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Review Paper

Pituitary tumors: diagnosis and treatment

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This article reviews the current general approach to the biochemical diagnosis and the treatment of pituitary tumors with special reference to medical treatment with dopamine agonists and somatostatin analogs. Dopamine agonists are the treatment of choice in patients with prolactin producing tumors. Octreotide is a major advance in the adjunctive treatment of growth hormone producing tumors. Trans-sphenoidal surgical decompression remains the primary treatment modality in gonadotrofinomas, clinically non-functioning pituitary tumors and thyroid stimulating hormone (TSH) producing tumors. Adrenocorticotrophin (ACTH) producing tumors are treated primarily by selective adenomectomy. The biochemical diagnosis of Cushing's syndrome is complex. Bilateral inferior petrosal sinus sampling for ACTH measurement is highly reliable in the differential diagnosis of ACTH dependent Cushing's syndrome, but needs expertise.

Key words: Bromocriptine, dopamine agonist, inferior petrosal sinus sampling, octreotide, pituitary tumor, somatostatin analog.

Introduction

Pituitary adenomas are the most frequently occurring tumor type in the sellar area. Prolactinomas represent approximately 40% of pituitary adenomas, gonadotrofinomas and clinically non-functioning tumors 30%, growth hormone producing tumors 20%, adrenocorticotrophin (ACTH) producing tumors 10%, whereas hypersecretion of thyroid stimulating hormone (TSH) by pituitary tumors occurs in less than 1% of cases. Clinical symptomatology is due to excess hormone secretion (prolactinoma syndrome, acromegaly, Cushing's disease, hyperthyroidism) and/or to a mass effect of the tumor (hypopituitarism, visual disturbances, cranial nerve palsies, headache) in patients with macroadenomas. The majority of prolactin producing tumors are microadenomas with a diameter of less than 10 mm and preferentially occur in women; macroprolactinomas are found more frequently in men. Most growth hormone producing tumors are macroadenomas of moderate size. ACTH producing tumors are microadenomas in at least 70% of cases. Gonadotrophin producing tumors, clinically non-functioning tumors and TSH producing tumors characteristically present as large slow growing adenomas. Neuroradiographic evaluation of pituitary tumors is carried out by computed tomography (CT) or magnetic resonance imaging (MRI). In macroadenomas both scans are equally diagnostic, but MRI offers more information on pituitary morphology and neighboring structures. MRI is also more sensitive in the detection of microadenomas. However, in general, both techniques fail to detect microadenomas with a diameter of less than 3 mm.

Recent studies have shown that most pituitary tumors are of monoclonal origin, strongly supporting somatic mutation of a single cell as a key event in neoplastic transformation.[1,2] This is illustrated by the finding of G-protein mutations in a subset of growth hormone producing tumors.[3] Attempts to correlate the presence of G-protein abnormalities with clinical features and growth hormone dynamics in acromegaly are still inconclusive.[4–6]

Optimal therapy of pituitary tumors theoretically includes total removal of the tumor with rapid normalization of hormone production without the induction of secondary pituitary insufficiency. The classic treatment modalities of neurosurgery and radiotherapy have been broadened by medical treatment during the last 20 years. This article will review the current general approach to the biochemical diagnosis and the treatment of pituitary tumors with special reference to medical treatment with dopamine agonists and somatostatin analogs.

Prolactin producing pituitary tumors

Prolactin secretion is primarily under the tonic inhibition of dopamine, synthesized in the hypothalamus and transported to the anterior pituitary via the hypothalamohypophyseal portal capillary system. Prolactin secretion is pulsatile. Exercise and stress stimulate prolactin secretion. Pregnancy is the most important cause of physiologic hyperprolactinemia. Estrogen induced hyperplasia of the lactotroph cell is associated with rising prolactin values, reaching peak levels in the third trimester. Post partum plasma prolactin values remain high for several months in breast feeding, with sharp surges in prolactin secretion occurring during suckling; in women who do not breast feed, plasma prolactin values decrease rapidly to normal values within 2 weeks. Although the upper limit of the normal range of plasma prolactin is often quoted at 0.36 U/l for men and 0.48 U/l for women (1 U = 25 μg), it really extends to 0.8 U/l because of the skewed normal distribution. Plasma prolactin values below 1 U/l are rarely of clinical significance.[7]

Pathological hyperprolactinemia is associated with inhibition of hypothalamic gonadotrophin releasing hormone (GnRH) pulse generator activity and, consequently, inhibition of pituitary gonadotrophin secretion by a mechanism involving hypothalamic dopaminergic and opiodergic signals. In addition, hyperprolactinemia may directly alter pituitary sensitivity to GnRH. The clinical picture is characterized by menstrual irregularities and amenorrhea in women, and by loss of libido and impotence in men. Gonadal steroid deficiency may induce osteoporosis. Due to a direct effect of prolactin on breast tissue, galactorrhea may be induced in both sexes.

Pathological hyperprolactinemia is usually caused by a prolactinoma or by the use of drugs, including dopamine depletors (reserpine, α-methyldopa) and dopamine receptor antagonists (phenothiazines, butyrophenones, metoclopramide and the antidepressant amoxapine). Other causes include hypothalamic lesions, primary hypothyroidism, chronic renal failure and liver failure.[8]

There is no generally accepted biochemical test method to differentiate between the various causes of hyperprolactinemia.

The estimated prevalence of prolactinomas is 1:1050 in women and 1:2800 in men.[9] Microprolactinomas are characterized clinically by the direct effect of hyperprolactinemia on gonadal function. The growth potency of microprolactinomas is small; progression to the development of macroprolactinomas is less than 5%.[10] Spontaneous remissions may occur.[11] Macroprolactinomas are characterized both by a direct effect of hyperprolactinemia on gonadal function and by a mass effect. Untreated macroprolactinomas continue to grow. In patients with prolactinomas the size of the tumor typically correlates well with the plasma prolactin level. However, clinically non-functioning pituitary tumors and other peripituitary masses may be associated with hyperprolactinemia as a result of stalk compression and functional disconnection of the pituitary from the hypothalamus. Extensive studies have shown that prolactin levels over 6 U/l almost always turn out to be associated with macroprolactinomas, whereas prolactin levels below 2.5 U/l are likely to be associated with a clinically non-functioning pituitary tumor or a peripituitary mass. Between these limits either diagnosis is possible.[12]

