



Diagnostic approach and testing for adrenal insufficiency in adults

This guide is a compilation of medical information regarding the initial diagnosis of Adrenal Insufficiency (AI). References are provided at the end of the document. An algorithm with page reference numbers is available [here](#). Our goal is to promote better understanding of these procedures and encourage open dialogue between patients and physicians.

The complexities of the endocrine system offer unique challenges for medical providers regarding approach and test interpretation. The Hypothalamus-Pituitary-Adrenal (HPA) axis has a central role in regulating many homeostatic systems in the body, including the metabolic, cardiovascular, immune, reproductive, and central nervous systems. Although adrenal insufficiency is a result of insufficient cortisol production by the adrenal glands, the dysfunction may have a secondary (pituitary) or tertiary (hypothalamic) origin. In addition, disorders such as Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME), recently renamed Systemic Exertion Intolerance Disease (SEID), and Fibromyalgia have their own diagnostic criteria with symptoms similar to adrenal insufficiency and care must be taken to distinguish between the illnesses to ensure the appropriate treatment is applied.

General Practitioners, (GP) and Nurse Practitioners, (NP) are typically the first medical provider patients consult. Although Addison's Disease (AD) from autoimmune cause remains rare, the numbers of AI patients are rising driven by multiple factors, including the increase of associated endocrinopathies (1), suppression from exogenous steroid treatment for a primary condition (2), and the prevalence of Endocrine Disrupting Chemicals (EDC's) in our environment. (3) The current shortage of endocrinologists is expected to increase, and patient wait times for appointments can be lengthy. (4) The average time from symptom onset to diagnosis is five years. (5) (6) Education of GP's and NP's regarding symptoms and initial testing procedures for AI may help reduce diagnostic times and the incidence of misdiagnosis.



Who should be tested for adrenal insufficiency

Disorders of the HPA axis often present with a variety of non-specific symptoms that develop over time. For example, body aches, fatigue, weakness, depression, and anxiety. We recommend physicians test for adrenal insufficiency in patients with non-specific symptoms severe enough to interfere with daily function.

The clinical presentation of adrenal insufficiency is variable, depending on whether the onset is acute, leading to adrenal crisis, or chronic, with symptoms that are more insidious and vague. The clinical diagnosis of chronic adrenal insufficiency is often more difficult than that of acute adrenal insufficiency. While the diagnosis may be obvious when classic symptoms and signs are present, early symptoms (such as fatigue and lassitude) are non-specific. (6) As a result, the possible presence of adrenal insufficiency is sometimes overlooked while other possibilities are pursued:

- Weight loss and gastrointestinal complaints may raise suspicion of gastrointestinal malignancy.
- Cutaneous hyperpigmentation is not always present. Hyperpigmentation is absent in some patients with primary adrenal insufficiency and in all patients with secondary (pituitary) and tertiary (hypothalamic) adrenal insufficiency. In addition, hyperpigmentation can be caused by antineoplastic, antimalarial, and other drugs, such as tetracyclines, phenothiazines, and zidovudine, and by heavy metals. The pigmentation of hemochromatosis is similar to that of adrenal insufficiency except that it seldom involves the mucous membranes.
- Patients with untreated adrenal insufficiency may have moderately elevated daytime serum thyroid-stimulating hormone (TSH) levels. Cortisol may play a role in regulating the normal circadian rhythm in TSH.
- In one study of 216 patients, 20 percent had symptoms for more than five years before diagnosis.

Even when the diagnosis appears obvious, endocrine evaluation is indicated to confirm the diagnosis and to determine the type of adrenal insufficiency and its cause. However, therapy should be started before the diagnosis is established in an acutely ill patient with possible adrenal crisis. (6)

Adrenal crisis

Patients with adrenal crisis often present with extreme weakness, confusion, nausea, and vomiting. Infections and trauma are major precipitating causes.

Acute adrenal insufficiency or adrenal crisis should be considered in any patient who presents with peripheral vascular collapse (vasodilatory shock), whether or not the patient is known to have adrenal insufficiency. Isolated corticotropin (ACTH) deficiency, although rare, should be considered in any patient who has unexplained severe hypoglycemia or hyponatremia. (6)

It is essential that treatment of patients who present in possible adrenal crisis not be delayed while diagnostic tests are performed. Blood for serum cortisol and serum chemistry should be drawn; some clinicians also draw and hold samples for later measurement of ACTH, renin, and aldosterone if the diagnosis of adrenal insufficiency is likely. Therapy should be initiated immediately with intravenous saline and dexamethasone. The short ACTH stimulation test can be performed after initiation of glucocorticoid treatment provided that:



- Glucocorticoid therapy has not been given for more than a few days, after which it could begin to suppress the hypothalamic-pituitary-adrenal axis and compromise the adrenal response.
- Neither hydrocortisone (cortisol) nor cortisone (which is converted to cortisol by the liver) is given because both are measured in cortisol radioimmunoassay. Dexamethasone, which is not measured in these assays, is the glucocorticoid of choice in these patients. It will, however, suppress ACTH and cortisol secretion. (6)

For more detailed information on adrenal crisis, please consult reference numbers 5, 8, and 18.

Diagnostic approach

History

Adrenal insufficiency is a disorder with multiple etiologies and history is an important factor in diagnosis. Physicians should consider testing for AI in patients with the following conditions in their patient profile or family history.

Causes of Primary adrenal insufficiency (9)

- Autoimmune disorders.
- Genetic diseases, such as Congenital adrenal hyperplasia, Neonatal and X-linked adrenoleukodystrophy, Familial glucocorticoid deficiency.
- Infections, such as Tuberculosis, HIV, Histoplasmosis, Cytomegalovirus, Cryptococcus, and fungal infections.
- Adrenal hemorrhage or thrombosis.
- Infiltrative disorders, such as Amyloidosis, Sarcoidosis, Hemochromatosis, and Metastatic disease.
- Drugs that inhibit cortisol biosynthesis, such as etomidate, ketoconazole, fluconazole, metyrapone, and suramin.
- Drugs that accelerate the metabolism of cortisol and most synthetic glucocorticoids by inducing hepatic mixed-function oxygenase enzymes, such as phenytoin, barbiturates, mitotane, and rifampin.

Causes of Secondary and Tertiary adrenal insufficiency (10)

- Glucocorticoid treatment for a primary condition
- Traumatic Brain Injury, (TBI).
- Pituitary irradiation/surgery.
- Brain/pituitary tumors.
- Pituitary necrosis or bleeding, postpartum pituitary necrosis (Sheehan's syndrome)
- Infiltrative disorders of the pituitary or hypothalamus, such as Sarcoidosis, Amyloidosis, Hemosiderosis, Metastatic cancer, and Lymphoma.
- Genetic cause, mutations in the POMC gene, TPIT gene mutations, and Familial cortisol-binding globulin (CBG, transcortin) deficiency.
- Isolated ACTH deficiency
- Drugs, such as high dose progestins or chronic administration of opiates.



