

Research paper

Is the 250 µg ACTH test a useful tool for the diagnosis of central hypoadrenalism in adult patients with pituitary disorders?

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ABSTRACT

OBJECTIVE: The diagnosis of hypothalamic-pituitary-adrenal insufficiency (HPAI) is a major clinical challenge. The gold standard procedure remains insulin tolerance test (ITT). This study aimed to evaluate the usefulness of standard-dose corticotrophin stimulation test (SDCT) in diagnosing HPAI. **DESIGN:** In this prospective study we performed SDCT and ITT in 55 consecutive patients (37F/18M) affected by pituitary disorders. **RESULTS:** A normal response to ITT was found in 44 patients, while HPAI was diagnosed in 11. Using ITT as reference test, the ROC curve showed that a cortisol value of 18 µg/dl (500 nmol/L) at 30 min or 21.8 µg/dl (600 nmol/L) at 60 min after SDCT represents the best compromise between sensitivity and specificity in diagnosing HPAI. Moreover, 30 min cortisol values >20.3 µg/dl (560 nmol/L) or 60 min cortisol values >24.1 µg/dl (665 nmol/L) exclude HPAI. Four out of 15 patients of Group A, previously non-respondent to SDCT, showed a normal response to a second SDCT. **CONCLUSIONS:** SDCT is not a reliable tool to identify HPAI, but it appears to be more useful in confirming the normality of HPA function. When SDCT fails to exclude HPAI, ITT should be performed. If ITT is contraindicated, retesting patients by SDCT is useful before starting an unnecessary replacement therapy.

Key words: Central hypoadrenalism, HPAI, ITT, SDCT

INTRODUCTION

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Central hypoadrenalism occurs in about one third of patients with hypothalamic-pituitary disorders,¹ and its diagnosis is necessary to allow a prompt replacement therapy in a potentially life-threatening condition. The insulin tolerance test (ITT) remains the gold standard procedure for the diagnosis of hypothalamic-pituitary-adrenal insufficiency (HPAI) where a cortisol peak greater than 18 µg/dl (500 nmol/L) is generally accepted to define a normal response.²⁻⁶ However, ITT is potentially hazardous, requires strict monitoring of the patients and is contraindicated in subjects with ischemic heart disease, arrhythmias or history of epilepsy.

In recent years different provocative tests, in particular the standard-dose (250 µg) corticotrophin stimulation test (SDCT) and low-dose (1 µg) corticotrophin stimulation test (LDCT), have been developed in order to provide a safe and effective alternative to ITT in the diagnosis of central hypoadrenalism. To date, a cortisol cut-off value from 18 to 23.5 µg/dl (500 to 650 nmol/L) at 30 min or 60 min after SDCT and from 18 to 21.7 µg/dl (500 to 599 nmol/L) at 30 min or peak levels after LDCT have been proposed to define a normal response.^{2-4,7-16} The diagnostic accuracy of SDCT at 30 min and 60 min cortisol values is considered similar.¹ Finally, data concerning the comparison between SDCT and LDCT are discordant, though LDCT seems to provide better sensitivity in diagnosing cortisol deficiency.¹

In a recent meta-analysis, Kazlauskaitė and colleagues analyzed results of 13 studies with at least ten subjects (total number of patients: 679) with suspected HPAI who underwent a reference test (ITT or metyrapone test), proposing a three-step approach for the assessment of HPA axis function.¹ In this approach, patients with basal cortisol levels (step 1) below 5 µg/dl (138 nmol/L) or 30 min cortisol levels after SDCT (step 2) below 16 µg/dl (440 nmol/L) are mostly affected by HPAI. Conversely, patients with basal cortisol levels above 13 µg/dl (471 nmol/L) or 30 min cortisol levels after SDCT above 30 µg/dl (833 nmol/L) can be considered normal. Intermediate values of basal cortisol (5-13 µg/dl, 138-471 nmol/L) and then 30 min cortisol levels after SDCT (16-30 µg/dl, 440-833 nmol/L) require ITT (step 3).