Clinical observation may be sufficient in some patients with microprolactinomas, but most have to be treated because of gonadal dysfunction with menstrual cycles occurring less than every 8 weeks, infertility or severe galactorrhea. Initial results of selective trans-sphenoidal adenomectomy are excellent, but the recurrence rate is high.[13] Medical treatment with dopamine agonists is now considered to be the first therapeutic approach.[14] Most experience has been obtained with the ergot derivative bromocriptine. Bromocriptine has been shown to be effective in normalizing plasma prolactin levels in over 90% of patients. Menses can be expected to return within 2 months in about 75% of patients. During the first 2–3 months of treatment the patients use barrier methods of contraception. Once plasma prolactin is normalized and no pregnancy is desired, the contraceptive pill may be used since during continued bromocriptine treatment estrogens do not cause progression of hyperprolactinemia or tumor growth.[15] The dose of bromocriptine needed to normalize plasma prolactin values is usually low (2.5–7.5 mg). Bromocriptine withdrawal after 1 year of treatment is associated with a remission in about 10% of patients.[16] Bromocriptine resistance is also observed in about 10% of patients.[17]

The results of neurosurgery in patients with macroprolactinomas are very poor and normoprolactinemia is reached only occasionally.[14] Dopamine agonists are the treatment of choice for patients with macroprolactinomas as both normalization of plasma prolactin levels and tumor size reduction are

observed in the majority of patients.[18–20] Meta-analysis of 271 patients with macroprolactinomas from 26 series receiving dopamine agonist therapy for at least 4 weeks showed that significant tumor shrinkage (greater than 25%) was observed in 215 cases (79%) and of 115 patients treated for longer than 1 year such shrinkage was shown in 83%. Of the 215 tumors that shrank by 25% or more, 58% attained a normal serum prolactin during the period of observation.[21] Of particular interest are the results of our study of 19 patients treated with 10–20 mg bromocriptine daily in four divided doses for a mean period of 4.4 years; plasma prolactin normalized in 17 patients, and tumor size reduction by more than 75% was observed in 17 patients and by 50–75% in two patients.[22] Both the relatively long observation period and the fixed dose regimen of bromocriptine regardless of the plasma prolactin lowering effect may have contributed to these excellent results. Tumor size reduction is due to a decrease in the volume of individual tumor cells due to a decrease in the size of the hormone-synthesizing apparatus. Tumor shrinkage may lead to a return of normal function in one or more endocrine axes and restoration of visual disturbances.[19–23] At this stage CT scans typically show hypodense tumor remnants and a hyperdense pituitary gland in an eccentric position connected with a displaced pituitary stalk.[23,24] The medical treatment of macroprolactinomas has clearly shown that hypopituitarism in patients with macroadenomas may be functional and therefore reversible.[25]

Withdrawal of dopaminergic therapy within 1 year in patients with macroprolactinomas is associated with a rapid rise of plasma prolactin levels and may lead to rapid tumor expansion.[20] Therefore, definitive ablative treatment by radiotherapy is advised once the macroadenoma has shrunk to within the confines of the pituitary fossa during bromocriptine treatment.[15] However, there is increasing evidence that long-term dopaminergic therapy may be curative in some patients.[26] Morphological studies carried out on tumor specimens after long-term bromocriptine treatment not only showed atrophic tumor cell nests, but also pycnosis, karyolysis, necrosis, fibrosis, hyalinosis and inflammatory cell infiltration suggestive of cytocidal effects.[27] Johnston *et al.*[28] studied the effect of dopamine agonist withdrawal in 15 patients who had been treated for macroprolactinomas for 1.5–7 (mean 3.7) years. Hyperprolactinemia redeveloped in 14 patients, but only one patient had a marginal increase in tumor size within 3 months. In another study persistent remission of hyperprolactinemia was reported in nine of 59 patients with macroprolactinomas treated for 4–10 years.[29] We studied the results of dopamine agonist withdrawal in 12 patients with macroprolactinomas who had been treated with bromocriptine for a mean period of 4.9 years (range: 3.5–7 years); a stable reduction of plasma prolactin levels was obtained in three patients followed up for 1 year and tumor re-expansion occurred in only one patient.[30] Thus, tumor re-expansion after withdrawal of long-term dopaminergic therapy is an exception, rather than a rule.

We propose to interrupt the medical treatment of macroprolactinomas after 4 years and to reinstitute medical treatment in patients with recurrent hyperprolactinemia and/or tumor re-expansion. This procedure may be repeated every 2 years.

Careful follow up is necessary in patients with persistent normoprolactinemia and no evidence of tumor re-expansion. In patients with microprolactinomas treatment may be interrupted for the first time after 2 years. Follow up is restricted to measurement of plasmaprolactin levels.

Bromocriptine has a relatively short duration of action and is usually given in two or three daily doses. Side-effects include dizziness, headache, nausea, vomiting, orthostatic hypotension and nasal congestion. The incidence and severity of these side-effects is greatly diminished by the gradual introduction of the drug over a period of days or weeks and by giving the drug in the middle of a meal.

Despite these precautions side effects remain bothersome in 5% of patients during chronic treatment. New formulations of the drug and new dopaminergic drugs have been developed to overcome these drawbacks. Parlodel LAR (long acting repeatable) is a repeatable depot injection of bromocriptine which may be given deep intramuscularly at monthly intervals.[15,31] Cabergoline is structurally related to bromocriptine and, due to its very long lasting prolacting lowering activity, the drug may be given orally once weekly.[32] CV 205-502, a nonergot dopamine agonist which specifically stimulates the D2 receptor, can be given orally once daily.[33,34] Both cabergoline and CV 205-502 are better tolerated than bromocriptine.[32,33] Recent studies also suggest that CV 205-502 may overcome bromocriptine resistance in about 50% of cases due to its higher affinity towards the D2 receptor.[35]

The usual strategy in the treatment of prolactinomas is to use the minimum dose of a dopamine

agonist required to keep plasma prolactin values within the normal range.[36] However, clinical studies after drug withdrawal showed that circulating prolactin levels tended to remain lower than those before treatment and these favorable effects were more evident in patients treated with the highest dose of the dopamine agonist.[28,29] Whether the dose of the dopamine agonist is a separate factor determining the chances for a permanent remission remains undecided at present.[30]

Growth hormone (GH) producing pituitary tumors

GH secreting pituitary tumors induce acromegaly. Recent epidemiologic studies have indicated an incidence rate of 3.3 per million per year and a prevalence rate of 66 per million.[37] There is also clear epidemiologic evidence for an increased mortality rate due to cardiovascular disease and cancer related mortality.[37,38] Hyperprolactinemia occurs in approximately one third of patients with GH secreting adenomas; prolactin is most frequently secreted from a mixed population of somatotroph and lactotroph tumor cells, but occasionally from a bihormonal somatomammotroph adenoma. The clinical picture of acral enlargement and soft tissue overgrowth produced by GH is mediated largely by somatomedins, especially somatomedin C or insulin-like growth factor 1 (IGF-1). The clinical diagnosis acromegaly is confirmed by an oral glucose tolerance test using 75–100 g of glucose. Normal subjects suppress GH to less than 2 mU/l (= 1 μg/l), but acromegalics do not.[39] Measurement of IGF-1 is also used in the diagnosis of acromegaly, but is particularly useful in the follow up after treatment as it represents the integrated 24 h GH secretion by the tumor.

