Long-Term Follow-Up in Adrenal Incidentalomas: An Italian Multicenter Study

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Context: The long-term consequences of subclinical hypercortisolism (SH) in patients with adrenal incidentalomas (AIs) are unknown.

Setting and Patients: In this retrospective multicentric study, 206 AI patients with a ≥5-year follow-up (median, 72.3 mo; range, 60–186 mo) were enrolled.

Intervention and Main Outcome Measures: Adrenocortical function, adenoma size, metabolic changes, and incident cardiovascular events (CVEs) were assessed. We diagnosed SH in 11.6% of patients in the presence of cortisol after a 1 mg-dexamethasone suppression test >5 μg/dL (138 nmol/L) or at least two of the following: low ACTH, increased urinary free cortisol, and 1 mg-dexamethasone suppression test >3 μg/dL (83 nmol/L).

Results: At baseline, age and the prevalence of CVEs and type 2 diabetes mellitus were higher in patients with SH than in patients without SH (62.2 ± 11 y vs 58.5 ± 10 y; 20.5 vs 6%; and 33.3 vs 16.8%, respectively; $P < .05$). SH and type 2 diabetes mellitus were associated with prevalent CVEs (odds ratio [OR], 3.1; 95% confidence interval [CI], 1.1–9.0; and OR, 2.0; 95% CI, 1.2–3.3, respectively), regardless of age. At the end of the follow-up, SH was diagnosed in 15 patients who were without SH at baseline. An adenoma size >2.4 cm was associated with the risk of developing SH (sensitivity, 73.3%; specificity, 60.5%; $P = .014$). Weight, glycemic, lipidic, and blood pressure control worsened in 26, 25, 13, and 34% of patients, respectively. A new CVE occurred in 22 patients. SH was associated with the worsening of at least two metabolic parameters (OR, 3.32; 95% CI, 1.6–6.9) and with incident CVEs (OR, 2.7; 95% CI, 1.0–7.1), regardless of age and follow-up.

Conclusion: SH is associated with the risk of incident CVEs. Besides the clinical follow-up, in patients with an AI >2.4 cm, a long-term biochemical follow-up is also required because of the risk of SH development. (***J Clin Endocrinol Metab*** **99: 827–834, 2014)**

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Abbreviations: AH, arterial hypertension; AI, adrenal incidentaloma; BMI, body mass index; CI, confidence interval; CT, computed tomography; CVE, cardiovascular event; DL, dyslipidemia; 1 mg-DST, 1 mg-dexamethasone suppression test; HPA, hypothalamic-pituitary-adrenal; LDL, low-density lipoprotein; OB, obesity; OR, odds ratio; ROC, receiver operating characteristic; SH, subclinical hypercortisolism; SN, sensitivity; SP, specificity; T2DM, type 2 diabetes mellitus; UFC, urinary free cortisol; ULN, upper limit of normal.

The wide use of radiological techniques has brought about the detection of incidental adrenal masses, named "adrenal incidentalomas" (AIs), in 1–4.2% of the general population (1, 2). Most of these masses are benign and nonfunctioning adenomas. However, the natural history of these tumors is only partially known (3–5).

In 5–20% of AI patients, a >1-cm adrenal mass enlargement occurs after a mean follow-up period of 4 years, regardless of adrenal function. Nevertheless, the risk of developing malignancy is low (<1/1000) (6–10). In 5–30% of AI patients, a condition of subclinical hypercortisolism (SH), defined by the presence of biochemical abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis without the signs and/or symptoms of overt cortisol hypersecretion, is present (11). The SH development has been described in up to 12% of AI patients after a mean follow-up period of 3 years (3, 12–15). A spontaneous normalization of adrenal function has been described in some AI patients with SH, whereas progression to Cushing's syndrome is rare (10, 14, 16, 17).

The diagnosis of SH is important because this condition has been associated with several cardiovascular risk factors, in particular obesity (OB), arterial hypertension (AH), type 2 diabetes mellitus (T2DM), dyslipidemia (DL), and osteoporosis (18–21). Some studies (22–26), but not all (27, 28), reported an improvement of these possible SH complications after adrenalectomy and their worsening in untreated patients. Therefore, the appropriate AI management and the utility of a long-term follow-up of these patients are debated (4, 11, 29–31). Even if SH has been associated with the risk of major cardiovascular events (CVEs) (32), large studies focused on "hard" end-points, such as major CVEs, are lacking (11).

