if present, depression is often agitated in nature. Some degree of psychiatric disturbance frequently persists after cure of Cushing's syndrome. Impairment in short-term memory and cognition is common and can persist for at least a year after treatment.[77] These effects are associated with a reduction in apparent brain volume that slowly reverses after correction of hypercortisolaemia.[78] Patients continue to have impaired quality of life even after resolution of cortisol excess[79–81] and should be counselled about this impairment.

Cortisol excess predisposes to hypertension and glucose intolerance. Patients with Cushing's syndrome are at increased cardiovascular risk, which might not return fully to normal after remission.[82–84] Hyperhomocysteinaemia and reduced serum folate concentrations present in active disease return to normal during remission,[85] suggesting that ongoing cardiovascular risk is not related to these factors. The adverse metabolic profile is also evident from imaging studies showing hepatic steatosis (20% of patients)[86] and increased visceral fat.[87]

Biochemical diagnosis of hypercortisolaemia

Diagnostic assessment is usually prompted by clinical suspicion, but in certain groups of patients without classic clinical features, screening might be warranted, such as in poorly controlled and hypertensive diabetic patients and men with unexplained osteoporosis (figure 1). Biochemical confirmation of the hypercortisolaemic state must be established before any attempt at differential

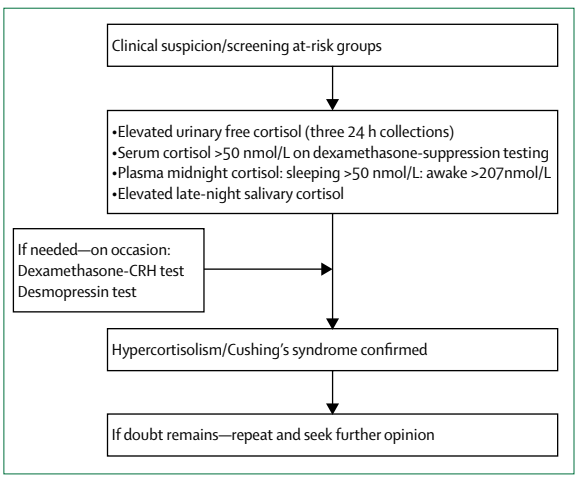

Figure 1: **Diagnosis of Cushing's syndrome**
CRH=corticotropin-releasing hormone.

diagnosis: failure to do so will result in misdiagnosis, inappropriate treatment, and poor management. Hypercortisolaemia is also seen in some patients with depression, alcoholism, anorexia nervosa, generalised resistance to glucocorticoids, and late pregnancy. However, by contrast with true endogenous Cushing's syndrome, the biochemical findings improves when the underlying disorder has resolved. Establishing a diagnosis of Cushing's syndrome on the rare occasion that it presents in pregnancy is a substantial challenge.[88,89]

No sole test is perfect; every one has different sensitivities and specificities and several are usually needed. Investigation should be done when there is no acute concurrent illness (eg, infection or heart failure) because these can cause false-positive results. Three main tests in use for diagnosis of Cushing's syndnrome are: 24-h urinary free cortisol, low-dose dexamethasone-suppression test, and assessment of midnight plasma cortisol or late-night salivary cortisol.

Urinary free cortisol

Measurement of urinary cortisol is a direct assessment of circulating free (biologically active) cortisol. Excess circulating cortisol saturates the binding proteins and is excreted in urine as free cortisol, accounting for its usefulness as a marker of hypercortisolaemia.[23] Values four-fold greater than the upper limit of normal are rare except in Cushing's syndrome.[90] A single measurement has low sensitivity for patients with intermittent hypercortisolaemia.[1,91] Low specificity is a common drawback, since in antibody-based assays the concentrations of urinary free cortisol overlap those seen in patients with other causes of hypercortisolaemia.[1,91] Use of high-performance liquid chromatography and tandem mass spectrometry might improve diagnostic accuracy, although substances such as digoxin and carbamazepine can produce peaks in the high-performance liquid chromatography assay that give falsely high values.[91] Moreover, if there is renal impairment with a glomerular filtration rate of less than 30·0 mL/min, or an incomplete collection, the urinary free cortisol concentration might be falsely low.[91] Review of the volume amount and correction for creatinine concentration might be helpful in assessing whether the collection is complete.

