The Diagnosis of Cushing’s Syndrome

Atypical Presentations and Laboratory Shortcomings

Marco Boscaro, MD; Luisa Barzon, MD; Nicoletta Sonino, MD

In the last 3 decades, there have been several advances in understanding the pathogenesis of Cushing’s syndrome and in testing for the diagnosis and differential diagnosis of its various forms. Advanced diagnostic techniques provide useful tools in discovering ectopic adrenocorticotropic hormone sources. However, the occurrence of unusual clinical presentations, laboratory shortcomings, and exogenous compound interference may lead to wrong conclusions. This article reviews the atypical presentations of hypercortisolism and some laboratory shortcomings that may confuse the diagnosis of Cushing’s syndrome. Comments and suggestions are given with the aim of helping the clinician avoid diagnostic mistakes. Arch Intern Med. 2000;160:3045-3053

Cushing’s syndrome is due to long-term glucocorticoid excess that may have various etiologies. Its pathogenesis can be divided into adrenocorticotropic hormone (ACTH) dependent (from a pituitary or ectopic source) and ACTH independent (Table 1). The most common form is pituitary-dependent bilateral adrenal hyperplasia, which is termed Cushing’s disease. There are, in addition, some nonendocrine disorders that may have clinical and biochemical features suggestive of hypercortisolism (pseudo-Cushing’s syndrome). Various tests proposed in the last 3 decades have been extensively reviewed and their diagnostic accuracy carefully discussed. Both urinary-free cortisol (UFC) and the overnight dexamethasone suppression tests are suggested for screening hypercortisolism, whereas the 48-hour low-dose dexamethasone suppression test (LDDST) (0.5 mg every 6 hours for 48 hours) is usually used to confirm this hypersecretion. Corticotropin measurement represents the first step in distinguishing ACTH-dependent from ACTH-independent hypercortisolism. If ACTH is below the limit of detection at 9 AM, especially by using a 2-site immunoradiometric assay method, an adrenal tumor is likely, and confirmatory tests may be helpful (responsiveness of cortisol to dexamethasone suppression and of ACTH and cortisol to corticotropin-releasing hormone [CRH] stimulation). Imaging techniques are generally required for localizing a tumor. In patients with suspected adrenal tumors, computed tomography (CT) or magnetic resonance imaging (MRI) may confirm the diagnosis and easily localize the mass. In cases with low ACTH levels, especially after CRH stimulation, other adrenal diseases should be considered, such as macronodular adrenal hyperplasia or adrenal hyperplasia due to stimuli other than ACTH. Morphofunctional analysis with radiocholesterol scintiscan may be helpful, especially in case of adrenal mass and hormonal tests that suggest a ACTH-dependent form. Indeed, an ipsilateral uptake at radiocholesterol scintiscan may confirm an autonomous adrenal adenoma and exclude macronodular adrenal hyperplasia. If ACTH levels are normal or high, an anterior pituitary corticotroph adenoma is likely, although ectopic ACTH (or CRH) production cannot be excluded. Therefore, the differential diagnosis between pituitary and ectopic ACTH production remains the major challenge. There is general consensus that the high-dose dexamethasone suppression test (HDDST) (2 mg every 6 hours for 48 hours) and the CRH stimulation test are the main tests used to differentiate pituitary from ectopic ACTH sources. Corticotropin-releasing hormone is a potent...
stimulus of pituitary ACTH, and it produces an exaggerated ACTH-cortisol response in most patients with a pituitary adenoma but not in those with ectopic ACTH production.

In cases with ambiguous dynamic tests and dubious pituitary and adrenal images, inferior petrosal sinus sampling (IPSS) seems to be a powerful tool in differentiating ectopic ACTH production from pituitary-dependent Cushing's disease. In addition to CT and MRI, new imaging techniques, such as somatostatin receptor scintigraphy, are available in an attempt to identify small masses with ectopic ACTH production.

Cushing's syndrome is characterized by an array of clinical features that easily suggest the presence of hypercortisolism (Table 2). However, the occurrence of any single feature ranges so widely among reported series that no single finding is a requisite for diagnosis. Moreover, some patients may present with only isolated symptoms. Even the most common findings, such as truncal obesity and hypertension, may be absent in some patients. The presence of only a few symptoms common to other diseases represents a challenge for the physician, since Cushing's syndrome may be misdiagnosed for a long time and patients might be treated in rheumatologic, psychiatric, or other clinics before the correct diagnosis is achieved. Atypical clinical presentations or forms of hypercortisolism without Cushing's syndrome, such as in patients with depression, further complicate the diagnosis.

