Pitfalls in the diagnosis and management of Cushing’s syndrome

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Despite many recent advances, the management of patients with Cushing’s disease continues to be challenging. Cushing’s syndrome is a complex metabolic disorder that is a result of excess glucocorticoids. Excluding the exogenous causes, adrenocorticotropic hormone–secreting pituitary adenomas account for nearly 70% of all cases of Cushing’s syndrome. The suspicion, diagnosis, and differential diagnosis require a logical systematic approach with attention paid to key details at each investigational step. A diagnosis of endogenous Cushing’s syndrome is usually suspected in patients with clinical symptoms and confirmed by using multiple biochemical tests. Each of the biochemical tests used to establish the diagnosis has limitations that need to be considered for proper interpretation. Although some tests determine the total daily urinary excretion of cortisol, many others rely on measurements of serum cortisol at baseline and after stimulation (e.g., after corticotropin-releasing hormone) or suppression (e.g., dexamethasone) with agents that influence the hypothalamic-pituitary-adrenal axis. Other tests (e.g., measurements of late-night salivary cortisol concentration) rely on alterations in the diurnal rhythm of cortisol secretion. Because more than 90% of the cortisol in the circulation is protein bound, any alteration in the binding proteins (transcortin and albumin) will automatically influence the measured level and confound the interpretation of stimulation and suppression data, which are the basis for establishing the diagnosis of Cushing’s syndrome. Although measuring late-night salivary cortisol seems to be an excellent initial test for hypercortisolism, it may be confounded by poor sampling methods and contamination. Measurements of 24-hour urinary free-cortisol excretion could be misleading in the presence of some pathological and physiological conditions. Dexamethasone suppression tests can be affected by illnesses that alter the absorption of the drug (e.g., malabsorption, celiac disease) and by the concurrent use of medications that interfere with its metabolism (e.g., inducers and inhibitors of the P450 enzyme system). In this review, the authors aim to review the pitfalls commonly encountered in the workup of patients suspected to have hypercortisolism. The optimal diagnosis and therapy for patients with Cushing’s disease require the thorough and close coordination and involvement of all members of the management team.

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mechanical effects of the macroadenoma on surrounding structures. Such effects include headaches, visual symptoms, and impairment of normal pituitary function.5

Even though the clinical, biochemical, and imaging characteristics of patients with Cushing’s disease have been well appreciated for decades, the diagnosis and management of this disease are often challenging.10,20,36 Patients with Cushing’s disease exhibit an increased mortality rate and increased morbidity secondary to chronic hypercortisolism.44 Deleterious effects of hypercortisolism include hypertension, obesity, osteoporosis, fractures, impaired immune function and wound healing, and glucose intolerance and diabetes.5,6,36,44 The high morbidity and mortality rates in those with this disease make it imperative to optimize the management of such patients.

Although patients with various illnesses may have cushingoid features, some features that should raise concern for true Cushing’s syndrome include proximal muscle weakness, multiple ecchymosis, prominent supraclavicular fat pads, violaceous striae, hypokalemia, unexplained osteoporosis, new-onset hypertension, and diabetes mellitus.10,44 These clinical manifestations are observed in most patients with hypercortisolism. The known common causes of hypercortisolism are listed in Table 1, with the most common being oral, intrarticular, intramuscular, inhalational, or topical administration of exogenous glucocorticoids used for the treatment of various illnesses.

Once the diagnosis of Cushing’s syndrome is clinically suspected, it should be confirmed or satisfactorily ruled out because of its association with increased risks of morbidity and mortality. The following 3 approaches are used to screen for hypercortisolism: assessment of cortisol secretion in a 24-hour period, documentation of the loss of normal diurnal variation in cortisol secretion (late-night salivary cortisol), and documentation of a loss of feedback inhibition of cortisol on the hypothalamic-pituitary-adrenal (HPA) axis (dexamethasone suppression testing). It is not unusual to require multiple tests to make the diagnosis. In this article, we review the pitfalls encountered in establishing the diagnosis of hypercortisolism in general and that of Cushing’s disease in particular and emphasize the limitations in interpreting the tests and biochemical and imaging studies commonly used.

### Overview of Biochemical Testing for Hypercortisolism

The evaluation and management of suspected Cushing’s syndrome should be performed by an experienced endocrinologist. There are a few conditions that are associated with some clinical features and at times even mild hypercortisolism but lack other features of Cushing’s syndrome.3,7,10,24,36,40,48 These conditions are referred to as pseudo-Cushing’s syndrome, which can be caused by stressful events, hospitalization, intense exercise, critical illness, pregnancy, depression, anorexia, alcohol dependence, and poorly controlled diabetes mellitus. Many patients with pseudo-Cushing’s syndrome present with some degree of hypercortisolism that may biochemically mimic that observed in patients with true Cushing’s syndrome.

