Cushing's syndrome

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Chronic exposure to excess glucorticoids results in diverse manifestations of Cushing's syndrome, including debilitating morbidities and increased mortality. Genetic and molecular mechanisms responsible for excess cortisol secretion by primary adrenal lesions and adrenocorticotropic hormone (ACTH) secretion from corticotroph or ectopic tumours have been identified. New biochemical and imaging diagnostic approaches and progress in surgical and radiotherapy techniques have improved the management of patients. The therapeutic goal is to normalise tissue exposure to cortisol to reverse increased morbidity and mortality. Optimum treatment consisting of selective and complete resection of the causative tumour is necessay to allow eventual normalisation of the hypothalamic-pituitary-adrenal axis, maintenance of pituitary function, and avoidance of tumour recurrence. The development of new drugs offers clinicians several choices to treat patients with residual cortisol excess. However, for patients affected by this challenging syndrome, the long-term effects and comorbidities associated with hypercortisolism need ongoing care.

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Introduction

Cushing's syndrome results from chronic exposure to excess glucocorticoids, which can be from either exogenous pharmacological doses of corticosteroids or from an endogenous source of cortisol. A century after the description of endogenous Cushing's syndrome, confirmation of hypercortisolism, identification of its causes, and achievement of optimum treatment is still challenging. Manifestations range from subclinical, cyclical, or mild to rapid-onset severe variants.[1–4] The variable biology of tumours explains the incomplete reliability of investigations, particularly in mild cases.[1–4] In this Seminar, we review progress on the pathophysiology, investigation, and therapy of endogenous Cushing's syndrome since the last update in this series.[4]

Epidemiology and causes

Cushing's syndrome has an estimated incidence of 0·2–5·0 per million people per year and a prevalence of 39–79 per million in various populations;[5–8] median age of onset/diagnosis was 41·4 years with a female-to-male ratio of 3:1. Studies suggest an increased but variable prevalence in people with uncontrolled type 2 diabetes, hypertension, or early-onset osteoporosis.[9–12]

Endogenous Cushing's syndrome is divided between adrenocorticotropic hormone (ACTH)-dependent (about 80%) and ACTH-independent (about 20%) causes (table 1). Among ACTH-dependent forms, pituitary corticotroph adenoma (Cushing's disease) is most common, outnumbering extrapituitary (ectopic) tumours that secrete ACTH by about seven-to-one;[2–4] up to 20% of ectopic ACTH tumours are still occult for many years.[13,14] Rarely, neuroendocrine tumours, medullary thyroid carcinoma, and phaeochromocytoma produce corticotropin-releasing hormone (CRH), leading to excess pituitary ACTH secretion.[2–4]

Cortisol excess from primary unilateral adrenal adenomas or carcinomas suppresses ACTH; these tumours account for about 20% of endogenous Cushing's syndrome cases.[2–4] Rarely, Cushing's syndrome is caused by primary bilateral macronodular adrenal hyperplasia (BMAH) or primary pigmented nodular adrenocortical disease (PPNAD) and its non-pigmented variant, isolated micronodular adrenocortical disease.[15,16]

Molecular pathophysiology

Corticotroph adenomas

The causes of anterior pituitary and Cushing's disease adenomas were unclear until the identification of molecular genetic abnormalities.[17] Cushing's disease rarely occurs in the context of germline mutations of the multiple endocrine neoplasia 1 (*MEN1*), aryl-hydrocarbon receptor-interacting protein (*AIP*; less than 5%), and *CDKN1B* (also known as p27Kip1) genes and other cyclin-dependent kinase inhibitor (*CDK1*) in MEN4 (an overlapping MEN syndrome with parathyroid, pituitary and other endocrine tumours) and *CDKN2C* (p18INK4c) and other *CDKI* genes in patients with MEN1-like or MEN4-like features (figure 1).[18–22] Other rare germline mutations include succinate dehydrogenase subunit.[23] Somatic *MEN1*, *PRKAR1A*, or *AIP* gene mutations do not seem to cause sporadic pituitary adenomas or Cushing's disease.[23–26]

Somatic mutations of the glucocorticoid receptor gene, dysfunction of proteins related to glucocorticoid-receptor function (Brg1 and HDAC2), and *TP53*-inactivating defects occur in Cushing's disease.[27–29]

Search strategy and selection criteria

We searched Medline from Jan 01, 2006, to Dec 30, 2014, in English and French with the following search terms: "Cushing's syndrome", "Cushing's disease", "hypercortisolism", "ectopic ACTH secretion". We selected publications from this period, but included pertinent older publications and review articles that provided comprehensive overviews beyond the scope of this Seminar. Considering the large number of publications on this topic and space restriction, we were unable to cite all pertinent studies and selected some articles which contributed to our knowledge.

	Proportion (%)	Age (peak)	Female:male	Features
ACTH-dependent	70–80	..	..	..
Cushing's disease	60–70	..	..	..
Corticotroph adenoma	60–70	3rd–4th decades	3–5:1	Roughly 50% non-visible on MRI
Corticotroph hyperplasia	Very rare	..	..	..
Ectopic ACTH*	5–10	..	..	..
Malignant neuroendocrine tumours	About 4	5th–6th decades	0·6–1:1	Might have very high ACTH
Benign neuroendocrine tumours	About 6	3rd–4th decades	..	Might respond to dexamethasone, CRH, desmopressin
Occult neuroendocrine tumours	About 2	..	..	..
Ectopic CRH	Very rare	..	..	Causes pituitary corticotroph hyperplasia
ACTH-independent	20–30	..	..	..
Unilateral adrenal	..	..	..	..
Adenoma	10–22	4th–5th decades	4–8:1	Most pure cortisol secretion
Carcinoma	5–7	1st, 5th–6th decades	1·5–3:1	Mixed cortisol and androgen frequent
Bilateral adrenal	1–2	..	..	..
Bilateral macronodular adrenal hyperplasia†	<2	5th–6th decades	2–3:1	Modest cortisol secretion compared with size; raised steroid precursors; might have combined androgen and mineralocorticoid cosecretion
Aberrant G-protein-coupled receptors	..	..	..	..
Autocrine ACTH production	..	..	..	..
Sporadic or familial (*ARMC5*)	..	..	..	..
Bilateral micronodular adrenal hyperplasias	<2	..	..	Adrenal size often normal
Primary pigmented nodular adrenocortical disease	Rare	1st–3rd decades	0·5:1 <12 years 2:1 >12 years	Frequent paradoxical increase of urine free cortisol with Liddle's oral dexamethasone suppression test
Isolated or familial with Carney complex	Rare	1st–3rd decades	..	..
Isolated micronodular adrenocortical disease	Very rare	Infants	..	Non-pigmented adrenal micronodules
Primary bimorphic adrenocortical disease	Very rare	Infants	..	..
McCune-Albright syndrome	Rare	Infants (<6 months)	1:1	Internodular adrenal atrophy
Bilateral adenomas or carcinomas	Rare	4th–5th decades	2–4:1	..

