The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline

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Co-sponsoring Association: European Society of Endocrinology

Objective: The objective of the study was to develop clinical practice guidelines for the diagnosis of Cushing's syndrome.

Participants: The Task Force included a chair, selected by the Clinical Guidelines Subcommittee (CGS) of The Endocrine Society, five additional experts, a methodologist, and a medical writer. The Task Force received no corporate funding or remuneration.

Consensus Process: Consensus was guided by systematic reviews of evidence and discussions. The guidelines were reviewed and approved sequentially by The Endocrine Society's CGS and Clinical Affairs Core Committee, members responding to a web posting, and The Endocrine Society Council. At each stage the Task Force incorporated needed changes in response to written comments.

Conclusions: After excluding exogenous glucocorticoid use, we recommend testing for Cushing's syndrome in patients with multiple and progressive features compatible with the syndrome, particularly those with a high discriminatory value, and patients with adrenal incidentaloma. We recommend initial use of one test with high diagnostic accuracy (urine cortisol, late night salivary cortisol, 1 mg overnight or 2 mg 48-h dexamethasone suppression test). We recommend that patients with an abnormal result see an endocrinologist and undergo a second test, either one of the above or, in some cases, a serum midnight cortisol or dexamethasone-CRH test. Patients with concordant abnormal results should undergo testing for the cause of Cushing's syndrome. Patients with concordant normal results should not undergo further evaluation. We recommend additional testing in patients with discordant results, normal responses suspected of cyclic hypercortisolism, or initially normal responses who accumulate additional features over time. **(*J Clin Endocrinol Metab* 93: 1526–1540, 2008)**

SUMMARY OF RECOMMENDATIONS

3.0 Diagnosis of Cushing's syndrome

Who should be tested

3.1 We recommend obtaining a thorough drug history to exclude excessive exogenous glucocorticoid exposure leading to iatrogenic Cushing's syndrome before conducting biochemical testing (1⊕⊕⊕⊕).

3.2 We recommend testing for Cushing's syndrome in the following groups:

- Patients with unusual features for age (*e.g.* osteoporosis, hypertension) (Table 1) (1⊕⊕◯◯)

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Abbreviations: CBG, Cortisol-binding globulin; DST, dexamethasone suppression test; HPA, hypothalamic-pituitary-adrenal; 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; LC-MS/MS, tandem mass spectrometry; LDDST, low-dose DST; 17OHCS, 17-hydroxycorticosteroid; SMR, standard mortality ratio; UFC, urine free cortisol.

- Patients with multiple and progressive features, particularly those who are more predictive of Cushing's syndrome (Table 1) (1⊕⊕◯◯)
- Children with decreasing height percentile and increasing weight (1⊕◯◯◯)
- Patients with adrenal incidentaloma compatible with adenoma (1⊕◯◯◯).

3.3 We recommend against widespread testing for Cushing's syndrome in any other patient group (1⊕◯◯◯).

Initial testing

3.4 For the initial testing for Cushing's syndrome, we recommend one of the following tests based on its suitability for a given patient (Fig. 1) (1⊕◯◯◯):

3.4.1 Urine free cortisol (UFC; at least two measurements)
3.4.2 Late-night salivary cortisol (two measurements)
3.4.3 1-mg overnight dexamethasone suppression test (DST)
3.4.4 Longer low-dose DST (2 mg/d for 48 h)

3.5 We recommend against the use of the following to test for Cushing's syndrome (1⊕◯◯◯):

- Random serum cortisol or plasma ACTH levels
- Urinary 17-ketosteroids
- Insulin tolerance test
- Loperamide test
- Tests designed to determine the cause of Cushing's syndrome (*e.g.* pituitary and adrenal imaging, 8 mg DST).

3.6 In individuals with normal test results in whom the pretest probability is high (patients with clinical features suggestive of Cushing's syndrome and adrenal incidentaloma or suspected cyclic hypercortisolism), we recommend further evaluation by an endocrinologist to confirm or exclude the diagnosis (1⊕◯◯◯).

3.7 In other individuals with normal test results (in whom Cushing's syndrome is very unlikely), we suggest reevaluation in 6 months if signs or symptoms progress (2⊕◯◯◯).

3.8 In individuals with at least one abnormal test result (for whom the results could be falsely positive or indicate Cushing's syndrome), we recommend further evaluation by an endocrinologist to confirm or exclude the diagnosis (1⊕◯◯◯).

Subsequent evaluation

3.9 For the subsequent evaluation of abnormal initial test results, we recommend performing another recommended test (Fig. 1, 1⊕◯◯◯).

3.9.1 We suggest the additional use of the dexamethasone-CRH test or the midnight serum cortisol test in specific situations (Fig. 1, 1⊕◯◯◯).

3.9.2 We suggest against the use of the desmopressin test, except in research studies, until additional data validate its utility (2⊕◯◯◯).

3.9.3 We recommend against any further testing for Cushing's syndrome in individuals with concordantly negative results on two different tests (except in patients suspected of having the very rare case of cyclical disease) (1⊕◯◯◯).

3.9.4 We recommend tests to establish the cause of Cushing's syndrome in patients with concordantly positive results from two different tests, provided there is no concern regarding possible non-Cushing's hypercortisolism (Table 2) (1⊕⊕◯◯).

3.9.5 We suggest further evaluation and follow-up for the few patients with concordantly negative results who are suspected of having cyclical disease and also for patients with discordant results, especially if the pretest probability of Cushing's syndrome is high (2⊕◯◯◯).

4.0 Special populations/considerations

4.1 Pregnancy: We recommend the use of UFC and against the use of dexamethasone testing in the initial evaluation of pregnant women (1⊕⊕⊕◯).

4.2 Epilepsy: We recommend against the use of dexamethasone testing in patients receiving antiepileptic drugs known to enhance dexamethasone clearance and recommend instead measurements of nonsuppressed cortisol in blood, saliva, or urine (1⊕⊕⊕◯).

4.3 Renal failure: We suggest using the 1-mg overnight DST rather than UFC for initial testing for Cushing's syndrome in patients with severe renal failure (2⊕◯◯◯).

4.4 Cyclic Cushing's syndrome: We suggest use of UFC or midnight salivary cortisol tests rather than DSTs in patients suspected of having cyclic Cushing's syndrome (2⊕◯◯◯).

4.5 Adrenal incidentaloma: We suggest use of the 1-mg DST or late-night cortisol test, rather than UFC, in patients suspected of having mild Cushing's syndrome (2⊕⊕◯◯).

METHOD OF DEVELOPMENT OF EVIDENCE-BASED RECOMMENDATIONS

The Clinical Guidelines Subcommittee of The Endocrine Society deemed detection and diagnosis of patients with Cushing's syndrome a priority area in need of practice guidelines and appointed a six-member Task Force to formulate evidence-based recommendations. The Task Force followed the approach recommended by the Grading of Recommendations, Assessment, Development, and Evaluation Group, an international group with expertise in development and implementation of evidence-based guidelines (1).

The Task Force used the best available research evidence that members identified and systematic reviews and metaanalyses of test accuracy to inform the recommendations (2). The Task Force also used consistent language and graphical descriptions of both the strength of a recommendation and the quality of evidence. In terms of the strength of the recommendation, strong recommendations use the phrase "we recommend" and the number 1, and weak recommendations use the phrase "we suggest" and the number 2. Cross-filled circles indicate the quality of the evidence, such that ⊕◯◯◯ denotes very low-quality evidence; ⊕⊕◯◯, low quality; ⊕⊕⊕◯, moderate quality; and ⊕⊕⊕⊕, high quality. A detailed description of this grading scheme has been published elsewhere (3).

