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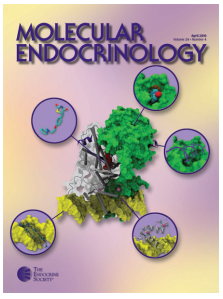

Use of the Desmopressin Test in the Differential Diagnosis of Pseudo-Cushing State from Cushing's Disease

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Context: The desmopressin (DDAVP) test has been proposed to discriminate Cushing's disease (CD) from pseudo-Cushing states (PC); however, current information on its value is scarce and contradictory.

Objective: The aim of the study was to assess the ability of the DDAVP test in distinguishing between these conditions, with emphasis on subjects with mild hypercortisolism.

Design and Setting: We conducted a retrospective/prospective study at the Division of Endocrinology, Polytechnic University of Marche, Ancona, Italy.

Patients: The study included 52 subjects with CD, 28 with PC, and 31 control subjects (CT).

Intervention(s): We performed the DDAVP test and standard diagnostic procedures for the diagnosis of Cushing's syndrome.

Main Outcome Measure(s): The diagnosis/exclusion of CD was measured.

Results: Interpretation of the DDAVP test based on percentage and absolute increment of cortisol and ACTH did not afford acceptable values of both sensitivity (SE) and specificity (SP). CD diagnosis based on simultaneous positivity for basal serum cortisol greater than 331 nmol/liter and absolute ACTH increment greater than 4 pmol/liter and its exclusion in subjects negative for one or both measures yielded an SE of 90.3% and an SP of 91.5%. The approach was also highly effective in distinguishing PC from: 1) CD with moderate values of urinary free cortisol (SE, 86.9%; SP, 92.8%); 2) CD with moderate values of serum cortisol after dexamethasone suppression (SE, 86.6%; SP, 92.8%); and 3) CD with moderate values of midnight serum cortisol (SE, 100%; SP, 92.8%).

Conclusions: Interpretation of the DDAVP test through a combination of parameters allowed effective discrimination of CD from PC, even in subjects with mild hypercortisolism. **(*J Clin Endocrinol Metab* 95: 1115–1122, 2010)**

Pseudo-Cushing state (PC) is caused by conditions (*e.g.* depression, alcoholism, polycystic ovary syndrome, severe obesity) that can activate the hypothalamic-pituitary-adrenal axis and is characterized by clinical and biochemical signs typical of Cushing's syndrome (CS) (1, 2).

However, the overlapping clinical features of the two conditions (3) and the similar values frequently determined in tests such as urinary free cortisol (UFC), serum cortisol after dexamethasone suppression, and midnight serum cortisol in the respective patients make it

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Abbreviations: Δ-ACTH, Absolute rise in plasma ACTH; AUC, area under the curve; AUC_{LR}, area under the ROC curve; BMI, body mass index; CD, Cushing's disease; CI, confidence interval; CS, Cushing's syndrome; CT, control; DA, diagnostic accuracy; DDAVP, desmopressin; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; OST, overnight dexamethasone suppression test; PC, pseudo-Cushing state; PPV, positive predictive value; ROC, receiver-operator characteristic; SE, sensitivity; SP, specificity; UFC, urinary free cortisol.

difficult to distinguish subjects with PC from CS patients (1, 2, 4).

One tool available to the clinician is the desmopressin (DDAVP) test. Albeit of little use in patients with adrenal CS or ectopic ACTH syndrome, the test has been proposed to be valuable in patients with mild hypercortisolism and normal ACTH levels in whom the differential diagnosis has narrowed to Cushing's disease (CD) or PC (3, 5, 6). Its ability to discriminate between CD and PC is related to the fact that it usually elicits a marked elevation of plasma ACTH and serum cortisol in most patients with CD—likely due to up-regulation of pituitary vasopressin receptors (V3) (7, 8)—but generally not in PC or in healthy subjects (3, 5).

Two studies investigating the scope for distinguishing CD from PC subjects by this test found a rise in plasma ACTH of at least 6 pmol/liter within 30 min of stimulation as the most effective diagnostic criterion (3, 5). However, the more recent study (3) described lower sensitivity values for this criterion (81.5 *vs.* 86.8%), especially in diagnosing CD with mild hypercortisolism (77.7 *vs.* 90%).

We report on the 10-yr application of the DDVAP test in distinguishing CD from PC at our center. The control group (CT) was made up of subjects with simple obesity, to make it comparable to CD and PC subjects in terms of body mass index (BMI). Test results were interpreted using published criteria (3, 5, 6) and then using a new method devised by our group to improve its diagnostic value. We also examined the ability of the test to distinguish CD with mild hypercortisolism from PC.

Subjects and Methods

Subjects

Subjects were 111 individuals consecutively admitted to our center between 1999 and 2008: 52 with a first diagnosis of active CD, 28 with PC, and 31 CT subjects. CD and PC subjects were admitted for suspected CD, which was then confirmed or excluded, respectively; they received clinical, instrumental, and biochemical evaluation as part of the diagnostic work-up, and their data were evaluated retrospectively. The CT subjects attended our center for diet counseling; they were recruited prospectively and underwent examination purely for research purposes.

The diagnosis of CD was based on clinical and biochemical data (9) and was subsequently confirmed on pituitary surgery and/or postoperative clinical and biochemical resolution of hypercortisolism. The diagnosis of PC (1) was based on common criteria (1, 3, 5, 10–12) that make it possible to exclude CD with a high degree of probability: 1) clinical and biochemical findings consistent with hypercortisolism; 2) identification of a clinical condition known to activate the hypothalamic-pituitary-adrenal axis; 3) a lack of progression of clinical and biochemical abnormalities during follow-up for at least 3 yr and/or normalization of biochemical abnormalities after treatment of the associated condition; and 4) normal pituitary findings on magnetic resonance imaging. Of the 28 subjects with PC, 16 had major depression (13); two had alcoholism (13); nine had both polycystic ovary syndrome (14, 15) and panic disorder (13); and one had bulimia nervosa (13).