- Correction of hypercortisolism (Cushing’s syndrome)
- Prader-Willi syndrome.

Clinical signs and symptoms

Some features of PAI, SAI, and TAI are identical, but others are distinctly primary or secondary attributes. For example, hyperpigmentation is seen with PAI, but not SAI and TAI. Patients who present with extreme fatigue and have other non-specific symptoms, hyperpigmentation may indicate PAI, while exogenous steroids for another disease may raise suspicion for SAI.

In addition, physicians should be aware that comorbidities may cause contradictory symptoms. For example, primary patients typically present with weight loss, while secondary patients often have weight gain due to other hormone imbalances or insulin resistance. (5) (8)

Symptoms of PAI

Weakness
Fatigue
Hyperpigmentation
Vitiligo
Nausea, abdominal pain
Vomiting
Weight loss
Hypotension
Amenorrhea
Volume depletion
Musculoskeletal complaints
Salt craving
Sleep disturbance
Diarrhea
Visual disturbance
Depression/anxiety
Heat intolerance

Symptoms of SAI/TAI

Weakness
Fatigue
Pale complexion
Cold (possible untreated thyroid issue)
Nausea, abdominal pain
Pain under ribs/mid-back
Weight gain/loss
Hypertension/Hypotension
Amenorrhea/Infertility
Cushingoid (exogenous steroids)
Musculoskeletal complaints
Apathy
Sleep disturbance
Diarrhea/constipation
Headaches/visual disturbance
Depression/anxiety



Pre-testing considerations

The following hormones or drugs may interfere with accurate test results.

- Glucocorticoids or corticosteroids in any form, including topical, inhaled, injected, and oral tablets/capsules.
- Birth control or other estrogens, including soybean food products and menopause formulas.
- Drugs that inhibit cortisol biosynthesis, such as etomidate, ketoconazole, fluconazole, metyrapone, and suramin.
- Drugs that accelerate the metabolism of cortisol and most synthetic glucocorticoids by inducing hepatic mixed-function oxygenase enzymes, such as phenytoin, barbiturates, mitotane, and rifampin.
- High dose progestins or chronic administration of opiates.

The diagnosis of adrenal insufficiency of any cause depends entirely upon the demonstration of inappropriately low cortisol production. Most tests use total serum cortisol as the diagnostic measurement. Caution should be taken in interpreting the results in patients with abnormalities of cortisol-binding globulin (CBG) or albumin, such as patients with cirrhosis or nephrotic syndrome, or those taking oral estrogens. In these settings, decreased or increased levels may lead to an incorrect diagnosis. Salivary or serum free cortisol have been suggested as alternatives but are not widely available, and criteria for response have not been developed. (6)

Salivary and circulating hormone levels have a diurnal rhythm. For certain hormones such as cortisol, the variations can be quite large, with increases to very high levels a few hours before awakening, pulsatile decreasing values throughout the day, and nadir values in the evening and early part of the sleep period. Time-specific normal ranges should be sought for these tests and test strategies should be planned with this in mind. As an example, measurement of late evening, but not early morning, serum cortisol levels is useful for the diagnosis of Cushing's syndrome, whereas measurement of early morning, but not late evening, serum cortisol levels is useful for the diagnosis of adrenal insufficiency. Clinicians should verify whether their patients have unusual sleep hour patterns (e.g., night shift work). (12)

Testing

The HPA axis operates on a negative feedback loop. When cortisol levels decrease, the hypothalamus secretes corticotropin releasing hormone (CRH). This stimulates the pituitary to produce adrenocorticotrophic hormone (ACTH), which in turn stimulates the adrenal glands to produce cortisol. When adequate cortisol levels are achieved the hypothalamus and pituitary decrease production in response. **Because of the dynamic nature of this relationship, both adrenal cortisol and pituitary corticotropin must be tested. Morning cortisol and ACTH baselines, drawn simultaneously and interpreted together, indicate the direction for further testing.**



Tests used to evaluate the HPA axis

The morning serum cortisol, and the ACTH stimulation test evaluate adrenal cortisol production, baseline, and stimulated levels respectively.

The ACTH stimulation test is used to confirm or exclude PAI if the baseline cortisol result is indeterminant. Lack of appropriate response may indicate adrenal atrophy in chronic SAI patients.

Adequate response to the ACTH stimulation test does not eliminate the possibility of secondary or tertiary AI.

The baseline serum ACTH evaluates pituitary corticotropin, (ACTH) levels.

The Metyrapone and the Insulin Tolerance Test (ITT), are used to measure stimulated pituitary corticotropin, (ACTH) production if SAI or TAI is suspected.

Confirmation of the clinical diagnosis of adrenal insufficiency is a three-stage process:

- Demonstrating inappropriately low cortisol secretion
- Determining whether the cortisol deficiency is dependent on or independent of corticotropin (ACTH) deficiency and evaluating mineralocorticoid secretion in patients without ACTH deficiency
- Seeking a treatable cause of the primary disorder (e.g., infiltrative process involving the adrenal glands or a pituitary adenoma compromising normal pituitary function) (6)

Although laboratory testing is essential to confirm the diagnosis of adrenal insufficiency, both laboratory and patient errors can cause misleading results. One way to minimize these errors is to make certain that the different tests are internally consistent. (6) *{See "Basic principles in the laboratory evaluation of adrenocortical function" Up to Date, Author: Lynnette K Nieman, MD Section Editor: André Lacroix, MD Deputy Editor: Kathryn A Martin, MD}* (12)

Further complicating the diagnosis is the fact that the most common cause of primary adrenal insufficiency, autoimmune adrenalitis, is a process that usually worsens over a period of many months or years. Two groups followed patients with adrenal autoantibodies but no evidence of adrenal insufficiency for three to five years and concluded that there were four stages in the development of (primary) adrenal insufficiency:

- Stage 1: High plasma renin activity and normal or low serum aldosterone
- Stage 2: Impaired serum cortisol response to ACTH stimulation
- Stage 3: Increased morning plasma ACTH with normal serum cortisol
- Stage 4: Low morning serum cortisol and overt clinical adrenal insufficiency

Thus, by the time the patient has developed low serum cortisol concentrations, adrenal destruction is essentially complete. (6)



Based on these diagnostic parameters we recommend the following initial tests.

To demonstrate inappropriately low cortisol secretion.

- The morning baseline serum cortisol, drawn between 8 and 9 am.

To determine if the cortisol deficiency is dependent on or independent of corticotropin (ACTH) deficiency.

- The baseline serum ACTH, drawn simultaneously with the morning cortisol.

To evaluate mineralocorticoid secretion.

- Aldosterone and plasma renin activity (PRA)

To evaluate organ function, electrolytes, hydration, and blood glucose levels.