The Task Force has confidence that patients who receive care according to the strong recommendations will derive, on average, more good than harm. Low- or very low-quality evidence usually leads to weak recommendations because of uncertainty

about the balance between risks and benefits; strong recommendations based on low-quality evidence usually indicate the panel's strong preference against the alternative course of action but are subject to change with new research. Given a weak recommendation, careful consideration of the patient's circumstances, values, and preferences is appropriate to determine the best course of action.

Linked to each *recommendation* is a description of the *evidence*, *values* that panelists considered in making the recommendation (when making these explicit was necessary), and *remarks*, a section in which panelists offer technical suggestions for testing conditions, dosing, and monitoring. These technical comments reflect the best available evidence applied to a typical patient. Often this evidence comes from the unsystematic observations of the panelists and should therefore be considered suggestions.

1.0 Definition, pathophysiology, and etiology of hypercortisolism

Cushing's syndrome comprises a large group of signs and symptoms that reflect prolonged and inappropriately high exposure of tissue to glucocorticoids (Table 1). Whereas the most common cause is iatrogenic from medically prescribed corticosteroids, endogenous Cushing's syndrome is an uncommon disorder. European population-based studies reported an incidence of two to three cases per 1 million inhabitants per year (4, 5). Excess cortisol production, the biochemical hallmark of endogenous Cushing's syndrome, may be caused by either excess ACTH secretion (from a pituitary or other ectopic tumor) or independent adrenal overproduction of cortisol.

Although Cushing's syndrome is clinically unmistakable when full blown, the spectrum of clinical presentation is broad, and the diagnosis can be challenging in mild cases. Few, if any, features of Cushing's syndrome are unique, but some are more discriminatory than others, including reddish purple striae, plethora, proximal muscle weakness, bruising with no obvious trauma, and unexplained osteoporosis (6–8). More often patients have a number of features that are caused by cortisol excess but that are also common in the general population, such as obesity, depression, diabetes, hypertension, or menstrual irregularity. As a result, there is an overlap in the clinical presentation of individuals with and without the disorder (Table 1). We encourage caregivers to consider Cushing's syndrome as a secondary cause of these conditions, particularly if additional features of the disorder are present. (see *Who should be tested* below.) If Cushing's syndrome is not considered, the diagnosis is all too often delayed.

In addition, overactivity of the hypothalamic-pituitary-adrenal (HPA) axis occurs without true Cushing's syndrome, so that there is an overlap between physiological and pathophysiological causes of hypercortisolism (Table 2). Thus, certain psychiatric disorders (depression, anxiety disorder, obsessive-compulsive disorder), poorly controlled diabetes mellitus, and alcoholism can be associated with mild hypercortisolism and may produce test results suggestive of Cushing's syndrome, including abnormal dexamethasone suppressibility and mildly elevated UFC (9). Circulating cortisol concentrations are usually normal (or slightly reduced) in obesity, but severe obesity can raise UFC. It is thought that higher brain centers stimulate CRH release in these conditions, with subsequent activation of the entire HPA axis (10). The negative feedback inhibition of cortisol on CRH and pituitary ACTH release partially restrains the re-

TABLE 1. Overlapping conditions and clinical features of Cushing's syndrome[a]

Symptoms	Signs	Overlapping conditions
Features that best discriminate Cushing's syndrome; most do not have a high sensitivity		
	Easy bruising	
	Facial plethora	
	Proximal myopathy (or proximal muscle weakness)	
	Striae (especially if reddish purple and > 1 cm wide)	
	In children, weight gain with decreasing growth velocity	
Cushing's syndrome features in the general population that are common and/or less discriminatory		
Depression	Dorsocervical fat pad ("buffalo hump")	Hypertension[b]
Fatigue	Facial fullness	Incidental adrenal mass
Weight gain	Obesity	Vertebral osteoporosis[b]
Back pain	Supraclavicular fullness	Polycystic ovary syndrome
Changes in appetite	Thin skin[b]	Type 2 diabetes[b]
Decreased concentration	Peripheral edema	Hypokalemia
Decreased libido	Acne	Kidney stones
Impaired memory (especially short term)	Hirsutism or female balding	Unusual infections
Insomnia	Poor skin healing	
Irritability		
Menstrual abnormalities		
In children, slow growth	In children, abnormal genital virilization	
	In children, short stature	
	In children, pseudoprecocious puberty or delayed puberty	

[a] Features are listed in random order.

[b] Cushing's syndrome is more likely if onset of the feature is at a younger age.

TABLE 2. Conditions associated with hypercortisolism in the absence of Cushing's syndrome[a]

Conditions
Some clinical features of Cushing's syndrome may be present
Pregnancy
Depression and other psychiatric conditions
Alcohol dependence
Glucocorticoid resistance
Morbid obesity
Poorly controlled diabetes mellitus
Unlikely to have any clinical features of Cushing's syndrome
Physical stress (hospitalization, surgery, pain)
Malnutrition, anorexia nervosa
Intense chronic exercise
Hypothalamic amenorrhea
CBG excess (increased serum but not urine cortisol)

[a] Whereas Cushing's syndrome is unlikely in these conditions, it may rarely be present. If there is a high clinical index of suspicion, the patient should undergo testing, particularly those within the first group.

sulting hypercortisolemia. As a result, the overlap in UFC excretion is limited to values up to about 4-fold normal.

2.0 Morbidity and mortality of Cushing's syndrome: rationale for diagnosis and treatment

The earliest reports of mortality in Cushing's syndrome likely described individuals with severe hypercortisolism, representing one end of the clinical spectrum. These reports documented a median survival of 4.6 yr, and in 1952 a 5-yr survival of just 50%, with most deaths caused by vascular (myocardial infarction, cerebrovascular accident) or infectious complications (11, 12). However, with modern-day treatments the standard mortality ratio (SMR) after successful normalization of cortisol was similar to that of an age-matched population during 1–20 yr of follow-up evaluation in one study (13). Because markers of cardiovascular risk remain abnormal for up to 5 yr after surgery, further studies are needed to assess long-term SMR (14). In patients who have persistent moderate hypercortisolism despite treatment, SMR is increased 3.8- to 5.0-fold, compared with the general population (4, 5). These data are consistent with the increased cardiovascular mortality and morbidity reported in patients with iatrogenic Cushing's syndrome secondary to the chronic use of synthetic corticosteroids (15).

Successful treatment of hypercortisolism reverses, but may not normalize, features of Cushing's syndrome. Bone mineral density and cognitive dysfunction improve after successful surgical treatment of Cushing's syndrome but do not normalize in all patients (16, 17). Additionally, quality of life improves after surgical treatment but remains below that of age- and gender-matched subjects for up to 15 yr (18). Indirect evidence supporting the need for intervention includes the finding that the risk of infection is lower in patients with mild to moderate, compared with severe, hypercortisolism (19).

There are limited and conflicting data regarding whether surgical treatment of patients with mild hypercortisolism in the setting of an adrenal incidentaloma is superior to medical treatment of comorbidities alone (20–23).

Although there are no formal controlled studies of consequences of cure in pediatric Cushing's syndrome, improvements in growth and body composition after treatment are reported in both patients with adrenal and those with pituitary causes (24, 25). Final stature in patients with endogenous Cushing's syndrome was reported to be disappointing (26), but more recent data showed that most patients reach a final height within their predicted parental target range (24).