Low-dose dexamethasone-suppression tests

Two tests are in widespread use: the overnight and the 48-h dexamethasone-suppression tests. In the overnight test, 1 mg of dexamethasone is given at 2300 h and the concentration of cortisol in serum measured the next day at 0800–0900 h. In the 48-h test, dexamethasone is given at the dose of 0·5 mg every 6 h for 2 days at 0900 h, 1500 h, 2100 h, and 0300 h with measurements of cortisol in serum at 0900 h at the start and end of the test. To exclude Cushing's syndrome, the concentration of cortisol in serum should be less than 50 nmol/L after either test.[1,91] The 48-h test, although more cumbersome than the overnight test, is more specific and with adequate regular instructions can be done by outpatients. In both tests, caution needs to be exercised if there is potential malabsorption of dexamethasone or if patients are on drugs that increase hepatic clearance of dexamethasone, such as carbamazepine, phenytoin, phenobarbital, or rifampicin.[92] Patients receiving oestrogen treatment, or who are pregnant, might have an increase in the amount of cortisol-binding globulin. Since commercial cortisol assays measure total cortisol, this could give a false-positive result on dexamethasone-suppression testing. Oral oestrogens need to be stopped for a period of 4–6 weeks so that cortisol-binding globulin can return to basal values. Furthermore, the cortisol assay should be known to be accurate at these low levels.

Some 3–8% of patients with Cushing's disease retain sensitivity to dexamethasone and show suppression of serum cortisol to less than 50 nmol/L on either test.[93,94] Additionally, a false-positive rate of up to 30% has been reported in other admitted patients and healthy individuals.[95] Thus, if clinical suspicion remains high, repeated tests and other investigations are indicated.

Midnight plasma cortisol or late-night salivary cortisol

Normal circadian rhythm of cortisol secretion is lost in patients with Cushing's syndrome. A single sleeping midnight plasma cortisol concentration of less than 50 nmol/L effectively excludes Cushing's syndrome at the time of the test and this might be especially helpful in patients in whom there has been incomplete suppression on dexamethasone testing. Concentrations of more than 50 nmol/L are noted in individuals with Cushing's syndrome, even those who suppress serum cortisol on low-dose dexamethasone testing,[96] but this cutoff lacks specificity because patients with acute illness also have values above this concentration. An awake midnight concentration of cortisol in plasma of more than 207 nmol/L differentiates between Cushing's syndrome

and other causes of hypercortisolaemia but can miss mild disease diagnosis in about 7% of cases.[97–99]

Late-night salivary cortisol

Reports have renewed interest in measurement of salivary cortisol concentrations for diagnosis of Cushing's syndrome. Salivary cortisol indicates the amount of free circulating cortisol, and its ease of collection and stability at room temperature make it a highly suitable screening procedure for outpatient assessment.[98,100–109] Diagnostic ranges vary between reports because of the different assays and the comparison groups used to set cutoff points. The test has a sensitivity and specificity of between 95% and 98%. Since salivary cortisol concentrations are an order of magnitude lower than those of serum cortisol, the performance of the local assay must be known and the appropriate cutoff point used. The test is of particular use in the assessment of cyclic Cushing's syndrome[108] and in children.[102,103]

Other tests

When doubt remains about diagnosis the dexamethasone-suppressed corticotropin-releasing hormone test[110,111] and the desmopressin test[112,113] are promising diagnostic procedures. However, their diagnostic accuracy needs further validation.

Establishing the cause of Cushing's syndrome

Once a diagnosis of Cushing's syndrome is established, the next step is to establish cause, which is best done in major referral centres (figure 2). Investigation will vary depending on availability of biochemical tests and imaging methods. The first step is to measure concentrations of corticotropin in plasma. Concentrations consistently lower than 1·1 pmol/L (5 pg/mL) indicate corticotropin-independent Cushing's syndrome and attention can be turned to imaging the adrenal gland with CT. Concentrations of corticotropin persistently greater than 3·3 pmol/L (15 pg/mL) almost always result from corticotropin-dependent pathologies and need investigation. Values between these two limits need cautious interpretation because patients with Cushing's disease and adrenal pathologies might have intermediate values.[1,91,114] Plasma should be separated rapidly and stored at –40°C to avoid degradation and a falsely low result. A positive corticotrophin-releasing hormone test shows an corticotropin-dependent hypercortisolism in a few patients with Cushing's disease with low baseline corticotropin plasma concentrations.