In the last few years, concerns about the risk of

unnecessary chronic glucocorticoid therapy in patients with pituitary disorders have been expressed, with regard to the difficulties in assessing a replacement therapy mimicking the circadian rhythm of cortisol secretion.¹⁷

The aim of this study was to verify the diagnostic performance of SDCT in a cohort of patients referred to our center for hypothalamic-pituitary disorders by considering ITT as gold standard procedure for HPAI diagnosis.

MATERIALS AND METHODS

Patients

The study included 55 patients affected by different hypothalamic-pituitary disorders (37F and 18M, mean age at diagnosis: 40.5 ± 10.6 yrs). Specifically, 19 patients had nonfunctioning pituitary adenomas (10 macroadenomas and 9 microadenomas, 7 of whom treated by transsphenoidal surgery, followed by radiotherapy in 2), 14 prolactinomas (7 macroadenomas and 7 microadenomas, 2 of whom had transsphenoidal surgery), 7 empty sella, 5 GH-secreting pituitary adenomas (4 macroadenomas and 1 microadenoma treated by transsphenoidal surgery followed by radiotherapy in 2), 3 lymphocytic hypophysitis, 2 Rathke's cleft cysts (1 treated by transsphenoidal surgery), 2 idiopathic hypopituitarism, 1 granulomatosis, 1 TSH-secreting macroadenoma and 1 ACTH-secreting microadenoma (both studied after a successful transsphenoidal surgery). At the beginning of the study, 13 patients had hypopituitarism (single deficit in 5 patients and multiple deficit in 8 patients). In detail, 10 patients showed GH deficiency, 8 patients hypogonadism, 3 patients diabetes insipidus and 2 patients hypothyroidism. All patients were evaluated at least two months after neurosurgery, were receiving appropriate replacement therapy for pituitary deficits and all nine patients receiving cortisol substitution stopped the treatment 24 h prior to the tests. In particular, the doses of cortisone acetate were stable between SDCT and ITT and no patient started replacement therapy between SDCT and ITT. Two female patients receiving estrogen replacement were tested after at least one month of treatment interruption. Patients taking steroids for unrelated diseases were excluded.

The Local Ethical Committee approved the study protocol and all patients gave their informed written consent to participate in the study.

Study protocol

All patients were evaluated in a fasting state at 08.00 h with an iv catheter inserted in a forearm vein and kept patent by slow saline infusion. In all cases, a SDCT followed by an ITT were performed, with a maximum interval of 6 weeks between tests (range: 2-6). In a subgroup of 15 patients, SDCT was repeated after a mean period of 6 months (range: 4-9). ACTH levels were assayed at baseline.

For the SDCT, 250 µg of tetracosactide (Synacthen 0.25 mg/1ml, Biofutura Pharma, Pomezia, Italy) was injected as a bolus intravenously. Blood samples for cortisol evaluation were taken at 0, 30 and 60 min. The choice of SDCT was made in order to avoid the possible inaccuracy in the dilution of the 250 µg vials, which is required for LDCT.

For the ITT, iv insulin bolus (Actrapid, Novo Nordisk, Denmark) at 0.1 U/kg body weight was administered. Blood samples for cortisol evaluation were taken at 0, 30, 45, 60, 90 and 120 minutes. Hypoglycaemia was considered adequate in the presence of blood glucose levels of less than 40 mg/dl (2.2 mmol/l). A peak cortisol level greater than 18 µg/dl (500 nmol/l) was considered normal.

Assays

All hormone measurements were performed in the same laboratory. Plasma ACTH levels (pg/ml) were measured by chemoluminescent immunometric assay (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) with an inter-assay coefficient of variation ranging from 6.1 to 10.0%, an intra-assay coefficient of variation ranging from 6.7 and 9.5% and sensitivity of 5 pg/ml.

Serum cortisol levels (µg/dl) were measured by electrochemiluminescence immunoassay (ECLIA) (Roche Cobas Cortisol, Roche Diagnostics, Mannheim, Germany) with an inter-assay coefficient of variation ranging from 1.4 to 1.6%, an intra-assay coefficient of variation ranging from 1.0 and 1.4% and lower detection limit of 0.018 µg/dl. Glucose was measured using the standard method.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences, version 12.0 (SPSS Inc, Chicago, IL, USA). All results are expressed as mean \pm SD unless otherwise stated. A Student's *t*-test was performed to compare continuous variables among groups when data were normally distributed. Categorical variables were compared by Fisher or χ^2 test, where appropriate. Linear regression analysis was used to determine correlation coefficients between different parameters.