Treatment of patients with moderate to severe Cushing's syndrome clearly reduces mortality and morbidity. Because Cushing's syndrome tends to progress and severe hypercortisolism is probably associated with a worse outcome, it is likely that early recognition and treatment of mild disease would reduce the risk of residual morbidity. However, no data addressing this assumption have been reported.

Our recommendations for testing for Cushing's syndrome are based on direct evidence from observational studies indicating a large treatment effect (which we have rated as low to moderate quality evidence) on morbidity and mortality in patients diagnosed with the condition. The next section of this document focuses on evidence that bears indirectly on these recommendations. The research in this area yields data on the likelihood of Cushing's syndrome in certain populations and on the accuracy of currently available tests in these populations. As a result, the majority of our recommendations are based on very low- to low-quality evidence. Higher-quality evidence to support testing should come from studies directly comparing the effect of testing strategies on patient-important outcomes. To date such evidence is not available in this field.

These guidelines focus on the more common clinical scenarios, with brief mention of conditions and situations that are rare or more complicated than space limitations allow; we hope that the reader will investigate these further.

3.0 Diagnosis of Cushing's syndrome

Who should be tested

3.1 We recommend obtaining a thorough drug history to exclude exogenous glucocorticoid exposure leading to iatrogenic Cushing's syndrome before conducting biochemical testing (1⊕⊕⊕⊕).

3.2 We recommend testing for Cushing's syndrome in the following groups:

- Patients with unusual features for age (*e.g.* osteoporosis, hypertension) (Table 1) (1⊕⊕◯◯)
- Patients with multiple and progressive features, particularly those that are more predictive of Cushing's syndrome (Table 1) (1⊕⊕◯◯)
- Children with decreasing height percentile and increasing weight (1⊕◯◯◯)
- Patients with adrenal incidentaloma compatible with adenoma (1⊕◯◯◯).

3.3 We recommend against widespread testing for Cushing's syndrome in any other patient group (1⊕◯◯◯).

3.1 Evidence

Features of Cushing's syndrome may occur as a result of exogenous glucocorticoid use. The severity of the Cushingoid features

depends on the potency of the preparation used, its dose, the route and duration of its administration, and whether concomitant medications prolong its half-life (27). A thorough drug history noting current or recent use of these medications, oral, rectal, inhaled, topical, or injected, should be obtained before embarking on any biochemical testing (28). In particular, glucocorticoid components of skin creams (including bleaching agents), herbal medications, "tonics," and joint or nerve injections may be overlooked. Megestrol acetate (medroxyprogesterone acetate) is a synthetic progesterone derivative that has glucocorticoid activity and in high doses may cause Cushing's syndrome (29). Our recommendation is based on high-quality evidence because it derives from the common observation that pursuing the alternative, testing to establish the diagnosis of Cushing's syndrome without first excluding exogenous glucocorticoid use, is associated with a very large risk of undesirable effects (including unnecessary testing and the associated consequences) without expectation of benefit.

3.2 Evidence

Cushing's syndrome is more likely to be present when a large number of signs and symptoms, especially those with high discriminatory index (*e.g.* myopathy, plethora, red striae, easy bruising, and thin skin in the young) are present (6, 8). However, there is a wide spectrum of clinical manifestations at any given level of hypercortisolism. Because Cushing's syndrome tends to progress, accumulation of new features increases the probability that the syndrome is present. A review of old photographs of the patient may help the clinician better appreciate whether physical changes have occurred over time.

In children, the sensitivity of combined reduced linear growth and increased weight is quite high. Although the probability of Cushing's syndrome has not been evaluated in a large number of children, clinical experience suggests that the specificity of these clinical features for the diagnosis is also very high (30). As a result, tests for Cushing's syndrome are not indicated in obese children unless their statural growth rate has slowed.

Clinicians often evaluate patients with an incidentally found adrenal nodule for autonomous adrenal cortisol excess. Such patients usually do not present with overt clinical features of Cushing's syndrome, but biochemical hypercortisolism is present in a large fraction (up to 10%). Bulow *et al.* (31) reported 2% prevalence of Cushing's syndrome; Libe *et al.* (32) reported 18%; Terzolo *et al.* (21) quoted 5–20%, depending on referral bias and diagnostic tests and criteria.

3.3 Evidence

Testing for Cushing's syndrome in certain high-risk populations has shown an unexpectedly high incidence of unrecognized Cushing's syndrome as compared with the general population. Although there are limited data on the prevalence of the syndrome in these disorders, the diagnosis should be considered.

In one study, 2–3.3% of patients with poorly controlled diabetes mellitus had surgically confirmed Cushing's syndrome or mild hypercortisolism. Most of these patients had unilateral adrenal adenomas (33). In another recent report, one of 99 patients with newly diagnosed diabetes mellitus had surgically proven Cushing's disease (34). Another study of 86 consecutive obese subjects referred to an endocrine clinic with diabetes mellitus, hypertension, and/or the polycystic ovary syndrome found a 5.8% incidence of Cushing's syndrome (35).

Screening studies of patients with hypertension reported a 0.5–1% prevalence of Cushing's syndrome (36, 37). Unsuspected Cushing's syndrome also was found in as many as 10.8% of older patients with osteoporosis and vertebral fracture in whom comprehensive testing was done for secondary causes (38). Unfortunately, there is little information on additional comorbidities and risk factors in these studies.

The few data on the outcome, after surgical remission of hypercortisolism, in patients with unsuspected Cushing's syndrome are mixed; hypertension and diabetes did not improve in all individuals (20–23).

Patients with familial disease that puts them at risk of Cushing's syndrome (*e.g.* Carney complex, multiple endocrine neoplasia-1) should be evaluated by an endocrinologist as part of a surveillance screening program.

3.3 Values

Because of the rarity of Cushing's syndrome, the high prevalence of conditions such as diabetes mellitus, obesity, and depression, and the limitations of the screening tests, the risk of false-positive test results is high. False-positive results, with their attendant costs, are reduced if case detection is limited to individuals with an increased pretest probability of having the disorder. The subsequent testing, labeling, and treatment may harm individuals with false-positive results and distract attention from the treatment of the conditions that prompted testing.

The proposed testing strategy places higher value on reducing the number of false-positive test results, particularly in patients with very mild disease in whom the benefits of intervention are unproven. Conversely, once the clinical scenario suggests a high pretest probability of the disorder, sensitivity needs to be high so that cases are not missed. This approach also seeks to use more convenient and less expensive tests.

Initial testing

3.4 For the initial testing for Cushing's syndrome, we recommend one of the following tests based on its suitability for a given patient (Fig. 1) (1⊕○○○):

3.4.1 UFC (at least two measurements)
3.4.2 Late-night salivary cortisol (two measurements)
3.4.3 1-mg overnight DST
3.4.4 Longer low-dose DST (2 mg/d for 48 h)

3.5 We recommend against the use of the following to test for Cushing's syndrome (1⊕○○○):

- Random serum cortisol or plasma ACTH levels
- Urinary 17-ketosteroids
- Insulin tolerance test
- Loperamide test
- Tests designed to determine the cause of Cushing's syndrome (*e.g.* pituitary and adrenal imaging, 8 mg DST).