ACTH=adrenocorticotropic hormone. CRH=corticotropin-releasing hormone.*Most frequent sources of ectopic ACTH syndromes are small cell lung carcinoma and neuroendocrine tumours of lung, thymus, and pancreas. Less frequent causes include medullary thyroid carcinoma, gastrinoma, phaeochromocytoma, prostate carcinoma, and several others. †In bilateral macronodular adrenal hyperplasia tissues, autocrine and paracrine ACTH might be produced and contribute to cortisol secretion. If confirmed by in-vivo studies, the ACTH-independent classification will need to be modified in the future.

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

Testicular orphan receptor 4 (TR4),[30] a nuclear receptor with unknown ligand, the pituitary transforming gene (*PTTG*),[31] and epidermal growth factor receptor[32] can be overexpressed in Cushing's disease tumours, suggesting potential causal and therapeutic roles (figure 1). In transgenic animals, administration of the CDK2/cyclin inhibitor R-roscovitin (targeting PTTG overexpression) or gefitinib, the epidermal growth factor receptor-tyrosine kinase inhibitor, inhibited corticotroph tumour growth and clinical or biochemical features of Cushing's syndrome.[31,32]

Further support for a role of epidermal growth factor receptor as a cause of Cushing's disease is provided by the identification of somatic mutations in the ubiquitin-specific peptidase 8 gene (*USP8*) in 35–62% of corticotroph adenomas from patients who had smaller and relatively more active tumours than those without *USP8* mutations.[33,34] Inactivation of *USP8* led to enhanced epidermal growth factor receptor deubiquitination, impairing its downregulation, increasing epidermal growth factor signalling and pro-opiomelanocortin (POMC) and ACTH synthesis.[33,34] Gefitinib decreased ACTH secretion in USP8-mutated human corticotroph cells in primary culture.[34]

Ectopic ACTH and CRH secretion

The molecular defects leading to ectopic ACTH secretion from neuroendocrine tumours and gastroenteropancreatic tumours are largely unknown. ACTH-secreting neuroendocrine tumours in MEN1 or MEN4 can harbour germline menin or other somatic mutations (figure 1). ACTH-producing phaeochromocytoma and medullary thyroid carcinoma might be part of MEN2 syndromes due to oncogenic *RET* mutations, but are usually sporadic.[13,14]

Adrenal adenomas

Mutations or activation of the cAMP-dependent or β-catenin signalling pathways are reported in adrenal adenomas.[35] Aberrant expression and function of various

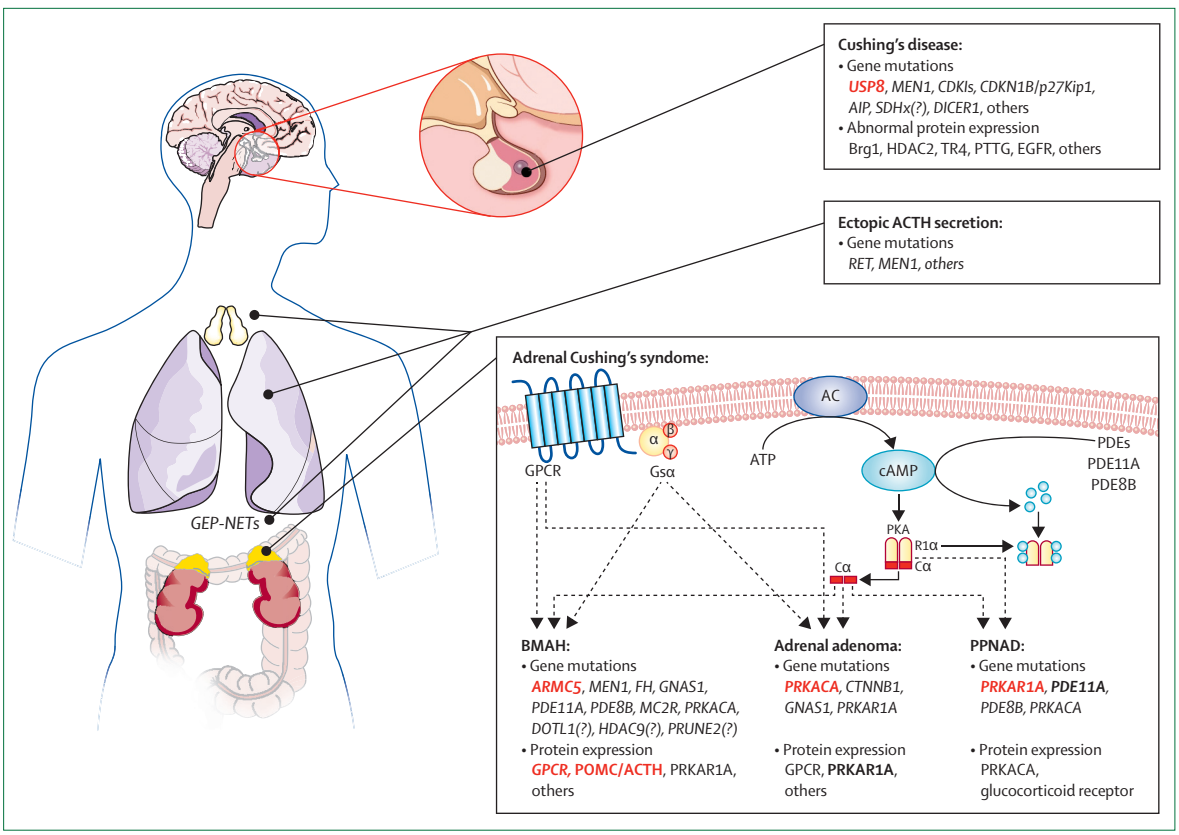

Figure 1: **Summary of genetic and molecular mechanisms implicated in Cushing's syndrome**
For each cause, the various genetic mutations or abnormal protein expression believed to play a part in the pathophysiology are shown. The most frequent mechanisms are highlighted in red; the well characterised mechanisms are highlighted in bold characters, and other potential mechanisms are in normal characters; a question mark shows an unconfirmed association or genetic predisposition. Please refer to the text for explanation of the various genetic defects under each diagnostic category. ACTH=adrenocorticotropic hormone. GPCR=G-protein-coupled receptor. AC=adenylate cyclase. BMAH=bilateral macronodular adrenal hyperplasia. PDEs=phosphodiesterases. PPNAD=primary pigmented nodular adrenocortical disease. PKA=protein kinase A. Cα=catalytic subunit of PKA. Rlα=type 1α regulatory subunit of PKA.