About 70–80% of acromegalics show a paradoxal rise of plasma GH after thyrotrophin releasing hormone (TRH) administration;[39] in these patients the TRH test is useful for the detection of residual pathological tissue after surgery.

The goal of treatment can be defined theoretically as cure of the disease by total elimination of the tumor with resolution of its mass effects, complete restoration of normal GH physiology including normalization of basal GH and IGF levels, restoration of normal GH secretory pulses including through concentrations below 2 mU/l, normalization of GH suppressibility by glucose, loss of abnormal responses to TRH, and a decrease of signs and symptoms of acromegaly to the greatest extent possible.[40] These rigid criteria for cure have been developed only recently with the availability of more sensitive assays for GH and concomitant assessment of IGF levels. For many years a basal GH concentration of 10 mU/l and a postglucose level of 4 mU/l or less were considered to be the desired goals. In a recent review of 1360 patients from 30 surgical series only 60% had GH levels below 10 mU/l after their operations.[41] Clinical improvement generally correlated with the degree of reduction of GH levels. Results are poor in patients with large tumors and initial GH levels greater than 100 mU/l. Even in those patients whose GH levels have been reduced to 5 mU/l or less, normal pulsatile GH secretion does not always occur and one in five patients still exhibit a GH response to TRH.[42] However, it is still unknown whether this finding is a predictor of tumor regrowth. However, occasional patients with postoperative GH levels below 5 mU/l will develop recurrences.[43] Despite these concerns, surgical therapy remains the primary choice of treatment in all patients who are acceptable surgical risks.

The main disadvantage of radiation therapy is the long duration required to achieve normal GH levels; even after 10 years elevated GH levels are present in 10–15% of patients.[44] In addition, a progressive loss of anterior pituitary function occurs in 15–50% of patients with a lag time of from less than 1 to 10 years.[45] It should be reserved for patients with persistent excess of GH after surgery and as a primary treatment option for the few patients who are not surgical candidates.

Bromocriptine has been widely used in treating acromegaly since 1974 and is a useful adjunctive therapy. GH secretion is suppressed presumably through a dopamine D2 receptor mechanism. The only factor predicting responsiveness is the coexistence of prolactin hypersecretion. Among 514 patients from 28 different series, basal GH levels decreased to less than 10 mU/l in 20%, whereas tumor shrinkage occurred in 10–15%, although 70% of patients had some improvement in clinical well-being.[46]

A major advance in the adjunctive treatment of acromegaly has been the use of octreotide, a cyclic octapeptide analog of the hypothalamic inhibitor of GH release, somatostatin. It is a better compound for clinical use than native somatostatin, as it has a long half-life in the circulation and inhibits GH preferentially over insulin. Although the response to octreotide is primarily dependent on the presence of somatostatin receptors in tumor tissue, the drug

also acts peripherally to inhibit secretion of IGF-1 and by stimulating the release of IGF-1 binding protein; since this protein has such a very high affinity for IGF-1, it may control access of IGF-1 to its receptor and thereby block IGF-1 induced biological effects.[47] A three times daily subcutaneous injection of 100 μg of octreotide is the mean optimal efficacious dose.[48] Long-term responsiveness can be predicted by the acute suppressive effects of a single 50 μg injection of octreotide.[48] Results of long-term treatment with octreotide in 189 acromegalic patients, published by the International Multicenter Acromegaly Study Group, show suppression of growth hormone to below 10 mU/l in 45% with IGF-1 values below 2 U/ml in 46%, whereas a reduction of pituitary size by at least 20% was obtained in 44% of patients. Most patients experienced some clinical improvement, and a reduction of GH and IGF-1 values.[49] There is some evidence that pretreatment of acromegalic patients with octreotide might improve the efficacy of trans-sphenoidal surgery, especially in patients with macroadenomas.[50] The effects of octreotide seem reversible as both pretreatment GH and IGF-1 levels reappear within days to weeks after stopping treatment; tumor size increases have also been noted within weeks of stopping the drug.[51] However, octreotide does not necessarily need to be used life long. The drug is more effective than bromocriptine and should be recommended until radiotherapy is effective in patients with persistent excess of GH after surgery.

Side effects of octreotide are related to its suppression of gastrointestinal motility and secretion. Flatulence and mild steatorrhea occur commonly but tend to decrease with continued therapy. Alterations in glucose metabolism are minor as octreotide suppresses insulin secretion less than it suppresses the insulin antagonists GH and glucagon. The side effect of greatest concern is cholelithiasis. Octreotide suppresses cholecystokinin secretion by the intestine and inhibits gallbladder contraction in response to cholecystokinin. Bile flow may also be inhibited. The incidence of gallstone formation is 40–50% during long-term treatment.[52]

Gonadotrofinomas and clinically non-functioning pituitary tumors

Gonadotroph adenomas are rare tumors occurring primarily in men over 50 years of age. In these patients hypersecretion of intact follicle stimulating hormone (FSH) may lead to hypertrophy of seminiferous tubules and bilateral testicular enlargement, whereas hypersecretion of intact luteinizing hormone (LH) is associated with a supranormal plasma testosterone concentration. About 30% of pituitary tumors are not associated with evidence of excess hormone secretion *in vivo*. Immunocytochemical as well as *in situ* hybridization and *in vitro* secretion studies have shown that most of these so called clinically non-functioning pituitary tumors contain and release gonadotrophins and their subunits.[53–55] The discrepancy between the findings of *in vitro* secretory activity and the absence of elevations in circulating hormone levels may be due to the fact that only a minority of tumor cells display hormone releasing activity.[56] Some of these tumors not only secrete gonadotrophins and their subunits, but also other anterior pituitary hormones, suggesting that clinically non-functioning pituitary tumors may represent neoplasms derived from precursor cells that can undergo differentiation towards several cell lines, but most often follow differentiation along the line of gonadotrophs.[57]

The tumors present clinically as sellar lesions with mass effect including hypopituitarism and should be differentiated from other sellar lesions. Free α-subunit concentrations are elevated in about one fifth of patients.[58] The secretion of α-subunit may be accompanied by secretion of intact hormones, the β-subunit of FSH (FSHβ) or, less often, the β-subunit of LH (LHβ). At present, FSHβ and LHβ are only assayed in research laboratories. Tumoral secretion of gonadotrophins can also be confirmed by a finding of abnormal responses to hypothalamic releasing hormones. Serum gonadotrophin or α-subunit responses to i.v. injection of TRH were found in 72% of patients.[55] An even higher response rate may be obtained if measurement of LHβ is included in the test.[59] The mechanism by which these tumors acquire receptors for TRH is unknown. Both measurement of the free α-subunit and the TRH test are also very useful in the reassessment of patients after treatment.

