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Overnight 1 mg dexamethasone suppression test and 24 h urine free cortisol—accuracy and pitfalls when screening for Cushing's syndrome

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Abstract
Diagnosis of Cushing's syndrome (CS) is often delayed due to variable clinical features and its rarity. Simple and accurate screening tests are required to enhance screening for hypercortisolism. Both overnight 1 mg dexamethasone suppression test (DST) and urinary free cortisol (UFC) demonstrate high sensitivity and specificity for the diagnosis of CS. However, each test has its own distinctive features, making it preferable in specific clinical conditions. This review will discuss the pitfalls for each of those tests.

Keywords Cortisol · Adrenal · Pituitary · Cushing's disease · UFC · DST

Introduction

To improve early diagnosis, therapy, and follow-up in patients with Cushing's syndrome (CS), tests with high sensitivity and specificity are of upper importance. A recent consensus statement [1] lists various options for the assessment of autonomous cortisol secretion, including late-night salivary cortisol, dexamethasone suppression testing, and urine free cortisol, the latter two being reviewed here in more detail.

Dexamethasone suppression test (DST)

DST assess the physiological suppression of endogenous cortisol by exogenous synthetic glucocorticoids (Fig. 1). Most studies report on the short-term test applying 1 mg dexamethasone between 11 pm and midnight, with subsequent blood collection for cortisol measurement next day between 8 to 9 am [2]. In a recent meta-analysis by Galm et al. DST demonstrated high sensitivity of 98.6%, with reasonable specificity of 90.6% [3]. In a re-analysis, further data on the cortisol assays used in individual studies was evaluated [4]. About 2/3 of studies used a pre-specified cutoff of 50 nmol/l to enhance sensitivity, as suggested by the Endocrine Society Practice Guideline [2]. Sensitivities were indeed high in a narrow range (88–100%, median 100%) irrespective of the assay system, contrasting with a wider range of somehow lower specificities (52–100%, median 91%). Offering excellent sensitivity with a well-established cutoff and little dependency on the assay system, as well as high reproducibility [5], DST appears to be especially suited as a screening parameter. Of note, post-DST cortisol was not associated with serum creatinine, but inversely correlated with BMI [5]. However, several studies have confirmed the use of 1 mg DST as an adequate screening test for hypercortisolism in patients with obesity [6, 7]. Post-DST cortisol was positively correlated with age [5, 8], but the clinical significance is currently unknown.

Pitfall 1—changes in transport proteins of serum cortisol

Increases or decreases of CBG and/or albumin result in parallel changes in total serum cortisol measured by current assay systems, without affecting the concentration of free cortisol (representing the active hormone):

– CBG and/or albumin ↑—> serum cortisol ↑: pregnancy, chronic active hepatitis, drugs (oral estrogens, SERMs, mitotane)

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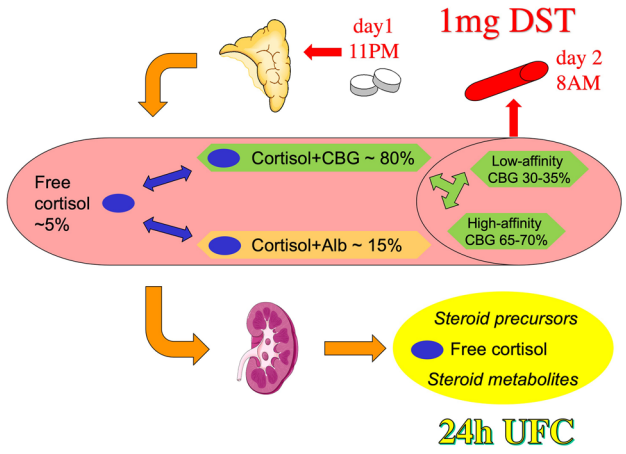

Fig. 1 Physiology and principles of testing for autonomous cortisol secretion. Approximately 5% of cortisol in serum is unbound, 10–15% is bound with low affinity to albumin, while the remaining is bound to CBG. The latter may be both a reservoir for cortisol and a modulator of cortisol release, by changes in cortisol binding affinity between a high- and a low-affinity conformation. Only unbound free cortisol is transferred into urine, mixed with other steroids and metabolites

– CBG and/or albumin ↓—> serum cortisol ↓: liver cirrhosis, hyperthyroidism, nephrotic syndrome, alcohol abuse, critical illness

Those alterations are clearly of clinical relevance, e.g. about 67% higher serum cortisol levels in subjects on oral estrogen compared to controls [9], resulting in 8/13 healthy female volunteers with inadequate suppression of cortisol by 1 mg dexamethasone while using oral contraceptives [10]. Alternatives are the use of tests investigating free cortisol like UFC or LNSC, or the withdrawal of oral estrogens. Withdrawal of contraceptives for 1 week reduced the number of false-positive results to 1/13, and 6 weeks after discontinuation, all tests were normal [10]. In the future, accuracy of the DST in women on oral contraceptives may be improved by measurement of free serum cortisol [11].

