

Chapter 7-CUSHING'S SYNDROME

Dr Petra Sulentic, Specialist in general medicine, KBC Sestre Milosrdnice, Zagreb, Croatia

Dr Damian G. Morris MBBS BSc PhD FRCP, Consultant Endocrinologist, The Ipswich Hospital, Suffolk IP4 5PN, UK

Professor Ashley Grossman BA BSc MD FRCP FMedSci, Professor of Endocrinology, Nuffield Dept. of Medicine, OCDEM, Churchill Hospital, University of Oxford Oxford OX3 7LJ, UK

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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 glycaemic 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, AETIOLOGY AND EPIDEMIOLOGY OF CUSHING 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 adrenocorticotropin (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). Any of the numerous synthetic steroids that have glucocorticoid activity, 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 aetiology 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 adrenal cortical hyperplasia and hypertrophy of adrenal gland. This results in an increased weight of 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 characterised by low levels of plasma ACTH, either because of adrenal glucocorticoid hypersecretion or secondary to the exogenous application 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 aetiology of disorder.

Table 1. Aetiology 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

Adrenal adenoma
 Adrenal carcinoma
 ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) – now known as bilateral macronodular hyperplasia (BMAH)
 AIMAH secondary to abnormal hormone receptor expression/function
 Primary pigmented nodular adrenal disease (PPNAD), associated with Carney complex or sporadic
 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 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 (2;3).

It presents much more commonly in women, and usually between 25 and 40 years of age.

It is almost always due to a corticotroph adenoma (4;5). Although apparent nodular corticotroph hyperplasia (in the absence of an CRH-producing tumour) has been described, it is rare in large surgical series (6;7), and its existence is still debated. The majority of tumours are intrasellar microadenomas (<1 cm in diameter), although macroadenomas account for approximately 5-10% of tumours, and extrasellar extension or invasion may occur. True pituitary corticotroph carcinomas with extra-pituitary metastases causing Cushing's syndrome have also rarely been described (8;9).

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 (10)

Up to 40 percent of patients 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; the feature that distinguishes this entity from ACTH-independent ones. (11-14).

Ectopic ACTH syndrome & 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 tumour types, which can broadly be divided into the group of highly malignant carcinomas and the more indolent group of neuroendocrine tumours, although this may be thought of as a continuum rather than as a binary separation. The most frequent cause is probably small-cell lung carcinoma, where it is estimated up to 12% of cases will have Cushing's syndrome (15). However, this may not be evident from series at endocrine centres where often more occult tumours are investigated (Table 2), and carcinoid (neuroendocrine) tumours tend to predominate. The ectopic ACTH syndrome is more common in men, and usually presents after the age of 40 years.

The ACTH precursor molecule, pro-opiomelanocortin (POMC) is not only expressed in normal pituitary but also in several normal extra pituitary tissues, as well as in some tumors (lung, testis) (16). The mechanism by which these non-corticotroph tumours express the pro-opiomelanocortin (POMC) gene is not fully understood but may be related to hypomethylation of the POMC promoter (17;18). In general, such tumours tend to produce higher amounts of POMC compared to ACTH, in contrast to the situation in Cushing's disease (16). As well as producing ACTH and POMC, these tumours may also produce other pre-ACTH precursor peptides, so -called "big" ACTH (19;20), which may potentially be helpful in the differential diagnosis of these tumours (21). However, assays for these are not routinely available in clinical practice.

Isolated ectopic CRH production is difficult to diagnose and exceedingly rare, with just over 20 cases described in the literature (22). In general, patients secreting CRH ectopically usually also secrete ACTH, rendering the distinction of little practical value.

Table 2. Aetiology of the ectopic ACTH syndrome in patients seen at St. Bartholomew's Hospital 1969-2001

Site of secretion	Female	Male
Bronchial carcinoid tumor	11	2
Small cell lung carcinoma	1	5
Medullary thyroid carcinoma		3
Pancreatic carcinoid tumor	1	2
Thymic carcinoid tumor		1
Disseminated carcinoid tumor		1
Mesothelioma	1	
Pancreatic carcinoma		1
Colonic carcinoma	1	
Phaeochromocytoma		1
Gall bladder carcinoma	1	
Total	16	16

ACTH-independent Cushing Syndrome

ACTH-independent causes of Cushing 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 (23). The incidence of adrenal cancer is approximately 0.2 per million per year (23). It is one and a half times more common in women, and has a bimodal age distribution, with peaks in childhood and adolescence, and late in life (1; 24).

ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) is a rare form of Cushing's syndrome with sometime huge nodular adrenal glands on imaging. Most cases are sporadic, but a few familial cases have been reported (25). AS ACTH has been found within these tumours, a better term is bilateral macronodular hyperplasia (BMAH). In most the aetiology 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. The best described example is food-dependent Cushing's syndrome, in which ectopic gastric inhibitory peptide (GIP) receptors on the adrenal glands respond to GIP released after a meal causing hypercortisolaemia (26). In one patient, treatment with octreotide ameliorated the syndrome (27). Abnormal

expression of vasopressin, β -adrenergic, luteinising hormone/human chorionic gonadotropin, serotonin, angiotensin, leptin, glucagon, IL-1, and TSH have also been described and functionally linked to cortisol production (28). BMAH tissue may express more than one of these aberrant receptors (29). More recently, around one-third of patients with BMAH have been found to show germline mutations of the tumour suppressor gene ARMC5, with the nodules demonstrating second independent hits in the same gene: familial forms of BMAH have been described.

Cushing's syndrome due to bilateral nodular adrenal disease can also be a feature of McCune-Albright syndrome (30). 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 at codon 201 of the α -subunit of the G protein stimulating cyclic adenosine monophosphate (cAMP) formation. This occurs in a mosaic pattern in early embryogenesis (31). 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 (32).

Primary pigmented nodular adrenal disease (PPNAD), otherwise known as micronodular adrenal disease, is another rare form of Cushing's syndrome. It is characterised by small or normal-size adrenal glands with cortical micronodules (average 2–3 mm) that may be dark or black in colour. The internodular cortex is usually atrophic, unlike in ACTH-dependent macronodular hyperplasia (33). Cases of PPNAD have been reported without Cushing's syndrome. Bilateral adrenalectomy is curative. Most cases 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 tumours; and acromegaly). Cushing's syndrome occurs in approximately 30% of cases of Carney Complex. The tumour suppressor gene PRKAR1A (type 1A regulatory subunit of protein kinase A) has been shown to be mutated in approximately half of patients with Carney complex. In isolated PPNAD, mutations in PRKAR1A and also the phosphodiesterase 11A (PDE11A) gene have demonstrated (34).

A missense mutation of the ACTH receptor resulting in its constitutive activation and ACTH-independent Cushing's syndrome has also been reported (35).

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 may produce hypercortisolaemia, usually in the context of ACTH-dependent disease after adrenalectomy (36–39). Ectopic cortisol production by an ovarian carcinoma has been reported (40).

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/amenorrhoea, are less prevalent. However, it is unclear as to whether these are true differences.

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 hypercortisolaemia. Both settle after resolution of the pre-disposing 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 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. 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. It is very helpful to notice 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 courses 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 type of steroid excess is determined by the underlying condition. Adrenal adenomas generally secrete glucocorticoids, but in case of an ACTH dependent disease or a carcinoma additional hyperandrogenism is common.

