

Screening and Diagnosis of Cushing's Syndrome

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The diagnosis of Cushing's syndrome is the most challenging problem in clinical endocrinology. Clinical manifestations of excessive glucocorticoid exposure (either endogenous or exogenous) are protean and may be quite subtle. The observation that patients who have incidentally discovered adrenocortical adenomas and subclinical hypercortisolism frequently have improvement in their diabetes, hypertension, and obesity after adrenalectomy, increases the importance of establishing the diagnosis of even mild Cushing's syndrome [1,2]. Because the clinical syndrome is not always obvious, a low index of suspicion is needed for screening and certainly mandated in high-risk patient populations.

Epidemiology

Most epidemiologic studies have suggested that spontaneous Cushing's syndrome is an unusual disorder. A population-based study in Denmark found that the diagnosis of endogenous hypercortisolism had been established in 166 patients in an 11-year period [3]. Ninety-nine patients had Cushing's disease; 48 had adrenal-dependent Cushing's disease; 16 had ectopic corticotropin (ACTH), and three patients were unclassified. This represented an incidence of 2 cases per 1 million inhabitants per year. The patients in this study who were not cured by surgical intervention or who had malignant disease had a poor prognosis with a standard mortality ratio

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of 3.8 to 5.0 compared with normal controls. This study was in agreement with a similar study conducted in Vizcaya, Spain, in 1994 [4]. In a Spanish population of 1.15 million, 49 patients were diagnosed with Cushing's disease in an 18-year period, yielding an incidence of 2.5 cases per 1 million inhabitants per year. The prevalence in this study population was documented at 39 cases per 1 million inhabitants at the time the study was conducted. This report also emphasized the poor prognosis in these patients, with a standard mortality ratio of 3.8. Although these data would suggest that spontaneous Cushing's syndrome is rare, other observational data and screening in high-risk populations suggest that the diagnosis frequently is overlooked. For example, the authors have evaluated 85 patients from the Milwaukee metropolitan area (population 1.5 million) over an 11-year period. Even if this represented all the patients in the area with Cushing's syndrome over that period of time (and the authors estimate that it probably represents 30% to 50%), this would represent an incidence of approximately 5 patients per million inhabitants per year.

Screening studies performed in high-risk populations recently have suggested an unexpectedly high incidence of occult Cushing's syndrome. In 1996, Leibowitz et al performed screening studies in 90 obese subjects who had poorly controlled diabetes mellitus (hemoglobin A1C > 9%) and found three patients (3.3%) who had surgically confirmed Cushing's syndrome [5]. More recently, Catargi et al [6] carefully studied 200 obese patients who had type 2 diabetes mellitus and hemoglobin A1C values greater than 8%. Four patients (2%) had definite Cushing's syndrome and seven had subclinical hypercortisolism with unilateral adrenal adenomas demonstrating uptake on iodocholesterol scintigraphy and elevated late-night cortisol levels. Another study performed in Turkey screened 100 consecutive obese subjects (body mass index > 25 kg/m²) and found 9 subjects who had surgically proven Cushing's syndrome (5 pituitary and 4 adrenal) [7]. Moreover, subclinical hypercortisolism has been shown in at least 10% of patients who have adrenal incidentalomas [8], a finding that is seen in approximately 2% of the adult population. These studies suggest that spontaneous Cushing's syndrome is more common than previously appreciated.

Who should be screened?

Endogenous hypercortisolism may occur at any age and usually has an insidious onset, with a usual duration of illness before clinical diagnosis of 3 to 5 years. The disorder appears to be more common in women, particularly in patients who have pituitary- and adrenal-dependent Cushing's syndrome; however, the ratio may be fairly equal in patients who have ectopic ACTH-dependent Cushing's syndrome [8]. The authors believe that screening tests should be performed in subjects who have relatively specific signs and symptoms of hypercortisolism or in patients who have clinical diagnoses that may be caused by endogenous cortisol excess.