The receiver operating characteristic (ROC) curve was obtained, using ITT as reference test, to assess the sensitivity and specificity of the SDCT in the diagnosis of HPAI at different cut-off values both for 30 and 60 min serum cortisol levels during the test. The area under the ROC curve (AUC) and pairwise comparison of ROC curves for 30 and 60 min serum cortisol levels was also calculated. Positive (PPV), negative (NPV) predictive value, sensitivity, specificity and accuracy were calculated using the number of patients true-positive (TP), true-negative (TN), false-positive (FP) and false-negative (FN). PPV (calculated as TP/(TP+FP) was defined as the likelihood that a subject with a positive test (peak cortisol below the specific cut-off point) is affected with central hypoadrenalism. NPV (calculated as TN/(TN+FN) was defined as the likelihood that a subject with a negative test (peak cortisol above the specific cut-off point) has a normal HPA function.

Values of $P < 0.05$ were considered statistically significant.

To assess reliability between stimulation tests, a limits of agreement plot as proposed by Bland and Altman was used (MedCalc Software Version 12.2.1). Limits of agreement between cortisol peak after SDCT and ITT were determined using 95% sample confidence intervals.

RESULTS

Of the 55 patients, 44 patients showed a normal response to ITT [peak cortisol levels greater than 18 µg/dl (500 nmol/L), Group A]. In the remaining 11 patients diagnosis of adrenal insufficiency was made (peak cortisol levels below 18 µg/dl, Group B).

Clinical and biochemical characteristics of the patients divided into Group A and Group B are shown in Table 1. Age, basal cortisol, ACTH levels and prevalence of other pituitary deficits were comparable between groups. Conversely, cortisol levels obtained via SDCT at 30 and 60 min were significantly lower in patients belonging to Group B with respect to Group A.

Analyzing SDCT data with the ROC curve, a cortisol value of 18 µg/dl (500 nmol/L) at 30 min pairs the highest sensitivity (64%) and specificity (64%), correctly identifying hypoadrenalism (30 min cortisol levels below 18 µg/dl) only in 30% of patients with positive tests (PPV) and correctly identifying normal HPA axis function (30 min cortisol levels above 18 µg/dl) in 88% of patients with negative tests (NPV) (accuracy 64%) (Figure 1A). Similarly, considering cortisol levels at 60 min, the ROC curve showed that the SDCT with a cut-off value of 21.8 µg/dl (600 nmol/L) achieved a sensitivity of 72% and a specificity of 61%, with a PPV of 32%, a NPV of 90% and accuracy of 64% (Figure 1B). The AUC values for cortisol levels at 30 and 60 min were comparable (0.690 vs 0.707, $P=0.8$).

Among patients of Group B, no patient showed cortisol levels higher than 20.3 µg/dl (560 nmol/L) and 24.1 µg/dl (665 nmol/L) at 30 and 60 min after SDCT, respectively (Figure 2). Therefore, the cut-off of 20.3 µg/dl at 30 min showed a 100% sensitivity and a 21% specificity, enabling identification of all patients with adrenal insufficiency but with a high rate of false positive results (PPV 24%, NPV 100%, accuracy 36%, Figure 2A). Similar data were obtained

with the cortisol value of 24.1 µg/dl at 60 min (PPV 25%, NPV 100%, accuracy 40%, Figure 2B).

Also considering a cut-off of 16 µg/dl (440 nmol/L), as proposed by Kazlauskaitė and colleagues,¹ SDCT correctly identified hypoadrenalism (30 min cortisol levels below 16 µg/dl) in only 43% of patients with positive tests (PPV), while correctly it identified normal HPA axis function (30 min cortisol levels above 16 µg/dl) in 83% of patients with negative tests (NPV), showing a sensitivity of 27% and specificity of 91%. As a whole, SDCT with a cut-off of 16 µg/dl failed to correctly detect 12 out of 55 patients (accuracy 78%). The performance of SDCT with these different cut-offs is summarised in Table 2.