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 hypercortisolaemia and additional mineralocorticoid effect, hypokalaemic alkalosis is found with peripheral oedema on clinical examination. The combination of rapid clinical deterioration, common hyperpigmentation, hypokalaemic alkalosis and clinical signs of mineralocorticoid excess should be indicative for suspicion of a small cell lung carcinoma secreting ACTH. In contrast, patients with ACTH producing carcinoids, because of the long duration of hypercortisolaemia 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, may complain of an abdominal pain accompanied with palpable tumour masses. In addition to hypercortisolism, they often secrete mineralocorticoids and androgens, therefore distinguishing them from benign adenomas which usually secrete cortisol alone.

In 10 percent of patients with adrenal incidentalomas, "subclinical" Cushing's can be found; this is characterised by mild hypercortisolism without other obvious clinical manifestations of Cushing's syndrome. As a result, not only clinical picture is dependable on the underlying condition and type of steroids, but also highly influenced by the sex of the patient.

Unlike 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 virilisation.

Obesity and weight gain are among the most common signs in Cushing 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 (41,42). Rarely, fat deposition in the epidural space can be manifest as a neurological deficit (43), while retroorbital deposition is noticeable as exophthalmos (44). In children, more generalised weight gain associated with growth retardation should highlight the possibility of the diagnosis (45). Other signs that are more discriminatory are proximal myopathy, osteoporosis, thin skin and easy bruising (46, 47).

Myopathy of proximal muscle of the lower limb and shoulder results from catabolic glucocorticoid effect. 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 hypokalaemia, as a result of concomitant mineralocorticoid activity; it is uncommon in pseudo- Cushing states. (1)

Osteoporosis occurs in approximately 50% of adult patients (48) 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 (49). Vertebral compression fractures leads 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 syndrome of chronic high dose glucocorticoid therapy (50). After successful treatment of the cause, bone density improves to a large extent (51; 52).

There are many changes in the skin and subcutaneous tissue, which are rarely seen in general population, suggesting the possibility of Cushing's syndrome. (1; 41). The result of hypercortisolaemia 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 and female gender. In addition, skin thickness may be preserved in women with hyperandrogenaemia related to Cushing's syndrome.

The classic plethora is not only a consequence of skin thinning but also of a loss of a facial subcutaneous fat. Because subcutaneous fat is also diminished, patients suffer easy

bruising, which often can be misinterpreted as senile purpura or even a coagulation disorder. Purple coloured "violaceous" striae greater than 1 cm in diameter are almost pathognomonic of Cushing's syndrome. Typically seen on the abdomen, they can also occur in other areas, such as the thighs, breasts and arms. More narrow and coloured striae are more commonly present, and should be differentiated from the typical healed silvery striae seen most commonly post-partum.

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 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, other forms of the ectopic ACTH syndrome, particularly associated with neuroendocrine tumours, may be clinically indistinguishable from patients with other forms of hypercortisolism (53).

Severe hirsutism and virilisation strongly suggest an adrenal carcinoma (54).

Hypercortisolism may suppress other pituitary hormones. In both men and women, hypogonadotropic hypogonadism is common and correlates with the degree of hypercortisolaemia (55; 56). Glucocorticoids inhibit gonadotrophin-releasing hormone pulsatility and the release of luteinising (LH) and follicle-stimulating hormone (FSH). Women experience menstrual irregularity, while both sexes have decreased libido. Gonadal dysfunction is reversible after correction of the hypercortisolaemia (55; 56). In addition, the coexistence of polycystic ovarian syndrome in Cushing's syndrome is more common (57). There is reduced GH secretion during sleep and blunted GH responses to dynamic stimulation tests (58). Thyrotropin-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 (59). This may not have a significant effect on free thyroid hormone levels during active hypercortisolaemia, but there is a significantly increased prevalence of autoimmune thyroid disease in patients successfully treated for Cushing's syndrome, and therefore it is important to follow them with serial thyroid function tests (60;61).

Hypokalaemic metabolic alkalosis is related to the degree of hypercortisolaemia and represents a mineralocorticoid action of cortisol at the renal tubule due to saturation of the enzyme 11 β -hydroxysteroid dehydrogenase type 2, which inactivates cortisol to cortisone and allows selective binding of aldosterone to mineralocorticoid receptor (62). In terms of hypersaturation, cortisol can now access the mineralocorticoid receptor and act as a mineralocorticoid. It occurs when urine free cortisol excretion is greater than about 4100 nmol per day (63). 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 characterised by insulin resistance and hyperinsulinaemia. Glucose intolerance is evident in 20%-30%, and overt diabetes mellitus in 30%-40% of patients, (64; 65). Glucocorticoids stimulate glycogen deposition, promote gluconeogenesis, inhibit glucose uptake in peripheral tissues, activate lipolysis and have a permissive effect on contra-regulatory hormones, glucagon and catecholamines. It has been suggested that 2-3% of overweight, poorly-controlled patients with diabetes may

have occult Cushing's syndrome, if investigated (66; 67). However, in the absence of clinical suspicion the percentage is probably lower (68; 69), and therefore it is probably not justified to screen for Cushing in poorly controlled diabetic patients unless other suggestive features are present (70). Hyperglycaemia becomes easier to control after treatment (71).

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 (72).

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 (73) as well as hypertension, glucose intolerance, overt diabetes mellitus, dyslipidaemia and visceral obesity. Overall, hypertension is common in patients with Cushing syndrome, (74). Severe hypertension with additional hypokalaemia is more prevalent in ectopic Cushing syndrome, usually best controlled with spironolactone (75) Cardiovascular risk markers continue to be present long after cure of the hypercortisolaemia (76) and cardiovascular risk remains increased (77; 78). Sympathetic autonomic function is also abnormal in patients with Cushing's syndrome (79), and the ECG abnormalities of a prolonged QTc dispersion (QTcd) and left ventricular hypertrophy have been identified as characteristic features in patients with Cushing's disease (80)

Hypercortisolaemia increases clotting factors including factor VIII, fibrinogen, and von Willebrand factor, and reduces fibrinolytic activity. 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 (81). Rates of thromboembolic events, either postoperatively or unrelated to surgery, are higher in patients with Cushing's syndrome than the estimated incidence in an age and sex matched control population, especially in the latter group unrelated to surgery (82).

Ophthalmic complications include glaucoma and exophthalmus due to retroorbital fat deposition. (83; 84). Cataract is rare, mostly a complication of diabetes (85).

Psychiatric symptoms such as insomnia, depression, anxiety, easy irritability, paranoid episodes, suicide or panic attacks are present in more than half of patients having any cause of Cushing's syndrome. (86-88). Cognitive defects as learning, cognition and impairment of short-term memory may be prominent. (89; 90). These changes are not always reversible with treatment.

In patients there is a greater frequency of infections because of inhibition of immune function by glucocorticoids by decreasing the number of CD4 cells, NK cells and inhibition in cytokine synthesis (91-93), with predominant effects on cell-mediated immunity (TH1 responses) . The most common are bacterial infections, and special attention should be pointed at a possibility of opportunistic pathogens, especially in cases of severe hypercortisolism. (94; 95)

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 hypercortisolaemia to allow accurate interpretation. Patients may 'cycle in' or 'cycle out' over periods of months or years; if at presentation they are eucortisolaemic, 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 occur with all causes of Cushing's syndrome (96).

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 pathognomonic ones, such as myopathy, 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.

As noted above, hypercortisolaemia 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.

It is important to realise 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 (1).