G-protein-coupled receptors (GPCR) are seen in adenomas,[36] but are more common in BMAH (figure 1).[37] Somatic mutations of *GNAS* (encoding Gsα, which activates adenylate cyclase) were reported in 5–17% of cortisol-secreting adenomas and β-catenin (*CTNNB1*) mutations in 16% of cortisol-secreting adenomas.[38–40] Somatic *PRKAR1A* mutations are noted in 20% of adenomas and losses at the *PRKAR1A* locus in 23% of adenomas.[41]

Three somatic mutations in the gene encoding the catalytic subunit of protein kinase A (*PRKACA*) were identified in 35–65% of adenomas from patients with overt Cushing's syndrome, but rarely in adenomas secreting less cortisol (figure 1).[38–40,42,43] The most frequent p.Leu206Arg mutation, located in the active cleft of the catalytic subunit, inactivates the site where the regulatory subunit RIIβbinds, resulting in increased protein kinase A (PKA) activity. The absence of *PRKACA* mutations in low cortisol-secreting adenomas might explain why they rarely progress to Cushing's syndrome over time.[42] Phosphodiesterase 11A (*PDE11A*) or phosphodiesterase 8B (*PDE8B*) genetic variants can predispose to adrenal adenomas, carcinomas, and BMAH.[44,45]

Adrenocortical carcinomas

Adrenocortical carcinoma usually presents with clinical hypercortisolism, either alone (45%) or with androgen overproduction (25%). Increased urinary metabolites of several androgens or glucocorticoid precursors are seen in most patients with adrenocortical carcinomas, suggesting decreased activity of steroidogenic enzymes.[46] The mechanisms regulating steroidogenesis in adrenocortical carcinomas are still unknown. Progress on the genetic causes of adrenocortical carcinomas is reviewed elsewhere.[47–49]

Bilateral macronodular adrenal hyperplasia

Bilateral adrenal hyperplasias are disorders with distinct epidemiology, histopathology, and molecular genetics. Bilateral adrenal hyperplasias are characterised by nodule diameter greater than 1 cm (macronodular) or less than 1 cm (micronodular).[50] The macronodular form, BMAH, was previously termed ACTH-independent macronodular adrenal hyperplasia. Although usually sporadic, familial forms of BMAH with autosomal dominant transmission have now been reported.[15]

BMAH is occasionally associated with MEN1, familial adenomatous polyposis, and fumarate hydratase gene mutations.[15,50,51] Several inactivating mutations of armadillo repeat containing 5 gene (*ARMC5*, chromosome 16p11.2) were identified in large families with BMAH and in up to 50% of patients with apparently sporadic BMAH.[52–56] In each macronodule examined, both alleles carried distinct *ARMC5* mutations (one a germline mutation and the other a distinct somatic mutation or deletion); by contrast, only the germline mutation was reported in internodular diffuse hyperplasia.[52] Nearly half of first degree relatives of patients with apparently sporadic cases of Cushing's syndrome carried the same *ARMC5* mutation and had unsuspected subclinical BMAH.[52,53] *ARMC5* is expressed in several human tissues. Its function is unknown but it might be a tumour suppressor gene; in-vitro inactivation of *ARMC5* reduced steroidogenesis whereas overexpression induced apoptosis and cell death.[52] Somatic mutations of *ARMC5* were also reported in meningiomas, which can occur in familial BMAH, suggesting that other tumours could result from mutations of *ARMC5*.[53,56] One study[40] identified somatic mutations in a histone H3 lysine 79 methyltransferase gene (*DOTL1*), in a histone deacetylase gene (*HDAC9*), or in a prune homologue 2 gene (*PRUNE2*). These findings suggest that in addition to the biallelic *ARMC5* mutations, other genetic events might play a part in BMAH nodule growth.

Steroidogenesis in BMAH is frequently regulated by the aberrant expression of one or several non-mutated GPCR (figures 1, 2),[37,51,57,58] including ectopic receptors for glucose-dependent insulinotropic peptide, β-adrenergic ligands, vasopressin (AVPR2 and AVPR3), and serotonin (5-HT7R). Other eutopic receptors show increased expression or activity including AVPR1, LHCGR, and 5-HT4R. A transcriptome study[59] in BMAH tissues reported additional aberrant GPCR for motilin (MLNR), g-aminobutyric acid (GABBR1), and α2A adrenergic (ADRA2A; 13 of 18). The mechanisms that bring about the aberrant GPCR adrenocortical expression and their relation to *ARMC5* mutations are unknown.

New paracrine regulatory mechanisms emerge from the demonstration of expression and secretion of POMC and ACTH in clusters of steroidogenic BMAH cells (figures 1, 2).[60] Tissues secreted ACTH in perifusion and in adrenal vein samples, although circulating ACTH concentrations were still low. Prohormone convertase 1 is expressed in BMAH, allowing POMC processing; however, ACTH secretion was not regulated by CRH, dexamethasone, or the glucocorticoid receptor antagonist mifepristone. Aberrant regulation by glucose-dependent insulinotropic peptide or the luteinising hormone/choriogonadotropin receptor (LH/CGR) led to release of ACTH, and cortisol increase was inhibited 40% by melanocortin 2 receptor (MC2R) antagonists.[60] Thus, cortisol production in BMAH seems to be controlled by

