Subclinical Cushing's Syndrome in Patients with Adrenal Incidentaloma: Clinical and Biochemical Features

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ABSTRACT

Incidentally discovered adrenal masses are mostly benign, asymptomatic lesions, often arbitrarily considered as nonfunctioning tumors. Recent studies, however, have reported increasing evidence that subtle cortisol production and abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis are more frequent than previously thought. The purpose of this study was to investigate the clinical and hormonal features of patients with incidentally discovered adrenal adenomas, in relation to their clinical outcome. Fifty consecutive patients with incidentally detected adrenal adenomas, selected from a total of 65 cases of adrenal incidentalomas, were prospectively evaluated. All of them underwent abdominal computed tomography scan and hormonal assays of the HPA axis function: circadian rhythm of plasma cortisol and ACTH, urinary cortisol excretion, 17-hydroxyprogesterone, androgens, corticotropin stimulation test and low-dose (2 mg) dexamethasone test. The patients were reevaluated at regular intervals (6, 12, and 24 months) for a median period of 38 months. Subtle hypercortisolism, defined as abnormal response to at least 2 standard tests of the HPA axis function in the absence of clinical signs of Cushing's syndrome (CS), was defined as subclinical CS. Mild-to-severe hypertension was found in 24 of 50 (48%) patients, type-2 diabetes in 12 of 50 (24%), and glucose intolerance in 6 of 50 (12%) patients. Moreover, 18 of 50 patients (36%) were diffusely obese (body mass index, determined as weight/height^2, > 25), and 14 patients (28%) had serum lipid concentration abnormalities (cholesterol ≥ 6.21 mmol/L, low-density lipoprotein cholesterol ≥ 4.14 mmol/L and/or triglycerides ≥ 1.8 mmol/L). Compared with a healthy population, bone mineral density Z-score, determined by the DEXA technique, tended to be slightly (but not significantly) lower in patients with adrenal adenoma (−0.41 SD). Endocrine data were compared with 107 sex- and age-matched controls, and patients with adenomas were found to have heterogeneous hormonal abnormalities. In particular, significantly higher serum cortisol values ($P < 0.001$), lower ACTH concentration ($P < 0.05$), and impaired cortisol suppression by dexamethasone ($P < 0.001$) were observed. Moreover, in patients with adenomas, cortisol, 17-OH progesterone, and androstenedione responses to corticotropin were significantly increased ($P < 0.001$, all), whereas dehydroepiandrosterone sulfate levels were significantly lower at baseline, with blunted response to corticotropin ($P < 0.001$, both). However, the criteria for subclinical CS were met by 12 of 50 (24%) patients. Of these, 6 (50%) were diffusely obese, 11 (91.6%) had mild-to-severe hypertension, 5 (41.6%) had type-2 diabetes mellitus, and 6 (50%) had abnormal serum lipids. The clinical and hormonal features improved in all patients treated by adrenalectomy, but seemed unchanged in all those who did not undergo surgery (follow-up, 9 to 73 months), except for one, who was previously found as having nonfunctioning adenoma and then revealed to have subclinical CS. In conclusion, an unexpectedly high prevalence of subtle autonomous cortisol secretion, associated with high occurrence of hypertension, diabetes mellitus, elevated lipids, and diffuse obesity, was found in incidentally discovered adrenal adenomas. Although the pathological entity of a subclinical hypercortisolism state remained mostly stable in time during follow-up, hypertension, metabolic disorders, and hormonal abnormalities improved in all patients treated by adrenalectomy. These findings support the hypothesis that clinically silent hypercortisolism is probably not completely asymptomatic. (*J Clin Endocrinol Metab* **85:** 1440–1448, 2000)

THE INTRODUCTION of diagnostic tools [such as ultrasound and computed tomography (CT)] in the last 25 yr and, more recently, of magnetic resonance imaging has increased the frequency of detection of adrenal neoplasms (1, 2). This evidence is in accordance with data from autopsy series documenting microscopic or macroscopic adrenal nodules in 2–9% of patients who had never had signs of adrenal dysfunction (3, 4). These incidentally discovered adrenal masses, the so called incidentalomas, are mostly benign and asymptomatic (2, 4, 5) and are often considered as nonfunctional tumors. They could actually be nonfunctioning or

produce adrenocortical hormones in amounts insufficient to cause a clinically apparent disease.

Several years ago, Ross and Aron (6) suggested that endocrine screening procedures, including urinary cortisol measurement and dexamethasone (DXM) testing, were not justified in patients with incidentalomas, given the low probability of cortisol production in such tumors. However, since 1990, an increasing body of evidence has emerged showing that subtle cortisol production and subsequent abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis are more frequent than previously thought. In the early 1990s, several authors have reported abnormalities in the HPA axis in 5–6% of adrenal incidentalomas (7, 8); but since 1992, the occurrence reported has increased to 12–16% of patients with incidentalomas (9–13). This entity has been defined as subclinical or preclinical Cushing's syndrome (CS). "Subclinical CS" is likely a more adequate definition, because the rate of

progression to overt CS is unclear and probably is very low (10, 14).

The true prevalence of autonomous cortisol production by incidentally discovered adrenal tumors is unknown and likely is underestimated because of the infrequent use of careful endocrine evaluation of incidentalomas in the older studies. However, different criteria have been adopted to define autonomous cortisol secretion in more recent studies (14, 15). The lack of both standard endocrine tests and criteria for the definition of subclinical CS limits the possibility of a correct evaluation and management of these patients. Moreover, recent evidence has shown a growing prevalence of autonomous cortisol production, emphasizing the importance of careful endocrine evaluation in all patients with apparently nonfunctional adrenal tumors such as incidentalomas.