In this study, we evaluate in a large population of AI patients with (SH+) and without SH (SH−), conservatively followed up for at least 5 years, the prevalence and the incidence of major CVEs and the changes of cortisol secretion and size of the adenoma over time.

Patients and Methods

Patients

We retrospectively analyzed the records regarding 1120 AI patients referred to the participating Endocrine Units between January 1996 and December 2012. Among these patients, 282 have been operated on for the presence of overt hypercortisolism or for adenoma size, 448 were not included due to the exclusion criteria and/or a follow-up of <5 years, and 184 were lost to follow-up. Eventually, 206 AI patients with a follow-up of ≥5 years and without signs of overt hypercortisolism were enrolled. Sixty-five patients had been included in a previous cross-sectional study (33). We excluded patients with psychiatric diseases and alcoholism, those taking drugs influencing cortisol and dexamethasone metabolism or cortisol secretion, and those with signs or symptoms of overt cortisol excess (ie, moon facies, striae rubrae, skin atrophy, or buffalo hump), and a history of malignancy, infections, adrenal hemorrhage, pheochromocytoma, primary hyperaldosteronism, and infiltrative disease potentially affecting the adrenal glands.

All AI were discovered by computed tomography (CT) scan, ultrasonography, or magnetic resonance imaging performed for unrelated diseases. Ultrasound findings were confirmed with unenhanced CT scan. All adrenal masses were ≥1 cm in diameter and displayed a CT pattern consistent with a benign adenoma, such as a homogeneous texture with low density (<10 Hounsfield units), regular and smooth margins, and size <6 cm (4, 11, 29). In patients with bilateral adenomas, the diameter of the largest adenoma was reported.

In all patients, we measured the following at baseline and at the end of follow-up: 24-hour urinary free cortisol (UFC), ACTH levels at 8 AM, and serum cortisol levels at 8 AM after 1 mg-dexamethasone suppression test (1 mg-DST). We diagnosed SH in the presence of 1 mg-DST cortisol levels >5 μg/dL (138 nmol/L) or in the presence of at least two of the following parameters: ACTH <10 pg/mL (2.2 pmol/L), increased UFC, and 1 mg-DST cortisol levels >3.0 μg/dL (83 nmol/L). Increased UFC levels were defined by values above the upper limit of normal (ULN) of each assay. On the basis of these criteria, at baseline 24 patients (11.6%) were SH+ and 182 were SH−. No SH+ patient showed a normalization of adrenal function over time, whereas 15 SH− patients (8.2%) became SH+ by the end of the follow-up. Because we could not establish the time of SH occurrence, these patients have been included in the SH+ group. Therefore, the SH+ and SH− groups were composed of 39 and 167 subjects, respectively.

Written informed consent was obtained from all subjects, and the study was approved by the local Ethics Committees.

Methods

Given the retrospective design of the study, there was no standardized protocol among the participating centers, and we report data at the beginning and the end of the follow-up. In all patients, the presence of OB, AH, T2DM, and DL and the prevalence of CVEs (coronary heart disease or ischemic/hemorrhagic stroke) were evaluated at baseline. The changes in weight, glucose and lipid metabolism, and blood pressure and the occurrence of CVEs were evaluated at the end of the follow-up. In the diabetic, dyslipidemic, and hypertensive patients, the prevalent and incident CVEs, blood pressure, and metabolic control were assessed by reports of the cardiologists and diabetologists who evaluated the patients annually. In the remaining patients, this information was obtained from reports by their general practitioners at the beginning and end of the follow-up. In the diabetic patients, the metabolic control was assessed by glycated hemoglobin.

AH was defined as the presence of systolic blood pressure ≥140 mm Hg, and/or diastolic blood pressure ≥90 mm Hg, and/or antihypertensive treatment (34). T2DM was diagnosed using World Health Organization criteria (35). DL was diagnosed in the presence of triglyceride levels ≥150 mg/dL (1.7 mmol/L) or high-density lipoprotein cholesterol levels <40 mg/dL (1.0 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women or if any specific treatment was given (36).

