CLINICAL STUDY

Post-surgical hypocortisolism after removal of an adrenal incidentaloma: is it predictable by an accurate endocrinological work-up before surgery?

Cristina Eller-Vainicher¹, Valentina Morelli¹, Antonio Stefano Salcuni¹, Massimo Torlontano², Francesca Coletti³, Laura Iorio⁴, Antonello Cuttitta⁵, Angelo Ambrosio⁵, Leonardo Vicentini⁶, Vincenzo Carnevale⁷, Paolo Beck-Peccoz¹, Maura Arosio^{1,3}, Bruno Ambrosi⁴, Alfredo Scillitani² and Iacopo Chiodini¹

¹Unit of Endocrinology and Diabetology, Department of Medical Sciences, University of Milan, Fondazione Policlinico IRCCS, Pad. Granelli, Via F. Sforza 35, 20122 Milan, Italy, ²Unit of Endocrinology, 'Casa Sollievo della Sofferenza' IRCCS, San Giovanni Rotondo, Foggia, Italy, ³Unit of Endocrinology, Ospedale San Giuseppe di Milano, Milan, Italy, ⁴Unit of Endocrinology, Department of Medical and Surgical Sciences, University of Milan, IRCCS Policlinico San Donato Institute, San Donato Milanese, Milan, Italy, ⁵Unit of Surgery, 'Casa Sollievo della Sofferenza' IRCCS, San Giovanni Rotondo, Foggia, Italy, ⁶Surgery Unit, Fondazione Policlinico IRCCS, Milan, Italy and ⁷Unit of Internal Medicine 'Casa Sollievo della Sofferenza' IRCCS, San Giovanni Rotondo, Foggia, Italy

(Correspondence should be addressed to I Chiodini at Unit of Endocrinology and Diabetology; Email: iacopo.chiodini@email.it)

Abstract

Objective: Few data are available regarding the need of steroid substitutive therapy after unilateral adrenalectomy for adrenal incidentaloma (AI). It is unknown whether, before surgery, the hypothalamic–pituitary–adrenal (HPA) axis secretion parameters can predict post-surgical hypocortisolism.

Aim: This study aimed to evaluate whether, in AI patients undergoing unilateral adrenalectomy, post-surgical hypocortisolism could be predicted by the parameters of HPA axis function.

Design: Prospective, multicenter.

Methods: A total of 60 patients underwent surgical removal of AI (surgical indication: 29 subclinical hypercortisolism (SH); 31 AI dimension). Before surgery, SH was diagnosed in patients presenting at least three criteria out of urinary free cortisol (UFC) levels > 60 µg/24 h, cortisol after 1-mg dexamethasone suppression test (1 mg-DST) > 3.0 µg/dl, ACTH levels < 10 pg/ml, midnight serum cortisol (MSC) > 5.4 µg/dl.

Two months after surgery, HPA axis function was assessed by low dose ACTH stimulation test or insulin tolerance test when needed: 39 patients were affected (Group B) and 21 were not affected (Group A) with hypocortisolism. The accuracy in predicting hypocortisolism of pre-surgical HPA axis parameters or their combinations was evaluated.

Results: The presence of > 2 alterations among 1 mg-DST > 5.0 µg/dl, ACTH < 10 pg/ml, elevated UFC and MSC has the highest odds ratio (OR) for predicting post-surgical hypocortisolism (OR 10.45, 95% confidence interval, CI 2.54–42.95, $P=0.001$). Post-surgical hypocortisolism was predicted with 100% probability by elevated UFC plus MSC levels, but not ruled out even in the presence of the normality of all HPA axis parameters.

Conclusion: Post-surgical hypocortisolism cannot be pre-surgically ruled out. A steroid substitutive therapy is indicated after unilateral adrenalectomy for SH or size of the adenoma.

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Introduction

In the recent years, the wider application and technical improvement of imaging diagnostic tools, led to the frequent finding of incidentally discovered adrenal masses, commonly defined as 'adrenal incidentalomas' (AI) (1–6). This term covers a wide range of disorders, adrenocortical adenoma being the most frequent. Most incidentalomas are clinically silent and non-functioning. However, in about 5–30% of patients, endocrine work-up reveals abnormalities of the

hypothalamic–pituitary–adrenal (HPA) axis function, in keeping with a subtle cortisol hypersecretion, defined as 'subclinical Cushing's syndrome' or 'subclinical hypercortisolism' (SH) (7, 8). The true SH prevalence is unknown, due to the lack of widely accepted biochemical criteria for defining this condition (7–11).