The purpose of the present study was to evaluate the presence of subclinical CS attributable to autonomous cortisol production in a group of consecutive patients with incidentally discovered adrenal adenomas and to follow the natural history of this pathological entity.

Subjects and Methods

Patients

From 1992 to 1998, 65 consecutive patients (41 women and 24 men; age range, 27–78 yr; mean, 54) with incidentally detected adrenal masses were prospectively evaluated. They were part of a group of 106 patients referred to the Department of Molecular and Clinical Endocrinology and Oncology of the "Federico II University", in Naples, for the evaluation of adrenal masses. All the incidentalomas were discovered by abdominal ultrasound or CT scan, performed for the evaluation of unrelated diseases, such as urinary tract infection, renal colic, or biliary colic, nonspecific abdominal pain, or during a common check-up. Patients with known extraadrenal malignancies or those with hypertension of possible endocrine origin (*i.e.* paroxysmal hypertension, hypertension resistant to treatment or associated with hypokaliemia) were excluded. None of the patients showed specific signs or symptoms of hormone production excess, nor were they on hormonal treatment. A group of 107 healthy subjects and patients with multinodular euthyroid goiter and TSH concentration within the normal range (0.8–3.2 mU/L) also entered the study as controls (65 women and 42 men; age range, 29–75 yr; mean, 52.2). All of them were normal at the physical examination, had a negative medical history, and were not taking any medication. The study was designed in accordance with the Declaration of Helsinki; all subjects and patients gave their informed consent to enter the study.

The 65 patients of the study group were all hospitalized. All standard blood analytes were determined by routine clinical laboratory methods. In patients who had previously undergone only abdominal ultrasound,

a CT scan was also performed. All CT scans were reviewed by the same radiologist. Fifty patients with adrenocortical adenoma were selected from those with adrenal incidentalomas. The selection was performed on the basis of an initial endocrine evaluation plus CT criteria suggesting the presence of a benign mass (16, 17), as follows: tumor size less than 3.5 cm with a round or oval shape, hypodense and homogeneous pattern with well-defined margins, and no or mild enhancement after iv contrast medium administration. Patients with pheochromocitoma (n = 5), ganglioneuroma (n = 2), carcinoma (n = 2), schwanoma (n = 1), cysts (n = 3), and asymptomatic metastasis of other tumors (n = 2) were excluded. The diagnosis of adenoma was then confirmed by histological findings in 19 patients. When the histological examination was not available, as in patients who had not undergone surgery, the diagnosis of adenoma was confirmed by follow-up data.

At entry, all patients underwent the following endocrine evaluation: baseline serum cortisol and plasma ACTH at 0800; 1600, and 2400 h (mean of at least two samples taken on different days); measurement of 24-h excretion of urinary free cortisol (UFF), serum basal 17-hydroxyprogesterone (17-OHP), Δ4-androstenedione, testosterone, and dehydroepiandrosterone sulfate (DHEAS); and a low-dose 2-mg DXM suppression test (orally, 0.5 mg, four times a day for 2 days, with measurement of serum cortisol and other steroids at 0800 h the following morning; UFF was also determined). In addition, 24-h urinary excretion of catecholamines and vanillylmandelic acid was determined, to exclude the presence of pheochromocytoma; and measurement of renin plasma activity and aldosterone in the upright posture was always performed to exclude an aldosterone-producing adenoma. Nevertheless, serum potassium values were normal in all patients in a salt-repleted state.

To better assess steroid biosynthesis, a long ACTH test was performed (250 μg of 1,24-ACTH; Synacthen, Ciba, Basel, Switzerland) in iv 5-h infusion, with blood sampling at time 0, 5, and 6 h for F, 17-OHP, Δ4-androstenedione, and DHEAS. In patients who failed to achieve serum cortisol suppression to below 3 μg/dL (83 nmol/L) after the administration of 2-mg DXM (9), a high-dose DXM test was performed (2 mg, four times a day for 2 days).

Two more parameters were considered for the assessment of F secretion rates in adenomas: the daily average cortisol, calculated as (F0800 + F1600 + F2400 h)/3; and the cortisol percent ratio (F% ratio), expressing F circadian rhythm abnormalities, calculated as (F 2400/F 0800) × 100.

To define the reference range for each variable determined, means ± 2 SD were calculated in the control group. The ACTH test was performed in 78 healthy subjects (41 women and 37 men; mean age, 49 yr), and means ± 3 SD were considered as cut off values for abnormal responses of each variable, given the great variability of steroid responses to ACTH stimulus. The results of adrenal adenomas are compared with those of the control group in Table 1.

Corticoadrenal scintigraphy

Corticoadrenal scintigraphy was performed using [^{75}Se]selenio-6α-methyl-19-nor-cholesterol (Scintadren; Amersham Pharmacia Biotech, Amersham, The Netherlands) in 33 patients. Scintigrams were obtained

