A diagnosis of Cushing syndrome, unless unequivocal, is often a challenge for the clinician. The signs and symptoms of Cushing syndrome are often subtle during the initial stage of the illness. Cushing syndrome may present as an episodic hypercortisolism that is associated with fluctuating clinical findings; however, due to the detrimental effects of prolonged hypercortisolism on body tissues, early detection of the disorder is essential. In this paper I will review the various screening tests used to determine hypercortisolism (Cushing syndrome) and the biochemical evaluation performed to identify a pituitary ACTH-secreting adenoma (Cushing disease).

**BACKGROUND**

Cushing syndrome has an incidence estimated to be approximately 10 per 1 million persons. The ACTH-dependent forms of this syndrome include the following: 1) pituitary corticotroph adenomas or Cushing disease; 2) syndrome of ectopic ACTH secretion by tumors originating in the lung, bronchus, thymus, or pancreas (pheochromocytomas, medullary thyroid carcinomas, and ovarian steroid-cell tumors); and 3) a rare ectopic CRH secretion that usually originates from pheochromocytomas, gangliocytomas, and paragangliomas. Cushing disease accounts for between 70 and 80% of ACTH-dependent forms of hypercortisolism and for 15% of all pituitary adenomas in adults. The ACTH-secreting tumors are four- to sixfold more prevalent in women and are found in patients who are predominantly between the ages of 20 and 60 years. The ACTH-secreting pituitary tumors represent approximately 55% of pituitary adenomas that are diagnosed in children 11 years or younger and approximately 33% of such tumors diagnosed in patients younger than 20 years of age. The disease is equally common in prepubertal boys and girls. Cushing disease is most often caused by a solitary intrasellar microadenoma. Pituitary microadenomas are identifiable in more than 90% of adults and in 80 to 85% of children and adolescent patients with Cushing disease. Those patients who do not have an identifiable adenoma may experience a primary hypothalamic dysfunction. Macroadenomas account for up to 10% of corticotropinomas, with invasiveness exhibited more frequently among younger patients. Nodular corticotroph hyperplasia without evidence of a CRH-secreting neoplasm has been reported in 2% of surgical cases or less. A subset of older patients with nonsuppressible, long-standing ACTH-secreting adenomas may present with a macronodular adrenal disease by autonomously secreting cortisol.

**Clinical Syndrome**

Patients with Cushing syndrome may present with various signs and symptoms (Table 1). Clinical features more suggestive of Cushing syndrome include facial plethora, increased supraventricular fullness, central obesity, proximal muscle weakness, cutaneous wasting (thickness of skin on the dorsal of the hand < 2 mm), purple striae wider than 1 cm, spontaneous ecchymosis, osteopenia, hypertension, and, in children, early or delayed puberty and growth retardation with delayed or advanced bone age. Other symptoms and signs of hypercortisolism include the following: papular acne, vellus hypertrichosis of the forehead and upper cheeks, decreased libido, impo-
tence, oligomenorrhea and amenorrhea, infertility, cuta-
neous and systemic fungal infections, poor wound heal-
ing, nephrolithiasis, polyuria, headaches, neuropsychiatric
problems ranging from major affective disorders to glob-
al psychological dysfunction, and, rarely, spinal epidural
lipomatosis.12,27 Photos of patients are substantially
absent in patients with ectopic ACTH or CRH secre-
tion.27 Ectopic ACTH secretion, however, typically presents in a boy
with hypokalemia and rapid onset of symptoms.9 Cyclic
or periodic Cushing syndrome, in which the exacerbation
of mild cushingoid features may parallel fluctuating hor-
monogenesis, is due to Cushing disease in 50% of cases.35

SCREENING FOR CUSHING SYNDROME

Excessive glucocorticoid levels in urine or blood are
factors leading to a diagnosis of Cushing syndrome. No
biochemical diagnostic test is perfectly accurate, however,
and thus the clinician often must resort to different tests to
distinguish Cushing syndrome from pseudo-Cushing syn-
drome (Table 2). Conditions that alter absorption or me-
tabolism of the synthetic glucocorticoid dexamethasone
will confound the results of the overnight DST; the low-
dose DST, and the high-dose DST. Dexamethasone levels
should be measured in conjunction with the morning cor-
tisol level following administration of dexamethasone.

