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Clinical Research Article

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Interpretation of Abnormal Dexamethasone Suppression Test is Enhanced With Use of Synchronous Free Cortisol Assessment

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Abbreviations: BMI, body mass index; DST, dexamethasone suppression test; IQR, interquartile ratio; OCP, oral contraceptive pill.

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Abstract

Context: Interpretation of dexamethasone suppression test (DST) may be influenced by dexamethasone absorption and metabolism and by the altered cortisol binding.
Objective: We aimed to determine the normal ranges of free cortisol during DST in participants without adrenal disorders and to identify the population of patients where post-DST free cortisol measurements add value to the diagnostic workup.
Design and Setting: Cross-sectional study conducted in a tertiary medical center.
Participants: Adult volunteers without adrenal disorders (n = 168; 47 women on oral contraceptive therapy [OCP], 66 women not on OCP, 55 men) and patients undergoing evaluation for hypercortisolism (n = 196; 16 women on OCP).
Measurements: Post-DST dexamethasone and free cortisol (mass spectrometry) and total cortisol (immunoassay).
Main Outcome Measures: Reference range for post-DST free cortisol, diagnostic accuracy of post-DST total cortisol.
Results: Adequate dexamethasone concentrations (≥0.1 mcg/dL) were seen in 97.6% volunteers and 96.3% patients. Only 25.5% of women volunteers on OCP had abnormal post-DST total cortisol (>1.8 mcg/dL). In volunteers, the upper post-DST free cortisol range was 48 ng/dL in men and women not on OCP, and 79 ng/dL in women on OCP.

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When compared with post-DST free cortisol, diagnostic accuracy of post-DST total cortisol was 87.3% (95% CI, 81.7-91.7); all false-positive results occurred in patients with post-DST cortisol between 1.8 and 5 mcg/dL. OCP use was the only factor associated with false-positive results (21.1% vs 4.9%, $P = 0.02$).

Conclusions: Post-DST free cortisol measurements are valuable in patients with optimal dexamethasone concentrations and post-DST total cortisol between 1.8 and 5 mcg/dL.

Key Words: mild autonomous cortisol secretion, diagnosis, accuracy, adrenal mass, adrenal adenoma

A 1-mg dexamethasone suppression test (DST) is a standard-of-care endocrine test recommended in the evaluation of adrenal masses and for patients suspected to have endogenous Cushing syndrome (1-3). The guidelines from the European Society of Endocrinology/European Network for the Study of Adrenal Tumours define autonomous cortisol secretion as post-DST total cortisol >5 mcg/dL and possible autonomous cortisol secretion as post-DST cortisol between 1.8 and 5 mcg/dL (2). Patients with abnormal post-DST cortisol are at higher risk for cardiovascular morbidity (4), fractures (5, 6), and frailty (7), and are recommended to undergo workup to evaluate for associated comorbidities both at baseline and follow-up (1, 2).

Interpretation of DST is affected by the dexamethasone absorption and metabolism, with several studies reporting a rate of 6% to 20% of false-positive results because of inadequate dexamethasone concentrations (8-10). Another source of false-positive results is the difference in the proportion of cortisol bound to corticosteroid binding globulin affecting total cortisol concentrations. Free cortisol represents approximately 5% to 6% of total cortisol. The majority of cortisol is bound to the corticosteroid binding globulin, and less so to albumin and erythrocytes. Several conditions, such as pregnancy, liver and renal failure, and medications, such as oral contraceptive therapy (OCP) may affect corticosteroid-binding globulin concentrations (11-16). This may lead to either falsely low or high total cortisol concentrations and affect interpretation with either over or under diagnosis of adrenal insufficiency or hypercortisolism, depending on the clinical scenario.

OCP therapy is most commonly associated with false positives during DST, and the guidelines recommend stopping OCP for 6 weeks before performing DST (3). This can be quite taxing for patients and can result in exacerbations of their underlying conditions, such as hyperandrogenism and menorrhagia, or can result in undesired pregnancy. The value of post-DST free cortisol measurement in saliva has been previously explored by Ueland et al, but included very few patients treated with OCP (8).

In this study, we aimed to determine the utility of free cortisol assessment during DST. Our objectives were: (1) to determine the normal ranges of free cortisol during DST in participants without adrenal disorders, including women taking OCP and (2) to identify the population of patients in whom post-DST free cortisol measurement add value to the diagnostic work up of hypercortisolism.

Materials and Methods

Participants

This study protocol was approved by the institutional review board of Mayo Clinic, Rochester. We conducted this prospective study at the Endocrine Testing Center, Mayo Clinic Division of Endocrinology, in Rochester, Minnesota, between January 2016 and August 2018 in adults ≥ 18 years of age. All subjects provided a written informed consent before participation.

In brief, this was a cross-sectional study with prospective consecutive enrollment of (1) volunteers without adrenal disorders and (2) patients being assessed for cortisol excess for clinical reasons. All participants underwent DST with measurements of total and free cortisol as well as dexamethasone concentrations. After establishing the normal ranges for post-DST free cortisol in volunteers, the cutoffs were applied to a cohort of patients evaluated in the endocrine clinic and being assessed with DST.