The primary form of therapy is trans-sphenoidal surgical decompression. Surgery results in improved vision and decreased headaches, but improvement of anterior pituitary function is uncommon. Complications are relatively frequent. In a recent study of 126 patients from Hardy's group one or more postoperative complications occurred in 42 patients (29%) and two patients died in the postoperative period.[60] Surgery is rarely

curative and usually followed by external pituitary irradiation, but there are no large uncontrolled studies of its effectiveness.[61] Effective medical treatment would be desirable, since preoperative tumor reduction could increase the chances of curative surgery.

Clinically non-functioning pituitary tumors have been shown to contain dopaminereceptors[62] and to respond to bromocriptine inhibition *in vitro*.[55] In a recent review of 84 patients from seven series only seven patients showed a small decrease in tumor size during bromocriptine treatment (8.3%), but 15.4% of those treated for longer than 1 year achieved shrinkage,[21] probably because the drug has a time-dependent increasing effect on the release and synthesis of gonadotrophins and α-subunit.[63] Failure to achieve major tumor size reduction may be due to the low percentage of cells showing secretory activity *in vitro*, the low density of membrane bound dopamine receptors, whereas these tumors may possess post-dopamine receptor defects that render the cells unable to respond to receptor activation.[21] Long-term treatment of five patients with CV 205-502 was characterized by a decrease of serum FSH and/or α-subunit concentrations in all cases, improvement of visual field defects in two cases and tumor shrinkage in one case.[64] These interesting results warrant further investigation on the use of CV 205-502 in the medical treatment of clinically non-functioning pituitary tumors.

Specific stomatostatin receptors have also been identified on the cell membranes of clinically non-functioning pituitary tumors.[65] We showed that octreotide was able to inhibit LH secretion in a man with an LH secreting pituitary tumor.[66] However, chronic octreotide treatment is associated with tumor size reduction in only a few patients with clinically non-functioning pituitary tumors.[67] The use of *in vivo* scintigraphy with radiolabeled somatostatin analogs has been proposed to identify patients positive for somatostatin receptors who might potentially benefit from octreotide treatment.[68] Recently, we treated four patients with clinically non-functioning pituitary tumors with a high dose of octreotide (1200 μg s.c.) for a period of 3–6 months; the presence of somatostatin receptors in the adenoma tissue was demonstrated *in vivo* using [^{111}In]octreotide scintigraphy in three of these patients. However, no tumor size reduction was demonstrated in any patient.[69] Future clinical trials should include the combined use of dopamine agonists and somatostatin analogs.

GnRH agonist analogs have been administered to a small number of patients in an attempt to produce gonadotroph desensitization, but a persistent agonist effect on tumoral hormone secretion was found in most cases.[70] The effects of GnRH antagonist analogs remain unknown. In summary, at present there is no effective medical treatment for clinically non-functioning pituitary tumors.

TSH producing pituitary tumors

Thyroid stimulating hormone (TSH) secreting pituitary tumors induce diffuse goiter and hyperthyroidism associated with either inappropriately 'normal' or elevated plasma TSH concentrations.[71] About 80 cases of this rare tumor have so far been described. In over 80% of patients hypersecretion of free α-subunit accompanies the production of TSH.[72] Both the finding of a non-suppressed plasma TSH level and of an elevated α-subunit/TSH molar ratio is useful diagnostically. About one third of patients also have excess production of other anterior pituitary hormones. In particular coexisting GH secretion has been reported frequently and those patients have clinical features of acromegaly. There is evidence for both dual secretion of two hormones from a single cell and isolated secretion from separate cell types.

Neurosurgery is the treatment of choice for TSH secreting pituitary tumors. Due to the large size of most tumors, the results are poor, even when neurosurgery is combined with radiotherapy.[71] Dopaminergic agonists may acutely reduce serum TSH in some cases, but longer-term therapy has generally been unsuccessful.[71] Octreotide is effective in controlling hypersecretion of TSH and the associated hyperthyroidism.[73,74] Tumor size reduction has been observed during long-term treatment in some patients,[75] but the long-term efficacy of octreotide treatment in patients with TSH secreting tumors remains to be determined. At present surgery remains the primary treatment modality for these tumors.

ACTH producing pituitary tumors

Cushing's syndrome is a corticosteroid excess syndrome usually (70%) due to increased ACTH secretion by a pituitary tumor (Cushing's disease). Cushing's syndrome may also be due to a cortisol producing adrenocortical tumor (20%) or ectopic ACTH secretion (up to 10%). Patients with Cushing's syndrome secondary to an adrenal tumor

are readily differentiated from other patients with Cushing's syndrome by undetectable plasma ACTH levels and the presence of an adrenal mass demonstrated by radiological imaging techniques. The principal problem is the differential diagnosis in ACTH dependent Cushing's syndrome as both the pituitary microadenoma in Cushing's disease and an occult ectopic source of ACTH in the ectopic ACTH syndrome may be exceedingly small and difficult to locate. Diagnostic difficulties may also arise in distinguishing Cushing's syndrome from obesity, depression and alcoholism, where the clinical and biochemical evidence can be confusing. Thus, it is essential first to confirm a diagnosis of Cushing's syndrome and second to establish the precise etiology. A myriad of tests have been developed in the diagnosis and differential diagnosis of Cushing's syndrome. Guidelines are based on meta-analysis of individual reports of small groups of patients. A detailed analysis is given in several recent reviews.[61,76,77] We will summarize the most important test procedures and focus on recent developments.

Several excellent screening tests are available. The overnight dexamethasone suppression test is carried out primarily in outpatients by oral administration of 1 mg dexamethasone at 11:00 p.m. and measuring plasma cortisol at 9:00 a.m. the following morning. Normal values are below 75 nmol/l in our laboratory. The test has a high sensitivity but rather low specificity. More specific is measurement of the 24 h urinary excretion of free cortisol, an indirect measurement of cortisol production. Normal values are below 300 nmol/24 h. Midnight plasma cortisol levels are elevated in Cushing's syndrome as a consequence of loss of diurnal secretion of cortisol. To confirm the diagnosis Cushing's syndrome the low dose dexamethasone suppression test is carried out by oral administration of 0.5 mg dexamethasone every 6 h for two consecutive days. Cushing's syndrome is characterized by failure to suppress urinary steroid metabolite excretion and plasma cortisol to very low levels.

