Screening Tests for Hypercortisolism in Patients With Adrenal Incidentaloma

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Abstract

Background: To compare the diagnostic performance of different first-line screening tests for subclinical hypercortisolism (SH) in patients with adrenal incidentaloma (AI).

Methods: We studied a series of patients with AI, with no clinical evidence of hormonal hypersecretion. For screening for SH, all patients performed 1-mg dexamethasone suppression test (1-mg DST), late night salivary cortisol (LNSC) and 24-h urinary free cortisol (UFC). A control group of patients with confirmed Cushing's syndrome (CS) was used to calculate the diagnostic performance of the screening tests.

Results: In the 83 patients with AI, morning cortisol after 1-mg DST was ≤ 1.8 μg/dL in 69.9%, 1.9 to 5 μg/dL in 26.5% and > 5 μg/dL in 3.6%. LNSC was elevated in 20.5% and all patients had normal UFC levels. In the control group, composed of 50 patients with confirmed CS, all patients who underwent 1-mg DST had cortisol levels > 1.8 μg/dL (1.9 to 5 μg/dL in 16.2% and > 5 μg/dL in 83.3%); LNSC was elevated in 93.8% and the UFC was increased in 85.4% of patients tested. Overall, for the screening of SH, the 1-mg DST presented a sensitivity and specificity of 100% and 69.9% with its lowest threshold (≤ 1.8 μg/dL) and 83.3% and 96.4% with its highest threshold (< 5 μg/dL). LNSC showed a sensitivity and specificity of 93.8% and 79.5% and the UFC of 85.4% and 100%, respectively.

Conclusions: The 1-mg DST at its lowest threshold presented the highest sensitivity in identifying SH, but its low specificity encourages us to consider UFC levels, to reduce false-positive test results.

Keywords: Adrenal incidentaloma; Hypercortisolism, Dexamethasone; Salivary cortisol

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Introduction

The widespread use of high resolution abdominal imaging procedures has led to an increasing prevalence of incidentally discovered adrenal masses [1, 2]. The majority of adrenal incidentalomas (AI) are clinically non-functioning, benign adrenocortical adenomas [3]. Even in the absence of overt Cushing's syndrome (CS), 5 to 30% of patients with AI present abnormalities of hypothalamic-pituitary-adrenal (HPA) axis and exhibit various patterns of autonomous cortisol production, suggesting the existence of different degrees of subclinical hypercortisolism (SH) [4-6].

The diagnosis of SH is important because although these patients do not present the typical stigmata of CS, they are exposed to the long-term consequences of continuous, endogenous cortisol secretion, and frequently display features of metabolic syndrome, such as hypertension, obesity, diabetes mellitus, and osteoporosis [5, 7-9].

The prevalence of SH varies from 5 to 20% among patients with AI, depending on diagnostic criteria used to define it [10-14]. The lack of a golden standard makes the diagnosis of subclinical hypercortisolism difficult. Previous studies have used different diagnostic algorithms to exclude SH, but a direct comparison between screening tests to evaluate their diagnostic accuracy has been scarcely studied [15].

The purpose of this study was to compare the diagnostic performance of different first-line screening tests to hypercortisolism in patients with AI.

Patients and Methods

To calculate the diagnostic performance of the screening tests for hypercortisolism, we performed a case-control study and compare patients with adrenal incidentaloma with a reference group of patients with confirmed CS.

Group 1 (AI patients)

From January 2015 to December 2017, 83 consecutive patients with adrenal incidentaloma referred to an endocrinology appointment in Centro Hospital do Porto were enrolled in the study.

The diagnosis of AI was based on the finding of an adrenal

Table 1. Clinical and Biochemical Characteristics of Patients With Adrenal Incidentaloma