Volunteers were enrolled through an advertisement placed online within the Mayo Health System. Each volunteer was interviewed to confirm absence of adrenal disorders and exogenous glucocorticoid use within 3 months and examined for clinical symptoms and signs of Cushing syndrome. Careful medication history was taken to exclude medications interfering with metabolism of dexamethasone. The type and dose of oral estrogen-containing therapy was recorded and pregnancy was excluded at time of participation.

Patients were consecutively recruited from Endocrinology clinic; all patients requiring DST as a part of their clinical care were offered enrollment in the study, and 96% of approached patients consented to participate in this study. Pertinent clinical information for this study was obtained through medical electronic record review and an interview.

Clinical evaluation

Medical record review was conducted to ascertain demographic parameters (sex, age, body mass index [BMI]), clinical indication for DST (in patient cohort), and whether oral contraceptive therapy was in use (in women, type and dose collected). All study participants were instructed to take 1 mg of dexamethasone at 11 PM the night before blood sample collection. Blood samples were obtained between 8 and 9 AM and analyzed for total cortisol, free cortisol, and dexamethasone concentrations.

Total cortisol concentration

Measurement of total cortisol used a competitive binding immune-enzymatic assay on the UniCel DxI 800 (Beckman Coulter, Brea, California). Total cortisol measurements are provided in milligrams per deciliter. Total cortisol after 1 mg DST in patients was considered normal if ≤1.8 mcg/dL. In patients, total cortisol >1.8 mcg/dL were considered to have hypercortisolism (mild hypercortisolism when total cortisol was 1.8-5 mcg/dL and significant hypercortisolism when >5 mcg/dL).

Dexamethasone concentration

Dexamethasone was measured by liquid/liquid extraction followed by high-performance liquid chromatography and detection by tandem mass spectrometry, which is commercially available through Mayo Clinic Laboratories and is validated by USA federal Clinical Laboratory Improvement Amendments requirements. Relevant details of the dexamethasone assay are included in supplemental Table 1. Lower limit of quantification was 0.1 mcg/dL. The threshold for dexamethasone concentration resulting in suppression of total cortisol was determined in healthy volunteers. Then, this threshold was applied to determine whether dexamethasone concentration was optimal for interpretation of DST in the patient group.

Free cortisol concentration

Free cortisol was measured by an assay developed in Mayo Clinic Laboratories, as previously reported (17). In brief, free cortisol was separated from its bound form without disrupting the equilibrium of the sample using a commercially available equilibrium dialysis plate (Thermo Fisher Scientific, Waltham, Massachusetts). Each sample well was separated by a vertical cylinder of dialysis membrane (molecular weight cutoff, 8000). Duplicate samples of 0.25 mL were added to each well. The adjacent chamber was filled with dialysis buffer. After equilibrium was reached, the dialysate was removed, and the d3-cortisol internal standard was added to the dialysate. The dialysate/internal standard mixture was analyzed using turbo flow liquid chromatography combined with atmospheric pressure chemical ionization and tandem mass spectrometry. Briefly, dialysate is injected onto a 0.5 × 50 mm HTLC C18XL column (Cohesive Technologies, Alpharetta, Georgia) before being transferred to a Zorbax XDB-C18, 4.6 × 50 mm (Agilent Technologies, Santa Clara, California). Cortisol and its internal standard were measured by multiple reaction monitoring (MRM) using the precursor ions of cortisol (m/z 363.2), its isotopic internal standard (m/z 366.2), and their respective primary collision-induced fragment/product ions (m/z121.0 for both) for sensitive and specific quantification on an AB 5000mass spectrometer (Sciex, Framingham, Massachusetts). A calibration curve generated from stripped serum spiked standard was included with each batch of patient specimens. Measurements are provided in nanograms per deciliter.

Data from volunteers without clinical evidence of hypercortisolism were used to develop a reference range for the free cortisol assay following the 1 mg DST. Reference ranges for women on OCP and men and women not on OCP were constructed after the exclusion of results when dexamethasone concentration was <0.1 mcg/dL and after excluding of outliers. The utility of free cortisol measurements in addition to total cortisol was then evaluated in the patient study population.

Statistical analysis

Data were analyzed using JMP 15.1.0 software (SAS Inc., Cary, North Carolina). Variables were assessed for normality by the Kolmogorov-Smirnov test. All continuous data are summarized as median and interquartile ranges (IQR), while categorical data are summarized as a number (percent). Subjects with missing data were excluded from analyses. Associations between variables were assessed using the Kruskal-Wallis test for continuous variables and the χ^2 test or Fisher exact test for categorical variables, as appropriate. Statistical significance was defined as $P < 0.05$.

Results

Volunteers

One hundred and sixty-eight volunteers participated in this study, including 113 women (67%), median age 29.5 (IQR 26, 40) years and 55 men (33%), median age of 30 (IQR 27, 40) years, Table 1. Among women participants, 47 (42%) were taking OCP with median estradiol dose 30 mcg (IQR 20, 35).