The 31 CT subjects were selected from among individuals with simple obesity (BMI > 30 kg/m^2) and no clinical or biochemical signs of CS or evidence of psychiatric disorders; all had normal UFC on three different collections, normal serum cortisol circadian rhythm, and serum cortisol after overnight low-dose (1 mg) dexamethasone suppression test (OST) below 50 nmol/liter (4, 9). They also had normal bone mineral density. No patient with adrenal incidentaloma was included. Subjects taking medications known to affect any parameter addressed in the study underwent washout before hospitalization.

The study was performed according to the Declaration of Helsinki and approved by the institutional ethics committee. All subjects undergoing testing at our center are asked to sign an informed consent form at admission. Some of the data were acquired in the framework of a research protocol that entailed an additional consent form.

Study protocol

All subjects underwent comprehensive physical examination and determination of: 1) serum cortisol circadian rhythm [awake midnight serum cortisol was measured after 24 h hospitalization (4, 16)]; 2) 24-h UFC (three collections); and 3) serum cortisol after OST. The DDAVP test was performed after overnight fasting by injecting 10 μg DDAVP (Minirin/DDAVP; Ferring Pharmaceuticals Ltd., Malmo, Sweden) as a slow iv bolus. An indwelling catheter was inserted in a forearm vein at 0800 h; blood samples were collected 15 min before the test, at 0 min (0830 h) and then at 10, 20, 30, 45, 60, 90, and 120 min. Samples for the other CS diagnostic procedures were collected from an indwelling venous catheter placed at least 1 h earlier.

Bone mineral density was assessed in all subjects by dual x-ray absorptiometry (DPX, software version 3.61; Lunar Radiation, Madison, WI).

Basal serum cortisol and basal plasma ACTH were the means of the two baseline values (-15 and 0 min) before DDAVP administration. The absolute rise in plasma ACTH after the DDAVP test was calculated according to the literature (3, 5), *i.e.* as the difference between time 0 and the highest value reached within 30 min (Δ-ACTH). UFC was the mean of the three samples.

All the presented results of CD and PC subjects were obtained during hospitalization for the diagnosis or exclusion of CD, whereas those of CT subjects were obtained during hospitalization for research purposes.

Assays

Chemiluminescent immunometric assays were used to measure plasma ACTH (Immulite; Diagnostic Products Corp., Los Angeles, CA) and serum cortisol and UFC (Advia Centaur; Bayer Diagnostics, Newbury, UK), the latter after urine extraction with dichloromethane. Method sensitivity was 0.99 pmol/liter for plasma ACTH and 11 nmol/liter for both urinary and serum cortisol; intraassay and interassay coefficients of variation were 3.4 and 4.8% for plasma ACTH and 4.4 and 6.0% for both urinary and serum cortisol, respectively. Normal ranges in our laboratory are 0–10 pmol/liter for plasma ACTH, 41–413

nmol/24 h for UFC, and 138–634 nmol/liter for morning serum cortisol (0830 h).

Statistical analysis

Shapiro-Wilk's test was applied to verify the normal distribution of the clinical and biochemical continuous variables. Values are expressed as mean ± SEM if normally distributed and as median (interquartile range) if not normally distributed.

The prevalence of clinical signs was analyzed by the χ^2 test or, where appropriate, with Fisher's exact test. The net integrated area under the curve (AUC) for plasma ACTH (AUC-ACTH) and serum cortisol (AUC-cortisol) responses to DDAVP was calculated using the trapezoidal method (17).

Comparisons between groups were made with Mann-Whitney's *U* test given the nonnormal distribution of the variables examined. Comparisons among groups were made with ANOVA followed by Fisher's least significant difference *post hoc* test for normally distributed values, and with Kruskal-Wallis test followed by Mann-Whitney's *U* test (if significant differences were detected) for not normally distributed variables; *P* values were corrected using the Bonferroni-Holm method (18).

Spearman correlation was performed. The biserial correlation coefficient was used to test for correlations between continuous and dichotomized variables after logarithmic transformation to obtain a normal distribution, as appropriate.

The diagnostic performance of the DDAVP test in subjects with mild hypercortisolism was further analyzed by creating an artificial "PC reference range" for UFC, OST serum cortisol, and midnight serum cortisol according to the classic method "mean ± 2 SD"; CD subjects were then assigned to one or more of three subgroups defined by: 1) UFC below the mean + 2 SD (771 nmol/24 h) of UFC values found in PC (Mild-UFC-CD); 2) OST serum cortisol values below the mean + 2 SD (458 nmol/liter) of OST serum cortisol found in PC (Mild-OST-CD); and 3) midnight serum cortisol values below the mean + 2 SD (428 nmol/liter) of midnight serum cortisol found in PC (Mild-Cort24-CD).

For this procedure, skewed variables were logarithmically transformed to obtain normality before statistical analysis and then back-transformed to their natural units as appropriate.

Sensitivity (SE), specificity (SP), positive likelihood ratio (LR+), negative likelihood ratio (LR−), positive predictive value (PPV), negative predictive value (NPV), and receiver-operator characteristic (ROC) curves were calculated according to standard statistical methods (19); diagnostic accuracy (DA) was calculated as the proportion of correctly rated patients out of the total number of patients tested. Exact 95% binomial confidence intervals (CIs) were computed for SE and SP (20).

