Defining non-functioning adrenal adenomas on the basis of the occurrence of hypocortisolism after adrenalectomy.

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Abstract

Background: In patients with adrenal incidentalomas (AI), there is uncertainty on how to rule out hypercortisolism. The occurrence of post-surgical (unilateral adrenalectomy) hypocortisolism (PSH) has been proposed as a proof of the presence of a pre-surgical hypercortisolism in AI patients. The aim of this study was to define the thresholds of cortisol level after 1 mg overnight dexamethasone suppression test (F-1mgDST), urinary free cortisol (UFC), midnight serum cortisol (MSC) and adrenocorticotroph hormone (ACTH) able to predict the absence of PSH in AI patients undergoing surgery.

Methods: In 60 patients who underwent AI excision, cortisol secretion was assessed by low-dose corticotropin stimulation test or insulin tolerance test, when needed. We searched for the lowest pre-surgical value of F-1mgDST, UFC and MSC and the highest value for ACTH in AI patients with PSH as indexes of normal cortisol secretion.

Results: the lowest values of F-1mgDST, UFC and MSC and the highest value for ACTH in PSH patients were 1.2 µg/dL (33 nmol/L), 10.4 µg/24h (29 nmol/24h), 1.2 µg/dL (33 nmol/L) and 26.9 pg/ml (6 pmol/L), respectively, but only F-1mgDST <1.2 µg/dL (33 nmol/L) was able to predict the absence of PSH. Among AI patients with F-1mgDST <1.2 µg/dL (33 nmol/L) no subjects had diabetes mellitus and/or metabolic syndrome and these subjects tended to have a better metabolic profile than those with F-1mgDST ≥1.2 µg/dL (33nmol/L)

Conclusion: in AI patients a F-1mgDST <1.2 µg/dL (33 nmol/L) rules out PSH and could be used to exclude hypercortisolism in AI patients.

Précis: In patients with adrenal incidentalomas the cortisol after 1 mg overnight dexamethasone suppression test <1.2 µg/dL (33 nmol/L) excludes hypercortisolism.

Key words: cortisol, 1-mg overnight dexamethasone suppression test, adrenal incidentalomas, hypocortisolism

Introduction

In the recent years several studies showed that in patients with incidentally discovered adrenal masses (AI) but without the classical signs and symptoms of cortisol excess, the presence of a less severe hypercortisolism (previously called autonomous cortisol excess, possible autonomous cortisol excess or subclinical hypercortisolism) was associated with an increased mortality, mainly due to an elevated risk of cardiovascular events (1-5). In fact, these findings were somewhat expected, since in the last decades many studies showed an increased frequency of cardiovascular risk factors (5) and their reduction after adrenalectomy (6) in AI patients with less severe hypercortisolism.

Nevetheless, several works reported an increased risk of cardiovascular disease (7-11) or diabetes mellitus (12) even in patients with non-functioning AI (NFAI). In keeping, recently, the mortality associated with the presence of NFAI has been found to be similar to that in AI patients with a less severe hypercortisolism (13), and a metanalysis showed that adrenalectomy could reduce the risk of hypertension and diabetes mellitus even in patients with NFAI (6).

The unexpected finding of the increased cardiovascular risk even in NFAI patients was suggested to be due to an exaggerated cortisol response to stress (11), an increased secretion of steroid precursors (14-16), a cyclic cortisol secretion (17) and to variations in the individual cortisol sensitivity (18). Alternatively, it has been proposed that these tumours have a subtle cortisol (hyper)secretion (19, 20), that cannot be disclosed by the currently used parameters of hypothalamic-pituitary-adrenal (HPA) axis activity. If this was the case, the current cut-offs used for the definition of hypercortisolism in AI patients may be inadequate. This idea is in keeping with the fact that cortisol after dexamethasone suppression test with a cut-off set at 1.4-1.5 µg/dL (39-41 nmol/L), rather than at 1.8 µg/dL (50 nmol/L, as currently used) had the highest accuracy in predicting cardiovascular risk (11, 21) or the incidence of diabetes mellitus (12) in AI patients.