The diagnosis of Cushing’s syndrome is made by demonstrating unequivocal evidence of hypercortisolism, usually through the combination of initial tests and confirmatory studies, if needed. The 3 approaches most often used to evaluate for hypercortisolism are: 1) assessing cortisol excretion in a 24-hour period, 2) documenting loss of feedback inhibition of cortisol on the HPA axis with dexamethasone suppression testing, and 3) documenting the loss of normal diurnal variation in cortisol secretion with late-night salivary cortisol measurement.10

In evaluating patients with clinical features suggestive of Cushing’s syndrome, one can start with any of the previously mentioned approaches.10 In general, 2 of the 3 tests are needed to confirm the diagnosis. The measurement of 24-hour urine cortisol excretion is the gold-standard test for diagnosing Cushing’s syndrome, because it provides an estimate of cortisol production, which is a central feature of Cushing’s syndrome. A 3-fold to 4-fold increase over normal values is diagnostic of Cushing’s syndrome; if this increase is present, no additional testing is required to confirm the diagnosis. For less dramatic increases in the urinary free-cortisol level, another diagnostic approach for hypercortisolism, such as the overnight dexamethasone suppression test or the measurement of late-night salivary cortisol concentration, is required.

Confirmatory testing is needed also when initial study results are suggestive but equivocal for hypercortisolism. If not performed as an initial study, the 24-hour urinary free-cortisol test remains the gold-standard confirmatory test. The standard (as opposed to the overnight) low-dose dexamethasone suppression test, also known as the Liddle test,31 may also be used as a confirmatory study. This test is performed by administering dexamethasone (0.5 mg) every 6 hours for 48 hours, which in normal subjects typically causes the suppression of serum cortisol levels to less than 1.8 μg/dl and of urinary cortisol-excretion levels to less than 20 μg/day, with higher levels being consistent with Cushing’s syndrome.10,17,21,29,47 Occasionally, the low-dose dexamethasone suppression test is combined with the administration of corticotropin-releasing hormone (CRH), and the rise in plasma ACTH and cortisol levels is used as

<table>
<thead>
<tr>
<th>TABLE 1. Common causes of Cushing’s syndrome</th>
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<tr>
<td>Cause</td>
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<tr>
<td>Endogenous Cushing’s syndrome</td>
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<tr>
<td>ACTH dependent</td>
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<td>ACTH-secreting pituitary adenomas</td>
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<td>Ectopic ACTH secretion by tumors</td>
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<td>Bilateral macronodular hyperplasia</td>
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<td>ACTH independent</td>
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<td>Adrenal adenoma</td>
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<tr>
<td>Adrenal carcinoma</td>
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<tr>
<td>Exogenous Cushing’s syndrome</td>
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<td>Administration of glucocorticoids (prednisone, dexamethasone, hydrocortisone)</td>
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<td>Administration of drugs w/ glucocorticoid activity (progestational agents, such as megestrol acetate)</td>
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— = not available.
a tool for establishing the diagnosis of hypercortisolism and differentiating patients with Cushing’s syndrome from those with pseudo-Cushing’s syndrome. In a recent study, Alwani et al. examined the performance of 4 tests and their ability to differentiate patients with Cushing’s syndrome from those with pseudo-Cushing’s syndrome. These tests include overnight dexamethasone suppression, 24-hour urinary free cortisol, late-night serum, or salivary cortisol concentration, and the combined dexamethasone suppression/CRH-stimulation test. They found that the combined dexamethasone suppression/CRH-stimulation test and the late-night cortisol concentration test provided a high diagnostic accuracy in differentiating patients with Cushing’s syndrome from those with pseudo-Cushing’s syndrome. Other tests, such as CRH stimulation alone or in combination with desmopressin, are used occasionally in cases with equivocal results. These tests should be performed by endocrinologists experienced in the management of patients with this condition.

After hypercortisolism is firmly established, the cause should be explored further to determine whether it is adrenal, pituitary, or ectopic. The timing of ACTH measurement is not important as long as the state of hypercortisolism is already established. For example, measurements of the plasma ACTH level can differentiate ACTH-dependent (inappropriately normal or elevated ACTH levels, as with a pituitary adenoma) from ACTH-independent (low [< 5 pg/ml] or undetectable ACTH levels, as with adrenal neoplasms) states of hypercortisolism. The ACTH level should be measured simultaneously with the cortisol level. Other approaches used to determine the cause of hypercortisolism include dynamic testing with higher doses of dexamethasone (e.g., 8 mg/day). The value of the high-dose (2 mg every 6 hours for 48 hours) dexamethasone suppression test is limited, however, because of the significant overlap in responses observed among patients with different forms of Cushing’s syndrome.