3.6 In individuals with normal test results in whom the pretest probability is high (patients with clinical features suggestive of

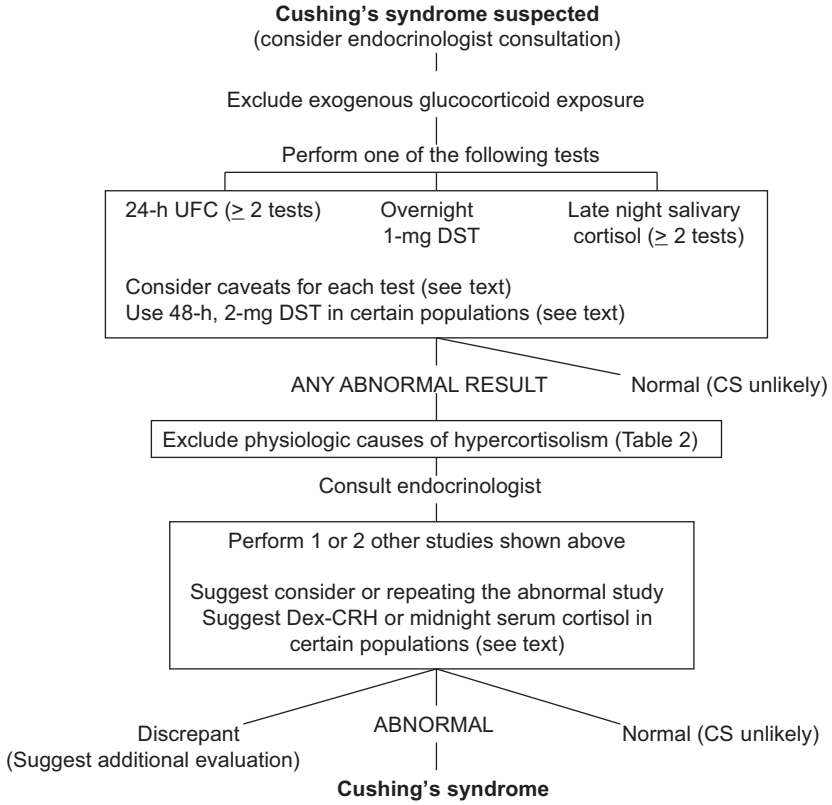

FIG. 1. Algorithm for testing patients suspected of having Cushing's syndrome (CS). All statements are recommendations except for those prefaced by suggest. Diagnostic criteria that suggest Cushing's syndrome are UFC greater than the normal range for the assay, serum cortisol greater than 1.8 μg/dl (50 nmol/liter) after 1 mg dexamethasone (1-mg DST), and late-night salivary cortisol greater than 145 ng/dl (4 nmol/liter).

Cushing's syndrome and adrenal incidentaloma or suspected cyclic hypercortisolism), we recommend further evaluation by an endocrinologist to confirm or exclude the diagnosis (1⊕○○○).

3.7 In other individuals with normal test results (in whom Cushing's syndrome is very unlikely), we suggest reevaluation in 6 months if signs or symptoms progress (2⊕○○○).

3.8 In individuals with at least one abnormal test result (for whom the results could be falsely positive or indicate Cushing's syndrome), we recommend further evaluation by an endocrinologist to confirm or exclude the diagnosis (1⊕○○○).

3.4 Evidence

In this section, we first discuss the testing strategies and then provide evidence for and remarks about each of the recommended tests that can be used to identify patients with Cushing's syndrome.

Nonendocrinologist clinicians may perform the initial evaluation for Cushing's syndrome (or refer to an endocrinologist). In this setting, the goal is to choose a test with a high sensitivity for the disorder; unfortunately, no test has optimally high specificity, so that false-positive results may occur. The four recommended tests have acceptable diagnostic accuracy when the suggested cutoff points are used (2, 30). If the initial testing results are normal, assuming that there is no reason to mistrust the result (see remarks below), then the patient is very unlikely to have Cushing's syndrome. Thus, the patient can be reassured and no further testing need be done; a recommendation to return in 6 months if symptoms progress ensures that evolving symptoms or new features will not be ignored.

In patients with a high pretest probability of Cushing's syndrome, to expedite diagnosis, the physician may elect to perform two tests simultaneously.

3.4 Remarks for all tests

Measurement of cortisol (urine, serum, or salivary) is the end point for each of the recommended tests. As with all hormone assays, the physician must be aware that several collection and assay methods are available for the measurement of cortisol, and results for a single sample measured in various assays may be quite different (39). Assays differ widely in their accuracy; results near the cutoff value on a single measurement often can be explained by assay variability. In particular, the expected salivary and serum concentrations in these tests are close to the functional limit of detection of the assays. Because precision deteriorates at these levels, assays should be chosen on the basis of their performance at this low range.

Normal ranges vary substantially, depending on the method used, so it is essential to interpret test results in the context of the appropriate normal range. Antibody-based immunoassays such as unextracted RIA and ELISA can be affected by cross-reactivity with cortisol metabolites and synthetic glucocorticoids. In contrast, structurally based assays such as HPLC and tandem mass spectrometry (LC-MS/MS) do not pose this problem and are being used with increasing frequency. However, there are also drugs (carbamazepine and fenofibrate) that may interfere with some of these chromatographic methods (Table 3), thereby causing falsely elevated values (40, 41). Upper limits of normal are much lower with HPLC or LC-MS/MS than in antibody-based assays. For example, urine cortisol values obtained using HPLC may be as low as 40% of the value measured by RIA (42, 43).

Estrogens increase the cortisol-binding globulin (CBG) concentration in the circulation. Because serum assays measure total cortisol, false-positive rates for the overnight DST are seen in 50% of women taking the oral contraceptive pill (44). Wherever possible, estrogen-containing drugs should be withdrawn for 6 wk before testing or retesting (45). Conversely, decreases in CBG or albumin, which occur in the critically ill or nephrotic patient, are associated with decreased serum cortisol values (39, 46).

Because the hypercortisolism of Cushing's syndrome can be variable, we recommend that at least two measurements of urine or salivary cortisol be obtained. This strategy increases confidence in the test results if consistently normal or abnormal results are obtained.

TABLE 3. Selected drugs that may interfere with the evaluation of tests for the diagnosis of Cushing's syndrome[a]

Drugs
Drugs that accelerate dexamethasone metabolism by induction of CYP 3A4
Phenobarbital
Phenytoin
Carbamazepine
Primidone
Rifampin
Rifapentine
Ethosuximide
Pioglitazone
Drugs that impair dexamethasone metabolism by inhibition of CYP 3A4
Aprepitant/fosaprepitant
Itraconazole
Ritonavir
Fluoxetine
Diltiazem
Cimetidine
Drugs that increase CBG and may falsely elevate cortisol results
Estrogens
Mitotane
Drugs that increase UFC results
Carbamazepine (increase)
Fenofibrate (increase if measured by HPLC)
Some synthetic glucocorticoids (immunoassays)
Drugs that inhibit 11β-HSD2 (licorice, carbenoxolone)

[a] This should not be considered a complete list of potential drug interactions. Data regarding CYP3A4 obtained from http://medicine.iupui.edu/flockhart/table.htm.

Remarks for dexamethasone tests

Variable absorption and metabolism of dexamethasone may influence the result of both the overnight 1-mg DST and the 48-h, 2 mg/d test. Drugs such as phenytoin, phenobarbitone, carbamazepine, rifampicin, and alcohol induce hepatic enzymatic clearance of dexamethasone, mediated through CYP 3A4, thereby reducing the plasma dexamethasone concentrations (Table 3) (47). Conversely, dexamethasone clearance may be reduced in patients with liver and/or renal failure. Dexamethasone levels show interindividual variation, however, even in healthy individuals on no medication.