	Unilateral adenoma	Bilateral adenoma	P
Age (years)[a]	66.9 ± 13.9	63.5 ± 8.0	0.406
Gender (N, % female)	45/63 (71.4%)	7/20 (35.0%)	< 0.001*
Adrenal mass size (mm)[b]	20 (8 - 45)	22 (10 - 53)	0.693
Cortisol after 1-mg DST (µg/dL)	1.6 (0.4 - 7.0)	1.4 (0.6 - 2.3)	0.216
LNSC (µg/dL)[b]	0.206 (0.005 - 1.140)	0.296 (0.050 - 0.621)	0.019*
UFC (nmol/24 h)[b]	28.3 (4.4 - 130.0)	48.0 (10.4 - 119.0)	0.192
BMI[b]	27.0 (20.3 - 42.4)	27.4 (21.2 - 33.0)	0.852
Obesity (BMI ≥ 30) (N, %)	18/61 (29.5%)	5/19 (26.3%)	0.503
Hypertension (N, %)	46/63 (73.0%)	14/20 (70.0%)	0.501
Diabetes mellitus (N, %)	36/63 (57.1%)	11/20 (55.0%)	0.534
Dyslipidemia (N, %)	20/63 (31.7%)	5/20 (25.0%)	0.392
Cardiovascular disease[c] (N, %)	8/49 (16.3%)	3/14 (21.4%)	0.658

[a]Normally distributed data are presented by mean and SD; [b]Non-normally distributed data are presented by median and range; [c]Coronary disease, previous stroke or peripheral artery disease. *P < 0.05 indicates statistically significant difference between unilateral and bilateral adenoma.

mass by an imaging workup of the abdomen, performed for the evaluation of unrelated diseases. At computed tomography (CT) all adrenal masses showed typical benign features: small size (< 60 mm), homogeneous and well-circumscribed, CT attenuation ≤ 10 Hounsfield units (HU) or contrast-enhanced washout CT within adenoma range [15, 16-18]. All patients repeated the CT 6 months after and no change in size or mass characteristics was observed.

None of the patients showed either signs or symptoms specific of cortisol excess or were medicated with drugs influencing cortisol and dexamethasone metabolism or cortisol secretion [19, 20]. Patients with previous or current history of malignancy known to metastasize in the adrenal glands were excluded.

Study protocol

Patients with AI underwent a standardized diagnostic protocol, including a detailed clinical, biochemical and hormonal evaluation. Screening for hypercortisolism included the evaluation of 24-h excretion of urinary free cortisol (UFC), morning (MSC) and late night salivary cortisol (LNSC) and overnight low-dose dexamethasone suppression test (1-mg DST) with measurement of serum cortisol at 8 am the following morning.

In patients with serum cortisol > 1.8 µg/dL after 1-mg DST, overt CS was subsequently excluded with basis on normal LNSC, UFC excretion, ACTH and 2-day low dose dexamethasone test.

The diagnosis of pheochromocytoma and primary hyperaldosteronism was excluded by determinations of plasma-free metanephrines 24-hour urinary metanephrines and catecholamines and plasma aldosterone/renin activity ratio (ARR) [15, 21, 22].

Group 2 (reference group (confirmed CS patients))

The reference group included 50 patients with confirmed CS (47 cases of Cushing's disease (CD) and three cases of ectopic CS. CS diagnosis was stablished with basis on at least two of the following: the lack of cortisol suppression below 1.8 µg/dL after the 1-mg DST, an elevated LNSC and elevated UFC levels (a mean of two collections). The diagnosis of CD was established in patients with normal or elevated serum ACTH levels and a high-dose dexamethasone test with ≥ 80% suppression of cortisol levels, an increase in ACTH of 50% and plasma cortisol of 20% in response to corticotropin-releasing hormone (CRH) stimulation test or a baseline central/peripheral ACTH ratio above 2 and after CRH stimulation above 3

Table 2. Comparative Results of Screening Tests for Hypercortisolism Between Patients With AI and Cushing Syndrome

	Cutoff	Adrenal incidentaloma[a]	Control group (Cushing syndrome)[a]	P
1-mg DST	≤ 1.8 µg/dL	58/83 (69.9)	0	< 0.001*
	1.9 - 5.0 µg/dL	22/83 (26.5)	6/36 (16.7)	
	> 5.0 µg/dL	3/83 (3.6)	30/36 (83.3)	
LNSC	> 0.350 µg/dL	17/83 (20.5)	15/16 (93.8)	< 0.001*
UFC	> 172 nmol/24h	0	35/41 (85.4)	< 0.001*

[a]Data are presented in proportion and %. *P < 0.05 indicates statistically significant difference between adrenal incidentaloma and the control group.

in the inferior petrosal sinus sampling (IPSS). Ectopic CS was confirmed by IPSS, indicating a non-pituitary ACTH source.