This article reviews the atypical presentations of hypercortisolism and some laboratory shortcomings that may confuse the diagnosis of Cushing's syndrome. Comments and suggestions are given with the aim of helping the clinician avoid diagnostic mistakes.

### Table 1. Causes of Cushing’s Syndrome*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients, %</th>
</tr>
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<tbody>
<tr>
<td>ACTH dependent</td>
<td></td>
</tr>
<tr>
<td>Pituitary-dependent Cushing’s syndrome (Cushing’s disease)</td>
<td>66.0</td>
</tr>
<tr>
<td>Ectopic ACTH syndrome (ie, bronchial, thymic, pancreatic carcinoids)</td>
<td>7.1</td>
</tr>
<tr>
<td>Ectopic corticotropin-releasing hormone syndrome</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Macronodular adrenal hyperplasia</td>
<td>1.6</td>
</tr>
<tr>
<td>Non-ACTH dependent</td>
<td></td>
</tr>
<tr>
<td>Adrenal adenoma</td>
<td>18.0</td>
</tr>
<tr>
<td>Adrenal carcinoma</td>
<td>6.2</td>
</tr>
<tr>
<td>Macronodular adrenal hyperplasia</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Micronodular adrenal hyperplasia (including the Carney complex)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Adrenal hyperplasia caused by other stimuli (ie, gastric inhibitory polypeptide, vasopressin, β-adrenergic agonists, interleukin 1, immunoglobulins)</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Data are based on the authors' patient population (N = 302). ACTH indicates adrenocorticotropic hormone.

### Table 2. Signs and Symptoms of Patients With Cushing’s Syndrome in the Authors’ Patient Population*

<table>
<thead>
<tr>
<th>Sign or Symptom</th>
<th>Frequency, %</th>
</tr>
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<tbody>
<tr>
<td>Truncal obesity</td>
<td>96</td>
</tr>
<tr>
<td>Facial plethora</td>
<td>82</td>
</tr>
<tr>
<td>Diabetes or glucose intolerance</td>
<td>80</td>
</tr>
<tr>
<td>Gonadal dysfunction</td>
<td>74</td>
</tr>
<tr>
<td>Hirsutism, acne</td>
<td>72</td>
</tr>
<tr>
<td>Hypertension</td>
<td>68</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>64</td>
</tr>
<tr>
<td>Skin atrophy and bruising</td>
<td>62</td>
</tr>
<tr>
<td>Mood disorders</td>
<td>58</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>38</td>
</tr>
<tr>
<td>Edema</td>
<td>18</td>
</tr>
<tr>
<td>Polydipsia, polyuria</td>
<td>10</td>
</tr>
<tr>
<td>Fungal infections</td>
<td>6</td>
</tr>
</tbody>
</table>

*N = 302; 48 males and 254 females; mean ± SD age, 39.5 ± 11.9 years; range, 11-75 years.

### Pituitary Incidentalomas

Patients with ectopic ACTH production may show biological characteristics, ACTH levels, dexamethasone suppressibility, and ACTH response to CRH similar to those with pituitary-dependent Cushing’s syndrome. The presence of incidentally discovered tumors of the pituitary gland in a healthy population ranges from 10% to 20%, hence, the possible coexistence of an ectopic source of ACTH with a pituitary incidentaloma should be considered. Moreover, many ectopic tumors remain occult even at an extensive imaging evaluation and may become evident years after the diagnosis of Cushing’s syndrome. Since pituitary microsurgery is the treatment of choice for Cushing’s disease, an accurate diagnosis is mandatory before transsphenoidal exploration of the pituitary gland.

Selective venous petrosal sinus sampling may be required for the differential diagnosis, whereas additional morphologic evaluation by new radiologic techniques, such as somatostatin receptor scintigraphy and positron emission tomography, may reveal the source of ectopic ACTH.

### Adrenal Incidentalomas

Since 1% to 8% of healthy subjects harbor nonfunctioning adrenal adenomas, some of which are large...
enough to be easily recognized by abdominal CT, a significant number of patients who undergo assessment for Cushing’s syndrome might be found to have an incidentaloma that has no relevance to the cortisol overproduction problem.

In case of increased cortisol production with borderline test results, adrenocortical scintigraphy, in addition to the CRH test and/or the HDDST, is a useful tool for assessing adrenal mass function. Bilateral radiocholesterol uptake may indicate ACTH-dependent hypercortisolism despite the presence of a unilateral adrenal mass.