Specific signs and symptoms

Signs and symptoms that should provoke a biochemical evaluation for possible Cushing's syndrome are shown in **Box 1**. Weight gain with redistribution of fat centrally affecting the face, neck, trunk, and abdomen is one of the most common clinical findings. Unfortunately, this type of weight gain is very common and may be indistinguishable from those patients who have the metabolic syndrome. The weight gain is often insidious, and frequent review of old photographs may help the clinician better appreciate the physical changes that may have occurred in patients who have weight gain. The physical changes that may occur in a patient over a period of 12 years are illustrated in **Fig. 1**. Although patients who have Cushing's syndrome may have the classic moon facies, the facial rounding can be quite subtle. The patient in **Fig. 1** was examined by many clinicians (including several endocrinologists) before the diagnosis of spontaneous Cushing's syndrome was considered. The presence of significant supraclavicular

Box 1. Who should be screened for Cushing's syndrome?*Signs and symptoms*

Central obesity with:

- Facial rounding with plethora
- Increased supraclavicular and dorsocervical fat
- Cutaneous wasting with ecchymoses
- Wide violaceous striae (greater than 1 cm)
- Proximal myopathy
- Increased lanugo hair
- Superficial fungal infections
- Growth retardation (in children)

Clinical diagnosis

Metabolic syndrome X

- Diabetes mellitus (Hgb A1C > 8%)
- Hypertension
- Hyperlipidemia
- Polycystic ovary syndrome (PCOS)

Hypogonadotropic hypogonadism

- Oligomenorrhea/amenorrhea/infertility
- Decreased libido and impotence

Osteoporosis (especially rib fracture)

- Patients aged < 65 y

Incidental adrenal mass



Fig. 1. Progression of facial features before (*left*) and after a period of 12 years (*right*) of Cushing's disease.

fullness and dorsocervical fat accumulation should generate a screening test for hypercortisolism.

The catabolic effects of glucocorticoid excess frequently lead to cutaneous wasting from atrophy of the epidermis and underlying connective tissue. These changes result in the thin appearance of the skin with the typical plethoric facial appearance and easy bruisability. The skin is fragile and, when removing adhesive tape, may peel off like damp tissue paper. Most women who have Cushing's syndrome have skin fold thickness less than 2 mm in the dorsum of the hand, compared with greater than 2 mm with PCOS [9]. The significant weight gain and the skin changes often result in violaceous depressed and wide striae (usually > 1 cm in diameter). These striae usually occur on the abdomen but also may occur over the breasts, hips, buttocks, thighs, and axilla. Striae usually are observed in patients who have significant hypercortisolism, and rarely in patients over the age of 40 [10]. Minor wounds or abrasions may heal poorly. In addition, because of the immunosuppressive effects of hypercortisolism, superficial mucocutaneous fungal infections such as tinea versicolor also may be seen [11]. Although androgen excess may be present and result in facial hirsutism, vellus hypertrichosis (lanugo hair) that is glucocorticoid-dependent is probably more common in women who have Cushing's syndrome. In addition, papular acne may occur in younger patients who have Cushing's syndrome.

The catabolic effects of hypercortisolism also may result in type II muscle fiber atrophy with significant weakness in the proximal musculature [12]. Patients frequently complain of difficulty climbing stairs or rising from a chair. The myopathy may be particularly problematic in older adults and

may be misdiagnosed as amyotrophic lateral sclerosis or multiple sclerosis. Body composition studies have demonstrated reduced body cell mass, indicating a true protein loss in these patients [13].

Glucocorticoid excess also is known to blunt somatic growth. The impairment of somatic growth is mostly caused by direct action of glucocorticoids in the growing long bones in children, arresting the development of epiphysial cartilage [14,15]. Hypercortisolism also blunts growth hormone secretion, but insulin-like growth factor-1 levels are usually normal [15]. Growth retardation associated with progressive, and frequently generalized, obesity is the hallmark of Cushing's syndrome in children. After correction of hypercortisolism, children who have retarded linear growth rate should be treated with growth hormone, because there is a limited window of opportunity to promote an increase in growth to obtain normal adult height [16]. Impaired growth hormone responses to provocative stimuli also are observed in adult patients who have Cushing's syndrome and may persist for up to 2 years after treatment [17]. The authors are unaware of studies evaluating the efficacy of growth hormone replacement therapy in these patients.

