

Screening and Diagnosis of Cushing's Syndrome

ABSTRACT

Cushing's syndrome (CS) results from sustained pathologic hypercortisolism. The clinical features are variable and the most specific features for CS include abnormal fat distribution, particularly in the supraclavicular and temporal fossae, proximal muscle weakness, wide purple striae, and decreased linear growth with continued weight gain in a child. Clinical presentation of CS can be florid and in this case the diagnosis is usually straightforward. However, the diagnosis can be difficult particularly in states of mild or cyclical or periodical hypercortisolism. Several tests based on the understanding of the physiologic characteristics of the hypothalamic-pituitary-adrenal axis have been used extensively to confirm the diagnosis of Cushing's syndrome, but none has proven fully capable of distinguishing all cases of CS from normal and/or pseudo-Cushing individuals. Three first-line diagnostic tests are currently used to screen for CS: measurement of free cortisol in 24-hour urine (UFC), cortisol suppressibility by low doses of dexamethasone (DST), and assessment of cortisol circadian rhythm using late-night serum and/or salivary cortisol. This paper discusses the effectiveness regarding best cut-off values, the sensitivity and the specificity of these tests to screen for CS. Late-night salivary cortisol appears to be the most useful screening test. UFC and DST should be performed to provide further confirmation of the diagnosis. (**Arq Bras Endocrinol Metab 2007;51/8:1191-1198**)

Keywords: Cushing's syndrome; Hypercortisolism; Salivary cortisol; Urinary free cortisol; Dexamethasone suppression; Circadian rhythm

RESUMO

Rastreamento e Diagnóstico da Síndrome de Cushing.

A síndrome de Cushing (SC) resulta de um hipercortisolismo patológico mantido. As manifestações clínicas são variáveis e os achados mais específicos para a SC incluem distribuição anormal de gordura, particularmente nas fossas supraclaviculares e temporais, fraqueza muscular proximal, estrias purpúreas largas e interrupção do crescimento linear com ganho contínuo de peso na criança. A apresentação clínica da SC pode ser florida e, neste caso, o diagnóstico é usualmente direto. Entretanto, o diagnóstico pode ser dificultado particularmente em estados de hipercortisolismo leve ou cíclico/periódico. Vários testes baseados na compreensão das características fisiológicas do eixo hipotálamo-hipófise-adrenal têm sido usados extensivamente para confirmar o diagnóstico da SC, mas nenhum deles mostrou-se totalmente capaz de distinguir todos os casos de SC dos indivíduos normais e/ou portadores de pseudo-Cushing. Três testes diagnósticos de primeira linha são atualmente empregados para rastrear SC: a medida do cortisol livre em urina de 24-horas (CLU), a supressão do cortisol por doses baixas de dexametasona (TSD) e a avaliação do ritmo circadiano do cortisol usando a dosagem do cortisol sérico ou salivar às 23-24 hs. Este artigo discute a efetividade com relação aos melhores valores de corte e a sensibilidade e especificidade destes testes no rastreamento da SC. O cortisol salivar às 23-24 hs parece ser o teste mais útil de rastreamento. O CLU e o TSD devem ser realizados na tentativa de fornecer confirmação adicional ao diagnóstico. (**Arq Bras Endocrinol Metab 2007;51/8:1191-1198**)

Descritores: Síndrome de Cushing; Hipercortisolismo; Cortisol salivar; Cortisol livre urinário; Supressão com dexametasona; Ritmo circadiano

revisão

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CUSHING'S SYNDROME (CS) results from lengthy and inappropriate exposure to excessive concentrations of circulating free glucocorticoids. CS may be caused by excess ACTH production (80–85%), usually by a pituitary corticotroph adenoma — Cushing's disease (CD), less frequently by an extrapituitary tumor (ectopic ACTH syndrome), or very rarely by a tumor secreting CRH (ectopic CRH syndrome). CS can also be ACTH-independent (15–20%) when it results from excess secretion of cortisol by unilateral adrenocortical tumors, either benign or malignant, or by bilateral adrenal hyperplasia or dysplasia (1,2). However, it is important to point out that the most common cause of Cushing's syndrome is use of supra-physiological amounts of exogenous glucocorticoids, including topical or inhaled corticosteroids (iatrogenic Cushing's syndrome). Therefore, the diagnosis of endogenous CS should begin with a careful case history and a thorough physical examination, looking for the characteristic features while excluding the use of oral, parenteral, inhaled, or topical corticosteroids.

The suspicion of CS in a patient arises in the presence of central obesity with supraclavicular fat accumulation, a cervical fat pad, thinned skin, purple striae, proximal muscle weakness, fatigue, high blood pressure, glucose intolerance, acne, hirsutism, and menstrual irregularity. Neuropsychological disturbances including depression, emotional irritability, sleep disturbances, and cognitive deficits are also frequently observed. Muscular atrophy and purple striae are particularly helpful stigmata in adults, whereas in children growth retardation is frequently present (1,2).