Overall, median post-DST total cortisol was 0.79 mcg/dL (IQR 0.60, 1.30), higher in women taking OCP compared with those who were not (median of 1.30 vs 0.72 mcg/dL in women not on OCP and 0.64 in men, $P = 0.002$), Table 1. Among 18 (11%) participants with post-DST total cortisol >1.8 mcg/dL, 12 (67%) were women taking OCP. About one-quarter of women taking OCP had abnormal post-DST cortisol when compared with other participants (26.5% vs 4.3%, $P < 0.001$). The dose of ethinyl estradiol was not associated with any differences in post-DST total or free cortisol, Table 2. After excluding women treated with OCP, no associations between post-DST total cortisol concentrations and sex, age, or BMI were found (data not shown).

Following the overnight 1 mg administration of dexamethasone, volunteers demonstrated dexamethasone concentrations at a median of 0.34 ng/dL (IQR 0.25, 0.44), with 2.4% having inadequate dexamethasone concentrations <0.1 ng/dL (Table 1, supplemental Fig. 1) (18).

Post-DST free cortisol concentration in volunteers was at a median of 24 ng/dL (IQR 19.3, 32.0), not significantly different among men, women not taking OCP, and women taking OCP, respectively (21 ng/dL, 22 ng/dL, 33 ng/dL;

Table 1. Dexamethasone suppression test in volunteers

	All volunteers (n = 168)	Women, taking OCP[a] (n = 47)	Women, no OCP (n = 66)	Men (n = 55)	*P* value
Age, y, median (IQR)	29.5 (26, 40)	27 (24, 30)	32 (27, 42.5)	30 (27, 40)	0.003
BMI, kg/m^2, median (IQR)	25.4 (22.8, 29.2)	24.5 (22.4, 28.3)	25.1 (22.6, 29.0)	26.9 (24.3, 30.5)	0.40
Estradiol dose, mcg, median (IQR)	-	30 (20, 35)	-	-	-
≥ 30 mcg, n (%)		34 (72.3%)			
Dexamethasone concentration,[b,c] mcg/dL, median (IQR)	0.34 (0.25, 0.44)	0.37 (0.24, 0.43)	0.30 (0.25, 0.40)	0.38 (0.25, 0.46)	0.31
Dexamethasone concentration[b,c] ≥0.1 mcg/dL, number (%)	162 (97.6%)	45 (95.7%)	65 (98.5%)	52 (98.1%)	0.32
Total serum cortisol,[b,c] mcg/dL, median (IQR)	0.79 (0.60, 1.30)	1.3 (1.1, 1.9)	0.72 (0.58, 0.88)	0.64 (0.51, 0.83)	0.02
Total serum cortisol > 1.8[b,c] mcg/dL, number (%)	18 (10.9%)	12 (25.5%)	3 (4.6%)	3 (5.7%)	0.001
Free serum cortisol,[b] ng/dL, median (IQR)	24 (19.3, 32.0)	33 (24.0, 47.0)	22 (19.0, 27.3)	21 (17.0, 26.0)	0.70

Abbreviations: BMI, body mass index; IQR, interquartile range; OCP, oral contraceptive pill.
[a]OCP use group included all women who received estrogen-containing oral contraceptive. Women who were on progestin-only contraceptive therapy (n = 2) were considered in the non-OCP group.
[b]Dexamethasone concentration, total serum cortisol, and free serum cortisol were collected after 1 mg oral dexamethasone suppression.
[c]Data unavailable for dexamethasone concentration in 2 volunteers and total serum cortisol in 3 volunteers.

Table 2. Dexamethasone suppression test in female volunteers treated with oral contraceptive therapy

	All women, taking OCP[a] (n = 47)	OCP with EE < 30 mcg (n = 13)	OCP with EE ≥ 30 mcg (n = 34)	*P* value
Age, years, median (IQR)	27 (24, 30)	26 (23, 27.5)	28 (25, 34)	0.06
BMI, kg/m^2, median (IQR)	24.5 (22.4, 28.3)	25.4 (22.5, 28.6)	24.5 (22.2, 28.2)	0.53
Dexamethasone concentration,[b,c] mcg/dL, median (IQR)	0.37 (0.24, 0.43)	0.37 (0.20, 0.45)	0.36 (0.26, 0.43)	0.92
Dexamethasone concentration,[b,c] ≥0.1 mcg/dL, number (%)	45 (95.7)	11 (84.6)	34 (100)	0.07
Total serum cortisol,[b,c] mcg/dL, median (IQR)	1.3 (1.1 ,1.9)	1.3 (0.9, 2.9)	1.3 (1.2, 1.9)	0.29
Total serum cortisol > 1.8,[b,c] mcg/dL, number (%)	12 (25.5)	3 (23.1)	9 (26.5)	0.81
Free serum cortisol,[b,d] ng/dL, median (IQR)	33 (24 ,47)	35 (22 ,53)	32 (25 ,45.5)	0.12
Free serum cortisol within reference range,[b,d] number (%)	44 (93.6)	11 (84.6)	33 (97.1)	0.18