By definition, AI patients are asymptomatic for the classic signs and symptoms of hypercortisolism. However, the occurrence of post-surgical hypocortisolism (PSH) has been proposed as an indirect proof of the presence of an autonomous cortisol secretion by these tumours (5, 22, 23).

Therefore, the aim of the present study was to define the absence of hypercortisolism in AI by assessing the cut-offs of F-1mgDST and other parameters of HPA axis activity able to predict the absence of PSH in AI patients undergoing surgery.

Patients and Methods

Patients

The current study is based on the population of a previous study aimed to evaluate whether, in AI patients undergoing unilateral adrenalectomy, PSH could be predicted by the parameters of HPA axis function (22). In that study, performed from December 2003 to December 2008, 319 consecutive AI patients with unilateral AI and without signs and/or symptoms specific of cortisol excess (moon facies, striae rubrae, skin atrophy, buffalo hump) have been enrolled. In all patients, a unilateral adrenal mass was incidentally found by non-invasive imaging methods of the abdomen performed for other causes. No subject had evidence of metastatic diseases. At computed tomography all adrenal masses were consistent with the diagnosis of adrenocortical adenomas (i.e: homogeneous, hypodense below 10 Hounsfield units and with well-shaped features). We excluded 61 patients taking drugs or affected with diseases influencing cortisol metabolism and/or secretion (i.e. rheumatologic or haematological diseases, depression, alcoholism, eating disorders, bowel diseases, thyrotoxicosis and chronic renal and/or hepatic disease) and 18 patients with pheochromocytoma (n=8) or aldosterone-secreting adenoma (n=10). Among the remaining 240 AI patients, 60 underwent surgical removal of the adrenal mass due to the presence of hypercortisolism and/or on the basis of the size of the lesion and were included in the study. Figure 1 summarizes the protocol used.

In all patients, at baseline, the following parameters have been measured at least once: basal morning (08.00 h) adrenocorticotroph hormone (ACTH), urinary free cortisol (UFC), midnight serum cortisol (MSC) and F-1mgDST levels. For each subject, the mean values of these parameters were reported, if more than one determination was available during the pre-surgical follow-up, which lasted from 15 days to 24 months.

In 31 patients, surgery was considered the best therapeutic option on the basis of the increasing dimensions (>1 cm increase during 12 months of follow up) or a size larger than 4 cm at the diagnosis. None of these patients had a clearly fluctuating cortisol secretion. In 29 patients, even

though completely asymptomatic for hypercortisolism, the surgical operation was decided on the basis of biochemical data strongly suggesting the presence of cortisol excess, such as the presence of ≥3 criteria out the following : 1) UFC levels >60 μg/24h (165 nmol/24h, the 97th percentile of a reference population for UFC), 2) F-1mgDST >3.0 μg/dL (83 nmol/L), 3) ACTH levels <10 pg/mL (2.2 pmol/L), iv) MSC >5.4 μg/dL (149 nmol/L), as suggested by other authors (24).

Laparoscopic or laparotomic adrenalectomy was decided by the endocrine surgeon, depending on the size of the adrenal adenoma and the patients' clinical characteristics. In all cases histology were consistent with adrenocortical adenoma. No patient had peri-operative and post-operative complications.

Since adrenal failure occurs is more frequent during stressful events, and following the recommendations of the Italian Association of Clinical Endocrinologists (24), we administered in all patients a precautionary steroid therapy during surgery with hydrocortisone [100 mg i.v., on the basis of the

anesthesiological protocol used in our Hospital, at the time of the study] and immediately after operation with cortisone acetate per os (at weight-related dosing ranging between 25 and 37.5 mg/day in 3 subdivided doses during the day corresponding to 20-30 mg/day hydrocortisone), in order to avoid the possible consequences of an undiagnosed hypoadrenalism (24). The starting cortisone acetate dose was weight-adjusted (corresponding to ~0.3 mg/kg per day hydrocortisone). The commonly used cortisone acetate dose was 25 mg/day (corresponding to hydrocortisone 20 mg/day, 51 patients), while higher doses of 31.3 and 37.5 mg/day (corresponding to 25 and 30 mg/day hydrocortisone) were used in 7 and 2 patients, respectively.

