**CUSHING’S SYNDROME**

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**ABSTRACT**

Cushing’s syndrome results from chronic exposure to excessive circulating levels of glucocorticoids. Cushing’s disease, pituitary-dependent Cushing’s syndrome, is the most common cause of endogenous hypercortisolism. The recommended screening tests include the 1mg overnight dexamethasone suppression test, late-night salivary cortisol (at least 2 samples), and 24-hour urinary free cortisol (at least two 24-hour collections). If the initial test is positive on 2 occasions the patient should be evaluated by an endocrinologist for further assessment. Plasma 09:00h ACTH measurement guides imaging and further investigations. If ACTH is elevated/inappropriately normal, MRI scanning of the pituitary should be performed, but if ACTH is suppressed imaging of the adrenals should follow. The corticotrophin releasing hormone (CRH) or desmopressin tests helps distinguishing pituitary from ectopic ACTH-dependent Cushing's syndrome, while bilateral petrosal sinus sampling remains the gold standard test and should be considered, if available, with the exception of the presence of a pituitary macroadenoma. It is prudent to perform a CT of the thorax, abdomen and pelvis in all patients. Transsphenoidal surgery is the first line treatment for Cushing’s disease, followed by radiotherapy as a second-line option. Adrenalectomy is the first-choice treatment for adrenal ACTH-independent Cushing’s syndrome and resection of the ACTH source should be performed for the ectopic ACTH-dependent Cushing’s syndrome, where possible. Bilateral adrenalectomy can always be considered as an option. Steroidogenesis inhibitors remain the most effective medical agents and are useful when surgery or the effects of radiotherapy are awaited or are unsuccessful.

**INTRODUCTION**

# Cushing’s syndrome results from chronic exposure to excessive circulating levels of glucocorticoids. It is now more than one hundred years since Harvey Cushing reported the classical clinical syndrome that bears his name. Even now, its investigation and management can vex the most experienced endocrinologist. It may be difficult to miss the diagnosis in its most florid form but, given the high prevalence of many of its non-specific symptoms such as obesity, muscle weakness, and depression, clinicians are now required to consider the diagnosis in its earlier manifestations. The plethora of investigations often needed for the diagnosis and differential diagnosis has grown over the intervening century, and require careful interpretation. In its severe form and when untreated, the metabolic upset of Cushing's syndrome is associated with a high mortality. However, more subtle excesses of cortisol may also have significant effects on glycemic control and blood pressure, and may therefore be an important cause of morbidity. Treatment is often complex and may require all the modalities of surgery, radiotherapy. and medical management.

**PATHOPHYSIOLOGY, ETIOLOGY, AND EPIDEMIOLOGY OF CUSHING’S SYNDROME**

In normal physiology the final product of the hypothalamo-pituitary-adrenal (HPA) axis is the glucocorticoid cortisol, secreted from the zona fasciculata of the adrenal gland under the stimulus of adrenocorticotrophin (ACTH) from the pituitary gland. ACTH is secreted in response to corticotrophin-releasing hormone (CRH) and vasopressin from the hypothalamus. Cortisol exerts negative feedback control on both CRH and vasopressin in the hypothalamus, and ACTH in the pituitary. In normal individuals, cortisol is secreted in a circadian rhythm; levels fall during the day from a peak at 07.00h-08.00h to a nadir at around midnight: they then begin to rise again at 02.00h.

It is the loss of this circadian rhythm, together with loss of the normal feedback mechanism of the hypothalamo-pituitary-adrenal (HPA) axis, which results in chronic exposure to excessive circulating cortisol levels and that gives rise to the clinical state of endogenous Cushing's syndrome (1, 2). Any of the numerous synthetic steroids that have glucocorticoid activity, if administered in excessive quantities, can give rise to exogenous Cushing's syndrome. This is the commonest cause of Cushing's syndrome seen in general clinical practice, usually due to treatment for chronic conditions such as asthma or rheumatological disease. The clinician needs to carefully search for exogenous exposure to topical, inhaled, or injected forms of corticosteroids.

The etiology of Cushing's syndrome can broadly be divided into two categories, ACTH-dependent and ACTH-independent (Table 1).

ACTH-dependent forms are characterized by excessive ACTH production, which stimulates all three layers of adrenal cortex and results in bilateral adrenocortical hyperplasia and hypertrophy of adrenal gland. This results in increased weight of the adrenals, which often show micronodular or sometimes macronodular changes. Circulating glucocorticoids are increased and often, to a lesser extent, are accompanied by a rise in serum androgens.

ACTH-independent forms constitute a heterogeneous group characterized by low levels of plasma ACTH, either because of adrenal glucocorticoid hypersecretion or secondary to the exogenous administration of glucocorticoids. Except for adrenal adenomas, which usually secrete only glucocorticoids, among the other endogenous adrenal entities there is usually also a rise in androgens and sometimes steroid precursors. The microscopic and macroscopic appearance of non-affected adrenal tissue mainly depends on the etiology of the disorder.

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| **Table 1. Etiology of Cushing's Syndrome** |
| **ACTH-dependent** |
| Pituitary-dependent Cushing's syndrome (Cushing's disease) |
| Ectopic ACTH syndrome |
| Ectopic CRH syndrome (very rare) |
| Exogenous ACTH administration |
| **ACTH-independent** |
| Adrenocortical adenoma |
| Adrenocortical carcinoma |
| ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) – now known as bilateral macronodular adrenocortical disease (BMAD)(3) |
| Idiopathic micronodular adrenocortical disease (i-MAD) |
| Primary pigmented (micro)nodular adrenocortical disease (PPNAD, <1cm nodules), associated with Carney complex (c-PPNAD) or idiopathic (i-PPNAD) |
| McCune-Albright syndrome |
| Exogenous glucocorticoid administration |

**ACTH-Dependent Cushing's Syndrome**

CUSHING’S DISEASE

Pituitary-dependent Cushing's syndrome, better known as Cushing's disease, is the most common cause of endogenous Cushing’s syndrome, accounting for 60-80% of all cases. Epidemiologic studies from Europe suggest an incidence of between 0.7 and 2.4 per million per year (4, 5). It presents much more commonly in women, usually between 25 and 40 years of age.

It is almost always due to a corticotroph adenoma (6, 7). Although apparent nodular corticotroph hyperplasia (in the absence of an CRH-producing tumor) has been described, it is rare in large surgical series (8, 9), and its existence is still debated.The majority of tumors are intrasellar microadenomas (<1 cm in diameter), although macroadenomas account for approximately 5-10% of tumors, and extrasellar extension or invasion may occur. True pituitary corticotroph carcinomas with extra-pituitary metastases causing Cushing's syndrome have also rarely been described (10, 11).

Despite much research, the molecular pathogenesis of corticotroph adenomas remains unknown, but the evidence supports the concept of primary pituitary rather than a hypothalamic disorder (12). However, recent data suggest that around one-third are due to a somatic mutation causing constitutive activation of USP8, a deubiquitinase which leads to increased expression of the EGF receptor on corticotrophs (13). Corticotroph adenomas could rarely be associated with familial syndromes such as MEN1, MEN2, Carney Complex, or familial isolated pituitary adenoma syndrome. Those are secondary to mutations in the menin gene (*MEN1*), the *RET* oncogene, PRKR*1A* and the *AIP* (gene coding for aryl hydrocarbon receptor-interacting protein) respectively (14). Very rarely, Cushing’s disease has been described in individuals with McCune-Albright and Beckwick-Wiedemann syndromes, where ACTH-independent CS is more common.

Up to 40 percent of older patients with long-existing Cushing’s disease develop ACTH-dependent macronodular adrenocortical hyperplasia. The adrenals tend to be enlarged, with occasional prominent nodules, but invariably with internodular hyperplasia (15, 16); the level of ACTH may be lower than anticipated, and recovery of the hypercortisolemia delayed after apparent removal of the pituitary tumor.

ECTOPIC ACTH SYNDROME AND ECTOPIC CRH TUMORS

Most other cases of endogenous ACTH-dependent Cushing’s syndrome, after excluding Cushing’ disease, are associated with non-pituitary tumors secreting ACTH, referred to as the ectopic ACTH syndrome. Ectopic sources of ACTH derive from a diverse group of tumor types, which can broadly be divided into the group of highly malignant carcinomas and the more indolent group of neuroendocrine tumors, although this may be thought of as a continuum rather than as a binary separation. This may not be evident from series at endocrine centers where often more occult tumors are investigated (Table 2), but bronchial neuroendocrine tumors tend to predominate and account for up to 25% of ectopic ACTH-dependent Cushing’s syndrome cases. The next in frequency is small-cell lung carcinoma, causing around 19% of ectopic Cushing's syndrome (17-19). Around 16% patients with an ectopic source of ACTH remain occult and require repeat imaging. The ectopic ACTH syndrome is more common in men, and usually presents after the age of 40 years, but should always be considered, even in children.

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| **Table 2. Etiology of the Ectopic ACTH Syndrome in Patients (17-19)** | |
| **Tumor type** | **Percentage of total Ectopic Cushing's syndrome cases reported in selected literature (n=398)** |
| Lung carcinoma | 18.8 |
| Bronchial neuroendocrine tumor | 25.4 |
| Thymic neuroendocrine tumor | 7.3 |
| Medullary cell carcinoma | 4.5 |
| Pancreatic or gastrointestinal NET | 11.8 |
| Phaeochromocytoma/paraganglioma | 3.8 |
| NET of unknown primary | 6.0 |
| Occult tumor | 16.1 |
| Miscellaneous malignant tumors | 6.3 |

NET - neuroendocrine tumor

The ACTH precursor molecule, pro-opiomelanocortin (POMC) is expressed not only in normal pituitary but also in several normal extra-pituitary tissues, as well as in some tumors (lung, testis) (20). The mechanism by which these non-corticotroph tumors express the *POMC* gene is not fully understood, but may be related to hypomethylation of the POMC promoter (21, 22). In general, such tumors tend to produce higher amounts of POMC compared to ACTH, in contrast to the situation in Cushing’s disease. As well as producing ACTH and POMC, these tumors may also produce other pre-ACTH precursor peptides, so-called "big" ACTH (23, 24), which may potentially be helpful in the differential diagnosis of these tumors (25). However, assays for these are not routinely available in clinical practice. Isolated ectopic CRH production is difficult to diagnose and exceedingly rare, with few confirmed cases described in the literature (26). In general, patients secreting CRH ectopically usually also secrete ACTH, rendering the distinction of little practical value.

**ACTH-Independent Cushing’s Syndrome**

ACTH-independent causes of Cushing’s syndrome, apart from exogenous glucocorticoids, encompass a heterogeneous group of diseases. The most common pathology is an adrenal adenoma or carcinoma. The latter may lack some of the classic histological features of malignancy, but can usually be differentiated on the basis of weight (more than 100g), nuclear pleomorphism, necrosis, mitotic figures, and vascular or lymphatic invasion. These features are incorporated in the Weiss score for the distinction between adenomas and carcinomas.

Adrenal adenomas occur most often around 35 years of age and are significantly more common in women, with an incidence of approximately 0.6 per million per year (5). The incidence of adrenal cancer is approximately 0.2 per million per year (5). It is one and a half times more common in women, and has a bimodal age distribution, with peaks in childhood and adolescence, and 40-50 years (1, 27). Approximately 50-60% of adrenocortical carcinomas secrete adrenal hormones of which the most common are glucocorticoids and adrenal androgens (28).

Bilateral macronodular adrenocortical disease (BMAD, previously known as ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH)) is a rare form of Cushing’s syndrome with sometimes huge nodular (>5cm) adrenal glands with more than 1cm nodules on imaging. Most cases are sporadic, but a few familial cases have been reported (29). In most the etiology is unknown, but in a few cases the nodules have been shown to express increased numbers of receptors normally found on the adrenal gland, or ectopic receptors that then can stimulate cortisol production. Most present as subclinical CS. The best described example is food-dependent Cushing’s syndrome, in which ectopic glucose-dependent insulinotropic polypeptide (GIP) receptors on the adrenal glands respond to GIP released after a meal causing hypercortisolemia (30). Treatment with octreotide may ameliorate the syndrome (31); however, the effect decreases after few months due to down-regulation of somatostatin receptors in the intestine (32). Abnormal expression of vasopressin, β-adrenergic, luteinizing hormone/human chorionic gonadotrophin, serotonin, angiotensin, leptin, glucagon, IL-1, and TSH have also been described and functionally linked to cortisol production (32). BMAD tissue may express more than one of these aberrant receptors (33). Around one-third of patients with BMAD have been found to show inactivating germline mutations of the tumor suppressor gene *ARMC5* (armadillo repeat containing protein 5), with each of the nodules demonstrating second independent hits in the same gene: familial forms of BMAD have been described (34). Heterozygous germline pathogenic variants in *KDM1A* gene encoding lysine-specific demethylase 1 have been reported in GIP-dependent Cushing’s syndrome in BMAD (35). In some individuals with BMAD, germline mutations in *MEN1, FH* (fumarate hydratase gene), and *ACP* (familial polyposis coli gene) have been found (36, 37)

Cushing’s syndrome due to bilateral nodular adrenal disease can also be a feature of McCune-Albright syndrome (38). The characteristic features are fibrous dysplasia of bone, café-au-lait skin pigmentation, and endocrine dysfunction: pituitary, thyroid, adrenal, or most commonly gonadal hyperfunction (precocious puberty). This condition is caused by an activating mutation in the *GNAS* gene encoding for the α-subunit of the G protein stimulating cyclic adenosine monophosphate (cAMP) formation. This occurs in a mosaic pattern in early embryogenesis (39). However, if this affects some adrenal cells the constitutive activation of adenylate cyclase leads to nodule formation and glucocorticoid excess. The normal adrenal cortex, where the mutation is not present, becomes atrophic (40, 41).

Primary pigmented nodular adrenal disease (PPNAD), otherwise known as micronodular adrenal disease, is another rare form of Cushing’s syndrome. It is characterized by small or normal-size adrenal glands with cortical micronodules (average 2–3 mm) that may be dark or black in color. The internodular cortex is usually atrophic, unlike in ACTH-dependent macronodular hyperplasia (42). Cases of PPNAD have been reported without Cushing’s syndrome. Bilateral adrenalectomy is curative. 70% of PPNAD occur as part of the Carney complex in association with a variety of other abnormalities, including myxomas of the heart, skin or breast, hyperpigmentation of the skin, and other endocrine disorders (sexual precocity; Sertoli cell, Leydig cell, or adrenal rest tumors; and acromegaly). Cushing’s syndrome occurs in approximately 30% of cases of Carney complex. The tumor suppressor gene *PRKAR1A* (type 1A regulatory subunit of protein kinase A) has been shown to be mutated in over 70% of patients with Carney complex. A few cases of pituitary corticotrophinoma have been identified in patients with Carney complex, one of them having both adrenal and pituitary Cushing’s syndrome (43, 44). In isolated PPNAD, mutations in *PRKAR1A* and also the phosphodiesterase 11A (*PDE11A*) gene have been demonstrated (45, 46).

A missense mutation of the ACTH receptor resulting in its constitutive activation and ACTH-independent Cushing’s syndrome has also been reported (47).

Other very rare causes of Cushing's syndrome have been reported: adrenal rest tissue in the liver, in the adrenal beds, or in association with the gonads which may produce hypercortisolemia, usually in the context of ACTH-dependent disease after adrenalectomy (48, 49). Ectopic cortisol production by an ovarian carcinoma has also been noted (50).

**Exogenous Cushing’s Syndrome**

The basis for iatrogenic Cushing’s syndrome was discussed earlier. The development of the features of Cushing’s syndrome depends on the dose, duration, and potency of the corticosteroids used in clinical practice. ACTH is rarely prescribed nowadays, but it will also result in Cushingoid features if administered long-term. Some features, such as an increase in intraocular pressure, cataracts, benign intracranial hypertension, aseptic necrosis of the femoral head, osteoporosis, and pancreatitis, are reported as more common in iatrogenic than endogenous Cushing’s syndrome, whereas other features, notably hypertension, hirsutism, and oligomenorrhoea/amenorrhea, are less prevalent. However, it is unclear as to whether these are true differences (51).

**Pseudo-Cushing's Syndrome**

Pseudo-Cushing's states are conditions in which a patient presents with clinical features suggestive of true Cushing's syndrome and with some biochemical evidence of hypercortisolemia. Both resolve after resolution of the predisposing condition. The pathophysiology has not clearly been established. Depression and alcohol abuse are the two most common such states (1).

**CLINICAL MANIFESTATIONS OF CUSHING’S SYNDROME**

The clinical manifestations in Cushing’s syndrome result from a chronic exposure to excess glucocorticoids and show a wide spectrum of abnormalities, from mild, subclinical disease to florid manifestations.

The classical impression of the disease in its most obvious form, as the association of gross obesity of the trunk with wasting of the limbs, facial rounding and plethora, hirsutism with frontal balding, muscle weakness, spontaneous bruising, vertebral fractures, hypertension and diabetes mellitus, is less commonly seen nowadays (Table 3) (52-54). More frequently, the clinical diagnosis may be equivocal because many symptoms common in Cushing's syndrome, including lethargy, depression, obesity, hypertension, hirsutism, and menstrual irregularity, are also very common in the general population. Therefore, it is useful to have an investigation strategy exploring the more *specific* features considering the diagnosis, most helpfully relating to the catabolic features of glucocorticoid excess. It is very helpful to notice the presence of several signs and symptoms, accompanied by a progressive course. Sequential photographs of the patient over many years can be extremely helpful in demonstrating progression to a Cushingoid state.

The clinical manifestations are usually determined by the duration and amplitude of glucocorticoid exposure, but in some aggressive cases of ectopic ACTH secretion, such as small cell carcinoma, symptoms of hypercortisolism are hard to detect because of the predominant malignant signs and symptoms such as weight loss and anorexia. The mean time to diagnosis of Cushing’s syndrome is reported as 34 months, and depends on the cause of glucocorticoid excess with shortest time to diagnosis in the ectopic Cushing’s syndrome (14 months), ACTH-independent CS (30months) and the longest with Cushing’s disease (38 months)(55).

The type of steroid excess is determined by the underlying condition. Adrenal adenomas generally secrete glucocorticoids, but in ACTH-dependent disease or a carcinoma hyperandrogenism is common.