Twenty-Four Hour UFC Level

Determination of a 24-hour UFC level by performing
HPLC or an RIA is the best screening test for Cushing
syndrome.28 Two to three 24-hour collections of urine en-
sure the increased accuracy of the test. The diagnosis is
unequivocal in a patient with classic features of the syn-
drome and four times the normal level of cortisol excre-
tion (~ 400 μg/day according to most RIAs).29 In adults,
normal values are less than 80 to 120 μg (220–330 nmol)
when assessed using an RIA and at least 50 μg when
performing HPLC.30 In children, the UFC level should
be corrected for the patient’s body-surface area. Normal
values are less than 70 μg/m² per day (37.5 ± 15.1
μg/m²/day for boys and 31.9 ± 17.6 μg/m²/day for
girls).12 In assessing women who are pregnant, one should
accept a higher upper limit of normal free cortisol. In-
creased fluid intake will augment the amount of UFC
excretion and renal insufficiency or a low urine volume
will lower the amount of cortisol that is excreted.27 The
test is not reliable when creatinine clearance is less than
20 ml/minute.28 The diagnostic sensitivity and specificity
of measurements provided by HPLC range from 95 to
100% in various series.24 False-positive results may oc-
cur in patients with pseudo-Cushing syndrome due to en-
dogenous depression, obsessive–compulsive disorder,
anxiety, chronic alcoholism, eating disorders, poorly con-
trolled diabetes mellitus, sleep apnea, or serious illness.26,28
Periodic endogenous hypercortisolism may vary in cy-
cle length from 12 hours to 85 days. If cyclic Cushing
syndrome is suspected based on the clinical findings and
the initial biochemical evaluation is nondiagnostic, a re-
peated evaluation between 3 and 6 months later is recom-
mended.38,39

Midnight Plasma and Salivary Cortisol Levels

Cortisol secretion normally follows a circadian rhythm,
peaking in the early morning and reaching its nadir some-
time in the late evening to a few hours past midnight.31 In
patients with Cushing syndrome cortisol secretion fails to
decrease during the normal nadir period. Measurement of
the plasma level of cortisol via an indwelling venous cath-
ter distinguishes pseudo-Cushing syndrome from Cushing
syndrome with a 95% diagnostic accuracy when 7.2
μg/dl (198 nmol/L) is used as a cutoff value.31 The over-
all test has a 5% false-negative rate.28

Measurement of late-night salivary cortisol (obtained at
bedtime, 11 p.m., or midnight) is as sensitive as and more
convenient than the plasma cortisol test, and obviates the
stress of venipuncture.11,33 A cutoff value of 0.27 μg/dl
(7.5 nmol/L) offers a diagnostic accuracy of 93%,11 which
is comparable to that of a midnight serum concentration of
cortisol (95.7%) and a UFC level (95.3%).32

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**TABLE 1**

Clinical signs and symptoms of Cushing syndrome

<table>
<thead>
<tr>
<th>Most Specific Signs &amp; Symptoms</th>
<th>Less Specific Signs &amp; Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>plethora</td>
<td>popular acne</td>
</tr>
<tr>
<td>supraclavicular &amp; dorsal fat pads</td>
<td>vellus hypertrichosis of face</td>
</tr>
<tr>
<td>central obesity</td>
<td>increased libido/impotence</td>
</tr>
<tr>
<td>proximal muscle weakness</td>
<td>oligomenorrhea/amenorrhea</td>
</tr>
<tr>
<td>cutaneous wasting</td>
<td>infertility</td>
</tr>
<tr>
<td>purple striae</td>
<td>cutaneous &amp; systemic fungal infections</td>
</tr>
<tr>
<td>spontaneous ecchymosis</td>
<td>poor wound healing</td>
</tr>
<tr>
<td>osteopenia</td>
<td>infertility</td>
</tr>
<tr>
<td>hypertension</td>
<td>headaches</td>
</tr>
<tr>
<td>early or delayed puberty (in children)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2**