Biochemical Studies Used to Establish State of Hypercortisolism and Their Limitations

Measurements of Random and Dexamethasone-Suppressed Serum Cortisol Levels

A random serum cortisol level is often obtained by primary physicians as a first step in the workup of patients suspected to have Cushing’s syndrome. In this respect, it is rarely useful and has many limitations that need to be addressed. A random serum cortisol concentration usually reflects the activity of the HPA function at the time of the measurement. The HPA activity at any point in time is an integration of all stimuli and inhibitory factors influencing the axis. For example, a stressed individual may likely have a transient elevation in his or her serum cortisol levels throughout the stressful event. The stress can be transient, such as with the phlebotomy, but it can also be more extensive as would be the case in a postoperative period. A practical example of this is critical illness, with which patients are known to have elevated cortisol levels throughout the illness. As will be discussed in the subsequent sections, critically ill patients demonstrate many of the biochemical features observed in patients with Cushing’s syndrome. Thus, the workup and evaluation for possible hypercortisolism should not be performed while patients are under stress.

Using standard and reliable methods for the measurement of serum cortisol concentrations is important for avoiding potential conflicting results. The direct immunoassays and enzyme immunoassays used in the past and still prevalent in some laboratories are fraught by several limitations, especially those that require proper extraction and prepurification. The newer high-pressure liquid chromatography (HPLC) or liquid chromatography tandem mass spectrometry assays are highly specific (especially liquid chromatography tandem mass spectrometry) and provide reliable measurements of cortisol in plasma and urine samples.

There are several other important factors that limit the interpretation of serum cortisol levels. One such serious limitation is the impact of changes in transcortin. All commercially available assays used for the determination of serum cortisol concentrations measure the total serum cortisol levels (protein-bound and free fractions). Because more than 90% of cortisol in the circulation is bound to either transcortin (75%) or albumin (15%–18%), an abnormality in either of these proteins would have a major impact on measured total cortisol concentrations. High estrogen states, such as that occurring with pregnancy or the administration of estrogen (e.g., in women using postmenopausal estrogen-replacement therapy or oral contraceptives), can lead to an increase in the transcortin level and the subsequent rise in measured serum cortisol concentration. A similar increase in transcortin levels is observed in patients with hepatitis, especially those with hepatitis C. Thus, total serum cortisol levels in patients with hepatitis C would be higher than expected as a result of the increase in transcortin and the transcortin-bound cortisol in the circulation.

An increase (e.g., with the use of estrogen) or a decrease (proteinuria) in binding proteins will accordingly alter serum cortisol levels and clearly confound tests that rely on measurements of the hormone in the circulation. Thus, the overnight dexamethasone suppression test gives misleading results in patients with such alterations in the binding protein. Such an effect can be observed in patients who are on oral contraceptives and are suspected to have hypercortisolism. In such patients, the serum cortisol level measured the morning after 1 mg of dexamethasone will be abnormally high and raise suspicion for Cushing’s syndrome, even though the patient may not have the disease. The opposite outcome might be observed in patients with proteinuria, in whom the levels of both binding proteins (transcortin and albumin) are low. In the latter instance, patients with true Cushing’s syndrome may have what seems to be a normal response to dexamethasone. As will be discussed in the next section, measurements of salivary cortisol concentrations may offer a good alternative approach in patients with binding-protein abnormalities. As will be discussed in a subsequent section, alterations in transcortin also influence the interpretation of serum cortisol measured after resection of an ACTH-secreting adenoma.
Salivary Cortisol Concentrations

In healthy persons, cortisol secretion exhibits a diurnal rhythm in which serum cortisol levels reach a nadir late at night and peak in the early morning. Because loss of this diurnal rhythm is a central feature of Cushing’s syndrome, measurement of the nighttime or late-evening cortisol level can be used as a diagnostic tool for patients suspected to have hypercortisolism.15,18,43 Earlier studies required patients to be admitted to the hospital to obtain late-night serum cortisol levels through an indwelling catheter. Over the past 15–20 years, the measurement of salivary cortisol concentration was introduced as an alternative approach to determining abnormalities in the diurnal rhythm of cortisol secretion.

Salivary cortisol is in equilibrium with the free cortisol in the circulation. Changes in the serum free-cortisol levels are paralleled, within minutes, by similar alterations in salivary cortisol concentrations such that one can use the latter as a surrogate of the former. The rate of saliva secretion does not affect the test. A midnight or late-night serum cortisol level in a healthy individual is expected to be quite low under nonstressful conditions and while asleep. However, in a person with Cushing’s syndrome, a loss of this nadir would be diagnostic. Thus, one can ask patients suspected of having Cushing’s syndrome to obtain a sample of saliva at late night and send it to the laboratory the next morning for a measurement of cortisol concentration. It is expected that in normal subjects, the salivary cortisol concentration will be at its lowest at 11 pm to midnight, provided the patient is relaxed, quiet, and ready to retire for the evening at the time the sample is obtained. Any activity at that time may alter the cortisol concentration and provide misleading data. Salivary or serum cortisol concentrations are expected to be increased in any healthy subject who is active or physically or emotionally stressed at the time the sample is obtained, irrespective of whether it was taken at midnight or in the morning. A common misleading result is often encountered in patients who are asked to obtain a sample at midnight but are unable to stay up that late. What some might do is set an alarm clock for midnight, go to sleep, wake up with the clock, and proceed to obtain the sample. The stress associated with awakening suddenly after an alarm is heard is strong enough to activate the HPA axis and result in an increase in cortisol secretion, which, because of the time it was collected, might be misinterpreted as evidence for hypercortisolism. Thus, detailed instructions need to be given to patients performing this test to avoid confounding results. Similarly, one has to be cautious in people with a blunted circadian rhythm, such as night-shift workers, critically ill patients, patients crossing time zones, and extremely depressed patients.