As regards the variables used singly, the cutoffs obtained from the ROC curves that offered the highest sum of SE and SP were adopted. The combination of basal serum cortisol and Δ-ACTH was tested by MultiRoc analysis (21), where the ROC curve was plotted based on the rule: "Diagnose as positive for the disease if basal serum cortisol is greater than a fixed cutoff point and Δ-ACTH is greater than all possible cutoff points." This was done for all possible basal serum cortisol cutoff points and yielded a ROC curve for each basal serum cortisol cutoff point. The procedure yielded the SE and SP of all possible cutoff combinations of basal serum cortisol and Δ-ACTH, from which the pair affording the highest sum of SE and SP was selected.

The diagnostic performance of the various tests was analyzed by comparing their SE values with the McNemar test; the same procedure was applied to compare the SP values. A logistic regression analysis was also performed for each test parameter and for the Δ-ACTH and basal serum cortisol combination: the predicted probabilities from the logistic regression models were used to calculate the area under the ROC curves (AUC_{LR}) (22) with 95% CIs (CI_{AUC}); the AUC_{LR} were compared according to DeLong *et al.* (23).

A two-step approach, previously used by Pecori Giraldi *et al.* (3), was applied to assess the diagnostic performance of the second-level tests (midnight serum cortisol and DDAVP) on the basis of different estimates of CD prevalence. The first step involved calculating the PPV of the first-level tests (UFC and OST serum cortisol) using the CD prevalence obtained from the literature. In the second step, the highest PPV obtained in the previous step was taken as the *a priori* CD prevalence on which the diagnostic performance of midnight serum cortisol and the DDAVP test were calculated. The resulting PPV, NPV, and DA were compared by the χ^2 test as follows: PPV *vs.* PPV; NPV *vs.* NPV; DA *vs.* DA. PPV, NPV, and DA ranges were obtained by applying the procedure using the endpoints of the CI for the SE and SP of the same tests.

Significance was set at $P < 0.05$. Statistical analyses were performed using SPSS 16 package (SPSS Inc., Chicago, IL).

Results

The demographic and biochemical data of the study subjects are shown in Table 1. The clinical data of CD and PC subjects are reported in Fig. 1.

TABLE 1. Demographic and biochemical data of the study subjects

Subjects	CD	PC	CT
n	52	28	31
Sex (males/females)	8/44	3/25	8/23
Age (yr)	38.1 ± 1.2	35 ± 2.2	35.6 ± 2.4
BMI (kg/m²)	32.2 ± 1.1	34.2 ± 1.5	33 ± 0.5
Basal plasma ACTH (pmol/liter)	14.1 (8.9 to 18.8)[c,d]	4.2 (3.1 to 5.3)	3.7 (2.3 to 4.7)
Basal serum cortisol (nmol/liter)	648.6 (554.7 to 872.1)[c,d]	496.8 (364.3 to 524.4)[a]	358.8 (331.2 to 441.6)
Midnight serum cortisol (nmol/liter)	632 (524.4 to 770)[c,d]	124.2 (80 to 220.8)	110.4 (82.8 to 115.9)
OST serum cortisol (nmol/liter)	552 (182.1 to 797.6)[c,d]	88.3 (35.8 to 107.6)[d]	27.6 (22 to 30.3)
UFC (nmol/24 h)	778 (484.2 to 1,545)[b,d]	526.9 (461.3 to 620.7)[d]	193.1 (140.7 to 242.7)
AUC-ACTH (pmol/liter · 120 min)	750.7 (161 to 1,554.3)[c,d]	80.8 (35 to 174.9)	186.4 (−5.6 to 306.9)
AUC-cortisol (nmol/liter · 120 min)	14,020.8 (4,680.9 to 32,140.2)[c,d]	414 (−13,391.5 to 9,510.9)	−372.6 (−12,356.5 to 7,038)

Values are expressed as mean ± SEM if normally distributed, and as median (interquartile range) if not normally distributed.

[a] $P < 0.05$ *vs.* CT; [b] $P < 0.01$ *vs.* PC; [c] $P < 0.001$ *vs.* PC; [d] $P < 0.001$ *vs.* CT; not significant unless specified.

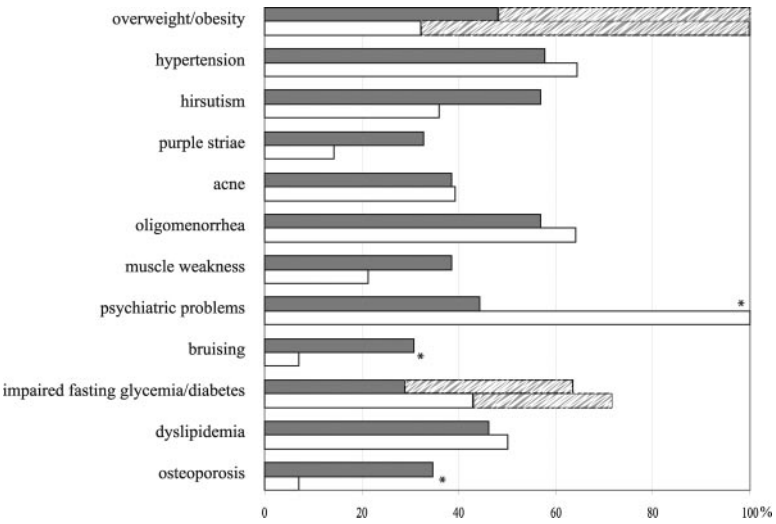

FIG. 1. Prevalence of signs and symptoms in CD patients (*gray bars*) and PC subjects (*white bars*). For overweight/obesity and impaired fasting glycemia/diabetes, *striped bar* indicates obesity (BMI > 30 kg/m^2) and diabetes (40) and *filled/white bar* overweight (BMI, 25–30 kg/m^2) and impaired fasting glycemia (40). *, Statistical significance ($P < 0.05$).