Abbreviations: BMI, body mass index; EE, ethinyl estradiol; IQR, interquartile range; OCP, oral contraceptive pill.
[a]OCP use group included all women who received estrogen-containing oral contraceptive. Women who were on progestin only contraceptive therapy (n = 2) were considered in the non-OCP group.
[b]Dexamethasone concentration, total serum cortisol, and free serum cortisol were collected after 1 mg oral dexamethasone suppression.
[c]Data unavailable for dexamethasone concentration in 2 volunteers and total serum cortisol in 3 volunteers.
[d]Reference range free cortisol ≤ 79 ng/dL for women on OCP.

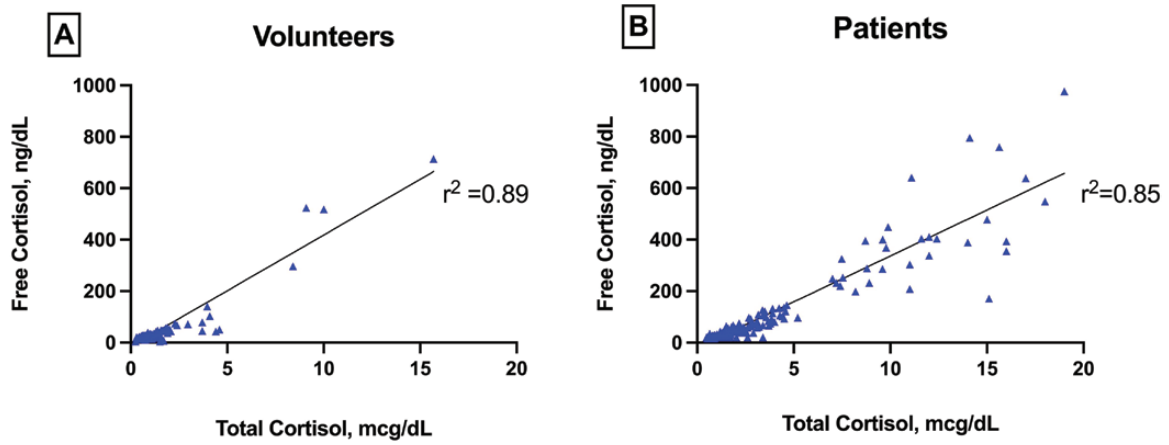


Figure 1. Post-dexamethasone suppression test: free cortisol vs total cortisol concentrations in (A) volunteers and (B) in patients undergoing evaluation for hypercortisolism.

$P = 0.70$), Table 1. Overall, post-DST free cortisol was strongly correlated with post-DST total cortisol, $R^2 = 0.89$ with a linear correlation (Fig. 1A).

Reference range for post-DST free cortisol were determined by first excluding any participants with inadequate dexamethasone concentrations (<0.1 ng/dL) (n = 4), and excluding outliers (n = 4); supplemental Fig. 2 (18). With this approach, the post-DST free cortisol maximum level was ≤ 48 ng/dL for men and women not on OCP and ≤ 79 ng/dL for women on OCP. The remaining participants' total cortisol and free cortisol concentrations had a strong correlation with binomial fit ($R^2 = 0.91$); supplemental Fig. 3 (18). This relationship was very strong for men and for women not taking OCP ($R^2 = 0.96$), but was more dispersed for women taking OCP ($R^2 = 0.63$) (data not shown).

Patients

One hundred and ninety-six patients participated in the study at a median age of 57 years (IQR 40, 66), consisting of 67% women (of whom 13 [10%] were taking OCP), Table 3. Indications for DST varied, including evaluation of adrenal mass (152, 77.6%), evaluation of pituitary mass (8, 4.1%), and evaluation for Cushing syndrome (24, 12.2%); among all patients, there were concerns for overt Cushing syndrome in 19 (9.7%).

Adequate dexamethasone concentrations were demonstrated in the majority of patients, suggestive of an optimal compliance and absorption/metabolism of dexamethasone, with similar rates to healthy volunteers (96.3% and 97.6%, respectively; $P = 0.56$). Patients with inadequate dexamethasone concentrations had higher post-DST total and free cortisol when compared with patients with adequate dexamethasone concentrations (supplemental Table 2) (18), without any patient characteristics differentiating the groups.

Among patients in whom DST was ordered as part of clinical care, 100 (51%) had hypercortisolism excluded (defined as post-DST total cortisol <1.8 mcg/dL), 65 (33%) had a post-DST total cortisol between 1.8 and 5 mcg/dL, and 31 (16%) had post-DST total cortisol >5 mcg/dL. Overall, post-DST total cortisol correlated with post-DST free cortisol (Fig. 1B); 100% of post-DST free cortisol was above upper limit of normal in patients with post-DST cortisol >5 mcg/dL, but in only 70.7% of those with post-DST cortisol between 1.8 and 5 mcg/dL (Table 3).