Just like others, this approach has limitations, some of which are serious.30,42,49 As discussed, any activity or excitement at the time the sample is obtained will lead to misleading high values that will raise concern for Cushing’s syndrome. Other confounders include blood contamination, smoking, chewing tobacco, and eating licorice. Licorice can inhibit 11β-hydroxysteroid dehydrogenase type 2, which normally converts cortisol into cortisone.41 Also, sex- and age-specific levels are not fully defined. Contamination with oral products or facial lotions containing a steroid and blood leakage with tooth-brushing can also lead to falsely elevated levels. Bovine hormones normally present in dairy products can cross-react with anti-cortisol antibodies and cause false results. Acidic or high-sugar foods can compromise assay performance by lowering the pH of the sample and influencing bacterial growth.

Twenty-Four-Hour Urinary Free-Cortisol Measurements

This test uses the principle of increased glucocorticoid synthesis with Cushing’s syndrome, and measurements of urinary excretion provide an integral estimate of that increase. Valid 24-hour sample collection requires adequate excretion of creatinine in the urine appropriate for age and sex (15–20 mg/kg in women and 20–25 mg/kg in men). The creatinine excretion progressively decreases after 50 years of age. To improve the yield and accuracy, most clinicians require 2–3 spaced-out collections. The test is difficult to do for many patients, especially those who are active and those who have evening or night jobs. Despite these and other limitations to be discussed, the test often provides definitive data about cortisol excretion and indirectly about its secretion rate.

There are, however, several pitfalls that should be avoided when evaluating such data. The major factor is the adequacy of the urine collection and whether it was a complete or incomplete sample (Table 2). Thus, measurement of urinary creatinine excretion in the same specimen can provide reassurance, because it is relatively constant (10%–15% variation between days), and its excretion is proportionate to muscle mass. Another potential confounding factor is a reduction in the glomerular filtration rate, which leads to lower than expected urinary free-cortisol data and may misclassify some patients performing the test. A urinary tract infection can lead to a decrease in the measured urinary free-cortisol excretion as a result of metabolism by the bacteria in the urine.

A false-positive result can occur with a high fluid intake of > 3.5 liters,33 because the associated high urine volume can affect cortisol reabsorption and/or its metabolism in the kidney.33 Similarly, a false-positive result can occur in those with one of many physiological conditions, such as those associated with pseudo-Cushing’s

| TABLE 2. Drugs and conditions that may influence 24-hour urinary free-cortisol excretion |
|-----------------------------------------------|-----------------------------------------------|
| Drugs/conditions that increase 24-hr excretion | Drugs/conditions that decrease 24-hr excretion |
| Exercise/stress | Proteinuria |
| Carbamazepine (if measured by HPLC) | Fenofibrate (if measured by HPLC) |
| Some synthetic glucocorticoids (immunoassays) | Drugs that inhibit 11β-hydroxysteroid dehydrogenase type 2 (licorice, carbenoxolone) |
| Polyuria | Conditions that decrease 24-hr excretion |
| Low glomerular filtration rate | Urinary tract infection |
syndrome, and in a person who has exercised during the collection period. Drugs or medications that inhibit 11β-hydroxysteroid dehydrogenase type 2 can similarly (Table 2) raise urinary cortisol excretion.41 Such drugs include carbadoxolone and glycyrrhetic acid,41 which is a steroid-like chemical that resides in the root of licorice. It is interesting to note that when HPLC is the assay used for the measurement of urinary free cortisol, some drugs may interfere in the determination and cause an increase in the reported measurement. Such drugs include fenofibrate32 and carbamazepine.19 However, the interference in the measurement of urinary free cortisol by carbamazepine can be avoided when the assay methods include mass spectroscopy in addition to HPLC.19,32 Some patients with Cushing’s syndrome may have a cyclic disease and therefore have a value in the normal range at times. Other rare patients may have a deficiency in one of the adrenal enzymes, such as 21 hydroxylase.11 One of the advantages of the 24-hour urinary free-cortisol test is its usefulness in patients whose transcorin level is increased as a result of using estrogen. In that setting, one can use 24-hour collection and avoid the need to discontinue estrogen therapy for more than 6 weeks before being able to rely on serum cortisol measurements.

**Overnight Dexamethasone Suppression Test**

The serum cortisol level measured the morning after a 1-mg dexamethasone dose given the night before is generally < 1.8 μg/dl in healthy subjects.19,21,47 Although a postdexamethasone level of > 5 μg/dl is clearly suggestive of Cushing’s syndrome, intermediate values require additional testing. The serum cortisol level in approximately 5%–8% of patients with Cushing’s syndrome is suppressed to < 1.8 μg/dl, and additional testing is required to confirm the diagnosis.