The Bland-Altman difference plot showed a bias of 0.6 (-0.47 to 1.66)% (mean [95% confidence interval]) with 95% limits of agreement -7.1 to 8.3, and no systematic bias was evident (not shown). Moreover, a statistically significant correlation was found between cortisol peak after SDCT and ITT ($r^2=0.51$, $p<0.001$).

With regard to basal cortisol levels, no patient with diagnosis of HPAI by ITT showed levels higher than 11.4 µg/dl (315 nmol/L). Conversely, four out of six patients with basal cortisol levels lower than 5 µg/dl (138 nmol/L) showed a normal response after ITT.

Finally, in 15 patients of Group A, previously non-responsive to SDCT, a second SDCT was performed after a mean period of 6 months. In 4 out of these 15 patients, 30 min cortisol levels >20.3 µg/dl (560 nmol/L) or 60 min cortisol levels >24.1 µg/dl (665 nmol/L) were found, thus confirming the as per ITT normal HPA function (Figure 3).

Table 1. Clinical and biochemical characteristics of patients with normal (Group A) or insufficient (Group B) cortisol response to insulin tolerance test (ITT). Response to ITT was considered normal if peak cortisol levels were >18 µg/dl (500 nmol/L)

	Group A, n=44	Group B, n=11	P
Age (yr)	41.0±10.8	38.5±9.7	0.49
Other pituitary deficits (%)	9 (20.5)	4 (36.4)	0.46
ACTH (pg/ml)	13.4±5.4	17.4±10.3	0.08
Basal cortisol (µg/dl)	9.0±2.6	8.6±2.6	0.67
Peak cortisol levels after ITT (µg/dl)	23.4±2.9	14.4±2.1	<0.001
30 min cortisol levels after SDCT (µg/dl)	18.8±2.3	16.8±3.0	0.016
60 min cortisol levels after SDCT (µg/dl)	22.5±2.8	20.2±2.8	0.019

Data are expressed as mean ± SD or absolute number with percentage in parenthesis.