The improvement/worsening of body weight was defined by a >5% change between baseline and the end of follow-up (37).

The improvement/worsening of arterial blood pressure was determined if nonhypertensive patients passed from a prehypertension category to another category or hypertensive patients passed from a hypertension grade to another grade (34), or if any antihypertensive treatment was modified. Fasting glucose and low-density lipoprotein (LDL)-cholesterol levels were considered improved/worsened if they passed from one category to another (36).

Serum and urinary samples were stored at −20°C until assayed. In all patients, ACTH and serum and urinary cortisol levels were measured in each institute using commercially available reagents. The intra- and interassay coefficients of variation were <10% for all the assays.

Because the normal values of UFC vary when different assays are used, UFC values were expressed as the percentage difference between the detected value and the ULN of each assay.

Statistical analysis

Statistical analysis was performed by SPSS version 18.0 (SPSS Inc). The results are expressed as mean ± SD, unless differently specified. Categorical variables were compared by χ^2 test. Continuous variables were compared among the different groups using one-way ANOVA or the Bonferroni test, as appropriate.

Bivariate associations were tested by either Pearson product moment or Spearman correlation, as appropriate.

The logistic regression analysis assessed the association between: 1) the CVE prevalence (dependent variable) and the SH presence and variables that were significantly different between SH+ and SH− patients at baseline; 2) the SH appearance (dependent variable) and variables that were significantly different (or tended to be different; ie, $P \leq .2$) between patients who remained SH− and patients who developed SH during follow-up; 3) the worsening of at least two parameters among body weight, blood pressure, glycemic and LDL cholesterol control (dependent variable), and the SH presence, age, and duration of follow-up; and 4) the incident CVEs (dependent variable) and the SH presence or of increased 1 mg-DST cortisol levels, or UFC or ACTH <10 pg/mL (2.2 pmol/L) and the variables that were significantly different (or tended to be different; ie, $P \leq .1$) between SH+ and SH− patients at follow-up.

The receiver operating characteristic (ROC) curve analysis assessed the cutoff of the variables with the best diagnostic accuracy (sensitivity [SN], specificity [SP], and positive and negative predictive values) for detecting SH− patients at risk for developing SH over time. The same analysis assessed the best cutoff(s) of the 1 mg-DST cortisol levels and their SN and SP for predicting the incident CVEs. *P* values <.05 were considered significant.

Results

Clinical and biochemical parameters of SH+ and SH− patients at baseline and at the end of follow-up are summarized in Table 1.

Baseline

Body mass index (BMI), gender, and the presence of OB, AH, and DL were comparable between SH+ and SH− patients. As expected, ACTH levels were lower, whereas UFC and 1 mg-DST cortisol levels were higher in the SH+ group than in the SH− group. In this latter group, 11 patients showed 1 mg-DST cortisol levels between 3.0 and 4.9 μg/dL (83–135 nmol/L), in the absence of other alterations of the HPA axis parameters.

Adenoma diameter and the prevalence of bilateral adenoma were greater in SH+ than SH− patients. The adenoma side was 58.8% right and 41.2% left.

Table 1. Clinical and Biochemical Parameters of Patients With and Without SH at Baseline and at End of Follow-Up