In interpreting data from this test, one needs to be aware of the following significant limitations:

1. Because the outcome of the test is decided by determining the serum cortisol level, one needs to address the limitations discussed earlier, specifically, the impact of alterations in binding proteins, such as in patients on estrogen therapy.
2. Poor sleep and excess activity after dexamethasone administration will result in the activation of HPA function such that it can override the attempted suppression by dexamethasone.
3. Poor absorption of dexamethasone, as would occur in patients with malabsorption or Celiac disease and others who have a rapid gastrointestinal transit time, will result in a lower concentration of dexamethasone than anticipated and subsequently lower degrees of suppression. To minimize the impact of this issue, one can measure the dexamethasone level simultaneously with the serum cortisol level to ensure adequate absorption.
4. Because dexamethasone is metabolized by the P450 enzyme system, any condition, particularly the concomitant use of other drugs or agents that alter this system, will accordingly increase or decrease its metabolism. Table 3 provides a list of commonly used drugs that are inducers or inhibitors of the P450 enzyme system. Such an effect alters the responsiveness of the HPA axis and obviously alters the result of the suppression test. Thus, patients on drugs that induce the P450 enzyme system will have a lower concentration of dexamethasone and therefore less cortisol suppression caused by using the 1-mg dose. In contrast, those who are receiving drugs that inhibit the P450 enzyme system will have more suppression that expected.

It is important to emphasize that one should not make the diagnosis of Cushing’s syndrome on the basis of the overnight dexamethasone suppression test only. That is, a positive test is not sufficient to establish the diagnosis of Cushing’s syndrome.

**Standard 48-Hour Dexamethasone Suppression Test**

Some endocrinologists prefer to use a protocol of 0.5 mg of dexamethasone given every 6 hours for 48 hours with measurements of serum and urinary free cortisol on the 2nd day of the test. This format of the test is known as the Liddle test,31 which was one of the earliest tests used to establish the diagnosis of Cushing’s syndrome.10,17,31 With a cut-off serum cortisol level of 1.8 μg/dl (50 nmol/L) measured on the 2nd day of the test, the test has a sensitivity of approximately 90%,10,17 but its specificity is not high. Most studies that have reported on the performance of this test did not measure serum dexamethasone levels, which could be a confounding factor. Most of the reports have shown a diagnostic accuracy similar to or slightly lower than that of the conventional overnight 1-mg dexamethasone suppression test.10,17 Because the test relies on measurements of serum cortisol, all the limitations listed earlier in that respect apply here. Similarly, with the use of dexamethasone, factors that influence its absorption and metabolism should be considered as potential confounders of the data generated. Similarly, the limitations in the determination of 24-hour urinary free cortisol discussed earlier are applicable here as well.

**Combined Dexamethasone Suppression/CRH-Stimulation Test Confirms State of Hypercortisolism**

This combined test was introduced in an attempt to
improve the sensitivity and specificity of the low-dose dexamethasone suppression test. Theoretically, patients without Cushing’s syndrome will not only have suppressed serum cortisol levels after dexamethasone administration (0.5 mg every 6 hours for 48 hours), but that level (i.e., cortisol) will also remain suppressed (< 1.4 μg/dl) despite stimulation with CRH. In contrast, patients with Cushing’s syndrome will have a rise in serum cortisol level with the administration of CRH despite being on dexamethasone. Initial experience with the test showed that it was effective in differentiating patients with pseudo-Cushing’s syndrome from those with Cushing’s syndrome. Subsequent experience showed the diagnostic accuracy of the test to vary, but it is generally lower than that in the initial report. When used, the test should be performed by an experienced team aware of its limitations and difficulties in interpreting the data. If used, we suggest that dexamethasone levels be determined for accurate interpretations of the results. The previously stated limitations that apply to serum cortisol measurements and dexamethasone absorption and metabolism certainly influence the outcome and confound data interpretation.

Tests Used to Establish the Cause of Hypercortisolism

Biochemical Studies

Once hypercortisolism is established, further studies need to be performed to explore the cause. Measurements of plasma ACTH can differentiate ACTH-dependent (ACTH levels are inappropriately normal or elevated [e.g., pituitary adenoma]) from ACTH-independent (ACTH levels are low/undetectable [e.g., adrenal neoplasms]) states of hypercortisolism. The timing of ACTH measurement is not important as long as the state of hypercortisolism is already established. Other approaches used to determine the cause of hypercortisolism include dynamic testing with dexamethasone alone or in combination with Metopirone. However, the value of these tests is limited by the degree of overlap in responses observed among patients with different forms of hypercortisolism. Similarly, the limitations of the other tests already discussed apply here as well. It is important to point out that the difficulty is often in distinguishing the hypercortisolism caused by an ACTH-secreting pituitary adenoma from that caused by ectopic ACTH secretion by slowly growing carcinoid tumors. These 2 forms of Cushing’s syndrome can be difficult to differentiate at times. In contrast, it is often easy to identify patients with ectopic ACTH secretion caused by more malignant tumors, such as lung cancer. In the latter instance, patients exhibit more metabolic abnormalities (metabolic alkalosis and hypokalemia) and present without the classical clinical features that are often associated with chronic states of hypercortisolism (e.g., central obesity, plethora, and osteoporosis).