**RARE FORMS OF HYPERCORTISOLISM**

**Macronodular Adrenal Hyperplasia**

Pituitary-dependent Cushing’s disease and, more rarely, cases of ectopic ACTH production may evolve during long periods into macronodular adrenal hyperplasia with autonomous adrenal nodules. In these cases, pituitary surgery may not be curative. At variance, some adrenal nodules have been noted to decrease in size after removal of the pituitary adenoma, confirming that they were still depending on ACTH control.

Evaluation of ACTH levels, ACTH responsiveness to CRH stimulation, and/or cortisol suppressibility by the HDDST demonstrates whether cortisol is under ACTH control. Adrenocortical scintiscan with radiocholesterol may give further information on the degree of autonomy of these adrenal nodules. Exclusive radiocholesterol uptake by an adrenal mass indicates adrenal autonomy and excludes ACTH-dependent Cushing’s syndrome.

**Pigmented Micronodular Adrenal Hyperplasia**

Another peculiar form of ACTH-independent hypercortisolism has been termed pigmented micronodular adrenal hyperplasia. Despite hormonal tests indicating autonomous adrenal cortisol secretion (ie, suppressed ACTH levels with no response to CRH stimulation), adrenal glands appear to be of normal size on CT scan. Only surgical examination shows numerous small nodular brown or black lesions in the adrenal glands. This rare and separate entity of hypercortisolism occurs primarily in childhood and may be part of a more complex clinical syndrome, the Carney complex, which includes myxomas of the heart, skin, or breast, pigmented skin lesions, endocrine tumors, and peripheral nerve tumors. Factors other than ACTH (ie, immunoglobulins) have been suggested to directly stimulate the adrenal glands.  

Diagnosis may be achieved by a careful clinical examination and, in isolated cases, by an accurate morphologic analysis of the adrenal glands by CT or MRI. It has been recently observed at dexamethasone suppression testing that these patients show a paradoxical cortisol increase.

**Abnormal Sensitivity to Glucocorticoids**

Clinical and laboratory features of Cushing’s syndrome have been reported in rare cases with low, undetectable, or normal cortisol levels, as we also observed in one of our patients. Despite undetectable cortisol levels, our patient had clinical features of severe Cushing’s syndrome, which were controlled only by treatment with a potent antiglucocorticoid, mifepristone. During this therapy, plasma cortisol levels remained undetectable, and ACTH levels, which were low before treatment, showed only a slight and transient increase (M.B. and N.S., unpublished data, 1995). Although in vitro studies demonstrated that tissues of these patients are abnormally sensitive to glucocorticoid activity, the pathophysiology of this disease is still under investigation.

Clinical and laboratory features may be similar to those of patients given high doses of synthetic glucocorticoids. Therefore, the differential diagnosis between patients with abnormal sensitivity to cortisol and those with factitious hypercortisolism may be difficult. An accurate medical history and clinical examination and even a meticulous search for synthetic glucocorticoids in urine by high-performance liquid chromatography may be required to exclude the effect of exogenous compounds.

**Ectopic ACTH Production by Pheochromocytomas**

Although rare, adrenal pheochromocytomas may cause Cushing’s syndrome by their ectopic ACTH production. The possibility of a pheochromocytoma should be investigated in patients with an adrenal mass and clinical and laboratory data consistent with ectopic ACTH production. These tumors must be properly recognized because of the risk of serious hypertensive crisis at surgery.

In patients with a CT scan consistent with adrenal pheochromocytoma, in addition to plasma catecholamines and/or urinary total metanephrine and catecholamine measurements, medullary scintiscan with iodine 123–or iodine 131–meta-iodobenzylguanidine can confirm this rare diagnosis.

**CUSHING SYNDROME IN CERTAIN POPULATIONS**

**Cushing’s Syndrome in Children**

The lack of classic features of hypercortisolism in children may delay diagnosis and treatment. However, high glucocorticoid levels strongly interfere with growth.

All children with increased weight, stunted linear growth, and no other relevant conditions should be tested by UFC measurements and/or a 1-mg overnight dexamethasone suppression test.

**Pregnancy**

Differential diagnosis between normal pregnancy and Cushing’s syndrome during pregnancy is often difficult. Cushing’s syndrome during pregnancy is rare but it should be considered in the presence of endocrine abnormalities. During pregnancy, maternal plasma cortisol levels (both bound and free) rise progressively, and in the third trimester, despite a preserved circadian periodicity, glucocorticoid feed-
back of the hypothalamic-pituitary-adrenal system is impaired, mimicking the presence of Cushing's syndrome.