Once a diagnosis of Cushing's syndrome has been made, the finding of undetectable plasma ACTH levels indicates that the patient suffers from a cortisol producing adrenocortical tumor. In patients with ACTH dependent Cushing's syndrome the differential diagnosis is carried out by a combination of suppression and stimulation tests. The high dose dexamethasone suppression test is performed by oral administration of 2 mg dexamethasone every 6 h for two consecutive days. In 85–90% of patients with Cushing's disease urinary steroid excretion and plasma cortisol levels are suppressed by at least 50%; however, a similar response is obtained in about 10% of patients with an ectopic ACTH syndrome, particularly in patients with carcinoid tumors. The corticotrophin releasing hormone (CRH) test involves the i.v. injection of 100 μg of ovine CRH and measurement of plasma cortisol at regular intervals for 2–3 h. A plasma cortisol response is obtained in about 90% of patients with Cushing's disease; responsiveness to CRH almost always excludes the ectopic ACTH syndrome. Diagnostic accuracy is about 95% when both tests are combined.

In the last decade the technique of bilateral inferior petrosal sinus sampling for ACTH measurement has been developed to improve the differential diagnosis of ACTH dependent Cushing's syndrome and for the preoperative location of pituitary microadenomas in one half of the pituitary gland. The procedure involves inserting catheters into both femoral veins and maneuvring their tips into the inferior petrosal sinuses bilaterally. Blood is obtained simultaneously from both sinuses and a peripheral vein for measurement of plasma ACTH. The procedure is repeated 2 and 5 min after i.v. injection of CRH. A central to peripheral ACTH gradient indicates that the ACTH causing the hypercortisolism is of pituitary origin; this gradient may be improved by CRH administration. The lack of a gradient is presumptive evidence that ACTH is being secreted from a non-pituitary site. The finding of an intersinus ACTH gradient suggests lateralization of a pituitary microadenoma to the site of the highest ACTH concentration. In the hands of experienced radiologists the technique has achieved an accuracy of 100% in the differentiation of Cushing's disease from the ectopic ACTH syndrome,[78,79] although the preoperative location of pituitary microadenomas is poorly predicted.[78,80] However, this time consuming invasive procedure has to be carried out many times to identify one occult ectopic ACTH producing tumor. The central obesity of patients with Cushing's syndrome makes the catheterization procedure technically more difficult and significant inguinal bleeding can occur after the procedure.[81] In one patient the right atrium was punctured.[81]

These drawbacks of the catheterization procedure may be overcome by improvements of biochemical function testing. Many years ago, we compared the oral high dose dexamethasone test with a 5-h i.v. dexamethasone test (1 mg/h) in seven patients with Cushing's disease. Infusion of

dexamethasone was associated with suppression of plasma cortisol levels in all cases including two who did not respond to oral dexamethasone administration.[82] The i.v. dexamethasone test has now been performed in 80 patients with Cushing's disease and, without any exception, suppression of plasma cortisol was obtained; the degree of suppression could be further improved by extending the test to 7 h (1 mg/h).[83] We propose that the 7-h i.v. dexamethasone test should replace the oral high dose dexamethasone test. Furthermore, Orth recently claimed that by simultaneously administering ovine CRH i.v. and arginine–vasopressin or lysine–vasopressin (10 pressor units i.m.) no false-negative responses have been observed in 42 patients with Cushing's disease.[81] These findings suggest that the combination of the 7-h i.v. dexamethasone test and a CRH–vasopressin test may virtually obviate the need of the catheterization procedure.

For years Cushing's disease was treated primarily by surgery of the adrenals. With the advent of the dissecting microscope, trans-sphenoidal microsurgery has become the treatment of choice over the last 15 years. The successfully treated patient is characterized by undetectable plasma cortisol levels in the immediate postoperative period and prolonged cortisol dependency, but ultimately the patient becomes cortisol independent with normal regulation of pituitary–adrenocortical function and no evidence of hypopituitarism. Few patients have been evaluated according to these criteria. With these restrictions in mind, initial remission rates of at least 75% are reported in most series.[61,84–86] Recurrences occur in 6–15% and increase with prolonged follow up. Persistent Cushing's syndrome and recurrences are particularly frequent in patients with macroadenomas and invasive tumors.[87] Treatment is either by re-exploration or external pituitary irradiation. Pituitary irradiation can be combined with the use of pharmacologic agents that block the adrenal system—such as metyrapone—until the effect of irradiation peaks.

Experimental medical treatment aiming to reduce pituitary ACTH secretion has been carried out as part of pathogenetic studies. Based on the view that Cushing's disease may be due to a defect of central neurotransmission,[88] neurotransmitter active drugs such as the dopamine agonist bromocriptine, the serotonin antagonist cyproheptadine and the γ-aminobutyric acid transaminase inhibitor sodium valproate have all been used in the medical treatment of Cushing's disease. The later finding that dopamine and cyproheptadine influence ACTH secretion via a direct effect on the corticotroph adenomas[89] was compatible with the view at that time that in a subset of patients Cushing's disease is of neurointermediate lobe origin[90] and those patients might respond to neurotransmitter active drugs. However, neither histological[91,92] nor immunocytochemical[93] or biochemical[94,95] evidence for the existence of such an entity was found. We now believe that heterogeneity of the normal anterior pituitary corticotroph, e.g. the presence of receptors for neurotransmitters on a subpopulation of these cells, is responsible for the fact that some of these cells respond to neurotransmitter active drugs.[92] From a practical point of view, results of medical treatment with neurotransmitter active drugs have been disappointing. Responsiveness is observed in a minority of patients and may be partial or temporary. At present drugs aiming to reduce pituitary ACTH secretion have no place in the medical treatment of Cushing's disease.