After exclusion of 7 (3.6%) patients with inadequate dexamethasone concentrations, a total of 24 (12.7%) patients demonstrated a discordance between the post-DST total and free cortisol (Fig. 2). Nineteen patients (21%) had abnormal post-DST total cortisol but had a post-DST free cortisol in the reference range (false-positive results), Table 4. The only patient characteristic associated with this discordance was OCP use (21.1% in the false-positive group vs 4.7%, $P = 0.02$); no patients with discordant results had clinical features consistent with overt Cushing syndrome. None of the patients with false-positive results had a post-DST total cortisol >5 mcg/dL, Table 4. When compared with the post-DST free cortisol concentrations, post-DST total cortisol had diagnostic accuracy of 87.3% (95% CI, 81.7-91.7), Fig. 2.

Discussion

We aimed to develop reference ranges for post-DST free cortisol concentrations in subjects without adrenal disorders and to determine the utility of post-DST free cortisol assessment in patients evaluated for cortisol excess. We found that the addition of post-DST free cortisol was particularly

Table 3. Characteristics of participants and the results of dexamethasone suppression test

	Volunteers (n = 168)	Patients (n = 196)	Patients based on post-DST total cortisol concentrations			
			Post-DST TC ≤ 1.8 mcg/dL (n = 100)	Post-DST TC > 1.8 mcg/dL (n = 96)	Post-DST TC 1.8-5 mcg/dL (n = 65)	Post-DST TC > 5 mcg/dL (n = 31)
Age, years, median (IQR)	29.5 (26, 40)	57 (40, 66)	56 (38.5, 63.8)	58 (42, 68)	61 (52, 71)	44 (30, 60)
Women, n (%)	113 (67.3)	132 (67.3)	60 (60)	72 (74.2)	46 (69.7	26 (83.9)
BMI, kg/m^2, median (IQR)	25.4 (22.8, 29.2)	31.0 (27.1, 37.5)	31.5 (28.1, 37.4)	30.9 (25.5, 37.5)	30.4 (25.1, 37.9)	30.9 (26.5, 36.6)
Oral estradiol use,[a] n (%)	47 (28.0)	13 (6.6)	5 (5.1)	8 (8.2)	4 (6.1)	4 (12.9)
Estradiol dose, mcg, median (IQR)	30 (20, 35)	30 (20, 35)	30 (20, 32.5)	25 (20, 35)	32.5 (22.5, 35)	20 (20, 31.3)
Dexamethasone concentration,[b,c] mcg/dL, median (IQR)	0.34 (0.25, 0.44)	0.46 (0.32, 0.62)	0.45 (0.33, 0.58)	0.47 (0.32, 0.73)	0.49 (0.33, 0.84)	0.45 (0.21, 0.63)
Dexamethasone concentration[b,c] ≥0.1 mcg/dL, n (%)	162 (97.6)	182 (96.3)	100 (100)	83 (92.2)	62 (95.4)	23 (87.1)
Total serum cortisol,[b,c] mcg/dL, median (IQR)	0.8 (0.6, 1.3)	1.8 (1.2, 3.4)	1.2 (0.8, 1.5)	3.4 (2.3, 8.5)	2.7 (2.1, 3.5)	11 (8.7, 15)
Free serum cortisol,[b,d] ng/dL, median (IQR)	24 (19.3, 32.0)	44 (20, 90)	20 (20, 38.4)	92 (55.2, 240.5)	60 (46, 92.6)	369 (259, 449)
Free serum cortisol above reference range,[b,d] n (%)	4 (4.2)	82 (41.8)	5 (5)	77 (79.4)	46 (70.7)	31 (100)
Discordant free to total cortisol, n (%)	11 (6.7)	24 (12.2)	5 (5)	19 (19.6)	19 (29.2)	0 (0)
Clinical evidence of overt Cushing syndrome, n (%)	0 (0)	19 (9.7)	0 (0)	19 (19.6)	5 (7.7)	14 (45.2)