- Comprehensive metabolic panel (CMP)

One report evaluated the utility of basal morning serum cortisol measurements in the diagnosis of adrenal insufficiency. Values below 5 mcg/dL (138 nmol/L) had almost 100 percent specificity, but only 36 percent sensitivity (as defined by a subnormal serum cortisol response to insulin-induced hypoglycemia). Using a higher serum cortisol of 10 mcg/dL (275 nmol/L) as the criterion for adrenal insufficiency increased the sensitivity to 62 percent, but reduced the specificity to 77 percent. * **Thus, a low morning serum cortisol concentration alone is not a reliable predictor of deficient adrenal function. (6) In all testing scenarios, a baseline ACTH should be drawn. (11)**

*Test **sensitivity** is the ability of a test to correctly identify those with the disease (true positive rate), whereas test **specificity** is the ability of the test to correctly identify those without the disease (true negative rate).

It should be noted that no test has 100% sensitivity and 100% specificity. Variability is present in patients with the same disease and results obtained must be interpreted in conjunction with the patients' history and clinical examination. Hence, interpretations of laboratory results are guidelines rather than absolutes in many cases. (11)

Interpretation

Morning cortisol and baseline ACTH.

In normal subjects, serum cortisol concentrations are higher in the early morning (about 6 AM), ranging from 10 to 20 mcg/dL (275 to 555 nmol/L), than at other times of the day. (6)

- If serum cortisol is inappropriately low and a simultaneous plasma ACTH concentration is very high, the patient has primary adrenal insufficiency.
- If both the serum cortisol and plasma ACTH concentrations are inappropriately low, the patient has secondary (i.e., pituitary disease) or tertiary (hypothalamic disease) adrenal insufficiency.



- In primary adrenal insufficiency, the 8 AM plasma ACTH concentration is high, sometimes as high as or higher than 4000 pg/mL (880 pmol/L); these patients have mineralocorticoid deficiency in addition to cortisol deficiency, plasma renin concentration or activity will be elevated, while aldosterone levels will be low, with increased serum potassium and decreased serum sodium levels.
- In contrast, plasma ACTH concentrations are low or low normal in secondary or tertiary adrenal insufficiency. The normal value at 8 AM is usually between 20 and 52 pg/mL (4.5 and 12 pmol/L) in a two-site chemiluminescent assay. Plasma levels of renin and aldosterone are usually unaffected in secondary or tertiary adrenal insufficiency, but mineralocorticoid deficiency can sometimes occur after very prolonged deficiency of ACTH. Serum sodium is often decreased with normal serum potassium secondary to increased vasopressin levels resulting from its decreased inhibition by cortisol. (6)

If cortisol and ACTH test results show a definitive diagnosis of adrenal insufficiency, glucocorticoid treatment should be initiated without delay. {Please see current guidelines; *Diagnosis and treatment of PAI. 2015 JCEM (7), Central Hypoadrenalism, 2014 JCEM (2)*}

In addition, patients should be educated about the need for stress and illness dosing, (17) (23) medical alert jewelry, and supplied with an emergency intramuscular injection in the case of adrenal crisis. (18) Physicians and patients also need to create an adrenal crisis plan in the event the patient needs to go to the emergency room (ER). A brief letter with detailed instructions, signed by the physician, can eliminate confusion in the ER.

Indeterminate results:

Most recommendations agree that a cortisol test result achieving the top reference number or above, indicates no dysfunction, and a result at or below the bottom reference number indicates definitive AI. However, test results between these two points, usually considered “normal values range” are indeterminate in many cases when profile, history, and clinical symptoms are considered. Therefore, some recommendations set a threshold within this “normal values range” to indicate further testing needed.

Examples:

0800 h basal serum cortisol, 165–680 nmol/L (6 - 25 mcg/dL) Serum cortisol <165 nmol/L, (6 mcg/dL) definite adrenal insufficiency; serum cortisol <300 nmol/L, (11 mcg/dL) adrenal insufficiency not excluded; serum cortisol >550 nmol/L, (20 mcg/dL) generally excludes primary adrenal insufficiency.

0800 h basal serum cortisol, 165–680 nmol/L (6 – 25 mcg/dL) Serum cortisol <100 nmol/L, (4 mcg/dL) definite adrenal insufficiency; serum cortisol 100–500 nmol/L, (4 – 18 mcg/dL) adrenal insufficiency not excluded; serum cortisol >500 nmol/L, (18 mcg/dL) excludes secondary adrenal insufficiency. *The Lancet 2014 (1)*

An early morning (8 am) plasma cortisol level lower than 3 µg/dL confirms adrenal insufficiency, whereas a value higher than 15 µg/dL makes the diagnosis highly unlikely. Cortisol levels in the range of 3 to 15 µg/dL may be seen in patients with primary, secondary, or tertiary adrenal insufficiency. *Cleveland Clinic (15)*



Cortisol secretion is episodic, and the normal ranges are broad. A single serum value, if it falls within the normal range, is inconclusive. An individual can have partial pituitary or adrenal insufficiency but maintain plasma ACTH and serum cortisol concentrations within their respective normal ranges. For these reasons, stimulation or suppression testing should be performed when there is doubt. Nevertheless, samples drawn at the appropriate time for the suspected endocrine dysfunction can be very helpful in excluding adrenal hypofunction or hyperfunction. (13)

- Patients with primary or secondary adrenal insufficiency have low early morning serum cortisol concentrations. If the value is greater than 10 mcg/dL (276 nmol/L), it is unlikely that the patient has clinically important adrenal insufficiency, whereas if it is less than 3 mcg/dL (83 nmol/L), the probability of adrenal insufficiency is high. Since serum cortisol is often undetectable one hour after the beginning of sleep, measurement at this time does not identify patients with adrenal insufficiency.
- Patients with congenital adrenal hyperplasia may have normal or low serum cortisol values (corresponding to simple virializing and "late-onset" CYP21A2 deficiency types) in the early morning.
- Most patients with Cushing's syndrome have early morning serum cortisol concentrations within or slightly above the normal range. In contrast, serum cortisol concentrations one hour after sleep are almost always high (greater than 7.5 mcg/dL [207 nmol]) and are often equal to the early morning values (i.e., they have an abnormal or absent circadian rhythm) (13)

Aldosterone and plasma renin activity (PRA)

Renin is an enzyme produced by the kidneys. It activates the hormone angiotensin, which stimulates the adrenal glands to produce aldosterone. Produced by the adrenal cortex, aldosterone regulates the retention of sodium and the elimination of potassium by the kidneys.