Early diagnosis is imperative during pregnancy, since Cushing’s syndrome is associated with a high rate of complications for both mother and fetus. The CRH stimulation test and morphologic evaluation by MRI at pituitary and/or adrenal level may be useful procedures to diagnose a pathologic hypercortisolism.

Pseudo-Cushing’s States

Patients without true Cushing’s syndrome may show symptoms and clinical features suggestive of hypercortisolism. The clinical and biochemical presentation of mild hypercortisolism is often indistinguishable from that seen in pseudo-Cushing’s states, such as obesity, depression, or alcoholism. Thus, in borderline cases, the clinician should rule out clinical conditions that mimic Cushing’s syndrome.

Obesity

Mild hypercortisolism, diabetes, and hypertension, often present in obese patients, may be suggestive of Cushing’s syndrome. The differential diagnosis may be difficult, especially in the case of obese patients with poor circadian rhythmicity and abnormal suppression of cortisol with dexamethasone.

In obese patients, 1 mg of dexamethasone administered overnight may fail to suppress plasma cortisol, suggesting a greater utility of UFC testing. In these cases, 3 UFC measurements in carefully performed 24-hour urine collections are usually sufficient to distinguish obesity from Cushing’s syndrome.

Depression

Patients with major depression may show some hormonal features of Cushing’s syndrome. In depressive states, cortisol production is slightly increased, with failure to suppress by the LDDST and occasionally increased UFC.

Although in depressed patients hypercortisolism is usually mild, with normal circadian cortisol rhythm, the differential diagnosis with Cushing’s syndrome may be arduous. An accurate examination of clinical features (ie, hypertension, thin skin, truncal obesity), the blood chemistry profile (ie, blood cell counts and glucose, potassium, coagulation tests), and urinary cortisol determinations may provide the correct diagnosis. Moreover, in patients with depression the ACTH response to the CRH test is blunted, whereas cortisol response is normal. Since depression is a common symptom of hypercortisolism and may precede the occurrence of Cushing’s syndrome, a careful follow-up is needed in all patients with depression who present with more pronounced endocrine abnormalities.

Alcohol

Alcohol excess in a minority of individuals can produce clinical features identical to those of Cushing’s syndrome. This picture may be associated with abnormalities of cortisol secretion (ie, loss of circadian rhythm, lack of normal response to the LDDST). The exact mechanism by which alcohol may influence cortisol secretion is still unclear. It has been hypothesized that alcohol intake may interfere in steroidogenic pathways, ie, by decreasing 11β-hydroxysteroid dehydrogenase activity.

The patient’s use of alcohol is extremely important in the initial assessment of subjects suspected of having Cushing’s syndrome. Since a derangement of γ-glutamyltransferase is usually observed in case of regular alcohol intake, testing liver function in all patients suspected of having Cushing’s syndrome is recommended. Normalization of clinical and biochemical features after ethanol use is withdrawn is the simplest way to avoid a false diagnosis.

CORTISOL ABNORMALITIES WITHOUT CUSHING’S SYNDROME

Familial Resistance to Glucocorticoids

High plasma and urinary cortisol levels not suppressed by dexamethasone have been described in patients who present with hypertension and hypokalemia but without other classic features of hypercortisolism. This condition, defined as familial glucocorticoid resistance, is an inherited, generalized but incomplete end-organ resistance to cortisol due to impaired glucocorticoid receptors. The mechanisms involved include decreased glucocorticoid receptor concentration and ligand-binding affinity, thermolability, and/or abnormal interactions with DNA. Glucocorticoid resistance may be also associated with conditions such as drug administration (ie, mifepristone, chemotherapeutic drugs) or pathological states (ie, ectopic ACTH syndrome, Nelson syndrome, hematologic malignant neoplasms). Although the increased activity of hypothalamic-pituitary-adrenal axis observed in these patients seems to be an adaptive condition, high ACTH levels cause clinical manifestations of hyperandrogenism and/or hypomineralocorticoidism by acting on other steroid hormone receptors, which, at variance with glucocorticoid receptors, maintain a normal sensitivity to their stimulus, ie, together with hypercortisolism without features of Cushing’s syndrome, increased adrenal androgens lead to hirsutism, menstrual irregularities, or sexual precocity, and high mineralocorticoid levels lead to hypertension and hypokalemia.