Assays

Hormones were measured in-house with commercially available reagents. Late night saliva samples were collected between 11 pm and midnight and morning saliva samples between 7 am and 8 am, using cotton swabs from Salivette® tubes (Sarstedt, Numbrecht, Germany). All patients received detailed and careful instructions on how to properly perform home sampling for LNSC. LNSC and serum cortisol were measured using an automated electrochemiluminescence immunoassay (Cobas e601, Roche Diagnostics, Mannheim, Germany). The cross-reactivity with dexamethasone was negligible. UFC was measured using a chemiluminescent microparticle immunoassay (ARCHITECT i2000SR, Abbott).

Statistical analysis was performed with SPSS® Statistics V.22. Data are presented as proportions, means (SD), or, in the case of variables that did not conform to a normal distribution, median (range). For independent samples, two-way comparisons for proportions were performed by Chi-square test (χ^2) for categorical variables and Mann-Whitney U test for continuous variables. Statistical significance was taken as two-tailed at the level of 0.05. All phases of preparation for the study were in line with the ethical and deontological principles regarding data collection and statistical analysis. All patient clinical data were anonymized and analyzed by an independent reviewer. For this type of study formal consent is not required.

Results

Patients with AI were predominantly female (61.9%), with a mean age of 66.2 ± 12.5 years. Most patients presented a unilateral adrenal mass (75.9%), with a median size of 20.5 mm (range 8.0 - 53.0mm), and only in three patients the size was higher than 40 mm (3.6%). Median BMI of AI patients was 27.8 (range 20.3 - 42.4), 28.8% were obese, 72.3% had hypertension, 56.6% had type 2 diabetes mellitus, 30.1% had dyslipidemia and 17.5% had documented atherosclerotic cardiovascular disease. Other clinical and biochemical characteristics of patients with AI are presented in Table 1.

In the AI group, morning cortisol levels after 1-mg DST were ≤ 1.8 µg/dL in 58 (69.9%) patients, between 1.9 and 5 µg/dL in 22 patients (26.5%) and > 5 µg/dL in three cases (3.6%). LNSC was elevated (> 0.350 µg/dL) in 17 patients (20.5%) and all patients had normal UFC levels (≤ 172 mol/24 h) (Table 2). In patients with bilateral adrenal incidentalomas, LNSC was more frequently elevated (40.0% vs. 15.9%; P < 0.05). Patients without cortisol suppression ≤ 1.8 µg/dL after 1-mg DST were more frequently hypertensive (88.0% vs. 65.5%; P < 0.05) and dyslipidemic (48.0% vs. 22.4%; P < 0.05) (Table 3).

The control group consisted of 50 patients with confirmed CS, with a mean age of 53.6 ± 16.0 years and the majority were female (76.0%). CS patients had significantly higher median levels of cortisol after 1-mg DST, LNSC and UFC (P < 0.001)

Table 3. Prevalence of Cardiovascular Risk Factors and Cardiovascular Disease According to the Results on the 1-mg DST and LNSC Tests

Cutoff	1-mg DST				LNSC		
	≤ 1.8 µg/dL	1.9 - 5.0 µg/dL	> 5.0 µg/dL	P	≤ 0.350 µg/dL	> 0.350 µg/dL	P
Hypertension (N, %)	38/58 (65.5%)	19/22 (86.4%)	3/3 (100%)	< 0.05*	48/66 (72.7%)	12/17 (70.6%)	0.540
Diabetes mellitus (N, %)	32/58 (55.2%)	13/22 (59.1%)	2/3 (66.7%)	0.643	39/66 (59.1%)	8/17 (47.1%)	0.267
Dyslipidemia (N, %)	13/58 (22.4%)	10/22 (45.5%)	2/3 (66.7%)	< 0.05*	19/66 (28.8%)	6/17 (35.3%)	0.402
BMI[b]	27.5 (20.3 - 39.9)	28.7 (20.4 - 42.4)	26.6 (25.0 - 27.9)	0.563	28.0 (20.3 - 42.4)	26.9 (20.4 - 33.0)	0.504
Obesity (BMI ≥ 30) (N, %)	15/55 (27.3%)	8/22 (36.4%)	0/3 (0%)	0.932	18/64 (28.1%)	5/16 (31.3%)	0.514
Cardiovascular disease[c] (N, %)	7/43 (16.3%)	2/18 (11.1%)	2/2 (100%)	< 0.05*	9/52 (17.3%)	2/11 (17.5%)	0.619