To evaluate for false-positive and negative responses, some experts have advocated simultaneous measurement of both cortisol and dexamethasone for these tests to ensure adequate plasma dexamethasone concentrations [>5.6 nmol/liter (0.22 μg/dl)] (48). However, given the limited availability outside the United States and cost of the dexamethasone assay, this otherwise desirable approach may not be feasible.

As noted above, false-positive rates for the overnight DST are seen in 50% of women taking the oral contraceptive pill because of increased CBG levels (44).

3.4.1 Evidence for use of UFC

The introduction of UFC represented a major advance over measurement of 17-hydroxycorticosteroids (17OHCS), which reflects both urine metabolites and cortisol. Because 17OHCS has high rates of false-positive and negative results, it is now rarely used.

Since the 1970s, experts have advocated the use of UFC for making the diagnosis of Cushing's syndrome (49, 50). UFC provides an integrated assessment of cortisol secretion over a 24-h period. It measures the cortisol that is not bound to CBG, which is filtered by the kidney unchanged. Therefore, unlike serum cortisol, which measures both CBG-bound and free hormone, UFC is not affected by conditions and medications that alter CBG. For example, healthy women taking oral estrogen may have increased CBG, and therefore high serum cortisol concentration, but their UFC remains normal. Because cortisol production is increased in Cushing's syndrome, the amount of unbound hormone is higher, resulting in elevated UFC values.

As with any other test, sensitivity and specificity of UFC are subject to the cutoffs selected. When the assay upper limit of normal is used as a criterion, the overall evidence supports the diagnostic accuracy of UFC in adults suspected of having Cushing's syndrome (2, 51). Sensitivity for Cushing's syndrome in pediatric patients is high (~89%) (30).

Thus, to achieve the goal of high sensitivity, we recommend using the upper limit of normal for the particular assay as the criterion for a positive test, provided the creatinine shows that the collection is complete and there is not excessive volume. For pediatric patients, the adult normal ranges may be used because most pediatric patients are of adult weight (*i.e.* > 45 kg).

At the recommended cutoff point, false-positive elevations of UFC may be seen in several conditions. High fluid intake (≥5 liters/d) significantly increases UFC (52). Any physiological or pathological condition that increases cortisol production raises UFC (Table 2). Therefore, in these conditions a normal result is more reliable than an abnormal one.

At the recommended cutoff point, false-negative results of urine cortisol collections also may occur. Because UFC reflects renal filtration, values are significantly lower in patients with moderate to severe renal impairment. A falsely low UFC can occur when creatinine clearance falls less than 60 ml/min, and UFC levels fall linearly with more severe renal failure (53). UFC can be normal if a patient has cyclic disease and collects urine when the disease is inactive. Finally, it may be normal in some patients with mild Cushing's syndrome, in whom salivary cortisol may be more useful (54).

3.4.1 Remarks for UFC

Sample collection and instructions

It is important to ensure that patients provide a complete 24-h urine collection with appropriate total volume and urinary creatinine levels. This may require patient education using both oral and written instructions. The first morning void is discarded so that the collection begins with an empty bladder. All subsequent voids throughout the day and night should be included in the collection, which is kept refrigerated (but not frozen), up to and including the first morning void on the second day. Once the bladder has been emptied into the collection on the second morning, the sample is complete.

Patients should be instructed not to drink excessive amounts of fluid and to avoid the use of any glucocorticoid preparations, including steroid-containing skin or hemorrhoid creams, during

the collection. Because UFC levels in a patient with Cushing's syndrome are variable, at least two collections should be performed, particularly in children in whom reproducibility can be low.

3.4.2 Evidence for late-night salivary cortisol

In healthy individuals with stable conventional sleep-wake cycles, the level of serum cortisol begins to rise at 0300–0400 h, reaches a peak at 0700–0900 h, and then falls for the rest of the day to very low levels when the person is unstressed and asleep at midnight (55). The loss of circadian rhythm with absence of a late-night cortisol nadir is a consistent biochemical abnormality in patients with Cushing's syndrome (56, 57). This difference in physiology forms the basis for measurement of a midnight serum or late-night salivary cortisol.

Biologically active free cortisol in the blood is in equilibrium with cortisol in the saliva, and the concentration of salivary cortisol does not appear to be affected by the rate of saliva production. Furthermore, an increase in blood cortisol is reflected by a change in the salivary cortisol concentration within a few minutes (58). Various methods have been used to measure cortisol in the saliva, resulting in different reference ranges and yielding differences in sensitivity and specificity (59–67). The best-validated assays used in the United States to measure salivary cortisol are an ELISA and an assay performed by LC-MS/MS (28). When these two assay techniques are used, normal subjects usually have salivary cortisol levels at bedtime, or between 2300 and 2400 h, of less than 145 ng/dl (4 nmol/liter). Using a variety of assays and diagnostic criteria, investigators from different countries have reported that late-night salivary cortisol levels yield a 92–100% sensitivity and a 93–100% specificity for the diagnosis of Cushing's syndrome (59–67). Overall, the evidence in adults suggests that the accuracy of this test is similar to that of UFC (2). This easily performed, noninvasive test has been used in children to differentiate patients with Cushing's syndrome from those with simple obesity. Investigators have reported high sensitivity (100%) and specificity (95.2%) for Cushing's syndrome in this setting (68).

The influence of gender, age, and coexisting medical conditions on the late-night salivary cortisol concentrations has not been fully characterized. It is important to note that the circadian rhythm is blunted in many patients with depressive illness and in shift workers (69, 70) and may be absent in the critically ill (71). Other populations may have a high percentage of false-positive results. For example, in a study of men aged 60 yr or older, Liu *et al.* (72) reported that 20% of all participants and 40% of diabetic hypertensive subjects had at least one elevated late-night salivary cortisol measurement. Using the upper reference range of each assay as the cutoff point, Baid *et al.* (28) measured bedtime salivary cortisol levels in a large number of obese subjects and found a specificity of only 85% when they used a RIA technique, but a better specificity of 92% when tandem mass spectrometry was used.

3.4.2 Remarks for late-night salivary cortisol

Most clinicians using the late-night salivary cortisol test ask patients to collect a saliva sample on two separate evenings between 2300 and 2400 h. Saliva is collected either by passive drooling into a plastic tube or by placing a cotton pledget (salivette) in the mouth and chewing for 1–2 min. The sample is stable at room or refrigerator temperature for several weeks and can be mailed to a reference laboratory. Reports show good correlation between salivary and simultaneous serum cortisol values in healthy volunteers (73, 74). When samples were obtained at the same sitting, those collected using the salivette device had lower cortisol concentrations than those collected from passive drooling, but they correlated better with total and free serum cortisol levels (74).

Several factors that affect the salivary cortisol test should be considered when evaluating the results. The salivary glands express 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts the biologically active cortisol to inactive cortisone (75). It is theoretically possible that individuals using licorice or chewing tobacco (both of which contain the 11β-hydroxysteroid dehydrogenase type 2 inhibitor glycyrrhizic acid) may have a falsely elevated late-night salivary cortisol. Patients who smoke cigarettes also have been shown to have higher late-night salivary cortisol measurements than do nonsmokers (76). Although the duration of this effect is not known, it seems prudent to avoid cigarette smoking on the day of collection. Direct contamination of the salivette by steroid-containing lotion or oral gels also may result in false-positive results. Because the test assumes a nadir of cortisol in the late evening, it may not be appropriate for shift workers or those with variable bedtimes, and the timing of the collection should be adjusted to the time of sleeping for those with bedtimes consistently long after midnight. Similarly, nocturnal salivary cortisols may be transiently abnormal in individuals crossing widely different time zones. Finally, stress immediately before the collection also may increase salivary cortisol physiologically; therefore, ideally, samples should be collected on a quiet evening at home (64).