Characteristics of plasma ACTH and serum cortisol response to and diagnostic performance of the DDAVP test

In CT and PC, basal serum cortisol exhibited a negative, significant correlation with AUC-ACTH (CT, r = −0.51, $P = 0.003$; PC, r = −0.37, $P = 0.04$) and Δ-ACTH (CT, r = −0.75, $P < 0.001$; PC, r = −0.39, $P = 0.03$). The correlations among these variables were not significant in CD.

Analysis of the ROC curve at several time points demonstrated that percentage and absolute increase in serum cortisol and percentage increase in plasma ACTH after the DDAVP test did not afford optimum values of both SE and SP (data not shown). Δ-ACTH analysis with the ROC curve showed that a cutoff of 6 pmol/liter was associated with the highest SE (75%) and SP (89.8%) (Table 2), in line with previous works (3, 5).

To improve the diagnostic performance of the DDAVP test, we devised a new interpretive approach that involves simultaneous determination of two parameters. CD diagnosis in subjects showing simultaneous basal serum cortisol greater than 331 nmol/liter and Δ-ACTH greater than 4 pmol/liter and its exclusion in those negative for one or both parameters yielded 90.3% SE and an SP of 91.5% (Fig. 2). The parameter combination yielded a significantly greater ($P < 0.05$) classification accuracy, *i.e.* AUC_{LR} (22), compared with the two parameters used singly (Table 2). In addition, the AUC_{LR} of the parameter combination was significantly greater ($P < 0.05$) or approaching significance ($P = 0.05$) compared with those of UFC, OST, and midnight serum cortisol (Table 2).

Diagnostic performance of the DDAVP test in patients with mild hypercortisolism

The performance of some tests in discriminating Mild-UFC-CD from PC, who had not significantly different UFC values (data not shown), is reported in Table 3. As shown by the AUC_{LR} comparison, the DDAVP test distinguished Mild-UFC-CD from PC more accurately than did OST and midnight serum cortisol (Table 3).

The method also enabled outstanding (AUC_{LR}: 0.90) (22) and significantly better ($P < 0.05$) discrimination

TABLE 2. Diagnostic performance of the tests used for CD diagnosis in all study subjects

	SE (CI) (%)	SP (CI) (%)	LR+	LR−	AUC_{LR} (CI_{AUC})
UFC: cutoff > 413 nmol/24 h[a]	78.8 (65.3–88.9)[g]	52.5 (39.1–65.7)[g]	1.65	0.40	0.85 (0.79–0.92)[i]
OST serum cortisol: cutoff > 50 nmol/liter[b]	90.3 (78.9–96.8)	66.1 (52.6–77.9)[g]	2.66	0.14	0.86 (0.77–0.94)[i]
OST serum cortisol: cutoff > 138 nmol/liter[b]	78.8 (65.3–88.9)[g]	94.9 (85.8–98.9)	15.45	0.22	0.86 (0.77–0.94)[i]
Midnight serum cortisol > 207 nmol/liter[c]	94.2 (84–98.7)	83.05 (71–91.5)[h]	5.54	0.06	0.86 (0.77–0.94)[j]
Basal serum cortisol > 572 nmol/liter[d]	73.1 (58.9–84.4)[g]	89.8 (79.1–96.1)	7.18	0.30	0.85 (0.77–0.92)[i]
DDAVP test: Δ-ACTH > 6 pmol/liter[d]	75 (61–85.9)[g]	89.8 (79.1–96.1)	7.35	0.27	0.86 (0.78–0.94)[i]
DDAVP test[e]: basal serum cortisol > 331 nmol/liter[f] and Δ-ACTH > 4 pmol/liter[f]	90.3 (78.9–96.8)	91.5 (81.3–97.1)	10.62	0.10	0.94 (0.90–0.99)

[a] Upper limit of the normal UFC range in our laboratory.

[b] Cutoff commonly used for CS diagnosis (9).

[c] Cutoff according to Papanicolaou *et al.* (16).

[d] Cutoff yielding the highest sum of SE and SP (ROC curve).

[e] CD diagnosis based on the presence of both parameters; absence of either or both excludes CD.

[f] Cutoff yielding the highest sum of SE and SP in combination with the cutoff of the other parameter.

Comparison of the SE and SP of the various tests with the SE and SP of the DDAVP test as interpreted by "basal serum cortisol > 331 nmol/liter and Δ-ACTH > 4 pmol/liter": [g] $P < 0.05$; [h] $P = 0.06$; not significant unless specified.

Comparison of the AUC_{LR} of the various tests with that of the DDAVP test as interpreted by the combination of basal serum cortisol and Δ-ACTH: [i] $P < 0.05$; [j] $P = 0.05$; not significant unless specified.