The activity of this system is regulated in part by the effective arterial blood volume, which is determined in normal subjects by sodium intake. Thus, interpretation of measurements of plasma renin activity and serum aldosterone requires knowledge of the supine or upright posture of the subject and the level of sodium intake, which, in the steady state, can be estimated from 24-hour urinary sodium excretion. The aldosterone/renin ratio is often required to interpret the integrity of this axis. (12)

In practice, for most situations where aldosterone and renin levels are to be assessed, most centers draw a morning ambulatory upright sample. (14)

Renin can be measured in terms of its enzymatic activity (plasma renin activity, PRA), or its mass (active renin concentration). If available, plasma renin activity, although more time consuming, is preferred over the direct active renin concentration for at least two reasons:

Plasma renin activity, unlike direct renin concentration, takes into account endogenous renin substrate (angiotensinogen) levels. This is important in the context of higher circulating estrogen (e.g., women taking exogenous estrogen) which stimulates production of renin substrate. The resulting rise in angiotensin II (via angiotensin I) suppresses renal production of renin enzyme through a negative feedback mechanism, and direct renin concentration falls. In the face of raised substrate but reduced enzyme concentrations, plasma renin activity (angiotensin I generated by unit of time) remains relatively constant. As a result, when screening women



receiving estrogen-containing preparations for the presence of primary aldosteronism by aldosterone/renin ratio testing, false positive ratios can occur when renin is measured as direct renin concentration, but not when it is measured as plasma renin activity.

A sustained effect of the pre-ovulatory surge in estrogen is also thought to be responsible for the reported occurrence of false positive aldosterone/renin ratios using direct renin concentration (but not plasma renin activity) during the luteal phase of the menstrual cycle. (14)

Factors that affect aldosterone/renin levels:

- Sodium intake
- Age
- Gender, menstrual phase, and pregnancy
- Time of day – Aldosterone and renin levels show a diurnal rhythm, being highest in the early morning upon awakening and falling during the day.
- Posture
- Medication use
- Chronic kidney disease

Race For more detailed information on factors that affect aldosterone/renin please consult reference number 14.

Adrenal diseases have a variable effect on renin levels, being determined primarily by whether the disease results in an increase or a decrease in the secretion of mineralocorticoids (which induce volume expansion, resulting in suppression of renin release).

Normal, morning plasma renin activity for seated subjects ranges from about 1 to 4 ng/mL per hour (0.8 to 3.0nmol/L per hour). Corresponding active renin concentrations are 8 to 35 mU/L. (14)

Interpretation in adrenal disease.

Renin levels are usually low in:

- Primary aldosteronism, in which aldosterone production is excessive (relative to body sodium status), and independent of its normal regulator, renin/angiotensin.
- Primary aldosteronism may be due to an aldosterone-producing adrenocortical tumor (adenoma or, rarely, carcinoma), bilateral adrenal hyperplasia, or glucocorticoid-remediable aldosteronism (familial hyperaldosteronism type I).
- Patients with 11-beta-hydroxylase or 17-alpha-hydroxylase deficiency (due to mutations in CYP11B1 and CYP17 respectively), which are hypertensive forms of congenital adrenal hyperplasia associated with excessive production of the mineralocorticoid deoxycorticosterone (DOC), driven by adrenocorticotrophic (ACTH, levels of which are high as a result of deficient cortisol production).
- Primary glucocorticoid resistance, which is again associated with ACTH stimulation and excessive DOC production causing hypertension.
- Patients with DOC-producing adrenal tumors.
- Ectopic ACTH syndrome, in which cortisol levels may be high enough to overwhelm the 11-beta-hydroxysteroid dehydrogenase type 2 enzyme (which normally, by converting cortisol to cortisone, prevents excessive stimulation of the mineralocorticoid receptor). In addition, the high levels of ACTH may lead to excessive production of DOC.



They are usually normal in:

- Secondary adrenal insufficiency (i.e., hypopituitarism or isolated ACTH deficiency).
- Cushing's syndrome, but they can be low when there is a marked degree of hypercortisolism.

They are usually high in:

- Primary adrenal insufficiency, including Addison's disease.
- Patients with congenital adrenal hyperplasia due to deficiencies in either steroid acute regulatory protein (due to mutations in StAR), side-chain cleavage enzyme (CYP11A1), 3-beta-hydroxysteroid dehydrogenase (HSD3B2), 21-hydroxylase (CYP21A2) or aldosterone synthase (CYP11B2) in which deficient production of steroids with mineralocorticoid activity leads to salt-wasting. (14)

Morning serum (and plasma) aldosterone concentrations range from 5 to 30 ng/dL (140 to 830 pmol/L) in seated normal subjects with unrestricted salt intakes. (14)

Interpretation in adrenal disease.

Serum aldosterone levels are usually low:

- In patients with 11-beta-hydroxylase or 17-alpha-hydroxylase deficiencies, which are associated with excessive production of DOC (and possibly corticosterone in the latter condition).
- In some patients with Cushing's syndrome, with the extent of suppression depending upon the degree of hypersecretion of cortisol and other salt-retaining steroids.

Serum aldosterone levels are usually low or undetectable:

- In primary adrenal insufficiency, and may be normal or low in patients with chronic corticotrophin deficiency.
- In patients with deficiencies of either steroid acute regulatory protein, side-chain cleavage enzyme, 3-beta-hydroxysteroid dehydrogenase, and aldosterone synthase, since aldosterone synthesis is directly impaired.

Levels are high in:

- Many patients with primary aldosteronism, but are often normal (inappropriately, in the face of renin suppression). (14)

Comprehensive metabolic panel (CMP)

Physicians are familiar with the comprehensive metabolic panel, a frequently ordered panel of fourteen tests. The CMP provides information about the status of a person's metabolism, including the health of the kidneys and liver, electrolyte, acid/base balance, as well as levels of blood glucose and blood proteins. Abnormal results, and especially combinations of abnormal results, can be useful in the diagnosis of adrenal insufficiency.

A singular abnormal test result may have multiple possible causes, however, combined indeterminate cortisol and ACTH results, abnormalities on the PRA or CMP, autoimmune or HPA axis



disorders in the patients' history, and non-specific symptoms, would indicate the need for further testing.

Testing to confirm diagnosis and origin:

A major problem with relying on unstimulated serum cortisol values as the basis for the diagnosis of adrenal insufficiency is that cortisol secretion is episodic. A single early morning serum cortisol value, if it falls within the range of normal, is inconclusive, although a patient with adrenal insufficiency is very unlikely to have a high-normal value. Furthermore, the normal range is broad, and a patient can have pituitary or adrenal insufficiency but maintain basal corticotropin (ACTH) and/or cortisol secretion within the range of normal. In patients with congenital adrenal hyperplasia, the range of basal concentrations of serum cortisol, 17hydroxyprogesterone, and other adrenal steroids overlap the normal range. For these reasons, dynamic function tests should be performed when there is doubt about the status of hypothalamic-pituitary-adrenal (HPA) function. (19)

To confirm or exclude primary adrenal insufficiency:

ACTH stimulation test

Several protocols have been used to assess the response to exogenous corticotropin (ACTH). The agent used is synthetic ACTH (cosyntropin), which has the full biologic potency of native ACTH. Short (one hour or less) tests involve administration of a single-dose in a "low" or "high" concentration and can be performed on an outpatient basis. Both tests result in supraphysiological plasma ACTH concentrations: about 60,000 pg/mL (1320 pmol/L) after the standard high-dose ACTH test and about 1900 pg/mL (41.8 pmol/L) after the low-dose test. There are no untoward side effects. Allergic reactions are almost unheard of with cosyntropin. Prolonged ACTH stimulation tests are seldom performed because measurements of plasma ACTH in conjunction with the low-dose ACTH test provide the necessary information.

