Cushing's disease: a comparison of pituitary corticotroph microadenomas and macroadenomas

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Abstract

Cushing's disease appears as a functionally heterogeneous disease, but criteria that are able to distinguish between different clinical forms remain elusive. We compared two subgroups of patients with proven Cushing's disease according to the size of the pituitary adenoma, evaluated by computed tomography or magnetic resonance imaging. Our series comprised 11 patients with a microadenoma and 10 with a macroadenoma (median volumes (range): 173 (13–270) and 3022 (500–10312) mm³ respectively; $P < 0.0001$). The clinical presentation was similar in the two groups, but the time elapsed before diagnosis was longer, and visual impairment was less frequent in the patients with a microadenoma (1.5 ± 0.8 years and 0%) than in those with a macroadenoma (0.7 ± 0.6 years and 40%; $P < 0.05$). Morning and evening peripheral concentrations of ACTH were greater in patients with macroadenoma (134 ± 78 and 130 ± 7 ng/l respectively) than in those with microadenoma (52 ± 28 and 56 ± 19 ng/l, $P < 0.05$). Hypokalaemia and lymphopenia were also more pronounced in patients with macroadenoma (3.4 ± 0.3 mmol/l and 1273 ± 401 lymphocytes/mm³) than in those with microadenoma (3.8 ± 0.3 mmol/l and 1852 ± 668 lymphocytes/mm³; $P < 0.05$), although morning and evening plasma cortisol concentrations were similar in both groups. In patients with macroadenoma, there was less relative nycthemeral variation of ACTH concentrations ($28 \pm 24\%$, compared with $62 \pm 39\%$ in those with microadenoma; $P < 0.05$), less suppression of plasma cortisol by high doses of dexamethasone ($-30 \pm 14\%$, compared with $-61 \pm 25\%$; $P < 0.05$), and a reduced concentration ratio of mean basal cortisol to ACTH (7 ± 3 , compared with 12 ± 5 ; $P < 0.05$). Plasma IGF-I concentration and the TSH peak response to TRH were significantly lower in patients with macroadenoma than in those with microadenoma ($0.4 \pm 0.2 \times 10^3$ IU/l and 2.3 ± 1.8 mIU/l, compared with $1.8 \pm 0.6 \times 10^3$ IU/l and 5.2 ± 1.6 mIU/l; $P < 0.05$). Thus, in comparison with microadenomas, corticotroph macroadenomas are characterized by a greater and more autonomous ACTH secretion, inducing more pronounced biological signs of hypercorticism, and are more often accompanied by visual field defects and impairment of other pituitary hormonal secretions.

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Introduction

Cushing's disease, or endogenous pituitary adrenocorticotrophic hormone (ACTH)-dependent hypercorticism, appears as a heterogeneous disease, and its diagnosis is often difficult (1). Efforts have been made to distinguish functionally or clinically relevant subgroups of Cushing's disease. The disease is of clonal origin in the majority of cases, and subtypes could result from the heterogeneity of normal corticotroph cells (2, 3). Classifications have been proposed on the basis of histological (4), radiological (5), biochemical (6) or functional criteria (7, 8), but none has so far been widely accepted.

A simple characteristic, the size of the pituitary adenoma, is widely used in the field of prolactinomas (9–11), but has not been considered for classifying

corticotroph adenomas. This probably reflects the rather low prevalence of corticotroph macroadenomas reported in the literature, although they account for 20% of Cushing's diseases (12). In this study, we have compared the clinical and biological characteristics of patients with corticotroph micro- and macroadenomas recently diagnosed in our institution.