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| **Table 3. Presenting features of patients with Cushing’s syndrome (43-45)** | |
| **Presenting features** | **Prevalence (% of patients)** |
| Weight gain/obesity | 81-97 |
| Muscle weakness/tiredness | 46-67 |
| Round face | 88-92 |
| Skin thinning | 84 |
| Easy bruising | 21-62 |
| Edema | 48-50 |
| Purple wide striae | 35-84 |
| Hirsutism | 56-81 |
| Acne | 19-64 |
| Female balding | 13-51 |
| Dysmenorrhea | 35-84 |
| Reduced libido | 33-100 (higher in men) |
| Hypertension | 68-90 |
| Mental health disorders | 26-62 |
| Recurrent infections | 14-25 |
| Diabetes/impaired glucose tolerance | 43-50 |
| Fractures | 21-56 |

It is important to observe that combinations of Cushingoid features very much depend on the natural course of its underlying cause.

Patients with the ectopic ACTH syndrome usually present with severe and rapidly developing metabolic signs, most prominently anorexia, myopathy, and glucose intolerance. Because of severe hypercortisolemia and additional mineralocorticoid effect, hypokalemic alkalosis is found with peripheral edema on clinical examination. The combination of rapid clinical deterioration, hyperpigmentation, hypokalemic alkalosis, and clinical signs of mineralocorticoid excess should be indicative for suspicion of a small cell lung carcinoma secreting ACTH, or a high-grade bronchial carcinoid or pancreatic neuroendocrine tumor. In contrast, most patients with ACTH-producing low-grade bronchial carcinoids, because of the long duration of hypercortisolemia before clinical presentation, tend to develop all of the typical Cushingoid features, complicating its differentiation from Cushing’s disease.

Patients with adrenal carcinomas have a rapid onset of symptoms, and may complain of abdominal pain accompanied with a palpable tumor mass. In addition to hypercortisolism, they often secrete mineralocorticoids and androgens, therefore distinguishing them from benign adenomas which usually secrete cortisol alone (56). In women with androgen secreting ACC acne and hirsutism is usually readily apparent. However, increasingly, these tumors are discovered incidentally after routine scanning for other reasons.

In 10 percent of patients with adrenal incidentalomas, “subclinical” Cushing’s syndrome (currently called mild autonomous cortisol secretion, MACS) can be found; this is characterized by mild hypercortisolism without very obvious clinical manifestations of Cushing’s syndrome (57).

Unlike in men, where the main source of androgens is the testes, in women a substantial proportion of circulating androgens are adrenal in origin, such that the signs and symptoms of adrenal hyperandrogenism are readily diagnosed by symptoms of hirsutism and acne, and signs of virilization.

Obesity and weight gain are among the most common signs in Cushing’s syndrome. The distribution of fat can be useful, as typically in Cushing's syndrome there is increased visceral adiposity giving rise to truncal obesity, fat deposition in the cheeks and temporal fossae ("moon face"), dorsocervical area ("buffalo hump"), and supraclavicular fat pads (52, 58). Rarely, fat deposition in the epidural space can be manifest as a neurological deficit (59), while retroorbital deposition is noticeable as exophthalmos (60). In children, more generalized weight gain associated with growth retardation should highlight the possibility of the diagnosis (2). Other signs that are more discriminatory are proximal myopathy, wide purple striae, osteoporosis, thin skin, and easy bruising. Based on the screening study of 369 individuals with obesity, or weight in the overweight range, there were no reported cases of Cushing’s syndrome (61). Therefore, screening patients with generalized obesity and no specific features of Cushing’s syndrome is generally not recommended.

Myopathy of the proximal muscles of the lower limb and shoulder results from a catabolic glucocorticoid effect and is reported in 40-70% of patients with active Cushing’s syndrome. When assessing for myopathy it is useful to ask questions about function, typically affected by proximal muscle weakness, such as climbing stairs or getting up from a chair. Formal testing can be of leg extension whilst sitting, or rising unaided from a squatting position. Muscle weakness can be exacerbated by hypokalemia, as a result of concomitant mineralocorticoid activity; it is uncommon in pseudo-Cushing’s states (1). The myopathy may not fully recover after cure of hypercortisolism has been achieved (62).

Osteoporosis occurs in approximately 50% of adult patients with Cushing’s syndrome (63) and can be assessed by formal bone densitometry, or from a history of fractures, typically vertebral due to the preferential loss of trabecular bone induced by glucocorticoids. Glucocorticoids inhibit osteoblast function (64). Vertebral compression fractures lead to height loss. Rib fractures are often painless, with typical radiographic appearance of exuberant callus. Also, osteonecrosis (aseptic necrosis) of the femoral head has been described, usually in relation to iatrogenic Cushing’s syndrome following chronic high-dose glucocorticoid therapy (65). After successful treatment of the cause, bone density improves to a large extent (66-68).

There are many changes in the skin and subcutaneous tissue, which are rarely seen in the general population, suggesting the possibility of Cushing’s syndrome (1, 52). The result of hypercortisolemia is thinning of the skin, which is best tested over the dorsum of the hand, visible as “cigarette paper” (Liddle’s sign), but it is helpful to consider the age and gender of the patient as natural atrophy increases with age. In addition, skin thickness may be preserved in women with hyperandrogenemia related to Cushing's syndrome. The classic plethora (facial redness) is not only a consequence of skin thinning but also of a loss of a facial subcutaneous fat. Because subcutaneous fat and elastic tissue is also diminished, patients suffer easy bruising, which often can be misinterpreted as senile purpura or even a coagulation disorder. Purple-colored "violaceous" striae greater than 1 cm in diameter are almost pathognomonic of Cushing's syndrome (Figure 1). Typically seen on the abdomen, they can also occur in other areas, such as the thighs, breasts and arms. Narrow and colored striae are more commonly present, and should be differentiated from the typical healed ‘pearl’ striae seen most commonly post-partum.

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**Figure 1. The wide purple striae on the abdominal wall due to Cushing’s syndrome (patient permission obtained).**

Increased fine non-pigmented vellus hair on the upper cheeks or forehead may be seen in Cushing’s syndrome, as well as more typical terminal hair hirsutism on the face and body, reflecting increased androgens. Cutaneous fungal infections as truncal tinea versicolor and onychomycosis are often found.

Skin hyperpigmentation is much more common in ectopic Cushing’s syndrome (most often from small cell lung carcinoma) than Cushing’s disease. It is also associated with the rapid onset of profound weakness, often with little or no weight gain, and an absence of a gross Cushingoid appearance. However, as noted above, other forms of the ectopic ACTH syndrome, particularly associated with neuroendocrine tumors, may be clinically indistinguishable from patients with other forms of hypercortisolism (69).

Severe hirsutism and virilization strongly suggest an adrenal carcinoma (70).

Hypercortisolism may suppress other pituitary hormones. In both men and women, hypogonadotrophic hypogonadism is common and correlates with the degree of hypercortisolemia (71). Glucocorticoids inhibit gonadotrophin–releasing hormone pulsatility and the release of luteinizing (LH) and follicle-stimulating hormone (FSH). Women experience menstrual irregularity, while both sexes have decreased libido. Gonadal dysfunction is reversible after correction of the hypercortisolemia (72). In addition, the coexistence of polycystic ovarian syndrome in Cushing’s syndrome is common (73). There is reduced GH secretion during sleep and blunted GH responses to dynamic stimulation tests (74). Thyrotrophin-releasing hormone and thyroid-stimulating hormone release has been shown to be disturbed, and in particular the nocturnal surge of thyroid-stimulating hormone is lost (75). This may not have a significant effect on free thyroid hormone levels during active hypercortisolemia, but there is a significantly increased prevalence of autoimmune thyroid disease in patients successfully treated for Cushing’s syndrome, and it is therefore important to follow them with serial thyroid function tests (76, 77).

Hypokalemic metabolic alkalosis is related to the degree of hypercortisolemia and represents a mineralocorticoid action of cortisol at the renal tubule due to saturation of the enzyme 11β-hydroxysteroid dehydrogenase type 2, which inactivates cortisol to cortisone and allows selective binding of aldosterone to the mineralocorticoid receptor (78). When this occurs, cortisol can now access the mineralocorticoid receptor and act as a mineralocorticoid. This hypersaturation occurs when urine free cortisol excretion is greater than about 4100 nmol per day (79). Therefore, although a more common feature of ectopic ACTH secretion, it may also occur in approximately 10% of patients with Cushing’s disease.

Cushing’s syndrome is characterized by insulin resistance and hyperinsulinemia. Glucose intolerance is evident in 20-64%, and overt diabetes mellitus in 30-47% of patients (80-83). Glucocorticoids stimulate glycogen deposition, promote gluconeogenesis, inhibit glucose uptake in peripheral tissues, activate lipolysis, and have a permissive effect on the counter-regulatory hormones, glucagon and catecholamines. An excess of cortisol also stimulates serum and glucocorticoids-inducible kinase-1 which raises the phosphorylation of the forkhead box protein O1 (FOXO1) in adipocytes, increasing insulin resistance (84). It has been suggested that 2-3% of overweight, poorly-controlled patients with diabetes may have occult Cushing’s syndrome (85, 86). However, in the absence of clinical suspicion the percentage is lower (87, 88), and therefore it is probably not justified to screen for Cushing’s in poorly-controlled diabetic patients unless other suggestive features are present (89). Hyperglycemia becomes easier to control after treatment of hypercortisolism and diabetes remits with cure of Cushing’s syndrome in the majority of patients (90). .

There is an increase in total cholesterol and triglyceride levels, and a variable effect on high-density lipoprotein (HDL). These changes are multifactorial, including cortisol effects on increased hepatic synthesis of very low density lipoprotein (VLDL), lipolysis, and free fatty acid metabolism (91, 92).

The major cause of mortality in Cushing’s disease are cardiovascular events, and patients exhibit direct markers of accelerated cardiovascular disease, including increased carotid artery intima-media thickness and atherosclerotic plaques (93) as well as hypertension, glucose intolerance, overt diabetes mellitus, dyslipidemia, and visceral obesity. Overall, hypertension is common in patients with Cushing’s syndrome (82). Severe hypertension with additional hypokalemia is more prevalent in ectopic Cushing’s syndrome, usually best controlled with spironolactone or related drug (94). Cardiovascular risk markers continue to be present long after cure of the hypercortisolemia (95) and the cardiovascular risk remains increased (96, 97). Sympathetic autonomic function is also abnormal in patients with Cushing's syndrome (98), and the ECG abnormalities of a prolonged QTc dispersion and left ventricular hypertrophy have been identified as characteristic features in patients with Cushing's disease (99).

Hypercortisolemia increases clotting factors including factor VIII, fibrinogen, and von Willebrand factor, and reduces fibrinolytic activity by elevated plasminogen activator inhibitor-1 and antiplasmin. This along with other risk factors such as obesity, surgery, and invasive investigative procedures, results in a significantly increased risk of thrombotic events in patients with Cushing's syndrome (100). Rates of thromboembolic events, either postoperatively or unrelated to surgery, are 18-fold higher in patients with Cushing’s syndrome than the estimated incidence in an age and sex matched control population (101, 102). Venous thromboembolism (VTE) has been reported in 20% of patients with Cushing’s syndrome who did not receive thromboprophylaxis, at a mean follow-up of 6-9 years (103). In contrast, VTE occurred in only 6% of patients who received a therapeutic dose of unfractionated heparin at least for 2 weeks after any surgery. The hypercoagulable state may persist even up to 12 months of Cushing’s syndrome remission, and some experts recommend thromboprophylaxis from 24 hours following surgery; however, there is no clear evidence substantiating the duration of thromboprophylaxis (104). The recent *Pituitary Society* guidelines recommended use of thromboprophylaxis with low molecular weight heparin in patients undergoing surgery for Cushing’s syndrome and having an additional risk for VTE such us previous VTE, use of estrogens, reduced mobility, and severe hypercortisolism; however, there is no consensus on the duration of VTE prophylaxis (105). This ranged from 2-14 days before surgery to 2 days - 3months after surgery.

Ophthalmic complications include glaucoma and exophthalmos due to retroorbital fat deposition (106). Cataract is rare, mostly a complication of diabetes.

Psychiatric symptoms such as insomnia, depression, anxiety, easy irritability, paranoid episodes, and attempted suicide or panic attacks are present in more than half of patients having any cause of Cushing’s syndrome (107, 108). Cognitive defects as learning, cognition, and impairment of short-term memory may be prominent (109, 110). These changes are not always reversible with treatment.

In patients with Cushing's syndrome there is a greater frequency of infections because of inhibition of immune function by glucocorticoids by decreasing the number of CD4 cells and NK cells and inhibition in cytokine synthesis (111), with predominant effects on cell-mediated immunity (Th1 responses). The most common infections are bacterial, and special attention should be pointed to the possibility of opportunistic pathogens, especially in cases of severe hypercortisolism (112).

Some cases of ACTH-dependent Cushing's syndrome occur in a periodic or cyclical form, with intermittent and variable cortisol secretion, the symptoms and signs waxing and waning according to the active periods of the disease. These patients can cause particular diagnostic difficulty, as it is imperative that the diagnostic tests are performed in the presence of hypercortisolemia to allow accurate interpretation. Patients may 'cycle in' or 'cycle out' over periods of months or years; if at presentation they are eucortisolemic, they will need regular re-evaluation usually with urinary free cortisol or late-night salivary cortisol to allow full investigation at the appropriate time. Cyclicity can in fact occur with all causes of Cushing’s syndrome (113).

**BIOCHEMICAL CONFIRMATION OF CUSHING’S SYNDROME**

As stated above, there are many clinical features in various combinations in Cushing’s syndrome, but a small number of relatively pathognomonic ones, such as myopathy, wide purple striae, skin thinning and bruising, usually suggest the need for biochemical investigation. The basis for establishing the diagnosis of Cushing’s syndrome is biochemical confirmation of hypercortisolism, prior to any test of the differential diagnosis in terms of a specific cause.

Hypercortisolemia together with the loss of the normal circadian rhythm of cortisol secretion, and disturbed feedback of the HPA axis, are the cardinal biochemical features of Cushing's syndrome. Almost all tests to confirm the diagnosis are based upon these principles. Furthermore, to screen for Cushing's syndrome, tests of high *sensitivity* should be used initially so as to avoid missing milder cases. Tests of high *specificity* can then be employed to exclude false positives. In moderate to high clinical probability of Cushing’s syndrome, 2-3 different screening tests should be used, while if the probability of CS is low 1 negative test such as an overnight dexamethasone suppression test is generally sufficient (105).

It is important to realize that the validation of the published test criteria employed have been on specific assays, and thus test responses should ideally be validated on the local assay used before the results can be interpreted in particular patients. This is aided by supra-regional and nationwide inter-assay quality control assurance programs (1).

Cortisol is normally secreted in a circadian rhythm, with the highest levels early in the morning (07.00-08.00h) and reaching their nadir levels at about midnight (<50 nmol/L or 1.8 μg/dL). In patients with Cushing’s syndrome the circadian rhythm is lost. However, many patients still maintain their morning values within the normal range, but have raised nocturnal levels, rendering midnight levels most useful diagnostically. The measurement of free serum cortisol is very challenging, so either levels of salivary cortisol or total serum cortisol are used. However, exogenous oral estrogens and some medical conditions (see below) will increase cortisol-binding globulin and therefore total cortisol levels. Hence, in all investigations relying on a serum cortisol assay that measures total cortisol, hormone replacement therapy or the oral contraceptive pill should be stopped 4-6 weeks prior to investigation, although it is likely that a shorter time off treatment may still be effective.

**Late Night Salivary Cortisol**

Late-night salivary cortisolmeasurement accurately reflects the plasma free cortisol concentration, because cortisol-binding globulin (CBG) is absent from saliva. Loss of the circadian rhythm of cortisol secretion by measuring late night-time salivary cortisol (best taken at bedtime as nadir salivary cortisol level is detected at the time of falling asleep) can be utilized as a sensitive screening test for Cushing’s syndrome. Due to the simple non-invasive collection procedure which can conveniently be performed at home, and the fact that salivary cortisol is stable for days at room temperature, it offers a number of attractive advantages over blood collection, particularly in children or in cyclical Cushing’s syndrome. Due to variability, taking at least 2 samples on different days is recommended and patients should be advised not to eat, drink, smoke or brush teeth at least 15 minutes before saliva collection. Understandably, this test should not be used in the night-shift workers and individuals with a variable work pattern. Assays using a modification of the plasma cortisol radioimmunoassay, enzyme-linked immunosorbent assay, or liquid chromatography tandem mass spectrometry are now widely available.

Over the past decade there has been considerable increasing interest in this test and it was used in 28% of patients with Cushing’s syndrome from a European registry of 1341 patients diagnosed in 2000-2016 and included in the ERCUSYN registry (114). It has been evaluated at a large number of centers worldwide. In a meta-analysis of multiple studies, in adult patients the sensitivity and specificity of the late-night salivary cortisol appears to be relatively consistent in different centers, and overall is 92% and 96% respectively (115). However, it should be noted that the diagnostic value cut-off varies between studies because of different assays and the comparison groups studied. Late-night salivary cortisol used as a screening test had a somewhat lower sensitivity of 88-89% in subjects from the ERCUSYN registry. Normal values also differ between adults and pediatric populations, and may be affected by other comorbidities such as diabetes (116), and the method by which the saliva is collected (117). Not surprisingly, this test performs less well in patients with ‘subclinical Cushing's syndrome’ (118). Salivary cortisol has also been evaluated as the endpoint for the overnight dexamethasone suppression test. This has the potential benefit in terms of convenience but requires further evaluation (119). Salivary cortisol has also been advocated as a sensitive tool to detect recurrence or treatment failure in patient’s post-pituitary surgery for Cushing's disease (120, 121).

In summary, late-night salivary cortisol appears to be a useful and convenient screening test for Cushing's syndrome, particularly in the outpatient setting. However, local normal ranges need to be validated based on the assay used and population studied.