In all patients, after about 60 days, the cortisol secretion was assessed by low dose corticotropin stimulation test (LDCT), after a 24-hours steroid therapy withdrawal (25). The timing of the assessment of HPA axis function had been decided to avoid the persistence of a post-operative stress condition, as in this setting LDCT has not been validated (26, 27). At the time of the study we used the cut-offs of 16 μg/dl (440 nmol/L) and 22 μg/dL (600 nmol/L) for diagnosing PSH and for ruling out PSH, respectively on the basis of data showing that LDCT stimulated cortisol values less

than 16 μg/dL (440 nmol/L) best predicted adrenal insufficiency, whereas values greater than 22 μg/dL (600 nmol/L) best predicted a normal adrenal function with a good accuracy (area under the curve 0.94, 95% confidence interval 0.90 – 0.94) (26)

In 21 patients with baseline cortisol >5 μg/dL (138 nmol/L) and stimulated cortisol levels >22 μg/dL (600 nmol/L), PSH was ruled out and the steroid substitutive therapy was withdrawn, with none of these patients having symptoms suggesting PSH. At variance, 34 patients with baseline cortisol <5 μg/dL (138 nmol/L) and/or stimulated cortisol levels <16 μg/dL (441 nmol/L) were considered affected with PSH. Five patients with baseline cortisol >5 μg/dL (138 nmol/L) and LDCT stimulated cortisol levels between 16 μg/dL (441 nmol/L) and 22 μg/dL (600 nmol/L) underwent insulin tolerance test (ITT) (27, 28). The latter subjects showed a reduced cortisol response after ITT [i.e stimulated cortisol levels <18 μg/dL (496 nmol/L), at any time during the test in the presence of symptomatic hypoglycaemia and plasma glucose <40 mg/dL (2.22 mmol/L)] (27, 28) and were, therefore, diagnosed as having PSH.

We decided to use an higher cut-off (i.e. 22 μg/dL, 600 nmol/L, rather than 18 μg/dL, 500 nmol/L) in order to improve sensitivity. Indeed, an higher cut-off may be needed in patients after withdrawal of inhalatory steroid therapy, who might be comparable to those with a previous endogenous mild hypercortisolism (29).

Overall, 39 patients were found to have PSH and were re-tested by LDCT every 6 months. The PSH lasted 6, 12, 18, 24 and 36 months in 6, 15, 9, 7 and 2 patients, respectively.

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

Methods

All samples were stored at –20°C until assayed. The assays used were the same in all centers. In all patients plasma ACTH levels (mean of 3 determinations at 20-minute intervals) were measured by IRMA (BRAHMS Diagnostica GmbH, Berlin, Germany) and serum cortisol and UFC levels (after dichloromethane extraction) were determined immunofluorimetrically by TDX-FLX Abbott, GmbH, Diagnostika kits (Wiesbaden-Delkenheim, Germany). The intra- and inter-assay

coefficients of variation for all assays were <5 and 10%, respectively. Low dose corticotropin stimulation and ITT tests were performed as described elsewhere (22)

We recorded the following data: weight, height, body mass index (BMI), presence of arterial hypertension, obesity, dyslipidaemia, diabetes mellitus and metabolic syndrome following the World Health Organization criteria published in 2001 (30), as at the time of the data collection those were the commonly used criteria for diagnosing diabetes mellitus. However, the criteria to diagnose diabetes have changed during the last 10 years. Therefore, in this analysis, we reassessed these diagnoses on the basis of the new criteria (31) and we did not find differences in the diagnostic classification.

Study Design and Statistical Analysis

We postulated that the presence of PSH is an indirect sign of a presurgical hypercortisolism. We firstly compared patients with and without PSH. Then, we searched for the lowest value of F-1mgDST, UFC and MSC and for the highest value for ACTH in patients with PSH. Since, by definition, in our cohort no patients with PSH, and therefore with presurgical hypercortisolism, had F-1mgDST, UFC and MSC below and ACTH above those values, we considered these cut-offs as those that possibly individuate AI patients without hypercortisolism. Then, we assessed the sensitivity (SN), specificity (SP), positive and negative predictive values (PPV and NPV, respectively) of these parameters for identifying the absence of PSH occurrence and considered them as possible indexes of the absence of hypercortisolism. Furthermore, we compared the clinical characteristics of patients grouped on the basis of the HPA axis parameters and their cut-off that resulted to be able to significantly rule out the PSH occurrence.