TABLE 1. Hormonal evaluation of adrenal adenomas and controls

Variable	Adrenal adenomas	Controls	P value
Cortisol 0800 (nmol/L)	521.5 ± 195.9	365.6 ± 132.4	$P < 0.001$
Average daily cortisol (nmol/L)	343.8 ± 112.8	273.7 ± 53	$P < 0.001$
Cortisol percent ratio (%)	31.1 ± 11.96	23.11 ± 12.27	$P < 0.001$
Urinary free cortisol (nmol/day)	372.2 ± 208	312.6 ± 145.9	NS
Cortisol after 2 mg DXM (nmol/L)	176.3 ± 136	30.8 ± 12.96	$P < 0.001$
UFF after 2 mg DXM (nmol/d)	139.1 ± 267	38 ± 21.2	$P = 0.001$
Cortisol post-1,24 ACTH[a] (nmol/L)	1615.6 ± 642.5	1064.4 ± 270	$P < 0.001$
ACTH 0800 (pmol/L)	2.55 ± 2.39	3.17 ± 1.62	$P < 0.05$
17OHP post-1,24 ACTH[a] (nmol/L)	37.94 ± 27.4	13.9 ± 6.05	$P < 0.001$
Δ4-androstenedione post-1,24 ACTH[a] (nmol/L)	19.2 ± 8.4	14.1 ± 6.28	$P < 0.001$
DHEAS (μmol/L)	2.08 ± 2.03	5.69 ± 3.53	$P < 0.001$
DHEAS post-1,24 ACTH[a] (μmol/L)	2.66 ± 2.3	8.02 ± 4.5	$P < 0.001$

Average daily cortisol means serum value (0800 + 1600 + 2400 h)/3; cortisol percent ratio was determined as serum cortisol (2400 h/0800 h) × 100; NS, not significant.

[a] Values expressed as a peak of response to stimulus after 300 or 360 min.

by crystal γ-camera on days 3 and 7 after radiotracer injection. All scintiscans were reviewed by the same operator.

Bone mineral density (BMD) was evaluated in 18 patients, by dual-energy x-ray absorptiometry (DEXA), using a QDR 1000 densitometer (Hologic, Inc., Waltham, MA). BMD was measured on the lumbar spine in patients under 65 yr and at the right femoral neck in those older than 65 yr. Because of budget restrictions, only 1 measurement site per person was chosen, *i.e.* the spine district, known to experience a greater damage with excess cortisol administration, was substituted by the femoral neck district in elderly subjects. The substitution was done on the basis of epidemiological data in Southern Italy, indicating that this region is generally more sensitive in the elderly than the lumbar spine, likely because of frequent rheumatological pathologies, such as ostheoarthritis, at the latter site (18). Individual BMD values were expressed as Z-score, which represents the difference between individual value and mean of the reference age- and sex- matched population, expressed as sᴅ. The reference population adopted was the international pooled sample provided by the densitometer manufacturer (19); their data, in fact, did not differ significantly from those obtained on a local sample in a prestudy performed when the machine was set (18).

All the patients were followed up at regular intervals at 6 and 12 months and then every year for a median period of 34 months (range, 6–85 months). The same endocrine and clinical evaluation and abdominal CT scan were performed at least twice in all patients, at 6-month intervals. A 2-yr follow-up period was completed in 29 patients. None of the patients developed clinical signs of hormonal excess during follow-up. Women of reproductive age were studied in the early follicular phase of the menstrual cycle (days 3–6).

Endocrine assessment

All hormone assays were performed in the same laboratory using commercially available kits. F, UFF, T, and DHEAS were tested by Immulite, solid-phase chemiluminescent enzyme immunoassay (DPC; Los Angeles, CA) and Δ4-androstenedione, 17-OHP by RIA (Diagnostic Systems Laboratories, Inc., Webster, TX). ACTH was tested by double-antibody ^{125}I RIA (DPC).

Statistical analysis

The results are expressed as mean ± sᴅ. The statistical analysis was performed by the Wilcoxon test for unpaired data. The ANOVA was used for comparison of mean hormone levels between groups. The nonparametric method (Mann-Whitney U-test) was used when Wilk-Shapiro's test was not consistent with the Gaussian distribution of the data, *i.e.* for the evaluation of 17-OHP and DHEAS values. Significance was considered to be at $P < 0.05$.

Subclinical hypercortisolism was considered in accordance with the recommendations of the National Italian Study Group on Adrenal Tumors (4, 20) (some authors of this study participated in that multicentric study, and their data were included in the papers): no clinical sign of hormone excess (including truncal obesity, thin extremities with muscular hypotrophy, moon face, nuchal gibbous, and cutaneous purple striae) in the presence of at least two abnormalities in HPA function, assessed by routine endocrine tests. A necessary condition was failure to achieve serum cortisol suppression to below 83 nmol/L (3 μg/dL) by 2-mg DXM. Other abnormalities included: 1) UFF exceeding mean + 2 sᴅ of the control group; 2) average daily cortisol exceeding mean + 2 sᴅ of controls; 3) F% ratio above mean ± 2 sᴅ of controls F% ratio; and 4) reduced ACTH levels.

No patient with bilateral mass had TC scan evidence of pituitary adenoma or endocrine pattern suggesting Cushing's disease (lack of F response to 2 mg DXM test but responsiveness to 8 mg DXM test).

Results

Clinical data

A diagnosis of adrenocortical adenoma was made in 50 patients [21 men and 29 women, 32–73 yr old (mean, 56.3 yr)]. In 19, the tumors were located on the left side; in 28, they were on the right side; 3 patients presented bilateral masses. The size, obtained by CT ranged from 1–6.5 cm (mean, 3.26 cm).