Nonspecific signs and symptoms

Cushing's syndrome is associated with a range of psychologic and cognitive problems (see article by Bourdeau et al elsewhere in this issue). Most patients who have Cushing's syndrome meet criteria for depression, and a few patients have other neuropsychiatric problems including mania, anxiety, and cognitive dysfunction [18]. Psychosis may even occur, and suicidal tendency often has been reported in patients who have endogenous hypercortisolism. Children who have Cushing's syndrome often exhibit obsessive-compulsive behavior and do extremely well in their schoolwork [19]. Cognitive dysfunction with reversible loss of brain volume also has been reported [20].

Less common and unappreciated clinical features of Cushing's syndrome may involve the eyes. In Cushing's original report, 4 of 12 patients were described with exophthalmos [21]. A recent study demonstrated that 45% of patients who have active Cushing's syndrome had exophthalmos (> 16 mm) compared with 20% of treated Cushing's syndrome patients and 2% of normal controls [22]. The proptosis in patients who have Cushing's syndrome is asymptomatic and presumably caused by retro-orbital fat accumulation. Two other unique eye findings have been reported in patients who have Cushing's syndrome. Lisch nodules, thought to be specific for neurofibromatosis, but rare in the general population, are melanocytic hamartomas of the iris. These yellow or brown, dome-shaped elevations projecting from the surface of the iris recently were observed in 2 of 14 consecutive patients evaluated for endogenous hypercortisolism [23]. It was speculated that the underlying mechanism leading to the overgrowth of the

melanocytes in the iris may be similar to the corticotroph adenomatous change in the pituitary. The same group also reported the presence of central serous chorioretinopathy in 3 of 60 consecutive patients who have Cushing's syndrome [24]. This unusual condition represents the accumulation of subretinal fluid at the posterior pole of the fundus, causing an area of retinal detachment and some decrease in visual acuity. These findings also have been reported in patients receiving high-dose glucocorticoid therapy.

Clinical diagnoses associated with hypercortisolism

There are a few clinical disorders that alone should stimulate a consideration for the presence of Cushing's syndrome (see **Box 1**). Cushing's syndrome results in the entire clinical spectrum of the metabolic syndrome including obesity, diabetes, hypertension, and gonadal dysfunction. This phenotype has a high prevalence, and, with the increasing recognition of mild or subclinical hypercortisolism, it is clear that these two syndromes are clinically indistinguishable. The higher mortality rate observed in patients who have Cushing's syndrome seems to be related to the cardiovascular complications associated with the metabolic syndrome [4] as discussed in the article by Pivonello et al elsewhere in this issue.

The insulin resistance that accompanies hypercortisolism results in a decrease in glucose use by peripheral tissues; impaired glucose tolerance may occur in 30% to 60% of patients, and frank diabetes may occur in 25% to 50% of patients [25]. As previously mentioned, 2% to 3% of patients who have poorly controlled type 2 diabetes may have unrecognized Cushing's syndrome [5,6]. Glucocorticoid excess also plays an essential role in the accumulation of abdominal body fat. Thus, obesity is an independent risk factor for reduced life expectancy and correlates well with all of the metabolic sequelae and atherosclerosis associated with the metabolic syndrome. The importance of cortisol in the generation of abdominal obesity recently has been highlighted by targeted overexpression of 11 β -hydroxysteroid dehydrogenase type 1 in transgenic mice [26]. This resulted in increased visceral fat, presumably because of local production of active glucocorticoid within the adipocyte.

Arterial hypertension occurs in at least 80% of patients who have Cushing's syndrome and is a major contributing factor to cardiovascular morbidity. Although the hypertension may be mild, organ damage such as cardiac hypertrophy with left ventricular concentric remodeling may occur as a result [27]. The mechanism for the hypertension presumably is related to the mineralocorticoid activity of cortisol. In the presence of severe hypercortisolism, there is a failure to completely metabolize cortisol to the inactive cortisone by the renal enzyme 11 β -hydroxysteroid dehydrogenase type 2. This allows cortisol binding to the mineralocorticoid receptor in the distal nephron, resulting in hypertension and hypokalemia. Other possible mechanisms of hypertension in patients who have Cushing's syndrome

include enhancement of the inotropic pressor effects of vasoactive substances including catecholamines, vasopressin, and angiotensin II, and possible suppression of vasodilatory mechanisms including nitric oxide and prostacyclin production [28].