Corticotropin-independent Cushing's syndrome

In corticotropin-independent Cushing's syndrome caused by an adrenal adenoma, carcinoma, or corticotropin-independent macronodular adrenal hyperplasia, the anatomical cause is invariably visible on imaging with CT.[115] In primary pigmented nodular adrenal disease, the adrenal glands can appear normal. Thus, in an established diagnosis of corticotropin-independent Cushing's syndrome with normal appearances of the adrenal glands on imaging, genetic testing for mutations of *PRKAR1A* or assessment of other features of Carney's complex (lentigines, myxoma) can be of benefit as a diagnostic procedure. Exogenous glucocorticoid ingestion should be reconsidered also in this setting.

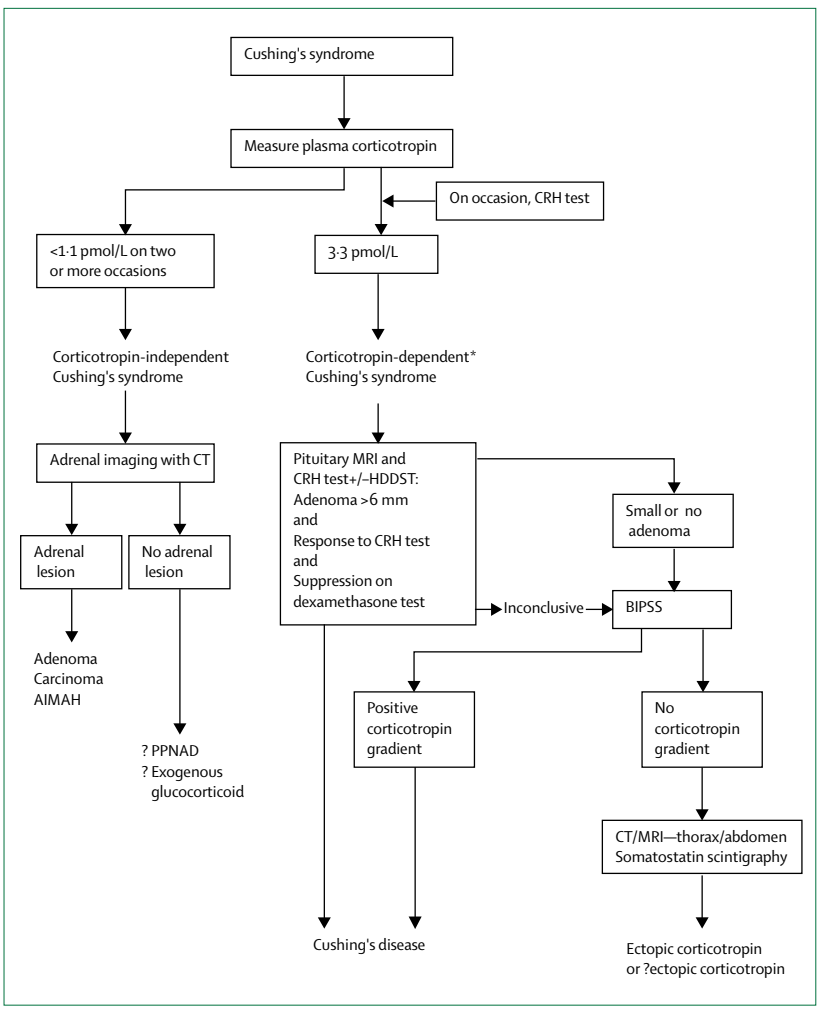

Figure 2: **Diagnosis of cause of Cushing's syndrome**
CRH=corticotropin-releasing hormone. AIMAH=corticotropin-independent macronodular hyperplasia. PPNAD=primary pigmented nodular adrenal disease. BIPSS=bilateral inferior petrosal sinus sampling. SCLC=small-cell lung cancer. HDDST=high-dose dexamethasone-suppression test. *If clear evidence of overt ectopic corticotropin (eg, SCLC) BIPSS might not be needed.

