

ENDOCRINE TESTING PROTOCOLS: HYPOTHALAMIC PITUITARY ADRENAL AXIS

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ABSTRACT

Abnormalities in the hypothalamic pituitary adrenal (HPA) axis are identified by a careful analysis of both direct and non-stimulated measurements of the hormones as well as provocative tests. Dynamic testing is useful to determine if elevated levels are suppressible and whether there is sufficient hormone reserve when low levels are measured under stimulation. A combination of all these analyses can distinguish between normal physiology and the consequences of clinical disease in the HPA axis. While clinical suspicion drives the testing performed, arrival at the correct diagnosis by laboratory testing is crucial for cure of the patient. Knowledge of the methodologies used in measuring cortisol and ACTH and associated hormones and binding proteins is essential for correct interpretation of the tests. In this review we compare methodologies available, sensitivity and specificity of the various assays and volumes of sample needed. There are at least 7 different types of dexamethasone suppression testing and they are compared and described in detail. Confirmation of the anatomic source of the hormone is necessary. Petrosal sinus sampling and adrenal vein sampling are reviewed and the clinical indications for

each discussed. Finally, once the endocrine diagnosis is reached based on endocrine testing, imaging studies are then reviewed which can confirm the endocrine diagnosis. An abnormality in the HPA axis is a laboratory diagnosis and radiologic imaging is reserved for the last step in the diagnosis of endocrine disease.

NONSTIMULATED HORMONE MEASUREMENTS

Overview

In evaluation of the hypothalamic pituitary adrenal (HPA) axis, static measurement of hormones is seldom useful due to the variable nature of cortisol and ACTH secretion in normal physiological states. In general, if one is suspicious of hypofunction of the HPA axis, then measurement of morning cortisol at 8 am when it is expected to be at its peaks is a good screening strategy. Depending on the result, this might need to be followed by dynamic testing to stimulate either adrenocorticotrophic hormone (ACTH) or cortisol for confirmatory purposes. On the other hand, if one is concerned about Cushing's syndrome (CS), an overproduction of cortisol or ACTH, then measurement of cortisol should be performed late at night, when it is expected to be at its nadir.

Alternatively, one could test cortisol's response to suppression with dexamethasone.

The American Endocrine Society Clinical Guidelines recommend one of the following tests for the initial CS testing: at least two measurements of urinary-free cortisol (UFC), two measurements of late night salivary cortisol (LNSC), 1 mg overnight dexamethasone suppression test (DST) or a longer low-dose DST (1). Cortisol measurement (serum, UFC or salivary) is the end point for each recommended test.

Despite recent literature reports describing utility of direct salivary and urine cortisol measurements in CS diagnosis (2-4), most clinicians prefer provocative testing due to the variable nature of cortisol and ACTH secretion in normal physiological states. Cortisol is secreted under the direction of ACTH and follows a diurnal variation, with peak values at 08:00 h and a nadir at 22:00 h. In CS, diurnal variation is lost and PM cortisol level is inappropriately elevated. Superimposed on this diurnal pattern are 8-10 pulsatile peaks released during the course of a 24-hour period. Therefore, depending on the instance when blood is sampled, there can be significant variation in the absolute values of ACTH and serum cortisol. Due to this variability of cortisol and ACTH levels, it may be challenging to distinguish pituitary-dependent Cushing's disease from pseudo-Cushing's states. Cunningham et al conducted a study where blood was sampled and cortisol measured every 20 to 30 minutes for 24 hours. The group demonstrated that both circadian and pulse amplitudes of cortisol secretion were decreased in Cushing's disease (5).

This section provides and overview of methodologies commonly used in clinical laboratories for direct determinations of cortisol and ACTH, regardless of whether they are a part of a provocative testing series or direct, non-stimulated hormone assessment.

Cortisol

Methods currently available for measuring serum cortisol levels include automated immunoassays and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

CORTISOL IMMUNOASSAYS (TOTAL CORTISOL)

Cortisol immunoassays are widely available, have been in use for a long time, and automated methods provide high throughput with minimal manual sample manipulations. Virtually all immunoassay methods are based on the competitive binding principle, where cortisol from the patient sample and exogenous, labeled cortisol compete for the binding sites available on the anti-cortisol antibody. The major difference between the assays is in the label design and chemistry enabling antibody-antigen binding. All currently available cortisol methods have limit of detection below 1 µg/dL, providing sufficient sensitivity to support interpretation of CS dynamic testing results.

A widely recognized disadvantage of immunoassays is a potential of interferences from auto-, anti-animal or heterophilic antibodies. In addition, the older generations of cortisol assays had significant cross-reactivity with other steroids, such as 6-β-hydroxycortisol or prednisolone, due to the use of less specific polyclonal antibody in the assay formulation. However, the majority of current immunoassay methods have transitioned to a more specific monoclonal antibody format, minimizing or eliminating cross-reactivity with other steroids. It should also be noted that some immunoassay vendors use biotinylated antibodies in their assay design. In these instances, biotin may interfere with the assay, causing spuriously elevated cortisol measurement. The presence and magnitude of interference is vendor-specific and the potential of biotin interference should be checked with the laboratory that performs the testing. In general, none of the assays manufactured

by Abbott use biotin in reagent formulation, while all assays manufactured by Roche do. The Roche cortisol assay should not be used to measure serum cortisol in a patient taking daily doses of biotin exceeding 5 mg, unless blood is obtained at least 8 hours following the last biotin ingestion.