**Urinary Free Cortisol**

Measurement of urinary free cortisol (UFC) is a non-invasive test that is most commonly used in the screening of Cushing's syndrome (performed in 78% of individuals in the ERCUSYN registry) (114). Under normal conditions, 5-10% of plasma cortisol is 'free' or unbound and physiologically active. Unbound cortisol is filtered by the kidney, with the majority being reabsorbed in the tubules, and the remainder excreted unchanged. As serum cortisol increases in Cushing’s syndrome, the binding capacity of CBG is exceeded and a disproportionate rise in UFC is seen. Thus, 24-hour UFC collection produces an integrated measure of serum cortisol, smoothing out the variations in cortisol during the day and night. In a series of 146 patients with Cushing's syndrome, UFC measurement was shown to have a sensitivity of 95% for the diagnosis (122). However, within this series 11% had at least one of four UFC collections within the normal range, which confirmed the need for multiple collections (at least 2-3 collections are recommended). Furthermore, this sensitivity figure is based on the more florid cases, and is likely to be much less for the more common subtle cases now being seen (123). In the ERCUSYN registry UFC was reported to show 86% sensitivity in adrenal and ectopic Cushing's syndrome and 95% in Cushing’s disease (114).

The major drawback of the test is the potential for an inadequate 24-hour urine collection, and written instructions must be given to the patient. Also, multiple collections reduce the possibility of overlooking episodic cortisol secretion. In addition, simultaneous creatinine excretion in the collection should be measured to assess completeness, and should equal approximately 1g/24 hours in a 70kg patient (variations depend on muscle mass). This should not vary by more than 10% between collections in the same individual (70). The cortisol to creatinine ratio in the first urine specimen can be used as a screening test, especially when cyclic secretion is suspected (124), with a cortisol to creatinine ratio over 25 nmol/mmol being suggestive of Cushing’s syndrome.

The 24-hour UFC is of little value in the differentiation from pseudo-Cushing's states (125, 126).

High-performance liquid chromatography or tandem mass spectrometry are now used to measure UFC, which overcomes the previous problem with conventional radioimmunoassays of cross-reactivity of some exogenous glucocorticoids and other structurally similar steroids (127). Drugs such as carbamazepine, digoxin, and fenofibrate may co-elute with cortisol during high-performance liquid chromatography and cause falsely elevated results (128).

In summary, UFC measurements have a high sensitivity if collected correctly, and several completely normal collections make the diagnosis of Cushing's syndrome very unlikely. Values greater than four-fold normal are rare except in Cushing's syndrome. For intermediate values the specificity is somewhat lower, and thus patients with marginally elevated levels require further biochemical assessment (1, 123). It is our opinion that the test is of little use for screening, and in general we rarely utilize it as a 1st line screening test nowadays.

**Low-Dose Dexamethasone Suppression Test (LDDST)**

This test works on the principle that in normal individual’s administration of an exogenous glucocorticoid results in suppression of the HPA axis, whilst patients with Cushing's syndrome are resistant, at least partially, to negative feedback. Dexamethasone is a synthetic glucocorticoid that is 30 times more potent than cortisol, and with a long duration of action. It does not cross-react with most cortisol assays. The original low-dose dexamethasone test (LDDST) described by Liddle in 1960 measured urinary 17-hydroxy-corticosteroid after 48 hours of dexamethasone 0.5mg 6 hourly (129). However, the simpler measurement of a single plasma or serum cortisol at 09.00h has been validated in various series, and gives the test a sensitivity of between 95% and 100% (123, 130).

The overnight dexamethasone suppression test (ONDST) was first proposed by Nugent *et al.* in 1965; this measures a 09.00h plasma cortisol after a single dose of 1mg dexamethasone taken at midnight (131), and is thus considerably easier to perform. Since then, various doses have been suggested for the overnight test, between 0.5 and 2mg, and various diagnostic cut-offs have been used (132, 133). There appears to be no advantage in discrimination between 1mg and 1.5mg or 2mg (134). Although higher doses have been tried, the increased suppression in some patients with Cushing's syndrome significantly decreases the sensitivity of the test (135). The 1mg ONDST was used in 60% of the subjects in the European registry of Cushing's syndrome (n=1341) and had the best performance among screening tests, with a sensitivity of 98-99% (114).

In a comprehensive review of the LDDST, both the original 2-day test and the overnight protocol appear to have comparable sensitivities (98-100%) using the criteria of a post-dexamethasone serum cortisol of <50nmol/L (1.8μg/dl) (136). However, the *specificity* is greater for the 2-day test (95-100%) compared to the overnight test (88%) (136).

If the ONDST test is used, we suggest that a dose of dexamethasone 1mg to be given at midnight and a threshold of cortisol <50nmol/L (1.8 μg/dl) at 09.00h will rarely lead to the diagnosis being missed, but false positives remain significant. In general, the overnight test is an excellent screening test, and we use the 48 hours LDDST as a confirmation test.

It may be useful to measure the dexamethasone level when ONDST is positive to exclude interference of other medications acting as CYP3A4 inducers causing fast metabolism of dexamethasone and subsequent false positive results (see below), although such measurements are not readily available. Another reason for the false positive results on LDDST is increasing cortisol-binding globulin (seen in pregnancy, estrogens users or chronic active hepatitis).

It should be noted that patients with PPNAD may show a paradoxical rise in cortisol levels to dexamethasone (137).

**Second Line Tests**

In some patients with equivocal results, other tests may be needed. The most useful of these are a midnight serum cortisol, LDDST as described above, and the dexamethasone-CRH test. Less reliable tests include the insulin tolerance test and the loperamide test (138). The desmopressin test is discussed below.

MIDNIGHT SERUM CORTISOL

Before the introduction of salivary cortisol, measurement of a midnight serum cortisol was the only reliable method used to determine loss of the circadian rhythm of cortisol secretion. It is still useful as a second-line test in cases of diagnostic difficulty. However, it is a burdensome test that requires that the patient should have been an in-patient for at least 48 hours to allow acclimatization to the hospital environment. The patient should *not* be forewarned of the test, and should be asleep prior to venipuncture, which must be performed within 5-10 minutes of waking the patient. A single sleeping midnight plasma cortisol <50nmol/L (1.8 μg/dL) effectively excludes Cushing's syndrome (139), but false positive results do occur, particularly in the critically ill, in acute infection, heart failure, and in the pseudo-Cushing's state associated with depression (140).

An awake midnight cortisol of greater than 207 nmol/L (7.5 mg/dL) was reported to show 94% sensitivity and 100% specificity for the differentiation of Cushing's syndrome from pseudo-Cushing's states (141). In the ERCUSYN cohort, 62% individuals with Cushing's syndrome had this test performed with a reported sensitivity of 96-99% (114).

This test has been currently replaced by LNSC and in most hospitals with high demand for the in-patient medical beds, investigations for CS are done mainly on an out-patient environment.

DEXAMETHASONE-CRH TEST

In 1993 the combined dexamethasone-CRH (Dex-CRH) test was introduced for the difficult scenario of the differentiation of pseudo-Cushing’s states (currently known as non-neoplastic hypercortisolism) from true Cushing’s syndrome in patients with only mild hypercortisolemia and equivocal physical findings (125). The theory is that a small number of patients with Cushing's disease as well as normal individuals will show suppression to dexamethasone, but those with Cushing's disease should still respond to CRH with a rise in ACTH and cortisol afterwards. In the original description of the test, dexamethasone 0.5 mg every 6 hours was given for eight doses, ending 2 hours before administration of ovine CRH (1 µg/kg intravenously) to 58 adults with UFC less than 1000 nmol/day (360 µg/day). Subsequent evaluation proved 39 to have Cushing’s syndrome and 19 to have a pseudo-Cushing’s state. The plasma cortisol value 15 minutes after CRH was less than 38 nmol/L (<1.4 µg/dL) in all patients with pseudo-Cushing’s states and greater in all patients with Cushing’s syndrome. A prospective follow-up study by the same group in 98 patients continued to show the test to have an impressive sensitivity and specificity of 99% and 96%, respectively (125). Importantly, in these two studies although eight of 59 patients with proven Cushing's disease showed suppression to dexamethasone, all were correctly characterized after CRH.However, the results from a number of other smaller studies have challenged the diagnostic utility of this test over the standard LDDST. Overall, in these reports the specificity of the LDDST in 92 patients without Cushing's syndrome was 79%, versus 70% for the Dex-CRH. The sensitivity in 59 patients with Cushing's syndrome was 96% for the LDDST versus 98% for the Dex-CRH (142). It is perhaps not surprising that the diagnostic utility of the Dex-CRH has altered with further studies at more centers. There are a number of reasons why there might be the case: variable dexamethasone metabolism in individuals; different definitions of patients with pseudo-Cushing's; different protocols and assays; and variable diagnostic thresholds. It is recommended that if this test is used, a dexamethasone level is measured at the time of CRH administration and the serum cortisol assay is accurate down to these low levels (89). We would not recommend its use, and indeed with the lack of availability of CRH currently, it is generally impossible to perform.

DESMOPRESSIN TEST

ACTH-secreting adenomas express V3 receptors therefore desmopressin increases ACTH and subsequently cortisol in patients with Cushing’s disease. The test involves intravenous injection of 10mcg of desmopressin and ACTH measurement every 15 minutes from -15minutes to 90minutes. The study of 173 subjects including 76 with Cushing’s disease, 30 with non-neoplastic hypercortisolism, 36 with obesity and 31 of controls proposed cut-off criteria for positive desmopressin test as ACTH increment of >6pmol/L (30ng/L) (143). Subsequently, another study of 52 patients with Cushing’s syndrome and 28 controls suggested new criteria with ACTH increment of 4pmol/L and basal cortisol above 331nmol/L providing sensitivity of 90.3% and specificity of 91.5% (144). The meta-analysis of 3 studies described use of desmopressin test in differentiation of Cushing’s disease and non-neoplastic hypercortisolism with cut-off for ACTH increment by 6 pmol/L in 2 studies and ACTH increment of 4 pmol/L and basal cortisol more than 331nmol/L gave pooled sensitivity of 88% and specificity of 94% (143-145). However, there was high patient selection bias and low certainty of evidence in that meta-analysis (145).

**DIFFERENTIAL DIAGNOSIS OF CUSHING’S SYNDROME**

Once Cushing's syndrome has been diagnosed, the next step is to differentiate between ACTH-dependent and ACTH-independent causes by measurement of plasma ACTH. Modern two-site immunoradiometric assays are more sensitive than the older radioimmunoassays and therefore provide the best discrimination. Rapid collection and processing of the sample is essential as ACTH is susceptible to degradation by peptidases so that the sample must be kept in an ice water bath and centrifuged, aliquoted, and frozen within 2 hours to avoid a spuriously low result. Measurements are usually taken on two different days to avoid misinterpretation because of the episodic secretion of ACTH. The circadian rhythm of ACTH in patients having Cushing’s syndrome is lost, as it is for cortisol measurement, and the optimal sample should be taken at 08.00-09.00h (146).

It is useful to repeat this test because patients with ACTH-dependent Cushing’s disease have been shown to have on occasion ACTH levels less than 10 ng/L (2 pmol/L) on conventional radioimmunoassay (147). The ACTH immunoassays can interfere with heterophilic antibodies or ACTH fragments and cases of falsely elevated ACTH have been reported using the Immulite ACTH assay (148). Therefore, if results are inconsistent or not in keeping with the clinical or imaging features, ACTH should be remeasured using an alternative immunoassay.

Consistent ACTH measurements of <10 ng/L (2 pmol/L) essentially confirm ACTH-independent Cushing's syndrome, and radiologic evaluation of adrenals is the next step in diagnosis. Conversely, if levels are consistently greater than 20-30 ng/L (4-6 pmol/L), Cushing's syndrome is ACTH-dependent, due to pituitary disease or ectopic ACTH secretion.

Intermediate levels are less discriminatory, but a lack of ACTH response to the CRH test or the desmopressin test (see below) may be particularly helpful in these intermediate cases.

**Investigating ACTH-Independent Cushing's Syndrome**

Imaging of the adrenal glands is the mainstay in differentiating between the various types of ACTH-independent Cushing's syndrome. High-resolution computed tomography (CT) scanning of the adrenal glands is the investigation of choice, is accurate for masses greater than 1 cm, and allows evaluation of the contralateral gland (149). MRI may be useful for the differential diagnosis of adrenal masses; the T2-weighted signal is progressively less intense in phaeochromocytoma, carcinoma, adenoma, and finally normal tissue (150).

Adrenal tumors typically appear as a unilateral mass with an atrophic contralateral gland (151). If the lesion is greater than 5 cm in diameter it should be considered to be potentially malignant until proven otherwise, and discussed in the local adrenal Multidisciplinary Team meeting (MDT). In comparison to carcinomas, adrenal adenomas are usually smaller and have a lower unenhanced CT attenuation value (usually <20HU) (152). Adrenal adenomas are homogeneous and hypointense on MRI T1-weighted images and iso- or hyperintense comparing to the liver on T2 images. Adrenal adenomas also demonstrate signal drop on out-of-phase MR imaging consistent with lipid-rich tissue. Signs of necrosis, hemorrhage and calcification are characteristics of both carcinoma and phaeochromocytomas, which can also co-secrete ACTH (153). Additional laboratory diagnostics reveal solely raised cortisol levels in adenomas, unlike additionally raised androgen levels in adrenocortical carcinomas. Bilateral adenomas can be present (154).

In PPNAD the adrenal glands appear normal or slightly lumpy from multiple small nodules, but are not generally enlarged (150, 155).

Exogenous administration of glucocorticoids results in adrenal atrophy and very small glands may be a clue as to this entity.

BMAD is characterized by bilaterally large (>5 cm) adrenals with a nodular configuration (15, 156).

Confusion can arise as the CT appearance of the adrenals in BMAD may be similar to the appearance seen in ACTH-dependent forms of Cushing's syndrome, where adrenal enlargement is present in 70% of cases (157), but the two can usually be distinguished by the ACTH level and the degree of adrenal enlargement. Some patients with Cushing's disease can also develop a degree of adrenal autonomy which can cause biochemical confusion (16).

**Identifying the Source in ACTH-Dependent Cushing's Syndrome**

This has been one of the most significant challenges in the investigation of Cushing's syndrome in the past, although advances over the last 15 years have greatly improved our diagnostic capability. Cushing's disease accounts for by far the majority of cases of ACTH-dependent Cushing's syndrome, between 85% and 90% in most series. In the European registry of Cushing’s syndrome (n=1341), 67% of cases were due to pituitary disease and, of ACTH-dependent Cushing's syndrome , 92% were of pituitary origin (54).This depends on gender, and in the series of 115 patients with ACTH-dependent Cushing's syndrome, of the 85 women, 92% had Cushing's disease; this percentage was 77% in the 30 men (158). Therefore, even before one starts investigation, the pretest probability that the patient with ACTH-dependent Cushing’s syndrome has Cushing's disease is very high, and any investigation must improve on this pretest likelihood. However, as transsphenoidal pituitary surgery is widely accepted as the primary treatment of Cushing's disease, testing should be designed to avoid inappropriate pituitary surgery in patients with ectopic ACTH production. Thus, any test should ideally be set with 100% specificity for the diagnosis of Cushing's disease.

Levels of serum cortisol and ACTH tend to be higher in the ectopic ACTH syndrome, but there is considerable overlap of values, producing poor discrimination (158, 159). Hypokalemia is more common in ectopic ACTH-dependent Cushing's syndrome than in patients with Cushing’s disease.

INVASIVE TESTING

*Bilateral Inferior Petrosal Sinus Sampling*

This is the "gold standard" test for distinguishing between Cushing's disease and an ectopic source of ACTH. However, most experts agree that if a pituitary macroadenoma (tumor of ≥ 10mm) is visualized on MRI and dynamic tests (hCRH/desmopressin) are consistent with Cushing’s disease, Bilateral inferior petrosal sinus sampling (BIPSS) is not necessary (105).The procedure involves placement of sampling catheters in the inferior petrosal sinuses that drain the pituitary. Blood for measurement of ACTH is obtained simultaneously from each sinus and a peripheral vein at two time points before and at 3-5 minutes and possibly also 10 minutes after the administration of 100mcg of human CRH if available, or nowadays 10mg desmopressin. A central (inferior petrosal) to peripheral plasma ACTH gradient of 2:1 or greater pre-desmopressin, or a gradient of 3:1 post-desmopressin (previously post-CRH), is consistent with Cushing's disease. The results from early series show these criteria to be 100% sensitive and specific for Cushing’s disease when CRH stimulation was used (160, 161). However, it is now clear that false negative tests and to a smaller degree false positive test results do occur (162-164). A meta-analysis including 23 studies and 1642 patients with ACTH-dependent Cushing's syndrome reported that IPSS had sensitivity of 94% and specificity of 89% with area under the ROC curve of 97% to diagnose Cushing’s disease again all with CRH stimulation (165).

In order to minimize these inaccuracies it is important to ensure the patient is actively hypercortisolemic (as above) at the time of the study (166), and that catheter position is confirmed as bilateral and any anomalous venous drainage noted by venography before sampling (167). There appears to be no discriminatory difference between ovine or human sequence CRH; however, as CRH is no longer available, desmopressin 10 μg shows similar efficacy (168). The study with 226 patients with Cushing’s disease and 24 patients with ectopic ACTH-dependent CS who underwent BIPSS with desmopressin stimulation achieved sensitivity of 97.8% and specificity of 100% when ACTH ration >2.8 was applied (169). However, it has been noted that for all with >6mm pituitary microadenoma on MRI baseline ACTH ratio of >1.4 distinguished all with Cushing’s disease without need for desmopressin stimulation. The meta-analysis of 11 studies including 611 patients compared BIPPS with CRH versus DDAVP stimulation and found no statistical difference in the results with pooled sensitivity of 96 % for desmopressin and 98% for CRH with 100% specificity (170). None of the studies using desmopressin reported subsequent hyponatremia when fluid restriction in the next 24hs has been followed.

It should be noted that the procedure is technically difficult, and should only be performed by radiologists experienced in the technique. The most common complications are transient ear discomfort or pain, and local groin hematomas. More serious transient and permanent neurological sequelae have been reported, including brainstem infarction, although these are rare (<1%), and most have been related to a particular type of catheter used (171, 172); if there are any early warning signs of such events the procedure should be immediately halted. Patients should be given heparin during sampling to prevent thrombotic events (82). There appears to be no advantage in trying to sample the cavernous sinus. Sampling of the internal jugular veins is a simpler procedure but is not as sensitive as BIPSS (173).