Statistical analysis was performed by SPSS version 26.0 statistical package (IBM SPSS, Milan, Italy). The results are expressed as mean ± SD, if not differently specified. The normality of data distribution was tested by Kolmogorov–Smirnov test.

The comparison of the continuous variables were performed using the Student's t-test or Mann–Whitney U-test as appropriate, while the categorical were compared by $\chi 2$ test or Fisher Exact test, as appropriate. The bivariate associations were tested by Spearman rho or Pearson correlation as appropriate.

P-values of less than 0.05 were considered significant.

Results

The clinical characteristics of patients with or without PSH occurrence are summarized in Table 1. The two groups were comparable for age, BMI, diameter of adenoma, duration of pre-surgical follow-up, mean hydrocortisone equivalent daily dose, indication for which surgery was chosen (i.e. size of adenoma or suspected subclinical hypercortisolism) and prevalence of hypertension, type 2 diabetes, obesity and metabolic syndrome. Among the HPA axis function's parameters, MSC levels were significantly higher in patients with PSH than in those without, whereas UFC, 1mg-DST and ACTH levels were comparable between the two groups. The lowest value of F-1mgDST, UFC and MSC and the highest value for ACTH in patients with PSH were 1.2 µg/dL (33 nmol/L), 10.4 µg/24h (29 nmol/24h), 1.2 µg/dL (33 nmol/L) and 26.9 pg/ml (6 pmol/L), respectively. The prevalence of post-surgical hypocortisolism was not different between patients selected for surgery by size criteria (18/31 58%) or because of hypercortisolism (21/29, 72.4%, P=0.287).

The number of subjects with F-1mgDST <1.2 µg/dL (33 nmol/L), UFC <10.4 µg/24h (29 nmol/24h), MSC <1.2 µg/dL (33 nmol/L) and ACTH >26.9 pg/mL (6 pmol/L) among patients with PSH and without PSH are also reported in Table 1. Among patients without PSH, 9, 2, 1 and 1 individuals showed F-1mgDST F-1mgDST <1.2 µg/dL (33 nmol/L), UFC <10.4 µg/24h (29 nmol/24h), MSC <1.2 µg/dL (33 nmol/L) and ACTH >26.9 pg/mL (6 pmol/L), respectively (Figure 2). As noted, the statistical significance was obtained only for F-1mgDST, probably due to the low number of patients with MSC levels below 1.2 µg/dL (33 nmol/L), UFC levels below 10.4 µg/24h (29 nmol/24h) and ACTH values above 26.9 pg/mL (6 pmol/L). Therefore, in this cohort we considered

F-1mgDST test as the only one with enough reliability to be investigated as possible marker of the absence of PSH occurrence after surgery.

By using a F-1mgDST cut-off set at 1.2 µg/dL (33 nmol/L, the lowest value in patients with PSH occurrence), we obtained a 100% SN and PPV (as expected) and a 42.9% SP and 76.5% NPV (accuracy 66.6%, p<0.0001). The comparison between patients grouped on the basis of a F-1mgDST cut-off ≥1.2 µg/dL or <1.2 µg/dL (33 nmol/L) is reported in table 2. Age, BMI, duration of pre-surgical follow-up, mean hydrocortisone equivalent daily doses, ACTH levels and diameter of the adenoma were comparable between the two groups, while F-1mgDST (by definition), UFC and MSC levels were higher in patients with F-1mgDST ≥1.2 µg/dL (33 nmol/L) than in those with F-1mgDST <1.2 µg/dL (33 nmol/L). This latter group showed a lower prevalence of dyslipidemia and tended to have a lower prevalence of diabetes mellitus than the former. Interestingly, among the 9 patients with F-1mgDST <1.2 µg/dL (33 nmol/L), no subjects were affected with diabetes mellitus and/or dyslipidemia and/or metabolic syndrome.