LC-MS/MS CORTISOL ASSAYS

The LC-MS/MS assays utilize liquid chromatography to separate cortisol from other serum/plasma components and tandem mass spectrometry to detect and quantify compounds of interest. LC-MS/MS based methods offer superior analytical sensitivity and specificity over immunoassays.

Serum Free Cortisol

In conditions where CBG concentrations are affected, such as pregnancy or critical illness for example, total serum cortisol may not always reflect the true pituitary-adrenal status. In these cases, assessment of serum free cortisol is preferred. Free serum cortisol concentration are directly measured by separating free serum cortisol fraction using equilibrium dialysis (6) or ultrafiltration (7, 8) followed by cortisol determination, usually performed using LC-MS/MS method. Alternatively, free serum cortisol can be estimated by calculating the ratio of serum cortisol and CBG to obtain serum cortisol index (6). Although not affected by CBG levels, free cortisol is also secreted in episodic fashion and thus not much more useful than random total serum cortisol levels in assessment of HPA axis functionality.

Urinary Free Cortisol

Cortisol is excreted in urine in an unbound (free) form and, like free serum cortisol is unaffected by fluctuations in CBG levels. Properly collected 24-hour urine specimens can be used to eliminate fluctuations that would affect serum cortisol levels, due to the

pulsatile nature of its release. Therefore, measurement of UFC from 24 hour urine collections has become a valuable diagnostic tool for evaluation of adrenal cortical function and it is one of the first line tests recommended for CS diagnostic testing (1). In the unstressed patient, with normal renal function, elevation of UFC in 24-hour urine specimen is usually sufficient to diagnose CS. A normal result is strong evidence against that diagnosis. Although this test has long been used, its utility in CS diagnosis still remains somewhat controversial. Studies show wide variability in clinical utility of UFC for diagnosis of CS with clinical sensitivity ranging from 53% to over 90% and specificity ranging from 79% to 90% (2, 3, 9). These differences are due to differences in study design, cut-off, and methodology used. Furthermore, in a careful study of normal subjects de Boss Kuil et al found that urinary excretion of free cortisol can differ by as much as 50% between the two consecutive urine collections, while the creatinine values can differ by as much as five fold (10). Since the ratio of free cortisol/creatinine also varies considerably (range 1.0-3.7; median 1.3), intra-variation in urinary cortisol excretion could not be attributed to variation in creatinine excretion. In addition to biological variation, other factors include difficulty in over or under collection of urine. Given such wide discrepancies in reported clinical sensitivity and specificity of UFC measurements and significant intra-individual UFC variability, this test may not be an ideal choice for initial screening of CS.

Methodology used for UFC quantitation is the same as for serum cortisol. In terms of specimen collection, an 8:00 AM to the following day's 8:00 AM collection is desirable. Samples should be refrigerated during collection and, while preservatives are not required, boric acid is usually acceptable. Quantitation of urine cortisol with a more sensitive and specific LC-MS/MS method is generally preferred over immunoassays. Typically, all the LC-MS/MS UFC assays involve a sample pretreatment with an organic solvent which removes the interfering substances. However, some

UFC assays immunoassays either do not include this pretreatment step or offer it as an optional step to the user. As a result, UFC reference ranges vary widely between the assay manufacturers, methodologies, and different laboratories. To increase sensitivity, it is recommended that the upper limit of normal for any UFC assay be used as a positive test (5). It would be thus incorrect to make a diagnosis of adrenal insufficiency relying solely on 24-hour urine collections.

Salivary Cortisol

Late-night (23:00-24:00 h) salivary cortisol (LNSC) is one of the first line tests used to screen for CS. Most studies report high diagnostic sensitivity of this test (80-90%), but there are discrepancies in reported specificities (70-90%), resulting mostly from difference in methodologies and populations studied (2, 3, 11-13). Interestingly, mass spectrometry assays demonstrate high sensitivity, but low specificity (75%) for the diagnosis of CS (11). One potential explanation, as postulated by Raff, is that higher analytical specificity of mass spectrometry actually leads to lower diagnostic specificity, suggesting that cortisol metabolites and precursors picked up by immunoassays may be diagnostically relevant (14). Kannankeril et al recently reported that LNSC has excellent negative predictive value (99.8%) but poor positive predictive value (16.8%) for diagnosis of ACTH-dependent CS (12). Thus, a negative LNSC can be used to rule out ACTH-dependent CS, but complementary tests of adrenal function are needed to establish the diagnosis.

Salivary cortisol concentration is not dependent on CBG and could therefore be useful during an ACTH stimulation testing in patients with increased CBG concentrations due to increased estrogen or decreased plasma binding globulins due to critical illness.

Similar to UFC, the assay methodology remains the same as serum cortisol with the differences in specimen collection.