Clinical presentation of CS can be florid and in this case the diagnosis is usually straightforward. However, the diagnosis can be difficult particularly in states of mild or cyclical or periodical hypercortisolism (3-5). CS diagnosis suspicion should also arise with a less complete picture, particularly if concomitant recent weight gain, impaired glucose tolerance, and high blood pressure are present (6). The epidemic of obesity and metabolic syndrome increased the number of patients with the Cushing's phenotype, which might also require screening for CS, especially if young and resistant to conventional treatment. In addition, two studies found that 2% to 3% of patients with poorly controlled type 2 diabetes mellitus may have unrecognized Cushing's syndrome (7,8). Many of adrenal incidentalomas demonstrate subtle autonomous secretion of cortisol (9,10). Subclinical Cushing's syndrome caused by adrenal incidentalomas is frequently associated with overweight and insulin resistance. A recent study to evaluate the prevalence of occult CS in

overweight, type-2 diabetic patients demonstrated a relatively high number of patients with occult CS (11). However, the authors suggested that further studies are needed to evaluate the impact of the cure of occult CS on obesity and diabetes mellitus in these patients. In addition to these conditions, screening might also be warranted in certain groups of patients without classic clinical features, such as hypertensive patients and men with unexplained osteoporosis (12). Therefore, the diagnosis of CS is often a challenge for clinicians due to the variable pattern and the nonspecificity of clinical manifestations.

Primary care physicians may suspect CS and proceed to the initial biochemical screening tests. However, due to the potential complexity of investigation, further evaluation and treatment of this syndrome should be conducted in specialized endocrinology referral centers. The choice of optimal laboratory screening procedures for patients in whom the suspicion of CS has arisen is not firmly established. In addition, in cases where the diagnosis of CS is suspected clinically but initial screening tests are normal, the patient should be reevaluated at a later date, and invasive procedures should be postponed.

BIOCHEMICAL DIAGNOSIS OF HYPERCORTISOLEMIA

Several tests based on the understanding of the physiologic characteristics of the hypothalamic–pituitary–adrenal axis have been used extensively to confirm the diagnosis of the Cushing syndrome, but none has proven fully capable of distinguishing all cases of CS from normal and/or pseudo-Cushing individuals. Among these, four diagnostic tests are currently used to screen for CS: measurement of free cortisol in 24-hour urine (UFC), cortisol suppressibility by low doses of dexamethasone, and assessment of cortisol circadian rhythm using late-night serum and salivary cortisol levels (6) and the dexamethasone-CRH test. Table 1 shows the diagnostic criteria and sensitivities and specificities for these tests.

Measurement of free cortisol in 24-hour urine

UFC is an integrated index of blood circulating free cortisol during 24-hour period. In contrast to plasma cortisol levels, which measure total cortisol, it is not affected by corticosteroid-binding globulin (CBG) levels (13). Urinary creatinine may also be measured with UFC; if glomerular filtration rate is less than 30

Table 1. Screening tests for Cushing's syndrome: diagnostic criteria, sensitivities and specificities for each test.

Screening test	Criteria	Sensitivity	Specificity
UFC (3 samples)	3-fold basal values	90–98%	45–95%
Plasma cortisol levels at 2300 h	< 1.8 µg/dL	93–100%	20–26%
Salivary cortisol levels at 2300 h	< 7.5 µg/dL	96–100%	88–100%
Plasma cortisol after 1 mg DST	< 250 ng/dL*	90–96%	96–100%
Plasma cortisol after 2 mg DST/2 days	< 1.8 µg/dL	91–97%	87–94%
Salivary cortisol after 1 mg DST	< 1.8 µg/dL	92–100%	92–100%
UFC after 2 mg DST/2 days	< 390 ng/dL	93–98%	94%
Plasma cortisol after low dose DST/CRH	< 10 µg/24h	97–100%	90–100%
DDAVP	< 1.4 µg/dL	100%	67–100%
	ACTH increase > 27 pg/mL or > 6 pmol/L	86.8%	90.7%

UFC (Urinary free cortisol): normal range: <80–120 µg/24 h or <220–330 nmol/ 24h (RIA); <50 µg/24 h or <138 nmol/24h (HPLC).

* Cut-off value (mean) based on several available studies (95%CI 153–346 ng/dL).

DST: dexamethasone suppression test, CRH: corticotrophin releasing hormone, DDAVP: Desmopressin.

To convert plasma cortisol from µg/dl to nmol/L, multiply by 27.6.

To convert salivary cortisol from ng/dl to nmol/L, multiply by 0.028.

mL/min, UFC can be normal despite the presence of excessive cortisol production. In children, the urinary cortisol excretion should be corrected for body surface area/1.72 m². Due to the possibility of intermittent hypercortisolism, up to three 24-h urine collections should be performed and if they are normal, CS is highly unlikely. On the other hand, values four-fold greater than the upper limit of normal strongly suggest Cushing's syndrome (12). Milder elevations of urinary cortisol can be found in conditions such as chronic anxiety, depression, and alcoholism, all of which are also known as pseudo-Cushing states (14), and in normal pregnant women.