It is unknown whether, after the surgical removal of an AI, steroid substitutive therapy is needed in all patients or only in those with a pre-surgical demonstration of SH. Indeed, some patients with AI develop post-surgical adrenal insufficiency, but others

do not (7, 10, 11). However, so far, no studies specifically investigated the possible association between the pre-surgical biochemical diagnosis of SH and the subsequent presence of post-surgical hypocortisolism. The prevalence of post-surgical hypocortisolism in patients, with and without SH, who underwent unilateral adrenalectomy for AI, varies depending on the criteria used to diagnose SH (12–18). In addition, most studies considered only post-surgical basal cortisol level or the presence of symptoms suggesting adrenal failure as a diagnostic tool for post-surgical hypocortisolism (12, 15, 17, 18). However, a proper approach includes evaluation of cortisol level in basal conditions plus stimulation tests, such as low dose ACTH test or insulin tolerance test (ITT), the latter being the 'gold standard' for the diagnosis of adrenal insufficiency (19–21). As a consequence of this lack of knowledge, there is no consensus regarding the management of patients who are undergoing unilateral adrenalectomy for AI. Indeed, some authors suggest to give glucocorticoid substitutive therapy only to patients with SH (5, 7), while others recommend a steroid coverage in all (10, 17).

In order to get further insight on this topic, the aim of this study was to assess in patients undergoing unilateral adrenalectomy for AI, the reliability of the currently used parameters for the biochemical diagnosis of SH for predicting post-surgical adrenal insufficiency.

Patients and methods

Patients

The study was performed in four referral Italian Endocrinology Units. From December 2003 to December 2008, we studied 319 consecutive patients affected with unilateral AI. Diagnosis of AI was based on the detection of a unilateral adrenal mass by non-invasive imaging methods of the abdomen, performed for unrelated disease. No subject had evidence of metastatic diseases. At computed tomography (CT), all adrenal masses were homogeneous, hypodense (unenhanced CT attenuation ≤ 10 Hounsfield units) and with well-shaped features consistent with the diagnosis of adrenocortical adenomas.

We excluded from the study: i) 61 patients due to the administration of drugs or presence of diseases known to affect corticosteroid metabolism or cortisol secretion (i.e. thyrotoxicosis, bowel diseases, chronic renal failure, chronic hepatic disease, depression, alcoholism, eating disorders, rheumatological or hematological diseases; ii) 18 patients diagnosed as affected with pheochromocytoma ($n=8$) or aldosteronoma ($n=10$); iii) signs or symptoms specific of cortisol excess (moon facies, striae rubrae, skin atrophy and buffalo hump).

Among the remaining 240 AI patients, 180 presenting normal HPA axis and small AI size were kept in follow-up. The remaining 60 AI patients underwent

surgical removal of the adrenal mass due to the presence of SH or on the basis of the size of the lesion, and were included in the study. No patient showed signs or symptoms specific of cortisol excess, and in all AI subjects, the determination of 24-h urinary catecholamines and aldosterone/plasma renin activity ratio excluded the presence of pheochromocytoma and aldosteronoma respectively.

In all patients, at baseline, we measured at least once: basal morning (0800 h) ACTH levels; urinary free cortisol (UFC) levels; midnight serum cortisol (MSC) levels; morning (0900 h) serum cortisol after 1-mg dexamethasone suppression test (1 mg-DST). For each subject, the mean values of these latter parameters were reported if more than one determination was available during pre-surgical follow-up. The period of follow-up before surgery lasted from 15 days to 24 months.

In 29 patients, the surgical operation was decided on the basis of biochemical data strongly suggesting SH, such as at least three criteria out the following four criteria: i) UFC levels $> 60 \mu\text{g}/24 \text{ h}$ (165 nmol/l), ii) 1 mg-DST $> 3.0 \mu\text{g}/\text{dl}$ (83 nmol/l), iii) ACTH levels $< 10 \text{ pg/ml}$ (2.2 pmol/l) and iv) MSC $> 5.4 \mu\text{g}/\text{dl}$ (149 nmol/l). The cut-off value of $60 \mu\text{g}/24 \text{ h}$ (165.6 nmol/d) for UFC corresponds to the 97th percentile of a reference population for UFC. The use of a 1 mg-DST cut-off of $3.0 \mu\text{g}/\text{dl}$ (83 nmol/l) and not $5.0 \mu\text{g}/\text{dl}$ (138 nmol/l) as recommended by NIH (5) was decided in order to increase sensitivity. The cut-off value of $5.4 \mu\text{g}/\text{dl}$ (149 nmol/l) for MSC has been proposed by Terzolo *et al.* for diagnosing SH (22).

In 31 patients, the surgical option was considered mandatory mainly on the basis of the increasing dimensions ($> 1 \text{ cm}$ increase during 12 months of follow-up) or a size larger than 4 cm at the diagnosis. None of these patients showed fluctuating SH.

