Concomitant Medication Use Can Confound Interpretation of the Combined Dexamethasone-Corticotropin Releasing Hormone Test in Cushing's Syndrome

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Context: The ability of combined dexamethasone-corticotropin releasing hormone (Dex-CRH) testing to distinguish pseudo-Cushing's syndrome (PCS) from Cushing's syndrome is controversial. One factor potentially impairing diagnostic efficacy is the concomitant use of commonly prescribed medications that may alter dexamethasone metabolism.

Objective: Our objective was to assess the diagnostic accuracy of the Dex-CRH test and evaluate the potential impact of concomitant drugs.

Design: The study was a retrospective one.

Participants: Participants included 101 patients [60 Cushing's disease (CD); 41 PCS] who underwent 112 Dex-CRH tests. Patients were divided into two groups, depending on use of medications potentially interfering with dexamethasone metabolism: 58 tests were classified as No Meds (32 CD; 26 PCS) and 54 as Meds (34 CD; 20 PCS). The latter group was further subdivided into patients taking one medication *vs.* those taking multiple medications.

Main Outcome Measures: Diagnostic accuracy of different serum cortisol and ACTH thresholds at baseline and 15 min after CRH injection was assessed.

Results: The specificity of a baseline post-low-dose-dexamethasone-suppressed test cortisol lower than 1.4 μg/dl (38 nmol/liter) was significantly higher in the No Meds *vs.* the Meds group ($P = 0.014$). Sensitivity and specificity using a post-CRH cortisol cutoff of 1.4 μg/dl (38 nmol/liter) were 93.1% (95% confidence interval = 88.4–97.8) and 92.3% (95% confidence interval = 87–97.6) in the No Meds group. The specificity of a cortisol lower than 1.4 μg/dl (38 nmol/l) at 15 min after CRH was significantly higher in patients taking only one medication *vs.* those on multidrug treatment ($P < 0.05$).

Conclusions: Medications commonly prescribed in hypercortisolemic patients undergoing Dex-CRH testing may contribute to the variable diagnostic accuracy of this test. Prospective studies to address this issue are needed. **(*J Clin Endocrinol Metab* 94: 4851–4859, 2009)**

Cushing's disease (CD) may be more common than previously assumed, and some have advocated more widespread screening for this disorder in specific patient groups (1, 2). Patients with obesity, diabetes mellitus, hypertension, polycystic ovarian syndrome, or osteoporosis may have an underlying tumor (3, 4). It is also clear that

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Abbreviations: AUC, Area under the ROC curve; CD, Cushing's disease; CI, confidence interval; CS, Cushing's syndrome; Dex-CRH, combined dexamethasone-corticotropin releasing hormone (Dex-CRH) testing; DST, dexamethasone suppression test; LDDST, low-dose DST; PCS, pseudo-Cushing's syndrome; ROC, receiver operating characteristic; UFC, urinary free cortisol.

some patients with psychiatric disease, alcoholism, central obesity, diabetes mellitus, and polycystic ovarian syndrome, the so-called pseudo-Cushing's syndrome (PCS), may demonstrate features of Cushing's syndrome (CS), including hypercortisolemia, without an underlying neoplasm. Therefore, it is essential to differentiate patients with true CS due to an underlying tumor from patients with PCS in these high-risk populations. The dexamethasone-suppressed CRH stimulation (Dex-CRH) test has been employed as such a diagnostic tool (5), although some reports indicate it is less useful than previously described (6–9). Its efficacy can be affected by several variables, including dexamethasone compliance, the type of CRH used, and patient comorbidities (2). An important potential confounder may be the effects of medications commonly used in these patients including antidepressants, antihypertensives, and lipid-lowering agents. These medications are also in wide general use; more than 10% of the U.S. population aged 18–64 are receiving antidepressants (10), and lipid-lowering agents are among the most widely prescribed drugs. Because some medications in these classes can potentially interfere with the CYP3A4 enzyme system, which regulates dexamethasone metabolism (11), they could potentially alter test results and affect diagnostic accuracy (2).

To determine the diagnostic accuracy of the Dex-CRH test and define whether concomitant medications affect its reliability, we retrospectively reviewed test results in 101 patients undergoing evaluation for possible CS.

Patients and Methods

Patients

Records were reviewed from 116 subjects who underwent Dex-CRH testing during their evaluation for CS at the Neuroendocrine Clinical Center of the Massachusetts General Hospital between May 1998 and June 2008. Dex-CRH testing was performed in patients with conflicting results on standard initial diagnostic evaluation for CS (2). All had clinical and biochemical features of hypercortisolism but needed an additional confirmatory test, due to a history of depression or alcoholism, or urinary free cortisol (UFC) from 1- to 3-fold above the upper limit of normal without classic physical features. The diagnosis of CD was made on the basis of clinical, biochemical, and radiological features and was confirmed by the histological identification of an ACTH-staining pituitary adenoma and/or evidence of clinical and biochemical remission after transsphenoidal surgery. A single patient had pathologically confirmed adrenal Cushing without completely suppressed ACTH levels. Because all other CS patients clearly had a pituitary source, this group will be termed CD.