A baseline inferior petrosal sinus (IPS) to peripheral prolactin ratio of >1.8 has been suggested as a confirmation of a successful catheterization (174). A multicenter study including 156 individuals with ACTH-dependent Cushing’s disease who underwent IPSS reported that IPS to peripheral ACTH to prolactin ratio of ≥1.4 improved further BIPSS performance in differentiating Cushing’s disease from ectopic ACTH-dependent CS with sensitivity of 96% and specificity of 100% (175), but not all are agreed that this extra level of analysis is worthwhile.

BIPSS has also been suggested to help to lateralize microadenomas within the pituitary using the inferior petrosal sinus ACTH gradient (IPSG), with a basal or post-stimulus inter-sinus ratio of at least 1.4 being the criteria for lateralization used in all large studies (161, 162, 176, 177). In these studies, the diagnostic accuracy of localization as assessed by operative outcome varied between 59% and 83%. This is improved if venous drainage is assessed to be symmetric (178). A study of 501 cases of Cushing’s disease showed that an interpetrosal ACTH ratio of ≥1.4 was achieved in 98% of patients but lateralized the lesion correctly in only 69% of subjects. A pituitary lesion was identified on the pre-operative MRI in 42% of patients in that study and, if seen, had a positive predictive value of 86% (179). Hence, the interpetrosal ratio can guide pituitary exploration in cases of a normal pre-surgery MRI scan. In this study, MRI was falsely positive in 12% of individuals.

An enhanced dynamic MRI has a better detection rate of pituitary microadenomas than conventional MRI and was reported to identify a pituitary lesion in 81% (83 out of 102) of patients with Cushing’s disease and lateralized correctly the pituitary adenoma in 62 out of 71 patients with histologically-proven Cushing's disease (180).

The accuracy of lateralization appears to be higher in children (90%), a situation where imaging is often negative (181). There is some discrepancy between studies as to whether CRH or desmopressin improve the predictive value of the test (182). If a reversal of lateralization is seen pre- and post-stimulus, the test cannot be relied upon (183).

NON-INVASIVE TESTS

*High Dose Dexamethasone Suppression Test*

As with the LDDST, the high dose dexamethasone suppression test (HDDST) was originally proposed by Liddle to differentiate between cortisol-secreting adrenal tumors and Cushing's disease (129). The HDDST’s role in the differential diagnosis of ACTH-dependent Cushing’s syndrome is based on the premise that most pituitary corticotroph tumors retain some albeit reduced responsiveness to negative glucocorticoid feedback, whereas ectopic ACTH-secreting tumors, like adrenal tumors, typically do not, with the exception of some neuroendocrine tumors, mainly bronchial (184, 185).

The test is performed according to the same protocol as the LDDST, either as 2mg 6 hourly for 2 days, or as an overnight using a single dose of 8mg of dexamethasone at 23.00h. The latter is more convenient for a patient because a single blood specimen is being tested on the next day at 08.00h. In most patients with pituitary-dependent Cushing’s syndrome, the final serum cortisol level is less than 5 mcg/dL (140 nmol/L). In normal subjects the level is usually undetectable (186).

Overall, only about 80% of patients with Cushing's disease will show a positive response to the test, defined by suppression of cortisol to less than 50% of the basal value. There are a high number of false positive tests (~10-30%) seen in ectopic Cushing’s syndrome (186-189). Shifting the criteria can only increase sensitivity with a loss of specificity, and vice-versa. Therefore, the test achieves worse discrimination than the pretest probability of Cushing's disease. In addition, one study has shown that suppression to HDDST can be inferred by a >30% suppression of serum cortisol to the 2-day LDDST (190). Therefore, we no longer recommend the routine use of the HDDST except when bilateral inferior petrosal sinus sampling is not available, and then only as part of a combined testing strategy with other tests. (see below).

The HDDST was performed in 30% of subjects (n=402) from the European registry of patients with Cushing's syndrome, with a cortisol reduction supporting the diagnosis of pituitary Cushing's syndrome in 92% and ectopic Cushing's syndrome in 93% of patients (specificity not given) (114). When used in individuals with negative IPSS, HDDST supported the diagnosis of pituitary disease in 100% and ectopic Cushing's syndrome in 82%.

The combined use of the HDDST and enhanced dynamic MRI of the pituitary was compared to BIPSS in 71 patients with histologically-proven Cushing's disease (180). The combination had a 98.6% positive predictive value (PPV) for Cushing's disease but a sensitivity of only 69.6%. In that study BIPSS alone had a similar PPV of 97%.

*The CRH Test*

Both ovine and human-sequence CRH are currently unavailable in most countries, and the test has been superseded by desmopressin. However, as it may become available in the future, the following section may be useful.

The use of the CRH (corticotrophin-releasing hormone) test for the differential diagnosis of ACTH-dependent Cushing's syndrome is based on the premise that pituitary corticotroph adenomas retain responsivity to CRH, while ectopic ACTH tumors lack CRH receptors and therefore do not respond to the agent. 100 µg of human sequence CRH (hCRH) is given as a bolus injection and the change in ACTH and cortisol measured. Human-sequence CRH has qualitatively similar properties to oCRH, although it is shorter-acting with a slightly smaller rise in plasma cortisol and ACTH in obese patients, and in those with Cushing's disease (191). This may be related to the more rapid clearance of the human sequence by endogenous CRH-binding protein (192).

Different centers have used differing protocols, including type of CRH and sampling time-points, and thus there is little consensus on a universal criterion for interpreting the test. In one of the largest published series of the use of oCRH, an increase in ACTH by at least 35% from a mean basal (-5 and -1 minutes) to a mean of 15 and 30 minutes after oCRH in 100 patients with Cushing's disease and 16 patients with the ectopic ACTH syndrome gave the test a sensitivity of 93% for diagnosing Cushing’s disease, and was 100% specific (193). Conversely, in the large series of the use of hCRH in 101 patients with Cushing's disease and 14 with the ectopic ACTH syndrome, the best criterion to differentiate Cushing's disease from ectopic ACTH syndrome was a rise in cortisol of at least 14% from a mean basal (-15 and 0 minutes) to a mean of 15 and 30 minutes, giving a sensitivity of 85% with 100% specificity. The best ACTH response was a maximal rise of at least 105%, giving 70% sensitivity and 100% specificity (158). In a multicentered analysis from Italy, both hCRH and oCRH were used in 148 patients with Cushing's disease and 12 with the ectopic ACTH syndrome. A maximal 50% increase in ACTH and cortisol levels were considered as consistent with Cushing's disease, excluding all patients with the ectopic ACTH syndrome and thus giving 100% specificity. The sensitivity and specificity for the ACTH response were comparable for the two types of CRH (sensitivity: 85% vs 87% for oCRH and hCRH respectively).

A CRH test was performed in 351 patients with ACTH-dependent Cushing's syndrome from the European registry of Cushing's syndrome, with a peak ACTH supporting the diagnosis of Cushing's disease in 90% of cases and ectopic Cushing's syndrome in 84% of patients (114). However, the sensitivity for the cortisol response was significantly greater with oCRH than with hCRH (sensitivity: 67% vs 50% for oCRH and hCRH respectively) (194). The authors do not report in this paper or an associated publication (27) whether time-point combinations other than the maximal were analyzed for the rise in cortisol. Indeed, our data showed that if the *maximal* rise in cortisol is used the sensitivity falls to 71% (158). These results again demonstrate that specific criteria need to be developed for each test, and cannot readily be extrapolated from other similar but non-identical agents.

In summary, the CRH test has been a useful discriminator between causes of ACTH-dependent Cushing's syndrome, particularly in a combined testing strategy with the HDDST or LDDST when diagnostic accuracy is greater than that of either test alone, yielding 98% to 100% sensitivity, and an 88% to 100% specificity (187, 190, 195). Which cut-off to use should be evaluated at individual centers, and caution should be exercised as there will undoubtedly be patients with the ectopic ACTH syndrome who respond outside these cut-offs. However, it should be remembered that responses to both CRH and high-dose dexamethasone are more frequently discordant in Cushing's disease due to a macroadenoma (196). Nevertheless, where BIPSS is unavailable, a response to both CRH (a rise) and the LDDST (a fall) renders an ectopic source extremely unlikely.

*Desmopressin Test*

Both vasopressin and desmopressin (a synthetic long-acting vasopressin analogue without the V1-mediated pressor effects) stimulate ACTH release in Cushing’s disease, probably through the corticotroph-specific V3 (or V1b) receptor. The study of 170 patients including 149 with Cushing’s disease reported that an ACTH increase after desmopressin by more than 32.4% provided sensitivity of 83% but specificity of 62%, which was inferior to HDDST (197). The meta-analysis of 11 studies using DDAVP in the differential diagnosis of ACTH-dependent Cushing’s syndrome reported that combination of an ACTH increase of >35% and a cortisol increase of >20% including 511 individuals had a pooled sensitivity of 88% and specificity of 74% to correctly diagnose Cushing’s disease (145).

Hexarelin, a growth hormone secretagogue, stimulates ACTH release probably occurs through stimulation of vasopressin release in normal subjects (198), and by stimulation of aberrant growth hormone secretagogue receptors in corticotroph tumors (199).

These peptides have been utilized in a similar manner to CRH to try and improve the differentiation of ACTH-dependent Cushing’s syndrome, but have unfortunately proved inferior (200-202).

A combined desmopressin and hCRH stimulation test initially looked promising (203), but further study of this combined test showed significant overlap in the responses (204). The inferior discriminatory value of these stimulants is most likely due to the expression of both vasopressin and growth hormone secretagogue receptors by some ectopic ACTH-secreting tumors (82, 205).

A retrospective study including 167 patients with Cushing’s disease and 27 patients with ectopic ACTH-dependent CS reported 100% positive predictive value for diagnosing Cushing’s disease when both CRH-stimulation test and DDAVP stimulation test were positive when the pituitary MRI scan and CT scan for ectopic source were negative. The positive test was defined as an ACTH increment of >33% and cortisol increment of >18% after administration of desmopressin and ACTH increment of 37% and cortisol >18% after administration of CRH (206). The negative predictive value was 100% when both tests were negative and pituitary MRI was negative but CT for ectopic source of ACTH positive. The authors concluded that this strategy would avoid IPSS in 47% of the patients.

**IMAGING**

## Pituitary

Imaging of the pituitary is an important part of the investigation of ACTH-dependent Cushing's syndrome to identify a possible pituitary lesion and to aid the surgeon during exploration. However, the results must be used in conjunction with the biochemical assessment as approximately 10% of normal subjects may have pituitary incidentalomas on MRI (207). Modern MRI techniques using T1-weighted spin echo and/or spoiled gradient recalled acquisition (SPGR, 1mm slice thickness) techniques will identify an adenoma in up to 80% of patients with Cushing’s disease (208). They provide greater sensitivity than conventional MRI but with more false positive results (208, 209). On MRI, 95% of microadenomas exhibit a hypointense signal with no post-gadolinium enhancement (Figure 2); however, as the remaining 5% have an isointense signal post-gadolinium, pre-gadolinium images are essential (210). The delayed pituitary microadenoma contrast washout was detected on FLAIR MRI as hyperintensity in 80% of patients with Cushing's disease and negative dynamic MRI (n=5) (211, 212).

If corticotroph microadenoma has not been clearly identified with modern MRI techniques, 11C-methionine PET co-registered with 3D gradient echo MRI may help in selected cases (213). The main limitation of this technique is short half-life of isotope of around 20min and it requires cyclotron on the site.

CT has a sensitivity of only approximately 40-50% for identifying microadenomas, and is thus significantly inferior to MRI (sensitivity 50-60%) (27, 214), and it should therefore be reserved for patients in whom MRI is contraindicated or unavailable. CT imaging typically shows a hypodense lesion that fails to enhance post-contrast.

Preoperative localization to one side of the pituitary gland by MRI had been advocated as better than BIPSS with a positive predictive value of 93% (163, 215). Other groups have found MRI less effective (162, 216). In addition, as noted above, we have found MRI often to be unhelpful in the pediatric age group, and BIPSS to be of significant value in these patients (181).

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**Figure 2. Magnetic resonance scan of the head with gadolinium showing left-sided pituitary hypointense microadenoma (white arrows) in 2 different patients (T1 image post-contrast).**

**Ectopic Tumors**

Visualizing an ectopic ACTH source can be a challenge, but in general patients should begin with imaging of the chest and abdomen with CT and/or MRI, bearing in mind likely sites (Table 2). The most common site of the secretory lesion is the chest, and although small cell lung carcinomas are generally easily visualized, small bronchial carcinoid tumors that can be less than 1cm in diameter often prove more difficult. Fine-cut high-resolution CT scanning with both supine and prone images can help differentiate between tumors and vascular shadows (1). MRI can identify chest lesions that are not evident on CT scanning, and characteristically show a high signal on T2-weighted and short-inversion-time inversion-recovery images (STIR) (217).

The majority of ectopic ACTH secreting tumors are of neuroendocrine origin and therefore may express somatostatin receptor subtypes. Therefore, the radiolabeled somatostatin analogue (111In-pentetreotide) scintigraphy may be useful to show either functionality of identified tumors, or to try and localize radiologically unidentified tumors (218). Undoubtedly this is a useful technique, but to date there are only sporadic reports that it identifies lesions not apparent using conventional imaging (219, 220). However, a lesion of uncertain pathology is more likely to represent a neuroendocrine tumor, and hence an ectopic source of ACTH, if somatostatin scintigraphy is positive. Unless the tumors are metabolically active, which is not usually the case, 18F-deoxyglucose positron-emission tomography (FDG-PET) does not generally offer any advantage over conventional CT or MRI (221, 222). However, 68Ga-DOTA-conjugated peptides (octreotide, lanreotide or octreotide) PET scanning, targeting SST receptors 1-5, is more sensitive than conventional octreotide scintigraphy and is indicated in the detection of primary occult neuroendocrine tumors (NETs) when conventional imaging modalities have failed (223). In a systematic review of small studies including a total of 77 patients with ectopic Cushing’s syndrome, the detection rate of the tumor was 70% for 68Ga-labelled peptide PET and 61% for 18F-FDG PET (224). Subsequent systematic review of 68Gallium-DOTATATE, DOTATOC, and DOTANOC positron emission tomography/computed tomography (68Ga-SSTR PET/CT) in detecting ectopic ACTH-secreting tumors had a pooled sensitivity of 64%, increasing to 76% in histologically confirmed lesions (225). 68Ga-somatostatin receptor analogues had better sensitivity in the diagnosis of bronchial carcinoids causing Cushing’s syndrome, while 18F-FDG PET appeared superior for small-cell lung cancers and other aggressive tumors (226).

**STRATEGY FOR THE DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS** **OF CUSHING’S SYNDROME**

There have been a number of international consensus statements published for the diagnosis and differential diagnosis of Cushing's syndrome, the latest on the diagnosis in 2021 (82, 89, 105). It is recommended that UFC (at least two measurements), the LDDST, or late-night salivary cortisol (two measurements) are used as the first line screening test. A second test should confirm abnormal results on one test (Figure 3).

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**Figure 3. Investigations algorithm for suspected Cushing’s syndrome; CS – Cushing’s syndrome, ONDST – overnight dexamethasone suppression test, UFC – urinary free cortisol, LDDST – low dose dexamethasone suppression test, HDDST – high dose dexamethasone suppression test, BIPSS – bilateral inferior petrosal sinus sampling, PPNAD – primary pigmented nodular adrenocortical disease, BMAD - bilateral multinodular adrenocortical disease.**

In patients with discordant results second-line tests should be used as necessary for confirmation. Once the diagnosis of Cushing’s syndrome is unequivocal, ACTH levels, the desmopressin test (combined with the results of the LDDST), together with appropriate imaging, are the most useful non-invasive investigations to determine the etiology. BIPSS is recommended in cases of ACTH-dependent Cushing’s syndrome where the clinical, biochemical, or radiological results are discordant or equivocal. However, in many centers where BIPSS is available and validated, the practice is to use this test in almost all cases of ACTH-dependent Cushing’s syndrome with the exception of corticotroph macroadenomas, although the Bordeaux group have indicated that the use of dynamic testing plus high-quality imaging can reduce the necessity for BIPSS to some 50% of cases (206).

**TREATMENT OF CUSHING’S SYNDROME**

Treatment should be directed toward resolving the primary cause of Cushing’s syndrome, presuming accurate differential diagnosis. Hypercortisolism, accompanied with fatal consequences if left untreated, should be controlled by all means. Whenever possible, surgery, regardless of etiology, presents a first-line treatment option aiming for a permanent cure and resolving the hypercortisolism together with its clinical consequences. However, the approach to the patient with Cushing’s syndrome is individual, so radiation therapy or even medical therapy as first-line treatment could be appropriate depending on etiology, clinical state, and the personal choice of a patient.

Following treatment, all of the signs and symptoms of adrenal deficiency should be promptly corrected with steroid replacement therapy. Associated medical disorders of Cushing’s syndrome such as diabetes mellitus, hyperlipidemia, osteoporosis, and hypertension should be treated, aiming to avoid permanent dependence on therapy after resolving the primary cause of Cushing’s syndrome.

It should also be emphasized that in severely-unwell patients the metabolic complications should be vigorously treated as a matter of priority, including hypokalemia, hypertension, and hyperglycemia. Any infections should be sought and treated, and some would advise prophylactic antibiotics (especially against pneumocystis infections) if the serum cortisol is especially high (>1000-1200 nmol/L). Most importantly, most centers would now advise immediate anti-coagulation with prophylactic low molecular weight heparin peri-operatively in all but the mildest cases or unless there are contraindications (227).

**Treatment of Cushing’s Disease**

First-line therapy almost always comprises transsphenoidal surgery (Figure 4). Patients with persistent Cushing’s post-operatively can be re-operated with a lower success rate than primary surgery and with higher rates of other pituitary hormonal deficiencies. Prior to repeated surgery it is wise to repeat diagnostic testing, especially if corticotrophinoma has not been found on pathologic examination, to exclude the possibility of missed ectopic ACTH syndrome. Besides re-operation, patients can be treated either by radiotherapy, medical therapy, or as a definitive solution to the hypercortisolism, bilateral adrenalectomy.