Corticotropin-dependent Cushing's syndrome

Overview

Differentiating between pituitary and non-pituitary sites of excess corticotropin secretion can be a considerable challenge in clinical endocrinology. Carcinoid tumours can be clinically indistinguishable from Cushing's disease and are frequently difficult to identify with imaging, especially if radiological (pituitary, thoracic, pancreatic) so-called incidentalomas complicate interpretation. As a result, biochemical assessment rather than imaging is used to

differentiate between pituitary and non-pituitary causes.[10,11] In women with corticotropin-dependent Cushing's syndrome, nine out of ten cases will be due to Cushing's disease. It is against this pretest likelihood that the performance of any test needs to be judged. The results of corticotropin-releasing hormone and dexamethasone tests and pituitary MRI should be judged together, and bilateral inferior petrosal sinus sampling is recommended unless there is a clear diagnosis (figure 2).

High concentrations of cortisol can either saturate the 11β-hydroxysteroid dehydrogenase type II enzyme in the kidney or decrease expression of this enzyme, allowing cortisol to act even more as a mineralocorticoid.[116] The most common cause of hypokalaemia is the ectopic corticotropin syndrome, but it is also present in patients with Cushing's disease who have very high cortisol production.[1]

Dynamic non-invasive tests

The high-dose dexamethasone suppression tests (2 mg given every 6 h for 48 h, or a single 8 mg dose) have been in widespread use for many years. The tests are based on the relative sensitivity of pituitary corticotrope adenomas to the effects of glucocorticoids, compared with the resistance shown by non-pituitary tumours. About 80% of patients with Cushing's disease will show suppression of amount of cortisol in serum to a value of less than 50% of the basal level.[1] The performance of the test is, therefore, less than the pretest likelihood of Cushing's disease and, thus, by itself the high-dose dexamethasone-suppression test has little diagnostic usefulness.[117] Moreover, if the 48-h low-dose dexamethasone-suppression test is used and if suppression of serum cortisol by more than 30% has already been shown, there is no further advantage of using the high-dose dexamethasone suppression test.[93] We would not recommend continued routine use of the high-dose dexamethasone-suppression test, except when bilateral inferior petrosal sinus sampling is not available.

In the corticotropin-releasing hormone test recombinant human or ovine-sequence corticotropin-releasing hormone is given as an intravenous bolus dose of either 1 μg/kg or, more usually, 100 μg. This dose stimulates corticotrope tumour cells in the pituitary gland to release corticotropin and hence raise cortisol concentrations in serum, although responses are uncommon in ectopic corticotropin syndrome. The ovine-sequence corticotropin-releasing hormone test has a sensitivity of 93% for Cushing's disease based on corticotropin responses at 15 and 30 min.[118] Using more detailed sampling (up to 90 min) and a stringent cutoff point of 50% increment in plasma corticotropin, ovine-sequence corticotropin-releasing hormone had a sensitivity of 86% for Cushing's disease.[119] This sensitivity also falls below the pretest likelihood, at least in women. An almost identical sensitivity is found for human sequence peptide sampling at the same timepoints.[120] Since the V3 receptor is expressed in pituitary and many ectopic tumours secreting corticotropin,[14,27,121,122] the desmopressin test is of restricted usefulness in the differential diagnosis of corticotropin-dependent Cushing's syndrome.[123,124] Similarly, a combined test with corticotropin-releasing hormone and desmopressin has been used,[125] but larger series have suggested that overlap remains between responses in patients with Cushing's disease and ectopic corticotropin syndrome.[126] Responses to both corticotropin-releasing hormone testing and the high-dose dexamethasone suppression test are also more frequently discordant in patients with Cushing's disease secondary to a pituitary macroadenoma.[18]