In patients with a bilateral pathology, the size of the largest adenoma was considered. Twenty-four patients (48%) had mild-to-severe hypertension (21), 12 (24%) had type-2 diabetes with fasting hyperglycemia (>126 mg/dL)(22), 6 patients had glucose intolerance (22), and 18 (36%) were diffusely obese [body mass index (BMI) determined as weight/height², BMI > 25]. Moreover, 14 patients (28%) had abnormal serum lipid concentrations (cholesterol ≥ 240 mg/dL (6.21 mmol/L), low-density lipoprotein cholesterol ≥ 160 mg/dL (4.14 mmol/L), and/or triglycerides ≥ 160 mg/dL (1.8 mmol/L). Compared with a sex- and age-matched healthy population, mean BMD Z-score was not significantly different (−0.41 sᴅ, 95% CI: −1.127 to 0.3115).

Corticoadrenal scintigraphy

The mapping showed an early (3 days) [75Se-19] norcholesterol uptake by all adenomas; in 14 of 33 patients, it was unilateral and concordant with the side of the lesion, suggesting an inhibited function of the other adrenal gland. The uptake was bilateral and symmetrical in 1 patient and bilateral, asymmetrical and prevalent on the side of the lesion in the remaining 18.

Endocrine data

The results of hormonal evaluation are summarized in Table 1. Compared with controls, patients with adenomas had significantly higher morning serum F, average F, and F% ratio ($P < 0.001$). In adenomas, a statistically significant difference was found also in morning ACTH levels, which were lower ($P < 0.05$) and in the degree of F suppression by 2 mg DXM, which was impaired ($P < 0.001$). Moreover, F, 17-OHP, and Δ4-androstenedione responses to corticotropin were also significantly increased ($P < 0.001$), whereas DHEAS was significantly lower at baseline and showed a blunted response to corticotropin ($P < 0.001$). Considering each single patient, 19 of them had average serum cortisol levels that exceeded the normal range, 8 had high F% ratios, and 11 patients had an increased UFF. DXM failed to suppress serum F levels in 16 patients; 15 subjects had low ACTH basal values. Frequent abnormalities were also observed in response to corticotropin administration: F and 17-OHP responses exceeded the normal range in 22 and 33 patients, respectively. On the other hand, DHEAS values ranged from normal to very low and were below control ranges in 24 patients; this was always associated with a blunted response to ACTH. A good correlation was found between the suppressibility of serum and urinary F by 2- and 8-mg DXM test.

Subclinical CS patients

Twelve (9 women, 3 men) of the overall series of 50 patients with adenoma were considered as having subclinical CS. The clinical and hormonal features are shown in Table 2. Six adenomas were located on the right side and 3 on the left; 3 patients had bilateral adenomas. The diameter ranged from 1.5–6.0 cm (mean, 3.4 cm). The patients' ages ranged between 47 and 72 yr (mean, 60.7yr). Six patients (50%) were diffusely obese; mild-to-severe systolic-diastolic hypertension (21) was found in 11 patients (91.8%), type-2 diabetes mellitus

TABLE 2. Clinical and hormonal features of patients with subclinical CS

Patient's number	Gender	Age	CT-characteristics		BMI	Hypertension	Diabetes mellitus	Lipids abnormalities	F% ratio	Average F	ACTH	Radiocholesterol uptake	Surgical treatment
			Tumor size (cm)	Tumor side									
1	M	59	4.5	Bilateral	33.3	Moderate	+	+	45.8	122	N	Unilateral Concordant	+
2	F	72	3.0	Right	22	Moderate	−	−	22.4	158.3	N	Bilateral Asymmetric	−
3	F	54	1.8	Left	42	Severe	−(*)	+	56.0	235.3	N	Bilateral Asymmetric	−
4	F	47	6.0	Right	25.4	No	−	−	27.1	105	N	Bilateral Asymmetric	+
5	F	47	1.5	Right	23	Severe	−	−	24.9	135.3	→	Bilateral Symmetric	+
6	F	70	5.0	Bilateral	30.46	Moderate	+	+	20.5	141.3	→	Bilateral Asymmetric	−
7	M	62	4.3	Right	25.75	Mild	+	−	22.6	130	→	Unilateral Concordant	−
8	M	65	3.0	Bilateral	22	Moderate	+	−	40.0	107	N	Bilateral Asymmetric	−
9	F	53	2.3	Left	21	Moderate	+	−	19.0	110	N	Unilateral Concordant	+
10	F	69	3.2	Left	23	Severe	+	+	36.0	59	N	Unilateral Concordant	+
11	F	73	3.7	Right	20.2	Moderate	−	+	43.4	134.8	→	Unilateral Concordant	−
12	F	58	2.5	Right	29.75	Mild	−	+	74.1	182.6	→	Unilateral Concordant	−

Average F was determined as serum cortisol 0800+1600 +2400/3; F% ratio was determined as serum cortisol (F2400/F0800) × 100; N, normal; ↓, low; +, present; −, absent; −(*), glucose intolerance.

(22) in 5 (41.8%), glucose intolerance with fasting hyperinsulinism (22) in 2, and abnormal lipids values in six (50%). No significant difference was found in BMD Z-score between subclinical CS and the remaining adenomas (data not shown).

More than 2 abnormalities in the HPA axis were found in 9 of 12 patients (75%). Their relative frequency is shown in Fig. 1. When considering the patients affected by subclinical CS and those by nonfunctioning adenomas as 2 separate groups, a significant difference was found in serum and urinary post-DXM F suppressibility, albeit to a lesser degree in subclinical CS ($P < 0.001$ and $P < 0,005$ respectively), and in morning ACTH values (Fig. 2a), also lower in the same group ($P < 0,05$). DHEAS also showed a more important impairment of the response to corticotropin stimulation in subclinical CS, compared with nonfunctioning adenomas ($P < 0,05$) (Fig. 2b). Nevertheless, no significant difference was found in 17-OHP and $\Delta4$-androstenedione values between the 2 groups, who were comparable in terms of age (60.75 ± 10.5 yr *vs.* 59.4 ± 9.7 yr).