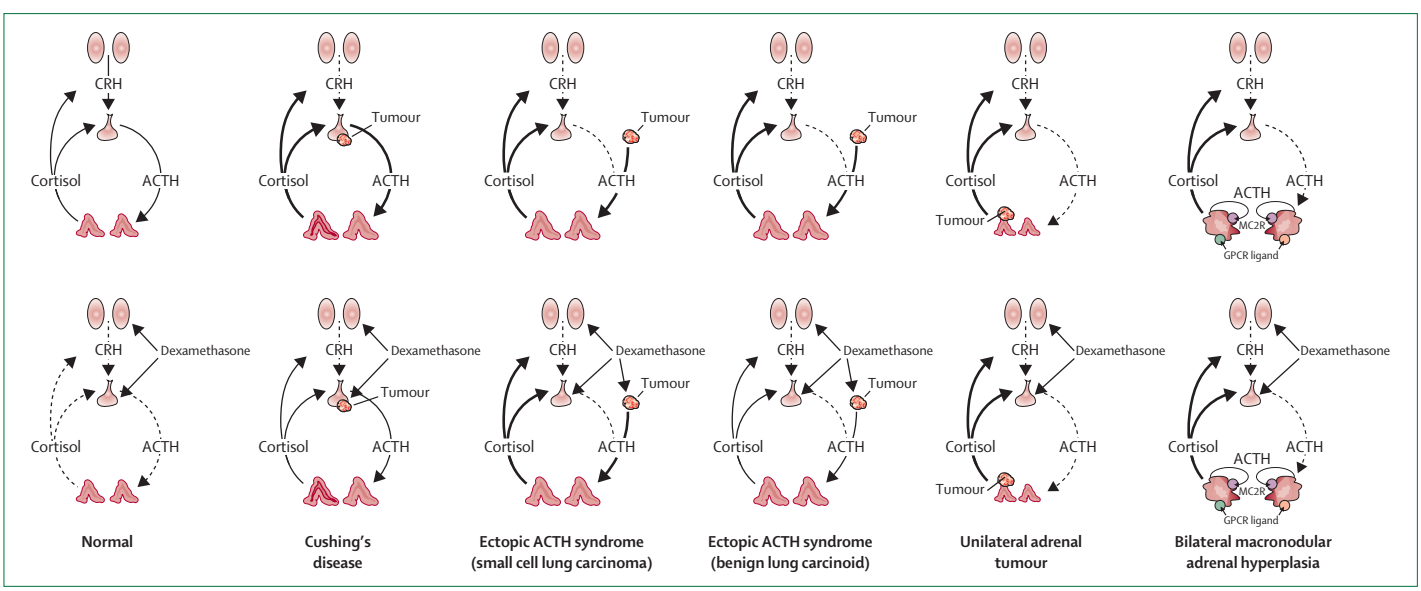

***Figure 2*: Differential regulation of the hypothalamic-pituitary-adrenal axis in healthy individuals and in the various causes of Cushing's syndrome**
In healthy people, stimulation of the hypothalamus by CNS centres regulates the secretion of CRH, which in turn increases ACTH secretion. ACTH stimulates adrenal secretion of cortisol, which, like exogenous low dose dexamethasone (lower panel), inhibits the secretion of both CRH and ACTH. In Cushing's disease, excess ACTH originating from the corticotroph tumour is partly resistant to excess glucocorticoid and high dose dexamethasone suppression, while CRH and healthy corticotropes are suppressed. Ectopic ACTH secretion from malignant tumours is usually autonomous from CRH and dexamethasone regulation, whereas ectopic ACTH originating from up to 50% of benign neuroendocrine tumours can be suppressed partly by high dose dexamethasone or respond to exogenous CRH or desmopressin, similar to Cushing's disease. In adrenal Cushing's syndrome caused by unilateral adrenal tumours, ACTH is suppressed by excess cortisol and is not modified by CRH or high doses of dexamethasone. In bilateral macronodular adrenal hyperplasia, cortisol can be regulated by the ligands of various aberrant hormone receptors (GPCR) stimulating paracrine ACTH production by adrenal hyperplasia cells acting on the ACTH receptor (MC2R); however, circulating ACTH concentrations are still low. Normal hormone secretion is shown by lines, suppressed secretion by thinner or dotted lines, and hypersecretion by thick lines. Modified from reference 169 by permission of Massachusetts Medical Society. ACTH=adrenocorticotropic hormone. CRH=corticotropin-releasing hormone. GPCR=G-protein-coupled receptor. MC2R=melanocortin 2 receptor.

both aberrant GPCR and autocrine ACTH, which amplifies the aberrant receptor effects (figure 2). To confirm the role of autocrine ACTH, we await demonstration that blockade of adrenal ACTH receptors reverses hypercortisolism in affected patients.[61] Although the previous term, ACTH-independent macronodular adrenal hyperplasia, is inappropriate from a causal perspective, circulating ACTH concentrations are low and still useful in diagnostic testing. This disease should be termed primary BMAH to distinguish it from secondary BMAH, which can occur after long-term stimulation by ACTH in Cushing's disease or ectopic ACTH secretion.[61]

Constitutive *MC2R* mutations are rare in BMAH.[15] In McCune-Albright syndrome, activating mutations of the Gsα subunit occur in a mosaic pattern in early post-zygotic embryogenesis, resulting in constitutive cAMP activation;[62] this might result in nodular adrenal hyperplasia and Cushing's syndrome in infants and children. In few adults with Cushing's syndrome without McCune-Albright syndrome, two different *GNAS* mutations at codon Arg201 were reported in BMAH nodules.[51,63]

Bilateral micronodular hyperplasia

PRKAR1A is the gene that most frequently causes PPNAD and Carney complex.[64] A phenotype–genotype study[64] identified more than 120 *PRKAR1A* mutations that included single-base substitutions and small deletions, insertions, or rearrangements spread along the gene, and a few large deletions. Most mutations were unique, but five were reported in unrelated pedigrees, and four led to isolated PPNAD.[64,65] Two mutations were associated with adrenocortical cancer that developed adjacent to PPNAD.[66,67] Patients' sex or puberty modify the expression of Cushing's syndrome in PPNAD; after adolescence, prevalence is higher in women than men, and by the age of 40 years more than 70% of female carriers of *PRKAR1A* defects manifested PPNAD, compared with 45% of men.[64,68] In rare cases of PPNAD without known mutations, germline duplications of the *PRKACA* gene increase adrenal tissue PKA activity and cause, in most cases, a PPNAD-like histology and clinical presentation.[42]

Very few infants have micronodular bilateral adrenal hyperplasia; they are mostly girls and have isolated micronodular adrenocortical disease with no other tumours.[69] In most patients, the molecular causes are unknown, and some carried *PDE11A* or *PDE8B* defects.[70,71] Patients with Beckwith-Wiedemann syndrome develop another distinct form of bilateral adrenal hyperplasia.[72] In Beckwith-Wiedemann syndrome as in PPNAD, McCune-Albright syndrome, and other micronodular forms of bilateral adrenal hyperplasia, persisting cells are probably derived from fetal adrenal precursors.[73]

Clinical symptoms and initial screening

Screening is recommended for individuals in whom a diagnosis of Cushing's syndrome is most likely: for example, patients with unusual age-related features (such as hypertension in young adults), many and progressive manifestations consistent with Cushing's syndrome, children with decreasing growth velocity and increasing weight, and patients with adrenal incidentaloma (1 mg overnight dexamethasone test; appendix).[1] For patients with unexplained severe features, such as resistant hypertension and osteoporosis, assessment is justified irrespective of age. Proximal muscle weakness, wide purple striae, and, in children, diminished growth seem more specific to Cushing's syndrome, but are noted in more severe cases.[1] The use of exogenous glucocorticoids (oral, inhaled, cutaneous, rectal, high-dose progestagens, and their potentiation by antiretroviral drugs) should be excluded.

See Online for appendix

Three screening tests are recommended: late night salivary cortisol, 24 h urine free cortisol (UFC) and the 1 mg overnight (or two-day, 2 mg) dexamethasone suppression test (appendix).[1] To optimise sensitivity, despite decreased specificity, guidelines recommend using the upper limit of the reference range for UFC and salivary cortisol and a cortisol concentration less than 50 nmol/L (1·8 μg/dL) after dexamethasone as the cut-off for healthy responses.[1]

Patients with a high pre-test probability of being affected, who have two abnormal screening test results, can be diagnosed with Cushing's syndrome.[1] Patients with possible cyclic hypercortisolism, minimum clinical features, or mixed or mildly abnormal responses might need repeated or additional testing and should be followed up with serial salivary cortisol or UFC concentrations for progression.

Some patients have physiological hypercortisolism and minimum features of Cushing's syndrome, but no tumour. These patients have been referred to as having pseudo-Cushing's syndrome based on the biochemical results, and various disorders (appendix) should be considered. Generally, hypercortisolism resolves as these disorders are treated or remit.

Establishing the cause of Cushing's syndrome

Cushing's syndrome with suppressed ACTH

Once Cushing's syndrome is diagnosed, the cause should be identified to decide/determine a specific treatment. Sustained excess of cortisol leads to CRH and ACTH suppression from healthy corticotroph cells (figure 2). Thus, the first step in the differential diagnosis of Cushing's syndrome is measurement of plasma ACTH;[2–4] however, various commercially available ACTH assays are imprecise in the low ranges and should be interpreted with caution.[74]

A decreased plasma ACTH concentration (<2·2 pmol/L or <10 pg/mL) in a patient with overt endogenous hypercortisolism usually suggests an adrenal cause[2–4] (figure 2 and figure 3). Intermediate ACTH values between 2·2 pmol/L and 4·4 pmol/L (10 pg/mL and 20 pg/mL) are less clear, but if Cushing's