Cortisol is normally secreted in a circadian rhythm, with the highest levels early in the morning and reaching their nadir levels at about midnight (<50 nmol/L; 1.8 μ g/dl). In patients with Cushing's syndrome the circadian rhythm is lost. However, most of the 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 being used. However, exogenous oestrogens 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 some time prior to investigation. We currently wait for a period of 4-6 weeks, although it is likely that a shorter time off treatment may still be effective.

Late night salivary cortisol

Salivary cortisol measurement 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 night-time salivary cortisol can be utilised 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. 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 has been evaluated at a large number of centres worldwide. In a recent meta-analysis of

these studies, in adult patients the sensitivity and specificity of this test appears to be relatively consistent at different centres, and overall is 92% and 96% respectively (97). However, it should be noted that the diagnostic value cut-off varies between studies because of different assays and the comparison groups studied. Normal values also differ between adults and paediatric populations, and may be affected by other comorbidities such as diabetes (98), and the method by which the saliva is collected (99). Not surprisingly, this test performs less well in patients with subclinical Cushing's syndrome (100). Salivary rather than serum cortisol has been evaluated as the endpoint for the low-dose dexamethasone suppression test (LDDST). This has the potential benefit in terms of convenience but requires further evaluation (101;102). Salivary cortisol has also been advocated as a sensitive tool to detect recurrence or treatment failure in patients post-pituitary surgery for Cushing's disease (103;104).

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 widely used in the screening of Cushing's syndrome. Under normal conditions, 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 measured. 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 (105). However, within this series 11% had at least one of four UFC collections within the normal range, which confirmed the need for multiple collections. Furthermore, this sensitivity figure is based on the more florid case, and is likely to be much less for the more common subtle cases now being seen.

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 in episodic cortisol secretion, sometimes seen in adrenal adenomas. 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 (54). Cortisol to creatinine ratio in the first urine specimen can be used as a screening test, especially when cyclic secretion is suspected (106), with the cortisol to creatinine ratio over 25 nmol/mmol being suggestible for Cushing's syndrome.

The 24 hour UFC is of little value in the differentiation from pseudo-Cushing's states (107;108), although obesity *per se* does not appear to confound the results (109).

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 (110). Drugs such as carbamazepine, digoxin and fenofibrate may co-elute with cortisol during high-performance liquid chromatography and cause falsely elevated results (111;112).

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 fourfold 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 investigation (1). It is our opinion that the test is of little use for screening, and in general we rarely utilise it nowadays.

Low-dose dexamethasone suppression test (LDDST)

This test works on the principle that in normal individuals 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 an extremely 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-OHCS after 48 hours of dexamethasone 0.5mg 6 hourly (113). However, the simpler measurement of a single plasma or serum cortisol has been validated in various series and gives the test a sensitivity of between 95% and 100% (114-116).

The overnight LDDST 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 (117), 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 (118-120). There appears to be no advantage in discrimination between 1mg and 1.5mg or 2mg (121). Although higher doses have been tried, the increased suppression in some patients with Cushing's syndrome significantly decreases the sensitivity of the test (122).

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 (1.8 µg/dL) (123). However, the *specificity* is greater for the 2-day test (95%-100%) compared to the overnight test (88%) (123).

If the overnight test is used, we suggest that a dose of dexamethasone 1mg at midnight and a threshold of <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 LDDST as confirmation.

It should be noted that patients with PPAD may show a paradoxical rise in cortisol levels to dexamethasone (124).

2nd line tests

In some patients with equivocal results other tests may be needed. The most useful of these are a midnight serum cortisol, and the dexamethasone-CRH test. Less reliable tests such as the insulin tolerance test (125), the loperamide test (126), and the desmopressin test (47) are not in widespread use.

Midnight serum cortisol

Before the introduction of salivary cortisol measurement 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, and we are content to use it as a major test to exclude Cushing's syndrome in problematic cases.

However, it is a burdensome test that requires that the patient should have been an in-patient for at least 48 hours to allow acclimatisation to the hospital environment. The patient should not be forewarned of the test, and should be asleep prior to venepuncture, which must be performed within 5-10 minutes of waking the patient. A single sleeping midnight plasma cortisol $<50\text{nmol/l}$ ($1.8\text{ }\mu\text{g/dL}$) effectively excludes Cushing's syndrome (116), 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 (127).

An awake midnight cortisol of greater than 207 nmol/l (7 mg/dL) was reported to show 94% sensitivity and 100% specificity for the differentiation of Cushing's syndrome from pseudo-Cushing's states (128).

The 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 from true Cushing's syndrome in patients with only mild hypercortisolaemia and equivocal physical findings (107). The theory being 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. 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 ($1\text{ }\mu\text{g/kg}$ intravenously) to 58 adults with UFC less than 1000 nmol/day ($360\text{ }\mu\text{g/day}$). 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 ($<1.4\text{ }\mu\text{g/dL}$) 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 (129). Importantly, in these two studies although eight of 59 patients with proven Cushing's disease showed suppression to dexamethasone, all were correctly characterised 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 (130). It is perhaps not surprising that the diagnostic utility of the Dex-CRH has altered with further studies at more centres. 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 (70).

The 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 a few hours to avoid a spuriously low result. Measurements are usually taken on two different days to avoid misinterpretation because of ACTH episodic secretion. 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. (131)

It is useful to duplicate 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 (132). Consistent ACTH measurements of <10 ng/L (2pmol/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 (4-6pmol/L), Cushing's syndrome is ACTH-dependent, due to pituitary disease or ectopic ACTH/CRH secretion.

Intermediate levels are less discriminatory, but a lack of ACTH response to the CRH 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 and is accurate for masses greater than 1 cm and allows evaluation of the contralateral gland (133). In certain circumstances MRI may be useful for the differential diagnosis of adrenal masses; the T2-weighted signal is progressively less intense in pheochromocytoma, carcinoma, adenoma, and finally normal tissue (134).

Adrenal tumours typically appear as a unilateral mass with an atrophic contralateral gland (135). If the lesion is greater than 5 cm in diameter it should be considered to be malignant until proven otherwise, and imaging characteristics should not be relied upon. In comparison to carcinomas, adrenal adenomas are usually smaller and have a lower unenhanced CT attenuation value. Signs of necrosis, haemorrhage and calcification are characteristics of carcinomas. (136). Additional laboratory diagnostics reveal solely raised cortisol levels in adenomas, unlike additionally raised androgen levels in carcinomas.

Bilateral adenomas can be present (135).

In PPAD the adrenal glands appear normal or slightly lumpy from multiple small nodules, but are not generally enlarged (137).

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

BMAH is characterised by bilaterally huge (>5 cm) adrenals with a nodular pattern (12; 138).

Confusion can arise as the CT appearance of the adrenals in BMAH may be similar to the appearance seen in ACTH-dependent forms of Cushing's syndrome, where adrenal enlargement is present in 70% of cases (139), 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 (11).

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. This depends on gender, and in our 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 (140). 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 (Fig 1) (140;141). Hypokalaemia is more common 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. 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 ovine or human CRH (IV 1 µg/kg or 100µg respectively). A central (inferior petrosal) to peripheral plasma ACTH gradient of 2:1 or greater pre-CRH, or a gradient of 3:1 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 (142;143). However, it is now clear that false negative tests and to a smaller degree false positive test results do occur (144-146).