Scintigraphy. An adrenocortical scintigraphy showed a unilateral uptake concordant with the side of the lesion in 50% of patients with subclinical CS; the uptake was bilateral and symmetrical in one and asymmetrical, prevalently on the side of the lesion, in the remaining patients.

Treatment and follow-up. Five of 12 patients with subclinical CS were treated by adrenalectomy, and the histological diagnosis was adenoma in all the cases. Median postoperative follow-up was 38 months (range, 12–63). In 2 patients (nos. 5 and 10), a transient adrenocortical insufficiency occurred that was treated by oral replacement therapy with cortone acetate. Patient no.1 was affected by transient dynamic adrenal insufficiency, defined as normal basal cortisol levels with blunted response (<500 nmol/L) (23) to corticotropin administration and was treated by replacement therapy only *in situ*ations of stress for a period of 12 months.

Three patients (nos. 5, 9, and 12), previously affected by arterial hypertension, became normotensive after surgery; and treatment was withdrawn. Normal arterial pressure persisted after the recovery of residual adrenal function in patient no. 5. A partial improvement in arterial pressure values was also present in patients 1 and 4, who thus required a smaller daily dose of antihypertensive therapy. A partial improvement in the initial glucose and lipid values was also found in patients treated by surgery. Two patients (nos. 9 and 10) were able to reduce their oral antidiabetic medications, and one (no. 1) was able to reduce the insulin daily dose. All these improvements persisted during the follow-up period.

Seven patients with subclinical CS did not undergo surgical treatment but were prescribed medical treatment for hypertension, diabetes, dislipidemia, and osteopenia, as appropriate. The clinical and hormonal features did not change substantially in six of them after a median follow-up of 28 months (range, 9–73 months). Patient no. 7 was initially diagnosed as having so-called nonfunctional adenoma; 6 months later, using the same endocrine investigation, the hormonal pattern fulfilled the criteria for the diagnosis of subclinical CS, despite an unchanged tumor size of 4.3 cm.
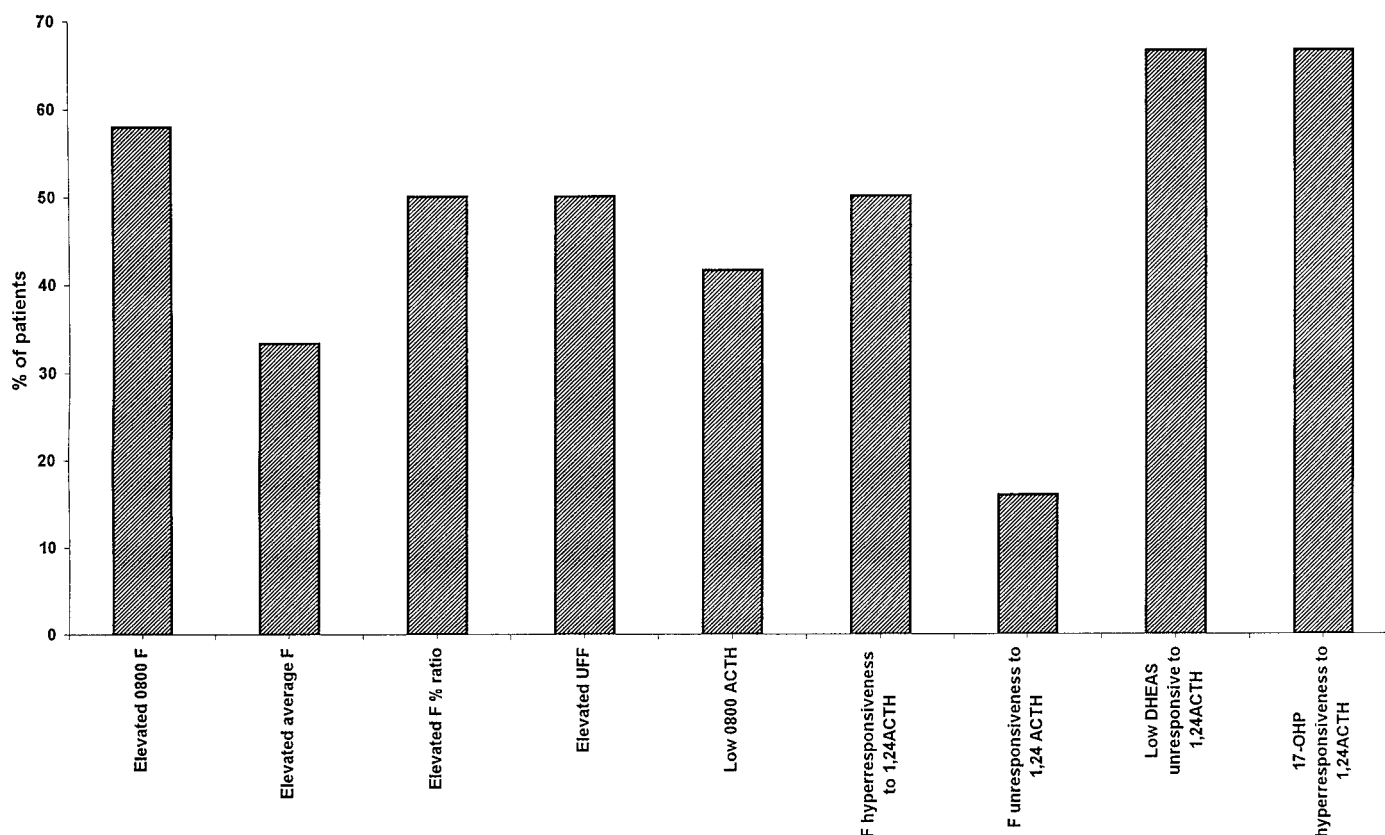
FIG. 1. Most common abnormalities in patients affected by subclinical CS. Average daily cortisol (F) was determined as (F0800 + F1600 + F2400)/3; F% ratio was determined as (F2400/F0800) ×100; F, DHEAS, and 17-OHP responses to 1,24-ACTH were evaluated at 300 and 360 min after administration.