Family studies demonstrated that the biochemical and clinical presentation is extremely various, depending on the degree of glucocorticoid resistance, and may differ even among members of the same kindred. Furthermore, the finding of high plasma and urinary cortisol levels not suppressible by classic doses of dexamethasone is relatively nonspecific, and currently there is no consensus on how to measure the response to glucocorticoids on an individual basis. An accurate medical history should exclude drug intake or clinical conditions associated with hypercortisolism.

Thyroid Disease

It is well established that thyroid disease can influence corticosteroid me-
tabolism. Hyperthyroidism is associated with increased catabolism of corticosteroids, the levels of which are elevated in urine, whereas the reverse occurs in hypothyroidism. Clinical examination and laboratory analysis, including measurement of thyroid hormones, can easily give a correct diagnosis.

**Anorexia Nervosa and Stress**

Other conditions, such as anorexia nervos₆₅ or stress,₅,₆₆ may be associated with hypercortisolism. Clinical features are so different from those of Cushing’s syndrome that the diagnosis of anorexia nervosa does not give difficulties. On the other hand, the transient states of high plasma cortisol levels produced by stressed conditions are inadequate to give clinical signs of hypercortisolism.

**FACTITIOUS CUSHING’S SYNDROME**

**Exogenous Glucocorticoids**

Long-term treatment with the glucocorticoids, such as dexamethasone or prednisone, may produce clinical features of hypercortisolism. Diagnosis may be suspected by findings of suppressed ACTH and cortisol with no response to CRH and ACTH, respectively. Patients taking hydrocortisone or cortisone, more similar to endogenous steroids, may show variable levels of cortisol in plasma or urine with suppressed plasma ACTH.

A careful medical history is necessary, because some patients take drugs that, unbeknown to them, contain steroids. Determination of synthetic glucocorticoids by gas chromatography or high-performance liquid chromatography should be performed whenever there is a clinical suspicion of steroid abuse.₇₇

**Exogenous ACTH**

Factitious hypercortisolism due to exogenous ACTH injections may be observed in patients with clinical and laboratory data similar to those with pituitary-dependent Cushing's syndrome.

Only a careful investigation of the patient’s history and a thorough search for injection sites may reveal this rare cause of hypercortisolism.

**SHORTCOMINGS IN LABORATORY TESTS USED TO INVESTIGATE CUSHING’S SYNDROME**

**Urinary-Free Cortisol**

Urinary-free cortisol is the most widely used assay to diagnose hypercortisolism. It has the advantage of simplicity and ability to provide an integrated measure of cortisol throughout 24 hours, whereas inaccurate urine collections and considerable variability of normal ranges among laboratories represent the major disadvantages. The sensitivity ranges from 95% to 100% and the specificity from 94% to 98%.₅,₉,₁₂

Because of the daily variability of cortisol secretion and the occurrence of inaccurate urine collection, 3 or more determinations are often needed. To assess the adequacy of 24-hour urine collection, creatinine should also be measured. Daily creatinine excretion is relatively constant and varies by less than 10% from day to day, thus providing some information about the accuracy of urine collection. Because UFC has no validity in patients with renal failure and glomerular filtration rate below 30 mL/min,₆₆ renal function should be verified before testing.

**Plasma Cortisol**

Plasma cortisol levels in healthy subjects show frequent fluctuations and circadian variations, with morning values higher than evening values. Although loss of rhythmicity of cortisol secretion is distinctive of all forms of Cushing’s syndrome, other conditions (ie, depression) may cause false-positive results. However, despite the disappearance of normal diurnal rhythm, fluctuations of plasma cortisol may still be present in Cushing’s syndrome, thus giving misleading results. Therefore, single morning or evening blood samples may fail to distinguish Cushing’s syndrome.₃

Three or more plasma samples during 24 hours are indicated to better identify cortisol hypersecretion. Because plasma cortisol concentrations in Cushing’s syndrome are higher than in healthy persons, especially in the late evening, plasma samples should also be collected at that time. Indeed, a retrospective study has suggested that a single plasma cortisol specimen taken at midnight during sleep can exclude Cushing’s syndrome with a sensitivity of 100% in comparison with the 98% sensitivity of the LDDST examined in parallel.₆₈,₆₉ Nonetheless, the practical difficulties in performing this test in an ambulatory setting may prevent its widespread acceptance.