[a]Normally distributed data are presented by mean and SD; [b]Non-normally distributed data are presented by median and range; [c]Coronary disease, previous stroke or peripheral artery disease. *P < 0.05 indicates statistically significant difference between patients with morning cortisol levels after 1-mg DST ≤ 1.8, between 1.9 - 5.0 and > 5.0 µg/dL.

Table 4. Comparison of Demographic and Biochemical Characteristics Between Patients With Adrenal Incidentaloma and Cushing Syndrome

	Adrenal incidentaloma	Control group (Cushing syndrome)	P
Age (years)[a]	66.2 ± 12.6	53.6 ± 16.0	< 0.001*
Gender (N, % female)	52 (62.7%)	38 (76.0%)	0.079
Cortisol after 1-mg DST (µg/dL)[b]	1.5 (0.4 - 7.0)	8.5 (2.0 - 41.0)	< 0.001*
MSC (µg/dL)[b]	0.512(0.005 - 1.430)	0.735 (0.266 - 2.21)	0.086
LNSC (µg/dL)[b]	0.224(0.005 - 1.140)	0.779 (0.192 - 9.890)	< 0.001*
LNSC/MSC ratio[b]	0.36 (0.49 - 4.23)	0.89 (0.49 - 1.92)	< 0.001*
UFC (nmol/24 h)[b]	30.0 (4.4 - 130.0)	275.0 (42.5 – 5,241.0)	< 0.001*

[a]Normally distributed data are presented by mean and SD; [b]Non-normally distributed data are presented by median and range. *P < 0.05 indicates statistically significant difference between adrenal incidentaloma and the control group.

and a higher LNSC/MSC ratio (P < 0.001) than patients with AI (Table 4). In this subgroup, all 36 patients who underwent 1-mg DST had cortisol levels > 1.8 µg/dL after 1-mg DST, six of which (16.2%) between 1.9 and 5 µg/dL and 30 (83.3 %) > 5 µg/dL; LNSC was elevated in 15 (93.8%) of the 16 patients who underwent the test and the UFC was increased in 35 (85.4%) of 41 patients tested (Table 2). The sensitivity, specificity, positive and negative predictive values and likelihood ratios for each one of the tests are presented in Table 5.

Discussion

The ideal diagnostic approach to a patient with an adrenal incidentaloma has been the subject of discussion over the last few decades. For several reasons, the diagnosis of SH is one of the biggest challenges for a clinician, especially in cases of mild hypercortisolism. First, cortisol secretion is a continuum with no clear separation from completely normal to increased levels, making it difficult to define cutoffs of indexes of cortisol secretion to most screening tests. Second, all currently available screening tests for the diagnosis of endogenous CS have limitations [23-26]. In order to standardize the clinical approach of these patients, the European Society of Endocrinology (ESE) has recently issued guidelines on the management of adrenal incidentalomas. The ESE panel recommends the use of the overnight 1-mg DST to exclude cortisol excess in all patients with AI, with basis on the simplicity of the test, the large evidence with its use and the physiological principle that in a patient with SH the mild adrenal cortisol secretion is not completely suppressed by a low dose of dexamethasone, but

may not be high enough to raise total urinary cortisol secretion. Serum cortisol levels after 1-mg DST lower than 1.8 µg/dL are recommended as a diagnostic criterion for the exclusion of autonomous cortisol secretion, whereas levels between 1.9 and 5.0 µg/dL should be considered as evidence of "possible autonomous cortisol secretion" and cortisol levels above 5.0 µg/dL should be taken as evidence of "autonomous cortisol secretion". In the cases with serum cortisol levels after 1-mg DST ≥ 1.8 µg/dL, the majority of the ESE Panel members preferred additional tests to better judge the degree of hypercortisolism, such as ACTH, 24 h UFC (and/or LNSC) and repetition of the 1-mg DST in 6 to 12 months [15].