The presumptive diagnosis of PCS was based on lack of conclusive biochemical testing at the initial evaluation, when results of first-line tests [UFC, late night salivary cortisol, overnight 1-mg dexamethasone suppression test (DST) or 48-h 2-mg DST] were not all consistent with CD as described in consensus guidelines (2). The diagnosis of PCS was confirmed by the improvement or the lack of progression of Cushingoid features during follow-up and absence of confirmation on repeated diagnostic testing.

Records were reviewed for presenting symptoms, medications, test results, depression (as indicated by use of antidepressants and/or formal psychiatric evaluation and therapy) and bone densitometry results, when available. Hepatic and renal diseases were excluded.

Detailed longitudinal records were available on 54 patients (30 CD; 24 PCS) followed regularly at our center. Sixty-two patients without recent care at our center were contacted and asked to fill out a questionnaire and to provide more recent tests of adrenal function. Fourteen patients (six CD; eight PCS) responded. Of the 48 patients who did not respond, the results of 33 were included on the basis of available data obtained after their initial evaluation. This group included 23 patients with CD, one with adrenal adenoma, and nine with PCS. The median follow-up in PCS patients who did not respond to the questionnaire was 12 months, with all patients except two being followed for 2–14 months after their test. Although we cannot rule out that some of them might have subsequently been diagnosed with CD, only two patients of 41 (4.8%) with PCS did not have any follow-up visit at our center after Dex-CRH testing.

Fifteen patients had no further testing or follow-up after their initial Dex-CRH test and were excluded from the analysis because the final diagnosis was unknown. This study was approved by the Institutional Review Board of Partners HealthCare.

We evaluated 112 tests on 101 patients (60 CD and 41 PCS). Eight patients had the test performed more than once.

Subgroups based on use of medications potentially influencing dexamethasone metabolism

Test results were categorized as Meds and No Meds, according to whether patients were receiving medications potentially interfering with dexamethasone metabolism (11), and further subdivided based on type of medications as listed in Table 1. Fifty-eight tests were categorized as No Meds (32 CD; 26 PCS) and 54 as Meds (34 CD, 20 PCS).

Diagnostic tests

Patients underwent initial hormonal evaluation for possible CD with measurement of serum cortisol (52 patients: 30 CD and 22 PCS), 24-h UFC (90 patients: 55 CD and 35 PCS), and plasma ACTH (72 patients: 44 CD and 28 PCS). UFC concentrations are expressed as the average of two measurements performed within 1 month before or after the Dex-CRH test. The overnight DST (1 mg) was performed in 28 patients.

The Dex-CRH test was performed as previously described (5). Women taking oral estrogen discontinued it for approximately 8 wk before the test (except two on conjugated estrogen). All tests were performed in an outpatient setting. Subjects self-administered 0.5 mg dexamethasone orally every 6 h for eight doses starting at 1200 h. They were instructed to return 2 d later at 0800 h. Intravenous ovine CRH (Ferring Pharmaceuticals, Inc., Tarrytown, NY) was administered at a dose of 1 μg/kg, maximum 100 μg, 2 h after the last dose of dexamethasone in fasted patients. Serum cortisol and plasma ACTH were measured at 0 and +15 min. Dexamethasone concentrations were

TABLE 1. Drugs taken by the Meds group that may potentially interfere with dexamethasone metabolism (11)

Class	Medications
SSRI/SNRI (33)	Sertraline, fluoxetine, paroxetine, trazodone, citalopram, bupropion, venlafaxine
Lipid-lowering agents (10)	Atorvastatin, simvastatin
Calcium channel blockers (12)	Verapamil, diltiazem, amlodipine, nifedipine, felodipine
Angiotensin (AT1type) receptor antagonists (3)	Irbesartan, Losartan
Atypical antipsychotics (5)	Olanzapine, quetiapine
Proton pump inhibitors (6)	Pantoprazole, lansoprazole, omeprazole
PPARγ antagonists (3)	Pioglitazone, rosiglitazone
Antiarrhythmics (2)	Quinidine
$\beta-$Adrenoceptor blockers (2)	Propranolol
Benzodiazepine sedatives (2)	Clonazepam
Anticonvulsants (2)	Tiagabine, topiramate

PPARγ , Peroxisome proliferator-activated receptor-γ ; SNRI, serotonin-noradrenaline reuptake inhibitors; SSRI, selective serotonin reuptake inhibitors. Number of tests performed on each class of medication is indicated in *parentheses*.

measured regularly starting in 2006 at −1 min before CRH injection and were available in 40 patients (18 CD; 22 PCS). Salivary cortisol levels were not routinely measured during the first part of the decade, so the number available was inadequate for analysis.