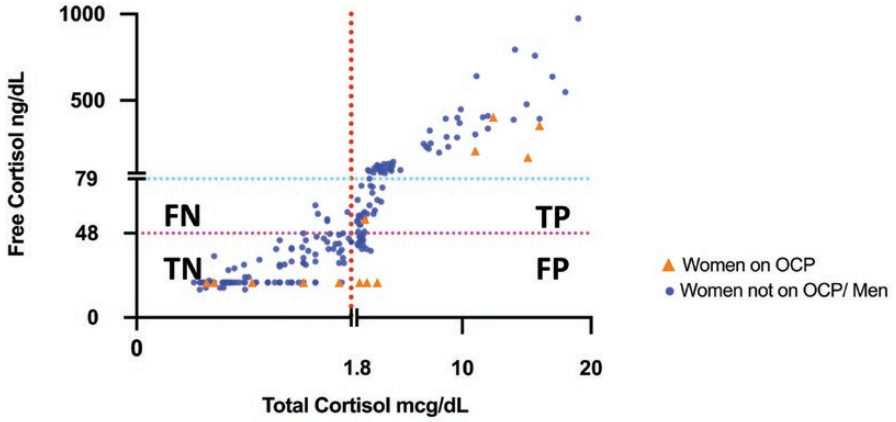
Abbreviations: BMI, body mass index; DST, dexamethasone suppression test; IQR, interquartile range; OCP, oral contraceptive pill; TC, total cortisol.
[a]OCP use group included all women who received estrogen-containing oral contraceptive. Women who were on progestin only contraceptive therapy (n = 2) were considered in the non-OCP group.
[b]Dexamethasone concentration, total serum cortisol, and free serum cortisol were collected after 1 mg oral dexamethasone suppression.
[c]Data unavailable for dexamethasone concentration in 2 volunteers and total serum cortisol in 3 volunteers.
[d]Reference range free cortisol for men and women not on OCP was ≤ 48 ng/dL and ≤ 79 ng/dL for women on OCP.

useful when total cortisol was between 1.8 and 5 mcg/dL, which allowed reclassification of 30% of patients with abnormal post-DST total cortisol as false positive. Post-DST free cortisol also allowed for enhanced interpretation of dexamethasone suppression testing in women taking OCP. We found that when compared with free cortisol, the diagnostic accuracy of post-DST total cortisol was 87%.

We observed adequate dexamethasone concentrations in 97.6% of volunteers and 96.3% of patients, suggesting that compliance was high and abnormal metabolism/absorption of dexamethasone was infrequent. Dexamethasone assays vary, with cutoffs suggestive of optimal concentrations ranging between 0.10 and 0.18 mcg/dL in other studies (8-10, 19, 20). Interestingly, other studies attributed 6% to 20% of false-positive post-DST total cortisol results to inadequate dexamethasone concentrations (Table 5), a much higher rate than what we observed. These differences may be due to retrospective nature of most other studies, differences in provided patient instruction on administration of dexamethasone, lack of optimal referent standard/control, and a smaller sample size with possible selection bias. Our findings suggest that routine measurement of dexamethasone concentrations may not be needed and should be reserved only when noncompliance or abnormal dexamethasone absorption or metabolism is suspected.

We have observed that post-DST total cortisol was normal in 75% of women treated with OCPs. Although the Endocrine Society guidelines recommend cessation of oral estrogens for 6 weeks before DST (2), our findings indicate that it is reasonable to consider completing DST in patients taking OCP, and considering an alternative option only in those with abnormal results. This approach would likely be less burdensome for patients and may reduce the risk of unwanted pregnancy or other symptoms for which OCPs were prescribed.

We found that both post-DST total and free cortisol concentrations are higher in women taking OCPs, though this difference is smaller for the free cortisol. This finding can be explained by the observation that approximately 80% and 90% of total cortisol transported by cortisol binding



Number	True positive	False positive	False negative	True negative	Total
	70	19	5	95	189
Diagnostic accuracy parameters % (95%CI)	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
	92.2 (85.1-97.8)	83.3 (75.2-89.7)	78.7 (70.9-84.8)	95.0 (89.0-97.8)	87.3 (81.7-91.7)

Figure 2. Diagnostic accuracy of post-DST free cortisol concentrations. True-positive result was defined as a case with increased post-DST free cortisol and total cortisol. True-negative result was defined as absence of normal post-DST free cortisol and total cortisol. False-positive result was defined as abnormal post-DST total cortisol, but normal post-DST free cortisol. False-negative result was defined as normal post-DST total cortisol, but abnormal post-DST free cortisol. Abbreviations: DST: dexamethasone suppression test; OCP: oral contraceptive pill.

Table 4. Characteristics of patients with adequate dexamethasone concentrations and false positive post-DST total cortisol

	All patients[a] (n = 189)	False positive results[b] (n = 19)	Concordant results[c] (n = 165)	*P* value
Age, y, median (IQR)	57 (40, 66)	57 (35, 64)	56 (39.5, 66.5)	0.99
Women, number (%)	126 (66.7)	13 (68.4)	111 (67.3)	0.92
BMI, kg/m^2, median (IQR)	31.0 (27.3, 37.5)	31.4 (25.5, 37.7)	31.0 (27.4, 37.5)	0.66
Oral estradiol use,[d] number (%)	12 (6.3)	4 (21.1)	8 (4.9)	0.02
Dexamethasone concentration,[e] mcg/dL, median (IQR)	0.47 (0.34, 0.63)	0.49 (0.41, 0.59)	0.46 (0.33, 0.63)	0.32
Total serum cortisol,[e] mcg/dL, median (IQR)	1.7 (1.2, 3.4)	2.2 (2.0, 2.4)	1.6 (1.1, 3.5)	0.27
Total serum cortisol,[c,d] number (%) 1.8-5 mcg/dL	62 (32.8)	19 (100)	43 (26.1)	<0.0001
> 5 mcg/dL	27 (14.3)	0 (0)	27 (16.4)	<0.0001
Free serum cortisol,[e] ng/dL, median (IQR)	43 (20, 79.5)	42 (39, 45.8)	43 (20, 92.3)	0.09
Clinical evidence of overt Cushing syndrome, number (%)	19 (10.0)	0 (0)	19 (11.2)	0.0001