Invasive testing

If a patient has corticotropin-dependent Cushing's syndrome, with responses on both dexamethasone suppression and corticotropin-releasing hormone testing suggesting pituitary disease, and their pituitary MRI scan shows an isolated lesion of 6 mm or more, most clinicians will diagnose Cushing's disease. A major drawback is that up to 40% of patients with proven Cushing's disease have normal pituitary MRI scans.[114] In these patients, sampling of the gradient of corticotropin from the pituitary gland to the periphery is the most reliable means of discriminating between pituitary and non-pituitary sources of corticotropin. Since the pituitary effluent drains via the cavernous sinuses to the petrosal sinuses and then jugular bulb, there is a gradient of the value of plasma corticotropin compared with the simultaneous peripheral sample when there is a central source of corticotropin. Bilateral inferior petrosal sinus sampling is a highly skilled and invasive technique, requiring placement of catheters in both inferior petrosal sinuses. Catheter position and venous anatomy are confirmed by venography, because non-uniform drainage is not uncommon. Diagnostic accuracy of the test needs corticotropin-releasing hormone to be given. A basal central:peripheral ratio of more than 2:1 or a ratio after stimulation with corticotropin-releasing hormone of more than 3:1 is consistent with Cushing's disease.[127] The combined data for many series suggest a sensitivity and specificity of 94%.[128] When corticotropin-releasing hormone is unobtainable or too costly, desmopressin offers a reasonable alternative, keeping in mind that few patients with ectopic corticotropin secretion have been studied in this way.

Although bilateral inferior petrosal sinus sampling remains the gold standard for differentiating between pituitary and non-pituitary sources of corticotropin, data have highlighted some of the potential pitfalls. In a series of 179 patients, two were noted to have responses consistent with Cushing's disease but ultimately turned out to have the ectopic corticotropin syndrome, while nine patients had a false-negative response, turning out to have Cushing's disease.[129] Small-series data have suggested that these false-negative responses can be identified by simultaneous sampling of prolactin to correct values in corticotropin.[130,131] It is possible that falsely positive results might be caused by inadequate

suppression of the normal corticotropes; the duration and amount of hypercortisolism should be assessed before the test.

In adults, bilateral inferior petrosal sinus sampling is only 70% accurate for lateralisation of the source of corticotropin within the pituitary gland,[1,91] but in children it can have greater accuracy for this purpose than MRI.[132] Sampling from the cavernous sinuses directly does not improve accuracy.[133]

Sampling from the internal jugular vein has been proposed as a simplified procedure compared with bilateral inferior petrosal sinus sampling. Direct comparison in the same patients has shown internal jugular vein sampling to be inferior to bilateral inferior petrosal sinus sampling.[134] This test can, however, have usefulness in centres with limited sampling experience, where bilateral inferior petrosal sinus sampling should be reserved for instances when the results are negative.[135]

Imaging

CT gives the best resolution of adrenal anatomy. In corticotropin-dependent Cushing's syndrome, nodules can arise, and adrenal hyperplasia is not always symmetrical, causing diagnostic confusion with a unilateral primary adrenal cause if the biochemistry is not strictly assessed. In 30% of patients with Cushing's disease, the adrenal glands appear normal, whereas in ectopic corticotropin the adrenal glands are virtually always homogeneously enlarged.[136]

Up to 40% of corticotrope adenomas causing Cushing's disease in adults are not visible on MRI scanning.[114] Those that are visible usually do not enhance with gadolinium on T_1-weighted imaging. Use of dynamic MRI with administration of intravenous contrast media and rapid sequence acquisition does not improve the overall diagnostic rate. However, spoiled gradient sequences might have high sensitivity in adults[137] and children.[138] There is also a 10% rate of pituitary incidentalomas in the normal population,[139] emphasising the need for careful biochemical discrimination of pituitary from non-pituitary sources of corticotropin. In the absence of a pituitary macroadenoma, an abnormal MRI scan is not conclusive evidence in favour of Cushing's disease.

Axial imaging with thin-cut multislice CT of the thorax and abdomen, MRI of the thorax, or both procedures, has the highest detection rate for ectopic corticotropin syndrome.[10–12] Most patients harbour small neuroendocrine tumours, which can express somatostatin receptors and might be disclosed on somatostatin-receptor scintigraphy. However, although standard somatostatin scintigraphy can confirm functionality for a lesion seen on axial imaging, it has only rarely been shown to disclose truly occult tumours that are not visible on CT.[1,10–12,140,141] Using higher than standard doses of radionucleotide might, in some cases, disclose lesions that were otherwise negative on imaging. In patients with recurrent disease, somatostatin scintigraphy can be useful for follow-up,[142] because it has a low false-positive rate.[143] PET with 18-fluorodeoxyglucose is of little benefit because these tumours are usually of low metabolic activity.[144] Although use of ^{11}C-5-hydroxytryptophan has been proposed as an universal imaging technique for neuroendocrine tumours, few patients have been studied[145] and further experience is needed to establish its usefulness. Despite detailed investigation, the cause of corticotropin production might remain occult in 5–15% of patients, and these patients need continued follow-up, this rate decreasing with time.[10,11]