Our findings confirm that adrenal autonomy is best assessed by the overnight 1-mg DST and the use of the lowest threshold proposed by ESE (a cortisol level ≤ 1.8 µg/dL) reaches the highest sensitivity (100%) to the diagnosis of CS. However, this cutoff exhibits a low specificity (69.9%) which emphasizes the need for further complementary tests to rule out false positive results. As expected, when the highest cutoff (a cortisol level ≤ 5.0 µg/dL) was considered the sensitivity was lower (83.3%) but the specificity increased to 96.4%. Previous studies have showed similar results, with reported sensitivities ranging from 75 to 100% and specificities from 67 to 71% when the lowest cutoffs were used (≤ 1.8 µg/dL) and if the highest cutoffs (≤ 5.0 µg/dL) were considered the sensitivity decreased to 44-58% and specificity increased to 83-100% [27-36]. These data suggest that cortisol levels after 1-mg DST > 5.0 µg/dL are effective to detect SH in patients with AI and levels ≤ 1.8 µg/dL are precise to rule out this diagnosis.

On the other hand, in this study, the UFC presented a maximum specificity (100%) for the diagnosis of SC but its

Table 5. Performance of Screening Tests for Hypercortisolism in a Group of Patients With Adrenal Incidentaloma

Test	Cutoff	SE (%)	SP (%)	PPV (%)	NPV (%)	LR[pos]	LR[neg]
1-mg DST	1.8 µg/dL	100	69.9	59.0	100	3.32	-
	5.0 µg/dL	83.3	96.4	90.0	93.0	23.1	0.173
LNSC	0.350 µg/dL	93.8	79.5	46.9	98.5	4.58	0.079
UFC	172 nmol/24h	85.4	100	100	93.3	-	0.146

SE: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative predictive value; LR[pos]: positive likelihood ratio; LR[neg]: negative likelihood ratio. LR[pos] cannot be calculated if SP = 100% and LR[neg] cannot be calculated if SE = 100%.

unsatisfactory sensitivity (85.4%) makes it unreliable for detecting subtle increases in cortisol secretion. Data previously reported from other series confirmed its lack of sensitivity for the detection of subclinical disorders of the HPA axis [28, 37, 38]. Therefore, UFC is not an adequate screening test for SH, but it can be useful in combination with other tests to increase the specificity.

Regarding the role of salivary cortisol in the screening of CS, in this series of patients LNSC levels and LNSC/MSC ratio were significantly higher in patients CS compare to patients with AI in whom CS was subsequently excluded by detailed clinical, biochemical and hormonal testing. The cutoff value validated by our laboratory ($\leq$ 0.350 µg/dL) presented a sensitivity of 93.8% and a specificity of 79.5% to the diagnosis of CS. Several authors showed an altered circadian cortisol secretion rhythm in AI patients with high midnight plasma and salivary cortisol levels [39-43]. In a meta-analysis of seven studies the estimated sensitivity and specificity of LNSC for the diagnosis of CS was 92% and 96%, respectively [44].

In this cohort of patients with AI, we found a high prevalence of hypertension (72.3%), type 2 diabetes mellitus (56.6%), dyslipidemia (30.1%) and obesity (28.8%). AI patients with cortisol levels after 1-mg DST $\geq$ 1.8 µg/dL had a significantly higher prevalence of hypertension, dyslipidemia and established cardiovascular disease. Reports from different research groups have consistently demonstrated an association between cortisol excess, hypertension, impairment of glucose metabolism, dyslipidemia and an increased risk of cardiovascular diseases [4, 12, 45-50]. These data support the concept that although SH is not associated with the typical clinical manifestations of overt cortisol excess, this condition may lead to long-term consequences of cortisol excess and an increased rate of several metabolic and cardiovascular co-morbidities, such as hypertension, impaired glucose metabolism and increased visceral fat.

Conclusions

The 1-mg DST at its lowest threshold ($\leq$ 1.8 µg/dL) shows a high sensitivity to rule out CS in patients with adrenal incidentaloma, however, its low specificity addresses the need for additional testing after a positive screening. Due to its high specificity, we suggest combine use of UFC to reduce the number of false-positive test results.

Conflict of Interest

The authors declare that they have no conflict of interest.

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