Imaging Studies

Imaging studies should be performed only after biochemical documentation of hypercortisolism is established. These studies offer no diagnostic information relevant to Cushing’s syndrome but instead aid in localizing any associated tumor. It is important to point out that tumors in the pituitary and/or adrenal glands can be seen incidentally without any associated biochemical or clinical features.

MRI of the Sella Turcica

Magnetic resonance images of the sella turcica should be obtained for patients with ACTH-dependent hypercortisolism. However, even in the hands of individuals with expertise in this area, MR images are interpreted as being normal in 40%–50% of patients with documented ACTH-secreting pituitary adenomas, primarily because of the small size of these adenomas. This makes differentiating patients with ACTH-secreting adenomas (75%) from others with ectopic ACTH secretion on the basis of MRI difficult. Dynamic contrast-enhanced pituitary MRI can improve detection in some cases, but it will not identify all potential adenomas. As stated above, the discussion of the value of MRI studies in patients with Cushing’s syndrome should be tempered by the fact that incidental pituitary masses are relatively common in asymptomatic healthy subjects, in whom the prevalence might be 10%–20%. Thus, imaging studies should not be done until the clinical and biochemical diagnosis is established. Even when the biochemical diagnosis is established, one cannot be confident that the mass is the source of ACTH excess. Resolution of the clinical and biochemical features of Cushing’s syndrome after tumor resection and/or demonstration of ACTH immunostaining in the tumor is the only way to confirm that it was the source of ACTH excess. A real example of this conundrum is presented in Fig. 1.

Inferior Petrosal Sinus Sampling

An approach used to evaluate for the central source of ACTH secretion in patients with completely negative pituitary MRI scans is bilateral inferior petrosal sinus catheterization with central and peripheral measurements of serum ACTH levels simultaneously from both sides before and after CRH stimulation. This test is technically difficult and should be performed only at experienced centers.

FIG. 1. Serial measurements of serum cortisol (CORT; solid line) and plasma ACTH (dotted line) levels obtained before and immediately after adenomectomy (time 0) in a 35-year-old woman with Cushing’s disease. At the first surgical procedure, an adenoma measuring 1.4 cm was removed; on subsequent testing, it proved to be not secreting ACTH. Re-exploration of the sella demonstrated the presence of a 2-mm adenoma on the other side of the pituitary that was removed successfully and on immunostaining was shown to be ACTH positive. Hypercortisolism developed soon after the second surgery, and remission was achieved.
Overview of the Procedure. The petrosal venous sinus drains the pituitary via the cavernous sinus. The inferior petrosal sinus sampling (IPSS) procedure is performed by inserting catheters in the inferior petrosal vein on both sides via the jugular or femoral veins. Samples for measurements of ACTH are obtained after placement of the catheters (baseline) and several times after the injection of CRH. CRH is used to maximize the gradient and minimize the impact of the pulsatile secretion of ACTH. Blood samples are drawn from the tips of the catheters and simultaneously from a peripheral line on each occasion. In patients with ACTH-secreting pituitary adenoma, the ratio of ACTH levels in the central over peripheral samples should be elevated, especially after stimulation with CRH; the ratio should exceed 3.** This approach, however, was noted to be reliable** but not more accurate than when the samples are obtained from the petrosal sinus.***

Difficulties and Limitations of the Procedure

Proper Diagnosis. The most important issue to emphasize in this respect is that this procedure should be done only if the diagnosis of ACTH-dependent hypercortisolism has already been unequivocally established biochemically. As a point of fact, if one were to perform the procedure on any healthy subject, he or she would have a central source of ACTH. That is, the central/peripheral ACTH levels will be > 2 at baseline and should increase to > 3 after CRH stimulation. We recently had the opportunity to review an example of the results of an IPSS procedure that was performed on a stressed subject who was suspected but never proven not to have Cushing’s syndrome. The data on such an example we saw for a second opinion after the sampling procedure are shown in Table 4. This sample patient has been followed for 16 years since the procedure and, at the time of this writing, has shown no evidence to indicate hypercortisolism.