All patients underwent surgical excision of the adrenal mass. Laparoscopic or laparotomic adrenalectomy was performed depending on the size of the adrenal adenoma and the clinical characteristics of subjects. No patient had peri-operative and post-operative complications. In all patients, the histological findings were consistent with adrenocortical adenoma.

Considering that adrenal failure occurs more frequently in the presence of stressful events, in all patients a precautionary steroid therapy during surgery with hydrocortisone (100 mg i.v.) and immediately after operation with cortisone acetate *per os* (at weight-related dosing ranging between 25 and 37.5 mg/day in three subdivided doses during the day) was administered, in order to avoid the possible consequences of an undiagnosed HPA insufficiency (23). The commonly used cortisone acetate dose was 25 mg/day (51 patients), while higher doses of 31.3 and 37.5 mg/day were used in seven and two patients respectively. After 2 months, cortisol secretion was assessed by low dose ACTH stimulation test (LDCT), after a 24-h steroid therapy withdrawal, as previously

described (19, 20). We decided to post-operatively re-evaluate the patients at 2 months in order to be sure that a post-operative stress condition was not still present. In stressful condition, indeed, the commonly used cut-off of LDCT for diagnosing adrenal insufficiency might be different (24) and, in acute illnesses, LDCT has not been validated (25). No patient presented with any withdrawal symptoms after discontinuing cortisone acetate after 24 h. Twenty-one patients with baseline cortisol $> 5 \mu\text{g/dl}$ (138 nmol/l) and stimulated cortisol levels $> 22 \mu\text{g/dl}$ (600 nmol/l) were considered not affected with adrenal insufficiency (Group A), while 34 patients with baseline cortisol $< 5 \mu\text{g/dl}$ (138 nmol/l) and/or stimulated cortisol levels $< 16 \mu\text{g/dl}$ (440 nmol/l) were considered hypoadrenal (Group B, Fig. 1). In patients with normal response to LDCT, steroid substitutive therapy was withdrawn. Subsequently, none of these patients presented with symptoms suggesting adrenal failure. Five patients with undefined response to LDCT, such as baseline cortisol $> 5 \mu\text{g/dl}$ (138 nmol/l) and stimulated cortisol levels between 16 and $22 \mu\text{g/dl}$ (440–600 nmol/l), underwent ITT (21, 25). All these latter subjects showed a reduced cortisol response after ITT, such as stimulated cortisol levels $< 18 \mu\text{g/dl}$ (500 nmol/l) at any time during the test in the presence of symptomatic hypoglycemia and plasma glucose $< 40 \text{ mg/dl}$ (2.2 mmol/l) (21, 25). They were, therefore, included in Group B that was finally constituted by 39 patients.

In 16 out of the 21 patients with normal cortisol secretion, basal cortisol levels measured before ACTH stimulation were $< 16 \mu\text{g/dl}$ (440 nmol/l), thus rendering the LDCT mandatory to exclude hypocortisolism.

Figure 1 Individual data of basal and cortisol levels after LDCT in 39 patients with post-operative adrenal insufficiency. LDCT, low dose ACTH stimulation test. Thirty-four patients with baseline cortisol $< 5 \mu\text{g/dl}$ (continuous line) and/or stimulated cortisol levels $< 16 \mu\text{g/dl}$ (dotted line) were considered hypoadrenal. Five patients with undefined response to LDCT, such as baseline cortisol $> 5 \mu\text{g/dl}$ and stimulated cortisol levels between 16 and $22 \mu\text{g/dl}$ (discontinuous line), underwent insulin tolerance test (ITT). All these latter subjects showed a reduced cortisol response after ITT and were considered affected with hypocortisolism.

Finally, after 6 months, all hypoadrenal patients were retested by LDCT, and six patients showed a normal cortisol response.

All subjects gave their witnessed informed consent before entering the study that was approved by local ethical committees and in accordance with Helsinki Declaration II.

Methods

Serum and urinary samples were collected and stored at -20°C until assayed. In all patients, plasma ACTH levels (mean of three determinations at 20-min intervals) were measured by immunoradiometric assay (BRAHMS Diagnostica GmbH, Berlin, Germany), and serum cortisol and UFC levels (after dichloromethane extraction) were determined immunofluorometrically by TDX-FLX Abbott, GmbH, Diagnostika kits. The assays used were the same in all centres. The intra- and inter-assay coefficients of variation for all assays were < 5 and 10% respectively.

LDCT was performed in the morning with patients fasting. Tetracosactrin (Synacthen, Novartis Pharma) was administered intravenously in a $1\text{-}\mu\text{g}$ dose, after being prepared using the method of Dickstein *et al.* (19). Serum cortisol was measured at baseline and at 30 min after the injection (19, 20).