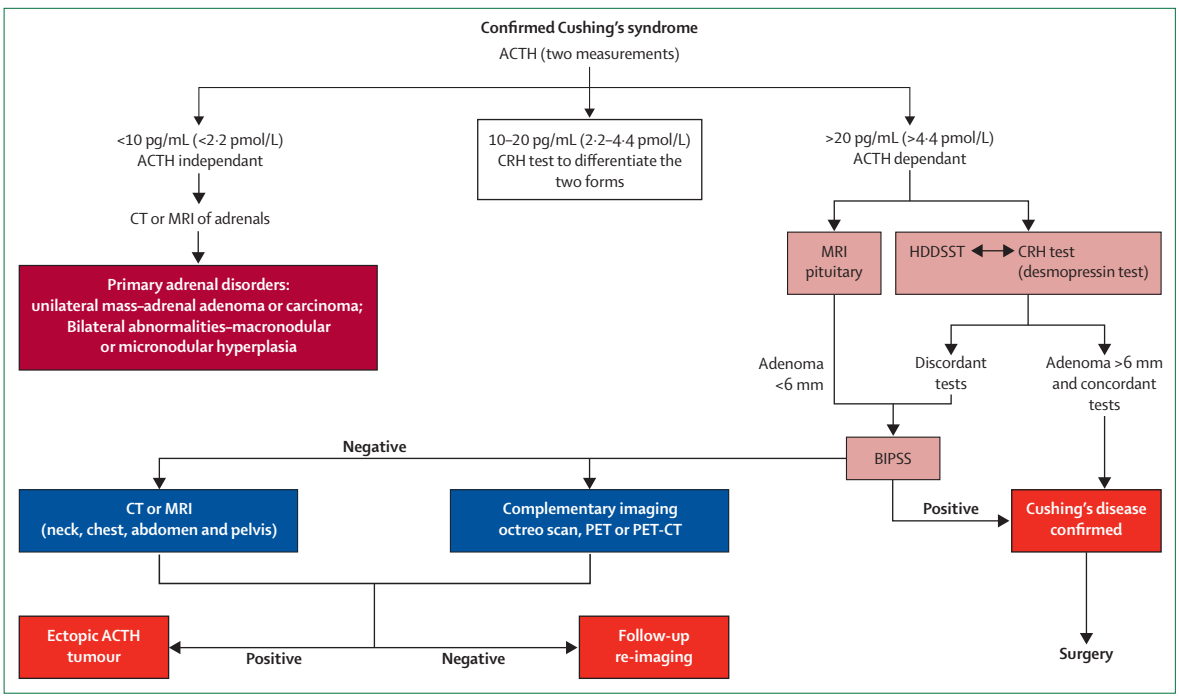

Figure 3: **Clinical decision-making flow chart for the differential diagnosis of confirmed Cushing's syndrome of different causes**
Modified from reference 170 by permission of The Endocrine Society Press. ACTH=adrenocorticotropic hormone. CRH=corticotropin-releasing hormone. HDDSST=high dose dexamethasone suppression test. BIPSS=bilateral inferior petrosal sinus sampling.

syndrome is moderate-to-severe, it is probably ACTH-dependent. When cortisol secretion is modest or cyclic, ACTH suppression might be incomplete; a CRH stimulation test might unmask ACTH responsiveness.[2–4] If ACTH is decreased, CT imaging of the adrenal glands can identify its cause, usually a unilateral adenoma (table 1).[2–4] A suspicious lesion (morphology or large size) or raised DHEAS concentrations increase the possibility of adrenal carcinoma; ^{18}F-fluorodeoxyglucose (FDG) PET scan and MRI might be needed before open adrenalectomy.[47]

In bilateral micronodular hyperplasias, the adrenal glands are not enlarged, but occupied by several small bead-like nodules on high resolution CT.[4,16] In PPNAD, cortisol is usually poorly stimulated by exogenous ACTH. A paradoxical UFC increase during sequential low and high doses of dexamethasone tests is common.[75]

In BMAH, plasma 17-OH-progesterone or urinary 17-OH-corticosteroids are proportionally more raised than urinary free cortisol (UFC) concentrations, as a result of abnormal activity of several steroidogenic enzymes.[51,57] Screening for aberrant GPCR with drugs that modulate the concentration of receptor ligands might help to confirm BMAH.[37,51,57,58] Adult family members of patients with BMAH should undergo screening for excess cortisol with the 1 mg overnight dexamethasone test; those with cortisol responses greater than 50 nmol/L (1·8 µg/dL) should have adrenal imaging.[37,53,55,56] Genetic testing might become available in the near future.[52–56]

ACTH-dependent Cushing's syndrome

Pituitary imaging is done only after biochemical confirmation of ACTH-dependent Cushing's syndrome (figure 3). T1-weighted spin echo MRI with gadolinium contrast identifies pituitary tumours in roughly 50% of patients with Cushing's disease.[2–4] About 10% of healthy individuals have incidental lesions up to 6 mm in diameter.[2–4] Finding a pituitary lesion less than or equal to 6 mm does not reliably identify Cushing's disease as the cause of Cushing's syndrome. Other MRI techniques might have better sensitivity: spoiled gradient recalled acquisition in the steady state technique (SPGR) had better sensitivity for detection of microadenoma than T1 spin echo imaging in adults (80% *vs* 49%) and children (68% *vs* 29%).[76,77]

No single best approach to testing patients with ACTH-dependent Cushing's syndrome exists. Because bilateral inferior petrosal sinus sampling (BIPSS) is invasive, some physicians prefer to schedule it only if other tests (such as high-dose dexamethasone, CRH, or desmopressin tests) are in favour of ACTH-dependent Cushing's syndrome and no pituitary adenoma is seen on MRI. Other physicians proceed directly to inferior petrosal sinus sampling (IPSS), some verify response to CRH or desmopressin to choose which to use during IPSS, whereas those without access to the procedure rely on non-invasive tests when a pituitary lesion is detectable on MRI. We recommend BIPSS in every patient with confirmed ACTH-dependent Cushing's syndrome in whom a pituitary lesion is less than 6 mm on MRI or in whom non-invasive tests are discordant (figure 3).

BIPSS is the gold standard test to identify a pituitary versus ectopic source of ACTH, with sensitivity and specificity of roughly 95%.[2–4] A pituitary source results in a central-to-peripheral ACTH gradient of more than two before CRH or desmopressin administration and more than three afterwards (figure 3). False-positive results can occur in patients with ectopic ACTH with cyclic or mild hypercortisolism without suppression of healthy corticotropes, or in CRH-producing tumours. Abnormal venous drainage or inability to cannulate the veins could cause false-negative results.[78] Measurement of prolactin (to normalise ACTH values) in cases without a gradient can confirm successful catheterisation.[79] In 396 patients, all ten with false-negative results (none with positive results) had peak petrosal sinus ACTH values of less than 400 pg/mL.[80] BIPSS has restricted value in predicting intra-pituitary tumour location; a side-to-side gradient of more than 1·4 correctly identified tumor localisation in only 69% of cases.[80]

Non-invasive tests are less accurate than BIPSS in identifying the source of ACTH, but they can contribute to confirming abnormal ACTH and cortisol regulation. Most Cushing's disease adenomas maintain sensitivity to CRH stimulation and suppression with high dose dexamethasone; most malignant tumours that ectopically produce ACTH are resistant to CRH, desmopressin, or dexamethasone administration, but some benign carcinoid tumours might be regulated in the same way as pituitary corticotroph tumours[2–4] (figure 2). Results are heterogeneous with human versus ovine CRH, different ACTH assays, and different criteria for ACTH and cortisol response. The two largest studies reported a sensitivity of 93%[81] and 70%[82] for the ACTH response to CRH, and both reported a specificity of 100%. For the cortisol response, sensitivity was 91% and 85%[81] and specificity was 88% and 100%[82] to detect Cushing's disease. By contrast with most healthy individuals, desmopressin can stimulate ACTH release from a high proportion of corticotroph adenomas via activation of vasopressin (AVPR2 and AVPR1B) receptors.[2–4,83] However, some ectopic ACTH-secreting tumours (mostly benign carcinoids) express those receptors and respond to desmopressin, so the test cannot always distinguish the ACTH source.[83]