Catheter Placement/Poor Sampling Time. It is clear that proper placement of the catheter tips (from which the blood samples are obtained) is extremely important. Improper placement of the catheter or asymmetrical or anomalous venous drainage can result in false-negative results. Although each invasive neuroradiologist’s skill may vary, an easy way to verify that the tip of the catheter is very close to the venous drainage of the pituitary is to measure other pituitary hormones in the blood samples obtained from the catheter tip. A convenient hormone that we and others advocate measuring is prolactin. Simultaneous measurements of prolactin and ACTH in central and peripheral blood samples have provided independent confirmation on catheter placement and consequently reduced the rate of false-negative results of the procedure. The ratio of central/peripheral prolactin concentrations should be at least 2 or higher. Any level below 2 (especially below 1.5) suggests poor placement of the catheter tip, which impacts the interpretation of the ACTH data. Another potential limitation that is often noted is poor sampling after CRH stimulation, which can occur as a result of several factors, including displacement of the catheter, thrombosis, and failure to obtain either a central or a peripheral sample. Poor catheter placement (far from the pituitary venous drainage) may give false-negative results. However, some studies suggest that this would be unlikely, because similar results can be obtained when the central blood samples are taken from the jugular bulb.

False-Positive Results. A false-positive result can be seen in any of the following settings:

1. Anybody with normal HPA function and who does not have Cushing’s syndrome would have a high central/peripheral ratio of ACTH. This is always true because the pituitary is the only source of ACTH in healthy subjects. To illustrate that point, we provide data on a patient who was evaluated for Cushing’s syndrome 2 days after knee surgery and was found then to have mild hypercortisolism and normal pituitary on MRI. IPSS revealed a central source of ACTH (Table 4; Patient 1). The patient was referred to us after the procedure for a second opinion, and observation was recommended. At the time of this writing, he has been well after 16 years of follow-up.

2. CRH-secreting tumors are a very rare cause of Cushing’s syndrome that is difficult to detect. Only a few cases have been reported, and most are single case reports, some of which are referenced here. The fact that some patients with this condition are diagnosed only at the postmortem examination reflects the difficulties in recognizing this disease entity. The tumors in some of these patients secrete ACTH as well, which makes them even harder to detect and differentiate from pituitary tumors. Although neuroendocrine

| Table 4. IPSS data in 2 patients with hypercortisolism |

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<th>Time (mins after CRH)</th>
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* Patient 1 had mild hypercortisolism noted soon after knee surgery. He had had no clinical or biochemical features to suggest Cushing syndrome after 16 years of follow-up.
† Patient 2 had classical features of ACTH-dependent hypercortisolism with normal pituitary MRI. He had a lung nodule that was resected and showed positive CRH-secreting cells. After lung-node resection, he developed adrenal insufficiency and had remission of Cushing’s syndrome.
tumors were the more likely source of CRH secretion, others, such as prostate cancer, were also reported.\(^9,28\) The secretion of CRH by an ectopic tumor, such as a pulmonary carcinoid, results in stimulation of the normal corticotrophs and leads to pituitary hyperplasia.\(^28\) Thus, if one were to perform the IPSS, one would find a clear central source of ACTH. An example of such IPSS findings is presented in Table 4. The patient with ectopic CRH secretion had clear clinical and biochemical evidence for ACTH-dependent hypercortisolism that was partially suppressed with dexamethasone and was not associated with any pituitary MRI abnormalities. Because of a greatly elevated serum chromogranin A level, a chest CT scan was obtained and showed a lung nodule that was biopsied and then resected, which resulted in the development of adrenal insufficiency and remission of Cushing’s syndrome. Immunostaining of the tumor showed cells staining for CRH.

3. Hypothalamic tumors that secrete CRH are rare and can easily be confused with ACTH-secreting adenoma.

4. Another cause for a false-positive IPPS result would be the presence of an ACTH-secreting pituitary adenoma in an unusual location such as the sphenoid sinus or elsewhere in the perisellar region. Rarely, a tumor can be located within the cavernous sinus, and although it is of sellar origin, it is likely to be missed during pituitary surgery.

**Adrenal CT/MRI**

Adrenal imaging studies are not needed for patients who have ACTH-dependent Cushing’s syndrome. They are, however, required for those with ACTH-independent Cushing’s syndrome. In patients with ACTH-independent hypercortisolism, a dedicated CT scan of the adrenal glands often shows a tumor (a smaller, homogeneous, more lipid-rich adenoma versus a larger, more irregular, vascular carcinoma). Although differentiating between a cortisol-secreting adrenal adenoma and a carcinoma is sometimes possible using clinical, biochemical, and imaging features, histological confirmation of capsular invasion is necessary for larger lesions. Patients with ACTH-dependent hypercortisolism have bilateral adrenal enlargement and at times may have unilateral or bilateral nodules, depending on the duration and severity of their disease. An occasional patient may have a dominant nodule that appears to function independently. In such patients, the ACTH levels are low to normal, and the clues to diagnosis are the presence of hyperplasia in the remaining limbs of that gland and a diffusely enlarged contralateral adrenal gland.

**Other Imaging Studies**

Because most ectopic ACTH sources are from the lungs, obtaining a CT scan of the chest is the best first step. Some authors recommend getting CT scans of the abdomen and neck as part of the workup also. Other suspected neuroendocrine tumors are best detected by octreotide (\(^1\)In-pentetreotide) scintigraphy, but this does not help with the differentiation when such a tumor produces ACTH. The false-negative scans may be a result of small size of the tumor or the insufficient expression of somatostatin receptors. It is important to emphasize the point that false-positive or true-positive tumors determined by octreotide scintigraphy should be confirmed by CT or MRI.