The hyperlipidemia seen in patients who have Cushing's syndrome appears to be similar to the dyslipidemia associated with the metabolic syndrome. There is an increase in very low density lipoprotein (LDL) and LDL with a decrease in high density lipoprotein (HDL) levels. This results in elevation of total triglyceride and cholesterol levels [29,30].

Less appreciated is the hypercoagulability in patients who have Cushing's syndrome leading to an increased risk for thromboembolic events mostly after surgery or during inferior petrosal sinus sampling [31]. Hypercortisolism stimulates the synthesis of several clotting factors such as fibrinogen by the liver and von Willebrand factor by endothelial cells. In addition, glucocorticoids also increase the synthesis of plasminogen activator inhibitor type 1, the main inhibitor of the fibrinolytic system [32]. The general consensus is that patients who have Cushing's syndrome should be given heparin during inferior petrosal sinus sampling and that anticoagulation should be considered in the postoperative period in many patients.

Because women who have Cushing's syndrome may present with menstrual irregularities or signs and symptoms of androgen excess, the diagnosis of PCOS often is considered. Kaltsas et al recently showed that most women who have Cushing's syndrome also have PCOS and suggested that women who have PCOS should undergo screening studies for hypercortisolism [33]. Interestingly, the menstrual irregularity in these women appears to be more closely related to the degree of cortisol excess than the actual circulating androgen concentrations [34]. Male patients frequently present with diminished libido or impotence associated with subnormal testosterone concentration. Hypogonadotropic hypogonadism is not an uncommon finding in men who have hypercortisolism and is underappreciated. Therefore, Cushing's syndrome should be considered in men who have hypogonadotropic hypogonadism, particularly if they have other phenotypic characteristics of hypercortisolism.

The diagnosis of Cushing's syndrome should be considered in patients who have unexplained osteoporosis, particularly in those younger than 65 years. Glucocorticoids influence bone and calcium homeostasis at many levels (see article by Shaker and Lukert elsewhere in this issue). Pathologic fractures may be the presenting feature of Cushing's syndrome, and rib fractures seem to be especially common. There is also an increased risk of nephrolithiasis in patients who have Cushing's syndrome. A recent study found kidney stones in 50% of patients who have active Cushing's syndrome, 27% of patients in remission, and 6.5% of controls [35]. Patients who have active Cushing's syndrome and nephrolithiasis had a significantly increased prevalence of arterial hypertension, hypercalciuria, hypocitraturia, and hyperuricosuria.

In light of the evidence that 6% to 10% of patients who have incidentally discovered adrenal masses (of at least 2 cm) have subclinical hypercortisolism, it seems obvious that screening studies for Cushing's syndrome are needed in this fairly common group of patients [36]. Information concerning evaluation and treatment of these patients is found in the article by Terzolo et al elsewhere in this issue.

Nonspecific routine laboratory abnormalities

There are no routine laboratory abnormalities that are specific for Cushing's syndrome. Thyrotropin (TSH) levels are lower in patients who have Cushing's syndrome and increase after remission [37]. Rarely, patients who have Cushing's syndrome will be referred to an endocrinologist because of a subnormal TSH (usually between 0.1 and 0.4). It is also well appreciated that successful cure of Cushing's syndrome may unmask a pre-existing autoimmune thyroid disorder with the appearance of hypothyroidism or hyperthyroidism [38]. Other more routine laboratory studies are rarely helpful. High normal values of hemoglobin, hematocrit, and red blood cell count may be seen because of androgen excess, but elevations into the polycythemic range are very rare. Leukocytosis, usually with depressed percentages of lymphocyte and eosinophils, may be seen occasionally. Electrolyte disturbances are quite uncommon with the exception of hypokalemia in patients who have prodigious hypercortisolism. Routine radiographs may be helpful in showing low bone density in the axial skeleton. Rarely, chest roentgenograms may show widening of the mediastinum because of mediastinal lipomatosis.