In order to minimise these it is important to ensure the patient is actively hypercortisolaemic (as above) at the time of the study (147), and that catheter position is confirmed as bilateral and any anomalous venous drainage noted by venography before sampling (148). For the diagnosis of Cushing's disease this test has a sensitivity of approximately 94% and specificity fractionally short of 100%. There appears to be no discriminatory difference between ovine or human sequence CRH. Recent data suggest that where CRH is unavailable desmopressin 10 µg may be used instead (149).

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 haematomas. More serious transient and permanent neurological sequelae have been reported, including brainstem infarction, although these are rare (<1%), and most have related to a particular type of catheter used

(150; 151); 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 (152). CRH itself is generally tolerated well, although patients may experience brief facial flushing and a metallic taste in the mouth. One case of CRH inducing pituitary apoplexy in a patient with Cushing's disease has been reported (153). 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 (154).

BIPSS has also been suggested to help to lateralise microadenomas within the pituitary using the inferior petrosal sinus ACTH gradient (IPSG), with a basal or post-CRH inter-sinus ratio of at least 1.4 being the criteria for lateralisation used in all large studies (143;144;155;156). In these studies the diagnostic accuracy of localisation as assessed by operative outcome varied between 59% and 83%. This is improved if venous drainage is assessed to be symmetric (157). The accuracy of lateralisation appears to be higher in children (90%), a situation where imaging is often negative (158). There is some discrepancy between studies as to whether CRH improves the predictive value of the test (159). If a reversal of lateralisation is seen pre- and post-CRH, the test cannot be relied upon (160).

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 tumours and Cushing's disease (113). The HDDST's role in the differential diagnosis of ACTH-dependent Cushing's syndrome is based on the same premise: that most pituitary corticotroph tumors retain some albeit reduced responsiveness to negative glucocorticoid feedback, whereas ectopic ACTH-secreting tumours like adrenal tumours typically do not, with the exception of some neuroendocrine tumors, mainly bronchial (161;162).

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-dependant Cushing's syndrome serum cortisol level is less than 5 mcg/dl (140 nmol/L). In normal subjects the level is usually undetectable. (113;163)

Overall, only about 80% of patients with Cushing's disease will show positive response of the test, defined by suppression of cortisol to less than 50% of the basal value. There are high number of false positives tests (~10-30%) seen in ectopic Cushing's syndrome (163-168). 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, a recent study has shown that suppression to HDDST can be inferred by a > 30% suppression of serum cortisol to the 2-day LDDST (169). 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 the CRH test (see below).

The CRH test

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 tumours lack CRH receptors and therefore do not respond to the agent.

CRH either 1 µg/kg or 100 µg synthetic ovine (oCRH) or 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 normal and obese patients, and in those with Cushing's disease (170); this may be related to the more rapid clearance of the human sequence by endogenous CRH-binding protein (171). The availability differs worldwide with oCRH predominant in North America but hCRH elsewhere.

Different centres 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. The best cortisol criterion proved less discriminatory (172). Conversely, in the largest 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 (140). In a multicentre 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). However, the sensitivity for the cortisol response was significantly greater with oCRH than with hCRH (sensitivity: 67% vs 50% for oCRH and hCRH respectively) (173). The authors do not report in this paper or an associated publication (24) whether time-point combinations other than the maximal were analysed for the rise in cortisol. Indeed, our data show that if the *maximal* rise in cortisol is used the sensitivity falls to 71% (140). 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 is 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 (165;169;174) Which cut-off to use should be evaluated at individual centres, 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 (175). Nevertheless, where BIPSS is unavailable, a response to both CRH (a rise) and the LDDST (a fall) renders an ectopic source extremely unlikely

Testing with other peptides

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. Hexarelin (a growth hormone secretagogue) stimulates ACTH release probably occurs through stimulation of vasopressin release in normal subjects (176), and by stimulation of aberrant growth hormone secretagogue receptors in corticotroph tumours (177). These peptides have been utilised in a similar manner to CRH to try and improve the differentiation of ACTH-dependent Cushing's syndrome, but have unfortunately proved inferior (178-180). However, in centres with no availability of CRH the desmopressin test may be an alternative. A combined desmopressin and hCRH stimulation test initially looked promising (181), but a further study of this combined test showed significant overlap in the responses (182). 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 tumours (152; 183).

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 (184).

Modern MRI techniques using T1-weighted spin echo and/or spoiled gradient recalled acquisition (SPGR) techniques will identify an adenoma in up to 80% of patients with Cushing's disease (24;185;186). They provide greater sensitivity than conventional MRI but with more false positive results (186;187). On MRI, 95% of microadenomas exhibit a hypointense signal with no post-gadolinium enhancement (Fig 2); however, as the remaining 5% have an isointense signal post-gadolinium, pre-gadolinium images are essential (188).

CT has a sensitivity of only approximately 40%-50% for identifying microadenomas, and is thus significantly inferior to MRI (sensitivity 50%-60%)(24;189;190), and it should therefore be reserved for patients in whom MRI is contraindicated or unavailable. Computerised tomography (CT) imaging typically shows a hypodense lesion that fails to enhance post-contrast.

Preoperative localisation to one side of the pituitary gland by MRI had been advocated as better than BIPSS with a positive predictive value of 93% (145;191). Other groups have found MRI less effective (144;192). In addition, as noted above, we have found MRI often to be unhelpful in the paediatric age group, and BIPSS to be of significant value in these patients (158).

Figure 2. Magnetic resonance imaging (MRI), showing a right sided hypointense (post-gadolinium) corticotroph adenoma (arrow).



Ectopic tumours

Visualising 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 visualised, small bronchial carcinoid tumours 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 tumours 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) (193).

The majority of ectopic ACTH secreting tumours are of neuroendocrine origin and therefore may express somatostatin receptor subtypes. Therefore the radiolabelled somatostatin analogue (^{111}In -pentetreotide) scintigraphy may be useful to show either functionality of identified tumours, or to try and localise radiologically unidentified tumours (194). Undoubtedly this is a useful technique, but to date there are only sporadic reports that it identifies lesions not apparent using conventional imaging (195;196). However, a lesion of uncertain pathology is more likely to represent a neuroendocrine tumour, and hence an ectopic source of ACTH, if somatostatin scintigraphy is positive.

Unless the tumours are metabolically active, which is not usually the case, 18-fluorodeoxyglucose positron-emission tomography (PET) does not generally offer any advantage over conventional CT or MRI (197;198). However, it is probable that ^{67}Ga -octreotide PET scanning is more sensitive than conventional octreotide scintigraphy.

Investigative 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 2008 (70;152). 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. One other of these tests should confirm abnormal results. 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 CRH test (combined with the LDDST or HDDST), together with appropriate imaging, are the most useful non-invasive investigations to determine the aetiology. 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 centres where BIPSS is available and validated, the practice is to use this test in almost all cases of ACTH-dependent Cushing's syndrome.

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 aetiology, 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 can be appropriate depending on aetiology, 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, osteoporosis and hypertension should be treated, aiming to avoid permanent dependence on therapy after resolving the primary cause of Cushing's syndrome.

Treatment of Cushing's disease

The first-line therapy almost always consists of transsphenoidal surgery. Patients with persistent Cushing's postoperatively, can be re-operated upon 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 corticotropinoma has not been found on pathologic examination, to exclude the possibility of a missed ectopic ACTH syndrome (6). Besides re-operation, patients can be treated either by radiotherapy, medical therapy, or as a definitive solution, bilateral adrenalectomy.