TRANSSPHENOIDAL SURGERY

According to the relevant 2021 consensus statement on the treatment of ACTH-dependent Cushing's syndrome (105, 228, 229), transsphenoidal surgery is widely regarded as the treatment of choice for Cushing’s disease (229). Besides the traditional microscopic approach there is an endoscopic approach which appears useful in patients with persistent or recurrent disease (230, 231) and is associated with a shorter hospital stay (232), and is now most often utilized. It is recommended to limit the number of surgeons performing transsphenoidal surgery to increase the number of operations performed per surgeon per year and have one dedicated surgeon per center for treatment of Cushing’s disease. The surgeons who have performed 200 transsphenoidal operations have the best outcomes and the lowest complication rates (233). The Pituitary Society consensus recommends that surgery for Cushing’s disease should be performed in the *Pituitary Tumor Centers of Excellence* when possible (105). The remission rate of Cushing’s disease due to pituitary microadenoma is similar for both techniques (around 80%, total n=6695), with better results in pituitary macroadenomas when using endoscopic approach (59.9% vs 76.3%) (234).

The procedure is not without risks, and in the European Cushing’s disease survey group of 668 patients, the perioperative mortality was 1.9%, with other major complications occurring in 14.5% (235). The frequency of reported adverse events varies widely: diabetes insipidus (AVP-deficiency, either temporary or permanent) (3-46%); hypogonadism (14-53%); hypothyroidism (14-40%); cerebrospinal fluid rhinorrhea (4.6-27.9%); severe growth hormone deficiency (13%); bleeding (1.3-5%); and meningitis (0-2.8%) (235-237).

Where an adenoma is apparent at transsphenoidal exploration, a selective microadenomectomy of tumor tissue is performed, and the surgeon may be guided by pre-operative imaging. However, where no tumor is obvious, and there is no concern regarding fertility, subtotal resection of 85-90% of the anterior pituitary gland should be considered, leaving a small part near the pituitary stalk. However, there is still a substantial and unpredictable risk of panhypopituitarism.

The overall remission rate combined for microadenomas and macroadenomas in various large series is in the order of 70-79%, although higher rates of approximately 90% can be achieved with microadenomas (8, 234-236, 238-242) (241). Remission rates are based on post-operative pathologic and biochemical results, although both can be equivocal. Half of all tumors cannot be pre-operatively visualized (243), and therefore parts of the tumor can be overlooked intra-operatively and left behind, affecting the surgical success rate (244). Adenomas can occur near or within the pituitary stalk, rarely in ectopic locations (245, 246), and may show signs of microscopic invasion (247).

Prognostic markers of long-term remission are patient age over 25 years, a microadenoma detected by MRI, lack of invasion of the dura or cavernous sinus, histological confirmation of an ACTH-secreting tumor, low post-operative cortisol levels, and long-lasting adrenal insufficiency (228, 241). (242, 248).

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**Figure 4.** **Management algorithm of Cushing’s disease; TSA, Transsphenoidal adenomectomy; CD, Cushing’s disease.**

Of patients achieving remission, some 10-15% of these will have a recurrence by 10 years and 20% by 20 years (249), this emphasizing the need for long-term annual follow-up based on the same diagnostic criteria as with initial diagnostics in the following order; salivary late-night cortisol, an overnight 1 mg dexamethasone suppression test, and lastly 24hours UFC (105). Special attention should be paid to patients with intermittent hypercortisolism (250). Transsphenoidal surgery is also a useful procedure in patients with Nelson’s syndrome to reduce tumor size, and ameliorate hyperpigmentation (251).

Thromboprophylaxis with low molecular weight heparin should be considered peri-operatively in all surgical procedures for Cushing's syndrome (100, 101, 105). The recent *Pituitary Society* guidelines recommended use of thromboprophylaxis with low molecular weight heparin in patients undergoing surgery for Cushing’s syndrome and especially having an additional risk for VTE such us previous VTE, use of estrogens, reduced mobility and severe hypercortisolism; however, there is no consensus on duration of VTE prophylaxis (105). Our practice is generally to consider LMW heparin prophylaxis in all patients, and to continue for some 2-3 months post-operatively.

POST-OPERATIVE EVALUATION AND MANAGEMENT

Many use glucocorticoid coverage for transsphenoidal surgery, tapering off within 1 to 3 days. Morning (09.00h) serum cortisol measurements are then obtained on day 4 or 5 post-operatively starting 20 hours after the last glucocorticoid administration, during which time the patient should be closely observed for the development of signs of adrenal insufficiency (252). However, where there is close post-operative supervision, it may be possible to assess early cortisol results in the absence of corticosteroid cover.

In the immediate post-operative period, there is a wide range of possible biochemical results. Post-operative hypocortisolemia (<50 nmol/L [1.8 µg/dL] at 09.00h) is probably the best indicator of the likelihood of long-term remission (253-255). However, detectable cortisol levels of less than 140 nmol/L (<5µg/dL) are also compatible with sustained remission (256-258). In cases of a mild or cyclic Cushing’s disease the normal corticotrophs may not be suppressed and sustain a normal cortisol level with a normal diurnal rhythm.

Higher post-operative cortisol levels are more likely to be associated with failed surgery; however, cortisol levels may sometimes gradually decline over 1-2 months reflecting gradual infarction of remnant tumor or a gradual loss of autonomy of the adrenal, reported in some 5% of patients (256, 259). Regardless of the possibility of this late remission, the approach should be individualized and additional testing done prior to 3 months if there is reason to believe in residual disease. Persistent cortisol levels greater than 140 nmol/L (>5 µg/dL) 3 months after surgery require further investigation. Persistent hypercortisolemia after transsphenoidal exploration should prompt reevaluation of the diagnosis of Cushing’s disease, especially if previous diagnostic test results were indeterminate or conflicting, or if no tumor was found on pathological examination.

The treatment options for patients with persistent Cushing’s disease include: repeat surgery, radiation therapy, and bilateral adrenalectomy. If immediate surgical remission is not achieved at the first exploration, early repeat transsphenoidal surgery including the endoscopic technique may be worthwhile in a significant proportion of patients (approximately 50%), at the expense of an increased likelihood of hypopituitarism (231, 260, 261). Repeat sellar exploration is less likely to be helpful in patients with empty sella syndrome or very little pituitary tissue on MRI scans. Patients with cavernous sinus or dural invasion identified at the initial procedure are not candidates for repeat surgery to treat hypercortisolism and should receive alternative therapy.

Patients who are hypocortisolemic should be started on glucocorticoid replacement. Eventually, hydrocortisone 15-20 mg total daily dose in three divided doses is the preferred choice by most. The largest dose (10 mg) should be taken before getting out of bed, and the last 5mg dose should be taken in early afternoon and no later than 18.00h because later administration of glucocorticoids may result in disordered sleep. This low dose of hydrocortisone should be used to avoid long-term suppression of the HPA axis. All patients receiving chronic glucocorticoid replacement therapy should be instructed that they are “dependent” on taking glucocorticoids as prescribed, and that failure to take or absorb the medication could lead to adrenal crisis and possibly death. They should be prescribed a 100mg hydrocortisone (or other high-dose glucocorticoid) intramuscular injection pack for emergency use. They should also obtain a medical information bracelet or necklace that identifies this requirement (*Medic-Alert* Foundation or similar). Education should stress the need for compliance with the daily dose of glucocorticoid; the need to double the oral dose for nausea, diarrhea, and fever; and the need for parenteral administration and medical evaluation during emesis, trauma, or severe medical stress.

The patient should be told to expect desquamation of the skin, and flu-like symptoms (malaise, joint aching, anorexia, and nausea) during the early post-operative months, and that these are signs that indicate remission. Symptoms can be especially prominent in patients with long-standing, severe Cushing’s syndrome. Some of these symptoms have been related to high levels of circulating interleukin-6 (262). Most patients tolerate these symptoms of glucocorticoid withdrawal much better if they are forewarned and alerted to their ‘positive’ nature. Signs of adrenal insufficiency, such as vomiting, electrolyte abnormalities, and postural hypotension, should be excluded (263). However, if patients develop severe symptoms of glucocorticoids withdrawal significantly affecting their quality of life, an initial higher dose of hydrocortisone replacement can be prescribed e.g. starting with double dose and tapering down to the total 20mg daily over 2-3 months (264).

Recovery of the HPA axis can be monitored by measurement of 09.00h serum cortisol after omission of hydrocortisone replacement for 20 hours. Because recovery after transsphenoidal surgery rarely occurs before 3-6 months and is common at 1 year, initial testing at 3-4 to 9 months is reasonable (122). If the cortisol is undetectable on 2 consecutive days, then recovery of the axis has not occurred and glucocorticoid replacement can be restarted. If the cortisol is >100nmol/L, adequate reserve of the HPA axis can be assessed using the insulin tolerance test (231), with a peak cortisol value of greater than 500 nmol/L (>18 µg/dL), indicating adequate reserve (265), although this value may need to be revised downwards with more recent assays. Many centers use the cortisol response to 250 µg synthetic (1-24) ACTH (Short Synacthen Test) as an alternative means of assessing HPA reserve (266, 267), but there is some controversy as to its reliability in this situation (267, 268) and it is certainly not recommended in the first 6 weeks post-surgery. If it is used instead of the insulin tolerance test, a 30-minute cortisol is most reliable (265), but the cut-off value for a ‘passed’ SST can vary between laboratories and assays (430-550nmol/L). Glucocorticoid replacement can be discontinued abruptly if the cortisol response is shown to be normal. Where recovery of the HPA axis is only partial on dynamic testing, but the 09.00h cortisol levels are above the lower limit of the normal range (200 nmol/L [7 µg/dL]), it is reasonable to slightly lower the hydrocortisone dose and repeat SST in 3-6 months unless symptoms of adrenal insufficiency occur. Patients need to continue to be aware of the continuing need for additional glucocorticoids at times of stress or illness and should be given a supply of oral hydrocortisone and an intramuscular injection pack. Assessment of adequate replacement of hydrocortisone dosing by measuring serum cortisol at various points throughout the day, ensuring that levels are always sufficient (>50 nmol/L [>1.8 µg/dL]) before each dose, is useful. This may mean that the peak levels after each dose appear to be unphysiological, but there is a trade-off between mirroring a normal physiologic rhythm as far as possible and the inconvenience of multiple dosing. Modified release hydrocortisone may provide more physiological replacement (269).

Two further conundrums may arise: the questions of recurrence and permanent lack of recovery of the axis. Life-long monitoring for recurrence of hypercortisolism is required (270). The evaluation of recurrence should start after the recovery of HPA axis has been confirmed and continue annually along clinical assessment. Patients who articulate that the Cushing’s syndrome has returned are often correct, even before physical and biochemical evidence are unequivocal. Investigation is warranted in a patient with these complaints or with recurrent physical signs characteristic of hypercortisolemia. Measurement of late-night salivary cortisol (at least 2 samples on different days) is most sensitive for detecting recurrence (105, 271), followed by 1mg dexamethasone suppression test and 24hours UFC (again at least 2-3 collections).

If recurrent Cushing’s disease is diagnosed, the therapeutic options are the same as for persistent disease. Repeat transsphenoidal surgery should be offered for recurrence of Cushing’s disease if tumor is visible on MRI, ACTH-staining from 1st operation confirmed a corticotroph adenoma or the initial IPSS was consistent with Cushing’s disease (272, 273). The remission rates from re-operation are reported between 37% and 88% with increasing risk of complications (241). Predictors of remission were post-operative cortisol of <55nmol/L (<2ng/dL), and operation for recurrence rather than persistent disease. It should be remembered when investigating recurrence that long-standing ACTH stimulation by a pituitary adenoma causing macronodular adrenal hyperplasia may subsequently involve semi-autonomous cortisol production (274).

The patient who has a subnormal cortisol response to ACTH 3 years after transsphenoidal surgery (in the absence of over-replacement) is likely to proceed to life-long ACTH deficiency, but this is also highly indicative of a lack of recurrence long-term.

Post-operatively, assessment for deficiencies of other pituitary hormones should also be sought, and the appropriate replacement regimen initiated as necessary, especially growth hormone deficiency in children.

Diuresis is common after transsphenoidal surgery and may result from intraoperative or glucocorticoid-induced fluid overload or may be due to AVP deficiency. For these reasons, assessment of paired serum and urine osmolality and the serum sodium concentration is essential. It is advisable to withhold specific therapy unless the serum osmolality is greater than 295 mOsm/kg, the serum sodium is greater than 145 mmol/L, and the urine output is greater than 200 mL/hour with an inappropriately low urine osmolality. Desmopressin (DDAVP, Ferring) 0.5-1 µg given subcutaneously will provide adequate vasopressin replacement for 12 hours or more.

Hyponatremia may occur in as many as 20% of patients within 10 days of surgery. This may be due to injudicious fluid replacement or the syndrome of inappropriate antidiuretic hormone secretion (SIAD) as is frequently seen after extensive gland exploration, and fluid intake should be restricted (275).

While transient central diabetes insipidus is common, in about 20% of operations (276), a small minority of patients proceed to permanent AVP deficiency, requiring long-term treatment with a vasopressin analogue. The state of permanent diabetes insipidus is usually accompanied by other anterior pituitary hormone deficiencies (277).

Many glucocorticoid-induced abnormalities, including hypokalemia, hypertension, and glucose intolerance, may be normalized during the post-operative period so that preoperative treatments for these need to be reassessed.

BILATERAL ADRENALECTOMY

Bilateral adrenalectomy is also an important therapeutic option in patients with ACTH-dependent Cushing’s syndrome not cured by other techniques, particularly in young patients desiring fertility where there are concerns over radiotherapy-induced hypopituitarism. However, it has the disadvantages of life-long glucocorticoid and mineralocorticoid replacement therapy, and increased peri-operative morbidity and mortality (although these complications should be extremely low following laparoscopic adrenalectomy in experienced centers). The incidence of adrenal crisis following bilateral adrenalectomy throughout life is reported higher than in patients with Addison’s disease or ACTH deficiency (9.3 events per 100 patients versus 3-6 events/100 patients) (278). In the post-operative period after bilateral adrenalectomy, the hydrocortisone dose should be maintained at 50mg of hydrocortisone four times a day by intravenous/intramuscular injection or 200mg per 24 hours in continuous intravenous infusion (279). When no complications are seen after 48 hours post-operatively, the dose of hydrocortisone is reduced to the double replacement dose (40 mg total/day). At this stage, fludrocortisone 100-200mcg daily orally should be introduced.

In addition, the development of Nelson’s syndrome in patients with ACTH-secreting pituitary adenomas occurs in between 28% and 53% of cases (280-283) at a mean time of 5.3 years following surgery. The chance of developing Nelson’s syndrome (later renamed as “corticotroph tumor progression after bilateral adrenalectomy”) appears to be greater if adrenalectomy is performed at a younger age, and if a pituitary adenoma is confirmed at previous pituitary surgery (280, 284). Prophylactic pituitary radiotherapy probably reduces the risk of developing Nelson’s syndrome (280). However, it may be best to hold radiotherapy in reserve and undertake regular MRI scanning of the pituitary, especially when imaging has originally not shown any clear tumor (285). The expert consensus recommends MRI scanning at 3 months then 12-monthly for 3 years after bilateral adrenalectomy and every 2-4 years afterwards (283). New or worsening skin hyperpigmentation should prompt ACTH measurement and pituitary MRI. The ACTH threshold proposed as a cut-off for diagnosis of Nelson’s syndrome is not agreed and varies between 200 and 700pg/mL (44-154pmol/L, taken before the morning dose of hydrocortisone) with progressive increase of ACTH being more indicative (283). Others have advocated unilateral adrenalectomy plus pituitary irradiation as an alternative to bilateral adrenalectomy, as it gives similar remission rates to primary transsphenoidal surgery (286), but this should be reserved for selected cases. Transsphenoidal surgery for corticotroph tumor progression should be considered as first-line treatment before extrasellar expansion occurs with radiotherapy as second-line treatment if appropriate following multi-disciplinary team discussion (283). There is no established medical treatment in Nelson’s syndrome, and single case reports of aggressive tumors suggest some response to temozolomide (287, 288). A recurrence of hypercortisolism following bilateral adrenalectomy due to growth of rest adrenal tissue with persistent ACTH stimulation is reported in <10% of cases (105).

PITUITARY RADIOTHERAPY

For patients in whom fertility does not represent an important issue and with uncertain preoperative localization, radiotherapy may be used as primary treatment, while in patients showing no signs of remission after transsphenoidal resection of a tumor, pituitary irradiation is one of the next treatment options. It may also be considered as primary therapy for children under age 18 years, because results are comparable to surgery (289, 290). Pituitary irradiation may also decrease the occurrence of Nelson's syndrome (“corticotroph tumor progression after bilateral adrenalectomy”) after medical or surgical adrenalectomy, but this has not been tested in a prospective randomized trial (291).

Primary pituitary radiotherapy for the treatment of Cushing’s disease in adults has been shown to produce rather poor long-term remission rates of around 50% (249, 292). In contrast, as a second-line therapy to failed pituitary surgery, better results are achieved with around 80% showing long-term remission as defined by the normalization of the clinical state and biochemical parameters (293, 294). In children, however, not only primary therapy shows better results with cure rate of 80%, but also they respond more rapidly, usually within 12 months (290), while remission in adults usually occurs by two years although it can take considerably longer. Medical therapy to control hypercortisolemia is usually utilized in the interim, and patients should be reassessed at least yearly (295). In order to evaluate results of pituitary irradiation, urinary free cortisol or several serum cortisol levels throughout the day are measured and medical therapy should be stopped for several consecutive days, followed upon patient education of early recognition signs and symptoms of adrenal insufficiency in outpatient conditions.

Conventional pituitary radiotherapy using a linear accelerator is delivered at a total dose of 45 to 50 Gy in 25 fractional doses over 35 days using a 3-or 5-field (opposed lateral fields and vertex field) technique. Side effects when given as primary therapy are rare, but there is significant risk of growth hormone deficiency occurring early in 36-68% of treated adults, while other anterior pituitary deficiencies may develop over time in around 20% of patients (96, 293, 296). There is some evidence of an increased risk of cerebrovascular complications, which is of concern particularly in younger patients (297), but not all studies agree and further studies are required (298). The incidence of ischemic infarcts after fractionated radiotherapy for pituitary adenomas was reported in the mean of 6.7% of patients (0-11.6%) in the systematic review of 11 studies including 4394 patients. Four studies on complications of gamma-knife surgery described no ischemic events (299) (see below). The risk of optic neuropathy is low and probably less than 1% as long as low-dose fractions are used. Although meningiomas and gliomas have been reported after pituitary radiotherapy (295, 300), a recent analysis suggests that external beam radiotherapy induces second tumors in around 4% of patients with pituitary tumors compared to 2% in controls (301) .