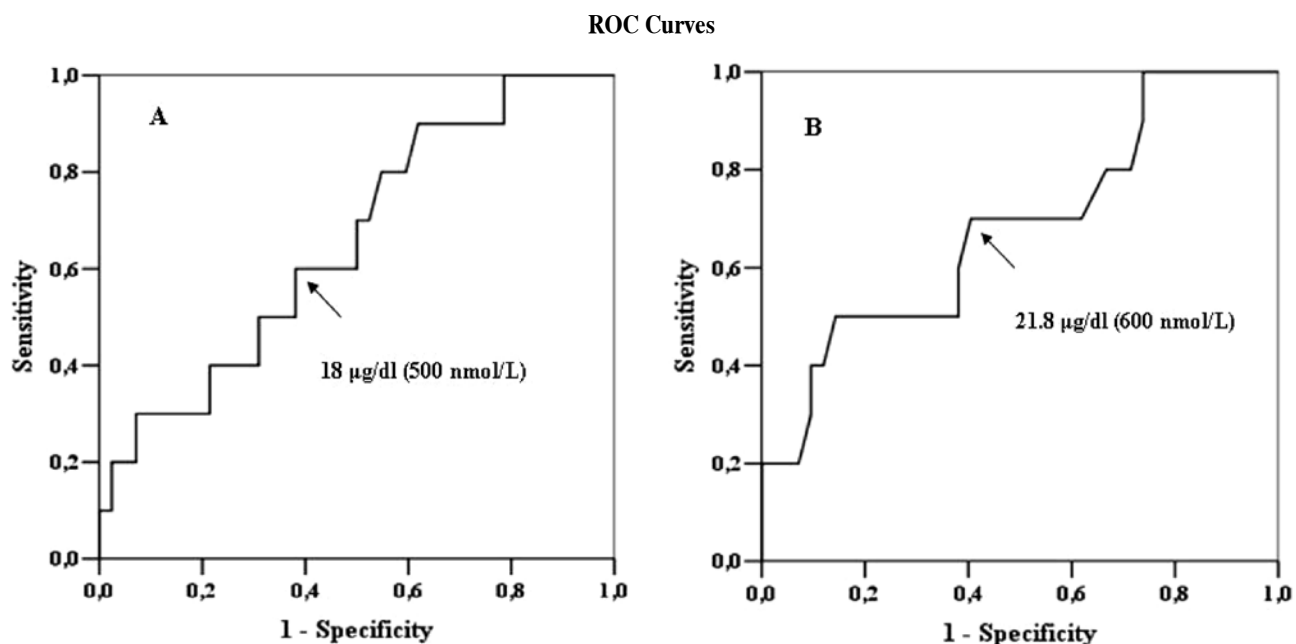


Figure 1. ROC curves for SDCT in the diagnosis of HPA insufficiency. A) ROC curve for 30-min cortisol levels at SDCT in identifying patients with an insufficient cortisol response after ITT. The arrow points at the 18 µg/dl (500 nmol/L) cut-off which is the cut-off with the best compromise between sensitivity and specificity. B) ROC curve for 60-min cortisol levels at SDCT in identifying patients with an insufficient cortisol response after ITT. The arrow points at the 21.8 µg/dl (600 nmol/L) cut-off which is the cut-off with the best compromise between sensitivity and specificity.

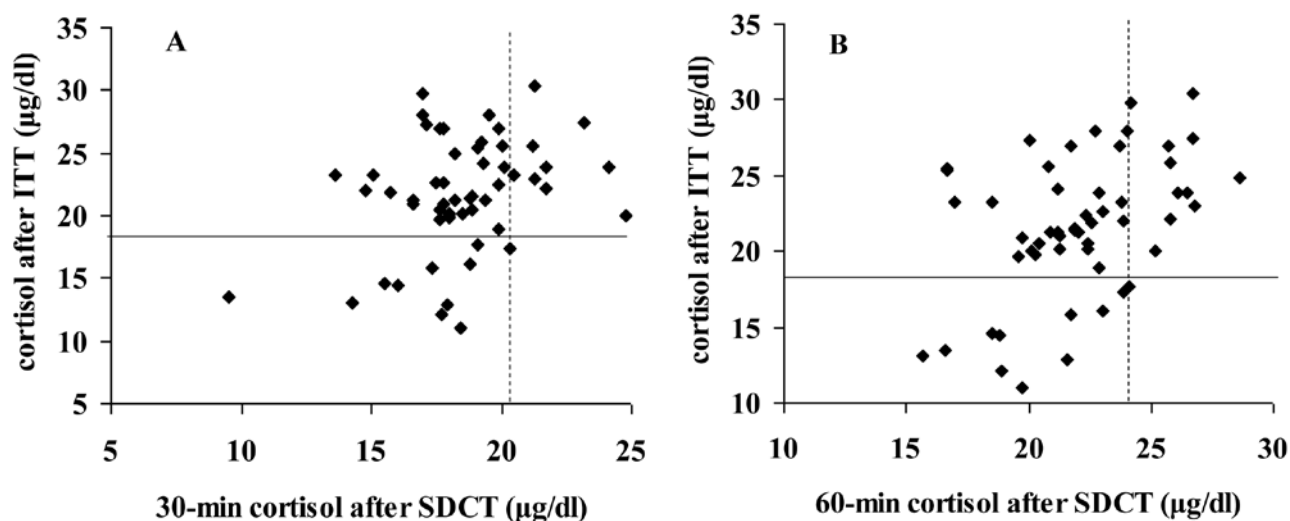
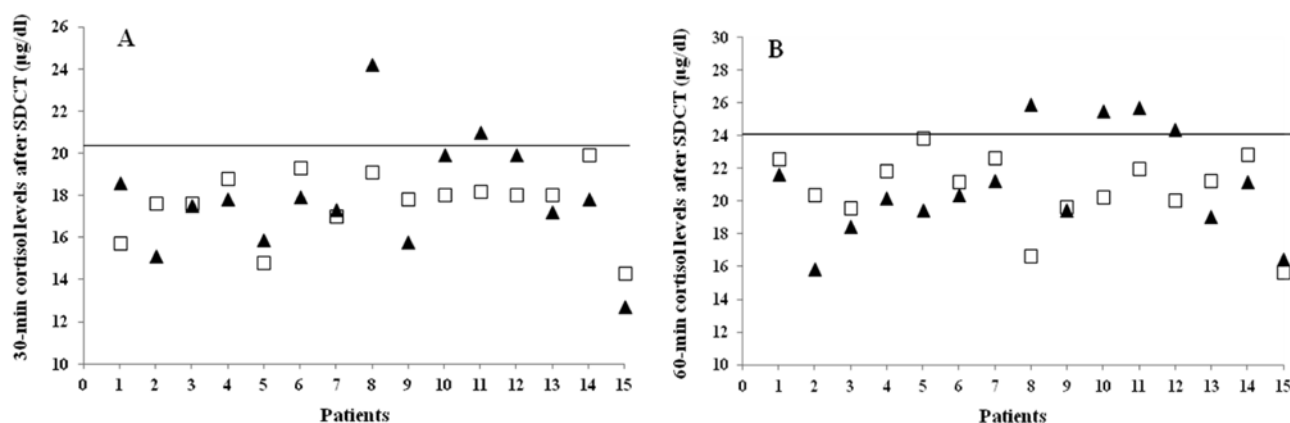