Transsphenoidal surgery

According to the relevant 2008 consensus statement on the treatment of ACTH-dependent Cushing's syndrome (199), transsphenoidal surgery is widely regarded as the treatment of choice for Cushing's disease (200). Besides the traditional microscopic approach there is an endoscopic approach which appears useful in patients with persistent or recurrent disease (201;202) and is accompanied with a shorter hospital stay (203). It is likely that the great majority of surgery will be endoscopic in the future.

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% (204). The frequency of reported adverse events varies widely: diabetes insipidus (either temporary or permanent) (3%-46%); hypogonadism (14%-53%); hypothyroidism (14%-40%); cerebrospinal fluid rhinorrhoea (4.6%-27.9%); severe growth hormone deficiency (13%); bleeding (1.3%-5%); and meningitis (0-2.8%) (204-207).

Where an adenoma is apparent at transsphenoidal exploration, a selective microadenomectomy of tumour tissue is performed, and the surgeon may be guided by pre-operative imaging. However, where no tumour is obvious, and there is no condition of fertility, subtotal resection of 85-90% of the anterior pituitary gland is done, leaving a small part near the pituitary stalk. The latter involves a substantial and unpredictable risk of future 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 (6;14;204;205;208). Remission rates are estimated upon postoperative pathologic and biochemical results, although both can be equivocal. Half of tumours cannot be preoperatively visualised (209), and therefore parts of the tumour can be overlooked intra-operatively and left behind affecting surgery success rate (210). Adenomas can rise near or within pituitary stalk (211), rarely in ectopic locations (212-214) and may show signs of microscopic invasion (215).

Prognostic markers of long-term remission are patients aged over 25 years, a microadenoma detected by MRI, lack of invasion of the dura or cavernous sinus, histological confirmation of an ACTH-secreting tumour, low postoperative cortisol levels and long-lasting adrenal insufficiency (199).

Of the patients achieving remission, about 10% of these will have a recurrence by 10 years and 20% by 20 years (216), and this emphasises the need for long-term annual follow-up based on the same diagnostic criteria as with initial diagnostics; salivary midnight cortisol, 24-hour urinary cortisol, and an overnight 1 mg dexamethasone suppression test results. Special attention should be paid to patients with intermittent hypercortisolism (217). A lower recurrence rate closely correlates with lower postoperative cortisol measurements as well as with complete postoperative recovery of HPA axis and responsiveness to insulin-induced hypoglycaemia (218)

Transsphenoidal surgery is also a useful procedure in patients with Nelson's syndrome to reduce tumour size, and ameliorate hyperpigmentation. (219).

Thromboprophylaxis with low molecular weight heparin should be considered perioperatively in all surgical procedures for Cushing's syndrome (81)

In cases where patients are treated with adrenal steroid inhibitors prior to surgery, glucocorticoid replacement therapy may be necessary preoperatively.

Post-operative evaluation and management

It is usual to give glucocorticoid cover for transsphenoidal surgery at initial daily doses of up to 400 mg hydrocortisone (4 mg dexamethasone), tapering off within 1 to 3 days. Morning (09.00h) serum cortisol measurements are then obtained for 3 consecutive days starting 24 hours after the last glucocorticoid administration, during which time the patient should be closely observed for development of signs of adrenal insufficiency (220). 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 postoperative period, there is a wide range of possible biochemical results, but elevated cortisol in 24-hour urine unequivocally favours operation failure with persistent disease. Postoperative hypocortisolemia (<50 nmol/L [1.8 µg/dL] at 09.00h) is probably the best indicator of the likelihood of long-term remission (218;221;222). However, detectable cortisol levels of less than 140 nmol/L (<5µg/dl) are also compatible with sustained remission (223;224). In cases of a mild or cyclic Cushing 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 tumour or a gradual loss of autonomy of the adrenal (223;225). Regardless of the possibility of this late remission, the approach should be individualised 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 (>5µg/dL) 3 months after surgery require further investigation.

Persistent hypercortisolaemia 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 tumour 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, at the expense of an increased likelihood of hypopituitarism (202;226;227). 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 hypocortisolaemic should be started on glucocorticoid replacement. Hydrocortisone 20 mg total daily dose in three divided doses is the preferred choice. The largest dose (10mg) should be taken before getting out of bed, and the last 5mg dose should be taken 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 will lead to adrenal crisis and possibly death. They should be prescribed a 100-mg 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, diarrhoea, 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 postoperative months, and that these are signs that indicate remission. Symptoms can be especially prominent in

patients with long-standing, severe Cushing's. Some of these symptoms have been related to high levels of circulating interleukin-6 (228). Most patients tolerate these symptoms of glucocorticoid withdrawal much better if they are forewarned and alerted to their positive nature. The glucocorticoid dose should not be increased in the absence of intercurrent illness based on these symptoms alone, because it constitutes iatrogenic hypercortisolism, but signs of adrenal insufficiency, such as vomiting, electrolyte abnormalities, and postural hypotension should be excluded (229).

Recovery of the HPA axis can be monitored by measurement of 09.00h serum cortisol after omission of hydrocortisone replacement. Because recovery after transsphenoidal surgery rarely occurs before 3-6 months and is common at 1 year, initial testing at 6 to 9 months is reasonable (230). 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 measurable, 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 on modern assays (232). Many centres use the cortisol response to 250 µg synthetic (1-24) ACTH as an alternative means of assessing HPA reserve (233;234), but there is some controversy as to its reliability in this situation (235;236). If it is used instead of the insulin tolerance test, a 30-minute cortisol of greater than 500 nmol/L is probably more reliable (232). 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 taper and stop the hydrocortisone 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. For patients with detectable but low 09.00h cortisol levels, the hydrocortisone replacement dose should be adjusted down if weight loss has occurred, and a slightly lower dose may be given. 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. In the future modified release hydrocortisone may provide more physiological replacement (237).

Two late conundrums may arise: the questions of recurrence and permanent lack of recovery of the axis. 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 hypercortisolaemia. UFC can be measured initially on dexamethasone 0.5 mg/day, if not yet weaned from glucocorticoids. Measurement of late-night salivary cortisol having omitted the afternoon dose of hydrocortisone may also be useful. However, ideally assessment of a cortisol circadian rhythm can be done as an inpatient having stopped the hydrocortisone completely.

If recurrent Cushing's disease is diagnosed, the therapeutic options are the same as for 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 (238).

If the UFC result is subnormal or low, the patient should be questioned about the actual

dose of glucocorticoid that has been taken. Often, patients take additional hydrocortisone, either because they discover that this decreases the symptoms of glucocorticoid withdrawal or because they have increased the dose “for stress,” often without following strict guidelines. These patients have a suppressed axis and very slow regression of Cushingoid features because of exogenous hypercortisolism. They require education and support along with reduction in the daily dose of hydrocortisone to recommended levels. 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.

Diuresis is common after transsphenoidal surgery and may result from intraoperative or glucocorticoid-induced fluid overload or may be due to diabetes insipidus. 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) 1 µg given subcutaneously will provide adequate vasopressin replacement for 12 hours or more. Hyponatraemia 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 (SIADH) as is frequently seen after extensive gland exploration, and fluid intake should be restricted (239). While transient central diabetes insipidus is common, in about 20% of operations (240), a small minority of patients proceed to permanent diabetes insipidus, requiring long-term treatment with a vasopressin analogue.