Assays

Plasma ACTH was measured using a commercial immunoluminescent kit (Immulite 2000; Siemens Medical Solutions Diagnostic, Los Angeles, CA). The intraassay variability was 6.7–9.5%, and interassay variability was 6.1–10.0%. Serum cortisol and UFC were measured using a chemiluminescent microparticle immunoassay (Abbott Diagnostics, Chicago, IL), with a total coefficient of variation of 2.5–7.7% and an intraassay coefficient of variation range of 2.1–6.1%. Normal ranges are 6–76 pg/ml for ACTH, 20–70 μg/24 h for UFC, and 5–25 μg/dl for serum cortisol. Undetectable post-Dex-CRH serum cortisol and plasma ACTH levels are reported as 0.1 μg/dl and 0.5 pg/ml, respectively. Earlier measurements were performed by RIA; assay characteristics have been previously described (12). Plasma dexamethasone levels were measured by Endocrine Sciences (Calabasas Hills, CA). The intraassay and interassay variabilities were 3.4 and 8.4%, respectively.

Statistical analysis

Quantitative outcomes are presented as mean ± SD; categorical outcomes are presented as frequency count and proportion (percent). To examine group differences, the independent-sample *t* test was used for continuous variables and χ^2 or Fisher's exact test used for categorical variables. Receiver operating characteristic (ROC) curve analysis was performed to examine the operating characteristics of Dex-CRH testing. Areas under the ROC curve (AUC) of the post-low-dose DST (post-LDDST), post-CRH cortisol, post-LDDST, and post-CRH ACTH levels were evaluated and compared (13). The effect of medication on diagnostic accuracy was examined by comparing the on- to off-medication sensitivity and specificity for each cutoff. A 95% confidence interval (CI) based on the large sample approximation was constructed for each sensitivity and specificity. Two sensitivities/specificities were considered statistically different if the 95% confidence intervals did not overlap. A test with *P* value <0.05 was considered statistically significant. All analyses were carried out using SAS version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Baseline clinical characteristics

Clinical characteristics of CD and PCS patients during their initial evaluation are shown in Table 2. Median follow-up for the entire group of 101 patients was 20.5 months [CD patients: median follow-up 20 months (range 0–120); PCS patients: median follow-up 28.5 months (range: 1–79); $P = 0.16$].

No significant differences in age and body mass index were observed between CD and PCS patients. Grade I, II, and III obesity were described in 14 (28%), 6 (12%), and 12 (24.5%) patients with CD *vs.* 10 (26%), 4 (10%), and 11 (29%) with PCS, respectively ($P = 0.63$).

Moon face ($P < 0.05$), buffalo hump ($P < 0.05$), erectile dysfunction ($P < 0.05$), and easy bruising ($P < 0.01$) were reported more frequently in CD than PCS patients, whereas headache ($P < 0.01$) and acne ($P < 0.01$) were more prevalent in patients classified as having PCS. The prevalence of depression was equal between groups; alcoholism was reported in two patients, both classified as PCS, and one of them had major depression with psychotic features (Table 2).

Results of Dex-CRH testing

Cortisol response

Mean cortisol and ACTH levels during the Dex-CRH tests are shown in Table 3; individual patient responses are shown in Fig. 1, A and B. CD patients had significantly higher hormone levels than PCS subjects both after LDDST (before CRH) and 15 min after CRH injection. Dexamethasone concentrations were available in 18 CD and 22 PCS patients and were comparable between groups (Table 3).

After the 48-h 2-mg/d LDDST and before CRH injection, 15 of 61 (24.6%) true Cushing's patients had serum cortisol suppressed to less than 1.4 μg/dl (38 nmol/liter), suggesting they did not have Cushing's (5). This yielded a post-LDDST sensitivity of 75.4% (95% CI 69.6–81.2%); six of these 15 subsequently stimulated above 1.4 μg/dl (38 nmol/liter) at 15 min after CRH and were correctly classified by Dex-CRH testing. Nine of 15 had a 15-min

TABLE 2. Patient demographic and clinical characteristics at the initial evaluation