The advantage of the high-dose test is that the cosyntropin can be injected IM as well as IV, because pharmacologic plasma ACTH concentrations are still achieved. The low-dose test has not been evaluated after IM injection and may not provide valid results by this route.

Plasma ACTH can also be measured in the basal sample, as in all ACTH stimulation tests. In healthy individuals, cortisol responses are greatest in the morning, but in patients with adrenal insufficiency, the response to cosyntropin is the same in the morning and afternoon. Thus, we recommend that the test be done in the morning to minimize the risk of misdiagnosis in a normal individual. (19)

- A standard high-dose test consists of measuring serum cortisol before, 30 and 60 minutes after intravenous (IV) injection of 250 mcg of cosyntropin; at this high dose, it can also be injected intramuscularly (IM).
- The low-dose ACTH test is performed by measuring serum cortisol before and 30 minutes after IV injection of 1 mcg cosyntropin.
- The current criteria used to indicate normal adrenal function are a minimum serum cortisol concentration ≥ 18 (low-dose test) or 18 to 20 (high-dose test) mcg/dL (500 to 550 nmol/L) before or after cosyntropin injection.



- A lower cutoff is used (≥ 16 mcg/dL [440 nmol/L]) if high-dose cosyntropin is administered IM.
- ACTH stimulation tests are not valid to evaluate hypothalamic-pituitary-adrenal (HPA) function if there has been a recent pituitary injury and insulin-induced hypoglycemia should be used in these circumstances.
- **Simultaneous determination of basal ACTH before performing the cosyntropin stimulation test determines if adrenal insufficiency is a primary adrenal disease (elevated basal ACTH values) or secondary to hypothalamic-pituitary deficiency (low basal ACTH value).** (19)

An adequate cortisol response to ACTH stimulation does not exclude secondary or tertiary AI. However, it can indicate adrenal atrophy in chronic adrenal insufficient patients, or suppression in patients being treated with drugs that affect the HPA axis, including chemotherapy.

Adrenal imaging (CT)

Not all adrenal disorders are visible on imaging.

21-hydroxylase antibody (21-OH AB)

The presence of 21-hydroxylase antibodies (greater than 1.0 U/mL) is indicative of primary adrenal insufficiency (Addison's disease) either isolated autoimmune adrenal insufficiency, or as part of autoimmune polyendocrine syndrome.

In males with adrenal insufficiency and 21-hydroxylase antibodies within the reference interval (less than 1.0 U/mL), X-Linked Adrenoleukodystrophy (X-ALD) {young males}, and X-Linked Adrenomyeloneuropathy (X-AMN) {adult males} should be excluded by using Very Long-Chain Branched Fatty Acids (VLCFA) in plasma for screening. (22)

Serum 17-hydroxyprogesterone (17-OHP) Non-Classic Congenital Adrenal Hyperplasia (NCCAH)

In adult women, the diagnosis of NCCAH is strongly suggested by a basal 17-hydroxyprogesterone value greater than 200ng/dL (6 nmol/L) and confirmed with an ACTH stimulation test. The biochemical criteria used for diagnosis in men are the same as those used for women. Adult men are typically asymptomatic but may be diagnosed during a family evaluation.

For initial screening, a morning (7:30 to 8 AM) serum sample for 17-hydroxyprogesterone concentration should be obtained during the follicular phase of the menstrual cycle if the woman is cycling regularly. For women with amenorrhea or infrequent menses, the sample can be drawn on a random day.

If the basal sample is >200 ng/dL (6 nmol/L), a high dose (250 mcg) ACTH stimulation test, the gold standard for diagnosis, should then be performed. The response to ACTH is exaggerated in NCCAH, and a serum 17-hydroxyprogesterone value exceeding 1500 ng/dL (43 nmol/L) confirms the diagnosis. Rarely, stimulated values at 60 minutes in affected patients range between 1000 ng/dL (30 nmol/L) and 1500 ng/dL (43 nmol/L), and this range overlaps with the carrier state; thus, genotyping is recommended if stimulated values are in this range.

When a lower post-ACTH cutoff for 17-hydroxyprogesterone (>1000 n/dL) is used, the false positive rate is high. In a study of 21 women with a stimulated serum 17-hydroxyprogesterone >1000 ng/dL (30 nmol/L), only 5 out of 21 cases had the diagnosis confirmed by 24-hour urinary steroid profiling and genotyping. Four others were heterozygotes, and 12 had no mutation. This



observation supports our approach to diagnosis; a 17-hydroxyprogesterone >1500 ng/dL (43 nmol/L) after ACTH stimulation confirms the diagnosis. For patients with equivocal results (values between 1000 ng/dL [30 nmol/L] and 1500 ng/dL [43nmol/L]), we perform genotyping to distinguish between a heterozygote carrier and an affected patient. (20)

For more detailed information on primary adrenal insufficiency, please consult reference numbers 1,6,7,9,12,13, 19, and 20. For more detailed information on non-classic congenital adrenal hyperplasia, please consult reference numbers 20 and 21.

To confirm or exclude secondary adrenal insufficiency:

ACTH reserve should be measured in patients with intermediate serum cortisol values. Several tests of ACTH reserve are available; each has advantages and disadvantages. We suggest metyrapone testing when a test of ACTH reserve is required. (16)

Metyrapone test

Metyrapone blocks the conversion of 11-deoxycortisol to cortisol by CYP11B1 (11-beta-hydroxylase, P-450c11), the last step in the synthesis of cortisol, and induces a rapid fall of cortisol and an increase of its immediate precursor 11-deoxycortisol in serum.

Because it is essentially devoid of glucocorticoid activity, 11-deoxycortisol does not inhibit adrenocorticotrophic hormone (ACTH) secretion. Thus, in healthy individuals, the fall in serum cortisol concentrations leads sequentially to decreased negative feedback at hypothalamic and pituitary levels, which increases corticotropin-releasing hormone (CRH) and ACTH secretion and adrenal steroidogenesis; the resultant secretion of cortisol precursors, in particular, 11-deoxycortisol (the substrate of CYP11B1), can be measured by radioimmunoassay, high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), or fast liquid chromatography-tandem mass spectrometry (LC-MS/MS) in blood or its metabolites in urine. (24)

The increase in serum 11-deoxycortisol concentrations provides an index of the increase in ACTH release; a failure of these values to rise can indicate either ACTH deficiency or primary adrenal disease. Thus, if the metyrapone test is abnormal, the ability of the adrenal gland to respond to exogenous ACTH must be assessed to distinguish between these disorders.