**Pitfalls in the Management of Cushing’s Syndrome**

The treatment of Cushing’s syndrome depends on the cause, certainty of the diagnosis, and the expertise of the managing team. The management of patients with this disease requires attention to details and integration of all clinical, biochemical, and imaging data. The specific management of patients with established Cushing’s syndrome is beyond the scope of this article. However, the pitfalls encountered in the diagnosis of the syndrome can also be applicable in patient management. One such consideration is the management of patients with ACTH-secreting pituitary adenomas in the immediate postoperative period.

Immediately after the successful removal of an adenoma, patients develop ACTH deficiency and require daily glucocorticoid-replacement therapy until endogenous ACTH production resumes, which can take as long as 1 year.

Although several methods have been used to define remission soon after the removal of ACTH-secreting adenomas, the simplest approach is to measure serum cortisol levels frequently after surgery.\(^1\) Because normal pituitary corticotrophs are suppressed in patients with ACTH-secreting adenomas, one can expect a rapid decline in plasma ACTH and cortisol levels immediately after adenomectomy. In such instances, the development of adrenal insufficiency is a good prognostic sign that indicates possible complete adenomectomy and a high likelihood for remission. For nearly 30 years, our protocol had been to observe patients in the immediate postoperative period, frequently measure their serum cortisol levels, and provide glucocorticoids only if they develop symptoms of adrenal insufficiency or if they clearly have low serum cortisol levels (< 3 μg/dl). Following this protocol, one can identify patients with complete resection of the adenoma and others with incomplete removal. On the basis of these data, one can intervene in the immediate postoperative period with a newer plan to manage the excess glucocorticoid secretion in those with persistent hypercortisolism. An example of this is demonstrated in Fig. 1, in a graph that shows the data of a 35-year-old woman with classic features of Cushing’s disease and a 1.4-cm pituitary mass in the immediate postoperative period. Removal of the mass did not resolve the hypercortisolism. Cells from the resected tumor mass stained positively for the alpha subunit and were negative for other hormones, including ACTH. Repeat pituitary surgery was performed within 3 days, and at that time, a 2-mm tumor was noted on the other side of the sella. Within 24 hours after the removal of this mass, the patient’s serum cortisol level decreased to < 2 μg/dl.

As discussed earlier, there are some limitations in interpreting serum cortisol levels in the immediate postoperative period. Failure to appreciate the impact of elevated transcortin levels on measured serum cortisol concentrations can lead to serious misinterpretation of the data. We have used perioperative cortisol and ACTH data of patients with ACTH-secreting adenomas to predict future...
recurrence of the disease.\(^1\) As illustrated in Fig. 2, the decline in serum cortisol levels after successful adenomec-
tomy in a patient with an elevated transcortin level will be slower than others with normal binding globulin levels, such that it may be misinterpreted as incomplete removal of the adenoma and might invite unnecessary therapeutic intervention. We found that patients who had a dramatic reduction in their serum cortisol levels (to < 3 μg/dL) have remissions. One would have predicted that the plasma ACTH levels would have been quite low by the time the serum cortisol concentrations were that low. However, when the plasma ACTH levels were measured in the post-
 operative period, we were initially surprised to find that, even though the serum cortisol concentrations had already dropped to < 3 μg/dL, the plasma ACTH levels were de-
tectable in most patients and in fact were inappropriately normal (20–50 ng/L) in some of them. With continued follow-up, we found that patients who had plasma ACTH levels greater than 20 ng/L in the immediate postopera-
tive period had recurrences of their disease over time, although their serum cortisol levels at that time were < 3 μg/
dl. Thus, recurrence of Cushing’s disease can occur within 2–5 years of a successful adenomectomy that resulted in clinical remission and in lowering the serum cortisol level to < 3 μg/dL.

Summary

The diagnosis and management of Cushing’s syndrome continue to be challenging and therefore require attention to many details and an appreciation of the limitations of the biochemical studies on which we rely. With many fac-
tors influencing the pituitary adrenal function, one should always interpret biochemical data with caution. Expertise and cooperation among the managing team certainly benefit patients with this disease.

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FIG. 2. Serial measurements of serum cortisol (solid line) and plasma ACTH (dotted line) levels obtained before and immediately after adenomectomy (time 0) in a 45-year-old woman with histologically documented ACTH-secreting pituitary adenoma who also had hepatitis C (left) and in another women who had also an ACTH-secreting adenoma but did not have hepatitis (right). The decreases in plasma ACTH levels were similar in both patients; low levels (< 10 ng/L) were reached within the first 12 postopera-
tive hours. In contrast, however, the decline in serum cortisol levels was much slower in the patient with hepatitis such that the level was < 3 μg/dL nearly 100 hours after adenomectomy compared with 30 hours in the patient without hepatitis.


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