The ITT was performed administrating short-acting regular insulin i.v. at 0.1 U/kg dose, and measuring serum glucose and cortisol levels at baseline and at 30, 45 and 60 min after the injection (21, 25).

Clinical examinations included weight and height with calculation of body mass index (BMI). The presence of blood hypertension, type 2 diabetes mellitus, obesity, dyslipidemia and metabolic syndrome was recorded, following the ATP-III criteria (26). In all patients, bone mineral density (BMD) was measured by Dual energy X-ray Absorptiometry (Hologic Discovery, Waltham, MA, USA) at spine (DXA L1–L4, *in vivo* precision 1.0%) and femur (*in vivo* precision 1.8 and 2.3% respectively).

Statistical analysis

Statistical analysis was performed by SPSS version 12.0 statistical package (SPSS Inc., Chicago, IL, USA). The results are expressed as mean $\pm$ s.d. if not differently specified.

Comparison of continuous variables between Group A and Group B was performed using Student's *t*-test, with log transformation of not normally distributed variables. Categorical variables between the two groups were compared by χ^2 test.

We calculated the specificity, sensitivity, positive and negative predictive value (PPV and NPV respectively), and accuracy of the most commonly used parameters or combination of parameters of HPA axis function measured before surgery in predicting post-surgical

adrenal insufficiency. Apart from criteria utilized to define SH before surgery (see methods section), we considered also 1 mg-DST $> 1.8 \mu\text{g/dl}$ (50 nmol/l) and $> 5.0 \mu\text{g/dl}$ (138 nmol/l), which are additional criteria commonly used for diagnosing hypercortisolism.

In all patients, logistic regression analysis assessed the association between the presence of post-surgical adrenal insufficiency (dependent variable, expressed as categorical variable) and the parameters or combination of parameters of HPA axis activity measured before surgery (independent variables, expressed as categorical variable, i.e. below or above the chosen cut-off), adjusting for age, BMI, duration of pre-surgical follow-up and indication for which surgery was decided.

P values of < 0.05 were considered significant.

Results

Clinical characteristics of patients with and without post-surgical hypocortisolism

The clinical characteristics of all patients and the comparison between patients who did not experienced post-surgical hypocortisolism (Group A) and those who did (Group B) are reported in Table 1.

Age, BMI, diameter of adenoma and duration of pre-surgical follow-up were not different between the two groups. Similarly, the indication for which surgery was chosen (i.e. size of adenoma or suspected SH) was comparable. The prevalence of patients with hypertension, type 2 diabetes, obesity, dyslipidemia and metabolic syndrome, and reduced BMD was also similar between patients with and without post-surgical

hypocortisolism. Among the HPA axis function's parameters, only MSC levels were different, being significantly higher in Group B than in Group A, whereas UFC, 1 mg-DST and ACTH levels were comparable. Twelve patients had both elevated UFC and elevated MSC, but only five patients showed a UFC higher than $90 \mu\text{g}/24 \text{ h}$ (248 nmol/l) and only two patients (included in Group B) of these latter showed MSC levels higher than $7.5 \mu\text{g/dl}$ (206 nmol/l). Our results were confirmed also after excluding these two patients from the analysis (data not shown).

The clinical characteristics of patients operated on for SH and for the size ($> 4 \text{ cm}$ or increasing $> 1 \text{ cm/year}$) of the adrenal mass were similar (Table 2).

One out of the three patients with completely normal HPA axis function and five out of nine patients showing only one altered parameter of HPA axis function before surgery developed post-operative hypocortisolism. Therefore, 6 out of 12 patients, in whom the presence of SH would have been excluded before surgery on the basis of biochemical parameters of HPA axis function, in fact, developed post-surgical hypocortisolism.

Eight patients who underwent surgery for SH had also an adrenal mass $> 4 \text{ cm}$ at diagnosis. Among these patients, five experienced post-surgical hypocortisolism, while three did not.

Diagnostic accuracy of various criteria of SH in predicting post-surgical hypocortisolism

The sensitivity, specificity, PPV and NPV, and accuracy for predicting post-surgical hypocortisolism of the various biochemical parameters measured before

Table 1 Pre-surgical clinical and biochemical features of patients with adrenal incidentalomas.