Theoretically, contamination with blood might increase salivary cortisol levels. Although Kivlighan *et al.* (77) reported that minor to moderate blood leakage as a result of vigorous tooth brushing had no effect on salivary cortisol values, the possible effect of gingivitis or oral sores or injury is not known.

3.4.3 Evidence for the 1-mg DST

In normal subjects, the administration of a supraphysiological dose of glucocorticoid results in suppression of ACTH and cortisol secretion. In endogenous Cushing's syndrome of any cause, there is a failure of this suppression when low doses of the synthetic glucocorticoid dexamethasone are given (78).

The overnight test is a simple outpatient test. Various doses of dexamethasone have been used, but 1 mg dexamethasone is usually given between 2300 and 2400 h, and cortisol is measured between 0800 and 0900 h the following morning. Higher doses (1.5 or 2 mg) do not significantly improve the accuracy of the test (49).

Researchers have used cutoff values for the suppression of serum cortisol from 3.6 to 7.2 μg/dl (100–200 nmol/liter) when measured by modern RIA (79). A widely cited normal response is a serum cortisol less than 5 μg/dl ($<$140 nmol/liter) (7, 80). Because some patients with Cushing's disease demonstrate suppressibility to dexamethasone, use of this diagnostic criterion

misclassified up to 15% of such patients as negative (81, 82). Therefore, to enhance sensitivity, experts have advocated requiring a lower cutoff for suppression of the postdexamethasone serum cortisol to less than 1.8 μg/dl (50 nmol/liter) to achieve sensitivity rates of greater than 95% (83). At the 1.8 μg/dl cutoff, the sensitivity is high with specificity rates of 80%; specificity increases to greater than 95% if the diagnostic threshold is raised to 5 μg/dl (140 nmol/liter) (7). Given our objective of using tests with high sensitivity at this stage, we recommend use of the more stringent cutoff of 1.8 μg/dl.

Overall, the evidence in adults indicates that in studies with low prevalence of Cushing's syndrome this test has similar performance as the others recommended for initial testing (2). Although the 1-mg overnight test is used as a screening test for pediatric patients, there are no specific data regarding its interpretation or performance in this population.

3.4.3 Remarks for the 1-mg DST

See the earlier comments under 3.4 *Remarks for dexamethasone tests*.

3.4.4 Evidence for the 48-h, 2 mg/d DST

Some endocrinologists prefer to use the 48-h, 2 mg/d low-dose DST (LDDST) as an initial test because of its improved specificity as compared with the 1-mg test. With adequate written instructions for the patient, the LDDST is easily performed in the outpatient setting.

As described above (*Section 1.0*), certain psychiatric conditions (depression, anxiety, obsessive compulsive disorder), morbid obesity, alcoholism, and diabetes mellitus can be characterized by overactivation of the HPA axis but without true Cushing's syndrome, *i.e.* hypercortisolism is not autonomous. In these conditions, UFC measurements are less useful as an initial test. The optimal test is the LDDST. Previous studies using various doses of dexamethasone and differing criteria for suppression suggest that at least 2 wk of abstinence from alcohol are needed to reduce the false-positive rate (84).

First described by Liddle (85) in 1960, the LDDST initially evaluated urinary 17OHCS as an indicator of cortisol suppression. However, using 17OHCS or UFC, sensitivity and specificity rates are less than 70–80%. Use of a serum cortisol end point is simpler and has higher diagnostic accuracy (78).

With a cutoff value for suppression of 50 nmol/liter (1.8 μg/dl), the initially reported sensitivity was greater than 95% for adult patients (86). With this approach, the sensitivity for Cushing's syndrome in 36 pediatric patients was 94% (87). With a slightly different protocol and a lower cortisol criterion [38 nmol/liter (1.4 μg/dl)], the sensitivity was 90% in another study (9).

Subsequent reports showed lower diagnostic accuracy of the LDDST (7, 88–90). Overall, in 92 patients without Cushing's syndrome, the specificity of the LDDST was 70% (95% confidence interval 69–87%). In 59 patients with Cushing's syndrome, sensitivity was 96% for the LDDST (91). The reasons for this apparent decrease in specificity are unknown. Serum dexamethasone levels were not evaluated; in healthy volunteers, dexamethasone levels 2 h after the last dose were 13.0 ± 6.1 μmol/liter (469.5 ± 220.4 μg/dl) (92).

Consequently, the overall evidence in adults indicates that this test has similar or slightly less diagnostic accuracy than the other tests recommended here for initial testing (2).

3.4.4 Remarks for the 48-h, 2 mg/d DST

In addition to the general remarks on dexamethasone tests presented in the *Initial testing* section, there are further considerations for the LDDST. Dexamethasone is given in doses of 0.5 mg for 48 h, beginning at 0900 h on d 1, at 6-h intervals, *i.e.* at 0900, 1500, 2100, and 0300 h. Serum cortisol is measured at 0900 h, 6 h after the last dose of dexamethasone. Yanovski *et al.* (9) proposed a different protocol: administering 48 h of dexamethasone at 6-h intervals but beginning at 1200 h and obtaining serum cortisol at 0800 h, exactly 2 h (rather than 6 h as in the usual protocol) after the last dexamethasone dose.

For pediatric patients weighing more than 40 kg, the initial adult protocol described above and the adult threshold for normal suppression [<50 nmol/liter (1.8 μg/dl)] are used. For patients weighing less than 40 kg, the dose is adjusted to 30 μg/kg·d (in divided doses) (87).

3.5 Evidence

The diagnostic accuracy of various other tests previously advocated for the diagnosis of Cushing's syndrome (urinary 17-ketosteroids, 1600 h or other random cortisol levels, and the insulin tolerance test) is too low to recommend them for testing (49). Other tests, such as the loperamide test, have insufficient evidence for their diagnostic accuracy. The response to those tests used specifically to establish the cause of Cushing's syndrome (*e.g.* pituitary, adrenal or thoracic imaging, plasma ACTH concentration, CRH stimulation test, 8 mg dexamethasone suppression test) may be both abnormal in healthy people and normal in patients with Cushing's syndrome and therefore are not helpful in establishing the diagnosis (78).

3.6–3.8 Evidence

Our recommendations for retesting patients with initially normal test results who develop new or progressive signs or symptoms of Cushing's syndrome comes from the panel's clinical observations and relate to the recognition that the patient's pretest probability of Cushing's syndrome would be higher on retesting and that hypercortisolism may have evolved concomitantly with the progression of the clinical syndrome, enhancing the likelihood that repeat tests would be positive.

Similarly, the recommendation to retest patients with suspected cyclic Cushing's syndrome comes from the recognition that these individuals may have normal test results when the disorder is quiescent (93).

The performance and interpretation of subsequent testing for Cushing's syndrome requires considerable expertise (both in the clinic and in the laboratory) and may be followed by either complex testing to establish its cause and surgical treatments or expert reassurance of patients that they do not have this condition. Because of this, it is the panel's observation that referral to endocrinology centers with expertise and interest in Cushing's syndrome in patients with abnormal initial testing is likely to be associated with better patient outcomes.