**Low-Dose Dexamethasone Suppression Test**

The overnight 1-mg dexamethasone suppression test may exclude the presence of Cushing’s syndrome when morning levels of plasma cortisol decrease to 1.38 nmol/L or less. It is the most frequently used screening test to rule out Cushing’s syndrome and has a sensitivity greater than 98%.₅,₉,₁₀,₁₁ The low-dose, 2-day, 2-mg dexamethasone suppression test is useful in confirming the diagnosis of hypercortisolism. A morning plasma cortisol level of 1.38 nmol/L or less after the patient has taken 8 doses of 0.5 mg of dexamethasone, every 6 hours, indicates a normal response. A reevaluation of this test revealed a sensitivity of 69% and specificity of 74% when standard criteria are used.₁₀ Owing to the practical difficulties in performing this test, it should be performed on hospitalized patients. Modifications of the dexamethasone test by administering the drug in terms of body weight and expression of urinary steroids in terms of grams of creatinine to improve accuracy have been proposed.₅

An accurate clinical history should be taken before performing this test to exclude interfering conditions that may give false-positive or false-negative results. Patients with psychiatric illness, obesity, alcoholism, stress, elevated corticosteroid-binding globulin (ie, pregnancy, estrogen treatment), glucocorticoid

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An accurate clinical history should be taken before performing this test to exclude interfering conditions that may give false-positive or false-negative results. Patients with psychiatric illness, obesity, alcoholism, stress, elevated corticosteroid-binding globulin (ie, pregnancy, estrogen treatment), glucocorticoid
and a specificity of 95% can be achieved when ACTH is tested. For cortisol, the sensitivity and specificity have been estimated at 91% and 95%, respectively.\(^7\)

There is general consensus that CRH stimulation together with the HHDST are the main tests to differentiate pituitary from ectopic ACTH sources. However, there are cases of ectopic ACTH production that show positive responsivity to CRH stimulation.\(^81,82\) Moreover, some of these patients may respond also to the HHDST,\(^81\) leading to a mistaken diagnosis of pituitary-dependent Cushing’s disease. At variance, negative results may be due to mistakes in handling CRH. In fact, the lyophilized peptide easily deteriorates if not carefully diluted immediately before testing.

Inferior Petrosal Sinus Sampling

Inferior petrosal sinus sampling allows the determination of the site of a ACTH-secreting lesion (pituitary vs ectopic).\(^19,83\) A central-to-peripheral ACTH gradient greater than 2 had a sensitivity of 95% and specificity of 100%.\(^19,84,85\) This procedure may also help localize the side of a ACTH-secreting pituitary adenoma. A gradient across both sides (left and right) greater than 1.4 may predict the exact localization of ACTH hypersecretion, with an accuracy varying between 55% and 80%.\(^83,86\) However, intersinus ACTH gradient may be absent in the case of an adenoma located in the median wedge of the adenohypophysis or in case of a primary diffuse corticotropic hyperplasia without adenoma. Moreover, marked anatomical variation in the number and size of anastomotic vessels between the inferior petrosal sinuses and vertebral, basilar, and epidural venous plexuses may cause misleading results.\(^87,88\) Therefore, it has been suggested that routine venography should be performed to correctly interpret the results of IPSS.\(^89\) Other reasons for incorrect diagnosis include the presence of an ectopic ACTH-secreting adenoma localized within the sphenoid sinus, producing a central-to-peripheral gradient similar to that of a pituitary-dependent form.\(^89\) At variance with

### Basal ACTH measurement

Basal ACTH measurement is the best way to discriminate between ACTH-dependent (normal or high ACTH levels) and ACTH-independent (suppressed ACTH) Cushing’s syndrome.\(^9,10\) especially by using the 2-site immunoradiometric assay, which shows high specificity and sensitivity and low detection limits.\(^78\) However, the 2-site immunoradiometric assay fails to recognize some ectopic sources that may secrete biologically active variants of ACTH. Therefore, the less specific radioimmunoassay might be more efficacious in detecting these ACTH-like forms.

The results may be falsely low if the assay, usually very precise, has gone awry or if blood has not been collected properly (the samples must be collected in plastic tubes containing EDTA as anticoagulant and aprotinin as proteinase inhibitor; the samples should be kept frozen until assay). In addition to the 2-site immunoradiometric assay, a radioimmunologic assay may be necessary in those cases in which clinical and laboratory data suggest the presence of ectopic ACTH production.