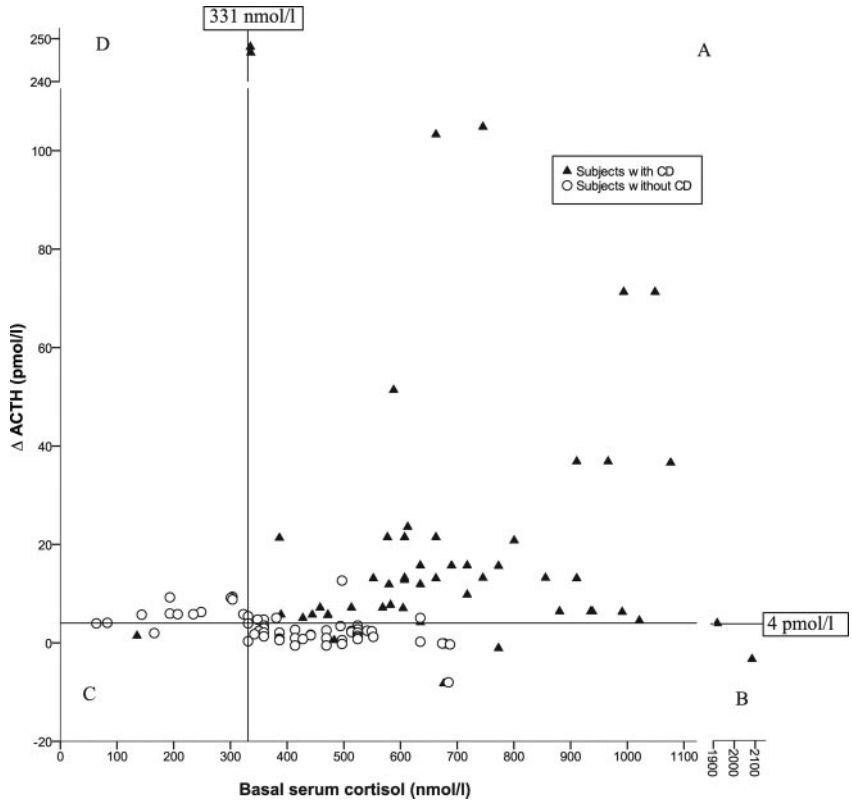

FIG. 2. Diagnostic performance of the DDAVP test interpreted according to the approach envisaging CD diagnosis in subjects with both basal serum cortisol greater than 331 nmol/liter and Δ-ACTH greater than 4 pmol/liter, and its exclusion in those negative for either or both parameters; the approach effectively discriminated patients with CD (quadrant A) from subjects without CD (quadrants B, C, D), with an SE of 90.3% and an SP of 91.5%.

of Mild-OST-CD from PC subjects (who had not significantly different OST serum cortisol; data not shown) compared with UFC and midnight serum cortisol (Table 3).

In addition, the AUC_{LR} of the DDVAP test was significantly higher ($P < 0.05$) than those of OST and UFC in discriminating Mild-Cort24-CD from PC, whose midnight serum cortisol did not differ significantly (data not shown) (Table 3).

Significant, positive correlations were noted in CD patients between OST serum cortisol and midnight serum cortisol ($r = 0.29$; $P = 0.035$), OST serum cortisol and UFC ($r = 0.34$; $P = 0.012$); and midnight serum cortisol and UFC ($r = 0.38$; $P = 0.005$). Significant correlations among these variables were never found in PC or CT subjects. In contrast, Δ-ACTH did not correlate significantly with the three measures in any of the subject groups (data not shown). OST serum cortisol, midnight serum cortisol, and UFC did not correlate significantly (biserial correlation) with CD exclusion (=0) or diagnosis (=1) based on the novel interpretive criteria of the DDAVP test in any of the groups studied (data not shown).

Comparison of PPV, NPV, and DA of second-level tests based on different estimates of CD prevalence

The PPV, NPV, and DA of the second-level tests calculated on the basis of two literature-derived estimates of CD prevalence are compared in Table 4.

The two-step method applied to assess the diagnostic performance of second-level tests involved calculating the PPV of the first-level tests with reference to the CD prevalence reported in the literature. The PPV of the first-level tests using 2/million inhabitants (3, 24) as the *a priori* baseline prevalence were: 3/million for UFC (cutoff, 413 nmol/24 h); 5/million for OST serum cortisol (cutoff, 50 nmol/liter); and 31/million for OST serum cortisol (cutoff, 138 nmol/liter). The highest PPV resulting from these calculations was the one obtained with OST serum cortisol (cutoff, 138 nmol/liter). This was used as the *a priori* CD prevalence to calculate the diagnostic performance of second-line tests and demonstrated that the DDAVP test interpreted according to our method achieved a significantly higher PPV and DA compared with midnight serum cortisol and Δ-ACTH greater than 6 pmol/liter (Table 4).

Application of the procedure using an estimate of CD prevalence of 10/million inhabitants (3, 25) showed that the DDAVP test interpreted with our method achieved significantly greater PPV and DA than midnight serum cortisol as well as higher PPV, NPV, and DA compared with the DDAVP test based on Δ-ACTH greater than 6 pmol/liter (Table 4).

Discussion

The scarce and inconsistent data on the clinical value of the DDAVP test in distinguishing CD from PC prompted us to share our 10-yr experience with the test and with a novel methodological approach devised by our group to improve its diagnostic performance, with emphasis on subjects with mild hypercortisolism, which raises the most difficult problems of differential diagnosis.

The commonly adopted interpretive criteria of the DDAVP test based on percentage and absolute rise in ACTH and cortisol proved ineffective in distinguishing our CD from PC patients. We tried to improve its diag-

TABLE 3. Diagnostic performance of the main tests in patients with mild hypercortisolism