Screening tests

The choice of tests in the initial evaluation of a patient suspected of endogenous hypercortisolism can be difficult. Even more problematic is the interpretation of the results of these tests, particularly if they are not in agreement with each other. This is particularly so in mild Cushing's syndrome; if the symptoms are subtle, the biochemical abnormalities are likely to be subtle as well. The authors recently published several extensive reviews of biochemical tests useful in the diagnosis of Cushing's syndrome [8,39–42]. Therefore, this section will focus on newer studies and conclude with an exposition on studies recently published that compare tests by modern statistical analyses.

Urine free cortisol

The measurement of free cortisol in a 24-hour urine collection has been the mainstay of the diagnosis of endogenous hypercortisolism [43]. The concept is that if the daily production of cortisol is increased, the free

cortisol filtered and not reabsorbed or metabolized in the kidney will be increased measurably. Mild Cushing's syndrome often results from small, but significant increases in nighttime cortisol secretion (Fig. 2). Because most of the cortisol secreted during any 24-hour period is usually between 4 AM and 4 PM, subtle increases in nighttime cortisol secretion may not be detected in standard 24-hour urine free cortisol measurements. It also requires an adequate urine collection that must be verified with a measurement of urinary creatinine.

The other problem with this test in the past was the different methods used to measure cortisol in urine. There are still laboratories that use direct immunoassay of cortisol without chromatographic separation. Although this assay lacks specificity, it may be useful if the patient is excreting an unusual pattern of cortisol metabolites that cross-react with the cortisol antibody. Most, however, consider the gold standard for urine free cortisol to involve some type of chromatographic separation, usually high-performance liquid chromatography (HPLC) [44–47]. Assuming the chromatography is done properly, the next issue is the method of detection. Immunoassay can be done after HPLC, although tandem mass spectrometry provides more specific results [45]. There are substances that will interfere with some of these chromatographic methods including carbamazepine [46] and fenofibrate [47].

Several recent studies found the sensitivity of urine-free cortisol (UFC) to range from 45% to 71% at 100% specificity [48–52]. That is, it is not uncommon for patients who have mild Cushing's syndrome to have at least one or more normal urine free cortisol measurements. Moreover, UFC may be increased in patients who have so-called "pseudo-Cushing's syndrome,"

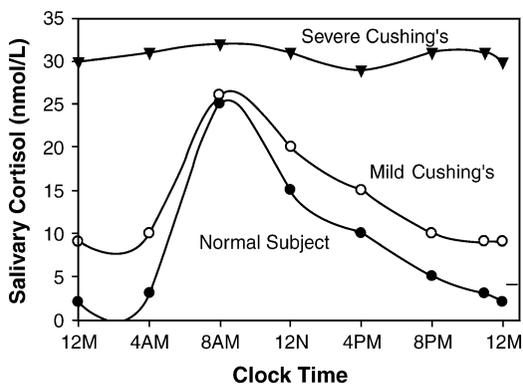


Fig. 2. Theoretical salivary cortisol levels during a typical day in a normal subject, a patient who has mild Cushing's disease, and a patient who has severe Cushing's syndrome. Note that the 11 PM–3 AM time period is the best to discriminate between mild Cushing's disease and normal subjects. For diagnostic purposes, salivary cortisol is usually sampled at 11 PM. (Reprinted from Raff H. Role of salivary cortisol determinations in the diagnosis of Cushing's syndrome. *Curr Opin Endocrinol Diabetes* 2004;11:271–5; with permission.)

which includes alcoholism, endogenous depression, and eating disorders [51,52]. When UFC is compared with other available screening tests using objective statistical analyses, its sensitivity and specificity are less than ideal (Table 1).

Circadian rhythm studies

One of the first biochemical disturbances that patients develop with mild Cushing's syndrome is a failure to decrease cortisol secretion to its normal nadir at night (see Fig. 2). This phenomenon has been exploited in the diagnosis of Cushing's syndrome with several different approaches.

Measurement of an elevated serum cortisol at midnight has a very high sensitivity and specificity for Cushing's syndrome of any etiology [51,53]. The main problem with this approach is the logistical problem of obtaining an unstressed blood sample in a routine clinical setting at midnight. This has made the widespread application of this test impractical.