	All patients (n=60)	Group A (n=21)	Group B (n=39)	*P values
Females/males	46/14	15/6	31/8	0.532
Age (years)	55.9 ± 10.8 (24–75)	57.2 ± 8.3 (33–75)	55.3 ± 12.0 (24–74)	0.505
BMI (kg/m^2)	28.2 ± 5.6 (17.4–41.0)	29.6 ± 5.4 (21.5–41)	27.4 ± 5.6 (17.4–41)	0.163
Reason for surgery (size of the mass/SH)	31/29	13/8	18/21	0.287
Pre-surgical follow-up (months)	5.9 ± 5.5 (0.5–24)	6.6 ± 6.0 (0.5–24)	5.5 ± 5.2 (0.5–19.6)	0.489
ACTH (pg/ml)	9.4 ± 5.2 (1.9–26.9)	9.0 ± 4.4 (5.0–23.8)	9.6 ± 5.7 (1.9–26.9)	0.665
1 mg-DST ($\mu\text{g/dl}$)	4.1 ± 3.1 (0.9–13.7)	3.2 ± 2.1 (0.9–9.0)	4.6 ± 3.5 (1.2–13.7)	0.098
UFC ($\mu\text{g}/24 \text{ h}$)	62.3 ± 41.7 (10.0–189.6)	51.3 ± 32.4 (10.0–120.0)	68.2 ± 45.2 (10.4–189.6)	0.136
Midnight serum cortisol ($\mu\text{g/dl}$)	5.1 ± 3.0 (1.0–12.5)	3.4 ± 2.0 (1.0–8.0)	6.0 ± 3.1 (1.2–12.5)	0.001
Diameter of adenoma (cm)	3.4 ± 1.2 (1.2–9.0)	3.2 ± 1.0 (1.5–5.7)	3.5 ± 1.3 (1.2–9.0)	0.340
Patients with type 2 diabetes mellitus (%)	14 (23.3)	5 (23.8)	9 (23.1)	1.000
Patients with arterial hypertension (%)	33 (55.0)	15 (71.4)	18 (46.2)	0.102
Patients with obesity (%)	25 (41.7)	10 (47.6)	15 (38.5)	0.587
Patients with dyslipidemia	22 (36.7)	10 (47.6)	12 (30.8)	0.263
Patients with metabolic syndrome	11 (18.3)	6 (28.6)	5 (12.8)	0.169
Patients with BMD T-score < -2.5 (%)	28 (46.7)	6 (28.6)	22 (56.4)	0.058

Group A, patients not affected with post-surgical adrenal failure; Group B, patients affected with post-surgical adrenal failure; BMI, body mass index; 1 mg-DST, serum cortisol after 1-mg dexamethasone suppression test (SI conversion factor 27.59: nmol/l); UFC, urinary free cortisol (SI conversion factor 2.76: nmol/24 h); ACTH (SI conversion factor 0.22: pmol/l); SH, subclinical hypercortisolism (suspected before surgery); BMD, bone mineral density. *Group B versus Group A.

Table 2 Clinical features of patients with adrenal incidentalomas operated on for the presence of subclinical hypercortisolism (SH) or for the size (>4 cm or increasing >1 cm/year) of the adrenal mass.

	Group SH (n=29)	Group size (n=31)	P value
Females/males	21/8	25/6	0.547
Age (years)	54.1 ± 10.6 (33–70)	57.7 ± 11.0 (24–75)	0.206
BMI (kg/m ²)	29.2 ± 6.1 (20.6–41)	27.2 ± 5.1 (17.4–41)	0.183
ACTH (pg/ml)	8.4 ± 4.8 (5.0–23.8)	10.4 ± 5.6 (1.9–26.9)	0.136
1 mg-DST (µg/dl)	5.2 ± 3.7 (1.1–13.7)	3.1 ± 2.0 (0.9–9.9)	0.006
UFC (µg/24 h)	69.3 ± 47.3 (10.0–180.0)	55.7 ± 35.2 (10.4–189.6)	0.212
Midnight serum cortisol (µg/dl)	6.2 ± 3.1 (1.0–12.5)	4.7 ± 3.5 (1.3–12.5)	0.175
Diameter of adenoma (cm)	2.8 ± 0.7 (1.2–3.7)	4.01 ± 1.3 (1.5–9.0)	0.0001
Patients with type 2 diabetes mellitus (%)	10 (34.5)	4 (12.9)	0.068
Patients with arterial hypertension (%)	14 (48.3)	19 (61.3)	0.437
Patients with obesity (%)	14 (48.3)	11 (35.5)	0.433
Patients with dyslipidemia	8 (27.6)	14 (45.2)	0.188
Patients with metabolic syndrome	6 (20.7)	5 (16.1)	0.745
Patients with BMD T-score < -2.5 (%)	17 (58.6)	11 (35.5)	0.120

Group SH, patients operated on for subclinical hypercortisolism (SH); Group size, patients operated on for size (>4 cm or increasing >1 cm/year) of the adrenal mass. BMI, body mass index; 1 mg-DST, serum cortisol after 1-mg dexamethasone suppression test (SI conversion factor 27.59: nmol/l); UFC, urinary free cortisol (SI conversion factor 2.76: nmol/24 h); ACTH (SI conversion factor 0.22: pmol/l); SH, subclinical hypercortisolism (suspected before surgery); BMD, bone mineral density.

surgery, alone or in combination, are reported in Table 3. Only the criteria V, XIII, XXI and XXII had an accuracy of more than 66% (i.e. correctly predicted post-surgical hypocortisolism in at least 2/3 of cases).