Materials and methods

Patients

We studied 10 patients suffering from Cushing's disease caused by a pituitary macroadenoma who were referred to our endocrinological centres between 1990 and 1996, and compared them with 11 patients with a

microadenoma observed during the same period. Seven other patients were not included in the analysis (one with malignant Cushing's disease, two with possible hypothalamic Cushing's disease, one with a combination of Cushing's disease with autonomous macronodular adrenal hyperplasia, and three because of insufficient case documentation). No silent corticotroph adenoma was included in the series. Hypercorticism was suspected on the basis of the medical history and physical examination, and confirmed by increased free cortisol excretion and failure to suppress plasma cortisol normally after administration of a low dose of dexamethasone (2 mg/day for 2 days). The pituitary origin of the hypercorticism was established on the basis of normal/high ACTH concentrations, adequate suppression of urinary 17-hydroxycorticosteroids by high-dose dexamethasone administration (8 mg/day for 2 days), or a high petrosal sinus to peripheral plasma ACTH gradient (1). All patients underwent pituitary surgery and Cushing's disease was confirmed either by histopathological examination or by favourable response to surgery. In these patients, there was no reason, either at initial evaluation or during follow-up, to suspect an ectopic ACTH syndrome. All the relevant data were collected before any analysis was performed.

Measurements of adenoma diameters were obtained by radiological examination of the pituitary (computed tomography or magnetic resonance imaging). Tumours in which the largest diameter was at least 10 mm were considered to be macroadenomas. Tumour volume was estimated by assuming the volume of a sphere: $4/3 \pi r^3$. Median (extreme) values were (173 (13–270) mm³ for microadenomas and 3022 (500–10 312) mm³ for macroadenomas ($P < 0.0001$).

Endocrine testing was performed according to standardized clinical procedures after informed consent had been given by the patient. The dexamethasone suppression test consisted of the oral administration of 0.5 mg (low-dose) or 2 mg (high-dose) dexamethasone every 6 h for 2 days. In the thyrotrophin-releasing hormone (TRH) stimulation test, 200 µg TRH (UCB, Brussels, Belgium) was administered i.v., and in the gonadotrophin-releasing

hormone (GnRH) stimulation test 100 µg GnRH (Wyeth, Louvain-la-Neuve, Belgium) was administered i.v. A corticotrophin-releasing hormone (CRF) stimulation test was performed during petrosal sinus sampling in 15 patients and consisted of i.v. administration of 100 µg ovine CRF (Bachem, Bubendorf, Switzerland).

Hormone assays

Plasma ACTH was measured by an immunoradiometric assay using two (N- and C-terminal) specific antibodies for human ACTH(1–39) (Nichols Institute, San Juan, CA, USA), so that falsely high values caused by the presence of high molecular weight precursors were unlikely. The sensitivity of this assay is 1 ng/l, the intra- and interassay coefficients of variation are 3 and 8% respectively, and it has been demonstrated to be free of any interaction with ACTH(11–24), ACTH(1–10), α - or β -melanocyte-stimulating hormone, β -lipotrophin or β -endorphin, and to display only minor interaction with ACTH(18–39). Plasma cortisol was measured by a specific radioimmunoassay (Cortisol assay kit, ICN Biomedicals, Asse, Belgium). The same assay was used for cortisoluria, after samples had been extracted and purified by high performance liquid chromatography. Other biological parameters were measured by classical methods.

Statistics

Results are presented as means $\pm$ s.d. Statistical analysis used Student's *t*-test whenever possible. Non-parametric variables were either log-transformed before the statistical analysis or submitted to the non-parametric Mann–Whitney test. Proportions were compared by Fisher's exact test. Relationships between parameters were assessed by the non-parametric Spearman's test (r_s). Values of $P < 0.05$ were assumed to be significant.

Results

The patients did not differ significantly in sex or weight (although the known predominance of microadenomas

Table 1 Clinical characteristics of patients with microadenoma or macroadenoma. Values are means $\pm$ s.d.

	Microadenoma	Macroadenoma
Age (years)	44 $\pm$ 15	55 $\pm$ 13
Sex (male/female)	1/10	4/6
Weight (kg)	73 $\pm$ 13	74 $\pm$ 14
Body mass index (kg/m ²)	25.4 $\pm$ 1.2	27.3 $\pm$ 6
Headache (%)	27	20
Mood disturbances (%)	36	40
Muscle weakness (%)	64	70
Systolic blood pressure (mmHg)	156 $\pm$ 15	166 $\pm$ 28
Diastolic blood pressure (mmHg)	100 $\pm$ 13	95 $\pm$ 15
Bruising (%)	73	50

Table 2 Main biochemical parameters in patients with a corticotroph micro- or macroadenoma. Values are ranges or means $\pm$ S.D.