The reference range for urine free cortisol levels depends on the type of assay used (15). Measurement of urinary cortisol by immunoassays (RIA or immuno-metric assays; normal range: < 80–120 µg/24h or < 220–330 nmol/24h) is influenced by various metabolites of cortisol and some synthetic glucocorticoids, whereas measurements using high-performance liquid chromatography (HPLC) allow the separation of various urinary glucocorticoids and metabolites (normal range: < 50 µg/24h or < 138 nmol/24h). Thus, HPLC or gas chromatography coupled with mass spectrometry provides the best specificity for measuring UFC (16,17) and is replacing older immunoassay methods because of its higher specificity. Differences in assay methodology may make interpretation of the data very challenging. Although HPLC has a high sensitivity and specificity, occasionally interfering sub-

stances, such as carbamazepine and digoxin, can also coelute with cortisol and produce false elevations of the UFC (18). The sensitivity of UFC for Cushing's syndrome may be only 45–71% at 100% specificity (19,20). Furthermore, pseudo-Cushing's states may have false-positive UFC testing (21,22). Therefore, although UFC may be useful to confirm Cushing's syndrome, its sensitivity and specificity are not optimal as an initial screening test and it cannot be considered as a universal single screening test for the detection of CS (6,23).

Low-dose dexamethasone suppression tests (DST)

Two dexamethasone-suppression tests (DST) are used for screen CS, the overnight and the 48-h DST. These tests depend on the concept that dexamethasone will suppress ACTH and cortisol release in normal subjects, while patients with corticotroph adenomas will not suppress below a specified cut-off. The overnight low-dose (1 mg) DST consists of the oral intake of 1 mg dexamethasone between 2300 and 2400 h, followed by measurement of fasting plasma cortisol between 0800 and 0900 h in the following morning. In the 48-h test, dexamethasone is given at the dose of 0.5 mg every 6 h for 2 days at 0900 h, 1500 h, 2100 h, and 0300 h with measurements of cortisol in serum at 0900 h at the start and end of the test. The original criterion for normal level of suppression was a plasma cortisol level below 5 µg/dL (138 nmol/liter) (24).

More recently, this cut-off level has been reduced to less than 1.8 µg/dL (50 nmol/liter) (2), greatly enhancing the sensitivity of the overnight DST, especially in patients with mild hypercortisolism. However, the cortisol assay must have a high sensitivity (1 µg/dL or 27.6 nmol/liter).

There are many interfering conditions in DST: decreased dexamethasone absorption, drugs enhancing hepatic dexamethasone metabolism, inducing the cytochrome P450-related enzymes (barbiturates, phenytoin, carbamazepine, rifampicin, meprobamate, aminoglutethimide, methaqualone), increased concentration of CBG (estrogen treatment, pregnancy) and pseudo-Cushing states (6). The 48-h test, although more cumbersome than the overnight test, is more specific. Because the 1 mg overnight DST is easy to perform and has low cost it is used as a first-line screening test in outpatient screening.

Some patients with Cushing's disease (3–8%) retain sensitivity to dexamethasone and show suppression of serum cortisol (25,26). Additionally, DST has a significant false positive rate, especially in chronically ill (23%) or obese patients (13%) and in patients with major depression (43%) or other psychiatric disorders (8–41%). A false-positive rate of up to 30% has been reported in other admitted patients and healthy individuals (27–29). Therefore, because of many interfering conditions and the significant variability of the biological behavior of corticotroph adenomas, neither the overnight nor 2-day DST test appears to be able to reliably rule out Cushing's syndrome using standard cut-offs for serum cortisol (12).

Late-night plasma cortisol

Physiological cortisol secretion is characterized by circadian rhythmicity. Serum cortisol concentration reaches its zenith in the morning (0600–0800 h) and its nadir in the night during the first half of normal sleep. Normal circadian rhythm of cortisol secretion is lost in patients with Cushing's syndrome and elevated late-night cortisol serum level has been reported to be the earliest and most sensitive marker of the disease (18). Thus, midnight serum cortisol has been demonstrated to be very effective in recognizing patients with mild signs and symptoms.