	Baseline		Follow-Up	
	SH− Group	SH+ Group[a]	SH− Group	SH+ Group[a]
n	167	39	167	39
Age, y	58.5 ± 10.1 (25–79)	62.2 ± 11 (25–78)[b]	65.3 ± 9.9 (35–86)	68.5 ± 11.0 (35–91)
Females	119 (71.3)	25 (64.1)		
BMI, kg/m^2	27.9 ± 5.0 (17.3–44.7)	28.3 ± 5.6 (19.4–47.0)	28.2 ± 5.4 (17.0–52.1)	29.2 ± 6.0 (19.3–49.6)
Obese subjects	46 (27.5)	13 (33.3)	54 (32.3)	18 (46.2)
Bilateral adenomas	18 (10.8)	11 (28.2)[b]	22 (13.2)	12 (30.8)[c]
Diameter of adenoma, cm	2.2 ± 0.7 (1.0–4.0)	2.8 ± 0.9 (1.5–6.0)[b]	2.5 ± 0.9 (1.0–6.0)	3.1 ± 1.0 (1.6–6.0)[b,c]
ACTH, pg/mL	16.1 ± 11.5 (2.0–78.0)	11.3 ± 6.0 (3.0–28.0)[b]	16.9 ± 10.6 (1.4–72.0)	8.6 ± 3.8 (3.0–19.8)[b,c]
1 mg-DST, μg/dL	1.6 ± 0.8 (0.2–4.9)	3.5 ± 2.1 (1.1–9.3)[b]	1.6 ± 0.8 (0.1–4.1)	4.9 ± 2.2 (1.5–10.4)[b,c,d]
UFC%	−42.0 ± 36.0 (−90.9 to 134.0)	−14 ± 68.5 (−85.7 to 266.0)[b]	−39.6 ± 34.4 (−93.3 to 111.4)	−14.1 ± 50.7 (−89.1 to 121.3)[b,c]
Hypertensive patients	90 (53.9)	26 (66.7)	105 (62.9)	34 (87.2)[b,c,d]
Diabetic patients	28 (16.8)	13 (33.3)[b]	37 (22.2)	17 (43.6)[b,c]
Dyslipidemic patients	70 (41.9)	21 (53.8)	90 (53.9)[b]	27 (69.2)[b]
Patients with prevalent CVEs	10 (6.0)	8 (20.5)[b]		

Data are expressed as mean ± SD (range) or absolute number (percentage). 1 mg-DST indicates serum cortisol levels after 1 mg-dexamethasone suppression test, and UFC% indicates the percentage difference between UFC levels and ULN values. SH is diagnosed in the presence of cortisol after 1 mg-DST >5 μg/dL (138 nmol/L) or of two out of the following three parameters: ACTH <10 pg/mL (2.2 pmol/L); 1 mg-DST >3 μg/dL (82.7 nmol/L); and high UFC levels. SI conversion factors: cortisol, × 27.59; ACTH, × 0.22.

[a] This group includes 15 SH− patients who became SH+ at the end of the follow-up. Because we could not establish the exact time of occurrence of SH, these patients have been included in the SH+ group also for baseline evaluations.

[b] $P < .05$ vs SH− at baseline.

[c] $P < .05$ vs SH− at follow-up.

[d] $P < .05$ vs SH+ at baseline.

Table 2. Comparison of the Clinical and Biochemical Characteristics at Baseline Between Patients Without SH and the 15 Patients Who Developed SH During Follow-Up, and at the End of Follow-Up Between the 15 Patients Who Developed SH During the Follow-Up and the 24 Patients With SH Diagnosed at Baseline

	Baseline		End of Follow-Up	
	Patients Without SH	Patients Who Developed SH During Follow-Up	Patients With SH Diagnosed at Baseline	Patients Who Developed SH During Follow-Up
n	167	15	24	15
Age, y	58.5 ± 10.1 (25–79)	59.3 ± 12.6 (40–76)	70.4 ± 10.5 (44–91)	65.5 ± 11.5 (35–83)
Females	119 (71.3)	13 (86.7)	12 (50)	2 (13.3)
Duration of follow-up, mo			79.5 ± 28.4 (60–178)	79.2 ± 20 (60–120)
BMI, kg/m^2	27.9 ± 5.0 (17.3–44.7)	28.0 ± 4.7 (20.2–36.3)	29.3 ± 6.5 (19.3–49.6)	29.1 ± 5.2 (20.7–38.5)
Obese subjects	46 (27.5)	6 (40)	10 (41.7)	8 (53.3)
Bilateral adenomas	18 (10.8)	4 (26.7)	7 (29.2)	5 (33.3)
Diameter of adenoma, cm	2.2 ± 0.7 (1.0–4.0)	2.8 ± 0.7 (1.5–4.0)[a]	3.1 ± 1.1 (1.6–6.0)	3.1 ± 0.9 (1.6–5.0)
ACTH, pg/mL	16.1 ± 11.5 (2.0–78.0)	11.0 ± 3.5 (4.0–15.0)	9.3 ± 3.7 (3.8–19.8)	7.4 ± 3.7 (3.0–16.0)
1 mg-DST, μg/dL	1.6 ± 0.8 (0.2–4.9)	1.9 ± 0.6 (1.1–3.2)	4.8 ± 2.1 (1.5–10.4)	4.9 ± 2.4 (1.5–9.9)
UFC%	−42.0 ± 36.0 (−90.9 to 134.0)	−40.4 ± 30.6 (−85.7 to 17.6)	−19.9 ± 49.6 (−89.1 to 79.1)	−4.8 ± 52.7 (−88.5 to 121.3)
Hypertensive patients	90 (53.9)	10 (66.7)	21 (87.5)	13 (86.7)
Diabetic patients	28 (16.8)	5 (33.3)	11 (45.8)	6 (40.0)
Dyslipidemic patients	70 (41.9)	8 (53.3)	17 (70.8)	10 (66.7)
Patients with prevalent CVEs	10 (6.0)	2 (13.3)	6 (25.0)	2 (13.3)