The state of permanent diabetes insipidus is usually accompanied with other anterior pituitary hormone deficiencies (241).

Many glucocorticoid-induced abnormalities, including hypokalaemia, hypertension, and glucose intolerance, may be normalised during the postoperative period so that preoperative treatments for these need to 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. In addition, the development of Nelson's syndrome in patients with ACTH-secreting pituitary adenomas occurs in between 8% and 38% of cases (242). The chance of developing Nelson's syndrome appears to be greater if adrenalectomy is performed at a younger age, and if a pituitary adenoma is confirmed at previous pituitary surgery (216;242). Prophylactic pituitary radiotherapy probably reduces the risk of developing Nelson's syndrome (243). 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 tumour (244). Others have advocated unilateral adrenalectomy plus pituitary irradiation as an alternative to bilateral adrenalectomy, as it gives similar

remission rates to primary transsphenoidal surgery (245), but this should be reserved for selected cases.

Pituitary radiotherapy

For patients in whom fertility represents an important issue and with uncertain preoperative localisation, radiotherapy is 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 18y, because results are comparable to surgery (246-248). Pituitary irradiation may also decrease the occurrence of Nelson's syndrome (large, locally invasive corticotrophinomas with hyperpigmentation) after medical or surgical adrenalectomy, but this has not been tested in a prospective randomised trial (249,250).

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% (216;251). 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 normalisation of the clinical state and biochemical parameters (252;253). In children, however, not only primary therapy shows better results with cure rate of 80%, but also they respond more rapidly, usually within three months (247), while remission in adults usually occurs by two years although it can take considerably longer. Medical therapy to control hypercortisolaemia is usually utilised in the interim, and patients should be reassessed at least yearly. (254). 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 linear accelerator is delivered at a total dose of 4500 to 5000 cGy 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, while other anterior pituitary deficiencies may develop over time.(77;252).

There is some evidence of an increased risk of cerebrovascular complications, which is of concern particularly in younger patients (255), but not all studies agree and further studies are required (256).

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, it is not clear whether the incidence is significantly greater than the background risk of developing such tumours (257;258).

Stereotactic radiotherapy using a gamma-knife or Cyber-knife ('radiosurgery') is used to optimise the tumour dose and minimise radiation to other areas by delivering a single high dose to a small tumour. This approach ensures that the daily dose to neural tissue does not exceed 180 cGy to avoid the complications of optic neuritis and cortical necrosis associated with larger total and fractional doses (259), 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 tumours (260;261). Most patients still develop endocrine deficiency in upcoming years after the treatment (262;263). 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 the radiosensitive tissues; optic chiasm or optic nerves, because of potential for blindness. Otherwise, if adenoma is not close to the optic pathway, it is considered to 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 55%, although follow-up times have not been as long as for conventional radiotherapy (269). It can also be used as salvage therapy in difficult tumours (265). Radiosurgery of the pituitary gland using proton beams has similar efficacy as second-line therapy (266), 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 small numbers of patients (267). As with other forms of radiotherapy, new hormone deficiencies are the major side-effect.

Medical therapy in Cushing's disease

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 commonly used therapy is adrenal enzyme inhibitors, but there are other possibilities. (please see below "Medical therapy in Cushing's syndrome).

Treatment for the Ectopic ACTH syndrome

If the ectopic ACTH-secreting tumour is benign and amenable to surgical excision, such as in a lobectomy for a bronchial carcinoid tumour, 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 tumours (268;269)

The course of the disease is mainly determined by the type of tumour, presence of metastases and degree of hypercortisolism. The lowest survival rate comes with small lung cancer, medullary thyroid cancer and gastrinomas (270;271).

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 (272;273). However, if significant metastatic disease is present, surgery is not curative, although it may still be of benefit in selected cases.

Regardless of the prognosis, control over hypercortisolism should be established medically either by inhibiting steroidogenesis, or performing mitotane induced medical adrenalectomy. If medical management fails, surgical bilateral adrenalectomy may be an option. Patients in whom control over hypercortisolism is established can develop thymic hyperplasia (274), which should be distinguished from tumour metastases or a primary thymic tumour. In cases where primary tumour origin remains unknown, adrenal inhibitors therapy is maintained as long as the patient goes to periodic re-examination for tumour localisation (270;271).

Ectopic CRH syndrome is rare and usually is associated with pulmonary carcinoid tumors, following the same therapeutic principles as ACTH secreting tumours (275)

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 (276; 277). Preoperatively, 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, eucortisolaemia can be achieved by using the appropriate receptor blockade (278; 279).

In adrenal adenomas, cure following surgery in skilled hands approaches 100% (280), and is associated with low morbidity and mortality (281).

Laparoscopic adrenalectomy, both unilateral and bilateral, has been shown in experienced hands to be a safe procedure and in many centres 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 (282).

In adrenal cancer; more aggressive surgical approaches probably account for the increase in life span reported in this disease (283;284). 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 (285-287).

Medical therapy of Cushing's syndrome

The role of medical treatment of Cushing's syndrome is an important one. It is the routine practice of many groups to pre-treat Cushing's syndrome patients prior to surgical treatment to reverse the hypercortisolaemia and its metabolic sequelae, and to hopefully reduce the complications of the definitive procedure. 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.

The most commonly used agents are adrenal enzyme inhibitors, but adrenolytic agents, pituitary-targeted therapies or glucocorticoid-receptor antagonists are also used. 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 within 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.

Adrenal enzyme inhibitors

These agents are primarily used as inhibitors of steroid biosynthesis in the adrenal cortex, and thus can be utilised in all cases of hypercortisolaemia 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. When used in combinations, they have a synergistic therapeutic effect, yet lowering the rate of side effects.

Metyrapone

Metyrapone acts primarily to inhibit the enzyme 11 β -hydroxylase, thus blocking the production of cortisol from 11-deoxycortisol in the adrenal gland (288). As a consequence to 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 some cross-reactivity from 11-deoxycortisol with some cortisol radioimmunoassays: this may result in an unnecessary increase in the metyrapone dose and subsequent clinical hypoadrenalism (289). It is preferable to measure the serum cortisol via liquid chromatography-tandem mass spectrometry in patients treated with metyrapone (290). The fall in cortisol is rapid, with trough levels at 2 hours post-dose, and in our unit we sometimes administer a test dose of 750 mg with hourly cortisol estimation for 4 hours (291). Maintenance therapy is usually in the range 500-6000 mg/day in 3-4 divided doses daily.

Metyrapone has been used to good effect to reduce the hypercortisolaemia in patients with Cushing's syndrome from adrenal tumours, 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. There have not been serious maternal or perinatal complications connected with the use of metyrapone in pregnant women, but question of safety remains open (292;293)

The principal side effects with metyrapone are hirsutism and acne (as predicted by the rise in adrenal androgens), dizziness and gastrointestinal upset. Because of the androgen effect the drug is not considered appropriate for the first-line therapy of long-term treatment in women (294;295). 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.

Hypokalaemia, oedema and hypertension due to salt retention because of mineralocorticoid activity of raised levels of deoxycorticosterone are infrequent (291), but may require cessation of therapy (296).

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 11 β -hydroxylase inhibition (297-299). It also inhibits 17-hydroxylase and 18-hydroxylase activity, amongst other enzymes (300). It has also been reported to have a direct effect on ectopic ACTH secretion from a thymic carcinoid tumour (301), 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 slower than metyrapone. The usual maximum dose is 400mgs.