Discussion

In AI patients, who underwent unilateral adrenalectomy, the PSH occurrence has been suggested to be an indirect proof of the presence of hypercortisolism before surgery (5, 22, 23). Therefore, the aim of the present study was to define how to exclude hypercortisolism in AI on the basis of the cut-off of F-1mgDST, MSC, UFC and ACTH able to predict in AI patients before surgery the absence of hypocortisolism after surgery. In our cohort, only F-1mgDST levels measured before surgery with a cut-off set at 1.2 µg/dL (33 nmol/L) could predict the PSH absence. Indeed, no AI patients with F-1mgDST below 1.2 µg/dL (33 nmol/L) experienced the PSH occurrence. Moreover, AI patients with F-1mgDST <1.2 µg/dL (33 nmol/L) tended to show a better metabolic profile as compared with those with F-1mgDST ≥1.2 µg/dL (33 nmol/L). These findings could suggest that in AI patients the F-1mgDST cut-off for defining the absence of hypercortisolism should be lowered to 1.2 µg/dL (33 nmol/L).

The present findings are in line with some previous data. Indeed, in AI patients cortisol after 2 days low dose (i.e. 2 mg/day) dexamethasone suppression test with a cut-off set at 1.4 µg/dL (39 nmol/L) and 1.1 µg/dL (30 nmol/L) had the higher accuracy in predicting cardiovascular risk and

insulin-resistance, respectively (11) and the F-1mgDST cut-off with the best conciliation between SN and SP for predicting cardiovascular events was found to be as low as 1.5 μg/dL (41 nmol/L) (21). Moreover, previous studies showed that in patients with NFAI (F-1mgDST <1.8 μg/dL, 50 nmol/L) the incidence of diabetes mellitus was increased as compared with patients without AI (12) and the removal of an NFAI can lead to an amelioration of blood pressure, diabetes mellitus and dyslipidemia (6, 21). Finally, a recent meta-analysis showed that the mortality was not differently increased in AI patients with autonomous cortisol secretion as compared with NFAI patients (13).

The finding in the present cohort of AI patients with F-1mgDST <1.2 μg/dL (33 nmol/L) being less frequently affected with dyslipidaemia and having a better metabolic profile is in agreement with the idea that at least some of the currently so called "non-functioning" adrenal adenomas, are, in fact, responsible of a subtle but significant degree of cortisol hypersecretion. The hypothesis that some AI patients, who are not considered as affected with hypercortisolism on the basis of the F-1mgDST level <1.8 μg/dL (50 nmol/L), may instead be hypercortisolemic, had already been proposed by other Authors in the past (19), but no suggestion on lowering the cut-off for F-1mgDST was given. Importantly, a lower cut-off of F-1mgDST has recently been proposed on the basis of data coming from subjects without AI and eucortisolemic (i.e. with F-1mgDST <1.8 μg/dL, 50 nmol/L). Indeed, we have recently reported that in postmenopausal women without hypercortisolism, an abnormal cortisol secretion is associated with hypertension (32) and the cut-off of F-1mgDST set at 0.9 μg/dL (25 nmol/L) may be used for predicting the presence of at least 2 among hypertension, diabetes mellitus and fragility fractures (33). In keeping, the role of cortisol secretion, even within the "normal" range, has been recently suggested as index of cardiovascular risk by three independent research groups (34-36).

It must be observed that the F-1mgDST cut-off set at 1.2 μg/dL (33 nmol/L), while possibly useful for ruling out PSH and, therefore, for predicting the absence of hypercortisolism in AI, cannot be used for predicting the certain PSH occurrence. Even in our cohort, many patients with F-1mgDST ≥1.2 μg/dL (33 nmol/L) did not develop PSH (22), as already reported even in patients with clinically overt ACTH-independent hypercortisolism (37). The different duration of the cortisol hypersecretion,

the different sensitivity to glucocorticoids and the different peripheral activation of cortisol may be all possible explanations of this apparently contradictory finding.