Stereotactic radiotherapy using a gamma-knife or Cyber-knife (‘radiosurgery’) is used to optimize the tumor dose and minimize radiation to other areas by delivering a single high dose (average dose of 20-25Gy) to a small tumor. This approach seeks to avoid the complications of optic neuritis and cortical necrosis associated with larger total and fractional doses (302), not to mention convenience for the patient receiving therapy in one treatment. It has been less well investigated so far, but has a number of theoretical advantages, including a possible reduction in risk of cerebrovascular disease. It is hard to make a direct comparison in effectiveness between methods because of the difference in size of the treated tumors (302, 303). Most patients still develop endocrine deficiencies in the years after treatment (304-306). Because of the high dose of delivered radiation, it is not suitable for large lesions because of the large volume of exposed tissue, or for lesions near to radiosensitive tissues, such as the optic chiasm or optic nerves (recommended at least 3-5mm distance), because of the potential for visual damage. Otherwise, if the adenoma is not close to the optic pathway, it may be superior to conventional fractionated therapy. Gamma knife radiosurgery is probably the most widely used of these techniques. As adjunctive therapy after failed transsphenoidal surgery it achieves biochemical remission in about 48-55%, although follow-up times have not been as long as for conventional radiotherapy (296, 306, 307). It can also be used as salvage therapy in difficult tumors (307, 308). Radiosurgery of the pituitary gland using proton beams has similar efficacy as second-line therapy (309), and while possibly more precise is not widely available. Cyber-knife radiotherapy for Cushing’s disease is less well described, but there are reports of some success in a small number of patients (310). As with other forms of radiotherapy, new hormone deficiencies are the major side-effect. It should be emphasized again that stereotactic radiotherapy cannot be used when the tumor is close to the optic chiasm. There is a difference in tolerance of radiation between cranial nerves, with optic nerves most sensitive. A dose above 8Gy should be avoided and a clearance of 3-5mm from the optic nerves is required, while for other cranial nerves doses of 19-23Gy are acceptable (311). Data on the use of proton beam therapy are sparse, but in time this may come to replace other forms of radiosurgery (312).

**Treatment for the Ectopic ACTH Syndrome**

If the ectopic ACTH-secreting tumor is non-metastatic and amenable to surgical excision, such as in a lobectomy for a bronchial carcinoid tumor, the chance of cure of Cushing’s syndrome is high.

Local radiotherapy following surgical resection of an ectopic ACTH-secreting source, may also be beneficial, particularly in non-metastatic thoracic carcinoid tumors (313, 314), but is not usually required. The course of the disease is mainly determined by the type of tumor, the presence of metastases, and degree of hypercortisolism. The lowest survival rate comes with small cell lung cancer, medullary thyroid cancer, and gastrinomas (17, 18). In patients with metastases solely in the liver, cryoablation, resection, or even liver transplantation can be curable. Prognosis is the best in patients younger than 50 years of age, with primary bowel or lung carcinoids (19, 315, 316). However, if significant metastatic disease is present, surgery is not curative, although it may still be of benefit in selected cases. Therapy for residual or metastatic disease should be based on current guidelines for neuroendocrine tumors (317).

Regardless of the prognosis, control of the hypercortisolism should be established medically by inhibiting steroidogenesis. If medical management fails, surgical bilateral adrenalectomy may be an option, but should be at least considered in the majority of cases where long-term treatment of the neuroendocrine tumor is considered. Patients in whom control over hypercortisolism is established can develop thymic hyperplasia (318), which should be distinguished from tumor metastases or a primary thymic tumor. In cases where primary tumor origin remains unknown, adrenal inhibitor therapy can be maintained as long as the patient undergoes to periodic re-examination for tumor localization (17, 18).

The ectopic CRH syndrome is rare and usually is associated with pulmonary carcinoid tumors, following the same therapeutic principles as ACTH-secreting tumors (319).

**Treatment of ACTH-Independent Cushing’s Syndrome**

Adrenalectomy is the treatment of choice for all cases of ACTH-independent Cushing’s syndrome. This is either unilateral in the case of an adrenal adenoma or carcinoma, or bilateral in cases of bilateral hyperplasia, either micronodular or macronodular. The only exception can be the case of milder hypercortisolism in macronodular hyperplasia, when unilateral adrenalectomy may provide hormonal control, at least temporarily (320, 321). Pre-operatively, adrenal enzyme inhibitor therapy can be used such that the clinical state of the patient is improved thus reducing the risk of complications. In cases where macronodular hyperplasia comes as a consequence of aberrant hormonal receptor expression, eucortisolemia can be achieved by using the appropriate receptor blockade (322, 323), but this is unlikely to be useful in the long-term.

In adrenal adenomas, cure following surgery in skilled hands approaches 100% (324), and is associated with low morbidity and mortality (325).

Laparoscopic adrenalectomy, both unilateral and bilateral, has been shown in experienced hands to be a safe procedure and in most centers has become the approach of choice for non-malignant disease. Its complication rate is lower than with the open approach, and the in-patient stay is significantly reduced (326, 327). A study comparing three surgical techniques (anterior laparoscopic, posterior laparoscopic, and robotic surgery) for bilateral adrenalectomy for Cushing’s syndrome showed similar morbidity in all approaches (328).

When the adrenal lesion is more than 6cm and suggestive of malignancy, open adrenalectomy remains the gold standard (329). In adrenal cancer, more aggressive surgical approaches probably account for the increase in life span reported in this disease (330). This approach may require multiple operations to resect primary lesions, local recurrences, and hepatic, thoracic, and, occasionally, intracranial metastases, and is usually accompanied by adjuvant mitotane, as discussed below. Overall, there is no significant evidence that radiotherapy improves survival in adrenocortical carcinoma, although in the literature there are sporadic reports that it may be helpful adjuvant treatment to radical surgery in selected cases and may decrease local recurrence (331-333).

**Medical Therapy of Cushing’s Syndrome**

Although the primary therapy of hypercortisolism in Cushing’s disease is surgical, medical therapy can be required in cases when surgery is delayed, contraindicated, or unsuccessful. The most common therapy is the use of adrenal enzyme inhibitors, less frequently somatostatin and dopamine receptor agonists and glucocorticoid receptor antagonists.

The role of medical treatment of Cushing’s syndrome is an important one. It is practice of many groups to pre-treat Cushing's syndrome patients with severe disease prior to surgical treatment to reverse the hypercortisolemia and its metabolic sequelae, and to hopefully reduce the complications of the definitive procedure. However, it is not routine practice for most patients with Cushing’s disease. Similarly, medical treatment is desirable in patients with Cushing's disease whilst awaiting for pituitary radiotherapy to take effect. In patients where surgery and/or radiotherapy have failed, medical management is often essential prior to (or long-term as an alternative to) bilateral adrenalectomy. Sometimes, in the occult ectopic ACTH syndrome, it may not always be possible to identify the source of secretion, and therefore medical management is desirable pending re-investigation. Finally, medical therapy is helpful as a palliative modality in patients with metastatic disease-causing Cushing's syndrome, at least in the short-term.

The most commonly used agents are adrenal enzyme inhibitors, but adrenolytic agents, pituitary-targeted therapies, or glucocorticoid-receptor antagonists are also used (Table 4). Drugs can be used in combinations in lower doses, aiming for side effect reduction with synergistic effects.

When determining the approach to treatment, the first step is to determine whether the final goal is reducing the level of serum cortisol to normal values or complete cortisol secretion blockade. The latter approach is convenient for patients with more variable secretion, while patients showing less variability can benefit more from lowering the values to the normal range and therefore avoiding the necessity of steroid replacement therapy, as well as a possibility of side effects connected to the higher dosages required with that strategy. A meta-analysis of 35 studies including 1520 patients reported pooled effectiveness of most commonly used medical agents in treatment of Cushing's syndrome, with mitotane being most effective in normalizing cortisol levels in 81.8% of patients and cabergoline (see below) being least effective and normalizing cortisol in 35.7% (334). However, as noted below, mitotane is not a simple drug to use or monitor, and generally it is reserved for adrenocortical carcinoma. The use of multiple agents achieved normalization of cortisol in 65.7% of patients.

ADRENAL ENZYME INHIBITORS

These agentsare primarily used as inhibitors of steroid biosynthesis in the adrenal cortex (Figure 5), and thus can be utilized in all cases of hypercortisolemia regardless of cause, but most commonly in ACTH-dependent forms, often with rapid improvement in the clinical features of Cushing's syndrome. The most commonly used agents are metyrapone, ketoconazole, and in certain circumstances etomidate. In the UK ketoconazole and metyrapone are licensed for the treatment of Cushing's syndrome, while mitotane is licensed for the treatment of hypercortisolemia due to adrenocortical carcinoma. The use of etomidate or mifepristone in Cushing's syndrome is off-license. Osilodrostat has also been approved for treatment of Cushing’s syndrome in USA and in Europe in 2020 and NICE-approved it in UK in 2021. However, the regulations could differ in different countries. When used in combinations, they have a synergistic therapeutic effect, lowering the rate of side effects.



**Figure 5. Steroidogenesis with main adrenal enzyme inhibitors point of action marked; SCCE – side-chain cleavage enzyme, HSD – hydroxysteroid dehydrogenase, OH – hydroxylase, DHEA – dehydroepiandrosterone, AR – aromatase, AS – aldosterone synthetase.**

*Metyrapone*

Metyrapone acts primarily to inhibit the enzyme 11β-hydroxylase, thus blocking the production of cortisol from 11-deoxycortisol in the adrenal gland (335) (Figure 5). As a consequence of the blockade of cortisol synthesis, levels of adrenal androgens and deoxycorticosterone rise. The subsequent elevation of 11-deoxycortisol can be monitored in the serum of patients treated with metyrapone. It should be noted that there may be cross-reactivity from 11-deoxycortisol with some cortisol radioimmunoassays: this may result in an unnecessary increase in the metyrapone dose and subsequent clinical hypoadrenalism (336). It is preferable to measure the serum cortisol via liquid chromatography-tandem mass spectrometry in patients treated with metyrapone (337). The fall in cortisol is rapid, with trough levels at 2 hours post-dose, and sometimes administration of a test dose of 750 mg with hourly cortisol estimation for 4 hours is performed, although not strictly necessary in our opinion (338). Maintenance therapy is usually in the range 750-6000 mg/day in 3-4 divided doses daily. Metyrapone has been used to good effect to reduce the hypercortisolemia in patients with Cushing's syndrome from adrenal tumors, the ectopic ACTH syndrome, and Cushing's disease. In the former, patients can be very sensitive to low doses of this agent, whilst in Cushing’s disease higher doses are often required. In Cushing's disease this can be due to the compensatory rise in ACTH in patients not having received pituitary radiotherapy. During short-term follow-up (1-16 weeks) of 54 patients with Cushing’s disease, cortisol normalized on the metyrapone treatment in 75% of participants and in 81% of 16 patients with adrenocortical carcinoma or adenoma (338). A subsequent multicenter study on 164 patients with Cushing’s disease reported that 43% achieved control of hypercortisolism at the mean of 8 months of treatment (339).A meta-analysis of 18 retrospective studies including patients with CD showed an average remission rate of 75.9% (31.3-83.2%) (334). The recent prospective study PROMPT including 50 patients with Cushing’s syndrome reported remission in 47% participants (340).

There have not been serious maternal or perinatal complications connected with the use of metyrapone in pregnant women, but the question of safety remains open (341-343). However, metyrapone and ketoconazole are the medications most commonly used in the treatment of Cushing’s syndrome in pregnancy (344).

The principal side effects with metyrapone are hirsutism and acne (as predicted by the rise in adrenal androgens) and reported by 70-83% of women. Dizziness and gastrointestinal upset occurring in 5% and 15% respectively. Because of the androgen effect the drug is not considered appropriate for the first-line therapy of long-term treatment in women (345, 346). However, it is hypoadrenalism that remains the most important potential problem, and careful monitoring of treatment and education of the patient is required. If there is uncertainty as to whether the measured cortisol is valid, and not over-estimated by cross-reactivity, it may be appropriate to consider a ‘block-and-replace’ regimen. Hypokalemia, edema, and hypertension due to salt retention because of mineralocorticoid activity of raised levels of 11-deoxycorticosterone are infrequent (338), but may require cessation of therapy (347).

*Ketoconazole*

Ketoconazole is an imidazole derivative originally developed as an oral anti-fungal agent. It is a potent inhibitor of sex steroids (androstendione and testosterone) production by its action on C17-20 lyase, and cortisol secretion by 11β-hydroxylase inhibition (348-350). It also inhibits 17-hydroxylase and 18-hydroxylase activity, amongst other enzymes (351). It has also been reported to have a direct effect on ectopic ACTH secretion from a thymic carcinoid tumor (352), and possibly corticotroph ACTH release.

The treatment for Cushing's syndrome is usually started at a dose of 200 mg twice daily, with an onset of action that is probably slower than metyrapone. The usual maximum dose is 400 mg three times a day. It has been used successfully to lower cortisol levels in patients with Cushing's syndrome of various etiologies including adrenal carcinoma, the ectopic ACTH syndrome, and invasive ACTH-producing pituitary carcinoma, with doses required between 200-1200 mg/day in up to 4 divided daily doses (353, 354), although 2-3 times daily is more usual. Although there have not been consequences on human fetuses, considering animal teratogenicity and toxicity the drug is not recommend for use during pregnancy (343, 355, 356). The normalization of cortisol levels was achieved in 71.1% of patients in pooled meta-analysis of all causes of Cushing's syndrome including 220 individuals and in 49% of patients with Cushing’s disease (334). A subsequent meta-analysis of 270 patients with CD treated with ketoconazole after failed transsphenoidal surgery included in 10 studies (all but 1 retrospective) reported control of hypercortisolism in 63% of individuals (95% CI 50-74%) (357).

The principal side effect of ketoconazole is hepatotoxicity (358, 359). A reversible elevation of hepatic serum transaminases occurs in approximately 5-20% of patients, with the incidence of serious hepatic injury at around 1 in 15,000 patients (360, 361). The hepatotoxicity appears to be idiosyncratic, but has been reported within first 4 weeks of the initiation of treatment in a patient with Cushing's syndrome and resolves within 2-12 weeks after dose reduction or discontinuation of treatment (361, 362). Prior to the start of therapy liver function tests should be performed. The alanine aminotransferase (ALT) level should be monitored weekly within the first month of therapy, then once a month in the following trimester and afterwards sporadically or when the dose is changed. If levels reach 3-times above the upper normal range, therapy should be discontinued. Other adverse reactions of ketoconazole include skin rashes and gastrointestinal upset, and one must always be wary of causing adrenal insufficiency (362-364).

Ketoconazole is a CYP3A4 inhibitor and increases the availability of medications metabolized by that enzyme. Hence, the reduction of the dose of affected medications maybe required. Ketoconazole is a mixture of levo- and dextro- enantiomeric forms. Currently, the levo-enantiomer of ketoconazole is less likely to be hepatotoxic than the racemic mixture (see below).

Due to its C17-20 lyase inhibition and consequent anti-androgenic properties, ketoconazole is particularly useful in female patients where hirsutism is an issue, which may be worsened with metyrapone. Conversely, gynecomastia and reduced libido in male patients may be unacceptable as a first-line long-term treatment and require alternative agents. However, replacement therapy is an option. On the other hand, women having lower levels of estradiol and testosterone do not experience clinically manifest disorder because of the usually present menstrual irregularity. Ketoconazole requires gastric acid for absorption, so should not be given with proton-pump inhibitors. One further advantage of ketoconazole is its inhibition of cholesterol synthesis, particularly LDL cholesterol (365), and in 34 patients with Cushing's syndrome the mean total cholesterol was reduced from 6.1 to 5.0 mmol/l on ketoconazole (363).

The triazole antifungal, fluconazole can also be effective in treatment of Cushing’s syndrome, but experience is limited to single case reports. They described an effective control of hypercortisolism on 200-1200mg daily dose of fluconazole (366, 367). Fluconazole was reported *in vitro* to be 40% less effective in inhibition of 11β-hydroxylase and 17-hydrohylase than ketoconazole (368). The side effects of fluconazole are similar to those of ketoconazole.

*Osilodrostat*

Osilodrostat is a novel steroidogenesis inhibitor. FDA approved osilodrostat for treatment of Cushing's disease in 2020 and NICE in 2021 in the UK. It is a selective inhibitor of 11β-hydroxylase, an aldosterone synthase and a non-steroidal aromatase. It causes a decrease in cortisol and aldosterone levels and an increase of 11-deoxycorticosterone and 11-deoxycortisol. Osilodrostat was evaluated in phase II trial as a potential anti-hypertensive agent in patients with primary hyperaldosteronism and essential hypertension (369). In 10-week study in patients with Cushing's disease (n=12) who were not cured by previous surgery, osilodrostat normalized urinary free cortisol (UFC) in 92% of subjects with more than 50% decrease in UFC in all participants (370). In 22-week phase II trial in patients with Cushing’s disease (n=19) and UFC >1.5 of the upper normal limit, osilodrostat (10-60mg/day) normalized UFC in 79% of patients. It also produced no significant change in blood pressure and an increase of ACTH 3-4-fold. Adrenal insufficiency was seen in 32% of subjects leading to the reduction of the dose, while an increase of testosterone and hirsutism was reported in around 30% of women (370). The phase III study was a double-blind randomized trial with a withdrawal phase after 24 weeks of treatment followed by continuation of osilodrostat mean dose of 5mg twice a day from 40 to 48 weeks (371). Fifty-three percent of participants in the osilodrostat arm (n=36) maintained UFC in the normal range without increasing the dose at 24 weeks, compared to 29% in the placebo group (n=35). Sixty-six patients were not randomized to withdrawal of treatment and continued osilodrostat due to higher cortisol levels. Of 137 individuals with Cushing's disease, 66% maintained UFC in the normal range after 48 weeks (6 months) (371). The extension study up to 70 months (6 years) showed maintained complete remission of hypercortisolism in 50-88% of participants and partial control in additional 18% of individuals (372, 373). The most frequent side effects included nausea (42%), headache (34%), fatigue (28%) and adrenal insufficiency (28%). Forty two percent of patients had reported hypertension and hypokalemia due to increased adrenal precursors and 11% of women noted increased hirsutism. The side effects related to hypoadrenalism reduced to 27.3% in the extension study (373).