	Normal range	Microadenoma		Macroadenoma	
		Value	<i>n</i>	Value	<i>n</i>
Potassium (mmol/l)	3.5–5	3.8 $\pm$ 0.3	10	3.4 $\pm$ 0.3*	10
Proteins (g/l)	65–80	70 $\pm$ 7	8	59 $\pm$ 4**	6
Albumin (g/l)	35–50	44 $\pm$ 4	7	35 $\pm$ 4**	5
LDH (IU/l)	100–340	437 $\pm$ 121	10	469 $\pm$ 118	10
CPK (IU/l)	10–170	114 $\pm$ 64	10	55 $\pm$ 26*	10
Haemoglobin (g/l)	120–170	147 $\pm$ 13	10	135 $\pm$ 11*	10
Neutrophils ($10^3/\text{mm}^3$)	1.6–7	5.1 $\pm$ 1.6	10	6.5 $\pm$ 2.2	10
Lymphocytes ($10^3/\text{mm}^3$)	0.8–5	1.8 $\pm$ 0.67	10	1.3 $\pm$ 0.40*	10

n, Number of patients; LDH, lactate dehydrogenase activity; CPK, creatine phosphokinase activity.

* $P < 0.05$, ** $P < 0.01$ compared with microadenoma group.

among women was less apparent for macroadenomas), and the clinical presentation of patients with either a micro- or a macroadenoma was also similar in terms of body mass index, headache, mood disturbances, bruising, muscular weakness or blood pressure (Table 1). Patients with a microadenoma tended to be younger (44 ± 15 years) than those with macroadenoma (55 ± 13 years; NS) and the diagnosis of their Cushing's disease was more delayed (time from reported onset of symptoms to diagnosis 1.5 ± 0.8 years compared with 0.7 ± 0.6 years; $P < 0.05$). Visual field defect was present in 40% of patients with macroadenomas, but none of those with microadenoma ($P = 0.05$) and electromyography tended more often to show a myogenous pattern in those with macroadenomas (67%, compared with 28% in patients with microadenoma; NS). There was no difference in the prevalence of osteoporosis estimated by densitometry or radiological evaluation. Biological signs of hypercorticism (hypokalaemia and lymphopenia) were significantly more pronounced in patients with a large tumour (Table 2). There was also a non-significant trend toward more pronounced eosinopenia and hyperglycaemia (data not shown). Biological parameters indicative of the general state of health, such as haemoglobinaemia, total proteinaemia, albuminaemia and calcaemia,

were also more significantly altered in patients with a macroadenoma.

Basal thyroid function was normal, except for a low-level tri-iodothyronine syndrome observed in patients with a macroadenoma (Table 3). The peak TSH-response to TRH was significantly lower in patients with a macroadenoma, and inversely correlated with the size ($\log \text{mm}^3$) of the adenoma ($r_s = -0.64$, $\log \text{mm}^3$ against mIU/l; $P < 0.05$). The plasma concentration of insulin-like growth factor-I was also significantly lower in the patients with macroadenoma (Table 3), and negatively correlated with adenoma size ($r_s = -0.75$, $\log \text{mm}^3$ against IU/l; $P < 0.05$). Basal concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were not significantly different between the two groups of patients stratified for age and sex, and the LH and FSH peak responses to GnRH were not different between the groups (data not shown). There was no difference in basal or TRH-stimulated concentrations of prolactin between the two groups of patients (data not shown).

Plasma ACTH concentrations, measured in the morning and in the evening, were significantly greater in those with macroadenoma (Fig. 1A, B). Twenty-four-hour cortisoluria and excretion of 17-hydroxycorticosteroids followed the same trend, although the difference did not

Table 3 Main hormonal values in patients with a corticotroph micro- or macroadenoma. Values are ranges or means $\pm$ S.D.