Patients	CD (n = 60)	PCS (n = 41)	Total (n = 101)	*P* value
Age (yr)	43.9 ± 15	42.1 ± 15.2	43.5 ± 14.8	0.56
Sex				<0.01[a]
Female	53 (88.3)	26 (63.4)	79 (77.4)	
Male	7 (11.7)	15 (36.6)	22 (22.6)	
BMI (kg/m^2)	35.1 ± 10.8	34.4 ± 7.8	34.6 ± 9.4	0.7
Central obesity	39 (67.2)	20 (48.8)	59 (58.4)	0.06
Diabetes	15 (25.4)	10 (24.4)	25 (24.7)	0.9
IGT	2 (3.4)	3 (7.3)	5 (5)	0.38
Hypertension	28 (48.2)	19 (46.4)	47 (46.5)	0.84
Depression	26 (44.8)	22 (53.6)	48 (47.5)	0.38
Osteoporosis	11 (47.8)	3 (33.3)	14 (43.7)	0.17
Moon face	31 (53.4)	12 (29.3)	43 (42.5)	0.015
Buffalo hump	22 (38)	6 (14.6)	28 (28)	0.012
Purple striae	7 (12)	5 (12.2)	12 (11.8)	0.98
Muscle weakness	21 (36.2)	8 (19.5)	29 (28.7)	0.07
Skin atrophy	6 (10.3)	2 (5)	8 (7.9)	0.33
Easy bruising	27 (46.5)	8 (19.5)	35 (34.6)	0.005
Hirsutism	29 (55)	13 (50)	42 (53.1)	0.11
Acne	12 (23)	17 (65)	29 (36.7)	<0.01
Oligomenorrhea	22 (41)	14 (53.8)	36 (45.5)	0.4
Erectile dysfunction	6 (86)	5 (33)	11 (50)	0.02
Headache	7 (12)	16 (39)	23 (22.7)	0.0018
Blurred vision	4 (6.9)	3 (7.3)	7 (7)	0.93

Age and body mass index (BMI) are expressed as mean ± SD; other values are expressed as number (%). *P* values are CD *vs.* PCS. Data on symptoms refer to 100 patients (59 CD and 41 PCS). Data on bone status refer to 32 patients. IGT, Impaired glucose tolerance.

[a] *P* value refers to comparison of females *vs.* males for each diagnosis and CD *vs.* PCS for each gender.

post-CRH serum cortisol of less than the 1.4 μg/dl (38 nmol/liter) historical cutoff (5), giving the Dex-CRH test an overall sensitivity of 86.3% (95% CI 82–90.7%). The difference between sensitivities before and after CRH was not significant.

Seven of 46 (15.2%) PCS patients had a post-LDDST serum cortisol level above 1.4 μg/dl (38 nmol/liter), suggesting the diagnosis of Cushing's. Fifteen minutes after CRH administration, one of those PCS patients had a lower serum cortisol of 1.2 (32 nmol/liter), whereas the other six still had serum cortisol over 1.4 μg/dl (38 nmol/liter), thereby incorrectly predicting true CD. Another patient, who had an initially suppressed post-LDDST baseline cortisol, was just above the cutoff point at 1.5 μg/dl (40.7 nmol/liter) after CRH injection. Overall, the specificity of the post-LDDST result overlapped that of the Dex-CRH test at the +15-min time point using a cutoff of 1.4 μg/dl (38 nmol/liter) (84.7%, 95% CI 79.3–90.2% for both tests). Progressive elevation of threshold values increased the specificity of both post-LDDST and Dex-CRH tests; a serum cortisol cutoff of 5 μg/dl (138 nmol/liter) allowed exclusion of all false-positive subjects even before CRH injection. As expected, however, this threshold increased false negatives; 34 Cushing's patients would have

TABLE 3. Mean cortisol and ACTH levels during the Dex-CRH test

	F at time 0	F at time +15 min	ACTH at time 0	ACTH at time +15 min	Dexamethasone (ng/dl)
CD	7.4 ± 8.2	12.5 ± 12.8	27.7 ± 26.7	56.5 ± 61.5	492.7 ± 174.54
PCS	0.51 ± 0.9	0.61 ± 0.99	3 ± 6.1	7.9 ± 1.17	423.13 ± 145.84
P value	<0.0001	<0.0001	<0.0001	<0.0001	0.17
CD Meds	7.12 ± 8.5	12.3 ± 11.6	25.9 ± 19	65.2 ± 75.1	489.6 ± 161.5
CD No Meds	7.6 ± 8	12.8 ± 14.2	30.2 ± 32.6	48.9 ± 45.9	553 ± 188.7
P value	0.80	0.88	0.54	0.32	0.5
PCS Meds	0.87 ± 1	0.89 ± 1.24	5.1 ± 8.7	7.1 ± 9.9	433.8 ± 161.5
PCS No Meds	0.23 ± 0.54	0.37 ± 0.7	1.4 ± 2.15	2.6 ± 5.2	412.4 ± 96.1
P value	0.016	0.077	0.04	0.056	0.74
Overall Meds	4.62 ± 7.32	8 ± 10.7	16.8 ± 27.8	41 ± 64.2	462.9 ± 172.7
Overall No Meds	4.27 ± 7	7.2 ± 12.2	17.2 ± 18.6	27 ± 40	456 ± 142.1
P value	0.8	0.7	0.93	0.18	0.90

Values are expressed as average ± SD. Units are micrograms per deciliter for cortisol, picograms per milliliter for ACTH, and nanograms per deciliter for dexamethasone. Each *P* value refers to the comparison of the above two lines. F, Serum cortisol.