Data are expressed as mean ± SD (range) or absolute number (percentage). 1 mg-DST indicates serum cortisol levels after 1 mg-dexamethasone suppression test, and UFC% indicates the percentage difference between UFC levels and ULN values. SH is diagnosed in the presence of cortisol after 1 mg-DST >5 μg/dL (138 nmol/L) or of two out of the following three parameters: ACTH < 10 pg/mL (2.2 pmol/L); 1 mg-DST >3 μg/dL (82.7 nmol/L); and high UFC levels. SI conversion factors: cortisol, × 27.59; ACTH, × 0.22.

[a] $P < .005$ vs SH− group.

Overall, 76.2% of patients had T2DM and/or AH and/or DL. SH+ patients were older and showed a higher prevalence of T2DM and CVEs than SH− patients. Therefore, we performed the logistic regression analysis including the presence of T2DM and age. This analysis showed that SH and T2DM were independently associated with the CVE presence (odds ratio [OR], 3.1; 95% confidence interval [CI], 1.1–9.0; and OR, 2.0; 95% CI, 1.2–3.3, respectively; $P < .05$), regardless of age (OR, 1.0; 95% CI, 1.0–1.1; $P = .553$). After excluding from the SH+ group the patients with a possible biochemically overt hypercortisolism (two subjects with UFC >100% of ULN and one patient with two or more altered parameters among 1 mg-DST cortisol levels >5 μg/dL [50 nmol/L], ACTH <5 pg/mL [1.1 pmol/L], and UFC >100% of ULN), the results did not change (data not shown).

Follow-up

Changes of cortisol secretion and adenoma size

The follow-up mean duration was 82.5 ± 32 months (median, 72.3 mo; range, 60–186 mo), comparable between SH+ and SH− patients.

In SH+ patients, 1 mg-DST cortisol levels increased during the follow-up, whereas ACTH and UFC levels were unchanged (Table 1). The adenoma diameter tended to increase over time. Five patients developed another adenoma at the contralateral adrenal gland (Table 1). A mass size increase ≥1 cm was observed in 8.3% of the patients, whereas an increase >2.5 cm was observed in 2.4% of the patients. The parameters of adrenal function at baseline were not associated with the diameter increase (data not shown).

Table 2 reports the comparison at study entry between SH− patients and the 15 patients who developed SH during follow-up. Table 2 also shows the comparison at the end of follow-up between the 15 patients who developed SH over time and the 24 patients with SH diagnosed at baseline. At the end of follow-up, the patients with SH diagnosed at baseline and those with SH diagnosed during follow-up were similar for clinical and biochemical characteristics. Among the 15 patients who developed SH during the follow-up, three subjects experienced an incident CVE. At baseline, two of these three patients showed 1 mg-DST cortisol levels >1.8 μg/dL (50 nmol/L) and an adenoma size >2.5 cm, one patient showed ACTH levels <10 pg/mL (2.2 pmol/L), and no one showed high UFC values.

At the study entry, the patients who developed SH during follow-up showed a larger size of adenoma ($P = .003$) and tended to be more frequently affected with bilateral adenomas as compared with SH− patients ($P = .08$). The cortisol secretion at baseline was comparable between SH− patients and subjects without SH at baseline who developed SH during follow-up, even if 1 mg-DST cortisol levels tended to be higher in the latter group ($P = .2$). However, at diagnosis among the 15 patients who developed SH during follow-up, nine (60.0%) showed 1 mg-DST cortisol levels ≤1.8 μg/dL (50 nmol/L).