	SE (CI) (%)	SP (CI) (%)	LR+	LR−	AUC_{LR} (CI_{AUC})
Mild-UFC-CD (n = 23) *vs.* PC (n = 28)					
OST serum cortisol: cutoff > 50 nmol/liter[b]	78.2 (56.3–92.5)	28.5 (13.2–48.6)[f]	1.09	0.76	0.68 (0.50–0.85)[h]
OST serum cortisol: cutoff > 138 nmol/liter[b]	65.2 (42.7–83.6)[g]	89.2 (71.7–97.7)	6.03	0.39	0.68 (0.50–0.85)[h]
Midnight serum cortisol > 207 nmol/liter[c]	86.9 (66.4–97.2)	64.2 (44–81.3)[f]	2.42	0.20	0.67 (0.51–0.83)[h]
DDAVP test[d]: basal serum cortisol > 331 nmol/liter[e] and Δ-ACTH > 4 pmol/liter[e]	86.9 (66.4–97.2)	92.8 (76.5–99.1)	12.06	0.14	0.89 (0.78–1)
Mild-OST-CD (n = 15) *vs.* PC (n = 28)					
UFC: cutoff > 413 nmol/24 h[a]	60 (32.2–83.6)	0 (0–10.1)[f]	0.6	***	0.54 (0.31–0.78)[h]
Midnight serum cortisol > 207 nmol/liter[c]	80 (51.9–95.6)	64.2 (44–81.3)[f]	2.23	0.31	0.65 (0.46–0.85)[h]
DDAVP test[d]: basal serum cortisol > 331 nmol/liter[e] and Δ-ACTH > 4 pmol/liter[e]	86.6 (59.5–98.3)	92.8 (76.5–99.1)	12.02	0.14	0.90 (0.77–1)
Mild-Cort24-CD (n = 6) *vs.* PC (n = 28)					
UFC: cutoff > 413 nmol/24 h[a]	33.3 (4.3–77.7)	0 (0–10.1)[f]	0.33	***	0.21 (0–0.46)[h]
OST serum cortisol: cutoff > 50 nmol/liter[b]	50 (11.8–88.1)	28.5 (13.2–48.6)[f]	0.69	1.75	0.26 (0–0.55)[h]
OST serum cortisol: cutoff > 138 nmol/liter[b]	16.6 (0.4–64.1)[g]	89.2 (71.7–97.7)	1.53	0.93	0.26 (0–0.55)[h]
DDAVP test[d]: basal serum cortisol > 331 nmol/liter[e] and Δ-ACTH > 4 pmol/liter[e]	100 (60.7–100)	92.8 (76.5–99.1)	13.8	0	0.95 (0.87–1)

[a] Upper limit of the normal UFC range in our laboratory.

[b] Cutoff commonly used for CS diagnosis (9).

[c] Cutoff according to Papanicolaou *et al.* (16).

[d] CD diagnosis based on the presence of both parameters; absence of either or both excludes CD.

[e] Cutoff affording the highest sum of SE and SP in combination with the cutoff of the other parameter.

Comparison of the SE and SP of the various tests with the SE and SP of the DDAVP test interpreted by "basal serum cortisol > 331 nmol/liter and Δ-ACTH > 4 pmol/liter": [f] $P < 0.05$; [g] $P = 0.06$; not significant unless specified.

Comparison of the AUC_{LR} of the various tests with that of the DDAVP test interpreted by the combination of basal serum cortisol and Δ-ACTH: [h] $P < 0.05$; not significant unless specified.

*** Impossible to compute.

nostic performance by applying a straightforward interpretive method that envisages simultaneous positivity for two DDAVP test parameters, *i.e.* a basal serum cortisol greater than 331 nmol/liter and Δ-ACTH greater than 4 pmol/liter. A diagnosis of CD based on positivity for both parameters and its exclusion in subjects who were positive for one or neither yielded 90.3% SE and 91.5% SP (Fig. 2 and Table 2).

TABLE 4. PPV, NPV, and DA of second-level tests compared on the basis of two different estimates of CD prevalence

	PPV (range)	NPV (range)	DA (range)
2/million[e]			
Midnight serum cortisol > 207 nmol/liter[a]	172[g] (26–1,879)	999,998 (999,998–999,998)	830,503[g] (710,001–915,012)
DDAVP test: Δ-ACTH > 6 pmol/liter[b]	228[g] (27–3,551)	999,991 (999,976–999,995)	897,995[g] (790,998–960,983)
DDAVP test[c]: basal serum cortisol > 331 nmol/liter[d] and Δ-ACTH > 4 pmol/liter[d]	330 (39–5,385)	999,996 (999,995–999,998)	915,200 (813,000–971,000)
10/million[f]			
Midnight serum cortisol > 207 nmol/liter[a]	861[g] (133–9,285)	999,989 (999,988–999,989)	830,517[g] (710,006–915,058)
DDAVP test: Δ-ACTH > 6 pmol/liter[b]	1,138[g] (134–17,473)	999,957[g] (999,881–999,977)	897,977[g] (790,992–960,918)
DDAVP test[c]: basal serum cortisol > 331 nmol/liter[d] and Δ-ACTH > 4 pmol/liter[d]	1,649 (194–26,245)	999,984 (999,973–999,988)	915,198 (812,999–970,997)

Data are expressed as number/million.

[a] Cutoff according to Papanicolaou *et al.* (16).

[b] Cutoff yielding the highest sum of SE and SP (ROC curve).

[c] CD diagnosis based on the presence of both parameters; absence of either or both excludes CD.

[d] Cutoff yielding the highest sum of SE and SP in combination with the cutoff of the other parameter.

[e] See Refs. 3 and 24.

[f] See Refs. 3 and 25.

Comparison of the PPV, NPV, and DA of the various tests with the PPV, NPV, and DA of the DDAVP test interpreted by "basal serum cortisol > 331 nmol/liter and Δ-ACTH > 4 pmol/liter": [g] $P < 0.05$; not significant unless specified.