Pitfall 2—concomitant medications may severely affect the accuracy of DST

Certain drugs may alter CYP3A4 activity and subsequently hepatic enzymatic clearance of dexamethasone:

– Induction of CYP3A4—> clearance of dexamethasone ↑—> false positive DST: anticonvulsants (phenytoin, phenobarbitone, and carbamazepine), pioglitazone, rifampin, primidone, alcohol
– Inhibition of CYP3A4—> clearance of dexamethasone ↓—> false negative DST: antidepressants (fluoxetine), diltiazem, cimetidine, itraconazole

Drugs taken by the patient can be checked for their effects on CYP3A4 by consulting a specific database, e.g. drug-interactions.medicine.iu.edu [12]. In a recent study, 8% of patients investigated for hypercortisolism demonstrated a significant association of CYP3A4 inducers with falsely elevated cortisol after DST [6].

Therefore, several studies investigated the use of parallel dexamethasone measurement, identifying 1.3 ng/ml (3.3 nmol/l) or 1.8 ng/ml (4.5 nmol/l) as minimal levels required for sufficient cortisol suppression [5, 13–15]. Interestingly, 2–4% of subjects were found with non-detectable dexamethasone levels suggesting non-compliance to ingest the test medication. Furthermore, 3–5% of subjects demonstrated detectable dexamethasone levels below the cut-off in combination with increased cortisol levels, resulting in false-positive classification. Dexamethasone levels were not influenced by age, sex, BMI, or nicotin consumption. However, dexamethasone clearance may be reduced in patients with liver or renal failure [2, 5, 15], and levels increased in patients with diabetes mellitus [15], at least theoretically resulting in false negative test results e.g. in patients with mild autonomy.

On the other hand, lower gastrointestinal absorption [16] or increased distribution due to low albumin binding [17] may result in low serum dexamethasone levels, resulting in false positive test results. Finally, increased or decreased CYP3A4 enzyme activity due to polymorphism may affect dexamethasone levels and thereby DST results [18]. The precise relevance of measuring dexamethasone remains to be established [11], with the use of this interesting method hindered by the limited availability of the method.

24 h urinary free cortisol (UFC)

UFC determines an integral of cortisol production over 24 h and may therefore be less prone to errors affecting single samples. Measurement is limited to free cortisol and therefore independent of changes in CBG (Fig. 1). In a recent meta-analysis on laboratory tests for the diagnosis of CS, UFC demonstrated sensitivity and specificity of 94.0 and 93.0%, respectively [3]. In a sub-analysis on assay characteristics, cutoff values varied considerably between 119 and 995 nmol/d over all studies [4], with a wide range of sensitivities (67–100%, median 93%) and specificities (33–100%, median 87%). Variability was much lower for single assays, emphasizing the need for assay-dependent reference ranges for correct interpretation of UFC samples.

Pitfall 1—interference with exogenous glucocorticoids

Of note, patients should be instructed to avoid any glucocorticoid preparations during collection, to prevent

any interference during measurement. As patients are not always aware of ingredients, they should be specifically questioned about the use of skin cremes, inhalations or intramuscular injections.

Pitfall 2—variability of UFC

Inherent variability of cortisol secretion as well as incorrect collection of UFC may impede correct interpretation of results. Complete collection should be confirmed by assessment of appropriate total urine volume and urinary creatinine levels [2]. Despite careful instruction of patients and written guidance, a relevant number of samples may still demonstrate reduced creatinine excretion possibly indicating insufficient urine collection [19]. Normalization of UFC to creatinine is not suitable to correct for collection errors, due to circadian changes in cortisol secretion in contrast to relatively stable creatinine excretion. Independent collection of at least two 24 h periods may be an alternative, as suggested by the Endocrine Society Practice Guidelines [2]. However, intra-individual day-to-day variations of UFC are high, with mean coefficients of variations up to 60% largely independent from sample numbers [19, 20]. It is currently unclear whether the mean or individual levels should be used for the assessment of CS.