*Levoketoconazole*

Levoketoconazole is a stereoisomer of ketoconazole and its efficacy and safety has been assessed in the SONICS study, phase III open-label trial of 94 individuals with Cushing's syndrome (85% with Cushing’s disease) and mean UFC 4.9 times upper normal range (374). The starting dose was 150mg twice a day and titrated up to a total daily dose of 1200mg aiming for normal UFC. Thirty-one percent maintained normal UFC by 6 months of treatment and 36% during maintenance phase. However, only 55 patients completed the maintenance phase and of those 61% were in remission (374). The phase III placebo-controlled randomized-withdrawal study, LOGICS, included 79 patients with Cushing’s syndrome on a levoketoconazole maintenance dose, 40.9% lost the control of hypercortisolemia comparing to the placebo arm where 95.5% became hypercortisolemic (375). Most common adverse effects were nausea (29-32%), headache (23-28%), and deranged liver function in 11-44% of participants. However, it remains to be seen whether it proves in practice to be less hepatotoxic than the racemic mixture. Levoketoconazole has been approved for treatment of Cushing’s syndrome in adults by the FDA but not currently by the EMA.

*Etomidate*

Etomidate is an imidazole-derived anesthetic agent which was reported to have an adverse effect on adrenocortical function in 1983 (376). Compared to the other imidazole derivative ketoconazole, etomidate more potently inhibits adrenocortical 11β-hydroxylase, has a similar inhibition of 17-hydroxylase, but has less of an effect on C17-20 lyase (377). At higher concentrations it also appears to have an effect on cholesterol side-chain cleavage (378, 379). Following their initial report in 1983, Allolio and colleagues showed that intravenous non-hypnotic etomidate dose (2.5 mg/hour) normalized cortisol levels in 5 patients with Cushing's syndrome of various etiologies (380). Since then, there have been a number of case reports on the use of etomidate in successfully reducing hypercortisolemia in seriously-ill patients with either Cushing's disease or the ectopic ACTH syndrome (381-384).

It is usually given at a dose of 2.5-3.0 mg/hour, which is adjusted based on the serum cortisol levels. It usually takes several hours for cortisol to be lowered to within the normal range (385). Etomidate is an effective agent that acts rapidly, but is limited in its use by the fact it has to be given parenterally and requires intensive care settings to safely manage and monitor cortisol and potassium levels 4-6 hours to adjust the infusion rate (386). Similar to metyrapone and osilodrostat, high levels 11-deoxycortisol may cross-react with many assays. A simultaneous infusion of hydrocortisone of 0.5-2 mg/h may be required to maintain normal cortisol levels. However, in this situation it may be lifesaving. The preparation available in the USA contains the vehicle propylene glycol with the potential for nephrotoxicity and lactic acidosis, as opposed to the preparation available in Europe which contains alcohol.

*Mitotane*

Mitotane (o’p'DDD), an isomer of DDD (belonging to the same family of chemicals as the insecticide DDT), was developed following the observation of adrenal atrophy in dogs administered DDD. Mitotane inhibits steroidogenesis by reducing cortisol and aldosterone production by blocking cholesterol side-chain cleavage and 11β-hydroxylase in the adrenal gland (387). It also acts as an adrenolytic drug, causing medical adrenalectomy, after being metabolized into an acyl chloride that binds in mitochondria and causes necrosis of adrenocortical cells (388).

Mitotane is used as a treatment for adrenocortical carcinoma and causes tumor regression and improved survival in some patients (389, 390). It has a beneficial effect on endocrine hypersecretion in approximately 75% of patients (391). It is also utilized in Cushing's syndrome of non-malignant origin, and in this regard lower doses can be utilized (up to 4 g/day), thus reducing the incidence of side effects, particularly gastrointestinal (392). At these lower doses the onset of the cortisol-lowering effect takes longer (6-8 weeks) than with higher doses. Mitotane should not be used in pregnant women, and reproductively active women must use reliable contraception while on therapy (393). A pooled meta-analysis of all causes of Cushing's syndrome in 173 patients reported the normalization of cortisol levels on mitotane treatment in 79.8% of all patients and in 81.8% of participants with Cushing’s disease (334).

The main side effect of mitotane treatment include nausea, vomiting and lethargy. One problem even with low-dose mitotane is the hypercholesterolemia (principally an increase in LDL-cholesterol), which appears to be due to the impairment of hepatic production of oxysteroids, normally a brake on the enzyme HMG CoA reductase (394). However, simvastatin, an HMG CoA reductase inhibitor, can reverse the hypercholesterolemia, and it or a similar agent should be used, if necessary, in patients treated with mitotane. Other side effects of mitotane include neurological disturbances; elevation of hepatic enzymes; hypouricemia; gynecomastia in men; and a prolonged bleeding time (391, 395). Most importantly, it elevates cortisol-binding globulin, such that levels of total serum cortisol are misleading. Control should be titrated using urinary free cortisol or salivary cortisol. Monitoring of serum levels of mitotane should be undertaken due to its narrow therapeutic window and the risk of toxicity. In the long-term, measurement of blood levels can allow dose titration and reduction as appropriate. A therapeutic level of 14-20 mg/L has been recommended for adrenocortical carcinoma, but lower levels can be sought for simple control of elevated cortisol levels. Mitotane is taken up by fatty tissues, sometimes being released gradually several months after discontinuing therapy, therefore requiring adjustments in glucocorticoid therapy dosage (396). Mitotane shows cytotoxic activity on both normal and tumorous tissue causing primary adrenal insufficiency and therefore requiring glucocorticoid replacement therapy. It tends to spare the zona glomerulosa, but in long-term use mineralocorticoid replacement is also needed (397). In general, despite effective in other forms of Cushing’s syndrome, its use has been limited outside of adrenocortical carcinoma, in which cases it has been shown to prolong life (390).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 4. Currently Available Medical Therapy for Cushing’s Syndrome (CS)** | | | | | |
| **Medication** | **Action** | **Dosage** | **Side effects** | **Contra-indications** | **Comments** |
| **Steroidogenesis inhibitors** | | | | | |
| Metyrapone | 11β-hydroxylase inhibitor | 250-1000mg  tds-qds, max 6g/day po | Nausea, vomiting, acne, hirsutism, hypo- or hypertension, oedema, hypokalemia | Pregnancy, breast-feeding, porphyria, severe liver impairment | 1st line treatment when available, avoid long-term use in young women |
| Ketoconazole | 11β-hydroxylase and 17,20-lyase inhibitor, | 200-400mg  tds po | Gynecomastia, alopecia, hypogonadism in men, hepatotoxicity, Gastrointestinal symptoms, rash | Liver impairment, pregnancy/  breast-feeding,  porphyria | Slow in onset of action, 1st line in children, stop PPI/H2-antagonist as gastric acid needed for absorption |
| Osilodrostat | 11β-hydroxylase inhibitor, | 2-7mg bd po | Hypertension, hypokalemia, hirsutism, asthenia, GI symptoms, adrenal insufficiency, headache | Pregnancy & breast feeding, | To use low dose in liver impairment,  Risk of increasing QT interval |
| Mitotane | Adrenolytic | 500-1000mg tds-qds, gradually increased from 500-1000mg/day to max 6g/day po | Gastrointestinal symptoms, deranged LFTs and TFTs, hyper-cholesterolemia, ataxia, orthostatic hypotension | Pregnancy/  breast-feeding,  stage 4-5 renal failure, severe liver impairment | Slow in action, hyperglycemia, mitotane level monitoring required, accumulates, now rarely used for CD, high rate of withdrawal due to intolerance |
| Etomidate | 11β-hydroxylase inhibitor | 0.01-0.5mg/kg/h iv | Sedation, nausea and vomiting, temporary uncontrolled muscle movements,  rash, angioedema | Pregnancy, breast-feeding, porphyria | Parenteral, rapid onset of action, anesthetic agent so ITU settings required, frequent monitoring of cortisol and K+ |
| **Modulators of ACTH release** | | | | | |
| Cabergoline | Dopamine agonist | 1-7mg/week po | postural hypotension, nausea, increased tendency of gambling, hallucinations, oedema, depression, possibility of heart valve sclerosis (only very high doses) | Porphyria, pregnancy, hyper-sensitivity to ergot derivates,  valvulopathy | Effective in <40% of patients, which wears off with time, cheap |
| Pasireotide | Somatostatin analogue | 600-900μg  twice daily sc | Hyperglycemia, cholelithiasis, diarrhea, headache | Severe liver impairment,  Avoid in poorly controlled diabetes | Effective only in mild CD, treatment of hyperglycemia frequently required |
| **Glucocorticoid receptor antagonist** | | | | | |
| Mifepristone | Glucocorticoid receptor antagonist | 300-1200mg daily po | nausea, vomiting, dizziness, headache, arthralgia, increased TSH, decreased HDL, endometrial thickening, rash, oedema | Severe asthma, porphyria, renal failure, severe liver impairment, breast-feeding | Cortisol and ACTH levels remain high so hypokalemia may persist, also anti-progesterone, monitoring difficult |
| **Investigational status in some countries** | | | | | |
| Levoketoco-nazole | 11β-hydroxylase and 17,20-lyase inhibitor | 300-1200mg, bd po | Headache, oedema, GI symptoms, increased liver enzymes, adrenal insufficiency | Liver impairment, pregnancy/  breast-feeding,  porphyria | FDA & EMA orphan drug status |

CBG – cortisol binding globulin, CD – Cushing’s disease, tds – 3 times a day; qds – 4 times a day, LFTs – liver function tests, TFTs – thyroid function tests, PPI – proton pump inhibitor, K+ - potassium, ACTH – adrenocorticotrophin hormone, po – orally, iv- intravenous, sc – subcutaneous, ITU – intensive care unit, FDA – Food and Drug Administration (USA), EMA – European Medicines Agency.

MODULATORS OF ACTH RELEASE

*Pasireotide*

Somatostatin receptors have been demonstrated on both corticotroph adenomas, and some ectopic ACTH-secreting tumors. However, although octreotide has been helpful in reducing ACTH and cortisol levels in selected case reports of ectopic ACTH-secreting tumors there has been much more limited success in patients with Cushing's syndrome probably through down-regulation of receptor sub-type 2 in these tumors by hypercortisolemia (398).

There has been renewed interest with the introduction of pasireotide, a somatostatin analogue with a broader spectrum of activity for somatostatin receptor subtypes, including type 5, which is not down-regulated during hypercortisolemia. Ever since this agent was shown *in vitro* to reduce human corticotroph proliferation and ACTH secretion (399, 400), there have now been a number of clinical trials published. In an initial phase II trial, pasireotide 600µg injected twice daily for 15 days reduced urinary free cortisol (UFC) levels in 76% of 29 patients and normalized levels in 17% (401). A multicenter phase III dose-randomized trial in 162 patients with either new, persistent, or recurrent Cushing's disease has shown at six months a reduction in UFC levels in 91 of 103 evaluable patients, with a median UFC reduction of 48%. Normalization of UFC levels were achieved in 14.6% of patients on the 600µg dose twice daily, and 26% of patients on the 900µg twice-daily dose. Patients who showed <50% reduction in UFC levels from baseline by month two were unlikely to show improvement by month 6 or 12.

The most clinically relevant adverse events were hyperglycemia (73%), with 46% developing frank diabetes mellitus related to decreases in both insulin and incretin secretion, and hypocortisolemia (8%) (401, 402). Other side effects included elevated liver enzymes, cholelithiasis, nausea and diarrhea at the rate expected from experience with other somatostatin analogues (402).

There is now also experience with pasireotide long-acting repeatable (pasireotide LAR), a monthly injection of 10 or 30mg, reporting around 41% of patients achieving normal UFC levels at 7 months of treatment and a similar safety profile to the subcutaneous form (403). More than a 20% reduction in size of the pituitary adenoma was described in 45% of patients and an increase by more than 20% in 10% of individuals (403). Long-term extension studies of monthly pasireotide showed improvement of cortisol levels up to 5 years (404). Pasireotide LAR decreased median volume of the corticotroph adenoma by 16.3-17.8% in 43-47% of patients (403).

Pasireotide is not recommended as a first-line treatment but can be considered as add-on therapy or second-line treatment if other medications are not tolerated. In cases where there is no clinical response, it should be discontinued.

Pasireotide at a lower dose of 250 µg three times daily has also been used in stepwise combination therapy with the dopamine agonist cabergoline (previously been demonstrated to have modest but variable efficacy as monotherapy in Cushing's disease (405), and ketoconazole. Pasireotide monotherapy induced normalization of UFC levels in 5 of 17 patients (29%). The addition of cabergoline normalized UFC levels in an additional 4 patients (24%). The further addition of ketoconazole in the remaining 8 patients induced normalization of UFC levels in 6 of these. Thus, in total, remission was achieved in 88% of patients using combination therapy out to 80 days of treatment (405). Therefore, pasireotide represents a potential new treatment for *mild* Cushing's disease or in combination therapy for individuals with higher hypercortisolemia, although the frequency of hyperglycemia is of major concern.

Corticotroph adenomas with *USP8* mutations had been reported to have higher SST5 receptor expression which may suggest higher response rate to pasireotide treatment in this subgroup (406, 407).

*Cabergoline*

The presence of dopamine receptors (D2) on around 80% of corticotroph adenomas supported the use of cabergoline in patients with Cushing’s disease (408). Cabergoline at a dose of 1-7mg weekly was reported to control hypercortisolemia due to Cushing’s disease in 25-40% of patients in small case series (409). A multicenter retrospective study of 53 patients treated with a median dose of 2.3mg/week normalized UFC in 40% of individuals in the first year of treatment, which was reduced to 23% at 32.5 months (410).

It is usually well tolerated and the most common side effects include nausea and dizziness. At the doses used for the treatment of pituitary tumors, the incidence of cardiac valve sclerosis and subsequent regurgitation was not increased in one large study, and therefore echocardiograms are not routinely needed unless high, long-term treatment is required (411). However, escape is seen in some patients, so the percentage of patients with long-term control is low. Another side effect is an impulse control disorder for which patients should be counselled before initiation of treatment (412).

*Temozolomide*

Temozolomide is an oral alkylating prodrug that is converted *in vivo* to the DNA repair inhibitor, dacarbazine. Traditionally, this chemotherapy agent has been used in the treatment of malignant gliomas, but recent evidence suggests it is also useful in selected aggressive pituitary tumors including corticotroph pituitary carcinomas (413, 414). Although, some reports suggested that the response to temozolomide in pituitary tumors can be predicted by low expression of the DNA repair enzyme O6-methylguanine-DNA-methyltransferase (MGMT), possibly related to MGMT gene promotor methylation (415, 416), not all studies have confirmed this (417, 418). However, the therapeutic response can usually be determined after 3 cycles of chemotherapy. Reported partial or complete response from case reports is around 80%, with improvement seen after 2 months with tumor size reduction from stable to 50% (419). Side effects include cytopenia, GI symptoms, headaches, hearing loss and dizziness.

OTHER AGENTS

*Retinoic Acid*

Retinoic acid has been found to inhibit ACTH-secretion and cell proliferation both *in vitro* in ACTH-producing tumor cell lines and cultured human corticotroph adenomas, and *in vivo* in nude mice (420). However, clinical trials in man are limited, and it is unlikely to be a major contributor to control.

*Rosiglitazone*

The thiazolidinedione rosiglitazone, a PPAR-γ agonist, was shown in supra-pharmacological doses to suppress ACTH secretion in human and murine corticotroph tumor cells. In addition, the development of murine corticotroph tumors, generated by subcutaneous injection of ACTH-secreting AtT20 cells, were prevented (421). It appears this is not specific to corticotroph adenomas, but also applies to other forms of pituitary tumor (422). However, the results in human subjects with Cushing's disease have been disappointing (423-425). This may be because doses used in the animal studies were much higher than the equivalent licensed dose in humans. Its use cannot be recommended.

*Receptor Antagonists to GIP, β-adrenergic and LH/hCG Receptors*

In the rare causes of Cushing’s syndrome due to bilateral macronodular adrenal hyperplasia (BMAD) and aberrant receptor expression of GIP, β-adrenergic and LH/hCG receptors, specific receptor antagonists may prove to be useful (426). Although octreotide has been shown to have a therapeutic response in GIP-related BMAD as mentioned above (31), others have found neither this somatostatin analogue nor pasireotide to be helpful in inducing a sustained response (427).

*Glucocorticoid Receptor Antagonist(s)*

Mifepristone (RU 486), is a potent antagonist of glucocorticoid and progesterone receptors that blocks the peripheral actions of glucocorticoids and progestogens (428, 429). As a consequence it also blocks glucocorticoid-induced negative feedback at the hypothalamo-pituitary level, inducing a rise in ACTH, arginine-vasopressin (AVP) and hence cortisol (430). It has occasionally been given to patients with all forms of Cushing's syndrome (431, 432), showing effectiveness in rapidly reducing symptoms of cortisol-induced psychosis (433, 434), and improving glycemic control and hypertension (432). Although, it has been proven to be effective in the treatment of hypercortisolemia-related symptoms and signs (431, 435), the major drawback is the lack of biochemical markers to assess either therapeutical effectiveness or possible hypoadrenalism. Adrenal insufficiency is challenging to treat, because the drug, besides blocking endogenous cortisol, also blocks the action of synthetic steroids as replacement therapy. Hypokalemia is a frequent problem due to the saturation of 11β-HSD type 2 and cortisol action on the mineralocorticoid receptor, although it responds well to spironolactone. The daily dose of mifepristone ranges between 300 and 1200mg. It showed a significant improvement of glucose and HbA1c in 60% of patients with impaired glucose tolerance or diabetes (432). Mifepristone could be used as add-on therapy for Cushing’s syndrome with associated hyperglycemia. Endometrial thickening and vaginal bleeding secondary to the anti-progestin effect are likely to be seen in women. However, a new derivative of mifepristone with less anti-progestogen blocking activity, relacorilant, is currently under trial.