The simultaneous presence of elevated UFC and MSC levels before surgery predicted with 100% probability the presence of hypocortisolism after surgery, but at the

expense of a very low sensitivity (30.8%). Conversely, the normality of all parameters of HPA axis activity before surgery could not rule out with 100% probability the appearance of post-surgical adrenal insufficiency. Interestingly, after excluding MSC, all combinations of the remaining parameters did not show enough reliability for predicting hypocortisolism after surgery.

Table 3 Diagnostic accuracy of various parameters and combinations of parameters of pituitary–adrenal axis activity in predicting the presence of hypocortisolism after surgery.

Criterion	Parameters	Sensitivity	Specificity	PPV	NPV	Accuracy
I	A	79.5	23.8	66.0	38.5	60.0
II	B	59.0	52.4	69.7	40.7	56.0
III	C	33.3	85.7	81.3	40.9	51.6
IV	D	48.7	81.0	82.6	45.9	60.0
V	E	64.1	81.0	86.2	54.8	70.0
VI	F	64.1	38.0	65.8	36.4	55.0
VII	A + F	56.4	47.6	66.7	37.0	53.3
VIII	B + F	41.0	66.7	69.6	37.8	50.0
IX	C + F	30.8	85.7	80	40	50.0
X	A + D	33.3	81.0	76.5	39.5	50.0
XI	B + D	23.1	90.5	81.8	47.4	46.6
XII	C + D	10.3	90.5	66.7	35.2	38.3
XIII	A + E	59.0	81.0	85.2	51.5	66.6
XIV	B + E	48.7	85.7	86.4	47.4	61.6
XV	C + E	33.3	95.2	92.9	43.5	55.0
XVI	D + E	30.8	100.0	100.0	43.8	55.0
XVII	2 out of A, D, F	69.2	42.9	69.2	42.9	60.0
XVIII	2 out of B, D, F	56.4	61.9	73.3	43.3	58.3
XIX	2 out of C, D, F	48.7	81.0	82.6	45.9	60.0
XX	2 out of A, D, E, F	82.1	33.3	69.6	50.0	65.0
XXI	2 out of B, D, E, F	74.4	57.1	76.3	54.5	68.3
XXII	2 out of C, D, E, F	69.2	76.2	84.4	57.1	71.7
XXIII	3 out of A, D, E, F	56.4	76.2	81.5	48.5	63.3
XXIV	3 out of B, D, E, F	46.2	85.7	85.7	46.2	60.0
XXV	3 out of C, D, E, F	38.5	85.7	83.3	42.9	55.0
XXVI	1 out of A, D, E, F	97.4	9.5	66.7	66.7	66.0

Data are expressed as percentage, with the exception of P values. A, 1 mg-DST > 1.8 µg/dl (50.0 nmol/l); B, 1 mg-DST > 3.0 µg/dl (83 nmol/l); C, 1 mg-DST > 5.0 µg/dl (138 nmol/l); D, elevated UFC > 60.0 µg/24 h (65 nmol/24 h); E, midnight serum cortisol > 5.4 µg/dl (149 nmol/l); F, ACTH < 10 pg/ml (2.2 pmol/l). PPV, positive predictive value; NPV, negative predictive value. Criteria with at least 66% of accuracy are reported in bold.

Table 4 Odds ratio for predicting post-surgical hypocortisolism after adjusting for confounding factors of the parameters or combination of parameters of hypothalamic–pituitary–adrenal axis secretion with at least 66% of diagnostic accuracy.