This study has several limits. Firstly, the low number of patients, in particular among those who did not experience PSH, suggests that the present findings should be taken with extreme caution. Secondly, we cannot completely exclude that some patients were affected with a cyclic form of hypercortisolism, thus rendering the F-1mgDST values less reliable. Thirdly, we should consider the possibility that in patients with F-1mgDST <1.2 μg/dL (33 nmol/L) the lack of PSH could be due to a short disease duration, that could not have been sufficient to induce the HPA axis impairment, rather than being an index of a normal cortisol secretion. However, the fact that the pre-surgical follow-up duration was not shorter in AI patients with F-mgDST <1.2 μg/dL (33 nmol/L) than in those with F-1mgDST ≥1.2 μg/dL (33 nmol/L) is against this possibility. We are aware that, nowadays, the recommended hydrocortisone-equivalent doses for the glucocorticoid substitutive therapy are slightly lower (i.e. hydrocortisone 15–20 mg/day or cortisone acetate 20–25 mg/day) than those used at the time of the study (38). This is due to the fact that immediately after the resolution of an adrenal hypercortisolism a substitutive dose of hydrocortisone of 12–15 mg/m^2/day has been recommended because it was enough to prevent adrenal crisis and to allow a recovery of the HPA axis (39, 40). Moreover, our protocol did not allow a step-by-step decrease in the dose of glucocorticoid substitution. Therefore, we cannot exclude that the high and not modifiable doses of glucocorticoid substitutive therapy might have influenced the present rate of post-operative hypocortisolism (i.e. 70.9%), which, however, was in keeping with that (i.e. 50-90%) reported in the literature (39). We cannot exclude also that the long PSH duration in some patients could have been influenced by the relatively high and not-modifiable dose of glucocorticoid substitutive therapy. However, the PSH duration in patients operated on for adrenal adenomas is reported to vary between 1 months and 50 months (39), figures that are in keeping with the present findings. In addition, we did not measure dexamethasone levels in blood, and, therefore, we cannot be sure that the HPA axis activity suppression was adequate in all patients. Finally, these results are derived from a cohort of patients operated on for adrenal adenomas and, therefore, the generalizability of these findings is limited

However, notwithstanding these limits, the present study suggests for the first time that the F-1mgDST cut-off below 1.2 µg/dL (33 nmol/L) is able to rule out the possible occurrence of hypocortisolism in AI patients undergoing the removal of the adrenal adenoma. A first consequence of the present findings is that in AI patients with F-1mgDST below 1.2 µg/dL before surgery, a post-operative glucocorticoid substitutive therapy is not needed. In addition, this F-1mgDST cut-off has the highest sensitivity to exclude hypercortisolism and, at least in AI patients, could define a normal cortisol secretion. Further studies with a thorough and prolonged endocrine workup before surgery in AI patients, are needed to confirm the need of lowering the currently used F-1mgDST cut-off for defining normal cortisol secretion.

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Figure 1 Legend

Flow chart reporting the inclusion criteria, reasons for adrenalectomy, assessments and protocol.

Footnotes: 319 consecutive patients with unilateral incidentally found adrenal adenoma (adrenal incidentaloma) and without signs and/or symptoms specific of cortisol excess (moon facies, striae rubrae, skin atrophy, buffalo hump) have been enrolled. We excluded 61 patients taking drugs or affected with diseases influencing cortisol metabolism and/or secretion (i.e. rheumatologic or haematological diseases, depression, alcoholism, eating disorders, bowel diseases, thyrotoxicosis and chronic renal and/or hepatic disease) and 18 patients with pheochromocytoma (n=8) or aldosterone-secreting adenoma (n=10). Among the remaining 240 patients, 60 underwent surgical removal of the adrenal mass on the basis of the size of the lesion or due to the presence of hypercortisolism [i.e. presence of ≥3 criteria out the following : 1) Urinary free cortisol levels >60 μg/24h (165 nmol/24h, the 97th percentile of a reference population), 2) cortisol after 1 mg-overnight dexamethasone suppression test >3.0 μg/dL (83 nmol/L), 3) adrenocorticotroph hormone levels <10 pg/mL (2.2 pmol/L), iv) midnight serum cortisol levels >5.4 μg/dL (149 nmol/L)].