### CRH Stimulation Test

Pituitary but not ectopic ACTH-secreting tumors usually have CRH receptors and exhibit exaggerated ACTH responses to CRH administration.\(^79\) A retrospective study in Cushing's syndrome indicated an ACTH increase of more than 35% compared with baseline as suggestive of a pituitary-dependent form. Based on this criterion, the diagnostic sensitivity and specificity of ACTH response for Cushing's disease was 93% and 100%, respectively.\(^80\) Unfortunately, the largest published series report only the use of ovine CRH, whereas there have been no large studies on ACTH response to human sequence CRH. Based on a survey of 10 published series, Kaye and Crapo\(^*\) developed their own criteria: a positive response would be a relative ACTH increase greater than 50% (or a cortisol increase greater than 20%); a negative response would be a relative ACTH increase less than 50% (or a cortisol increase less than 20%). Using these criteria, a sensitivity of 86% and a specificity of 95% can be achieved when ACTH is tested. For cortisol, the sensitivity and specificity have been estimated at 91% and 95%, respectively.\(^7\)

### Inferior Petrosal Sinus Stimulation

According to the original criteria established by Liddle (50% suppression),\(^70\) the HDDST reduces plasma cortisol levels in only 50% of patients with Cushing’s disease. Therefore, owing to an unacceptably low sensitivity and specificity,\(^50\) it seems to be of limited value in the differential diagnosis of Cushing’s syndrome. However, considering new criteria of suppression (ie, suppression of UFC to less than 10% of baseline for pituitary-dependent Cushing’s disease), it reduces a sensitivity of 70% and a specificity of 100%.\(^67,73,74\) This test also seems to be of limited value in recognizing an ectopic source, since 10% of ectopic tumors show suppression on the HDDST, whereas in some pituitary ACTH-secreting tumors there may be no suppression.\(^76\)

Besides the CRH stimulation test, the HHDST is considered the most useful tool in distinguishing pituitary from ectopic ACTH-dependent Cushing’s syndrome. A very high-dose dexamethasone suppression test (32 mg in 24 hours) has been recently reported to be more effective, and it has been recommended in nonsuppressible pituitary-dependent Cushing’s syndrome.\(^77\)
previous data, it has been recently reported that the accuracy of IPSS is not altered by surgery.91 Healthy individuals or patients with pseudo-Cushing’s syndrome may also display an intersinus ACTH concentration gradient.92,93

This procedure is expensive, and its morbidity is not negligible.94 It requires experienced teams in specialized centers, since inaccuracy in any step of this delicate procedure may generate data that are useless. Therefore, it should be preserved only for patients with imaging that shows a pituitary adenoma but with discordant hormonal test results and all patients with ACTH-dependent Cushing’s syndrome suspected of having an ectopic source.

INFLUENCE OF EXOGENOUS COMPOUNDS

Estrogen

After estrogen administration, corticosteroid-binding globulin levels rise, with a concomitant increase of total plasma cortisol levels. However, the free or unbound fraction of plasma cortisol levels rise, with a concomitant increase of total plasma cortisol levels. Therefore, UFC assay is suggested for testing patients treated by these compounds.

Glycyrrhetinic Acid

Glycyrrhetinic acid, derived from glycyrrhizic acid, is considered an agent able to induce pseudohyperaldosteronism59 and to increase urinary cortisol. This compound, by inhibiting 11β-hydroxysteroid dehydrogenase, which converts cortisol to cortisone, causes cortisol excess especially in the kidney, where the enzyme activity is high. As a consequence, cortisol activity may rise little if at all.95 Thus, women taking estrogens as contraceptives may not experience the stigmata of hypercortisolism, although their plasma cortisol levels are comparable with those of patients with Cushing’s syndrome.

Free plasma cortisol and its circadian variation, 24-hour UFC excretion, and results of the LDDST are usually normal, demonstrating a normal pituitary-adrenal axis.5,10 Therefore, UFC assay is adequate in excluding cortisol hypersecretion in these subjects.

Drugs

Pharmacologic agents such as phenytoin may accelerate the metabolism and catabolism of cortisol by inducing liver enzyme activities. Besides the effects of this compound on plasma cortisol levels, phenytoin might influence dexamethasone suppression tests by increasing its hepatic conjugation and biliary excretion.96 Other drugs such as barbiturates97 and rifampin98 may give similar problems.

Since in all these cases free cortisol levels are normal, UFC assay is suggested for testing patients treated by these compounds.