	OR	95% CI	P
Criterion V: E (presence/absence)	9.54	2.26–40.13	0.002
Age (1 year increase)	1.01	0.95–1.08	0.800
BMI (1 kg/m ² increase)	0.92	0.82–1.04	0.172
Diameter of adrenal adenoma (1 cm increase)	1.32	0.74–2.37	0.349
Pre-surgical follow-up (1 month increase)	1.00	0.90–1.12	0.954
Criterion XIII: A + E (presence/absence)	8.06	1.89–34.57	0.005
Age (1 year increase)	1.01	0.95–1.08	0.791
BMI (1 kg/m ² increase)	0.92	0.82–1.03	0.149
Diameter of adrenal adenoma (1 cm increase)	1.36	0.75–2.44	0.310
Pre-surgical follow-up (1 month increase)	0.99	0.89–1.11	0.895
Criterion XXI: 2 out of B, D, E, F (presence/absence)	4.95	1.43–17.08	0.011
Age (1 year increase)	1.00	0.94–1.05	0.855
BMI (1 kg/m ² increase)	0.92	0.83–1.03	0.139
Diameter of adrenal adenoma (1 cm increase)	1.36	0.76–2.43	0.303
Pre-surgical follow-up (1 month increase)	0.97	0.88–1.08	0.621
Criterion XXII: 2 out of C, D, E, F (presence/absence)	10.45	2.54–42.95	0.001
Age (1 year increase)	1.01	0.94–1.07	0.861
BMI (1 kg/m ² increase)	0.91	0.81–1.02	0.110
Diameter of adrenal adenoma (1 cm increase)	1.41	0.77–2.60	0.266
Pre-surgical follow-up (1 month increase)	0.97	0.87–1.09	0.645

A, 1 mg-DST > 1.8 µg/dl (50 nmol/l); B, 1 mg-DST > 3.0 µg/dl (83 nmol/l); C, 1 mg-DST > 5.0 µg/dl (138 nmol/l); D, elevated UFC > 60.0 µg/24 h (165 nmol/l); E, midnight serum cortisol > 5.4 µg/dl (149 nmol/l); F, ACTH < 10 pg/ml (2.2 pmol/l). CI, confidence interval.

The presence of at least two alterations among 1 mg-DST > 5.0 µg/dl (138.0 nmol/l), elevated UFC, elevated MSC and ACTH < 10 pg/ml (2.2 pmol/l) was associated with the highest odds ratio for predicting post-surgical hypocortisolism (criterion XXII in Table 4). Even if of no great value for the individual patient, this finding is interesting since this association was independent of age, BMI, size of the adenoma and duration of pre-surgical follow-up. This latter variable has been added since a longer exposure to a cortisol excess may lead to a higher HPA axis inhibition.

Since an oral replacement dose of > 25 mg/day of cortisone acetate could be suppressive in some patients, we repeated our analyses, considering only patients who were given 25 mg/day of cortisone acetate (51 patients). The results of the analyses in this subgroup did not differ from those obtained considering the whole sample of 60 patients (data not shown).

Discussion

To our knowledge, this is the first study specifically aimed to assess the diagnostic accuracy of the most commonly used parameters or combination of parameters of HPA axis function in predicting post-surgical hypocortisolism, in patients who underwent surgical excision of an incidentally discovered adrenal mass. We found that MSC as single parameter, the simultaneous presence of altered MSC and 1 mg-DST (> 1.8 µg/dl, 50 nmol/l), or of at least two altered parameters among MSC, UFC, ACTH and 1 mg-DST

(> 3.0 µg/dl, 83 nmol/l, or > 5.0 µg/dl, 138 nmol/l) suggests the possible presence of post-surgical adrenal failure. Indeed, we demonstrated that any patient with elevated UFC and MSC levels has a 100% probability to develop post-surgical adrenal insufficiency. Conversely, no parameters or combination of parameters have enough diagnostic accuracy to reliably exclude the possibility of post-surgical hypocortisolism, which can occur in up to the 50.0% of patients with no or only one altered parameter of HPA axis secretion.

It is widely recognized that post-surgical adrenal failure may be a life-threatening condition, but no widely accepted guidelines are available regarding the need of substitutive steroid therapy after surgical removal of an AI (10, 11). Indeed, some authors recommend a substitutive steroid therapy in all patients (10, 17), while others only in those with pre-surgically suspected SH (5, 7, 18). This discordance depends on the fact that the prevalence of post-surgical hypocortisolism has been differently estimated in the different series, even in the more recent studies (12–18). In our opinion, this could be firstly due to the characteristics of the disease *per se*. It is likely, indeed, that in SH, the slightly elevated and/or intermittent cortisol hypersecretion may be not reliably mirrored by the commonly employed biochemical markers (11, 22, 27, 28) and that the difference in the individual hypothalamic–pituitary sensitivity to cortisol may play a crucial role in the presence of a mild hypercortisolism (29). Secondly, the available studies are often not comparable due to the different biochemical criteria used to define SH.