Biochemical diagnostic tests for Cushing syndrome

screening
1) measurement of 24hr UFC in 2 or 3 collections (≥2 of 3 screens; cortisol excretion >normal value & unequivocal diagnosis if cortisol is >4-fold the normal value)
2) measurement of midnight plasma cortisol (cutoff value 7.2 μg/dl) & salivary cortisol (cutoff value 0.27 μg/dl) levels
3) 1-mg DST (cutoff value for cortisol 2 μg/dl)
4) low-dose DST (cutoff value for cortisol 2 μg/dl)
If patient has clinical features of hypercortisolism but testing is equivocal, repeat Steps 1 & 2 in 3 months.
differential diagnosis of Cushing syndrome in the presence of hypercortisolism
1) dexamethasone-suppression–CRH stimulation test (cutoff value for cortisol 1.4 μg/dl)
2) plasma ACTH (values >10 pg/ml are suggestive of ACTH-dependent disease; values <5 pg/ml are suggestive of ACTH-independent disease)
3) high-dose DST (≥50% suppression of UFC & 17-hydroxysteroid are suggestive of Cushing disease)
4) 8-mg DST (≥50% decrease in plasma cortisol values is suggestive of Cushing disease)
differential diagnosis of ACTH-dependent Cushing syndrome
bilat simultaneous IPS (IPS/P ratio >3 following CRH is consistent with Cushing disease)
Biochemical investigation of Cushing disease

One-Milligram Overnight DST

In patients with Cushing syndrome suppression of cortisol secretion fails following overnight or low-dose dexamethasone administration.34 In patients with Cushing disease, the set point for ACTH secretion is higher than normal. Thus, low doses of dexamethasone fail to suppress ACTH secretion.36 Dexamethasone has a half-life of approximately 5 hours in plasma and between 36 and 54 hours in tissue.40 This test consists of administering 1 mg dexamethasone (in children 15 μg/kg body weight)30 at 11 p.m. and measuring the serum cortisol level at 8 a.m. the next morning. Following dexamethasone administration, a normal plasma cortisol level is less than 2 μg/dl (50 nmol/L); concentrations higher than 10 μg/ml (275 nmol/L) are strongly suggestive of Cushing syndrome and values between 2 and 10 μg/dl (138–276 nmol/L) are equivocal.36 This test has a modest diagnostic accuracy due to the occurrence of false-positive results (15–20%)20 and a sensitivity as low as 55% in cases of mild hypercortisolism.3

The Low-Dose DST

The low-dose DST consists of oral administration of 0.5 mg dexamethasone every 6 hours for 48 hours. The plasma cortisol level is measured at baseline and 48 hours after the first dose of dexamethasone. Plasma cortisol levels lower than 2 μg/dl (50 nmol/L) reportedly have a sensitivity rate of approximately 97%.36

Differential Diagnosis of Cushing Syndrome

Once the diagnosis of Cushing syndrome has been established, the clinician must seek to identify the cause of excess cortisol secretion. Dynamic biochemical tests help distinguish corticotropin-secreting adenomas, which retain their responsiveness to both suppression by corticosteroids and stimulation by CRH from ectopic ACTH-secreting tumors, which usually function autonomously.