Table 3. Clinical Presentations and Laboratory Shortcomings That May Confuse the Diagnosis of Cushing’s Syndrome*

<table>
<thead>
<tr>
<th>Presentation or Shortcoming</th>
<th>Diagnostic Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical clinical presentations</td>
<td>Timed UFC</td>
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<tr>
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<td>CRH test, HDDST, Octreoscan, PET</td>
</tr>
<tr>
<td>Pituitary incidentalomas</td>
<td>CRH test, HDDST, adrenal radiocholesterol scintiscan</td>
</tr>
<tr>
<td>Adrenal incidentalomas</td>
<td>ACTH, CRH test, HDDST, adrenal radiocholesterol scintiscan</td>
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<tr>
<td>Rare forms</td>
<td>Clinical examination, adrenal CT or MRI, LDDST</td>
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<tr>
<td>Macronodular adrenal hyperplasia</td>
<td>Meta-iodobenzylguanidine scintigraphy</td>
</tr>
<tr>
<td>Pigmented micronodular adrenal hyperplasia</td>
<td>Medical history and examination, urinary steroid profile</td>
</tr>
<tr>
<td>Abnormal sensitivity to glucocorticoids</td>
<td>Medical history and examination, urinary steroid profile</td>
</tr>
<tr>
<td>Ectopic ACTH from pheochromocytoma</td>
<td>Meta-iodobenzylguanidine scintigraphy</td>
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<tr>
<td>Cushing’s syndrome in certain population</td>
<td>ACTH test, MRI</td>
</tr>
<tr>
<td>Cushing’s syndrome in children</td>
<td>UFC, 1 mg dexamethasone suppression test</td>
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<tr>
<td>Pregnancy</td>
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<tr>
<td>Pseudo-Cushing’s states</td>
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<tr>
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<tr>
<td>Depression</td>
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<td>Alcohol excess</td>
<td></td>
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<tr>
<td>Cortisol abnormalities without Cushing’s syndrome</td>
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<td>Familial resistance to glucocorticoids</td>
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<td>Thyroid disease</td>
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<td>Stress</td>
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<td>Factitious Cushing’s syndrome</td>
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<tr>
<td>Glucocorticoid use</td>
<td>Medical history, urinary steroids by HPLC</td>
</tr>
<tr>
<td>ACTH use</td>
<td>Medical history</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
</tr>
<tr>
<td>UFC</td>
<td>Check for the accuracy of urine collection (ie, urinary creatinine measurement)</td>
</tr>
<tr>
<td>Plasma cortisol</td>
<td>Multiple samples during 24 h or midnight sample</td>
</tr>
<tr>
<td>Dexamethasone suppression tests</td>
<td>Exclude interfering factors (psychiatric illness, obesity, alcoholism, stress, pregnancy, estrogen treatment, glucocorticoid resistance, etc.)</td>
</tr>
<tr>
<td>Basal ACTH</td>
<td>Check for proper collection of samples</td>
</tr>
<tr>
<td>CRH stimulation test</td>
<td>Avoid methodologic mistakes (storage, dilution of CRH)</td>
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<tr>
<td>IPSS</td>
<td>To be performed in selected cases and in specialized centers</td>
</tr>
<tr>
<td>Influence of exogenous compounds</td>
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<tr>
<td>Estrogen</td>
<td>UFC</td>
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<tr>
<td>Drugs (phenytoin, barbiturates, rifampin, spironolactone)</td>
<td>UFC</td>
</tr>
<tr>
<td>Glycyrrhetinic acid</td>
<td>Multiple plasma cortisol samples during 24 h</td>
</tr>
</tbody>
</table>

*UFC indicates urinary-free cortisol; CRH, corticotropin-releasing hormone; HDDST, high-dose dexamethasone suppression test; PET, positron emission tomography; ACTH, adrenocorticotropic hormone; CT, computed tomography; MRI, magnetic resonance imaging; LDDST, low-dose dexamethasone suppression test; HPLC, high-performance liquid chromatography; and IPSS, inferior petrosal sinus sampling.
be increased in the kidney and in urine but not in plasma.

Urinary cortisol might be misleading in testing patients who are taking liquorice\(^1\); therefore, in these cases adrenal function should be tested by measuring plasma cortisol rather than urinary cortisol.

**CONCLUSIONS**

Cushing’s syndrome remains an extremely complex endocrine condition. The choice of tests, the integrity of the specimens, the quality of endocrine assays, and a close dialogue among clinicians and laboratory clinicians are key factors for optimal patient care. The general clinician, keeping in mind the diagnostic traps described herein, may correctly assess most patients as to the diagnosis of Cushing’s syndrome (Table 3) and refer to an experienced endocrinologic center cases that need thorough further investigation.

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