Figure 2. Comparison between cortisol response to SDCT and ITT. A) Peak serum cortisol response to ITT vs 30-minutes serum cortisol response to SDCT. Continuous line represents cut-off limit for the diagnosis of hypoadrenalism after ITT (18 µg/dl, 500 nmol/L). Broken line represents cut-off with 100% sensitivity for the diagnosis of hypoadrenalism after SDCT (20.3 µg/dl, 560 nmol/L). B) Peak serum cortisol response to ITT vs 60-minutes serum cortisol response to SDCT. Continuous line represents cut-off limit for the diagnosis of hypoadrenalism after ITT (18 µg/dl, 500 nmol/L). Broken line represents cut-off with 100% sensitivity for the diagnosis of hypoadrenalism after SDCT (24.1 µg/dl, 665 nmol/L).

Table 2. Specificity, sensitivity, PPV, NPV and accuracy of 30 and 60 min cortisol levels after SDCT with different cut-offs

	30 min cortisol levels after SDCT			60 min cortisol levels after SDCT	
	Cut-off 16 µg/dl	Cut-off 18 µg/dl	Cut-off 20.3 µg/dl	Cut-off 21.8 µg/dl	Cut-off 24.1 µg/dl
Specificity (%)	91	64	21	61	25
Sensitivity (%)	27	64	100	72	100
PPV (%)	43	30	24	32	25
NPV (%)	83	88	100	90	100
Accuracy (%)	78	64	36	64	40

**Figure 3.** Modification of cortisol response to SDCT over time in a subgroup of 15 patients. A) 30-min serum cortisol levels after first (grey squares) and repeated SDCT (black triangles). Continuous line represents cut-off with 100% sensitivity for the diagnosis of hypoadrenalism after SDCT (20.3 µg/dl, 560 nmol/L). B) 60-min cortisol levels after first (grey squares) and repeated SDCT (black triangles). Continuous line represents cut-off with 100% sensitivity for the diagnosis of hypoadrenalism after SDCT (24.1 µg/dl, 665 nmol/L).

DISCUSSION

Central adrenal deficiency in patients with pituitary disorders is a life-threatening condition and requires a chronic replacement therapy. However, the diagnosis of this condition is still controversial. Among the different tests proposed, ITT is considered the gold standard and a cortisol peak greater than 18 µg/dl (500 nmol/L) is widely accepted as defining a normal response, though some authors have reported a poor reproducibility of cortisol response to ITT in subjects with hypopituitarism,¹⁸ as well as some cases of misclassification in patients with clinically manifest hypoadrenalism.¹⁹

In recent years different provocative tests, in particular the standard-dose (250 µg) corticotrophin stimulation test (SDCT) and low-dose (1 µg) corticotrophin stimulation test (LDCT), have been developed in order to provide a safe and effective alternative

to ITT in the diagnosis of central hypoadrenalism. However, the diagnostic performance of these tests depends on the value of cortisol cut-off, which as yet remains uncertain.