Abbreviations: BMI, body mass index; DST, dexamethasone suppression test; EE, ethinyl estradiol; IQR, interquartile range; OCP, oral contraceptive pill.
[a]Included only patients with dexamethasone concentration ≥ 0.1 mcg/dL (n = 7 excluded).
[b]False-positive results were defined as those with abnormal post-DST total cortisol suggesting hypercortisolism, but normal post-DST free cortisol.
[c]Concordant results were defined as those where presence or absence of hypercortisolism with DST was concordant whether interpreted with total cortisol or with free cortisol.
[d]OCP use group included all women who received estrogen-containing oral contraceptive. Women who were on progestin only contraceptive therapy (n = 2) were considered in the non-OCP group.
[e]Dexamethasone concentration, total serum cortisol, and free serum cortisol were collected after 1 mg oral dexamethasone suppression.

Table 5. Summary of studies assessing adequate dexamethasone concentration for dexamethasone suppression test

Clinical study	Study type	Participants	Dexamethasone cutoff used, mcg/dL	Interpretation
Ueland et al (2017) (8)	Prospective	101 healthy volunteers (2% OCP use) 201 patients, consecutively enrolled (7% OCP use)	0.13	20% false-positive rate attributed to inadequate dexamethasone concentration
DeGraaf et al (2019) (19)	Retrospective	1901 samples consecutively examined, unknown clinical scenario	0.16	10% absolute reduction in "positive" DSTs when lower limit of dexamethasone concentration was raised from 2.5th percentile to 5th percentile
Ceccato et al (2020) (9)	Retrospective Prospective	125 patients 75 patients, consecutively enrolled	0.18	6% had inadequate dexamethasone concentration Up to 40% of patients with post-DST cortisol > 5 mcg/dL had inadequate dexamethasone concentrations
Roper et al (2021) (10)	Retrospective	63 patients with 70 samples	0.14, 0.18	14% false-positive rate attributed to inadequate dexamethasone concentration
Vogg et al (2021) (20)	Retrospective	400 patients, 100 overt CS, 200 excluded CS, 100 adrenal incidentalomas and possible glucocorticoid autonomy (4.5% OCP use)	0.10	7% had inadequate dexamethasone concentration < 2.5th, and only 4% had undetectable levels (suggesting noncompliance)
Current study (Genere et al)	Prospective	168 healthy volunteers (28% OCP use) 196 patients, consecutively enrolled (7% OCP use)	0.10	3% false-positive rate attributed to inadequate dexamethasone concentration

Abbreviations: CS, Cushing syndrome; DST, dexamethasone suppression test; OCP, oral contraceptive pill.

globulin, whereas a minority is free cortisol (6%) (21, 22). The impact of oral estrogen on total cortisol concentrations is mediated through stimulation of the cortisol binding globulin; however, free cortisol is still affected proportionally, and to a smaller degree. These minimal increases in circulating free cortisol are likely not clinically significant given the absence of symptomatic hypercortisolism in women taking OCP.

When the post-DST free cortisol cutoffs derived from the volunteers without adrenal disorders were used in the interpretation of DST results in the clinical setting, the sensitivity of post-DST total cortisol was found to be 92%, specificity was 83%, positive predictive value was 79%, and negative predictive value was 95%, with an overall diagnostic accuracy of 87%. Notably, all false-positive results (abnormal post-DST total cortisol, normal post-DST free cortisol) occurred in patients with post-DST total cortisol between 1.8 and 5 mcg/dL, and results were concordant when cortisol was >5 mcg/dL. Therefore, free cortisol testing is likely unnecessary with post-DST total cortisol >5 mcg/dL. After excluding patients with inadequate dexamethasone concentrations, the only factor that we found to be associated with false-positive results was OCP use. We have not found any association between age, sex, or BMI and false-positive results. No other studies reported on the combined interpretation of the serum post-DST free and total cortisol concentrations.

The strengths of our study included a cross-sectional design with a prospective consecutive enrollment, standardized instructions and conduct of DST, a relatively large sample size, and inclusion of women treated with OCP (something that has not been previously addressed). The limitations of this study include a single institution design at a referral center that led to inclusion of a higher proportion of patients with hypercortisolism (than what would be observed in primary care setting). Volunteers were not assessed with a full battery of testing for hypercortisolism, and it is possible that a small minority of volunteers may have had an adrenal disorder that was missed by the interview or clinical examination. Our volunteer cohort was also younger than patient cohort, and future investigations may be helpful to understand the applicability of the reference range to an older population. Future studies should include a prospective multicenter design, inclusion of a higher number of volunteers, and cross-validation of assays used in different laboratories. In addition, although we established the free cortisol cutoffs based on a cohort of volunteers without adrenal disorders, the accuracy of these cutoffs, as well as clinical significance of any discordance between free cortisol and total cortisol, should be further investigated.