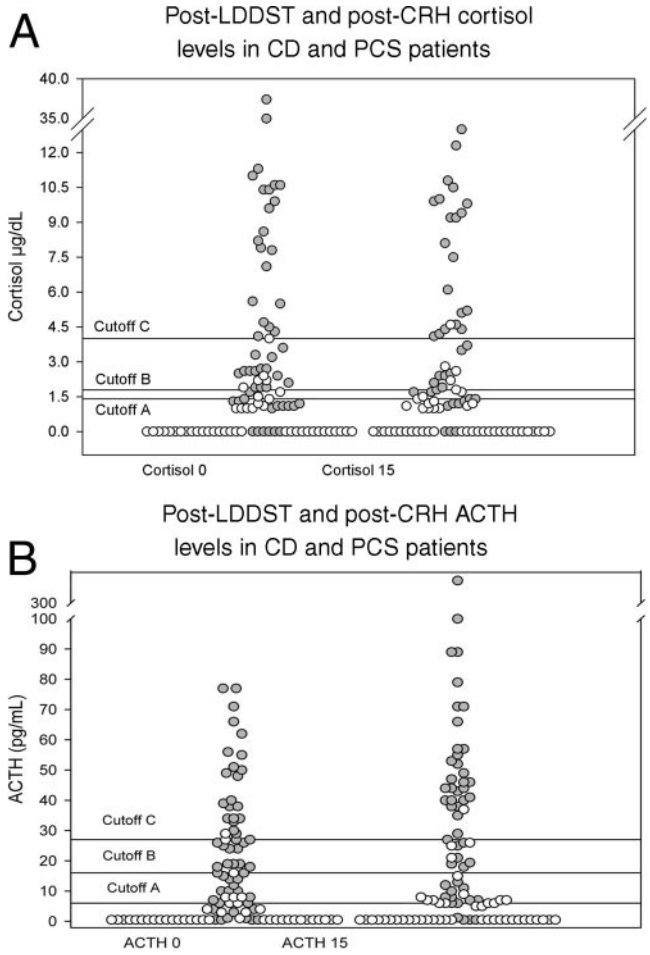

FIG. 1. A, Individual cortisol responses in patients with CD (●) and PCS (○) at baseline post-LDDST (cortisol 0) and 15 minutes post-CRH (cortisol 15). Cutoff A = 1.4 μg/dl (38 nmol/L); cutoff B = 1.8 μg/dl (50 nmol/L); cutoff C = 4 μg/dl (110 nmol/L). B, Individual ACTH responses in patients with CD (●) and PCS (○) at baseline post-LDDST (ACTH 0) and 15 minutes post-CRH (ACTH 15). Cutoff A = 6 pg/ml (1.3 pmol/L); cutoff B = 16 pg/ml (3.5 pmol/L); cutoff C = 27 pg/ml (5.9 pmol/L).

been misclassified after the LDDST because they had a baseline value less than 5 μg/dl. Nine of these would have moved to the correct diagnosis of CD after CRH injection, but 25 remained misclassified after the combined test, because they showed serum cortisol levels below 1.4 μg/dl (38 nmol/liter). This yields a post-LDDST sensitivity using a cutoff point of less than 5 μg/dl of 44.2% (95% CI 36.8–51.7%) and Dex-CRH sensitivity with the 1.4 μg/dl cutoff of 62.1% (95% CI 55.6–68.7%) (*P* value not significant) (Fig. 1A).

ROC analysis showed that the AUC of post-LDDST and post-CRH cortisol were not significantly different (0.898 *vs.* 0.928 μg/dl, respectively; $P = 0.084$) (graph not shown).

ACTH response

Dex-CRH testing specificity increased when using ACTH rather than 15-min post-CRH cortisol levels. Using the cutoff proposed by Erickson *et al.* (6) [27 pg/ml (5.9 pmol/liter)], the sensitivity and specificity were 75.4% (95% CI 69.4–81.5%) and 91.3% (95% CI 87.1–95.5%), respectively. Using the cutoff proposed by Gatta *et al.* (9) [16 pg/ml (3.5 pmol/liter)], the sensitivity and specificity were 63.1% (95% CI 56.2–70.2%) and 97.8% (95% CI 95.7–100%), respectively (Fig. 1B).

On the ROC curve, the AUC of post-LDDST and post-CRH ACTH were not significantly different. Likewise, differences between them and the AUC of post-LDDST and post-CRH cortisol were not significant (data not shown).

Effect of medications on the Dex-CRH test

The diagnostic accuracy of the Dex-CRH test in the Meds and No Meds groups is shown in Table 4. Both the sensitivity and specificity of the test in No Meds patients were higher than in the Meds group. The specificity using the standard post-LDDST (pre-CRH) cortisol threshold of 1.4 μg/dl (38 nmol/liter) was significantly higher in No Meds *vs.* Meds group ($P = 0.014$). Using a serum cortisol cutoff of 1.4 μg/dl (38 nmol/liter) 15 min after CRH, the No Meds and Meds group specificity was comparable ($P = 0.10$). Using a serum cortisol threshold of 1.8 μg/dl (50 nmol/liter) 15 min after CRH, the difference between the No Meds and Meds specificity approached significance ($P = 0.08$).