The present study, by evaluating in a homogeneous series of patients with unilateral AI the diagnostic accuracy of the commonly used biochemical criteria of SH in predicting adrenal insufficiency after adrenalectomy, clearly supports the need of a precautionary steroid therapy in patients who undergo adrenalectomy for SH and/or for the size (>4 cm or increasing >1 cm/year) of the adrenal mass, regardless for the presence of a normal cortisol secretion before surgery. This is further confirmed by the lack of difference in the prevalence of post-surgical hypocortisolism among patients operated on for the size of the adenoma or for a suspected SH. In keeping with this, we did not find any correlation between the occurrence of hypocortisolism and the size of the adenoma. This finding is in contrast with the study of Mitchell and co-authors (18) who found that patients with adenoma larger than 5.3 cm of diameter were at greater risk for post-surgical hypocortisolism. However, it is likely that this discordance depends on the fact that the criterion used for diagnosing hypocortisolism in that study (i.e. only the measurement of basal cortisol levels) was different from those used in the present one (i.e. LDCT test and ITT).

As far as the assessment of adrenal function is concerned, it is known that LDCT test does not have 100% sensitivity, but for screening purposes, it is much more convenient and safer than metyrapone or ITT ones (21, 25). It must also be considered that in the present study, we decided to re-evaluate adrenal function 2 months after surgery, in order to be sure that a possible stressful condition was not still present, which could render the results of LDCT difficult to interpret. On the basis of the present data, however, 35% of patients were shortly given an unnecessary steroid treatment. Therefore, it is possible to argue that an earlier post-operative assessment would be more appropriate in order to save a substantial number of patients from unnecessary steroid therapy. On the other hand, some patients may experience a transient hypocortisolism, which recovers within 2 months after surgery. Thus, evaluating cortisol secretion 2 months after operation may have led to ignore some cases of transient hypocortisolism. It must also be considered that, the specificity of the ACTH stimulation test is not 100% and, therefore, the incidence of adrenal insufficiency could have been overestimated, and, in turn, the specificity of the preoperative tests underestimated.

The lack of association in the present study between the presence of post-operative adrenal failure and the possible chronic complications of subtle cortisol excess (i.e. metabolic syndrome and osteoporosis) may be considered surprising. Indeed, if the presence of post-surgical hypocortisolism has to be considered a reliable index of pre-surgical subtle hypercortisolism, it would be expected that the complications of cortisol excess would be more frequent in subjects experiencing post-operative adrenal insufficiency than in those

not experiencing it. Indeed, some of the previous cross-sectional studies suggested that also a subtle hypercortisolism may be associated with an increased prevalence of metabolic syndrome and osteoporosis (30–39), which are common, though not specific, features of overt cortisol excess. However, although the glucocorticoid sensitivity may be different in different tissues (29), it is conceivable that in some patients, this subtle cortisol hypersecretion may be enough to determine end-organ consequences but not to constantly suppress HPA axis function and, therefore, to lead to hypocortisolism after surgery. It must also be considered that the lack of association between the post-surgical hypocortisolism and the possible clinical consequences of SH could be attributed to the small sample size. Moreover, this lack of association can also be explained considering that 2 months of exogenous glucocorticoid substitutive therapy, especially if at higher dosing and with three daily doses regimen, could have suppressed HPA axis function at least in some patients. However, after the exclusion of the nine patients assuming the higher doses of cortisone acetate, the lack of association between the presence of post-operative adrenal failure and the chronic complications of cortisol excess persisted. Therefore, on the basis of the present data, it is also possible to argue that the presence of post-surgical hypocortisolism might be a specific but not a sensitive enough index of SH. Studies assessing the correlation between possible clinical scores for subtle cortisol excess, and the parameters or combination of parameters of HPA axis secretion are needed in order to better characterize the SH condition.

Notwithstanding the possible limitation in considering post-surgical hypocortisolism as a clinical marker of SH, the present study suggests that MSC might be of some help to suspect SH, as already pointed out by other authors (22). Indeed, the determination of MSC levels as a single parameter shows an acceptable specificity (81%) in predicting post-surgical adrenal insufficiency. In addition, all the other combination of parameters with good diagnostic accuracy included also the determination of MSC levels. On the other hand, the limit of MSC is related to its low sensitivity (64.2%). Therefore, the finding of MSC in the normal range cannot rule out the occurrence of post-operative hypocortisolism.

In conclusion, the present study shows that in patients who undergo excision of an AI, post-surgical hypocortisolism can be predicted by determining MSC alone or in combination with the other biochemical parameters of cortisol secretion, but it cannot be ruled out with the 100% probability before surgery. Therefore, a precautionary steroid substitutive therapy is indicated in all AI subjects who undergo unilateral adrenalectomy for SH and/or for the size of the adrenal mass regardless for the presence of a normal cortisol secretion before surgery.

Declaration of interest

C Eller-Vainicher, V Morelli, A Stefano Salcuni, M Torlontano, F Coletti, L Iorio, A Cuttitta, A Ambrosio, L Vicentini, V Carnevale, M Arosio, A Scillitani, I Chiodini have nothing to disclose.

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