Various protocols with oral or intravenous high-dose dexamethasone suppression tests (HDDST) are used.[2–4] With more than 50% suppression of cortisol concentrations to suggest Cushing's disease, up to 30% of patients have false results for the 8 mg overnight test or the oral 2-day 8 mg HDDST (figure 2).[13,14,84] Although a positive response to both CRH and HDDST suggests Cushing's disease,[85] discordant results are recorded in up to 65% of patients.[2–4]

Thin-cut CT or MRI of thorax and abdomen and scintigraphic studies localise tumours in 70–90% of ectopic ACTH cases.[13,14] Neuroendocrine tumours express somatostatin receptors and can be identified with indium[111]. In-pentetreotide scintigraphy (octreoscan), with a sensitivity ranging from 25% to 80% depending on dose and use of single-photon emission tomography and CT fusion.[13,14,86] Hypercortisolism can suppress tumoral somatostatin subtype 2 receptor concentrations, potentially causing false-negative results; negative octreoscan can become positive after medical control of hypercortisolism.[87] ^{18}F-FDG PET has little additional value. ^{18}-L-3,4-dihydroxyphenylalanine-PET and ^{11}C-5-hydroxy-tryptophan-PET might better detect ACTH-producing neuroendocrine tumours, but no large studies have been reported.[88,89] Chromogranin A, 5-hydroxy-indolacetic acid, calcitonin, and gastrin might point to ectopic ACTH tumours.[13,14]

Treatment

Initial pituitary surgery for Cushing's disease

Transsphenoidal selective tumour resection (TSS) is the optimum initial treatment of Cushing's disease, meeting all therapeutic goals.[3,4] TSS might not be feasible in patients with high anaesthesia risk, or with invasive macroadenomas. The success of TSS depends on the surgeon's expertise because tumours might be small, difficult to recognise, or have dural invasion; piecemeal resection seems less successful than the histological pseudocapsule technique.[90] TSS complications (hypopituitarism, diabetes insipidus, syndrome of inappropriate antidiuretic hormone secretion, and visual loss) are more likely to occur with macroadenomas or extensive pituitary exploration.[2–4]

After successful tumour resection, concentrations of ACTH and cortisol are low and glucocorticoid replacement is needed. No consensus on the criteria for remission exists, or whether perioperative glucocorticoids change the results.[2–4] Most studies show prolonged remission when postoperative (within 7 days) cortisol concentrations are less than 138 nmol/L (<5 μg/dL) or if UFC concentrations are less than 28–56 nmol/day (<10–20 μg/day).[2–4] Patients in remission have gradual resolution of the signs of Cushing's syndrome and slow recovery of hypothalamic-pituitary-adrenal axis (HPA) function for a year or more. A combined 1 mg overnight dexamethasone test followed by desmopressin test (10 μg intravenous) was an early predictor of recurrence when both ACTH and cortisol increased by more than 50% (100% sensitivity and 89% specificity).[91]

Immediate and long-term remission rates decrease in the presence of a macroadenoma, dural or cavernous sinus invasion, postoperative eucortisolism (in the absence of preoperative or postoperative medical treatment), and absence of tumour on MRI or ACTH-positive tumour on pathology.[3,92] Long-term recurrence rates are as high as 34%.[2–4,93,94] Homozygosity for fibroblast growth factor receptor 4 (*FGFR4*) Gly388 allele and *FGFR4* overexpression were associated with raised frequency of persistence and recurrence.[95] All patients should have ongoing surveillance and might need additional treatment.

A longitudinal study[96] showed that enhancement of midnight salivary cortisol precedes increase of urinary cortisol concentrations in patients who eventually relapse; in view of the variability of late night salivary cortisol, a screening strategy with three or four samples collected on successive days was recommended in one study.[97] Overnight 1 mg dexamethasone suppression tests with or without desmopressin administration might be helpful during long-term follow-up.[91]

Second pituitary surgery is a good option when residual tumour is visible or has regrown but is not invasive.[2–4] Hypopituitarism is more probable after additional TSS, and resection success rates are lower (50–73% *vs* 81%), especially if no tumour is identified and hemihypophysectomy is done.[98] Successful tumour resection is more likely when the initial exploration was incomplete.

Medical therapy

Indications for medical treatment of Cushing's syndrome include acute complications of hypercortisolism (psychosis, and infection); surgery pretreatment in severe cases if surgery is delayed; hypercortisolism after unsuccessful surgery, while awaiting control from radiotherapy; unresectable or metastatic tumours; and hypercortisolism due to an occult ectopic ACTH-producing neuroendocrine tumour.[99] Treatments include steroidogenesis inhibitors, tumour-directed drugs, and glucocorticoid receptor antagonists;[99] a combination of drugs might be necessary to achieve eucortisolism.[100] Treatment should be individualised, considering patient characteristics, drug efficacy, and side-effects (table 2).[99,101]

The imidazole antifungal ketoconazole can decrease cortisol production at doses of 400–1200 mg/day.[102] The most important side-effects of ketoconazole are hepatotoxicity (monitoring of liver enzymes is needed), gastrointestinal complaints, and hypogonadism (in men).[99,102] Unfortunately, ketoconazole has been withdrawn from the European market. Metyrapone selectively inhibits 11β-hydroxylase at doses between 0·5 and 6 g/day; adverse events include hirsutism, hypertension, hypokalaemia, and oedema.[99,103] The availability of these drugs differs between countries. A pilot study[104] with the investigational 11β-hydroxylase inhibitor LCI699 showed encouraging efficacy in the short-term therapy of patients with Cushing's disease.[104] Mitotane is mainly used for treatment of adrenal carcinoma,[47,48] but it also inhibits many steroidogenic enzymes. In view of its serious side-effects (neurological and gastrointestinal), mitotane is less often used for benign tumours.[105] Etomidate can be given intravenously in the intensive care setting to rapidly decrease cortisol concentrations.[106]

Corticotroph adenomas can express dopamine (D2) and somatostatin receptors, which can be targeted with specific agonists.[107,108] The D2-receptor agonist cabergoline, at doses of 0·5–7 mg/week, induces long-term biochemical remission in about 30% of patients, although escape occurs.[109–111] Side-effects include nausea, headache, and dizziness; cardiac valve fibrosis, seen at higher doses, was not noted in patients given present doses.[112] This treatment represents an off-label use of the drug.