As shown in Table 4, PCS patients in the No Meds group had significantly lower serum cortisol and ACTH

TABLE 4. Comparison between No Meds and Meds tests using different cutoff points

Test	Cutoff[a]	Sensitivity % (95% CI)			Specificity % (95% CI)		
		Meds	No Meds	*P* value	Meds	No Meds	*P* value
Post- LDDST cortisol	1.4 (38)	73.3 (64.7–81.9)	85.7 (78.9–92.5)	0.77	70 (59–81)	96.1 (92.4–99.9)	0.014
Post-LDDST cortisol	1.8 (50)	70 (61–79)	85.7 (78.9–92.5)	0.61	80 (70.7–89.3)	96.1 (92.4–99.9)	0.08
15 min post-CRH cortisol	1.4 (38)	88.2 (82.6–93.9)	93.1 (88.4–97.8)	0.88	75 (64.8–85.2)	92.3 (87–97.6)	0.10
15 min post-CRH cortisol	1.8 (50)	82.3 (75.5–89.1)	89.6 (83.9–95.4)	0.81	80 (70.6–89.3)	96.1 (92.4–99.9)	0.08
15 min post-CRH cortisol	2.5 (70)	70.5 (62.2–79)	86.2 (79.6–92.8)	0.60	90 (83.2–96.8)	96.1 (92.4–99.9)	0.87
15 min post-CRH ACTH	16 (3.5)	75 (66.3–83.7)	84.6 (77.3–91.9)	0.76	85 (76.8–93.2)	96.1 (92.4–99.9)	0.77
15 min post-CRH ACTH	27 (5.9)	64.2 (54.4–74.2)	73 (63.8–82.3)	0.75	95 (90.1–99.9)	100	0.90

[a] Units are micrograms per deciliter (nanomoles per liter) for cortisol and picograms per milliliter (picomoles per liter) for ACTH.

levels at baseline after LDDST compared with PCS patients in the Meds group, suggesting that the medications may have altered the cortisol and ACTH response. In contrast, in CD patients, no difference was found when cortisol or ACTH levels at either baseline or after CRH administration were compared with levels in CD patients with or without medications. No difference in dexamethasone concentrations between No Meds and Meds patients was observed overall or when either CD or PCS subjects were compared (Table 3). Lower dexamethasone concentrations were observed in false-positive as compared with false-negative patients, but the difference was not significant ($P = 0.17$). Indeed, although dexamethasone levels were available in only three false-positive patients, they were, on average, lower than the values previously reported in 20 healthy volunteers (325 ± 119.5 *vs.* 469.5 ± 220 ng/dl) (14).

Test performance differences based on number of medications

The Meds group was divided into three subgroups according to whether patients were taking one (group 1: 23 tests, 16 CD and seven PCS), two (group 2: 20 tests, 14 CD and six PCS), or more than two medications (group 3: nine tests, two CD and seven PCS). Overall, the specificity of a post-LDDST cortisol was 100% in group 1 *vs.* 57.1% in group 3 ($P = 0.05$). When patients with CD and PCS were analyzed separately, statistically significant results were obtained in PCS patients only.

Both post-CRH cortisol and ACTH levels were significantly lower in group 1 than group 3 (0.3 ± 0.5 in group 1 *vs.* 1.3 ± 1 μg/dl, $P < 0.05$; and 2.3 ± 3.2 in group 1 *vs.* 15.1 ± 12 pg/ml in group 3; $P < 0.05$). The specificity of a post-CRH cortisol equal to 1.4 μg/dl (38 nmol/liter) was significantly higher in group 1 than group 3 (100 *vs.* 42.8%, 95% CI 20.9–64.8%, $P < 0.05$, respectively). Both sensitivity and specificity for post-LDDST ACTH equal to 16 pg/ml (3.5 pmol/liter) overlapped between group 1 and group 3 PCS patients. The specificity of a post-CRH ACTH cutoff of 16 pg/ml (3.5 pmol/liter) was 71.4% (95% CI 53.2–89.7%) in group 1, whereas it was 0% in group 3, because all seven PCS had values above the threshold.

Test performance differences based on type of medications

The accuracy of tests in patients on only antidepressants (22 tests) was compared with those taking only cardiometabolic medications (lipid-lowering and/or antihypertensive agents, 17 tests). In the PCS group, no difference was observed in either cortisol or ACTH response between patients taking antidepressants *vs.* cardiometabolic medications. Dexamethasone levels in PCS patients on lipid-lowering/antihypertensive agents were significantly lower than in those on antidepressants (265.6 ± 109 *vs.* 457.33 ± 32 ng/dl, $P = 0.04$), although the patient numbers were small. No difference in specificity was observed between the two groups for any previously established threshold.