In the present study, considering ITT as a reference test, ROC analysis showed that cortisol levels of 18 µg/dl (500 nmol/L) at 30 min and 21.8 µg/dl (600 nmol/L) at 60 min after SDCT reached the best compromise between sensitivity and specificity, although they correctly identified hypoadrenalism only in 30% of patients with positive tests. Therefore, in our patients the accuracy of SDCT in diagnosis of central hypoadrenalism was poor. The diagnostic discrimination of 30 and 60 min cortisol levels was similar, according to data of previous studies.¹ Furthermore, although 30 min cortisol levels after SDCT appear to be sufficient to evaluate the HPA axis, different thresholds should be considered when 60 min cortisol

levels after SDCT are used.²⁰

A recent revision of the available studies suggested that a 30 min. cortisol response to SDCT lower than 16 µg/dl (440 nmol/L) or higher than 30 µg/dl (833 nmol/L) strongly supports or excludes diagnosis of HPAI, respectively, while intermediate values are diagnostically inconclusive and require additional assessment with ITT.¹ Considering these cut-off values for SDCT, we would have diagnosed ACTH insufficiency in 7 out of 55 patients, while the other 48 subjects would have shown intermediate values of 30 min cortisol (16-30 µg/dl). Therefore, according to these criteria for SDCT, no patient of the present cohort would have received a diagnosis of normal HPA function. This is in strong contrast with the results obtained by retesting the patients with ITT, since a normal cortisol response to ITT was achieved in 44 patients and notably in 4 out of 7 patients with a peak cortisol level after SDCT below 16 µg/dl. Moreover, although the choice of a 30 min cut-off as low as that proposed by Kazlauskaite and colleagues permits achievement of the highest accuracy (78%), it leads to an incorrect diagnosis of central hypoadrenalism in the majority of patients analysed, which fail SDCT but show a normal response after ITT.

In our cohort of patients 30 min cortisol levels higher than 20.3 µg/dl (560 nmol/L) or 60 min cortisol levels higher than 24.1 µg/dl (665 nmol/L), we were able to exclude the presence of HPAI with a sensitivity of 100%. Although obtained in a limited number of patients, these results are in agreement with those of other authors, suggesting that these cut-offs allow reasonable exclusion of the disease.^{9,11,21} Obviously the choice of these cut-offs dramatically decreases the specificity (21% and 25%, respectively) of SDCT, correctly identifying only a quarter of subjects with normal HPAI function.

Moreover, 4 out of 15 patients (27%) with a normal response to ITT but indeterminate cortisol levels after SDCT (30 min cortisol levels lower than 20.3 µg/dl or 60 min cortisol levels lower than 24.1 µg/dl) showed a normal HPA function when retested by SDCT after a short period.

This finding may be of clinical relevance in the presence of any contraindication to ITT or for those Centers poorly experienced with this procedure. In

these cases, the repetition of SDCT over time should be considered before initiation of a chronic replacement therapy.

As reported for the performance of SDCT, in the present study the importance of basal cortisol levels in HPAI diagnosis was limited, since 4 out of 6 patients showing basal cortisol levels lower than 5 µg/dl (138 nmol/L) had a normal response to ITT. Conversely, basal cortisol levels greater than 11.4 µg/dl (315 nmol/L) seemed to suggest a normal HPA axis function.

It must be stressed that the aim of this study was to evaluate the diagnostic performance of the SDCT in the diagnosis of central hypoadrenalism in a limited cohort of patients with pituitary disease referred to our center and not to define new cut-offs for basal or after SDCT cortisol values. Indeed, in addition to the limitation due to the low number of patients of the present series, it is well known that cortisol measurement suffers from assay variability, leading to different cut-off values and diagnostic parameters.²² Moreover, the cut-off of 18 µg/dl (500 nmol/L) used to define a normal response to ITT, based on cortisol response to surgical stress,²³ should be confirmed in each Endocrine Unit with modern assay methods.

In conclusion, we observed that despite the choice of a strict cut-off value as 16 µg/dl, SDCT did not allow correct identification of patients with HPAI, leading to an overestimation of the frequency of HPAI. Nevertheless, according to the present data we suggest that SDCT, together with basal cortisol levels, may represent a valid procedure for patients with suspected central hypoadrenalism, with the main aim of excluding ACTH deficiency. When SDCT fails to rule out HPAI, ITT should be performed. If ITT is contraindicated, retesting patients by SDCT at 6-month intervals is useful in order to avoid unnecessary cortisol replacement therapy.

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