Corticotroph tumour cells express mainly somatostatin receptor subtype 5 (sst_5), but also sst_2.[107] Pasireotide is a multisomatostatin receptor ligand with affinity for sst_1, sst_2, sst_3, and mostly sst_5 receptor subtypes.[113] A trial[114] of 162 patients showed that subcutaneous pasireotide at a dose of 600 µg or 900 µg twice a day normalised UFC production in 15% (n=12) of patients receiving the 600 µg dose and 26% (n=21) of patients receiving the 900 µg dose. Hyperglycaemia via inhibition of incretins and insulin release[114,115] occurred in 73% (n=118) of patients. The use of daily pasireotide for treatment of

	Dose	Main side-effects
Pituitary tumour-directed drugs		
Pasireotide	750–2400 µg per day subcutaneously injected	Hyperglycaemia, gastrointestinal complaints, and gall stones
Cabergoline	Up to 7 mg per week orally	Gastrointestinal complaints, dizziness, headache, and possible risk of cardiac valvulopathy
Retinoic acid*	10–80 mg per day orally	Arthralgia, dryness of mouth and conjunctiva, headache, and gastrointestinal complaints
Steroidogenesis inhibitors		
Metyrapone	0·5–4·5 g per day orally	Gastrointestinal complaints, rash, hirsutism, hypertension, and hypokalaemia
Ketoconazole	400–1600 mg per day orally	Gastrointestinal complaints, gynaecomastia, hypogonadism, hepatotoxicity
Mitotane	3–5 g per day orally	Gastrointestinal complaints, gynaecomastia, hepatotoxicity, hypercholesterolaemia, adrenal insufficiency, and neurotoxicity
Etomidate	0·1–0·3 mg/kg/h intravenously	Gastrointestinal complaints, myoclonus, and pain at injection site
LCI699*	4–100 mg per day orally	Gastrointestinal complaints, fatigue, headache, dizziness, arthralgia, and hypokalaemia
Glucocorticoid receptor antagonists		
Mifepristone	300–1200 mg per day orally	Clinical adrenal insufficiency, endometrial hyperplasia, hypertension, oedema, and hypokalaemia

*Retinoic acid and LCI699 were only assessed in two pilot clinical studies with seven and 12 patients respectively.

Table 2: **Summary of drugs for Cushing's syndrome**

Cushing's disease when surgery is not an option is approved by regulatory agencies in Europe, the USA, and Canada; a monthly formulation is under assessment.

Other strategies have been assessed in preliminary studies. In view of the frequent coexpression of D2 and sst_5 receptors on corticotroph adenomas,[107] combined targeting of both receptors was assessed in 17 patients started on pasireotide with sequential addition of cabergoline and ketoconazole if UFC concentrations did not normalise.[116] Biochemical remission was achieved in 88% (n=15) of patients with three drugs.[116] Combination of cabergoline (2–3 mg/week) with low-dose ketoconazole (200–400 mg)[111,117] or daily oral doses of retinoic acid[118] normalised UFC concentrations in some patients.

Mifepristone is a glucocorticoid and progesterone receptor antagonist that can ameliorate the signs and symptoms of Cushing's syndrome.[119,120] Because it increases ACTH and cortisol concentrations in patients with Cushing's disease,[120] clinical cortisol-dependent variables (hyperglycaemia and hypertension) should be used to adjust the dose. The absence of a measurable marker greatly restricts the ability to judge overtreatment or undertreatment. Adverse events include symptoms of cortisol insufficiency (fatigue, nausea, vomiting, arthralgias, and headache), increased mineralocorticoid effects (hypertension, hypokalaemia, and oedema), and antiprogesterone effects (endometrial thickening).[120] Mifepristone is approved in the USA for the treatment of hyperglycaemia related to Cushing's syndrome in non-surgical candidates.

Radiotherapy

Pituitary radiotherapy is a good primary therapy for non-surgical candidates and is a second-line approach for persistent or recurrent disease after TSS, particularly when the tumour is invasive and not surgically resectable.[2–4] Conventional fractionated external beam radiotherapy delivers 1·7–2 Gγ daily for a total dose of 45 Gγ. Intensity-modulated radiotherapy (IMRT) allows further dose adjustment for tumour contours and spares nearby crucial structures. Conventional radiotherapy results in remission in roughly 50–83% of patients, from 6 to 60 months after treatment, but usually within 2 years; two-thirds of patients develop pituitary hormone deficiency.[3,121] Radiosurgery, which delivers radiation in a single setting, achieved remission in 54–83% of patients followed up for 5 years.[122] Hypopituitarism is common after radiosurgery, whereas cranial nerve damage is rare.[122] A restored diurnal rhythm is not necessarily achieved, so late night cortisol concentration should not be a criterion for remission.

Bilateral adrenalectomy

Bilateral adrenalectomy is the definitive treatment for Cushing's syndrome when rapid eucortisolism is necessary or when other therapies have failed.[2–4,123,124] Candidates for this treatment might include premenopausal woman who desire pregnancy soon after correction of Cushing's syndrome. Laparoscopic adrenalectomy has decreased the morbidity of this procedure.[123,124] Patients who undergo adrenalectomy need life-long glucocorticoid and mineralocorticoid replacement and individuals must be educated to avoid acute adrenal insufficiency episodes.

Up to 8–25% of patients could develop corticotroph tumour progression (tumour appearance or enlargement of more than 2 mm on MRI) after bilateral adrenalectomy for Cushing's disease.[2–4,125] Corticotroph tumour progression was reported in 25 of 53 patients without previous radiotherapy; only four patients developed a macroadenoma and one developed a pituitary apoplexy.[125] Patients with high postoperative plasma ACTH concentrations are more likely to develop tumour progression. Some investigators use plasma ACTH concentrations of more than 500 pg/mL (100 pmol/L) with hyperpigmentation as criteria for Nelson's syndrome diagnosis.[126] Patients adrenalectomised for Cushing's disease should be followed up regularly with pituitary MRI and measurement of ACTH concentrations; trans-sphenoidal surgery should be done before development of macroadenoma.[2–4] Routine pituitary radiotherapy after adrenalectomy is controversial, but does not seem necessary because close monitoring with MRI is available;[2–4] this procedure is recommended for patients with non-resectable tumours.[3,4,125,126] Long-term cabergoline or long-acting pasireotide were occasionally effective in reducing ACTH concentrations and tumour size.[127,128]

Treatment of primary adrenal causes

Surgical therapy

Minimally invasive unilateral adrenalectomy is the standard of care for cortisol-secreting unilateral adenomas.[129] Laparoscopic procedures are safe, effective, and less expensive than open adrenalectomy.[129] Open adrenalectomy is recommended if adrenocortical cancer is suspected.[47,48] Glucocorticoid replacement therapy is needed until the hypothalamic–pituitary–adrenal axis recovers. In non-resectable adrenal cancer with Cushing's syndrome, medical therapy with steroidogenesis inhibitors and mitotane is used.[47,48,99]

Bilateral adrenalectomy is the usual treatment for patients with BMAH and PPNAD with overt Cushing's syndrome.[2–4,15,16,124] In patients with BMAH and mildly increased cortisol production (UFC less than twice the upper limit of normal), unilateral adrenalectomy might be effective;[15,130] eventual increased contralateral secretion might necessitate a completion adrenalectomy.