When only CD patients were considered, baseline post-LDDST ACTH levels on antidepressants trended lower than on lipid-lowering/antihypertensive agents (18 ± 14 *vs.* 33.8 ± 23.1 pg/ml, $P = 0.053$). Dexamethasone levels were comparable. When sensitivity of the post-LDDST ACTH cutoff equal to 16 pg/ml (3.5 pmol/liter) was used, the difference in sensitivity between the two medication groups trended positive [50% (95% CI 33.5–66.5%) on antidepressants *vs.* 87.5% (95% CI 75.5–99.5%) on lipid-lowering/antihypertensive drugs, $P = 0.08$].

Other diagnostic tests

The diagnostic accuracy of the recommended first-line screening tests was compared with the post-Dex-CRH 1.4 μg/dl (38 nmol/liter) cutoff at 15 min. Results are shown in Table 5.

Discussion

We have shown that the diagnostic accuracy of Dex-CRH testing in patients with suspected CS, not on potential confounding medications, is high with a sensitivity and

TABLE 5. Comparison between first-line screening tests and the Dex-CRH test

Test	Cutoff	Sensitivity % (95% CI)	*P* value	Specificity % (95% CI)	*P* value
15 min post-CRH cortisol	1.4 μg/dl (38 nmol/liter)	86.3 (82–90.7)		84.7 (79.3–90.2)	
UFC	70 μg/24 h (193 nmol/liter)	94.5 (91.5–97.6)	0.13	40 (30.2–49.8)	<0.001
UFC	> 2-fold upper limit of normal	45 (39.8–50.6)	<0.001	68 (60.8–75.4)	0.83
UFC	> 3-fold upper limit of normal	20 (15–24)	<0.001	91 (86.8–95.4)	0.4
Post-LDDST cortisol	1.8 μg/dl (50 nmol/liter)	91 (84.7–97.1)	0.88	66 (45.8–87.5)	0.73
Post-LDDST cortisol	5 μg/dl (138 nmol/liter)	81.8 (73.3–90.4)	0.88	100	0.78

P value refers to the comparison between each test and the gold standard criterion of serum cortisol 15 min after CRH.

specificity of more than 92%, but we also demonstrate that the test is less reliable in patients on medications that could potentially affect dexamethasone metabolism. This effect is also seen with simple DSTs, because we found that the baseline post-LDDST cortisol with a threshold of 1.4 μg/dl (38 nmol/liter) yielded a 96.1% specificity in patients not on confounding medications, significantly greater than the 70% seen in those on medications. The specificity of a 15-min post-CRH cortisol level greater than 1.4 μg/dl was significantly higher in patients taking only one, rather than multiple, medications.

PCS patients on multiple medications showed significantly higher post-CRH cortisol and ACTH levels, suggesting inadequate suppression by dexamethasone. This could be due to increased dexamethasone clearance. Although dexamethasone levels in this group were inconclusive, they were available in less than half of the patients, because they were not routinely performed until 2006. Alternatively, those patients requiring multiple concomitant medications may have more hyperactivity of the hypothalamic-pituitary-adrenal axis, producing resistance to equivalent levels of dexamethasone.

Of all reports published to date on the Dex-CRH test, only two confined the analysis to patients taking no drugs known to interfere with dexamethasone clearance (6, 8). Martin *et al.* (8) found 67 and 88% specificities for post-CRH cortisol cutoffs of 1.4 μg/dl (38 nmol/liter) and 1.8 μg/dl (50 nmol/liter), respectively, in 36 subjects, whereas we reported 92.3 and 96.1% for the two thresholds in the No Meds subgroup. Erickson *et al.* (6) observed 95% sensitivity and 97% specificity using a post-CRH ACTH cutoff of 27 pg/ml (5.9 pmol/liter) in 51 patients, whereas they were 73 and 100%, respectively, using that cutoff in our series of 58 tests without potentially interfering medications. These discrepancies may be accounted for by differences in hormone assays, patients, or protocol characteristics. In particular, Martin *et al.* (8) used human-sequence CRH, which is known to have a lower stimulatory effect than the ovine CRH we administered. Moreover, they gave patients an additional ninth dose of dexamethasone 2 h before CRH.

It is well known that medications affect the metabolism of dexamethasone, inducing or inhibiting the cytochrome CYP3A4 and ultimately impairing the reliability of those tests, including the Dex-CRH, which rely on this glucocorticoid to suppress the pituitary-adrenal axis (2). The use of antidepressant, antipsychotic, lipid-lowering, and antihypertensive agents is increasing in the general population. It has been reported that antidepressant prescriptions in the United States noninstitutionalized population rose from 154.1 to 169.9 million during the period 2002–2005 (15). We found that the use of such medications is especially prevalent among patients being evaluated for CS, with 48.2% of patients taking medications that might affect dexamethasone metabolism.