The CRH stimulation Test

Approximately 85% of patients with Cushing disease respond to ovine CRH with an increase in plasma levels of ACTH and cortisol. Only 5% of patients with ectopic ACTH-secreting tumors respond.20 The CRH (1 μg/kg or 100 μg) is intravenously administered in the morning; this illicit an increase in plasma ACTH or cortisol levels in patients with Cushing disease, but no response in patients with ectopic ACTH secretion.30 A rise in plasma ACTH values greater than 35% (measured at 15 and 30 minutes postinjection) compared with baseline values yields a 100% rate of specificity and a 93% rate of sensitivity. An increase of at least 20% in the cortisol level measured 30 and 45 minutes after CRH administration yields a specificity of 88% and a sensitivity of 91% in the diagnosis of Cushing disease.36 When the CRH stimulation test is performed in conjunction with the DST, nondiagnostic results from both tests rule out a diagnosis of Cushing disease with a diagnostic accuracy greater than 98%.29

Dexamethasone Suppression–CRH Stimulation Test

The dexamethasone–CRH test distinguishes patients with pseudo-Cushing syndrome from those with Cushing syndrome. Integrating the low-dose DST with the CRH test (described below) significantly increases its diagnostic accuracy.28 This test is performed by oral administration of 0.5 mg, dexamethasone every 6 hours, providing eight doses beginning at noon and ending at 6 a.m. Corticotropin-releasing hormone (Acthrel; Ferring Pharmaceuticals, Inc., Tarrytown, NY), 1 μg/kg body weight, is given intravenously 2 hours after the last dose, and the level of cortisol is measured just before CRH administration and 15 minutes later. A plasma level of dexamethasone should be recorded before the CRH test is given to confirm the patient’s normal metabolism. A plasma cortisol level of 1.4 μg/dl (38.6 nmol/L) or greater supports the diagnosis of Cushing syndrome.22 Furthermore, Isidori and colleagues37 have demonstrated that more than a 30% suppression of serum cortisol during the low-dose DST and/or more than a 20% increase in cortisol during the CRH test had significantly higher rates of sensitivity (97%) and specificity (94%) than either the high-dose DST or the CRH test alone in the differential diagnosis of ACTH-dependent Cushing syndrome. Thus, the differential diagnosis between Cushing disease and ectopic ACTH secretion can be performed with a high accuracy by combining the results of the formal 2 mg/day 48-hour low-dose DST and the CRH test for serum cortisol.35

Plasma ACTH

The advent of a sensitive and specific two-site immunometric assay for plasma ACTH has facilitated the diagnosis of Cushing disease.7 Adrenocorticotropic hormone has a short plasma half-life, necessitating that samples be kept in an ice water bath, centrifuged, separated into aliquots, and frozen within a few hours to avoid obtaining spuriously low results.27 Simultaneous plasma cortisol levels should be determined.1 Using an immunometric assay, plasma ACTH levels measuring more than 10 pg/ml (2.2 pmol/L) and ACTH levels higher than 20 pg/ml (4.5 pmol/L) are indicative of an ACTH-secreting neoplasm.27 Patients with ectopic ACTH syndrome generally have very high plasma ACTH values, although these values may overlap with those seen in patients with Cushing disease.10 In patients with Cushing disease, 50% have a 9 a.m. plasma ACTH level within the normal reference range of 9 to 54 pg/ml (2–12 pmol/L) and the remaining patients have a slightly elevated ACTH level.36 Due to the loss of circadian rhythm, however, nighttime ACTH secretion is abnormal. A midnight plasma ACTH level greater than 23 pg/dl (5 pmol/L) confirms the presence of an ACTH excess.37 Plasma ACTH levels are suppressed when the source of the hypercortisolism is an adrenal cortisol-secreting tumor or a micronodular or macronodular adrenal disease.28 Subnormal daytime plasma ACTH levels that are lower than 5 pg/ml (1.1 pmol/L) are usually present in patients with ACTH-independent Cushing syndrome.10