The recommendation to perform additional testing in patients with discordant results derives from the knowledge that some patients with Cushing's syndrome, usually those with mild or cyclic disease, may have discordant results. Also, some patients without Cushing's syndrome may have only a minimally abnormal but discordant result. The distinction between these groups is difficult, and there is no one correct diagnostic strategy. The test results' validity should be evaluated in light of the caveats mentioned for specific patient situations and for each test and assay. For example, an abnormal UFC may not be accepted if the specimen volume and creatinine suggest overcollection. Underlying disorders that may cause mild hypercortisolism (Table 2) should be considered and testing repeated when these are treated or resolved. Postponing additional testing to allow progression of clinical and biochemical features may be useful. The patient should be reassured that this poses minimal risk in the setting of mild hypercortisolism.

Subsequent evaluation

3.8 For the subsequent evaluation of abnormal test results from one of the high-sensitivity tests, we recommend performing another recommended test (Fig. 1, 1⊕○○○).

3.8.1 We suggest the additional use of the dexamethasone-CRH test or the midnight serum cortisol test in specific situations (Fig. 1, 1⊕○○○).

3.8.2 We suggest against the use of the desmopressin test, except in research studies, until additional data validate its utility (2⊕○○○).

3.8.3 We recommend against any further testing for Cushing's syndrome in individuals with concordantly negative results on two different tests (except in patients suspected of having the very rare case of cyclical disease) (1⊕○○○).

3.8.4 We recommend tests to establish the cause of Cushing's syndrome in patients with concordantly positive results from two different tests, provided there is no concern regarding possible non-Cushing's hypercortisolism (Table 2) (1⊕⊕○○).

3.8.5 We suggest further evaluation and follow-up for the few patients with concordantly negative results who are suspected of having cyclical disease and also for patients with discordant results, especially if the pretest probability of Cushing's syndrome is high (2⊕○○○).

3.8 Remarks

If the initial test result is abnormal, further evaluation by an endocrinologist will ensure that the disorder is confirmed or refuted and that the possibility of a false-positive result will be considered.

Conversely, in cases in which there is a high pretest probability of Cushing's syndrome but a normal initial test, use of an additional alternative test has the potential benefit of disclosing those with milder disease.

3.8.1 Evidence for the 48-h, 2 mg/d LDDST with CRH

In an effort to improve the sensitivity of the 48-h, 2 mg/d test, researchers developed a combined CRH stimulation test. In theory, dexamethasone suppresses serum cortisol levels in individuals without Cushing's syndrome as well as a small number of those with Cushing's disease, but if given CRH, patients with Cushing's disease should respond with an increase in ACTH and cortisol. The test is done by administering the 48-h 2 mg/d DST, followed by administration of CRH (1 μg/kg, iv) 2 h after the last dose of dexamethasone. Cortisol is measured 15 min later.

The initial report of this strategy showed high diagnostic accuracy (92, 94). All eight of 59 patients with proven Cushing's disease who suppressed pre-CRH cortisol to less than 1.4 μg/dl (<38 nmol/liter; sensitivity 86%) were properly characterized after CRH administration.

Subsequent reports showed lower diagnostic accuracy of both the DST and the combined test (7, 88–90). Overall, in 92 patients without Cushing's syndrome, the specificity of the LDDST was 70% (95% confidence interval 69–87%), compared with a 60% specificity for the dexamethasone-CRH test (95% confidence interval 59–79%). In 59 patients with Cushing's syndrome, sensitivity was 96% for the LDDST and 98% for the dexamethasone-CRH test.

The reasons for the differences in the responses to the LDDST and the combined test are not clear. As discussed above, any dexamethasone test may give either false-positive or false-negative results in conditions that alter the metabolic clearance of the agent; additionally, differences in the performance of cortisol assays may contribute.

3.8.1 Remarks for the dexamethasone-CRH test

The dexamethasone-CRH test can be useful in patients with equivocal results for UFC. A dexamethasone level should be measured at the time of CRH administration to exclude a false-positive result, and the serum cortisol assay must be accurate at these low levels of detection. Additionally, it is possible that the 2-h time interval between dexamethasone and CRH administration is critical so that compliance must be assured.

In the United States, ovine-sequence CRH is available commercially (ACTHREL; Ferring Corp., Malmo, Sweden) with Food and Drug Administration-approved labeling for the differential diagnosis of Cushing's syndrome. In Europe, the human-sequence peptide is in widespread use (Ferring) but has lower stimulatory effect than the ovine-sequence CRH (95).

3.8.1 Evidence for the midnight serum cortisol test

As noted above, the nocturnal nadir of serum cortisol values is lost in patients with Cushing's syndrome, forming the basis of this test. Because the test is cumbersome to perform, we do not suggest its use in initial testing for Cushing's syndrome. However, the test may be useful in specific situations detailed below. Midnight serum cortisol may be assessed in the sleeping or awake state, using different diagnostic criteria. As with all tests, use of a higher diagnostic criterion is associated with reduced sensitivity but increased specificity.

Sleeping midnight serum cortisol

In one study, a single sleeping serum cortisol greater than 1.8 μg/dl (>50 nmol/liter) had high sensitivity (100%) for the diagnosis of Cushing's syndrome (96). More recent larger studies confirm the poor specificity for this criterion (20.2%), with a cutoff point of 7.5 μg/dl having higher specificity (87%) (7).

In 105 children with Cushing's syndrome, measurement of sleeping midnight cortisol had higher sensitivity than UFC (99 *vs.* 88%) (30).

When used in patients with a high clinical index of suspicion of Cushing's syndrome and who had normal UFC and full suppression on dexamethasone testing, a sleeping midnight serum cortisol of greater than 1.8 μg/dl or an awake value of greater than 7.5 μg/dl increases the probability of Cushing's syndrome (96). Conversely, where there is a low clinical index of suspicion, such as in simple obesity, but lack of suppression on dexamethasone testing and mildly elevated UFC, a sleeping midnight serum cortisol less than 1.8 μg/dl effectively excludes Cushing's syndrome at the time of assessment (7). The midnight serum cortisol test also has utility in the context of failure of suppression on dexamethasone testing due to anticonvulsant medication, in which a sleeping midnight serum cortisol less than 1.8 μg/dl has been used to exclude Cushing's syndrome (97). It is likely that similar values for awake measurements would have similar utility, but this has not been tested directly. Overall, the evidence in adult patients for the midnight serum cortisol accuracy is limited and inconsistent across studies, with at least one study showing that this test can enhance the accuracy of the UFC and 1-mg dexamethasone tests (2).

Awake midnight serum cortisol

Sampling for midnight serum cortisol when the patient is awake is far easier. Initial studies suggested that an awake midnight serum cortisol greater than 7.5 μg/dl (>207 nmol/liter) had a sensitivity and specificity greater than 96% (98, 99). However, when applied to an obese cohort, the specificity was only 83% (100). In an effort to improve on specificity, higher cutoff points have been advocated, inevitably at the cost of sensitivity: values of serum midnight cortisol greater than 8.3–12 μg/dl had 90–92% sensitivity with specificity of 96% (63, 101).

3.8.1 Remarks for the midnight serum cortisol test

The sleeping midnight cortisol requires inpatient admission for a period of 48 h or longer to avoid false-positive responses due to the stress of hospitalization; this approach may not be possible in some practice settings. If a sleeping value is desired, the blood sample must be drawn within 5–10 min of waking the patient, or through an indwelling line, to avoid false-positive results (96).