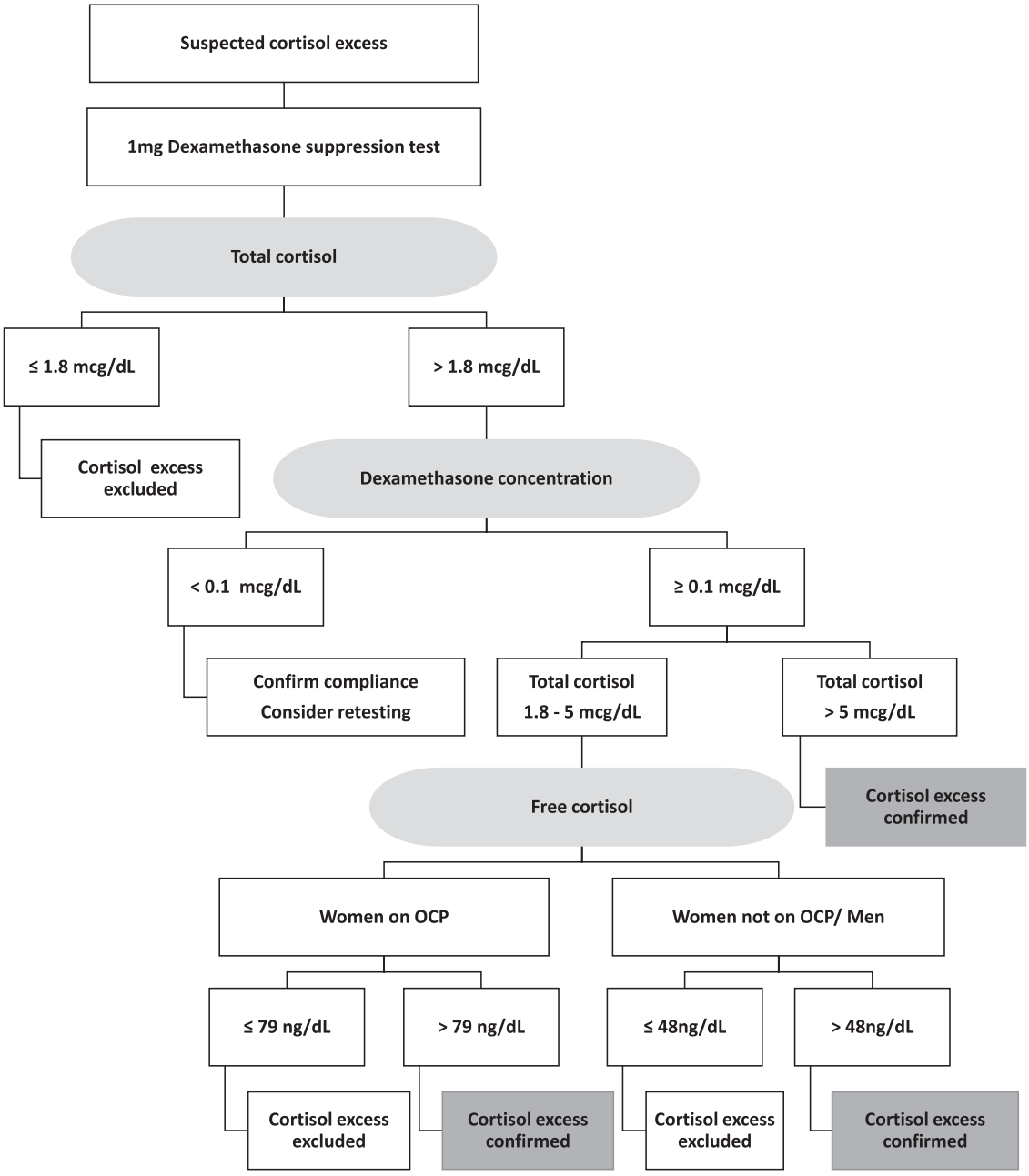


Figure 3. Suggested algorithm for the 1 mg overnight dexamethasone suppression test.

Clinical Implications

Based on the results of this study, we suggest a sequential approach to DST in clinical practice (Fig. 3). After providing optimal instructions, we propose that DST is performed in patients suspected to have cortisol excess regardless of special circumstances, such as OCP use. If post-DST total cortisol is >1.8 mcg/dL, we suggest measurements of dexamethasone concentrations on the stored serum. In patients with abnormal DST and adequate dexamethasone concentrations, we suggest proceeding with the free cortisol measurements only in the group of patients with post-DST total cortisol between 1.8 and 5 mcg/dL (because no false-positive results were found when post-DST total cortisol was >5 mcg/dL); Fig. 3. In conclusion, we found that post-DST free cortisol evaluation is particularly useful in patients with adequate dexamethasone concentrations and mildly abnormal post-DST total cortisol between 1.8 and 5 mcg/dL.

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