Newell-Price et al. (30) were the first authors to propose this screening test. They collected blood sample in 150 patients with CS and in 20 healthy subjects, in a sleeping state. The test achieved 100% sensitivity using a cut-off value of 1.8 µg/dL (50 nmol/L) but the specificity was not tested. Other authors also assessed patients, but in a nonsleeping state, and found higher

cut-off values (7.5 µg/dL or 207 nmol/L) with good sensitivity (90.2–96%) and high specificity (96.5–100%) (21,31). Therefore, a single sleeping midnight plasma cortisol concentration of less than 1.8 µg/dL effectively excludes CS. Concentrations of more than 1.8 µg/dL are noted in individuals with CS and in patients with acute illness. An awake midnight concentration of cortisol in plasma of more than 7.5 µg/dL obtained in a nonsleeping state had 97% sensitivity to distinguish between CS and pseudo-CS in the largest series up to now reported but can miss mild disease diagnosis in about 7% of cases (21). More recently, Reimondo et al., (32) obtained 100% sensitivity with a threshold value of 1.5 µg/dL, but the specificity was only 68.2% at that level. The best cut-off value found with ROC analysis was 4.0 µg/dL, which is remarkably greater than that proposed by the recent consensus statement (6). Recently, in a large series of Cushing's syndrome suspects, urinary free cortisol and cortisol suppression after 1 mg dexamethasone achieved satisfactory specificity. Conversely, serum cortisol concentrations at midnight should be interpreted with caution, especially with the 1.8 µg/dL cut-off (33). Besides controversial cut-off values, sleeping midnight blood sample collection for serum cortisol is not a stress-free sample and is not realistic under normal conditions of clinical practice, which make this test suboptimal for most clinicians (32).

Late-night salivary cortisol

Cortisol concentrations in saliva are independent of flow rate and transcortin fluctuations and reflect those in the free fraction of plasma (34,35). Salivary samples are obtained by noninvasive stress-free procedures, easier to collect, has stability at room temperature, and may be collected many times a day (36). The importance of salivary cortisol in the evaluation of the pituitary-adrenal function, specifically in CS, has been demonstrated more than two decades ago using a competitive assay (37,38). Because of the continuing need for improved noninvasive means of distinguishing pseudo-Cushing from CS, salivary cortisol is a highly suitable screening procedure for outpatient assessment. Indeed, it has now been convincingly demonstrated in more than 10 studies in adults (20,22,31, 32,39–42) and children (40,43,44) that an elevated late-night salivary cortisol sampling is an excellent substitute for increased midnight serum cortisol in the diagnosis of Cushing's syndrome. It could be particularly useful in investigating patients with cyclical CS with repeated evening measurements over time. Late-night salivary cortisol has also been shown to be useful to identify patients with pseudo-Cushing's syndrome

that is the presence of the cushingoid habitus associated with alcohol intake, type 2 diabetes, and major depression (22,31). However, there are several other conditions such as hypertension, advanced age, and psychiatric diagnoses that may lead to false-positive late-night salivary cortisol results (45).

Diagnostic ranges vary between reports because of the different assays and the comparison groups used to set cut-off points. Table 2 shows an overview of late-night salivary cortisol studies conducted in adult controls and CS patients. In the majority of previous papers, salivary cortisol was measured by radioimmunoassay (RIA). Recently the U.S. Food and Drug Administration approved a new enzyme-linked immunosorbent assay (EIA) developed by Diagnostic Systems Laboratories (DSL) for salivary cortisol (46). Using this assay, Raff et al. (47) compared in the same sample the measurement of salivary cortisol obtained by this method and also by RIA. Saliva samples, collected at 2300 h, were sent to their laboratory to screen patients for Cushing's syndrome. Other set of samples was obtained at 2300 h from healthy elderly and from apparently healthy individuals. The important finding was that EIA and RIA gave different results on the same sample. The EIA showed consistently higher salivary cortisol values than the RIA. The RIA results were much closer to the expected value of an independently created cortisol stock solution diluted in saliva. These data suggest that salivary cortisol measured by RIA reflects more accurately the free level of circulating cortisol.

Another immunoassay has been commercialized from ROCHE Elecsys immunoassay system (Salimetrics EIA) (48). The salivary cortisol measurement using this assay was also compared with the measure-

ment by the DPC RIA (46). The authors observed that Salimetrics EIA yielded results very close to those obtained with the DPC RIA, particularly in the critical diagnostic range of 0.3–10.0 nmol/L (11–357 ng/dL). These results are in contrast to those previously obtained by EIA from DSL method (47). The only relevant disadvantage of the Salimetrics EIA was that the highest calibrator concentration (49.7 nmol/L) is considerably lower and frequent dilution of high-concentration samples from patients with Cushing's syndrome are necessary. The advantages of the Salimetrics EIA compared with the DSL EIA are: it produces results not different from those obtained with the DPC RIA in the clinically important range, and it is FDA-cleared for in vitro diagnostic use.

More recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) measurement of salivary cortisol has been also available commercially (49,50). RIA and LC-MS/MS measurement of salivary cortisol and their accuracy of reference ranges were compared, in a cross-sectional prospective study of outpatients, in healthy volunteers and obese subjects with cushingoid features (49). This study demonstrated an important rate of abnormal bedtime salivary cortisol, measured in two different commercial assays and evaluated with laboratory provided normative ranges. The authors suggested that although a normal bedtime salivary cortisol result is useful to exclude Cushing's syndrome, an abnormal salivary cortisol value should not be used alone to establish the diagnosis of Cushing's syndrome. In a recent paper, Liu et al. (45) evaluated salivary cortisol in 206 male veterans with an enzyme immunoassay whose normal range was based on 73 healthy lean subjects. In that report, 20% of all participants and 40% of diabetic, hypertensive

Table 2. Overview of studies conducted in control subjects and Cushing's syndrome patients using late-night (2300 h) salivary cortisol.

Study	Cortisol assay	Detection limit (ng/dL)	Control subjects	Cushing's syndrome	Cut-off values
Laudat et al. (1988)	CPB assay	57	78–146	1278 ± 178	328
Raff et al. (1998)	RIA	14	43 ± 3.8	821 ± 150	130
Castro et al. (1999)	RIA	62	95 ± 8	924 ± 94	280
Papanicolaou et al. (2002)	RIA	100	260 ± 20	630	415
Raff et al. (2003)	EIA	10.7	43 ± 3.6	728 ± 464	153
Putignano et al. 2003	RIA	50	180 ± 20	970 ± 130	350
Yaneva et al. (2004)	RIA	30	79 ± 59	1210 ± 2031	200
Viardot et al. (2005)	RIA	29	75 (29–153)	674 (250–1824)	220
Vogeser et al. (2006)	EIA	36	178 (50–596)	NA	318
	LC-MS/MS	20	65–157		
Baid et al. (2007)	RIA	50	142 ± 218	NA	NA
	LC-MS/MS	4	46 ± 70		

CBP: competitive binding assay, RIA: radioimmunoassay, EIA: enzyme immunoassay, LC-MS/MS: liquid chromatography-tandem mass spectrometry; NA: not available. Salivary cortisol levels (ng/dL) are presented as mean ± SD or range, in control subjects and Cushing's syndrome patients.

subjects at least 60 yr of age had an abnormal 2300-h salivary cortisol level. At the time of publication, no participant had been diagnosed with Cushing's syndrome. Substantially lower concentrations were found with isotope dilution LC-MS/MS compared to RIA. Similar results were also observed comparing LC-MS/MS and the Roche Cobas cortisol immunoassay results (49,50).

Because salivary cortisol measurement is increasing in popularity, it is important to be aware of the different results generated by the commercially available methods and to interpret the published reference intervals appropriately. Therefore, the normal reference ranges are assay-dependent and should be validated for each laboratory. Nevertheless, late-night salivary cortisol measurement can be recommended as a first-line diagnostic test for CS in both low-risk and high-risk (pseudo-Cushing states) patients. Given its convenience and diagnostic accuracy (table 1), with sensitivity and specificity of between 95% and 98%, this test may profitably be added to traditional screening procedures, such as UFC and 1 mg DST.

So far, the majority of bedtime salivary cortisol measurement data were obtained by RIA. A critical analysis of these RIA data (table 2) indicates that among several available studies the cut-off value of late-night salivary cortisol levels, reliable to segregate pseudo Cushing from CS, was 250 ± 104 ng/dL (mean \pm SD, ranging from 130 to 415, 95%CI 153–346 ng/dL). Therefore, in the presence of bedtime salivary cortisol levels higher than 350 ng/dL, the diagnosis of CS is quite confident. On the other hand, in the presence of values lower than 150 ng/dL, the CS diagnosis is unlikely. However, if the bedtime salivary cortisol levels are in the gray area (> 150 and < 350 ng/dL), it is suggested that salivary cortisol sampling and other screening tests should be repeated. Until widely acceptable thresholds are generated, we suggest that each center should establish its own reference range and cut-off points (12,23,31).

Other second-line diagnostic tests to screen for CS

The dexamethasone/corticotropin-releasing-hormone (DST-CRH) test was designed to take advantage of the physiologic impact of glucocorticoid-negative feedback on the hypothalamic-pituitary-adrenal axis, as well as the sensitivity of the pituitary-adrenal system to hypothalamic stimulation with CRH. There are occasional patients in whom the screening tests described above are equivocal (figure 1). The combined DST-CRH test has been used in these condi-

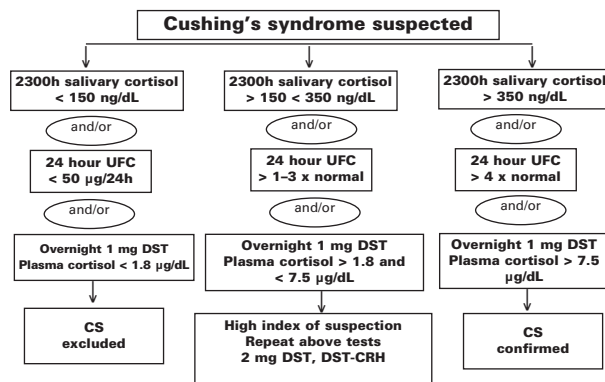


Figure 1. Algorithm for establishing the diagnosis of Cushing's syndrome. DST: dexamethasone suppression test, UFC: urinary free cortisol, DST-CRH: dexamethasone-corticotropin-releasing hormone test.

tions. The DST-CRH test was first shown by the NIH center to be highly accurate in distinguishing CS from pseudo-CS (51), mainly mild Cushing's disease from normal physiology (52).