References

1. Alexander JM, Biller BMK, Bikkai H, *et al.* Clinically nonfunctioning pituitary tumors are monoclonal in origin. *J Clin Invest* 1990; **86**: 336–40.
2. Herman V, Fagin J, Gonsky R, *et al.* Clonal origin of pituitary adenomas. *J Clin Endocrinol Metab* 1990; **71**: 1427–33.
3. Landis CA, Masters SB, Spada A, *et al.* GTPase inhibiting mutations activate the α-chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature* 1989; **340**: 692–6.
4. Landis CA, Harsh G, Lyons J, *et al.* Clinical characteristics of acromegalic patients whose pituitary tumors contain mutant Gs protein. *J Clin Endocrinol Metab* 1990; **71**: 1416–20.
5. Spada A, Arosio M, Bochicchio D, *et al.* Clinical, biochemical and morphological correlates in patients bearing growth hormone-secreting pituitary tumors with or without constitutively active adenylyl cyclase. *J Clin Endocrinol Metab* 1990; **71**: 1421–6.
6. Klibanski A. Editorial: further evidence for a somatic mutation theory in the pathogenesis of human pituitary tumors. *J Clin Endocrinol Metab* 1990; **71**: 1415A–C.
7. Hartog M, Hull MGR. Hyperprolactinomia. Common and treatable. *Br Med J* 1988; **297**: 701–2.
8. Vance ML, Evans WS, Thorner MO. Diagnosis and treatment. Drugs five years later. Bromocriptine. *Ann Int Med* 1984; **100**: 78–91.
9. Miyai K, Ichihara K, Kondo K, *et al.* Asymptomatic hyperprolactinaemia and prolactinoma in the general population. Mass screening by paired assays of serum prolactin. *Clin Endocrinol* 1986; **25**: 549–54.
10. Koppelman MCS, Jaffe MJ, Rieth KG, *et al.* Hyperprolactinaemia, amenorrhoea and galactorrhoea, a retrospective assessment of twenty-five cases. *Ann Intern Med* 1984; **100**: 115–121.

11. Schlechte J, Dolan K, Sherman B, *et al.* The natural history of untreated hyperprolactinemia: a prospective analysis. *J Clin Endocrinol Metab* 1989; **68**: 412–8.
12. Leading article. Hyperprolactinaemia: when is a prolactinoma not a prolactinoma? *Lancet* 1987; **ii**: 1002–4.
13. Schlechte JA, Sherman BM, Chapler FK, *et al.* Long-term follow-up of women with surgically treated prolactin-secreting pituitary tumors. *J Clin Endocrinol Metab* 1986; **62**: 1296–301.
14. Lamberts SWJ, Validation of the role of bromocriptine in the primary treatment of prolactinomas. *Neth J Med* 1988; **32**: 107–11.
15. Cunnah D, Besser M. Management of prolactinomas. *Clin Endocrinol* 1991; **34**: 231–5.
16. Moriondo P, Travaglini P, Nissim M, *et al.* Bromocriptine treatment of microprolactinomas: evidence of stable prolactin decrease after drug withdrawal. *J Clin Endocrinol Metab* 1985; **60**: 764–72.
17. Pellegrini I, Rasolonjanahary R, Gunz G, *et al.* Resistance to bromocriptine in prolactinomas. *J Clin Endocrinol Metab* 1989; **69**: 500–9.
18. Scotti G, Scialfa G, Pieralli S, *et al.* Macroprolactinomas: CT evaluation of reduction of tumor size after medical treatment. *Neuroradiology* 1982; **23**: 123–6.
19. Wass JAH, Williams J, Charlesworth M, *et al.* Bromocriptine in management of large pituitary tumours. *Br Med J* 1982; **284**: 1908–11.
20. Molitch ME, Elton RL, Blackwell RE, *et al.* Bromocriptine as a primary therapy for prolactin-secreting macroadenomas: results of a prospective multicenter study. *J Clin Endocrinol Metab* 1985; **60**: 698–705.
21. Bevan JS, Webster J, Burke CW, *et al.* Dopamine agonists and pituitary tumor shrinkage. *Endocr Rev* 1992; **13**: 220–40.
22. Verlaat JW van't, Croughs RJM, Hendriks MJ, *et al.* Results of primary treatment with bromocriptine of prolactinomas with extrasellar extension. *Can J Neurol Sci* 1990; **17**: 71–3.
23. Verlaat JW van't, Croughs RJM, Hendriks MJ, *et al.* Bromocriptine treatment of prolactin secreting macroadenomas: a radiological, ophthalmological and endocrinological study. *Acta Endocrinol* 1986; **112**: 487–93.
24. Hendriks MJ. Computed tomography of the pituitary gland. An anatomical and pathological study. *Thesis.* Utrecht 1988.
25. Leading article. Reversible hypopituitarism. *Lancet* 1991; **337**: 276.
26. Faglia G. Should dopamine agonist treatment for prolactinomas be life-long? *Clin Endocrinol* 1991; **34**: 173–4.
27. Gen M, Uozumi T, Ohta M, *et al.* Necrotic changes in prolactinomas after long-term administration of bromocriptine. *J Clin Endocrinol Metab* 1984; **59**: 463–70.
28. Johnston DG, Hall K, Kendall-Taylor P, *et al.* Effect of dopamine agonist withdrawal after long-term therapy in prolactinomas. Studies with high-definition computerised tomography. *Lancet* 1984; **ii**: 187–92.
29. Winkelmann W, Allolio B, Heesen D, *et al.* Impaired regulation of prolactin in patients with persisting normoprolactinaemia after withdrawal of long-term therapy with dopamine agonists. *Adv Biosci* 1988; **69**: 321–4.
30. Verlaat JW van't, Croughs RJM. Withdrawal of bromocriptine after long-term therapy for macroprolactinomas; effect on plasma prolactin and tumour size. *Clin Endocrinol* 1991; **34**: 175–8.
31. Verlaat JW van't, Lancranjan I, Hendriks MJ, *et al.* Primary treatment of macroprolactinomas with Parlodel LAR®. *Acta Endocrinol* 1988; **119**: 51–5.
32. Ferrari C, Mattei A, Melis GB, *et al.* Cabergoline: long-acting oral treatment of hyperprolactinemic disorders. *J Clin Endocrinol Metab* 1989; **68**: 1201–6.
33. Homburg R, West C, Brownell J, *et al.* A double blind study comparing a new non-ergot, long-acting dopamine agonist, CV 205-502, with bromocriptine in women with hyperprolactinaemia. *Clin Endocrinol* 1990; **32**: 565–71.
34. Verlaat JW van't, Croughs RJM, Brownell J. Treatment of macroprolactinomas with a new non-ergot, long-acting dopaminergic drug, CV 205-502. *Clin Endocrinol* 1990; **33**: 619–24.
35. Brue T, Pellegrini I, Gunz G, *et al.* Effects of the dopamine agonist CV 205-502 in human prolactinomas resistant to bromocriptine. *J Clin Endocrinol Metab* 1992; **74**: 577–84.
36. Luzzi A, Dallabonzana D, Oppizzi G, *et al.* Low doses of dopamine agonists in the long-term treatment of macroprolactinomas. *New Eng J Med* 1985; **313**: 656–9.
37. Bengtsson BA, Eden S, Ernest I, *et al.* Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. *Acta Med Scand* 1988; **223**: 327–35.
38. Klein I, Parveen G, Gavaler JS, *et al.* Colonic polyps in patients with acromegaly. *Ann Intern Med* 1982; **97**: 27–30.
39. Abboud CF, Laws ER. Diagnosis of pituitary tumors. In: Young FF, Klee GG, eds. *Diagnostic evaluation of endocrine disorders I. Endocrinol Metab Clin N Am* 1988; **17**: 241–80.
40. Frohman L. Clinical Review 22. Therapeutic options in acromegaly. *J Clin Endocrinol Metab* 1991; **72**: 1175–81.
41. Laws ER, Carpenter SM, Scheithauer BW, *et al.* Long-term results of trans-spenoidal surgery for the management of acromegaly. In: Robbins R, Melmed S, eds. *Acromegaly: a century of scientific and clinical progress.* New York: Plenum Press 1987: 241–8.
42. Losa M, Oeckler R, Schopohl J, *et al.* Evaluation of selective transsphenoidal adenomectomy by endocrinological testing and somatomedin-C measurement in acromegaly. *J Neurosurg* 1989; **70**: 561–7.
43. Serri O, Somma M, Comtois R, *et al.* Acromegaly: biochemical assessment of cure after long-term follow-up of transsphenoidal selective adenomectomy. *J Clin Endocrinol Metab* 1985; **61**: 1185–89.
44. Littley MD, Shalet SM, Swindell R, *et al.* Low-dose pituitary irradiation for acromegaly. *Clin Endocrinol* 1990; **32**: 261–70.
45. Kliman B, Kjellberg RN, Swisher B, *et al.* Long-term effects of proton beam therapy for acromegaly. In: Robbins RJ, Melmed S, eds. *Acromegaly: a century of scientific and clinical progress.* New York: Plenum Press 1987: 221–8.
46. Barkan AL. Acromegaly, diagnosis and therapy. *Endocrinol Metab Clin* 1989; **18**: 277–310.
47. Editorial, Octreoitide steaming ahead. *Lancet* 1992; **339**: 837–9.
48. Lamberts SWJ. Somatostatin analogs: their role in the treatment of growth hormone hypersecretion and excessive body growth. *Growth Regulation* 1991; **1**: 3–10.
49. Vance ML, Harris AG. Long-term treatment of 189 acromegalic patients with the somatostatin analog octreotide. Results of the International Multicenter Acromegaly Study Group. *Arch Intern Med* 1991; **151**: 1573–8.
50. Barkan AL, Lloyd RV, Chandler WF, *et al.* Preoperative treatment of acromegaly with long-acting somatostatin