The metyrapone test is a very sensitive test of pituitary adrenocorticotrophic hormone (ACTH) secretory reserve found to be more sensitive than insulin-tolerance test in certain studies and slightly less in other studies. It depends upon the release of pituitary ACTH secretion from negative feedback inhibition by cortisol; hypocortisolemia is a much less powerful stimulus to ACTH release than hypoglycemia or other stresses. Thus, a patient with partial hypopituitarism may maintain normal daily ACTH and cortisol secretion and respond to insulin-induced hypoglycemia with a normal increase in ACTH and cortisol secretion, yet be unable to increase ACTH secretion normally when cortisol biosynthesis is blocked by metyrapone. Conversely, a patient who responds normally to metyrapone almost always responds normally to hypoglycemia or other stresses. (24)

When used to diagnose Hypoadrenalism, a normal response to the metyrapone tests indicates an intact hypothalamic-pituitary-adrenal (HPA) axis; such a patient does not have any form of adrenal insufficiency and requires no further investigation. An abnormal test could reflect either primary or



secondary adrenal insufficiency. Distinction between the two can be determined by finding a high basal or stimulated plasma ACTH concentration, which would indicate primary adrenal insufficiency.

The metyrapone test has been performed initially as a two or three-day test based on 24-hour levels of 17-hydroxycorticosteroids (17-OHCS); this has been replaced now by an overnight single-dose test based on blood levels of 11-deoxycortisol. It cannot be performed in a patient who is taking any glucocorticoid. Metyrapone is currently available in North America through its distributor HRA Pharma (Paris, France) via its specialty pharmacy Direct Success Inc. with order forms available on the web (www.metopirone.us) or by phone at 1-855-674-7663; order forms can be faxed to 1-855-674-6767. (24)

Overnight single-dose metyrapone test.

The single-dose test is performed by oral administration of metyrapone (30 mg/kg, (2.2lbs.) body weight, or 2 grams for <70 kg, (<154lbs) 2.5 grams for 70 to 90 kg, (154-198lbs) and 3 grams for >90 kg, (>198lbs) body weight at midnight with a glass of milk or a small snack. Serum 11-deoxycortisol and cortisol are measured between 7:30 and 9:30 AM the next morning; plasma adrenocorticotrophic hormone (ACTH) can also be measured.

A normal response to the overnight single-dose test consists of:

- An 8 AM serum 11-deoxycortisol concentration of 7 to 22 mcg/dL (200 to 660 nmol/L).
- A serum cortisol concentration at 8 AM of less than 5 mcg/dL (138 nmol/L) confirms adequate metyrapone blockade and thereby documents compliance and normal metabolism of metyrapone. (24)

Serum 11-deoxycortisol concentrations less than 7 mcg/dL (210 nmol/L) with concomitantly suppressed cortisol values indicate adrenal insufficiency. However, in one study, the sum of 11-deoxycortisol and of cortisol >15 mcg/dL (450 nmol/L) following single dose overnight metyrapone yielded better diagnostic accuracy than using 11-deoxycortisol levels alone. In a study of 31 patients with various hypothalamic-pituitary-adrenal (HPA) axis abnormalities, comparing insulin tolerance test (ITT) with overnight metyrapone test, a cut-off of 144 nmol/L for 11-deoxycortisol yielded the highest sensitivity of 82.4 percent to detect patients responding normally to ITT, but only 64.3 percent of those with sub-normal response to ITT.

The ACTH response to metyrapone can distinguish between primary and secondary insufficiency. In general, patients with partial secondary adrenal insufficiency have ACTH responses from 10 to 200 pg/mL (2 to 44 pmol/L), while patients with primary adrenal insufficiency have higher responses. However, healthy individuals have an ACTH response of 42 to 690 pg/mL (9 to 210 pmol/L). Because of this overlap, the ACTH response alone cannot be used to distinguish between healthy individuals and those with adrenal insufficiency. (24)

The rise in serum 11-deoxycortisol concentrations may be exaggerated in women taking an oral contraceptive and patients with hypothyroidism, hypoglycemia, diabetes mellitus, congestive heart failure, obesity, and chronic renal failure. (24)

Settings in which false positive results can be obtained:

- Unappreciated recent exposure to synthetic glucocorticoids by any route can result in a subnormal response as a result of suppression of the corticotropes.



- One of the more common causes of a false positive result is unusually rapid clearance of metyrapone from the plasma, resulting in inadequate blockade of cortisol biosynthesis. This is manifested by a serum cortisol concentration greater than 7.5 mcg/dL (210 nmol/L) in the sample drawn at 8 AM in the overnight test. Cortisol levels measured by conventional immunoassays can be falsely elevated by the interference of increased 11-deoxycortisol levels induced by metyrapone; the wider availability of liquid chromatography-tandem mass spectrometry (LC-MS/MS) steroid assays should facilitate better interpretation of the results.

Rapid metyrapone clearance occurs in about 4 percent of normal subjects. Metyrapone is metabolized by hepatic cytochrome P-450 enzymes that are induced by many of the same drugs that increase steroid metabolism (e.g., phenobarbital, phenytoin, rifampin, and mitotane). As a result, these drugs should be stopped well before the metyrapone test is performed. (24)

Metyrapone administration may result in hypotension, nausea, and vomiting in patients with adrenal insufficiency; as a result, it should not be utilized in patients suspected of having severe adrenal insufficiency. Metyrapone can also cause dizziness, sedation, allergic rash or rarely decreased white blood cell count or bone marrow suppression. (24)

Insulin-induced hypoglycemia test (Insulin Tolerance Test, ITT)

The rationale for this test is that hypoglycemia induced by insulin administration is a sufficient stress to stimulate ACTH and therefore cortisol secretion. (16)

Advantages:

- The results correlate relatively well with the serum cortisol response to surgical stress.
- This test evaluates the integrity of the full hypothalamic-pituitary-adrenal axis as hypoglycemia acts centrally to stimulate hypothalamic corticotropin-releasing hormone (CRH) release and, therefore, ACTH release
- The degree of hypoglycemia can easily be quantified

Disadvantages:

- **Hypoglycemia can be dangerous in elderly patients and those with cardiovascular or cerebrovascular disease or a seizure disorder, and that constant monitoring is required during the first hour after the administration of insulin.** The monitoring is necessary to detect neuroglycopenic symptoms, which should be treated with intravenous glucose.
- Compared with other tests for the diagnosis of adrenal insufficiency, it is more difficult to perform, has some risk, and is more expensive. (25)

Procedure:

The patient fasts for at least eight hours before the test and must remain supine during the procedure. A clinician must be present at all times. A syringe containing 50 percent glucose solution should be at the bedside. An intravenous line is established and insulin is injected intravenously. The usual dose is 0.15 U/kg, but different doses may be indicated in certain patients:



- In patients thought to have hypopituitarism or primary adrenal insufficiency, the insulin dose is decreased to 0.1 U/kg because these conditions may be associated with decreased release of other counterregulatory hormones such as epinephrine and growth hormone.
- In patients with obesity, diabetes mellitus, suspected acromegaly or Cushing's syndrome, the dose is increased to 0.25 U/kg because insulin resistance is likely.
- In premenopausal women, the test can be performed at any phase of the menstrual cycle, as there are no cycle effects on the hypothalamic-pituitary-adrenal axis response to insulin-induced hypoglycemia. (25)

Blood is obtained for bedside measurement of serum glucose, and for laboratory measurement of serum glucose and cortisol assays (and for growth hormone, if indicated) immediately before insulin is injected and every 15 minutes thereafter. The last sample is obtained when adequate hypoglycemia, defined as 35 mg/dL (1.9 mmol/L) or less, is achieved. At that time, patients should have some symptoms of hypoglycemia, either of sympathetic discharge or of central nervous system glucose deprivation, such as simply falling asleep.