A solution to this sampling problem is the measurement of salivary cortisol at bedtime as a surrogate for serum cortisol [40,41,50,52,54–57]. The authors' initial study with this test demonstrated a sensitivity and specificity of approximately 95% [54]. There now have been many major studies validating this approach to screen for Cushing's syndrome [49,50,52,54–57], and this method will continue to increase in popularity as salivary cortisol assays become readily available [58,59]. A potential drawback of this test, however, is its specificity. There are many factors that can elevate cortisol secretion falsely at bedtime including proximal stress, sleep disturbances, psychoneuroendocrine factors, and contamination of the saliva sample [40]. Therefore, whereas 24-hour UFC lacks reliable sensitivity, salivary cortisol under certain circumstances may be too sensitive.

Another approach has been to perform urine collections for the measurement of free cortisol just from the overnight period [60]. The concept is that if one collects urine during and just after the circadian nadir, the sensitivity of the test might be improved. It also would require only one or two urine collections. This approach requires a very accurate

Table 1
Diagnostic characteristics of different biochemical tests at 100% sensitivity or 100% specificity

Diagnostic characteristic	LDDST: cortisol		24-h UFC [48]	Nighttime: cortisol	
	Serum [48]	Salivary [49]		Serum [48,51]	Salivary [50]
Sensitivity at 100% specificity	54%	NC	71%	75%, 96%	93%
Specificity at 100% sensitivity	41%	93%	73%	77%	96%

Abbreviation: NC, not calculated (sensitivity was 100% at 93% specificity).
Data from Refs. [48–51].

measurement of urinary creatinine to which the free cortisol measurement is normalized.

Suppression tests

The authors have reviewed the physiologic basis of using the sensitivity to glucocorticoid negative feedback to diagnose endogenous hypercortisolism [39]. Briefly, the theory is that a small enough dose of dexamethasone will not inhibit ACTH release from corticotroph adenomas or occult ectopic ACTH-secreting tumors, while suppressing ACTH from normal pituitary tissue. (Obviously, ACTH-independent [adrenal] Cushing's syndrome should be unaffected by dexamethasone administration). It is now clear that neither the overnight nor the 2-day, low-dose dexamethasone suppression tests (LDDST) are of sufficient reliability to be used to rule out Cushing's syndrome [61–63]. The authors recently demonstrated that 18% of patients who have proven Cushing's disease suppressed serum cortisol to the standard cut-off of 5 µg/dL (135 nmol/L), while 8% showed suppression to less than 2 µg/dL (< 54 nmol/L) [62]. The performance of the 2-day LDDST was even worse. Therefore, there was no cut-off that identified all patients who have Cushing's syndrome.

Most reference laboratories with high volumes use direct serum cortisol assays using platforms that employ chemiluminescent or electrochemiluminescent immunoassays. It recently has been shown that the performance of different assays introduces significant intermethod variability when performing the LDDST to detect patients who have mild or preclinical Cushing's syndrome [64]. Because a reliable LDDST requires accuracy at low serum cortisol concentrations, variability between reference laboratories is likely to introduce even more uncertainty in the usefulness of this test. This variability would not be included in the lack of sensitivity and specificity identified in a well-controlled study in which the same assay methodology is used.

Despite these limitations, the overnight LDDST remains widely employed. A recent consensus statement recommended that patients who have plasma cortisol greater than 1.8 µg/dL (50 nmol/L) after overnight 1 mg dexamethasone administration merit further evaluation [65]. It is predicted that this more stringent criterion will yield a diagnostic sensitivity of 95% to 98% [62], but that specificity (ie, false-positives) will suffer as a result.

Stimulation tests

The secretion of ACTH from the pituitary, in addition to being under negative glucocorticoid feedback control, is stimulated primarily by hypothalamic corticotropin-releasing hormone (CRH) and, to a lesser extent, arginine vasopressin (AVP). The CRH test has been used to attempt to identify patients who have mild ACTH-dependent Cushing's syndrome

[66]. The theory is that corticotroph adenomas will display exaggerated ACTH responses compared with normal subjects as long as they continue to express receptors for CRH. Furthermore, a V2 analog of vasopressin, DDAVP (desmopressin), also has been used to attempt to identify patients who have mild Cushing's disease [58,59]. The theory is that corticotroph adenomas will have an exaggerated response to vasopressin compared with normal subjects. Neither test appears to possess the adequate sensitivity or specificity to merit their expense [66–68].