Management

Medical therapies to lower cortisol

Metyrapone, ketoconazole, and mitotane can all be used to lower cortisol by directly inhibiting synthesis and secretion in the adrenal gland.[2,19] Metyrapone and ketoconazole are enzyme inhibitors and have rapid onset of action, but frequently control of hypercortisolism is lost with corticotropin oversecretion in Cushing's disease (known as escape). These drugs are not usually effective as the sole long-term treatment of the disorder, and are used mainly either in preparation for surgery or as adjunctive treatment after surgery, pituitary radiotherapy, or both procedures.[2] Mitotane acts as an adrenolytic drug with delayed onset but longlasting action, but there is no escape occurrence. Medical treatment can also be used in patients who are unwilling or unfit to undergo surgery. These drugs have gastrointestinal side-effects, and with ketoconazole, hepatocellular dysfunction is frequently noted and rare cases of hepatic failure described.[146] Treatment can be used long term for patients with ectopic corticotropin secretion, but some centres opt for adrenalectomy in that setting.[10,147] For acute control of severe hypercortisolaemia when the oral route is not available, the short-acting anaesthetic agent etomidate can be very useful,[148,149] including in children.[150] In patients with corticotropin-independent macronodular adrenal hyperplasia, cortisol secretion can be controlled by blocking the aberrantly expressed receptor—eg, propranolol use with aberrant β adrenergic receptor expression—or suppressing the ligand of the illegitimate receptor by giving somatostatin analogues in gastric inhibitory peptide-responsive corticotropin-independent macronodular adrenal hyperplasia or leuprolide in luteinising hormone-dependent Cushing's syndrome.[13,41,45,151]

New therapies to reduce corticotropin

There has been renewed interest in use of agents that might directly inhibit the secretion of corticotropin by corticotrope tumours. The peroxisome proliferator activated receptor γ agonist rosiglitazone reduced corticotropin and cortisol concentrations and prevented tumour growth in an animal model of Cushing's disease.[152] Although human pituitary corticotrope tumours express peroxisome proliferator activated receptor γ,[153] studies in patients with Cushing's disease have, unfortunately, been almost uniformly disappointing. Rosiglitazone achieved

only short-term control of cortisol, with later escape.[154,155] Similarly, the PPAR γ agonist pioglitazone (at licensed doses) did not affect corticotropin concentrations.[156] Rosiglitazone at 1·5 times licensed dose did not decrease the high amounts of corticotropin caused by corticotrope tumour progression after bilateral adrenalectomy (Nelson's syndrome).[157] Although these data are disappointing, it might be that higher doses or more potent agonists are needed, but at present the use of PPAR γ ligands cannot be recommended. Corticotrope tumours may also express the dopamine 2 receptor, and short-term administration of cabergoline at a dose of 1–3 mg per week can reduce hypercortisolism in up to 40% of cases,[158] but larger studies are needed. A newer somatostatin analogue, SOM-230, reduces corticotropin secretion in cell-culture models and in culture of human corticotrope tumour cells.[159] The results of first trials in human beings are awaited: preliminary results look encouraging.[160] In ectopic corticotropin syndrome, occasionally the somatostatin analogues octreotide and lanreotide directly inhibit corticotropin secretion,[140,141] or their combined use with high-dose cabergoline might be of benefit.[161] Finally, preliminary data in an animal model suggest that retinoic acid might cause direct inhibition of corticotropin secretion from corticotrope tumours.[162]