The same endocrine pattern suggestive of autonomous F secretion was confirmed after 6 other months. Tumor size increased by about 0.3 cm in patient numbers 3 and 5, over a period of 24 and 12 months, respectively, and by about 0.5 cm in patient no. 9; it did not change in others. The increase in tumor size was not associated with any significant modification in steroid secretion pattern.

Among the 13 patients in the group with nonfunctioning adenomas treated by adrenalectomy because of the large tumor size, 2 presented adrenocortical insufficiency, lasting 6 and 15 months, respectively; and 2 others, a dynamic insufficiency lasting 6 and 38 months, respectively. Before surgery, all of these patients presented some abnormalities in F, DHEAS, and 17-OHP secretion; and 2 of them had a scintigraphic pattern of unilateral uptake (1 patient with overt insufficiency and another with dynamic insufficiency), whereas a bilateral asymmetrical concordant pattern was present in 2 other patients (1 with overt and 1 with dynamic insufficiency). Improvement in clinical features was also observed in these patients after surgical treatment, to the extent that antihypertensive and antidiabetic therapy could be reduced in more than half of them; 2 patients even became normotensive. All the improvements persisted during the follow-up period.

Concerning endocrine data, normalization of most abnormalities (including baseline F, UFF, average daily F and F% ratio, post-DXM F suppressibility and 1,24-stimulated F, and 17-OHP values) were observed in all patients treated surgically who did not suffer from adrenal insufficiency, independent of whether they had subclinical CS or a nonfunctioning adenoma; DHEAS values, instead, continued to be low. In all but one patient affected by adrenal insufficiency, 17-OHP response to 1,24-ACTH became normal after recovery of the adrenal function. Only patient no. 1, who had a bilateral lesion (Table 2), after the first period of dynamic insufficiency subsequent to a unilateral adrenalectomy that removed only the larger tumor, showed again an increased response of 17-OHP to 1,24-ACTH in the absence of other hormonal abnormalities.

During the median follow-up of 26 months, no substantial changes in clinical and endocrine features were observed in those patients with nonfunctioning adenoma who were not treated by surgery. Tumor size increased from 0.2–0.9 cm in four of them over the period from 6–22 months and was unchanged in others. One patient underwent surgical treatment because of an increase in tumor size, by about 0.9 cm, that exceeded 3.5 cm in diameter. No increase in tumor size was associated with any significant steroid secretion change.

Discussion

The definition of adrenal incidentaloma is applied to an increasing amount of different pathologies; most of them are benign and nonfunctioning adrenal adenomas (5, 11, 12, 24). However, careful diagnostic assessment of adrenal inciden-
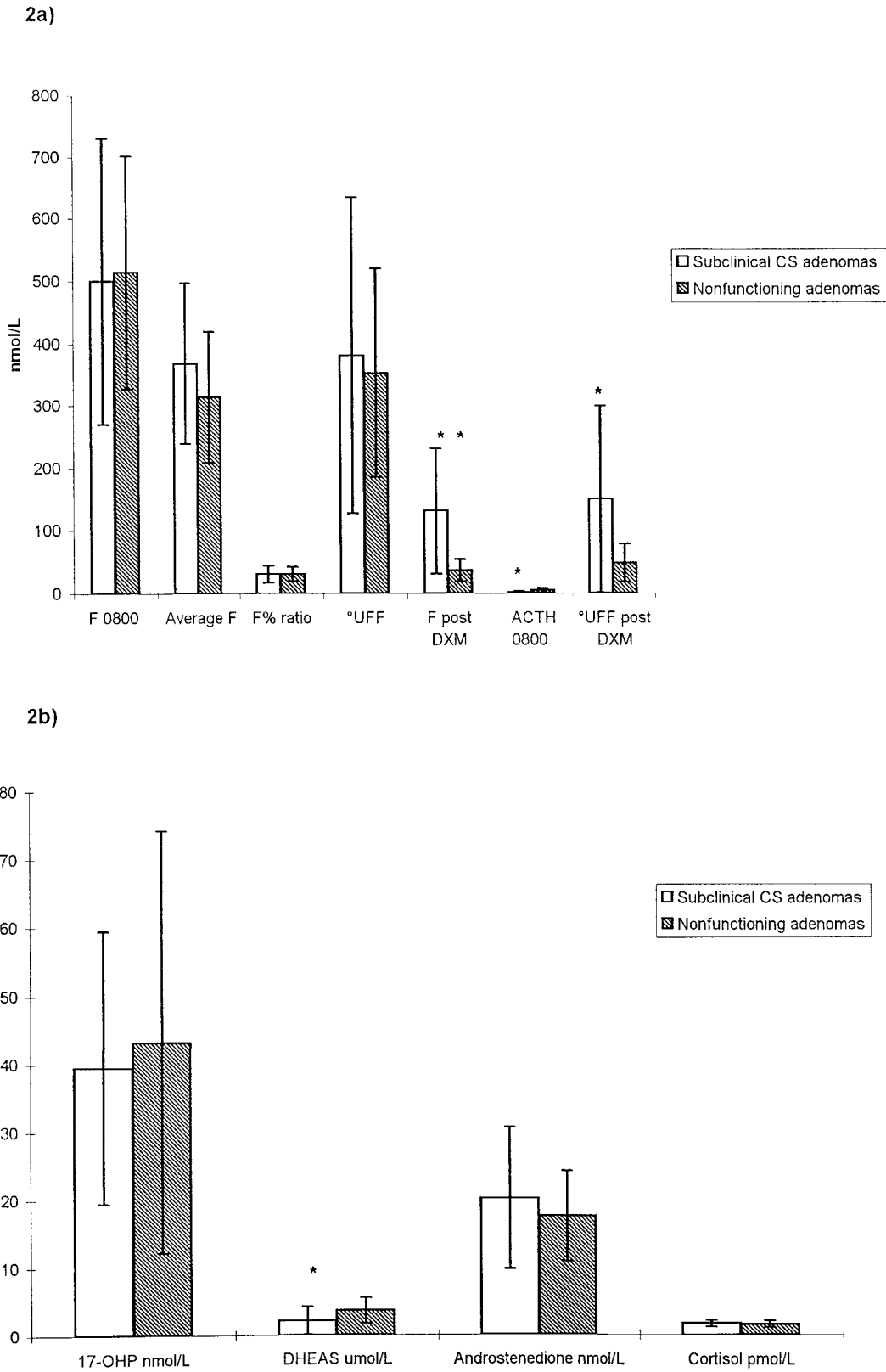
2a)