The test is performed by giving dexamethasone orally 0.5 mg every 6 h for 48 h, starting at 1200 h, and then administering ovine-sequence CRH (1 µg/kg) iv at 0800 h (2 h after the last dose of dexamethasone). The plasma cortisol value 15 min after CRH is greater than 1.4 µg/dL (38 nmol/liter) in patients with CS, but remains suppressed in normal individuals and in patients with pseudo-CS (51). Criteria for a normal ACTH response have not been firmly established. The DST-CRH test is expensive and time-consuming. Caution is needed in interpreting this test since it requires highly sensitive cortisol assays and the precision of the majority of cortisol assays in routine use at the this cut-off level is poor. Furthermore, abnormal DST-CRH testing has been found in patients with anorexia nervosa (53) suggesting that non-Cushing's related increases in hypothalamic-pituitary-adrenal axis activity will lead to false positive results.

Because desmopressin (1-deamino-8D-arginine vasopressin, DDAVP), a vasopressin analogue, stimulates ACTH release in patients with CS but not in the majority of normal, obese, and depressed subjects, it has been used to investigate its ability to discriminate CS from pseudo-Cushing states (54,55). In these studies, a plasma peak ACTH increase equal to or greater than 6 pmol/L (27 pg/mL), indicated by ROC analysis, allowed the best discrimination between patients with CS and the other groups, with a sensitivity of 86.8%, a specificity of 90.7%, and a diagnostic accuracy of 89%. Therefore, when doubt remains about diagnosis, the DST-CRH test and the

desmopressin test can be used. However, their diagnostic accuracy needs further validation.

In conclusion, different approaches have been developed over the years, but all of them have demonstrated some limitations, and none of the proposed screening tests can detect all cases of CS. The three commonly performed diagnostic tests for CS — UFC, low doses DST, and late-night plasma or salivary cortisol — are complementary. Figure 1 summarizes a suggested approach to screening for Cushing's syndrome. In patients with mild or intermittent hypercortisolism, any of these tests may yield "normal" results and be misleading. Because of the high sensitivity and ease with which repeated measurements can be performed, late-night salivary cortisol appears to be the most useful screening test. UFC and DST should be performed to provide further confirmation of the diagnosis.