It has been used successfully to lower cortisol levels in patients with Cushing's syndrome of various aetiologies including adrenal carcinoma, the ectopic ACTH syndrome, and invasive ACTH-producing pituitary carcinoma, with doses required between 200-1200mg/day in up to 4 divided daily doses (302-304), although 2-3 times daily is more usual. Although there has not been consequences on human fetuses, considering animal teratogenicity and toxicity the drug is not recommended for use during pregnancy (305; 306).

The principal side effect of ketoconazole is hepatotoxicity (307;308). A reversible elevation of hepatic serum transaminases occurs in approximately 5%-10% of patients, with the incidence of serious hepatic injury at around 1 in 15,000 patients (309). The hepatotoxicity appears to be idiosyncratic, but has been reported within 7 days of the initiation of treatment in a patient with Cushing's syndrome (310). Prior to the start of therapy liver function tests should be performed. The alanine aminotransferase (ALT) level should be monitored weekly within first month of therapy, then once a month in the following trimester and afterwards sporadically. 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 (311), and one must always be wary of causing adrenal insufficiency (310;312).

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, gynaecomastia 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 already usually present menstrual irregularity. It requires gastric acid for absorption, so should never be given with proton-pump inhibitors. One further advantage of ketoconazole is its inhibition of cholesterol synthesis, particularly LDL cholesterol (313), and in 34 patients with Cushing's syndrome the mean total cholesterol was reduced from 6.1 to 5.0 mmol/l on ketoconazole (311).

The triazole antifungal fluconazole can also be effective, but experience is limited. (314)

Etomidate

Etomidate is an imidazole-derived anaesthetic agent which was reported to have an adverse effect on adrenocortical function in 1983 (315). Compared to the other imidazole derivative ketoconazole, etomidate more potently inhibits adrenocortical 11 β -hydroxylase, has a similar inhibition of 17-hydroxylase, but has less of an effect on C17-20 lyase (316). At higher concentrations it also appears to have an effect on cholesterol side-chain cleavage (317;318).

Following their initial report in 1983 (319), Allolio *et al* have shown that low-dose intravenous non-hypnotic etomidate (2.5mg/hour) normalised cortisol levels in 5 patients with Cushing's syndrome of various aetiologies (320). Since then, there have been a number of case reports on the use of etomidate in successfully reducing hypercortisolaemia in seriously-ill patients with either Cushing's disease or the ectopic ACTH syndrome (321-325).

It is usually given at a dose of 2.5 - 3.0mg/hour, which is adjusted based on the serum cortisol levels. It usually takes about 10 hours for cortisol to be lowered to within normal range (326). Etomidate is an effective agent that acts rapidly, but is limited in its use by the fact it has to be given parenterally.

However, in this situation it may be life saving. The preparation available in the USA contains the vehicle propylene glycol with the potential for nephrotoxicity, as opposed to the preparation available in Europe, which contains alcohol.

Adrenolytic therapy

Mitotane (o'p'DDD), an isomer of the insecticide 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 11 β -hydroxylase in the adrenal gland (327). It also acts as an adrenolytic drug, causing medical adrenalectomy, after being metabolised into an acyl chloride that binds in mitochondria and causes necrosis of adrenocortical cells (328,329)

Mitotane is used as a treatment for adrenal carcinoma and causes tumour regression and improved survival in some patients (330,331), and has a beneficial effect on endocrine hypersecretion in approximately 75% of patients (332). It is also utilised in Cushing's syndrome of non-malignant origin, and in this regard lower doses can be utilised (up to 4 g/day), thus reducing the incidence of side effects, particularly gastrointestinal (289;333). 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 use reliable contraception while on therapy. (334)

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 Co A reductase (335). However, simvastatin, an HMG Co A reductase inhibitor, can reverse the hypercholesterolemia, and it or a similar agent should be used if necessary in patients treated with mitotane (335). Other side effects of mitotane include neurological disturbance; elevation of hepatic enzymes; hypouricaemia; gynaecomastia in men; and a prolonged bleeding time (332;336). Most importantly, it elevates cortisol-binding globulin, such that levels of total serum cortisol are misleading. Control should be titrated on urinary free cortisol or salivary cortisol.

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. Mitotane is taken up by fatty tissues; sometimes being released gradually several months after discontinuing therapy, therefore requiring adjustments in glucocorticoid therapy dosage (337).

Mitotane shows cytotoxic activity on both normal and tumorous tissue causing primary adrenal insufficiency and therefore requiring glucocorticoid replacement therapy. It tends to spare zona glomerulosa, but in a long-term use, mineralocorticoid replacement is also needed. (338)

In general, despite effective in other forms of Cushing syndrome, its use has been limited outside of adrenal carcinoma, in which cases it has recently been shown to prolong life (331).

Other medical agents

Somatostatin receptors have been demonstrated on both corticotroph adenomas, and some ectopic ACTH-secreting tumours. However, although octreotide has been helpful in reducing ACTH and cortisol levels in selected case reports of ectopic ACTH-secreting tumours there has been much more limited success in patients with Cushing's syndrome probably through down-regulation of receptor sub-type 2 in these tumours by hypercortisolaemia (339).

Recently, there has been renewed interest with the introduction of pasireotide (SOM230), a somatostatin analogue with a broader spectrum of activity for somatostatin receptor sub-types, including type 5, which is not downregulated during hypercortisolaemia. Ever since this agent was shown in vitro to reduce human corticotroph proliferation and ACTH secretion (340;341), 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 UFC levels in 76% of 29 patients and normalised levels in 17% (342). A multicentre phase III dose-randomised trial in 162 patients with either new, persistent, or recurrent Cushing's disease has presented 12 month results. At six months there was a reduction in UFC levels in 91 of 103 evaluable patients, with a median UFC reduction of 48%. Normalisation of UFC levels was 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 hyperglycaemia (39%), with 18% developing frank diabetes mellitus related to decreases in both insulin and incretin secretion, and and hypocortisolaemia (8%) (343;344).

Pasireotide should be given in a doze 600µg subcutaneously twice daily, with a possibility of increasing the dosage to 900µg twice daily after couple of months of therapy. In cases where there is no clinical response, it should be discontinued.

Pasireotide at a lower dose of 250µg 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 (345;346), and ketoconazole. Pasireotide monotherapy induced normalisation of UFC levels in 5 of 17 patients (29%). The addition of cabergoline normalised UFC levels in an additional 4 patients (24%). The further addition of ketoconazole in the remaining 8 patients induced normalisation of UFC levels in 6 of these. Thus in total, remission was achieved in 88% of patients using combination therapy out to 80 days treatment (347).

Therefore pasireotide represents a potential new treatment for Cushing's disease, although the frequency of hyperglycaemia is of major concern.

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 tumours including corticotroph pituitary carcinomas (348;349). Although, some reports suggested that the response to temozolomide in pituitary tumours can be predicted by low expression of the DNA repair enzyme O6-methylguanine-DNA-methyltransferase (MGMT), possibly related to MGMT gene promotor methylation (350;351), not all studies have confirmed this (352;353). However, the therapeutic response can usually be determined after 3 cycles of chemotherapy.

Retinoic acid has been found to inhibit ACTH-secretion and cell proliferation both *in vitro* in ACTH-producing tumour cell lines, and cultured human corticotroph adenomas, and *in vivo* in nude mice (354). However, clinical trials in man are limited.