Combined testing

Different diagnostic tests can be performed on separate occasions to attempt to improve the overall reliability compared with each test alone [39]. Because each of the standard tests for the diagnosis of Cushing's syndrome has merit but also some weaknesses, another approach to improve overall performance could be to perform two tests simultaneously in each patient. Recent examples of this are the combination LDDST–CRH test and the combination of late-night salivary cortisol and LDDST.

Low-dose dexamethasone suppression–corticotropin-releasing hormone test

The logic of the LDDST–CRH is that one can improve the discriminatory potential of each test for mild Cushing's disease by performing them simultaneously. The theory is that only abnormal corticotrophs will respond to CRH while suppressed with dexamethasone [69,70]. In this test, starting at noon, dexamethasone (0.5 mg) is administered every 6 hours for a total of eight doses, with the last given at 6 AM before dynamic studies. CRH (1 µg/kg) is then given intravenously at 8 AM with measurement of cortisol and ACTH every 15 minutes for 1 hour. A serum cortisol greater than 1.4 µg/dL (> 38.6 nmol/L) is considered abnormal. There are no currently accepted criteria for an abnormal plasma ACTH response to LDDST–CRH.

The initial studies with the LDDST–CRH test were promising but required great attention to detail and compliance with the rather sophisticated protocol. Its main strength was its ability to distinguish mild Cushing's syndrome from the so-called pseudo-Cushing's syndrome. The criteria for pseudo-Cushing's syndrome included a failure for symptoms to progress for 17 months. It would be interesting to see if any of those patients from 1993 [69] subsequently have been discovered to have Cushing's disease. A recent study in patients who have anorexia nervosa found about half with abnormal LDDST–CRH tests, raising a concern that it does not reliably discriminate Cushing's syndrome from every form of endogenous pathophysiologic activation of the hypothalamic-pituitary-adrenal axis [71].

The LDDST–CRH test requires measurement of serum cortisol, thereby using endogenous adrenocortical amplification of an ACTH signal for success [69–71]. To accomplish this, the test requires a very sensitive serum

cortisol measurement that is not the standard reference laboratory assay. It also, again, raises the possibility of variable reliability between reference laboratories [64]. The LDDST–CRH test is very expensive in terms of material and labor, and it usually is reserved only for patients who have mild Cushing’s syndrome or to confirm the diagnosis when other screening tests are equivocal [39].

Late-night salivary cortisol–low-dose dexamethasone suppression

When the authors first published results with late-night salivary cortisol to screen for Cushing’s, they hypothesized that the high sensitivity of the test combined with better specificity of the LDDST might be exploited by a combined test [54]. The theory is that almost all patients who have Cushing’s syndrome have elevated late-night salivary (or serum) cortisol, but a fair number of patients who have pseudo-Cushing’s syndrome do also (ie, low specificity). The LDDST, however, while having low sensitivity, may have a better specificity. Castro et al recently evaluated this concept by showing an increase in specificity from 88% with late-night salivary cortisol alone to 100% using the combined test but only when using nonobese subjects as the control group [49]. The problem was that the specificity for Cushing’s syndrome compared with obese patients who presumably did not have it was not increased by the combined test. In a follow-up study, it was suggested that perhaps a higher dose of dexamethasone might improve the specificity of the test [72]. Most importantly, they clearly showed that measurement of salivary cortisol after an overnight LDDST was significantly better than measuring serum cortisol, presumably because salivary cortisol is a much better estimate of free, biologically active cortisol [40].

Comparison of diagnostic characteristics

Because each diagnostic test for Cushing’s syndrome has liabilities, it is helpful to perform an objective comparison using well-defined diagnostic criteria. Table 1 focuses on several recent studies that meet the evidence-based criteria of comparing several tests within one group of patients, and performing careful step-wise analysis of sensitivity versus specificity. This table shows the sensitivity at a cut-off that provides 100% specificity, and the specificity at a cut-off that provides 100% sensitivity. Obviously, there is always a tradeoff between the two. Generally, high sensitivity is preferred for screening tests, but combinations of tests may improve both criteria.