In all patients, after about 60 days, the cortisol secretion was assessed by low dose corticotropin stimulation test (LDCT), In 21 patients with baseline cortisol >5 μg/dL (138 nmol/L) and stimulated cortisol levels >22 μg/dL (600 nmol/L), post-surgical hypocortisolism (PSH) was ruled out and the steroid substitutive therapy was withdrawn (PSH absent). At variance, 34 patients with baseline cortisol <5 μg/dL (138 nmol/L) and/or stimulated cortisol levels <16 μg/dL (441 nmol/L) were considered affected with PSH (PSH present). Five patients with possible PSH [i.e. baseline cortisol >5 μg/dL (138 nmol/L) and LDCT stimulated cortisol levels between 16 μg/dL (441 nmol/L) and 22 μg/dL (600 nmol/L)] underwent insulin tolerance test (ITT). All these latter subjects showed a reduced cortisol response after ITT [i.e stimulated cortisol levels <18 μg/dL (496 nmol/L)] and were, therefore, diagnosed as having PSH.

Overall, 39 patients were found to have PSH and were re-tested by LDCT every 6 months. The PSH lasted 6, 12, 18, 24 and 36 months in 6, 15, 9, 7 and 2 patients, respectively.

Figure 2 Legend

Presurgical levels of cortisol after 1 mg overnight dexamethasone suppression test (F-1mgDST) and midnight serum cortisol (MSC) in 60 patients with adrenal incidentalomas (AI) who underwent adrenalectomy.

Footnotes: all AI patients with a post-surgical hypeocortisolism (PSH) occurrence (black diamonds) showed F-1mgDST ≥1.2 µg/dL (33 nmol/L, vertical line) or MSC ≥1.2 µg/dL (33 nmol/L, horizontal line). In our cohort, 9 and 1 patients without PSH (empty circles) showed F-1mgDST or MSC <1.2 µg/dL (33 nmol/L).

Table 1. Pre-surgical clinical and biochemical features of patients with adrenal incidentalomas and without (PSH-) or with post-surgical hypocortisolism (PSH+)

	Group PSH+ n = 39	**Group PSH- n = 21**	**P value**
Females	31	15	0.532
Age (yrs)	55.3 ± 12.0 (24 - 74)	57.2 ± 8.3 (33 - 75)	0.505
BMI (kg/m^2)	27.4 ± 5.6 (17.4 – 41)	29.6 ± 5.4 (21.5 – 41)	0.163
Reason for surgery (size of the mass/SH)	18/21	13/8	0.287
HC doses (mg/day)	20.9±2.6 (20 – 30)	20.9±2.3 (20 – 30)	0.932
ACTH (pg/mL)	9.6 ± 5.7 (1.9 – 26.9)	9.0 ± 4.4 (5.0 – 23.8)	0.665
ACTH >26.9 pg/mL (%)	0 (0.0)	1 (1.7)	0.650
F-1mg-DST (µg/dL)	4.6 ± 3.5 (1.2 – 13.7)	3.2 ± 2.1 (0.9 – 9.0)	0.098
F-1mg-DST <1.2 µg/dL (%)	0 (0.0)	9 (42.9)	<0.0001
UFC (µg/24hrs)	68.2 ± 45.2 (10.4 – 189.6)	51.3 ± 32.4 (10.0 - 120.0)	0.136
UFC <10.4 µg/24hrs	0 (0.0)	2 (9.5)	0.119
MSC (µg/dL)	6.0 ±3.1 (1.2 – 12.5)	3.4 ± 2.0 (1.0 – 8.0)	0.001

MSC <1.2 µg/dL	0 (0.0)	1 (4.8)	0.350
Diameter of adenoma (cm)	3.5 ± 1.3 (1.2 – 9.0)	3.2 ± 1.0 (1.5 – 5.7)	0.340
Pts with type 2 diabetes mellitus (%)	9 (23.1)	5 (23.8)	1.000
Pts with arterial hypertension (%)	18 (46.2)	15 (71.4)	0.102
Pts with obesity (%)	15 (38.5)	10 (47.6)	0.587
Pts with dyslipidemia (%)	12 (30.8)	10 (47.6)	0.263
Pts with Metabolic Syndrome (%)	5 (12.8)	6 (28.6)	0.169