The adoption of this method and the improved diagnostic performance were not accidental, but rather stemmed from two observable correlations, *i.e.* a negative significant correlation of basal serum cortisol with Δ-ACTH and with AUC-ACTH both in CT and in PC (but not in CD). Such findings, together with the data listed in Table 1, clearly indicate that the negative feedback is normal in CT subjects, that it is preserved to a fair extent in PC patients, and that it is lost in CD. This key observation and the resulting correlations account for the very high DA associated with the simultaneous presence of basal serum cortisol greater than 331 nmol/liter and Δ-ACTH greater than 4 pmol/liter (Table 2) because CT and PC subjects with basal serum cortisol greater than 331 nmol/liter tended to have Δ-ACTH below 4 pmol/liter as a result, whereas those with basal serum cortisol below 331 nmol/liter usually had Δ-ACTH greater than 4 pmol/liter. In CD patients, loss of the cortisol-induced negative feedback resulted in the simultaneous presence of basal serum cortisol greater than 331 nmol/liter and Δ-ACTH greater than 4 pmol/liter.

The negative significant correlations between basal serum cortisol and AUC-ACTH noted in our CT and PC subjects, also described previously (5), are explained by the fact that in physiological conditions the hypothalamic synthesis of vasopressin (26, 27) and vasopressin-induced ACTH secretion (28–32) are inhibited by cortisol levels. However, PC subjects have a reduced negative feedback compared with healthy individuals (33, 34); this was apparent in our study population, where these subjects exhibited a weaker correlation between basal serum cortisol and Δ-ACTH compared with the CT group. In contrast, CD patients did not show a correlation between basal serum cortisol and Δ-ACTH and AUC-ACTH, in line with their loss of the negative feedback. However, the hyperresponsiveness of plasma ACTH and serum cortisol to the DDAVP test in CD patients is not determined exclusively by loss of the negative feedback, but is also determined by the overexpression of V3 receptor (7, 8), although the underlying mechanisms are still unclear (35).

Testing the effectiveness of the DDAVP test in subjects with mild hypercortisolism, where the differential diagnosis of CD from PC is even more challenging, involved comparing the diagnostic performance of the various tests in discriminating Mild-UFC-CD, Mild-OST-CD, and Mild-Cort24-CD subjects from PC individuals. Again, the DDAVP test interpreted according to our method did so most effectively, and the relevant LR+ and LR− values confirmed that it is more useful (36) to make a diagnosis than all first- and second-level tests (Table 3).

The poor diagnostic performance of UFC, OST, and midnight serum cortisol is related to their positive correlations in CD, whereby a low value on one of these parameters is generally associated with low values on the other two, resulting in a low SE of these tests in identifying Mild-UFC-CD, Mild-OST-CD, and Mild-Cort24-CD (Table 3). In contrast, Δ-ACTH and the exclusion/diagnosis of CD based on our methodological approach did not correlate with UFC, OST, serum cortisol, or midnight serum cortisol in CD, PC, or CT subjects (see *Results*), highlighting the independence of the DDAVP test on measures of hypercortisolism such as UFC, OST, serum cortisol, and midnight serum cortisol and explaining its superior diagnostic performance compared with the three tests in subjects with mild hypercortisolism (Table 3).

Given the use of knowing the diagnostic effectiveness of the DDVAP test with different CD prevalences, we calculated the dependent prevalence parameters, PPV, NPV, and DA, of the second-level tests based on two literature-derived estimates. Our method proved yet again more effective than midnight serum cortisol and a Δ-ACTH greater than 6 pmol/liter (Table 4).

Clearly, these data are to be taken with caution and need confirmation before they can be introduced into clinical practice, especially given the small number of subjects with mild hypercortisolism, particularly Mild-Cort24-CD, in our sample. However the DDAVP test had a consistently excellent diagnostic performance in all three subgroups exactly because of its independence of the measures of hypercortisolism and of the mutual link of these measures in CD patients, as already reported in the literature (37–39).

In conclusion, the study demonstrated that the DDAVP test can effectively be interpreted by evaluating a combination of parameters; notably, this approach achieves the differential diagnosis of CD from PC even in subjects with mild hypercortisolism.

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References

1. **Newell-Price J, Trainer P, Besser M, Grossman A** 1998 The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev 19:647–672
2. **Boscaro M, Arnaldi G** 2009 Approach to the patient with possible Cushing's syndrome. J Clin Endocrinol Metab 94:3121–3131
3. **Pecori Giraldi F, Pivonello R, Ambrogio AG, De Martino MC, De Martin M, Scacchi M, Colao A, Toja PM, Lombardi G, Cavagnini F** 2007 The dexamethasone-suppressed corticotropin-