2b)

FIG. 2. Endocrine data of patients with adrenal adenomas associated with subclinical CS and nonfunctioning ones. a, Comparison between parameters in cortisol and ACTH secretion. Average F was determined as (F0800 + F1600 + F2400)/3; F% ratio was determined as (F2400/F0800) ×100 and is expressed in percent. °UFF is expressed in nmol/24 h. b, Responses to corticotropin administration in subclinical CS and nonfunctioning adenomas. All values are expressed as maximum peak at 300 and 360 min after 1,24-ACTH stimulus. *, $P < 0.05$; **, $P < 0.001$ for subclinical CS *vs.* nonfunctioning adenomas.

talomas must be performed to exclude the presence of malignant and/or functioning lesions.

The diagnosis of adenoma in patients not treated surgically, made on the basis of CT criteria, was confirmed by scintigraphic uptake pattern (25) and by follow-up data.

Fifty patients with incidentally discovered adrenal adenomas evaluated in this study presented a wide spectrum of abnormalities in steroid secretion and HPA axis function. Nevertheless, in only several of them, the biochemical findings consistently pointed to a subtle functional autonomy of cortisol production, defined as subclinical CS. Previous studies had used variable criteria for such definition, reporting an occurrence of subclinical CS that ranged from 5–19% (8, 9, 12, 13, 26, 27). The criteria for the diagnosis of subclinical CS suggested by the National Italian Group of Study on Adrenal Tumors were used in the present study (20); two abnormal HPA axis routine tests were required to reduce the possibility of such classification on the basis of a false positive result. A necessary condition was repeated failure in adequate cortisol suppression by DXM, considered as the hallmark of abnormal steroid secretion with high negative predictive value when the suppression is normal (10, 28). Impaired F suppression was revealed at least twice in all patients considered to be affected by subclinical CS; the initial findings were confirmed after a 6-month interval. Although quite strict criteria were used for the definition of subclinical CS, an unexpectedly high prevalence (24%) was observed in our patients affected by adrenal adenoma. The degree of autonomous cortisol production ranged from slightly abnormal to completely pathological and was biochemically similar to the pattern of overt CS caused by adrenal adenoma in two patients (nos. 3 and 12).

Steroid abnormalities may be an expression of a partial autonomous cortisol production and/or dysregulation of intratumoral and intraadrenal enzymes. Variable expression of functional ACTH receptors and cytochrome P450 activities were described (29, 30). However, the most common endocrine abnormalities in this study were found in 17-OHP and DHEAS values. The opposite behavior observed for 17-OHP (increased accumulation) and DHEAS (decreased accumulation) seems to be attributable to a dysregulation of cytochrome P450C17 with 17-hydroxylase and 17,20-lyase activities. The impaired activity of the latter may perhaps be an intrinsic intraadrenal adaptory mechanism, limiting the overproduction of androgens (31, 32). Cytochrome P450c17, common to adrenal gland and gonads, is likely to be regulated mainly on the Δ5-pathway in adrenals and on the Δ4-pathway in gonads (31). Whereas DHEAS and 17-OHP secretion abnormalities similar to those found by us have been reported in other studies (3, 4, 13, 20, 27), a significant reduction in basal Δ4-androstenedione concentrations was reported in a study on 32 incidentally discovered adenomas that included 4 subclinical CS patients, compared with 14 controls (13). The difference disappeared after a short corticotropin test (30 min). Contrarily, we did not find any difference in baseline A4-androstenedione values between adenomas and controls, but only in peak response to a long ACTH test, which is considered to be the most effective stimulus on adrenal steroidogenesis, especially on androgens (33). Moreover, the 2 groups of patients considered were

not comparable at all: our group of patients with adenomas was characterized by a male-to-female ratio of 0.72; whereas the smaller group in Ambrosi's study (13) was composed prevalently of women (M/F ratio, 0.39), and the men studied were older. However, further data are needed to elucidate whether abnormalities in Δ4-androstenedione secretion ratio are frequent and have some pathological meaning in adrenal adenomas. Moreover, concentrations of other precursors of the glucocorticoid and mineralocorticoid pathways (such as progesterone, 11-deoxycortisol, and 11-deoxycorticosterone) have also been studied, had their accumulation described in incidentalomas (34–36), and may have a possible role in determining hypertension and metabolic abnormalities (37).