The first characteristic that is clear from this table is that the LDDST using the measurement of serum cortisol had the poorest performance of all of the tests [48]. Again, either sensitivity or specificity can be improved at the expense of the other by adjusting cut-off levels. Of all the tests, nighttime salivary cortisol has the highest sensitivity and specificity [50,55]. It is important to point out that the authors purposefully did not use their own

data in this analysis; these were independent evaluations of these tests with no apparent bias. It is also important to note that UFC performed better than the LDDST but still not as well as night-time salivary cortisol.

Probably the most important information for clinical endocrinologists is the practical nature of measuring salivary cortisol at 11 PM. There are now several assay methods available and reference laboratories that routinely perform this analysis [58,59]. The patient obtains the saliva at home thereby minimizing extraneous stress. The approach does not require the cumbersome collection of complete 24-hour urine samples, nor does it require taking (and absorbing) dexamethasone at the correct time. Salivary cortisol can be assessed in small children and the elderly without difficulty [40]. The authors have found this approach to be extremely useful and expect that its use will become accepted widely with the increased need for inexpensive, convenient, and reliable ways to screen the increasingly obese population for Cushing's syndrome.

Diagnostic strategy

Fig. 3 shows a strategy for screening patients for Cushing's syndrome. Salivary cortisol is obtained at 11 PM, usually on at least two separate nights. If both results are below the reference range (less than 4.3 nmol/L), Cushing's syndrome is unlikely. If both results are above twice the reference range cut-off (8.6 nmol/L), Cushing's syndrome is likely, particularly if the sampling times are verified, and other confounding factors (like contami-

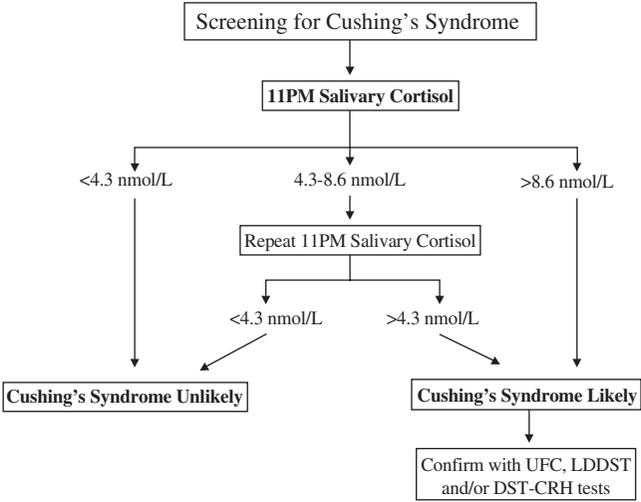


Fig. 3. A simple paradigm for screening patients for Cushing's syndrome using late-night salivary cortisol (cutoff values from Ref. [39]). This approach takes advantage of the excellent sensitivity of this test.

nation with steroid hand creams) are excluded. If the results are equivocal or not consistent between the two samples, two additional salivary cortisol samples should be obtained. It is also important to verify consistently abnormal salivary cortisol levels with other tests as indicated to confirm the diagnosis before entering the differential diagnostic strategy (described by Lindsay and Nieman elsewhere in this issue).

Summary

The recognition of mild or subclinical Cushing's syndrome and the protean nature of its clinical presentation are changing the diagnostic approach. Recent screening studies in high-risk populations have suggested that spontaneous Cushing's syndrome is more common than appreciated, and its incidence/prevalence has been underestimated. The authors believe that patients who have specific signs and symptoms or clinical diagnoses should be considered for screening (see [Box 1](#)). Currently, late-night salivary cortisol measurements provide the best sensitivity with reasonable specificity to recommend it as the initial screening test. In fact, trying to diagnose mild Cushing's syndrome without measuring late-night salivary cortisol may be like trying to diagnose mild primary hypothyroidism without obtaining a TSH. Despite their limitations, urine free cortisol and LDDST will continue to be used to confirm the presence and magnitude of endogenous hypercortisolism.

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