Pitfall 3—lack of subgroup-specific reference ranges

UFC levels are 1.4–1.5 × higher in males compared to females, as demonstrated in a large number of studies (summarized in [4]). Dissociation in cortisol secretion rates appears after the age of 11–12 years and is therefore probably related to puberty [21]. Beginning from that age, reference ranges for UFC should therefore be reported separately for both sexes, possibly increasing the accuracy of UFC for the diagnosis of CS. In contrast, the effects of BMI on UFC are controversial. Whereas one study described an inverse relation between BMI and UFC [22], another study demonstrated a U-shaped relationship between BMI and UFC, with highest levels in patients with anorexia nervosa, a nadir in the overweight-mild obese group, and again increasing levels with severe obesity [23]. Others have not found any consistent dependency of UFC on BMI [19, 24]. Age probably has an even lesser effect on UFC. Whereas one large epidemiological study did not find any significant effects [24], the Baltimore Longitudinal Study of Aging demonstrated a U-shaped pattern across the life span [25]. Therefore, the need for age- and/or BMI-dependent references ranges remains an open question.

Pitfall 4—alterations in UFC by other factors

Experimental water loading increased UFC with significant correlation to urine volume, when performed during the high secretory activity of the adrenal gland [26]. In the same line, urine volume was a significant predictor of UFC in a large epidemiological study comparing two different sampling periods [24]. Polydipsia/polyuria may therefore result in false-positive UFC results. Patients should be instructed to avoid uncommon high fluid intake during morning. Increased UFC levels in adult patients with urine output exceeding 3 l/d should be interpreted with caution. In contrast, salt restriction resulted in significantly lower UFC levels [27], with the potential of false-negative results when screening for CS, and should therefore be avoided during UFC collection.

Renal impairment is another condition with diminished UFC levels. Creatinine clearance (CrCl) and UFC levels are clearly associated, with significant reductions of UFC in patients with moderate renal impairment (CrCl: 20–60 ml/min), and even more with severe renal impairment (CrCl: < 20 ml/min) [28]. Therefore, the use of other screening parameters is suggested for patients with reduced kidney function (CrCl < 60 ml/min).

Pregnancy may resemble some of the clinical features of CS but represents a major challenge for any diagnostic test [29]. During the first, second, and third trimester, UFC values by LC–MS/MS were 1.7×, 2.4×, and 3.1 × higher in comparison to a control group, respectively, without any differences in urine volume or creatinine levels [30]. When measured by an immunoasssays, pregnancy UFC levels were increased by an additional 30–35%, potentially indicating interference with cortisol metabolites. Therefore, UFC is of limited value to evaluate CS during pregnancy without gestation-specific reference values.

Personal preference

For initial evaluation I usually rely on late-night salivary cortisol (LNSC) which is covered in a separate review in this special issue of PITUITARY. With the availability of well-established cut-offs for the assay used at our center, LNSC represents a highly sensitive and specific test, without requiring the patient to attend the clinic at specific time points [4]. Saliva sampling is routinely repeated on a second day to account for variations by stress and inherent day-to-day variability in cortisol secretion. Collecting early morning samples in addition to late night samples offers some kind of control: sufficiently high early-morning samples indicate correct collection procedure. Using a commercial collection vial improved acceptance by the patients and laboratory technicians. Handling of the collection vials is explained

in detail: patients receive written instructions with pictures explaining the sampling procedure, to avoid food intake and teeth brushing at least 15 min prior to sampling, and to collect late-night samples following a stress-free evening. As salivary cortisol is stable at room temperature for at least a week, samples can be returned by mail.

In patients with high clinical suspicion of hypercortisolism or positive LNSC, we rely on DST as second test at our center. Post-DST cortisol below 50 nmol/l allows reliable exclusion of hypercortisolism, when taking into account potential pitfalls described above. In our experience, post-DST cortisol > 94 nmol/l is highly specific for hypercortisolism and should clearly trigger further investigation [31]. With post-DST cortisol between 50 and 94 nmol/l, we may offer further work-up or repeat testing after 3–6 months, depending on clinical suspicion. As specificity of 1 mg DST appears to be preserved even in patients with severe obesity, we do not increase the dose of dexamethasone depending on BMI [7]. False-positive results do occur in our practice in women on oral contraceptives. Collecting post-DTS salivary cortisol instead or in addition to serum cortisol is one potential solution we offer to our patients. However, cutoffs need to be established for each assay system [4, 32]. Alternatives are the use of tests investigating free cortisol like UFC or LNSC, or the withdrawal of oral estrogens for at least 1 week [10]. However, the latter is frequently disliked by our patients.

For the author of this review, UFC remains the least preferable initial choice, due to the potential for collection errors, frequent rejection by patients, and the large number of cofounding variables. In our practice, therefore, UFC is usually reserved for follow-up of patients on medical treatment, as most registration trials still relied on UFC, or for patients with discordant results on prior testing procedures.

Conclusion

DST and UFC have been extensively studied as screening tests for the diagnosis of CS. The preference for initial evaluation will likely depend on local experience and availability of accurate assays. Peculiarities of each test make it preferable in specific clinical conditions. To choose the most appropriate test in individual patients, an expert endocrinologist should be consulted.

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Declarations

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