High-Dose DST

When plasma ACTH levels are higher than 10 pg/ml,
the source of ACTH secretion—pituitary or ectopic—
must be localized. Secretion of ACTH by corticotropin-
omas is usually inhibited by high-dose glucocorticoid
therapy. The high-dose DST is performed by collecting a
24-hour baseline urine sample of free cortisol and 17-hy-
droxy steroid, administering 2 mg of dexamethasone orna-
ly every 6 hours for 2 days (in children 80–120 μg/kg/day
divided into four doses every 6 hours or a maximum of 2
mg every 6 hours for 2 days),20,36 and repeating the 24-
hour urine collection during the last 24 hours of the test.
The criterion of 69% suppression from the baseline value
of 24-hour UFC is required to yield a specificity of 100%
in the diagnosis of Cushing disease.23,27 Urinary levels
of 17-hydroxysteroid are similarly suppressed in 85% of
patients with Cushing disease.20 Paradoxical responses
to dexamethasone indicate the presence of either micro-
nodular adrenal disease or ACTH-independent Cushing
syndrome.21

Eight-Milligram Overnight DST

The 8-mg overnight DST is widely used because of its
convenience. It consists of measuring a baseline plasma
cortisol level followed by oral administration of 8 mg
dexamethasone at 11 p.m. A second specimen of plasma
is obtained 9 hours later, at 8 a.m., and the cortisol level is
measured. A decrease in the plasma level of cortisol that is
50% or greater—the criterion for Cushing disease—
yields a diagnostic accuracy comparable to that provided
by the high-dose DST.23,27

Bilateral Simultaneous Inferior Petrosal Sinus Sampling

Inferior petrosal sinus sampling for ACTH has emerged
as the most accurate and reliable means of distinguishing
pituitary from nonpituitary ACTH-dependent Cushing
syndrome.4,8,10,14,17,19 The IPSS should be reserved for pa-
patients with classic clinical and biochemical Cushing dis-
 ease in whom magnetic resonance imaging findings are
non diagnostic or equivocal, for patients with equivocal
results from suppression and stimulation tests,26 and for
patients whose clinical presentation is consistent with ec-
topic ACTH secretion.9 In experienced hands, the diag-
nostic accuracy of IPSS approaches 80 to 100%.37 The
procedure must be performed when cortisol levels in the
peripheral circulation are elevated to suppress the normal
corticotroph population of the anterior pituitary. The mid-
night plasma cortisol level or the amount of UFC ex-
creation should thus be measured immediately before
IPSS.27 Peripheral CRH levels should be measured rou-
tinely to exclude the possibility of a nonpituitary CRH-
secreting neoplasm as the source of hypercortisolism.9
Concentrations of ACTH are greater in inferior petrosal
sinus samples obtained from patients with Cushing dis-
ease and increase after CRH administration. Corticotro-
pin-reducing hormone significantly reduces the number of
false-negative basal results.27 An IPS/P greater than 3 fol-
lowing administration of CRH is considered consistent
with Cushing disease. When ACTH secretion is ectopic,
values of this hormone in inferior petrosal sinus and per-
ipheral specimens are similar and do not increase after
CRH is given.27 Most patients with ectopic ACTH syn-
drome have an IPS/P less than 2 and, rarely, certain
patients have ratios between 2 and 3. Bilateral simultane-
ous sampling is essential because the maximal basal non-
dominant IPS/P is less than 2 in more than 50% of patients
with Cushing disease and remains less than 2 after admin-
istration of ovine CRH in 33% of cases.8 Lateralization of
the pituitary microadenoma is defined by an ACTH IPS
gradient greater than 1.4, before and after CRH stimula-
tion, with positive predictive values of 74 and of 83%,
respectively.10,17 Midline adenomas may cause misleading
lateralization gradients.17 The rate of correlation of the
ACTH IPS gradient with operative outcome ranges from
47 to 75%.4,57 The IPSS has been associated with morbid
and even fatal complications, including deep vein throm-
bolus, pulmonary emboli, and brainstem vascular dam-
age.3,4,17 The use of intravenous heparin during the proce-
dure is advocated to help prevent thrombosis.37

Sampling of the cavernous sinus has yielded a 20%
false-negative rate3 and has a higher incidence of occlu-
sive events.27 Jugular venous sampling is easier to perform
and has a sensitivity of 88% and a specificity of 100%
when the interpretation criteria is the same as those for
IPSS. This approach may be used as an initial procedure
with a referral for IPSS when results are nondiagnostic.4

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