	Normal range	Microadenoma		Macroadenoma	
		Value	<i>n</i>	Value	<i>n</i>
Basal TSH (mIU/l)	0.2–3.5	1.3 $\pm$ 0.1	10	0.9 $\pm$ 0.9	9
Stimulated TSH (mIU/l)	2.5–30	5.2 $\pm$ 1.6	3	2.3 $\pm$ 1.8**	7
Total T_4 (nmol/l)	58–161	92 $\pm$ 19	8	75 $\pm$ 28	9
Total T_3 (nmol/l)	1.38–3.08	1.43 $\pm$ 0.29	8	0.98 $\pm$ 0.42**	9
IGF-I (IU/l)	300–2000	1800 $\pm$ 600	5	400 $\pm$ 200**	5

T_4 , thyroxine; T_3 , tri-iodothyronine; IGF, insulin-like growth factor.

** $P < 0.01$ compared with microadenoma group.

Figure 1 Plasma concentrations of ACTH in patients with Cushing's disease cause by micro- (μ A) or macroadenoma (MA), at 0800 h (A; $P < 0.01$ between groups) or at 2000 h (B; $P < 0.05$ between groups), and relative daily variations in plasma concentrations of ACTH (defined as maximal ACTH minus minimal ACTH, divided by mean ACTH) (C; $P < 0.05$ between groups). D: Linear relationship between the logarithm of tumour volume (estimated from computed tomography/magnetic resonance imaging measurements) and mean plasma concentration of ACTH ($r_s = 0.66$, $P < 0.01$). Individual values ($\bullet$) and means (continuous lines) are shown. The broken lines in (D) represent confidence intervals.

Table 4 Parameters of the ACTH-adrenal axis in basal conditions and during endocrine testing. Values are means $\pm$ s.d.

	Normal values	Microadenoma		Macroadenoma	
		Value	n	Value	n
Morning cortisol (nmol/l)	400 $\pm$ 140	600 $\pm$ 206	11	670 $\pm$ 189	10
Evening cortisol (nmol/l)	160 $\pm$ 80	587 $\pm$ 188	11	697 $\pm$ 264	10
Urinary 17-OHC (mg/24 h)					
Men	17 $\pm$ 4	26 $\pm$ 15	10	34 $\pm$ 23	8
Women	12 $\pm$ 4	26 $\pm$ 15	10	34 $\pm$ 23	8
Urinary cortisol (μ g/24 h)	≤ 40	399 $\pm$ 334	11	465 $\pm$ 402	10
Basal ACTH, dominant petrosal sinus (ng/l)	—	269 $\pm$ 164	10	1610 $\pm$ 1702	6
Stimulated ACTH, dominant petrosal sinus (ng/l)	—	2558 $\pm$ 2454	9	7370 $\pm$ 4593**	6
Peripheral ACTH response to CRF (%)	—	283 $\pm$ 233	9	261 $\pm$ 342	6
Plasma cortisol change after HDDST (%)	—	-61 $\pm$ 35	9	-30 $\pm$ 14*	5
17-OHC change after HDDST (%)	—	-43 $\pm$ 27	9	-24 $\pm$ 27	5
Cortisoluria change after HDDST (%)	—	-48 $\pm$ 27	9	-74 $\pm$ 20	5

n, Number of patients; 17-OHC, urinary 17-hydroxycorticosteroids; HDDST, high-dose dexamethasone suppression test.

* $P < 0.05$, ** $P < 0.01$ compared with microadenoma group.

reach significance, and there was no difference in plasma cortisol concentrations in the two groups of patients (Table 4). A relationship was demonstrated between the size of the adenoma and mean plasma ACTH concentration ($r_s = 0.66$, $P < 0.005$; Fig. 1D). The relative daily variations of plasma concentrations of ACTH (defined as maximal ACTH minus minimal ACTH, divided by mean ACTH) was significantly blunted in patients with a macroadenoma ($P < 0.05$, Fig. 1C), as was the ratio of mean cortisol to mean ACTH concentrations (12 ± 5 in those with microadenoma and 7 ± 3 in those with macroadenoma; $P < 0.01$). This ratio, which is an indirect index of endogenous glucocorticoid feed-back, was inversely correlated to the size of the adenoma ($r_s = -0.55$, $\log \text{mm}^3$ against ratio; $P < 0.05$).