Because the SH− patients who became SH+ during follow-up were different (or tended to be different) for

tumor size, frequency of bilateral adenoma, and 1 mg-DST cortisol levels as compared with subjects who remained SH−, we performed a logistic regression analysis to assess the independent associations between these variables and the risk of SH occurrence. The analysis showed that adenoma diameter was associated with the risk of SH development (OR, 2.97; 95% CI, 1.37–6.44; $P = .006$), regardless of the presence of a bilateral adenoma (OR, 2.94; 95% CI, 0.75–11.51; $P = .123$) and 1 mg-DST cortisol levels (OR, 1.16; 95% CI, 0.61–2.23; $P = .653$).

The ROC curve analysis showed that the cutoff of the adenoma size with the best diagnostic accuracy for detecting patients at risk for SH development was 2.4 cm (SN, 73.3%; SP, 60.5%; positive predictive value, 14.3%; negative predictive value, 96.2%; $P = .014$). Indeed, only four of the 105 patients (3.8%) without SH at baseline and with an adenoma <2.4 cm developed SH during follow-up. On the other hand, 11 of the 77 patients (14.3%) without SH at baseline and with an adenoma ≥2.4 cm, in fact, developed SH during follow-up. Among the 15 patients who developed SH during follow-up, a diameter increase of >1 cm was found in only one subject.

Cardiovascular and metabolic outcome

The occurrence of CVEs and changes in body weight, blood pressure, glycemic and LDL cholesterol control in patients with and without SH at the end of follow-up are reported in Table 3.

In the SH+ group, the prevalence of AH significantly increased during follow-up. At the end of follow-up, SH+ patients showed a higher prevalence of AH and T2DM than SH− patients, whereas the prevalence of dyslipidemia and obesity was comparable. The annual rate of CVEs (2.2% in the whole population) was higher in SH+ (3.1%) than in SH− patients (1.2%; $P = .004$).

Weight, glycemic control, and LDL cholesterol worsened in 26, 25, and 13% of patients, respectively, without any difference between SH+ and SH− patients. Blood pressure control tended to worsen mainly in the SH+ group, rather than in the SH− group, even if statistical significance was not reached ($P = .07$). The worsening of at least two metabolic complications was higher in SH+ than in SH− patients (53.8 vs 25.7%; $P = .001$, respectively). The presence of SH was associated with the worsening of at least two metabolic complications (OR, 3.32; 95% CI, 1.6–6.9; $P = .002$), regardless of age (OR, 1.03; 95% CI, 0.99–1.06; $P = .08$) and the duration of follow-up (OR, 1.01; 95% CI, 1.0–1.02; $P = .08$).

At the end of follow-up, the SH+ patients experienced a higher number of CVEs than the SH− patients. Seven (32%) of the 22 incident CVEs occurred in patients with prevalent CVEs at baseline and 15 (68%) in patients without prevalent CVEs.

The logistic regression analysis showed that the presence of SH was significantly associated with the occurrence of incident CVEs (OR, 2.7; 95% CI, 1.0–7.1; $P = .04$), but not with the worsening of blood pressure control (OR, 1.3; 95% CI, 0.5–3.2; $P = .634$) and the duration of follow-up (OR, 1.0; 95% CI, 0.99–1.01; $P = .842$). The SN and SP of the SH diagnosis in predicting the incident CVEs were 36.4 and 83.2%, respectively.

The 1 mg-DST cortisol levels tended to be associated with the occurrence of incident CVEs (OR, 1.3; 95% CI, 1.0–1.6; $P = .06$), but not with the worsening of blood pressure control (OR, 1.5; 95% CI, 0.6–3.7; $P = .414$) and the duration of follow-up (OR, 1.0; 95% CI, 1–1.1; $P = .708$), whereas ACTH and UFC levels were not (data not shown). The ROC curve analysis confirmed this association (Figure 1) and showed that the cutoffs of 1 mg-

Table 3. Occurrence of CVEs and Changes in Body Weight, Blood Pressure, Glycemic and LDL Cholesterol Control in Patients With and Without SH at the End of Follow-Up