releasing hormone stimulation test and the desmopressin test to distinguish Cushing's syndrome from pseudo-Cushing's states. Clin Endocrinol (Oxf) 66:251–257
4. **Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM** 2008 The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 93:1526–1540
5. **Moro M, Putignano P, Losa M, Invitti C, Maraschini C, Cavagnini F** 2000 The desmopressin test in the differential diagnosis between Cushing's disease and pseudo-Cushing states. J Clin Endocrinol Metab 85:3569–3574
6. **Malerbi DA, Fragoso MC, Vieira Filho AH, Brenlha EM, Mendonça BB** 1996 Cortisol and adrenocorticotropin response to desmopressin in women with Cushing's disease compared with depressive illness. J Clin Endocrinol Metab 81:2233–2237
7. **Arnaldi G, de Keyzer Y, Gasc JM, Clauser E, Bertagna X** 1998 Vasopressin receptors modulate the pharmacological phenotypes of Cushing's syndrome. Endocr Res 24:807–816
8. **de Keyzer Y, René P, Beldjord C, Lenne F, Bertagna X** 1998 Overexpression of vasopressin (V3) and corticotrophin-releasing hormone receptor genes in corticotroph tumours. Clin Endocrinol (Oxf) 49:475–482
9. **Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, Fava GA, Findling JW, Gaillard RC, Grossman AB, Kola B, Lacroix A, Mancini T, Mantero F, Newell-Price J, Nieman LK, Sonino N, Vance ML, Giustina A, Boscaro M** 2003 Diagnosis and complications of Cushing's syndrome: a consensus statement. J Clin Endocrinol Metab 88:5593–5602
10. **Yanovski JA, Cutler Jr GB, Chrousos GP, Nieman LK** 1993 Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. A new test to distinguish Cushing's syndrome from pseudo-Cushing's states. JAMA 269:2232–2238
11. **Gatta B, Chabre O, Cortet C, Martinie M, Corcuff JB, Roger P, Tabarin A** 2007 Reevaluation of the combined dexamethasone suppression-corticotropin-releasing hormone test for differentiation of mild Cushing's disease from pseudo-Cushing's syndrome. J Clin Endocrinol Metab 92:4290–4293
12. **Erickson D, Natt N, Nippoldt T, Young Jr WF, Carpenter PC, Petterson T, Christianson T** 2007 Dexamethasone-suppressed corticotropin-releasing hormone stimulation test for diagnosis of mild hypercortisolism. J Clin Endocrinol Metab 92:2972–2976
13. **American Psychiatric Association** 1994 Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Press
14. **Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group** 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81:19–25
15. **Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group** 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41–47
16. **Papanicolaou DA, Yanovski JA, Cutler Jr GB, Chrousos GP, Nieman LK** 1998 A single midnight serum cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing states. J Clin Endocrinol Metab 83:1163–1167
17. **Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH** 2003 Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28:916–931
18. **Holm S** 1979 A simple sequentially rejective multiple test procedure. Scand J Statist 6:65–70
19. **Barry HC, Ebell MH** 1997 Test characteristics and decision rules. Endocrinol Metab Clin North Am 26:45–65
20. **Glantz SA** 2005 Primer of biostatistics. 6th ed. New York: McGraw-Hill Professional; 242–244
21. **Shultz EK** 1995 Multivariate receiver-operating characteristic curve analysis: prostate cancer screening as an example. Clin Chem 41: 1248–1255
22. **Hosmer DW, Lemeshow S** 2000 Applied logistic regression. 2nd ed. New York: John Wiley and Sons; 160–164
23. **DeLong ER, DeLong DM, Clarke-Pearson DL** 1988 Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44:837–845
24. **Carpenter PC** 1988 Diagnostic evaluation of Cushing's syndrome. Endocrinol Metab Clin North Am 17:445–472
25. **Harris PE, Bouloux PM, Biller BM** 2002 Endocrinology in clinical practice. London: Martin Dunitz; 62
26. **Erkut ZA, Pool C, Swaab DF** 1998 Glucocorticoids suppress corticotropin-releasing hormone and vasopressin expression in human hypothalamic neurons. J Clin Endocrinol Metab 83:2066–2073
27. **Itoi K, Mouri T, Takahashi K, Murakami O, Imai Y, Sasaki S, Yoshinaga K, Sasano N** 1987 Suppression by glucocorticoid of the immunoreactivity of corticotropin-releasing factor and vasopressin in the paraventricular nucleus of rat hypothalamus. Neurosci Lett 73:231–236
28. **McFarlane A, Coghlan J, Tresham J, Wintour EM** 1995 Cortisol feedback in adrenalectomized adult sheep. Am J Physiol 269:E10–E17
29. **Shipston MJ, Antoni FA** 1991 Early glucocorticoid feedback in anterior pituitary corticotrophs: differential inhibition of hormone release induced by vasopressin and corticotrophin-releasing factor in vitro. J Endocrinol 129:261–268
30. **Nicholson SA, Gillham B** 1989 Glucocorticoids act rapidly in vitro to attenuate second messenger responses to ACTH secretagogues in rats. J Endocrinol 122:545–551
31. **Schwartz J, Ash P, Ford V, Raff H, Crosby S, White A** 1994 Secretion of adrenocorticotrophin (ACTH) and ACTH precursors in ovine anterior pituitary cells: actions of corticotrophin-releasing hormone, arginine vasopressin and glucocorticoids. J Endocrinol 140: 189–195
32. **Durand P, Cathiard AM, Dacheux F, Naaman E, Saez JM** 1986 In vitro stimulation and inhibition of adrenocorticotropin release by pituitary cells from ovine fetuses and lambs. Endocrinology 118: 1387–1394
33. **Juruena MF, Cleare AJ, Pariante CM** 2004 The hypothalamic pituitary adrenal axis, glucocorticoid receptor function and relevance to depression. Rev Bras Psiquiatr 26:189–201
34. **Young EA, Veldhuis JD** 2006 Disordered adrenocorticotropin secretion in women with major depression. J Clin Endocrinol Metab 91:1924–1928
35. **Pecori Giraldi F, Marini E, Torchiana E, Mortini P, Dubini A, Cavagnini F** 2003 Corticotrophin-releasing activity of desmopressin in Cushing's disease: lack of correlation between in vivo and in vitro responsiveness. J Endocrinol 177:373–379
36. **Zakowski L, Seibert C, VanEyck S** 2004 Evidence-based medicine: answering questions of diagnosis. Clin Med Res 2:63–69
37. **Findling JW, Raff H** 2001 Diagnosis and differential diagnosis of Cushing's syndrome. Endocrinol Metab Clin North Am 30:729–747
38. **Findling JW, Raff H, Aron DC** 2004 The low-dose dexamethasone suppression test: a reevaluation in patients with Cushing's syndrome. J Clin Endocrinol Metab 89:1222–1226
39. **Friedman TC** 2006 An update on the overnight dexamethasone suppression test for the diagnosis of Cushing's syndrome: limitations in patients with mild and/or episodic hypercortisolism. Exp Clin Endocrinol Diabetes 114:356–360
40. **American Diabetes Association** 2006 Diagnosis and classification of diabetes mellitus. Diabetes Care 29(Suppl 1):S43–S48