Pts: patients. PSH: post-surgical hypcorticortisolism; PSH-: absence of PSH; PSH+: presence of PSH; MSC: midnight serum cortisol. UFC: urinary free cortisol; F-1mgDST: cortisol after 1 mg overnight dexamethasone suppression test. HC: hydrocortisone dose during substitutive therapy period. For plasma cortisol multiply x 27.56 to convert from µg/dL to nmol/L. For 24h urinary cortisol multiply x 2.756 to convert from µg/24h to nmol/24h. For ACTH multiply x 0.22 to convert from pg/mL to pmol/L

Table 2. Pre-surgical clinical and biochemical features of patients with adrenal incidentalomas and with cortisol after 1 mg overnight dexamethasone suppression test below 1.2 µg/dL or ≥1.2 µg/dL

	F-1mgDST <1.2 µg/dL n=9	**F-1mgDST ≥1.2 µg/dL n=51**	**P value**
Females	6 (66.7)	40 (78.4)	0.44
Age (yrs)	56.6±4.5 48 – 63	55.9±11.6 24 – 75	0.86
BMI (kg/m^2)	26.6±2.9 21.5 – 31.3	28.4±6.0 17.4 – 41.0	0.38
Duration of pre-surgical follow up (months)	8.4±7.4 1.0 – 24.0	5.5±5.0 0.5 – 19.6	0.142
HC doses (mg/day)	20.0±0.0 (20 – 20)	21.1±2.5 (20 – 30)	0.21
ACTH (pg/mL)	10.1±6.0 5.0 – 23.8	9.3±5.2 1.9 – 26.9	0.69
F-1mg-DST (µg/dL)	1.1±0.1 0.9 – 1.1	4.6±3.2 1.2 – 13.7	0.001
UFC (µg/24hrs)	36.6±17.7 10.0 – 59.0	66.8±43.2 10.4 – 189.6	0.04
MSC (µg/dL)	3.0±1.6 1.0 – 5.5	5.4±3.1 1.2 – 12.5	0.03
Diameter of adenoma (cm)	3.4±1.2 1.5 – 5.7	3.5±1.3 1.2 – 9.0	0.85
Pts with type 2 diabetes mellitus (%)	0 (0.0)	14 (27.5)	0.07
Pts with arterial hypertension (%)	3 (33.3)	30 (58.8)	0.15
Pts with obesity (%)	3 (33.3)	22 (43.1)	0.72
Pts with dyslipidemia (%)	0 (0.0)	22 (43.1)	0.02
Pts with Metabolic Syndrome (%)	0 (0.0)	11 (21.6)	0.19

Pts: patients. MSC: midnight serum cortisol. UFC: urinary free cortisol; F-1mgDST: cortisol after 1 mg overnight dexamethasone suppression test. For plasma cortisol multiply x 27.56 to convert from µg/dL to nmol/L. HC: hydrocortisone dose during substitutive therapy period . For 24h urinary cortisol multiply x 2.756 to convert from µg/24h to nmol/24h. For ACTH multiply x 0.22 to convert from pg/mL to pmol/L

Figure 1

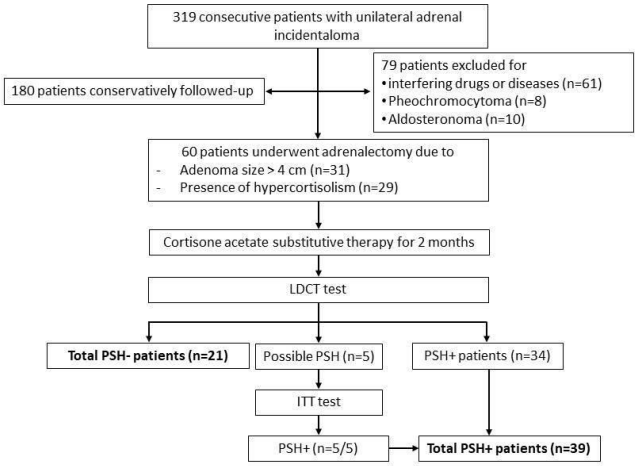

Figure 2