	SH− Group	SH+ Group	*P*
n	167	39	
Duration of follow-up, mo	83.2 ± 33.6 (60–186)	79.4 ± 25.2 (60–178)	.826
New CVE	14 (8.4)	8 (20.5)	.040
New CVE in CVE− patients at baseline	11 (6.6)	4 (10.0)	.343
Increased body weight[a]	40 (24.0)	13 (33.3)	.229
Worsened blood pressure control[b]	52 (31.1)	18 (46.2)	.070
Worsened glycemic control[c]	39 (23.4)	12 (30.8)	.334
Worsened LDL[c]	20 (12.0)	7 (17.9)	.303

Data are expressed as mean ± SD (range) or absolute number of patients (percentage). CVE− indicates patients without previous CVEs.

[a] Body weight is considered improved or worsened in the presence of at least a 5% variation with respect to baseline (37).

[b] Blood pressure level was considered improved or worsened if it passed from one category to the other, in agreement with the guidelines of the European Societies of Hypertension and Cardiology (34).

[c] Fasting glucose and LDL-cholesterol levels were considered improved or worsened if they passed from one category to the other, in agreement with the Adult Treatment Panel III criteria (36).

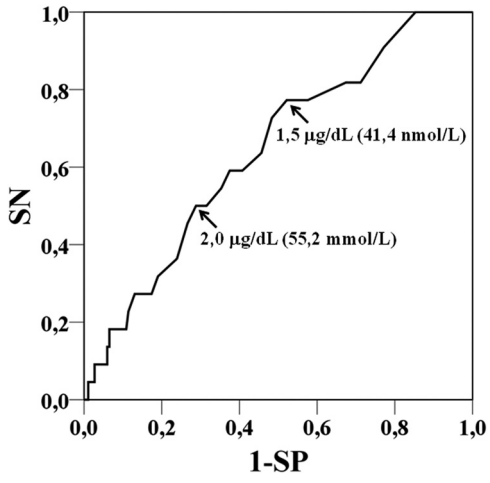

Figure 1. ROC curve for cortisol levels after 1 mg-DST in predicting the occurrence of incident CVEs. The cutoffs of 1 mg-DST cortisol levels with the best compromise between SN and SP in predicting the occurrence of a new CVE are set at 1.5 μg/dL (41.4 nmol/L; SN, 77.3%; SP, 50%) and at 2.0 μg/dL (55.2 nmol/L; SN, 50%; SP, 68.5%), as indicated by the arrows.

DST cortisol levels with the best compromise between SN and SP in predicting the occurrence of a new CVE were set at 1.5 μg/dL (41.4 nmol/L; SN, 77.3%; SP, 50%) and at 2.0 μg/dL (55.2 nmol/L; SN, 50%; SP, 68.5%).

Discussion

In our large population of AI patients conservatively followed up for ≥5 years, we observed that 8.2% of the patients developed SH over time, the risk being particularly increased in patients with an adenoma size ≥2.4 cm. The CVE prevalence was higher in SH+ than SH− patients, regardless of age and the presence of T2DM. The incident CVEs were higher in SH+ than SH− subjects, independent of the duration of the follow-up.

The finding that 8.2% of AI patients develop SH over time is in keeping with previous studies with a shorter follow-up. Indeed, the SH occurrence has been described in up to 12% of AI patients after a mean follow-up period of 3 years (3, 12–15, 17). In accordance with some (7, 27) but not all (15, 16) authors in our series, no patients showed a normalization of the adrenal function. Because SH is clinically silent, we cannot establish the time of the appearance of the biochemical alterations.

It has been suggested that tumors of ≥3 cm are more likely to develop hyperfunction than smaller tumors (5, 38). In keeping with this suggestion, in the present study, the only variable associated with the risk of SH appearance was adenoma size. Indeed, the 14.3% of patients with an adenoma ≥2.4 cm developed SH over time, whereas this risk was low in patients with adenomas of <2.4 cm.