In patients with a macroadenoma, petrosal sinus sampling revealed a larger ACTH response to CRF in the dominant sinus compared with that in patients with microadenoma, but this was not so in the peripheral plasma (Table 4). Basal and stimulated central/peripheral ACTH gradients were always superior to 2 and 3, respectively, indicating the pituitary origin of hypercorticism. The two gradients were not significantly different between the two groups (basal gradients 11 ± 8 and 9 ± 6 , stimulated gradients 49 ± 50 and 74 ± 93 in nine patients with microadenoma and six with macroadenoma respectively; NS).

High doses of dexamethasone suppressed the plasma cortisol concentrations significantly less in patients with large tumours (Table 4). A plasma cortisol suppression greater than 50% was observed in six of nine of the patients with a microadenoma, but in none of those with a macroadenoma ($P < 0.05$). There was no significant difference between the groups, however, when the suppression of urinary 17-hydroxycorticosteroids or cortisoluria by high doses of dexamethasone were compared (Table 4): urinary 17-hydroxycorticosteroid suppression greater than 50% was observed in five of nine patients with a microadenoma and one of five with a macroadenoma. A cortisoluria suppression greater than 50% was observed in five of nine patients with a microadenoma and in three of five patients with a macroadenoma.

Discussion

Our results demonstrate that corticotroph micro- and macroadenomas differ significantly on functional grounds. The distinction between the two forms of adenoma has been used widely for many years in relation to lactotroph adenomas. It is useful in the diagnosis of prolactinoma, in which the concentration of prolactin is related to the size of the adenoma (9). Furthermore, the responses of micro- and macroprolactinomas to dopaminergic agonists differ (11), as does the *in vitro* reactivity of cells obtained from surgical specimens from micro- and macroprolactinomas (10).

In Cushing's disease, however, no relationship between the size of the adenoma, the severity of hypercorticism and the clinical presentation has previously been described in man (13), although such relationships have been reported in dogs (14, 15).

We have now shown here that ACTH concentrations are greater in patients with a corticotroph macroadenoma than in those with a microadenoma. Corticotroph macroadenomas have previously been considered to be associated with a more indolent disease (13), although to date this has not been systematically investigated, but other data from six previously published series (5, 6, 16–19) confirm our present observations (Table 5). Our results also suggest that corticotroph macroadenomas are less responsive to suppression by high-dose dexamethasone. Similar conclusions may be drawn from human data reported by Burke & Beardwell (20), who used the high-dose dexamethasone suppression test, and from animal data reported by Kooistra *et al.* (21). In contrast, Lamberts *et al.* (5) found a greater suppression of cortisol after 1 mg dexamethasone in patients with radiologically demonstrated lesions of the sella turcica (admittedly, an indirect index of macroadenoma) than in patients with a radiologically intact sella. This last observation is difficult to reconcile with our results, although the tests are somewhat different. We believe that additional arguments favouring a more autonomous secretion of ACTH by macroadenomas can be derived from the lower daily variation in ACTH plasma concentrations and the higher ratio of mean plasma cortisol to mean plasma ACTH that we observed in patients with a large tumour. Finally, new data have recently been reported from a small series of large pituitary corticotroph adenomas by Ikeda *et al.* (22). The authors observed greater plasma concentrations of ACTH, absent circadian cortisol rhythm and blunted ACTH/cortisol responses to CRF and dexamethasone tests in large corticotroph tumours. Taken together, these data are consistent with our own observations (greater ACTH concentrations, reduced circadian rhythm of ACTH and blunted response to dexamethasone).