For over a decade, the Dex-CRH test was considered an excellent tool to discriminate CS from PCS. The original report by Yanovski *et al.* (5) observed 100% sensitivity and 100% specificity for 15 min post-CRH plasma cortisol greater than 1.4 μg/dl (38 nmol/liter) in a population evaluated prospectively for hypercortisolism with a UFC less than 3-fold above the normal range. More recent studies have not confirmed these results and have recommended different thresholds to not misclassify patients. Indeed, when the historical cortisol cutoff of 1.4 μg/dl (38 nmol/liter) was applied, specificities ranged from 50–76% (6–9), all lower than the 96.1% demonstrated in our No Meds group. This raises the possibility that concomitant medication use might have lowered the diagnostic accuracy in some of those studies. Thus, the utility of the Dex-CRH test is still under debate.

A post-CRH cortisol cutoff equal to 4 μg/dl (110 nmol/liter), which was associated with 86% specificity and 100% sensitivity in one previous report (9), yielded a very high specificity in both our whole series and the No Meds group (97.8 and 100%, respectively) in the face of a reduced sensitivity (69.6 and 68.7%, respectively) (data not shown).

Discrepancies between the results obtained in our series and others may relate to the fact that our cohort has more than twice as many subjects as previous reports. In addition, differences in the hormone assays and type of CRH (ovine *vs.* human) cannot be ruled out.

At baseline, a post-LDDST cortisol cutoff equal to 1.4 μg/dl (38 nmol/liter) yielded a sensitivity that was slightly, although not significantly, lower than that observed in all the groups analyzed, confirming previous reports (2). One possible explanation could be a difference in timing of the dexamethasone administration. An alternative possibility is that differences in patient populations may account for different sensitivities between the classic LDDST and the Dex-CRH test, particularly because the latter test is typically performed in patients with mild cortisol elevation. It is important to note that nine CD patients of those 15 who would be misclassified as PCS at baseline were properly diagnosed after CRH administration, confirming the role of the Dex-CRH test in decreasing false-negative results.

When specificities of post-LDDST and post-CRH cortisol cutoffs of 1.4 μg/dl (38 nmol/liter) were compared within all three groups analyzed (*i.e.* whole series, Meds, and No Meds), a substantial overlap was demonstrated. Moreover, the specificity of both tests was higher in either the whole series or the No Meds group than in previous reports (2, 6, 7, 9). Indeed, in other studies, CRH admin-

istration was associated with a clinically relevant increase in the number of false positives. Pecori Giraldi *et al.* (7) found that three of nine PCS patients who were misclassified as having CD after the Dex-CRH test had normal suppression at baseline, whereas the remaining six had lack of inhibition after LDDST as well. In our study, only one patient among those who had normal post-LDDST suppression was misclassified as having CD at the 1.4 μg/dl (38 nmol/liter) cutoff after CRH injection.

Thus, although we did not find the same perfect diagnostic accuracy as Yanovski *et al.* (5), we confirm that the Dex-CRH test has an acceptable reliability, especially in terms of specificity, and may be a more valuable diagnostic tool than a standard LDDST to differentiate between hypercortisolemic states and true CS in a carefully selected group of patients, particularly those not taking any medications that might affect CYP3A4. Further studies are required to establish how long a patient should be off such medications before performing the test.

Limitations of our study include retrospective design, relatively small sample size, inherent potential inaccuracies of historical data collection, and the low number of dexamethasone measurements performed. In addition, short follow-up in PCS, although not statistically different from CD, and some missing data because of loss of follow-up in the overall series can be considered limitations as well. The lack of cortisol-binding globulin measurements is also a limitation. However, because oral estrogen increases hepatic production of cortisol-binding globulin and therefore has the potential to raise serum cortisol, Dex-CRH testing was performed after withdrawal of estrogen for approximately 8 wk in all patients except two. If salivary cortisol levels had been available over the entire decade, they would have been interesting to compare with other tests.

It is conceivable that a patient classified as PCS may actually have CD. This is especially true if they had a cyclical pattern of cortisol hypersecretion and were evaluated during an off cycle. The median follow-up of 28.5 months in the PCS group argues somewhat against this.

Although dexamethasone levels did not appear significantly different between Meds and No Meds patients, this may relate to the small number of assays available in each group. Lower dexamethasone concentrations were observed in false-positive as compared with false-negative patients, which may explain the equivocal performance of the test in these individuals, most of whom were taking some of the more common medications that influence cytochrome activity. It was not possible to specify the impact of each class of drugs on dexamethasone metabolism because medications within the same class can have different potencies, and many patients included in our study were on a multidrug regimen.

In conclusion, our study shows that several common medications, including selective serotonin reuptake inhibitors, serotonin-noradrenaline reuptake inhibitors, and calcium channel blockers may contribute to the variable diagnostic accuracy of the Dex-CRH test. The use of confounding medications may explain some of the differences in the previously reported diagnostic accuracy of this test. Clinicians should carefully consider all potential confounders, including drugs, in each patient being evaluated for hypercortisolemia, to maximize diagnostic efficacy. Furthermore, prospective studies regarding the medications, doses, and durations that may interfere with diagnostic testing in CS will be important.

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