Young children may have their cortisol nadir earlier than midnight. In children, precatheterization is essential so that a sleeping sample for serum cortisol can be obtained.

3.8.2 Remarks for the desmopressin stimulation test

The desmopressin stimulation test involves measurement of plasma ACTH just before and 10, 20, and 30 min after iv administration of 10 μg 1-desamino-8-D-arginine vasopressin. In general, patients with Cushing's disease show an increase in ACTH, but those with other causes of Cushing's syndrome or those without Cushing's syndrome do not respond (7, 22, 102). The sensitivity for patients with Cushing's disease was 82–87%; when other patients with Cushing's syndrome were included, the sensitivity was 63–75%. The specificity ranged from 85 to 91%. Until additional data validate the utility of the test in a larger population of patients with all causes of Cushing's syndrome, it seems prudent to restrict this test to research studies.

4.0 Special populations/considerations

4.1 Pregnancy: We recommend the use of UFC and against the use of dexamethasone testing in the initial evaluation of pregnant women (1⊕⊕⊕◯).

4.2 Epilepsy: We recommend against the use of dexamethasone testing in patients receiving antiepileptic drugs known to enhance dexamethasone clearance and recommend instead measurements of nonsuppressed cortisol in blood, saliva, or urine (1⊕⊕⊕◯).

4.3 Renal failure: We suggest using the 1-mg overnight DST rather than UFC for initial testing for Cushing's syndrome in patients with severe renal failure (2⊕◯◯◯).

4.4 Cyclic Cushing's syndrome: We suggest use of UFC or midnight salivary cortisol tests rather than DSTs in patients suspected of having cyclic Cushing's syndrome (2⊕◯◯◯).

4.5 Adrenal incidentaloma: We suggest use of the 1-mg DST or late-night cortisol test, rather than UFC in patients suspected of having mild Cushing's syndrome (2⊕⊕◯◯).

4.1 Evidence for choice of tests in pregnant women

Screening for hypercortisolism is more difficult in pregnancy, particularly in the second and third trimesters. UFC excretion is normal in the first trimester; however, it increases up to 3-fold by term to overlap values seen in women with Cushing's syndrome (103). Thus, only UFC values in the second or third trimester greater than 3 times the upper limit of normal can be taken to indicate Cushing's syndrome. Serum cortisol circadian variation is preserved in normal pregnancy, albeit with a higher midnight nadir. Whereas loss of circadian variation is characteristic of Cushing's syndrome, diagnostic thresholds for evening serum or salivary cortisol in pregnant patients are not known (103, 104). Furthermore, suppression of serum and urinary cortisol by dexamethasone is blunted in pregnancy (105). Thus, dexamethasone testing has an increased potential for false-positive results in pregnancy.

4.2 Evidence for choice of tests in patients receiving anticonvulsants

As discussed above (see 3.4 *Remarks for dexamethasone tests*), commonly used anticonvulsant medications, including phenytoin, phenobarbitone, and carbamazepine, induce hepatic enzymatic clearance of dexamethasone, mediated through CYP 3A4, and may cause false-positive responses on testing. There are, however, no data to guide the length of time needed after withdrawal of such medication to allow dexamethasone metabolism to return to normal, and such a medication change may not be clinically possible. Switching to nonenzyme-inducing medication may correct this situation, but an alternative and more practical approach is to use another test, such as assessment of midnight salivary or serum cortisol, to exclude Cushing's syndrome in these patients (97).

4.3 Evidence for choice of tests in chronic renal failure

As noted above (see 3.4.1), excreted urine cortisol values decrease below creatinine clearance of 60 ml/min and are quite low,

below 20 ml/min (53). Although the cortisol circadian rhythm was present in one study, neither serum nor salivary midnight cortisol concentrations have been reported in this population (106). However, serum free cortisol values measured over a 24-h period were reported to be elevated (106). As a result, a normal (low) midnight cortisol value probably excludes Cushing's syndrome, but the diagnostic threshold for either serum or salivary cortisol is not known. The absorption and metabolism of 1 mg dexamethasone, as well as the cortisol response, have been reported to be both normal and abnormal (107–109). Responses to administration of 3 and 8 mg dexamethasone were normal in some but not all patients (106, 108). In the absence of additional data, a normal response to 1 mg dexamethasone is likely to exclude Cushing's syndrome, but an abnormal response is not diagnostic.

4.4 Evidence for choice of tests in cyclic Cushing's syndrome

Rarely patients have been described with episodic secretion of cortisol excess in a cyclical pattern with peaks occurring at intervals of several days to many months (93). Because the DST results may be normal in patients who are cycling out of hypercortisolism, these tests are not recommended for patients suspected of having cyclic disease. Instead, measurement of UFC or salivary cortisol may best demonstrate cyclicity. In patients for whom clinical suspicion is high but initial tests are normal, follow-up is recommended with repeat testing, if possible to coincide with clinical symptoms.

4.5 Evidence for choice of tests in adrenal incidentaloma

UFC appears to be less sensitive than the 1-mg DST or late-night cortisol for the identification of Cushing's syndrome in this population (20–23). There is no consensus on the best algorithm or the best diagnostic criterion for the 1-mg DST. A suppressed ACTH or dehydroepiandrosterone sulfate concentration supports the diagnosis of Cushing's syndrome in patients with adrenal masses (20–23). Measurement of ACTH or dehydroepiandrosterone sulfate is not part of initial diagnostic evaluation of a patient presenting with clinical features of Cushing's syndrome, but it may indicate subtle adrenal hyperfunction in this specific population.

5. Future directions and recommended research

The evidence on which many of these recommendations have been made is of low to very low quality because there are limited data linking diagnostic strategies to patient outcomes as much of the work has focused on developing, validating, and ascertaining diagnostic test performance. This focus may be due to the rarity of the disease and the availability of diverse diagnostic methods. In addition, published data, which are often from larger tertiary referral centers, might be biased toward more diagnostically challenging cases, higher pretest probability, and greater disease severity. Such bias may result in an overly sanguine view of the diagnostic performance of these tests, particularly compared with their expected performance in unselected populations in usual clinical practice. These issues highlight the need for further research and for improvements in the research methods used to determine whether testing will lead to improved patient outcomes.

Investigation in the following areas would significantly improve the future care of patients with hypercortisolism:

1. *Pooled information*. A commitment from endocrinologists supported by national and international endocrine organizations and funding agencies to establish databases of consecutive patients tested for Cushing's syndrome allowing for prospective pooling of the diagnostic test information. This pooled information would help to define discriminatory symptoms and signs and provide data on the most accurate testing strategies.

2. *Standardization of assays*. The diagnosis of Cushing's syndrome is critically dependent on the quality and performance of cortisol assays, be they from serum, saliva, or urine and measured by RIA, ELISA, or LC-MS/MS. Clinicians need a greater appreciation of the robustness (or otherwise) of their particular assay and its variance from published cutoff data. National laboratories of excellence might be used as referral centers in difficult cases; approval by the health authorities/insurance companies for such use would be important.

3. *Improved clinical outcome data and targeted clinical trials*. Initial testing for hypercortisolism may be desirable to the extent that its results will favorably affect outcomes that matter to patients. There is a pressing need to investigate outcomes in patients cured of Cushing's syndrome with modern-day practice. In particular, there are conflicting data on the need to treat mild or so-called subclinical Cushing's syndrome, notably in patients with adrenal incidentalomas. Appropriately powered and rigorously designed randomized clinical trials to compare diagnostic-treatment strategies should be established to inform clinicians and patients on optimal management.

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