- Almost all patients have some degree of perspiration. If the patient does not perspire, the adequacy of the stress stimulus must remain suspect irrespective of the serum glucose concentration.
- Most patients also have a hyperactive precordium (but not tachycardia or hypotension, because they are supine), and feelings of hunger, drowsiness, detachment, or anxiety. The last is common and sometimes severe, and many patients find this an unpleasant experience.
- Hypoglycemia usually occurs 30 to 45 minutes after insulin injection. If adequate hypoglycemia is not achieved, a second similar dose of regular insulin should be injected intravenously. Adequate hypoglycemia should be achieved within the ensuing 30 to 45 minutes.
- Ideally, an automated glucose oxidase analyzer should be available at the bedside. Unfortunately, most glucose oxidase strips, whether read visually or with a glucose meter, are inaccurate at low serum glucose concentrations, tending to underestimate them, leading to premature termination of the test. The final, definitive blood sample for measurement of cortisol should be obtained 5 to 10 minutes after the patient begins to perspire or, if it can reliably be measured, when the serum glucose falls below 35 mg/dL (1.9 mmol/L). Some have recommended a serum glucose nadir of 45 mg/dL (2.4 mmol/L) or less, which appears to be adequate in most patients. (25)

Patients with primary or secondary adrenal insufficiency or long-standing diabetes mellitus have an impaired compensatory response to hypoglycemia.

- **Therefore, the test should be stopped when the serum glucose concentration falls to or below 35 mg/dL (1.9 mmol/L), by infusing 10 percent glucose solution in addition to giving sweetened orange juice or cola by mouth.**
- A 50 percent glucose solution should be infused over a period of one minute (slowly, because of its hypertonicity) if seizure, chest pain, confusion, disorientation, or other potentially serious complications occur; this will result in hyperglycemia within 30 seconds.
- Blood should be drawn for measurement of cortisol when glucose is given, and 15 minutes later, as levels continue to increase despite increasing serum glucose concentrations. (25)



The impact of glucose infusion on cortisol values was illustrated in a study of hypoglycemic symptoms in 16 healthy subjects who underwent two insulin tolerance tests. Each received 500 mL of a 5 percent glucose or a 0.9 percent saline solution in a randomized fashion when the blood glucose fell to less than 40 mg/dL (2.2 mmol/L). The duration and severity of symptoms were reduced significantly by glucose infusion and peak cortisol levels were similar in the two tests. Although such an approach has not been validated in patients with adrenal insufficiency, it may be useful in patients with pituitary disease who may have diminished counter-regulatory responses. (25)

Normal values

The criteria for a normal serum cortisol response ranged from 18 to about 22 mcg/dL (500 to 600 nmol/L) in multiple studies using different assays and various methods to establish a normal reference range. Ideally, reference ranges would be determined locally but this is rarely done in practice. If serum cortisol reaches this level, it is unimportant whether hypoglycemia was adequate. On the other hand, failure to reach this level is indicative of an inadequate response only if the serum glucose fell to 35 mg/dL (1.9 mmol/L) or less. If this was not achieved, the stimulus was inadequate, and the test must be repeated. It is the serum cortisol concentration that is achieved rather than the increment that is important.

The serum cortisol increase parallels that of plasma ACTH up to a serum cortisol concentration of about 22 mcg/dL (607 nmol/L), corresponding to a plasma ACTH concentration of approximately 75 pg/mL (16 pmol/L), which is the acute maximally stimulating plasma concentration of ACTH. (25)

Plasma ACTH can also be measured. The normal plasma ACTH response has not been carefully defined, but the peak concentration should exceed 150pg/mL (33 pmol/L).

Serum growth hormone should also increase; its measurement provides another index of anterior pituitary function. (25)

An inadequate cortisol response can be due to hypopituitarism of any etiology, including hypothalamic CRH deficiency, isolated ACTH deficiency, partial or pan hypopituitarism, and acute or chronic administration of synthetic glucocorticoids; it can also be due to primary adrenal insufficiency when serum cortisol rather than plasma ACTH values are used as the end-points of the test. However, false negative results in patients who have partial adrenal insufficiency can occur because hypoglycemia is such a potent stimulus that it may obscure a partial defect.

- Hypoglycemia is a much stronger stimulus of ACTH secretion than is hypocortisolemia (as induced by metyrapone). Consequently, patients may have a normal response to hypoglycemia, but an inadequate response to metyrapone, which can detect subtle defects in ACTH secretion. The reverse is almost never true.

Although insulin-induced hypoglycemia is a valid, and perhaps the most rational, test of hypothalamic-pituitary-adrenal response to stress, there is little, if any, reason for performing the test except in patients with suspected recent corticotropin (ACTH) deficiency (e.g., recent pituitary surgery) or in patients with suspected growth hormone and ACTH deficiency. (25)

Cosyntropin (ACTH) stimulation test



The rationale for the administration of cosyntropin, (synthetic ACTH) is that the adrenal glands atrophy when they have not been stimulated for a prolonged period; as a result, they do not secrete cortisol normally in response to a bolus dose of ACTH. The test is usually performed by administering 0.25 mg (25 units) of cosyntropin (synthetic ACTH 1-24) intramuscularly or intravenously and measuring serum cortisol 60 minutes later. A serum cortisol concentration of ≥ 18 mcg/dL (497 nmol/L) is considered a normal response.

In practice, this test is not often useful because a patient who has such severe ACTH deficiency that the adrenal glands do not respond normally to cosyntropin will also probably have an 8 to 9 AM basal serum cortisol value that is ≤ 3 mcg/dL (83 nmol/L) and therefore will not need a test of ACTH reserve. *On the other hand, a patient who has partial ACTH deficiency may respond normally to cosyntropin and requires a test of ACTH reserve.*

A low-dose cosyntropin stimulation test has been proposed as yielding fewer falsely normal results, but several studies show that this test has the same pitfalls as the standard dose test. We do not recommend either the standard or low-dose test. (16)

Pituitary imaging (MRI)

Not all pituitary disorders will appear on imaging

For more detailed information on secondary adrenal insufficiency, please consult reference numbers 2,8,10, 12,13, 15, 16, and 19.