REFERENCES

- Orth DN. Cushing's syndrome. **N Engl J Med** 1995;332:791-803.
- Newell-Price J, Trainer P, Besser GM, Grossman AB. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. **Endocr Rev** 1998;19:647-72.
- Atkinson AB, McCance DR, Kennedy L, Sheridan B. Cyclical Cushing's syndrome first diagnosed after pituitary surgery: a trap for the unwary. **Clin Endocrinol** 1992;36:297-379.
- Arnaldi G, Mancini T, Kola B, Appolloni G, Freddi S, Concutoni C, et al. Cyclical Cushing's syndrome in a patient with a bronchial neuroendocrine tumor (typical carcinoid) expressing ghrelin and growth hormone secretagogue receptors. **J Clin Endocrinol Metab** 2003;88:5834-40.
- Gunther DF, Bourdeau I, Matyakhina L, Cassarino D, Kleiner DE, Griffin K, et al. Cyclical Cushing syndrome presenting in infancy: an early form of primary pigmented nodular adrenocortical disease, or a new entity? **J Clin Endocrinol Metab** 2004;89:3173-82.
- Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, et al. Diagnosis and complications of Cushing's syndrome: a consensus statement. **J Clin Endocrinol Metab** 2003;88:5593-602.
- Leibowitz G, Tsur A, Chayen SD, Salameh M, Raz I, Cerasi E. Pre-clinical Cushing's syndrome: an unexpected frequent cause of poor glycaemic control in obese diabetic patients. **Clin Endocrinol** 1996;44:717-22.
- Contreras LN, Cardoso E, Lozano MP, Pozzo J, Pagano P, Claus-Hermberg H. Deteccion de sindrome de Cushing preclinico en pacientes con sobrepeso y diabetes mellitas tipo 2. **Medicina** 2000;60:326-30.
- Reincke M, Nieke J, Krestin GP, Saeger W, Allolio B, Winkelmann W. Preclinical Cushing's syndrome in adrenal "incidentalomas": comparison with adrenal Cushing's syndrome. **J Clin Endocrinol Metab** 1992;75:826-32.
- Terzolo M, Pia A, Ali A, Osella G, Reimondo G, Bovio S. Adrenal incidentaloma: a new cause of the metabolic syndrome? **J Clin Endocrinol Metab** 2002;87:998-1003.
- Catargi B, Rigalleau V, Poussin A, Ronci-Chaix N, Bex V, Vergnot V, et al. Occult Cushing's syndrome in type-2 diabetes. **J Clin Endocrinol Metab** 2003;88:5808-13.
- Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. **Lancet** 2006;367(9522):1605-17.
- Kaye TB, Crapo L. The Cushing syndrome: an update on diagnostic tests. **Ann Intern Med** 1990;112:434-44.
- Boscaro M, Barzon L, Sonino N. The diagnosis of Cushing's syndrome: atypical presentations and laboratory shortcomings. **Arch Intern Med** 2000;160:3045-53.
- Murphy BE. Urinary free cortisol determinations: what they measure. **Endocrinologist** 2002;12:143-50.
- Turpeinen U, Markkanen H, Valimaki M, Stenman UH. Determination of urinary free cortisol by HPLC. **Clin Chem** 1997;43:1386-91.
- Lin CL, Wu TJ, Machacek DA, Jiang NS, Kao PC. Urinary free cortisol and cortisone determined by high performance liquid chromatography in the diagnosis of Cushing's syndrome. **J Clin Endocrinol Metab** 1997;82:151-5.
- Findling JW, Raff H. Newer diagnostic techniques and problems in Cushing's disease. **Endocrinol Metab Clin North Am** 1999;28:191-210.
- Gorges R, Knappe G, Gerl H, Venz M, Stahl F. Diagnosis of Cushing's syndrome: re-evaluation of midnight plasma cortisol vs. urinary free cortisol and low-dose dexamethasone suppression test in a large patient group. **J Endocrinol Invest** 1999;22:241-9.
- Viardot A, Huber P, Puder JJ, Zulewski H, Keller U, Muller B. Reproducibility of nighttime salivary cortisol and its use in the diagnosis of hypercortisolism compared with urinary free cortisol and overnight dexamethasone suppression test. **J Clin Endocrinol Metab** 2005;90:5730-6.
- Papanicolaou DA, Yanovski JA, Cutler GB, Chrousos GP, Nieman LK. A single midnight cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing's states. **J Clin Endocrinol Metab** 1998;83:1163-7.
- Papanicolaou DA, Mullen N, Kyrou I, Nieman LK. Nighttime salivary cortisol: a useful test for the diagnosis of Cushing's syndrome. **J Clin Endocrinol Metab** 2002;87:455-521.
- Findling JW, Raff H. Cushing's Syndrome: important issues in diagnosis and management. **J Clin Endocrinol Metab** 2006;91:3746-53.
- Liddle GW. Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. **J Clin Endocrinol Metab** 1960;20:1539-60.
- Isidori AM, Kaltsas GA, Mohammed S, Morris DG, Jenkins P, Chew SL. Discriminatory value of the low-dose dexamethasone suppression test in establishing the diagnosis and differential diagnosis of Cushing's syndrome. **J Clinical Endocrinol Metab** 2003;88:5299-306.
- Odagiri E, Naruse M, Terasaki K, Yamaguchi N, Jibiki K, Takagi S, et al. The diagnostic standard of preclinical Cushing's syndrome: evaluation of the dexamethasone suppression test using various cortisol kits. **Endocrine J** 2004;51:295-302.
- Chriquer RS, Elias LL, da Silva IM Jr, Vieira JG, Moreira AC, de Castro M. Glucocorticoid sensitivity in young healthy individuals: in vitro and in vivo studies. **J Clin Endocrinol Metab** 2005;90:5978-84.
- Crapo L. Cushing's syndrome: a review of diagnostic tests. **Metabolism** 1979;28:955-77.
- Findling JW, Raff H, Aron DC. The low-dose dexamethasone suppression test: a reevaluation in patients with Cushing's syndrome. **J Clin Endocrinol Metab** 2004;89:1222-6.
- Newell-Price J, Trainer P, Perra L, Wass J, Grossman A, Besser M. A single sleeping midnight cortisol has 100% sensitivity for the diagnosis of Cushing's syndrome. **Clin Endocrinol** 1995;43:545-50.
- Putignano P, Toja P, Dubini A, Pecori G, Corsello SM, Cavagnini F. Midnight salivary cortisol versus urinary free and midnight serum cortisol as screening tests for Cushing's syndrome. **J Clin Endocrinol Metab** 2003;88:4153-7.
- Reimondo G, Allasio B, Bovio S, Paccotti P, Angeli A, Terzolo M. Evaluation of the effectiveness of midnight serum cortisol in the diagnostic procedures for Cushing's syndrome. **Eur J Endocrinol** 2005;153:803-9.
- Giraldi FP, Ambrogio AG, De Martin M, Fatti LM, Scacchi M, Cavagnini F. Specificity of first-line tests for the diagnosis of Cushing's syndrome. Assessment in a large series. **J Clin Endocrinol Metab** 2007;[Epub ahead of print]
- Walker RF, Riad-Fahmy D, Read GF. Adrenal status assessed by direct radioimmunoassay of cortisol in whole saliva or parotid saliva. **Clin Chem** 1978;24:1460-3.