analog SMS 201-995: shrinkage of invasive pituitary macroadenomas and improved surgical remission rate. *J Clin Endocrinol Metab* 1988; **67**: 1040–8.
51. Charest L, Comtois R, Beauregard H, *et al.* Growth hormone rebound after cessation of SMS 201-995 treatment in acromegaly. *Can J Neurol Sci* 1989; **16**: 442–5.
52. Daughaday WH. Octreotide is effective in acromegaly but often results in cholelithiasis. *Ann Int Med* 1990; **112**: 159–60.
53. Jameson JL, Klibanski A, Black PM, *et al.* Glycoprotein hormone genes are expressed in clinically nonfunctioning pituitary adenomas. *J Clin Invest* 1987; **80**: 1472–8.
54. Asa SL, Gerrie BM, Singer W, *et al.* Gonadotropin secretion *in vitro* by human pituitary null cell adenomas and oncocytomas. *J Clin Endocrinol Metab* 1986; **62**: 1011–9.
55. Kwekkeboom DJ, de Jong FH, Lamberts SWJ. Gonadotropin release by clinically nonfunctioning and gonadotroph pituitary adenomas *in vivo* and *in vitro*: relation to sex and effects of thyrotropin-releasing hormone and bromocriptine. *J Clin Endocrinol Metab* 1989; **68**: 1128–35.
56. Yamada S, Asa SL, Kovacs K, *et al.* Analysis of hormone secretion by clinically nonfunctioning human pituitary adenomas using the reverse hemolytic plaque assay. *J Clin Endocrinol Metab* 1989; **68**: 73–80.
57. Asa SL, Cheng Z, Ramyar L, *et al.* Human pituitary null cell adenomas and oncocytomas *in vitro*: effects of adenohypophysiotropic hormones and gonadal steroids on hormone secretion and tumor cell morphology. *J Clin Endocrinol Metab* 1992; **74**: 1128–34.
58. Oppenheim DS, Kana AR, Sangha JS, *et al.* Prevalence of alpha-subunit hypersecretion in patients with pituitary tumors: clinically nonfunctioning and somatotroph adenomas. *J Clin Endocrinol Metab* 1990; **70**: 859–64.
59. Daneshdoost L, Gennarelli TA, Bashey HM, *et al.* Recognition of gonadotroph adenomas in women. *N Eng J Med* 1991; **324**: 589–94.
60. Comtois R, Beauregard H, Somma M, *et al.* The clinical and endocrine outcome to transsphenoidal microsurgery of nonsecreting pituitary adenomas. *Cancer* 1991; **68**: 860–6.
61. Klibanski A, Zervas NT. Diagnosis and management of hormone-secreting pituitary adenomas. *N Eng J Med* 1991; **324**: 822–31.
62. Bevan JS, Burke CW. Non-functioning pituitary adenomas do not regress during bromocriptine therapy but possess membrane-bound dopamine receptors which bind bromocriptine. *Clin Endocrinol* 1986; **25**: 561–72.
63. Kwekkeboom DJ, Hofland LJ, van Koetsveld PM, *et al.* Bromocriptine increasingly suppresses the *in vitro* gonadotropin and α-subunit release from pituitary adenomas during long-term culture. *J Clin Endocrinol Metab* 1990; **71**: 718–24.
64. Kwekkeboom DJ, Lamberts SWJ. Long-term treatment with the dopamine agonist CV 205-502 of patients with a clinically non-functioning, gonadotroph, or α-subunit secreting pituitary adenoma. *Clin Endocrinol* 1992; **36**: 171–6.
65. Ikuyama S, Nawata H, Kato K, *et al.* Specific somatostatin receptors on human pituitary adenoma cell membranes. *J Clin Endocrinol Metab* 1985; **61**: 666–71.
66. Vos P, Croughs RJM, Thijssen JHH, *et al.* Response of luteinizing hormone secreting pituitary adenoma to a long-acting somatostatin analogue. *Acta Endocrinol* 1988; **118**: 587–90.
67. Liuzzi A, Dallabonzana D, Oppizzi G, *et al.* Is there a real medical treatment for 'non secreting' pituitary adenomas? In: Faglia G, Beck Peccoz P, Ambrosi B, *et al.*, eds. *Pituitary adenomas: new trends in basic and clinical research. Proceedings of the 5th European workshop on pituitary adenomas.* Amsterdam: Excerpta Medica 1991: 383–90.
68. Faglia G, Bazzoni N, Spada A, *et al. In vivo* detection of somatostatin receptors in patients with functionless pituitary adenomas by means of a radioiodinated analog of somatostatin ([^{123}I]SDZ 204-090). *J Clin Endocrinol Metab* 1991; **73**: 850–6.
69. de Bruin TWA, Kwekkeboom DJ, van't Verlaat JW, *et al.* Clinically nonfunctioning pituitary adenoma and octreotide: response to long-term high dose treatment, and studies *in vitro*. *J Clin Endocrinol Metab* 1992; in press.
70. Oppenheim DS, Klibanski A. Medical therapy of glycoprotein hormone-secreting pituitary tumors. *Endocrinol Metab Clin N Am* 1989; **18**: 339–58.
71. Smallridge RC. Thyrotropin-secreting pituitary tumors. *Endocrinol Metab Clin N Am* 1987; **16**: 765–92.
72. Klibanski A, Zerras NF. Diagnosis and management of hormone-secreting pituitary adenomas. *New Eng J Med* 1991; **324**: 822–31.
73. Comi RJ, Gesundheit N, Murray L, *et al.* Response of thyrotropin-secreting pituitary adenomas to a long-acting somatostatin analogue. *New Eng J Med* 1987; **317**: 12–7.
74. Beck-Peccoz P, Mariotti S, Guillausseau, *et al.* Treatment of hyperthyroidism due to inappropriate secretion of thyrotropin with the somatostatin analog SMS 201-995. *J Clin Endocrinol Metab* 1989; **68**: 208–14.
75. Beckers A, Abs R, Mahler Ch, *et al.* Thyrotropin-secreting pituitary adenomas: report of seven cases. *J Clin Endocrinol Metab* 1991; **72**: 477–83.
76. Kay TB, Crapo L. The Cushing syndrome: an update on diagnostic tests. *Ann Int Med* 1990; **112**: 434–44.
77. Trainer PJ, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome. *Clin Endocrinol* 1991; **34**: 317–30.
78. Oldfield EH, Doppman JL, Nieman L, *et al.* Petrosal sinus sampling with and without corticotropin-releasing hormone for the differential diagnosis of Cushing's syndrome. *New Eng J Med* 1991; **325**: 897–905.
79. Findling JW, Kehoe ME, Shaker JL, *et al.* Routine inferior petrosal sinus sampling in the differential diagnosis of adrenocorticotropin (ACTH)-dependent Cushing's syndrome: early recognition of the ectopic ACTH syndrome. *J Clin Endocrinol Metab* 1991; **73**: 408–13.
80. Tabarin A, Greselle JF, San-Galli F, *et al.* Usefulness of the corticotropin-releasing hormone test during bilateral inferior petrosal sinus sampling for the diagnosis of Cushing's disease. *J Clin Endocrinol Metab* 1991; **73**: 53–9.
81. Orth DN. Corticotropin-releasing hormone in humans. *Endoc Rev* 1992; **13**: 164–91.
82. Croughs RJM, Docter R, de Jong FH. Comparison of oral and intravenous dexamethasone suppression tests in the differential diagnosis of Cushing's syndrome. *Acta Endocrinol* 1973; **72**: 54–62.
83. Biemond P, de Jong FH, Lamberts SWJ. Continuous dexamethasone infusion for seven hours in patients with the Cushing syndrome. A superior differential diagnostic test. *Ann Int Med* 1990; **112**: 738–42.
84. Atkinson AB. The treatment of Cushing's syndrome. *Clin Endocrinol* 1991; **34**: 507–13.
85. Guilhaume B, Bertagna X, Thomsen N, *et al.* Transsphenoidal pituitary surgery for the treatment of Cushing's