To confirm or exclude tertiary adrenal insufficiency:

The corticotropin-releasing hormone (CRH) test has been proposed for the diagnosis of secondary adrenal insufficiency and for the distinction between secondary or tertiary adrenal insufficiency. The rationale for the latter use is that a maximally stimulating dose of exogenous CRH will stimulate adrenocorticotrophic hormone (ACTH) secretion by anterior pituitary corticotrophs if they are intact and the problem lies in the hypothalamus, but will not stimulate ACTH secretion if the pituitary is damaged. The test is primarily used in patients without the expected elevation in basal plasma ACTH levels in the presence of adrenal insufficiency, indicating that they have a hypothalamic or pituitary cause rather than primary adrenal disease. The CRH plus vasopressin tests have not been evaluated in patients with adrenal insufficiency. (26)

The CRH test is expensive; the cost for the CRH dose alone is over \$300 (United States). Most investigators use an increase in either plasma ACTH or plasma cortisol concentration as the criterion for response. Multiple blood samples must be obtained (at least one basal sample and at least two and as many as eight post-CRH samples). As a result, the test should probably be performed only by specialists in carefully selected patients. In the United States, ovine CRH is available and carries US Food and Drug Administration (FDA) labeling for the differential diagnosis of Cushing's syndrome. In Europe, the human analog is available. (26)

For more detailed information on Tertiary adrenal insufficiency, please consult reference numbers 1, 2, 10, 15, 16, and 26.



Further testing

The hypothalamus and pituitary glands control production of both adrenal and thyroid hormones. SAI and TAI patients often have thyroid disorders, as well as other hormone deficiencies.

The use of thyroid hormones is contraindicated in patients with uncorrected adrenal insufficiency. Thyroid hormones increase tissue demand for adrenocortical hormones and may precipitate an acute adrenal crisis. In patients with controlled adrenal insufficiency or other endocrine disorders, therapy for these concomitant disorders may need to be adjusted following thyroid replacement, since hypothyroidism often obscures or diminishes the signs and symptoms of these conditions. Clinical monitoring of thyroid and adrenal function as well as other appropriate endocrine function is recommended during thyroid hormone therapy. (drugs.com levothyroxine disease interactions)

Hypothyroidism in patients who have pituitary or hypothalamic disease is the result of thyrotropin (TSH) deficiency and, therefore, unlike in patients who have thyroid disease, an elevated serum TSH concentration cannot be used to make the diagnosis of hypothyroidism. The serum TSH concentration is usually not low either, except when the hypothyroidism is treated. Screening for hypothyroidism in patients with pituitary or hypothalamic disease is therefore performed by measuring thyroxine, either total thyroxine (T4) and triiodothyronine (T3) uptake or free T4. (16)

For this reason, we suggest the following tests to evaluate the hypothalamus-pituitary-thyroid (HPT) axis when SAI or TAI is suspected.

Thyroid panel

Thyroid antibodies

Physicians should also consider testing gonadotropins, growth hormone, and prolactin, if indicated. (16)

Summary

- Disorders of the HPA axis often present with a variety of non-specific symptoms that develop over time. For example, body aches, fatigue, weakness, depression, and anxiety. We recommend physicians test for adrenal insufficiency in patients with non-specific symptoms severe enough to interfere with daily function.
- Adrenal insufficiency is a disorder with multiple etiologies and history is an important factor in diagnosis.
- The clinical presentation of adrenal insufficiency is variable, depending on whether the onset is acute, leading to adrenal crisis, or chronic, with symptoms that are more insidious and vague.
- It is essential that treatment of patients who present in possible adrenal crisis not be delayed while diagnostic tests are performed. Blood for serum cortisol and serum chemistry should be drawn; some clinicians also draw and hold samples for later measurement of ACTH, renin, and aldosterone if the diagnosis of adrenal insufficiency is likely. Therapy should be initiated immediately with intravenous saline and dexamethasone.



- Some features of PAI, SAI, and TAI are identical, but others are distinctly primary or secondary attributes. For example, hyperpigmentation is seen with PAI but not SAI and TAI. In addition, physicians should be aware that comorbidities may cause contradictory symptoms. For example, primary patients typically present with weight loss, while secondary patients often have weight gain due to other hormone imbalances or insulin resistance.
- Other hormone treatments, supplements, or drugs may interfere with accurate test results.
- Confirmation of the clinical diagnosis of adrenal insufficiency is a three-stage process:
 1. Demonstrating inappropriately low cortisol secretion
 2. Determining whether the cortisol deficiency is dependent on or independent of corticotropin (ACTH) deficiency and evaluating mineralocorticoid secretion in patients without ACTH deficiency
 3. Seeking a treatable cause of the primary disorder (e.g., infiltrative process involving the adrenal glands or a pituitary adenoma compromising normal pituitary function)
- Based on these diagnostic parameters we recommend the following initial tests.
 1. To demonstrate inappropriately low cortisol secretion. The morning baseline serum cortisol, drawn between 8 and 9 am.
 2. To determine if the cortisol deficiency is dependent on or independent of corticotropin (ACTH) deficiency. The baseline serum ACTH, drawn simultaneously with the morning cortisol.
 3. To evaluate mineralocorticoid secretion. Aldosterone and plasma renin activity (PRA)
 4. To evaluate organ function, electrolytes, hydration, and blood glucose levels. Comprehensive metabolic panel (CMP)
- Morning cortisol and ACTH baselines, drawn simultaneously and interpreted together, indicate the direction for further testing.
- If serum cortisol is inappropriately low and a simultaneous plasma ACTH concentration is very high, the patient has primary adrenal insufficiency.
- If both the serum cortisol and plasma ACTH concentrations are inappropriately low, the patient has secondary (i.e., pituitary disease) or tertiary (hypothalamic disease) adrenal insufficiency.
- Most recommendations agree that a cortisol test result achieving the top reference number or above, indicates no dysfunction, and a result at or below the bottom reference number indicates definitive AI. However, test results between these two points, usually considered “normal values range” are indeterminate in many cases when profile, history, and clinical symptoms are considered.
- Indeterminate results require dynamic testing to confirm or exclude diagnosis.



- To confirm or exclude Primary adrenal insufficiency:
 1. ACTH stimulation test
 2. 21-hydroxylase antibody (21-OH AB)
 3. Serum 17-hydroxyprogesterone (17-OHP)
 4. Adrenal imaging

- To confirm or exclude Secondary adrenal insufficiency:
 1. Metyrapone stimulation test
 2. Insulin Tolerance Test (ITT)
 3. Pituitary imaging

- To confirm or exclude Tertiary adrenal insufficiency:
 1. CRH test

- Further testing if SAI/TAI is suspected:
 1. Thyroid panel
 2. Thyroid antibodies

- Other hormone deficiencies to consider in SAI/TAI patients:
 1. Gonadotropins
 2. Growth hormone
 3. Prolactin

Compiled by:

Maria Stewart, Director, Adrenal Insufficiency Coalition.

Chief Editor: Desley Rolph, Associate Director, Adrenal Insufficiency Coalition.

Deputy Editor: Michelle Dalton, Adrenal Insufficiency Coalition.

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