However, although at baseline 1 mg-DST cortisol levels were suppressed (≤1.8 μg/dL; 50 nmol/L) in about half of patients deemed to develop SH, two of the three subjects who developed SH and showed an incident CVE had baseline 1 mg-DST cortisol levels >1.8 μg/dL (50 nmol/L) and an adenoma size >2.5 cm. These findings suggest that, even in the absence of a SH diagnosis, the presence of an adenoma size ≥2.4 cm, particularly if associated with 1 mg-DST cortisol levels >1.8 μg/dL (50 nmol/L), is important for an appropriate biochemical/clinical follow-up.

In keeping with previous studies (3, 7, 8), the 8.3% of patients had an increase of adenoma size of ≥1 cm; only 2.4% of cases showed a diameter increase >2.5 cm, and none was malignant.

The novelty of the study is the evaluation of the prevalence and incidence of "hard" end-points such as the major CVEs after a long-term follow-up. As already described (32), we found that SH is associated with prevalent CVEs, but for the first time we demonstrate that SH patients are also at risk of incident CVEs. Even if the AH worsening could have been a risk factor for incident CVEs, the worsening of blood pressure levels was not associated with the incident CVEs when adjusting for the duration of the follow-up and for the 1 mg-DST cortisol levels or the SH presence. However, because SH itself may lead to the AH worsening, it is difficult to discriminate the possible independent roles of cortisol hypersecretion and blood pressure levels in determining the CVE risk in these patients.

Our findings may contribute to defining the utility of long-term follow-up of AI patients and to suggesting adequate management (4, 11, 29). As widely recommended on the basis of previous smaller series (6), long-term radiological follow-up should not be routinely undertaken. However, follow-up of the cardiovascular risk should be done in these patients. The rate of CVEs in SH+ patients (3.1%) in the present study, similar to that reported in the populations at risk (39), is not surprising, considering the high prevalence of patients with T2DM and/or AH and/or DL (76.2%) in our population.

This study suggests that the occurrence of CVEs or worsening of cardiovascular risk prompts an endocrine re-evaluation (40) and that SH patients should be carefully followed up. The use of 1 mg-DST cortisol levels as a single parameter to predict the CVE risk seems to have an acceptable SN (77%) if a low cutoff is chosen (ie, 1.5 μg/dL [41.4 nmol/L]) but a low SP (50%). The use of a combination of different HPA axis parameters, such as the one we chose in the present study, increases the SP (83.2%). Unfortunately, data regarding midnight serum cortisol levels were not available because this requires hospital admission (11), which is not widely available in Italy. In

the absence of prospective trials, surgery is suggested in younger SH patients showing diseases potentially attributable to SH (ie, AH) that are of recent onset or resistant to adequate medical treatment (4, 30). The present findings demonstrate that the deterioration of metabolic and cardiovascular status is fueled by SH. It is possible that in these patients surgery could avoid the CVE occurrence.

We disclose the limitation of the small number of SH patients, which severely affects the power for low-frequency events and for teasing out the influence of cortisol. Moreover, the retrospective design of the study and the lack of a standardized protocol among the participating centers did not allow us to obtain potentially interesting data (ie, waist circumference) and might have led to a possible selection bias toward patients at better prognosis. It is likely that patients who were perceived to be at higher CVE risk or who presented with larger masses underwent adrenalectomy. However, this bias may have paradoxically reinforced the importance of our findings. Indeed, although the patients included in the study may have theoretically had a low CVE risk, in fact the incident CVEs were increased. Another limitation of this study is related to the SH diagnosis (11), which is defined by arbitrary criteria. It is possible that some patients classified as not having SH might, in fact, have a mild hypercortisolism. This may explain why CVEs also occur in SH− patients. In keeping with this explanation, 11 SH− patients had 1 mg-DST cortisol levels between 3 and 4.9 μg/dL (83–135 nmol/L), in the absence of other alterations of the HPA axis activity parameters. Finally, although we used a uniform definition of SH, the lack of centralization of the hormone measurements is another limitation of the study because ACTH and cortisol assays are largely inaccurate at the low ends (11, 41).

In conclusion, the study provides two major findings. First, in AI patients without SH, a clinical and biochemical long-term follow-up is recommended for the risk of SH development, especially in patients with an adenoma of $\geq$2.4 cm. Second, in AI patients with SH, the increased risk of incident CVEs has to be taken into account in addressing the treatment of choice.

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