disease: results in 64 patients and longterm follow-up studies. *J Clin Endocrinol Metab* 1988; **66**: 1056–64.
86. Mampalam TJ, Tyrrell B, Wilson CB. Transsphenoidal microsurgery for Cushing's disease. A report of 216 cases. *Ann Int Med* 1988; **109**: 487–93.
87. Lüdecke DK, Heinrichs M, Saeger W. Recurrences after transnasal adenomectomy in Cushing's disease. In: Faglia G, Beck-Peccoz P, Ambrosi B, *et al.*, eds. *Pituitary adenomas: new trends in basic and clinical research. Proceedings of the 5th European workshop on pituitary adenomas.* Amsterdam: Excerpta Medica 1991: 337–48.
88. Krieger DT. Lack of responsiveness to L-dopa in Cushing's disease. *J Clin Endocrinol Metab* 1973; **36**: 277–84.
89. Ishibashi M, Yamaji T. Direct effects of thyrotropin releasing hormone, cyproheptadine and dopamine on adrenocorticotropin secretion from human corticotroph adenoma cells *in vitro*. *J Clin Invest* 1981; **68**: 1018–27.
90. Lamberts SWJ, de Lange SA, Stefanko SZ. Adrenocorticotropin-secreting pituitary adenomas originate from the anterior or the intermediate lobe in Cushing's disease. Differences in the regulation of hormone secretion. *J Clin Endocrinol Metab* 1982; **54**: 286–91.
91. McNicol AM, Teasdale GM, Beastall GH. A study of corticotroph adenomas in Cushing's disease: no evidence if intermediate lobe origin. *Clin Endocrinol* 1986; **24**: 715–22.
92. Croughs RJM, Koppeschaar HPF, van't Verlaat JW, *et al.* Bromocriptine-responsive Cushing's disease associated with anterior pituitary corticotroph hyperplasia or normal pituitary gland. *J Clin Endocrinol Metab* 1989; **68**: 495–8.
93. Coates PJ, Doniach I, Wells C, *et al.* Peptides related to alpha-melanocyte-stimulating hormone are commonly produced by human pituitary corticotroph adenomas: no relationship with pars intermedia origin. *J Endocrinol* 1989; **120**: 531–6.
94. Koppeschaar HPF, Croughs RJM, Thijssen JHH, *et al.* Response to neurotransmitter modulating drugs in patients with Cushing's disease. *Clin Endocrinol* 1986; **25**: 661–7.
95. Croughs RJM, Thijssen JHH, Mol JA. Absence of detectable immunoreactive alpha melanocyte stimulating hormone in plasma of various types of Cushing's disease. *J Endocrinol Invest* 1991; **14**: 197–200.

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