The thiazolidinedione rosiglitazone, a PPAR- γ agonist, has been shown in supra-pharmacological doses to suppress ACTH secretion in human and murine corticotroph tumour cells. In addition, the development of murine corticotroph tumours, generated by subcutaneous injection of ACTH-secreting AtT20 cells, were prevented (355). It appears this is not specific to corticotroph adenomas, but also applies to other forms of pituitary tumour (356). However, the results in human subjects with Cushing's disease have been disappointing (357-360). 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, and indeed for other reasons it has now been withdrawn from the market.

In the rare causes of Cushing's syndrome due to bilateral macronodular adrenal hyperplasia (BMAH) and aberrant receptor expression of GIP, β -adrenergic and LH/hCG receptors, specific receptor antagonists may prove to be useful (361). Although octreotide has been shown to have caused a therapeutic response in GIP-related AIMAH as mentioned above (27), others have found neither this somatostatin analogue or pasireotide to be helpful in inducing a sustained response (362).

Mifepristone (RU 486) as a potent antagonist of glucocorticoid and progesterone receptors, blocks the peripheral actions of glucocorticoids and progestogens (363; 364). 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 (365). It has, occasionally been given to patients with all forms of Cushing's syndrome, (366;367), showing effectiveness in rapid reducing symptoms of cortisol-induced psychosis (368;369), and the metabolic benefit of glycemic control and hypertension have been established (367). Although, it has proven to be effective in the treatment of hypercortisolaemia symptoms and signs (370;371), 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. Hypokalaemia is a frequent problem, although it responds well to spironolactone.

Monitoring Treatment

It is important to monitor all patients on medical therapy for Cushing's syndrome, 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. We use the mean of 5 serum cortisol measurements across the day, although others favour measurement of urinary free cortisol (UFC). A mean serum cortisol between 150 and 300 nmol/l (5.5-11 ug/dl) corresponds to a normal cortisol production rate (372), and this range should be the aim of therapy. As mentioned above, a liquid chromatography tandem mass spectrography cortisol assay is preferable in patients on metyrapone.

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, which therefore cannot stand for correct measurements. Because it raises the level of cortisol binding globulin, level of total serum cortisol is inappropriate for monitoring of cortisol secretion, as it can be two to threefold elevated. (373; 374). Alternated levels of globulins explain why replacement dosage of steroids needs to be increased in case of an adrenal insufficiency.

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 plasma levels of cortisol are generally normal but with some radioimmunoassays may be increased (375;376). ACTH levels are increased (377). Glomerular filtration rates of less than 30 mL/min result in decreased cortisol excretion and spuriously low UFC values (378). The ACTH and cortisol responses to ovine CRH may be suppressed in patient with renal failure except for those undergoing continuous ambulatory peritoneal dialysis (379). The metabolism of dexamethasone is normal in chronic renal failure, but the oral absorption can be altered in some patients. There is reduced degree of suppression of cortisol by dexamethasone suggesting a prolonged half-life of cortisol. Normal suppression to the overnight 1-mg LDDST is uncommon, and the 2-day LDDST does better in this regard (375;380).

Paediatric Cushing's syndrome

The most common presentation of Cushing's syndrome in children is growth retardation, whilst weight increases (45). However, one proviso is that patients with virilising adrenal tumours may show growth acceleration (381). Other virilizing signs such as acne and hirsutism are seen in approximately 50% of patients regardless of aetiology (45). Hypertension and striae are seen in approximately 50% of cases (382). Muscle weakness may be less common in the paediatric patient due to increased exercise (383). Psychiatric and cognitive changes may affect school performance; however, children may show "compulsive diligence" and actually do quite well academically (384). Headaches and fatigue are common (45). 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. 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 virilisation 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 (385).

As mentioned previously, late night salivary cortisol measurement has particular logistic benefits in children (101; 386). Serum midnight cortisol measurements in inpatients has

high sensitivity (387). UFC should be corrected for body surface area (388). 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 (389). As in adults there is good correlation between the cortisol suppression on the LDDST and the HDDST for the differential diagnosis and thus the latter is not necessary (390). 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 lateralisation of the pituitary tumour (385). MRI is at least as useful as CT in the evaluation of adrenal causes (391).

Transsphenoidal surgery is the treatment of choice in children with Cushing's disease, with similar rates of remission as in adults (292). Conventional radiotherapy after non-curative transsphenoidal surgery performs even better than in adults, with reported remission rates as high as 100%, with remission occurring within 12 months (393). 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 (394). Normalisation of reduced bone mineral density can also be achieved (395). 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 (292,293). 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 cases (48% percent adenoma and 10% carcinoma) followed by Cushing's disease and bilateral adrenal hyperplasia, and rarely ectopic disease (292;293;396;397). 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.

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. In contrast to patients with pathologic hypercortisolism, levels of urinary 17-OH-corticosteroid excretion are within normal range and the cortisol diurnal rhythm is maintained, but with a higher nadir (396). UFC excretion is normal in the first trimester and then rises up to three-fold by term (398). Suppression to dexamethasone testing is blunted, especially after the first trimester (399). Plasma ACTH levels are slightly decreased in the beginning of the pregnancy, but later tends to rise, partially because of placental ACTH secretion.

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, 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. Therefore, the differential diagnosis regarding the possible aetiology 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 possibility of ACTH-independent cause. High-dose dexamethasone test may be useful to distinguish adrenal cause, because women with adrenal causes tend to suppress, while those with Cushing's disease do not (397;400-404).

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 this drug.

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. BIPSS with appropriate additional radiation protection for the fetus should be reserved only for cases where diagnostic uncertainty remains.

Maternal hypocortisolism is associated with 70% hypertension, preeclampsia, 25% diabetes mellitus, renal failure, and rarely death (292)

Although the fetus is partially protected from maternal hypercortisolism by placental 11-beta-hydroxysteroid dehydrogenase, which converts 85% of cortisol to inactive cortisone (405), the untreated condition is associated with miscarriage, premature delivery and neonatal adrenal insufficiency (292).

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 prevent stillborn deliveries. (396)

Medical treatment carries potential risk 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 pituitary radiation effect. Metyrapone is probably the adrenolytic agent of choice, although an association with pre-eclampsia has been reported. Ketoconazole has been utilised successfully in a small number of patients but is teratogenic in animals and therefore should be used with caution.

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 (406)

Even today, patients with severe hypercortisolism can be fatally in jeopardised by increased coagulability consequences or opportunistic infections (95;407;408), emphasising the need for taking over the hormonal control 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 occur over time. (409). 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 is not significantly different from that in the general population (23;401;408;411). However, another population based study suggested that mortality is marginally increased (3), while even more recently a very significantly increased mortality was shown even in patients who remained cured. This is perhaps not surprising given that 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 (77). 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 (412). 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 paediatric Cushing's disease is excellent if treated at centres with appropriate experience (382; 413).

Cushing's syndrome results in significant impairment in quality of life (414), psychiatric symptoms (415) and cognitive deficits (416; 417), as previously noted. However, in general these are only partially improved with treatment, but 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 hypercortisolaemia (418;419). Bisphosphonate treatment may induce a more rapid improvement in bone mineral density (420), 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 (52;421-423). 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. Tumours that produce ectopic ACTH tend to have a poorer prognosis, compared with tumours from the same tissue that do not produce ACTH. Small cell lung cancer, islet cell tumours and thymic carcinoids (424) 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 (15), although currently a survival in terms of months should be expected.

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