Hypertension, diabetes, hyperlipidemia, and diffuse obesity have frequently been observed in patients with adrenal adenomas, the prevalence being even higher in those with subclinical CS. Incidentalomas are frequently seen in patients with other diseases, which accounts for their higher prevalence, compared with the general population. In spite of similar BMI in patients with subclinical CS and nonfunctioning adenomas (26.5 ± 6.8 *vs.* 25.6 ± 5.9), the occurrence of diabetes and abnormal lipids levels was higher in the group of subclinical CS patients (50% *vs.* 26% and 50% *vs.* 21%, respectively). The difference was even greater for hypertension, which occurred in 91.8% of patients with subclinical CS and in 34% of the remaining patients. The high prevalence of hypertension and metabolic disorders in patients with subclinical CS suggests that the clinically silent hypercortisolism is probably not completely asymptomatic. Variable extent and duration of subtle cortisol autonomous secretion may influence the clinical features. This hypothesis is supported by the fact that hormonal and clinical features improved in all patients after surgical treatment. This improvement, described also by other authors (9), was persistent during the follow-up period, even after resolution of the biochemical adrenal insufficiency.

With regard to the scintigraphic pattern, unilateral concordant or bilateral asymmetrical concordant uptake of radiocholesterol in this study predicted neither degree of endocrine abnormalities nor adrenocortical insufficiency after surgical treatment. This is in agreement with some previous studies (14, 30), although several others have found some degree of relationship between radiocholesterol uptake and the functional characteristics of adrenocortical lesions (3, 8, 25). In a recent study, adrenal scintiscans were performed in 136 patients affected by adrenal incidentaloma, indicating a significant positive correlation between cortisol secretion rate abnormalities and radiotracer uptake (38). Such correlation was not sought in our study, our study population being relatively small; however, unilateral concordant uptake was present in both patients with subclinical CS and postsurgical insufficiency, and in those without significant steroidogenic abnormalities or with no insufficiency after surgical approach. Furthermore, adrenal insufficiency was present in 1 woman with a unilateral adenoma associated with subclinical CS (patient no. 5) and a scintigraphic pattern of bilateral symmetrical radiocholesterol uptake. The follow-up data confirmed that the concordant uptake, however, provided the classification of adrenal mass as benign (25, 38). Moreover, none of the abnormalities in cortisol production

were reliable in predicting which patient will need glucocorticoid replacement therapy after surgical treatment of the adenoma. Thus, all patients who undergo adrenalectomy should be screened for residual adrenal function.

There are several facts suggesting that the entity of subclinical CS remains mostly stable in time: first, unchanged clinical and endocrine features in patients with subclinical CS not treated surgically over the follow-up period; second, their age (60.75 + 10.5 yr), which differed significantly from those of the 8 patients with overt CS caused by adrenal adenoma and who were a part of the initial series of 106 patients admitted to our department for the evaluation of adrenal mass (33 ± 6.4 yr, data not shown). The hypothesis that the majority of patients with subtle hypercortisolism will never develop a clinically overt disease is also supported by the relatively infrequent occurrence of CS (10). Because the previous data about the effects of subtle hypercortisolism and its natural history are still unclear and the follow-up periods of such patients in various studies are relatively short, there was insufficient evidence of surgical treatment usefulness in such patients to recommend it for all of them. For this reason, in our study, surgical treatment was restricted to patients with large tumors (diameter > 3.5cm) and to those with recent, severe hypertension. All patients, whether treated surgically or not, are in follow-up under conservative treatment for their current symptoms.

On the other hand, hypertension, insulin resistance, unfavorable lipid profile, and abdominal/visceral obesity are well-known symptoms of overt CS and are related to increased risk of cardiovascular disease. Metabolic consequences of more subtle but likely, prolonged effects of hypercortisolism on the cardiovascular system in subclinical CS might also be present. The groups of patients with subclinical CS are still too small, dishomogeneous, and studied in different ways, making it impossible to draw any reasonable conclusion about the risk. If the high prevalence of hypertension and metabolic disorders found in our study is to be confirmed by others, surgical treatment should possibly be recommended to almost all patients with a subclinical CS.

Despite the abnormal biochemical markers of bone turnover in patients with adrenal incidentalomas documented by previous studies (39, 40), our BMD data are consistent with the lack of significant bone loss in patients with adrenal adenoma, even when associated with subclinical CS.

In conclusion, abdominal CT scans and at least a minimal biochemical evaluation, including determination of serum electrolytes, lipids, glycemia, catecholamines urinary excretion, baseline ACTH and steroids (F, 17-OHP, DHEAS, UFF), and DXM suppression test (low-dose, 2-mg test), should be performed in all patients with adrenal incidentalomas to determine the functional autonomy and biological behavior of the lesion. As to the surgical treatment of incidentalomas, this should still be reserved for patients with large tumors, imaging characteristics suggestive of malignancy, recent severe hypertension, evidence of increase in tumor size, or overt hypersecretion during follow-up, until the discrepancy between the clear improvement in clinical and endocrine features in surgically treated patients and apparent stability of those treated pharmacologically is elucidated. However,

all patients treated by surgery should be screened for adrenal insufficiency.

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