*Relacorilant (CORT125134)*

Relacorilant is a glucocorticoid receptor inhibitor with no effect on the progesterone receptor. A phase II study (GRACE) included 130 patients with Cushing’s syndrome and type 2 diabetes and/or hypertension. Half of the patients receiving higher doses (range of 100mg-400mg daily) of relacorilant for 16 weeks and the HbA1c was reduced by ≥0.5% or the dose of insulin/sulfonylurea reduced by ≥25%. A reduction of systolic BP by at least 5mmHg was reported in 64% of participants receiving a higher dose of medication. A Phase III multicenter, placebo-controlled randomized withdrawal trial is still on-going and expected to be completed in 2024 ([clinicaltrials.gov](http://www.clinicaltrials.gov) code: [NCT03697109](https://clinicaltrials.gov/ct2/show/NCT03697109)).

It should be noted that the use of all these novel agents may be limited by their expense and availability.

MONITORING TREATMENT

It is important to monitor all patients on medical therapy for Cushing’s syndrome in order to assess the effectiveness of treatment, and in particular to avoid adrenal insufficiency. Serum cortisol level and/or urine cortisol level are used in order to estimate steroid inhibitor therapy. One way is to assess the mean of 5 serum cortisol measurements across the day, although others favor measurement of urinary free cortisol (UFC). A mean serum cortisol between 150 and 300 nmol/L (5.5-11 μg/dL) corresponded to a normal cortisol production rate (436), and this range should be the aim of therapy, although this figure may be an overestimate as it is based on older cortisol assays. As mentioned above, a liquid chromatography tandem mass spectrography cortisol assay is preferable in patients on metyrapone, osilodrostat and etomidate.

When mitotane is used, only measurement of 24-hour urinary free cortisol reflects therapy effectiveness and concentration of serum free cortisol, because mitotane reduces 17-OHCS excretion. Because it raises the level of cortisol binding globulin (CBG), the level of total serum cortisol is inappropriate for monitoring of cortisol secretion, as it can be two to threefold elevated (437, 438). The high level of CBG explains why replacement dosage of steroids needs to be increased in cases of adrenal insufficiency, although there is also a contribution from increased hepatic steroid metabolism.

**CUSHING’S SYNDROME IN SPECIFIC GROUPS**

**Chronic Renal Failure**

Cushing’s syndrome in the setting of chronic renal failure is poorly described, but may pose diagnostic difficulties. In chronic renal failure serum levels of cortisol are generally normal but with some radioimmunoassays may be increased (439, 440). ACTH levels are increased (441). Glomerular filtration rates of less than 30 mL/min result in decreased cortisol excretion and spuriously low UFC values (442). The ACTH and cortisol responses to CRH may be suppressed in patients with renal failure, except for those undergoing continuous ambulatory peritoneal dialysis (443). The metabolism of dexamethasone is normal in chronic renal failure, but the oral absorption can be altered in some patients. There is a reduced degree of suppression of cortisol by dexamethasone suggesting a prolonged half-life of cortisol. Normal suppression during the overnight 1-mg LDDST is uncommon, and the 2-day LDDST is better in this regard (439, 444).

**Pediatric Cushing’s Syndrome**

The most common presentation of Cushing’s syndrome in children is growth retardation, with weight increases (445). However, one proviso is that patients with virilizing adrenal tumors may show growth acceleration (446). Other virilizing signs such as acne and hirsutism are seen in approximately 50% of patients regardless of etiology (445). Hypertension and striae are seen in approximately 50% of cases (447). Muscle weakness may be less common in the pediatric patient due to increased exercise (448). Psychiatric and cognitive changes may affect school performance; however, children may show “compulsive diligence” and actually do quite well academically (449). Headaches and fatigue are common (445). Cushing’s disease accounts for the between 75% and 80% of Cushing’s syndrome in older children, but before the age of 10 years ACTH-independent causes of Cushing’s syndrome are more common (450). Cushing’s disease has a male predominance in pre-pubertal children. Two causes of ACTH-independent Cushing’s syndrome, McCune-Albright syndrome and PPNAD, are typically diseases of childhood or young adults. Signs of virilization in the very young (<4 years) suggest adrenal carcinoma. Ectopic secretion of ACTH occurs rarely in the pediatric population and is usually due to bronchial or thymic carcinoids (2).

As mentioned previously, late night salivary cortisol measurement has particular logistic benefits in children (451, 452). Serum midnight cortisol measurements in in-patients has high sensitivity (453). UFC should be corrected for body surface area (454). The standard 2-day LDDST adult protocol can be used in children weighing 40kg or more, otherwise the dexamethasone dose is adjusted to 30µg/kg/day (455). As in adults, there is a good correlation between the cortisol suppression on the LDDST and the HDDST for the differential diagnosis and thus the latter is unnecessary (456). Although it can be argued that the ectopic ACTH syndrome is so rare in children that BIPSS is not necessary, it does add reassurance in those with a negative pituitary MRI, which is the case in more than 50% of cases. In addition, BIPSS has arguably better accuracy in lateralization of the pituitary tumor (385). MRI is at least as useful as CT in the evaluation of adrenal causes (457).

Transsphenoidal surgery is the treatment of choice in children with Cushing's disease, with similar rates of remission as in adults in expert hands (458). Conventional radiotherapy after non-curative transsphenoidal surgery performs even better than in adults, with reported remission rates as high as 100%, with remission usually occurring within 12 months (459). Following pituitary surgery, plus or minus radiotherapy, the incidence of growth hormone deficiency is high, but prompt diagnosis and treatment with human growth hormone ensure acceptable growth acceleration and catch-up growth, although an abnormal body composition often persists (460). Normalization of reduced bone mineral density can also be achieved (384). Adrenalectomy is first-line therapy in ACTH-independent Cushing's syndrome.

**Cushing’s Syndrome in Pregnancy**

Cushing’s syndrome in pregnancy is fortunately rare, because ovulatory disorders and consequently infertility constitute the clinical picture in 75% of untreated patients with Cushing’s syndrome (341, 342). The epidemiology in pregnant women is different to that in the non-pregnant population, in that pregnant patients show a 60% prevalence of ACTH-independent Cushing's syndrome (48% adenoma and 10% carcinoma) followed by Cushing’s disease and bilateral adrenal hyperplasia, and rarely ectopic disease (342, 343, 461). The onset of adrenal-dependent Cushing’s syndrome may relate to the aberrant expression of LH receptors on the tumor, cross-reacting with hCG. The diagnosis is challenging because of the symptoms and signs common to both Cushing’s syndrome and normal physiological changes in pregnancy; such as weight gain, fatigue, striae, hypertension, and glucose intolerance. In addition, the hormonal changes, which occur during pregnancy may confuse the interpretation of the biochemical test procedures (343).

Total serum cortisol levels increase in pregnancy, as a result of induced production of corticosteroid-binding globulin by estrogens, beginning in the first trimester and peaking at 6 months, with a decrease only after delivery. Levels of free cortisol are also raised. Late night salivary cortisol levels are 2-fold higher in normal pregnancy. In contrast to patients with pathologic hypercortisolism, levels of urinary 17-OH-corticosteroid excretion are within the normal range and the cortisol diurnal rhythm is maintained, but with a higher nadir (461). UFC excretion is normal in the first trimester and then rises up to three-fold by term (462). Suppression to dexamethasone testing is blunted, especially after the first trimester (135). Plasma ACTH levels are slightly decreased in the beginning of the pregnancy, but later tends to rise, partially because of placental ACTH and CRH secretion. The circadian rhythm of cortisol is usually maintained in the first 2 trimesters of pregnancy and becomes blunted in the 3rd trimester.

In general, biochemical evaluation follows the same principles as with the non-pregnant patients. However, there are no agreed guidelines in interpreting results of hormonal measurements in pregnant Cushing’s patients, considering normal physiological deflection of cortisol metabolism in pregnant women. As mentioned above, UFC excretion is normally increased, so if there is less than a 3-fold rise it cannot be diagnostic, and the dexamethasone response is blunted therefore cannot be used as screening test because of the possibility of a false positive result. Late night salivary cortisol is an alternative screening test for pregnant women and probably the most reliable investigation (463, 464). In pregnant women with Cushing’s syndrome, higher cut-offs for LNSC are suggested, depending on the trimester, of 7, 7.2 and 7.9nmol/L for the 1st, 2nd and 3rd trimester respectively (465). Therefore, the differential diagnosis regarding the possible etiology of Cushing’s syndrome can be quite demanding. If suppressed, levels of ACTH can point to adrenal origin, but lack of suppression does not eliminate the possibility of ACTH-independent cause. The high-dose dexamethasone test may be useful to distinguish an adrenal cause, because women with adrenal causes tend not to suppress, while those with Cushing’s disease do (461, 466, 467). As an initial evaluation the basal levels of ACTH and the high-dose dexamethasone test may be performed. Furthermore, due to the high prevalence of primary adrenal disease, it is reasonable to perform an abdominal ultrasound at an early stage.

The CRH test has also been used to identify patients with Cushing's disease, and there is no evidence of harm both in animal studies and the small number of pregnant patients studied with CRH. There are 2 case reports of desmopressin test being carried out in pregnancy with significant ACTH increment suggesting Cushing’s disease, later confirmed on post-TSS histology (468, 469).

MRI without gadolinium enhancement is considered safe in the third trimester, and its use in combination with the non-invasive tests above should be able to resolve most diagnostic issues. Current guidelines allow use of contrast only if it is going to change fetal or maternal outcomes (470). BIPSS with appropriate additional radiation protection for the fetus should be reserved only for the rare cases where diagnostic uncertainty remains. Ultrasound of the adrenals can be used as a first-line imaging in ACTH-independent Cushing's syndrome.

Maternal hypercortisolism is associated with 40-70% hypertension, 14-26% preeclampsia, 25-37% diabetes mellitus, 5% osteoporosis and fractures, 3% cardiac failure, 4% mental health disorders and rarely (2%) death (471, 472).

Although the fetus is partially protected from maternal hypercortisolism by placental 11-B-hydroxisteroid dehydrogenase type 2, which converts 85% of cortisol to inactive cortisone (405), the untreated condition is associated with miscarriage, premature delivery, and neonatal adrenal insufficiency (472).

Because of both maternal and neonatal risk, definitive surgical treatment of adrenal or pituitary disease is recommended to achieve eucortisolemia. The second trimester is probably the safest time for adrenal surgery or transsphenoidal operation, although adverse fetal outcomes after the successful treatment may still persist, such as intrauterine growth restriction and premature birth, but it does appear to prevent stillborn deliveries (472) (396).

Medical treatment carries potential risks to the fetus and should be considered only as second-line therapy when the benefit outweighs the risk, and generally only as an interim measure to operation or awaiting the pre-pregnancy pituitary radiation effect. Metyrapone is probably the adrenolytic agent of choice, although an association with pre-eclampsia has been reported (343). Ketoconazole has been utilized successfully in a small number of patients but is teratogenic in animals, and therefore should be used with caution. Cabergoline is probably a safe potential treatment option for mild hypercortisolism during pregnancy.

## Pseudo-Cushing’s Syndrome

Pseudo-Cushing’s states (PCS) or more recently called non-neoplastic hypercortisolism are conditions which cause increased cortisol production, manifest with some features of Cushing’s syndrome but are reversible by resolution of the causal state. Distinguishing pseudo-Cushing’s state from a true Cushing’s syndrome could often be challenging even for the endocrinologist. The detailed history taking is the key in diagnosis of PCS.

The states causing PCS could be physiological or related to other disorders. The physiological ones include severe persistent stress (emotional or related to severe illness), major surgery, persistent strenuous exercise or prolonged fasting/eating disorders. Non-physiological causes of PCS are alcoholism, severe depression or anxiety, poorly controlled diabetes, polycystic ovaries syndrome or obesity (473).

In alcoholism the majority of individuals have facial plethora, proximal weakness, central obesity or hypertension but rarely have purple striae (474). The hypercortisolism results from the elevation of CRH and stimulation of the HPA axis, an increased activity of 11B-HSD type 1, and reduced cortisol clearance due to liver disease (475). The abstinence from alcohol for more than 1 month resolves hypercortisolism.

In severe depression hypercortisolism is seen in 20-30% of patients but clinical features of Cushing’s syndrome are usually rare. The hypercortisolism is due to stimulation of HPA axis and reduced activity of 11B-HSD type 2 (476). Successful treatment of depression resolves the hypercortisolism.

Poorly controlled type 2 diabetes, polycystic ovary syndrome, and obesity may also be associated with increased cortisol levels and lack of suppression on overnight dexamethasone suppression test. Although the majority of individuals with those disorders have hypertension, hyperlipidemia and other features of metabolic syndrome, they are unlikely to have proximal myopathy, purple striae or bruising (473, 475).

In anorexia nervosa cortisol levels are often increased but features of hypercortisolism are absent. High levels of CRH but normal ACTH, reduced cortisol clearance and usually preserved cortisol circadian rhythm are reported in eating disorders (477).

As discussed in the second line investigations for Cushing’s syndrome, a mid-night cortisol, LDDST-CRH test and desmopressin test were helpful differentiating Cushing’s syndrome from pseudo-Cushing’s states. Overnight dexamethasone suppression test usually fails in most of patients with pseudo-Cushing’s states with specificity of 58%. The LDDST has slightly better specificity of 74% (473).

An awake midnight cortisol of greater than 207 nmol/L (7.5 mg/dL) was reported to show 94% sensitivity and 100% specificity for the differentiation of Cushing's syndrome from pseudo-Cushing's states (141).

Due to shortage of CRH, desmopressin test is the next line test. The study of 173 subjects including 76 with Cushing’s disease, 30 with non-neoplastic hypercortisolism, 36 with obesity and 31 of controls proposed cut-off criteria for positive desmopressin test as ACTH increment of >6pmol/L (30ng/L) (143). Subsequently, another study of 52 patients with Cushing’s syndrome and 28 controls suggested new criteria with ACTH increment of 4pmol/L and basal cortisol above 331nmol/L providing sensitivity of 90.3% and specificity of 91.5% (144). The meta-analysis of 3 studies described use of desmopressin test in differentiation of Cushing’s disease and non-neoplastic hypercortisolism with cut-off for ACTH increment by 6 pmol/L in 2 studies and ACTH increment of 4 pmol/L and basal cortisol more than 331nmol/L gave pooled sensitivity of 88% and specificity of 94% (143-145). However, there was high patient selection bias and low certainty of evidence in that meta-analysis (145).

**PROGNOSIS AND COURSE AFTER EFFECTIVE TREATMENT**

Before treatment was readily available, the mortality rate for Cushing’s syndrome was 50% after the first symptoms appeared, mainly due to cardiovascular, thromboembolic, infectious or hypertensive complications (478).

Even today, patients with severe hypercortisolism have a raised mortality rate due to increased coagulability and it’s the consequences or opportunistic infections (112, 479, 480), emphasizing the need for controlling the hormonal situation as soon as possible. The prognosis is mainly a reflection of the underlying condition. The life expectancy of patients with non-malignant causes of Cushing's syndrome has improved dramatically with effective surgical and medical treatments.

Even when cured by strict criteria, Cushing’s disease may often recur over time (481). From a number of studies in patients with Cushing’s disease treated in the era of transsphenoidal surgery, it initially appeared that after curative transsphenoidal surgery long-term mortality was not significantly different from that in the general population (480, 482). However, another population-based study suggested that mortality is marginally increased (4),while even more recently a very significantly increased mortality was shown even in patients who remained cured. A large European Registry of 1564 patients with Cushing’s syndrome, including 1045 patients with Cushing's disease, reported a 3.1% 90-day mortality in this group generally (483). The main cause of death was progression of the main disease (36%), infections (31%), and cardio- and cerebro-vascular disease (17%). As expected, the highest mortality was in individuals with ectopic Cushing's syndrome (20.2%), 2.2% in patients with Cushing's disease and 1.6% in those with ACTH-independent Cushing's syndrome. However, a large-scale meta-analysis showed that patients with Cushing’s disease who were cured at their *first* operation showed a normalized standardized mortality ratio, further emphasizing the importance of this modality of treatment and the necessity for an experienced surgeon. Nevertheless, while abdominal obesity may improve, hypertension and insulin resistance leading to increased cardiovascular risk with evidence of atherosclerotic disease persists when measured 5 years after remission of Cushing’s disease (96). It is therefore important to aggressively treat associated conditions such as hypertension and diabetes, even when the Cushing’s *per se* has been controlled. Unlike some signs and symptoms that disappear gradually over the next year after successful treatment, co-morbidities such as diabetes mellitus and hypertension improve, but may not resolve completely, requiring further aggressive treatment. There is also some evidence that the outcome from Cushing's disease may be worse in males (53). Some of the signs and symptoms of Cushing’s syndrome are expected to disappear gradually over the following year after the treatment; skin thickness improves in weeks, but for some it may take longer, as does muscle strength.

The outcome of pediatric Cushing’s disease is excellent if treated at centers with appropriate experience (447, 484).

Cushing's syndrome results in significant impairment in quality of life (485, 486), psychiatric symptoms (487), and cognitive deficits (488), as previously noted. However, in general these are only partially improved with treatment, and often do not resolve completely in either children or adults.

There is some evidence that deficits in bone mass may be partially reversed after treatment of hypercortisolemia (489, 490). Bisphosphonate treatment may induce a more rapid improvement in bone mineral density (491), and should be considered (along with calcium and vitamin D supplements), but it is unclear whether they are needed for the majority of patients with osteoporosis. Osteoporosis starts to improve after 6 months, with rapid improvement over the next two years, but with the possibility of residual disease to some extent (492). However, in general the prognosis is good without any specific treatment, and the care should be expectant.

The prognosis of the potentially malignant causes of Cushing's syndrome is more variable. Adrenal cancer associated with Cushing's syndrome has an extremely poor prognosis. Tumors that produce ectopic ACTH tend to have a poorer prognosis, compared with tumors from the same tissue that do not produce ACTH. Small cell lung cancer, islet cell tumors, and thymic carcinoids illustrate this phenomenon: up to 82% of patients with small cell lung cancer and Cushing’s syndrome were reported to die within 2 weeks from the start of chemotherapy (493), although currently a survival in terms of months should be expected.

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