Table 5 Plasma ACTH concentrations reported in the literature in patients with corticotroph microadenoma or macroadenoma. Values are means $\pm$ s.d., or median (extreme values).

Reference	Microadenoma		Macroadenoma	
	ACTH (ng/l)	<i>n</i>	ACTH (ng/l)	<i>n</i>
5	52 ± 14	13	73 ± 6	9
16	55 ± 37	5	336 ± 295	3
17*	52 ± 48	57	89 ± 12	9
18	9 ± 8	16	16 ± 10	4
19	86 ± 69	20	158 ± 152	2
6	7 (2–25)	27	23 (10–66)	6

*Values extrapolated from a figure.

suppression test). Furthermore, Ikeda *et al.* (22) had the opportunity to perform a histopathological study that demonstrated a different ultrastructure of the secretion apparatus in corticotrophs from large tumours. This is an additional argument in favour of the concept of intrinsic differences between corticotroph micro- and macroadenomas.

We can only speculate on the mechanisms whereby macroadenomas might secrete ACTH more autonomously. Gibson *et al.* (6) proposed that macroadenomas preferentially secrete biologically inactive precursors of ACTH that could interfere with ACTH measurements and the secretion of which is not regulated like that of ACTH. Our results do not support this hypothesis. First, our assay, using two specific antibodies against the two end-residues of mature ACTH, is less likely to give falsely increased measurements in the presence of a high molecular weight precursor. Second, our patients with a macroadenoma exhibited more pronounced biological signs of hypercorticism, such as hypokalaemia and lymphopenia, thus adding weight to the hypothesis of a larger secretion of bioactive ACTH in these patients. Another hypothesis is that the microenvironment of the tumour (hypoxia, accessibility to regulating agents, exposure to auto/paracrine factors, etc.) is different in small and large adenomas. In prolactinomas, however, it has been shown that the *in vitro* reactivity of cells obtained from micro- and macroadenomas differed, suggesting a more intrinsic cellular defect (10). It is possible that different mutations are responsible for the clonal diseases leading to the development of small or large tumours, or that a similar mutation affects corticotroph cells with different potentialities (3). Kooistra *et al.* (21) recently reported that, in dogs with pituitary-dependent hypercorticism, there was an inverse relationship between glucocorticoid feedback and the size of the pituitary gland. These authors hypothesized that various mutations were responsible for the escape of the adenoma from glucocorticoid feedback, and for various patterns of tumour growth.

From a clinical standpoint, the presentation of patients with micro- and macroadenomas was not strikingly different. Patients with a macroadenoma were somewhat older, however, and the diagnosis was made more rapidly in their case, probably as a consequence of the more pronounced biological consequences of the hypercorticism. This more prominent biological alteration was also reflected in lower proteinaemia and albuminaemia, which are non-specific but suggestive features of general health impairment. As expected, those manifestations that were related mainly to a mass effect of the tumour, such as visual field defects or impairment of other pituitary secretions, were present only in patients with macroadenoma. In the series of patients studied by Ikeda *et al.* (22), there was a non-significant trend toward more frequent myopathy, obesity and mental disturbances, but lower blood pressure, in those with a macroadenoma.

Corticotroph macroadenomas have been under-reported in the endocrinological literature. Their true frequency can be estimated to be about 20% of all Cushing's diseases (12, 23). In this series, we observed a similar number of patients with a micro or a macroadenoma. This might be explained by the particular referral of those with larger tumours to our specialized centres, by the exclusion from the study of patients with hypercorticism of uncertain origin, which are more likely to include small pituitary adenomas, and by the relatively limited number of patients considered.

In conclusion, corticotroph microadenomas and macroadenomas differ in terms of the importance and the degree of autonomy displayed by their ACTH secretion, leading to more pronounced signs of hypercorticism in patients with a macroadenoma. The two types of corticotroph tumours certainly deserve to be considered independently when the normal values are being established in dynamic tests, in physiological or pathological studies, or in the search for the causative mutation(s) leading to Cushing's disease.

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