The role of aberrant GPCR in BMAH allows for specific pharmacological therapies that might avoid bilateral adrenalectomy. Blockade of postprandial GIP release with octreotide[37,131] or pasireotide[131] led to transient improvement, but eventual escape. In catecholamine-dependent BMAH, β blockers achieved long-term control of Cushing's syndrome.[37,132,133] In luteinising hormone or

human chorionic gonadotropin-dependent BMAH and Cushing's syndrome or androgen excess, suppression of luteinising hormone with leuprolide acetate controlled steroidogenesis and avoided bilateral adrenalectomy.[134,135] No tumour regression occurred, however, because other proliferative factors might be present.[52,136,137] Development of specific corticotropin receptor (MC2R) antagonists might offer future targeted treatment for hypercortisolism and prevention of disease progression in familial BMAH with paracrine ACTH secretion.[60,61]

Ectopic ACTH syndrome

Ideally, ectopic ACTH-secreting neuroendocrine tumours should be resected. Possible metastases should be biopsied to establish histological diagnosis and guide management. When complete surgical resection is impossible, tumour chemotherapy, steroidogenesis inhibitors, mifepristone, or bilateral adrenalectomy can control Cushing's syndrome.[3,4,13,14,99] Medical therapy to inhibit ectopic ACTH production with dopamine agonists or somatostatin analogues might be useful, either alone or in combination, although treatment escapes occur.[138,139]

Paediatric Cushing's syndrome

About 10% of cases of Cushing's syndrome occur in children, with a female-to-male predominance as in adults; in very young children, a male-to-female predominance might exist.[140,141] The most common symptom of paediatric Cushing's syndrome is weight gain, but a unique feature is the effect on linear growth; the combination of weight gain and decreased height velocity is pathognomonic for paediatric Cushing's syndrome. Other manifestations include facial plethora, headaches, hypertension, hirsutism, virilisation, or delayed sexual development. Acne, violaceous striae, bruising, and acanthosis nigricans are also common. The investigation and treatment of paediatric Cushing's syndrome are similar to those in adults.[140,141]

Before the age of 7 years, adrenal causes of Cushing's syndrome (adenoma, carcinoma, or bilateral hyperplasia) are most common, whereas Cushing's disease accounts for roughly 75% of Cushing's syndrome after that age.[140] Pituitary blastomas are rare aggressive tumours of the neonatal pituitary that can produce ACTH and severe Cushing's disease in neonates as a result of germline *DICER1* mutations.[142] Cushing's syndrome is present in a third of paediatric adrenal cancers; most occur before the age of 5 years, with a female-to-male predominance. Micronodular bilateral hyperplasias (PBAD, PPNAD, and isolated micronodular adrenocortical disease) more frequently cause Cushing's syndrome in children than in adults.[140] Ectopic ACTH accounts for less than 1% of Cushing's syndrome in adolescents; neuroblastomas and ganglioneuromatous tumours can rarely secrete ACTH in young infants. Cushing's syndrome can be the first sign of a mutation in a tumour-predisposing gene in a child; for example, *MEN1* and *AIP* mutations were reported in children with Cushing's disease with no known family history of MEN1 or acromegaly.[143] Likewise, Cushing's syndrome can be the first sign of Carney complex or McCune-Albright syndrome.[16,50]

Cyclical Cushing's syndrome

Cyclical Cushing's syndrome has been reported from all causes of the syndrome.[144] Irrespective of age, PPNAD and isolated micronodular adrenocortical disease are often cyclic.[16,69,140] Frequent midnight salivary cortisol and UFC measurements over a long period, sometimes years, might be needed to confirm the diagnosis.[145] Measurement of cortisol in scalp hair shows the historical timeline of cyclic hypercortisolism and could be available in future.[146]

Cushing's syndrome in pregnancy

Pregnancy occurs rarely in Cushing's syndrome, probably because hypercortisolism inhibits ovulation. Cushing's syndrome increases the risk of fetal abortion, perinatal death, premature birth, intrauterine growth retardation, hypertension, diabetes, and pre-eclampsia.[147,148] Diagnosis is complicated by the overlap of UFC concentrations between patients with Cushing's syndrome and healthy pregnant women, and the normal increase in corticosteroid-binding globulin that occurs in pregnancy, which raises cortisol concentrations; criteria for the 1 mg dexamethasone suppression test in pregnancy are not available. Salivary (free) cortisol might be useful.[149] Adrenal causes are more common, but ACTH concentrations are not uniformly suppressed.[147,148] Exacerbation or transient Cushing's syndrome during pregnancy can partly regress post partum if caused by aberrant adrenal luteinising hormone or human chorionic gonadotropin expression.[37,134] TSS or adrenalectomy are preferred treatments, except perhaps late in the third trimester when metyrapone can be used cautiously; remission might not improve the fetal prognosis.[147,148]

Mortality, morbidity, and prognosis of Cushing's syndrome

Mortality in Cushing's syndrome (non-malignant causes) is increased, with a standard mortality ratio (SMR) roughly between 2·0 and 4·0; cardiovascular deaths are most common.[5,7,150–153] Patients with persistent Cushing's disease after pituitary surgery had a SMR between 4·0 and 5·0.[5,7,150,151] Long-term remission improves but does not restore normal SMR in some studies,[7,150–153] but not all.[5,154] Patients with benign adrenal Cushing's syndrome had normal[8,153] or increased SMR.[5,151] Persistent comorbidities despite remission could increase mortality.[155] An increased risk of cardiovascular events and mortality is present in patients with adrenal incidentaloma and a modest increase in cortisol secretion.[156,157]

Chronic hypercortisolism leads to multisystem morbidities.[155] Many cardiovascular risk factors (obesity, hypertension, diabetes, and dyslipidaemia) predispose to myocardial infarction, left ventricular dysfunction, and

cerebrovascular disease.[147,155,158–160] Cushing's syndrome creates a hypercoagulable state due to an activated coagulation cascade and impaired fibrinolysis;[155] patients with non-malignant Cushing's syndrome have a more than ten-fold increased risk of developing venous thromboembolic disease.[161]

Chronic brain exposure to excess cortisol causes structural changes in cerebral areas and affects brain functions.[155,162–165] Major depression and anxiety disorders are common, but acute psychosis is rare.[162,163] Cognitive deficits include memory dysfunction, poor visual memory, impaired decision making, and sleep disturbances.[162–165] The immunosuppressive effects of Cushing's syndrome increase susceptibility to opportunistic infections and sepsis.[155]

These comorbidities might not normalise after successful treatment.[155] Cardiovascular risk factors persist in 40–60% of patients[155,158,159] and psychopathology and neurocognitive dysfunction improve incompletely.[162–165]

Quality of life is severely impaired in Cushing's syndrome owing to physical (eg, fatigue and changed appearance) and psychological (eg, emotional instability and cognitive deficits) factors.[155,164–166] In many patients, including children and young adults, quality of life is still impaired after remission. This impairment might reflect residual comorbidities[155,167] or hypopituitarism in Cushing's disease.[168]

Conclusions

Patients with Cushing's syndrome need complex investigations and long-term follow-up by an experienced multidisciplinary team to identify and correct the cause of their syndrome, monitor possible recurrence, ensure adequate hormonal replacement, and treat the psychological and multi-organ consequences of exposure to excess glucocorticoids.

Contributors

All authors contributed to the literature review and writing of specific sections of this Seminar. AL contributed the initial draft of table 1, figure 2, and figure 3. RAF contributed to table 2, CAS contributed the initial draft of figure 1, and LKN contributed to the appendix. AL was responsible for the integration of the various sections and references, and all authors revised and approved the final version of the Seminar.

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