Surgery

Tumour-specific surgery

Several series, including many within the past 5 years, have shown the results and long-term follow-up of trans-sphenoidal surgery for Cushing's disease.[18,163–179] Trans-sphenoidal surgery offers the potential for a selective microadenectomy of the causative corticotrope adenoma leaving the remaining pituitary function intact. Taking all published series together, the quoted initial remission rate is between 60% and 80% (<15% for macroadenomas[18]) but with a relapse rate of up to 20% when followed up for many years. It is probable that these variations result from varying surgical skill and from controversy about the characterisation of remission or continuing disease in the postoperative period. If there is clear persistent disease postoperatively, immediate reoperation might be of benefit.[180,181] Patients who are hypocortisolaemic in the immediate postoperative period need glucocorticoid treatment until the hypothalamo-pituitary-adrenal axis recovers full activity usually 6–18 months after surgery. On long-term follow-up (10 years), however, the overall remission rate is about 60%, whereas on careful endocrine testing in some series, there can be deficiencies of other pituitary hormones in up to 50% of cases.[172] Although long-term remission is most probable when postoperative concentration of cortisol in serum is low (<50 nmol/L), there is no threshold value that fully excludes possible recurrence. These data emphasise the ongoing need for alternative therapies directed against the pituitary gland.

Resection of the tumour producing corticotropin ectopically is optimum treatment for this cause of Cushing's syndrome. Unfortunately, this goal often is precluded by metastatic or occult disease, which is then treated medically or by adrenalectomy.[10,11]

Adrenal surgery

Laparoscopic surgery is now the treatment of choice for unilateral adrenal adenomas.[182–190] Prognosis after removal of an adenoma is good, although, by contrast, the outlook is almost uniformly poor in patients with adrenocortical carcinomas. These latter tumours frequently present with metastases and are characterised by a dismal 5-year survival. They are not usually radiosensitive or chemosensitive and the most important predictor of outcome in this disease is the ability to do a complete resection.[191]

In any cause of corticotropin-dependent Cushing's syndrome, total bilateral adrenalectomy induces a rapid resolution of the clinical features. After surgery, patients need lifelong treatment with glucocorticoids and mineralocorticoids. With low morbidity associated with laparoscopic adrenal surgery, this approach is being considered more frequently, and possibly even as main treatment in some individuals with Cushing's disease, especially when disease is severe or because of patient preference. A major concern after bilateral adrenalectomy in patients with Cushing's disease is the development of Nelson's syndrome—a locally aggressive pituitary tumour that secretes high concentrations of corticotropin, resulting in pigmentation. Whether the tumour progression is a result of the lack of cortisol feedback after adrenalectomy, or whether the progression results from corticotrope tumours that were programmed to behave in an aggressive manner from the beginning, is controversial.[192] The tumour itself might be treated with further surgery or radiotherapy.[193] Some clinicians advocate pituitary radiotherapy at the time of adrenalectomy to reduce the risk of this syndrome,[194] but others have not confirmed this finding.[192]

Pituitary radiotherapy

Persisting hypercortisolaemia after trans-sphenoidal surgery can be treated with pituitary radiotherapy. Conventional fractionated radiotherapy is a very effective means of treatment but is associated with long-term hypopituitarism,[195] and can be very delayed in effectiveness, although it tends to be more rapidly curative in children.[196] Use of stereotactic radiosurgery has also been reported.[197,198] Despite enthusiasm for the gamma knife, a relapse rate of up to 20% after treatment has been shown,[199] which does not compare favourably with conventional radiotherapy. It might, however, be more rapidly effective.

Conclusions

Diagnosis and management of Cushing's syndrome remains a considerable challenge. Our understanding of the pathogenesis has advanced, but mainly with respect to

the very rare causes of Cushing's syndrome, although the underlying pathogenesis of the most common cause—Cushing's disease—remains to be elucidated. Cushing's syndrome can be present in up to 2% of patients with poorly controlled type 2 diabetes, and has great implications for screening of this at-risk population. Measurement of cortisol in saliva has emerged as a promising screening tool, and might be especially suited for this purpose. In view of the complexity of diagnosis, differential diagnosis, and further management, patients presenting with Cushing's syndrome warrant referral to major centres. The outcome of treatment for the most common cause of Cushing's syndrome—Cushing's disease—remains disappointing, and further developments are needed in this area.

Conflict of interest statement

We declare that we have no conflict of interest.

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