35. Umeda T, Hiramatsu R, Iwaoka T, Shimada T, Miura F, Sato T. Use of saliva for monitoring unbound free cortisol levels in serum. **Clin Chim Acta** 1981;110:245-53.
36. Santiago LB, Jorge SM, Moreira AC. Longitudinal evaluation of the development of salivary cortisol circadian rhythm in infancy. **Clin Endocrinol** 1996;44:157-61.
37. Luthold WW, Marcondes JAM, Wajchenberg BL. Salivary cortisol for the evaluation of Cushing's syndrome. **Clin Chim Acta** 1985;151:33-9.
38. Laudat MH, Cerdas S, Fournier C, Guiban D, Guilhaume B, Luton JP. Salivary cortisol measurement: a practical approach to assess pituitary-adrenal function. **J Clin Endocrinol Metab** 1988;66:343-8.
39. Raff H, Raff JL, Findling JW. Late-night salivary cortisol as a screening test for Cushing's syndrome. **J Clin Endocrinol Metab** 1998;83:2681-6.
40. Castro M, Elias PCL, Quidute ARP, Halah FP, Moreira AC. Outpatient screening for Cushing's syndrome: the sensitivity of the combination of circadian rhythm and overnight dexamethasone suppression salivary cortisol tests. **J Clin Endocrinol Metab** 1999;84:878-82.
41. Trilck M, Flitsch J, Ludecke DK, Jung R, Petersenn S. Salivary cortisol measurement — a reliable method for the diagnosis of Cushing's syndrome. **Exp Clin Endocrinol Diabetes** 2005;113:225-30.
42. Yaneva M, Mosnier-Pudar H, Dugue M-A, Grabar S, Fulla Y, Bertagna X. Midnight salivary cortisol for the initial diagnosis of Cushing's syndrome of various causes. **J Clin Endocrinol Metab** 2004;89:3345-51.
43. Martinelli CE, Sader SL, Oliveira EB, Daneluzzi JC, Moreira AC. Salivary cortisol for screening of Cushing's syndrome in children. **Clin Endocrinol** 1999;51:67-71.
44. Gafni RI, Papanicolaou DA, Nieman LK. Nighttime salivary cortisol measurement as a simple, noninvasive, outpatient screening test for Cushing's syndrome in children and adolescents. **J Pediatr** 2000;137:30-5.
45. Liu H, Bravata DM, Cabaccon J, Raff H, Ryzen E. Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans. **Clin Endocrinol** 2005;63:642-9.
46. Raff H, Homar PJ, Skoner DP. New enzyme immunoassay for salivary cortisol [Letter]. **Clin Chem** 2003;49:203-4.
47. Raff H, Homar PJ, Burns EA. Comparison of two methods for measuring salivary cortisol [Letter]. **Clin Chem** 2002;48:207-8.
48. Chiu SK, Collier CP, Clark AF, Wynn-Edwards KE. Salivary cortisol on ROCHE Elecsys immunoassay system: pilot biological variation studies. **Clin Biochem** 2003;36:211-4.
49. Baid SK, Sinaii N, Wade M, Rubino D, Nieman LK. Radioimmunoassay and tandem mass spectrometry measurement of bedtime salivary cortisol levels: a comparison of assays to establish hypercortisolism. **J Clin Endocrinol Metab** 2007;92:3102-7.
50. Vogeser M, Durner J, Seliger E, Auernhammer C. Measurement of late-night salivary cortisol with an automated immunoassay system. **Clin Chem Lab Med** 2006;44:1441-5.
51. Yanovski JA, Cutler GB Jr., Chrousos GP, Nieman LK. Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. **JAMA** 1993;269:2232-8.
52. Yanovski JA, Cutler GB Jr., Chrousos GP, Nieman LK. The dexamethasone suppressed corticotropin-releasing hormone stimulation test differentiates mild Cushing's disease from normal physiology. **J Clin Endocrinol Metab** 1998;83:348-52.
53. Duclos M, Corcuff J-B, Rober P, Tabarin A. The dexamethasone-suppressed corticotropin-releasing hormone stimulation test in anorexia nervosa. **Clin Endocrinol** 1999;51:725-31.
54. Coiro V, Volpi R, Capretti L, Caffarri G, Chiodera P. Desmopressin and hexarelin tests in alcohol-induced pseudo-Cushing's syndrome. **J Intern Med** 2000;247:667-73.
55. Moro M, Putignano P, Losa M, Invitti C, Maraschini C, Cavagnini F. The desmopressin test in the differential diagnosis between Cushing's disease and pseudo-Cushing states. **J Clin Endocrinol Metab** 2000;85:3569-74.

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