ACTH

ACTH measurements, while subject to the same circadian variability as cortisol (actually it is the variability of the ACTH that is directly responsible for the variability of the cortisol), are not subject to the effects of CBG. Values of ACTH > 100 pg/ml in the setting of possible adrenal insufficiency are usually suggestive of primary adrenal insufficiency, while values >500 pg/ml are diagnostic. Low concentrations of plasma ACTH are not diagnostic, except for the undetectable levels observed in patients with cortisol producing adrenal adenomas. Plasma ACTH concentration is also low in patients taking exogenous steroids.

Unlike widely available cortisol assays, the availability of clinical ACTH assays is limited. All currently available methods are immunoassays based on the “sandwich” principle, where two antibodies that recognize different ACTH epitopes are utilized. The first antibody, designated as capture antibody, detects one specific site on ACTH molecule and is used to pull the antigen from the patient’s plasma. The second antibody that detects a different ACTH epitope is then used to “sandwich” the antigen and generate a signal.

As is the case with any immunoassay, ACTH assays are susceptible to heterophilic antibody interferences. Several cases have been described in literature where aberrant, falsely elevated ACTH results were inconsistent with clinical picture and lead to unnecessary testing, misdiagnosis, and in some cases surgical interventions. These cases emphasize the importance of interaction between clinicians and the laboratory to identify any interference present and ensure that each patient is appropriately managed (15, 16). In addition, just as is the case with cortisol

immunoassays, some vendors use biotinylated antibodies in the capture antibody design. Unlike cortisol, however, biotin interference may result in falsely decreased ACTH levels. The two most commonly used ACTH assays are manufactured by Siemens and Roche. Siemens ACTH assay is not affected by biotin, while the recommendation for Roche ACTH assay is not to use the test in patients ingesting >5 mg biotin daily, unless at least 8 hours had elapsed following the last biotin dose (cf. Roche Elecsys ACTH Package Insert V 12.0, 2020-11).

The preferred specimen for ACTH is EDTA plasma. ACTH is heat labile, and if not collected and preserved on ice, will lead to proteolysis, which can reduce the plasma concentration leading to falsely lower values.

Miscellaneous Non-Stimulated Measurements

CORTISOL BINDING GLOBULIN (CBG)

As mentioned earlier, the majority of cortisol (~92%) is bound to CBG, a serum protein. CBG levels increase in pregnancy and patients on oral contraceptives or supplemental estrogen. CBG is decreased in hyperinsulinemic states, nephrotic syndrome, starvation, severe illness, and chronic liver disease. This test is useful for the assessment of unexpected serum cortisol values. It is offered by large reference laboratories and uses a radioimmunoassay method.

11-DEOXYCORTISOL (COMPOUND S)

This is the immediate precursor of cortisol and is typically increased when ACTH is elevated or in 11 beta-hydroxylase deficiency. The method for 11-deoxycortisol measurement is now available by LC-MS/MS technology and is offered by most reference laboratories.

ANTI-ADRENAL ANTIBODIES

The measurement of anti-adrenal antibodies has been suggested to be useful in detecting early evidence of adrenal insufficiency, before cortisol values are decreased even in response to stimuli. The only test currently clinically available is a test that detects 21-hydroxylase autoantibodies, which are present in the common autoimmune form of Addison's disease (17). This test is offered by major reference laboratories and is based on the radioimmunoassay format.

CORTICOTROPHIN RELEASING HORMONE (CRH)

Serum concentration of CRH is markedly elevated in pregnancy, presumably due to the production of CRH by the placenta. High levels are associated with high levels of CRH binding protein. Although mentioned as useful in the diagnosis of ectopic CRH syndromes, little data is available in this regard. CRH testing is not commonly done and we have not been able to find a commercial laboratory that is currently performing this test.

DYNAMIC TESTING

Glucocorticoid Deficiency

Adrenal insufficiency is a life-threatening disorder and prompt diagnosis is important because adequate hormonal replacement therapy can be lifesaving.

Despite that more than 35 years have elapsed since the initial description of the use of the insulin tolerance test (ITT) to diagnose adrenocortical deficiency (18), and more than 200 scientific publications in this area, clinicians today still argue as to which is the most sensitive and specific test to diagnose adrenocortical deficiency. The ITT is still regarded as the gold standard upon which to compare all other tests of HPA axis function. Unfortunately, this test has a considerable spectrum of intra-individual and inter-individual variation (19, 20). Therefore, when comparing other tests to the "gold standard", if the

standard is not reliable, how can one determine the effectiveness of the other forms of testing? The problem lies in the ability of a single laboratory to know what the values are for their tests. Therefore, ranges from an ITT test response in normal subjects performed in one laboratory may not be normal for another laboratory. Taking this into account there are some general guidelines that are available for evaluating patients with suspected adrenal insufficiency.

PRIMARY ADRENAL INSUFFICIENCY

High Dose ACTH Stimulation Test

WHEN TO USE THIS TEST: Patients acutely ill in the hospital or clinic who present with signs and symptoms suggestive of primary adrenal insufficiency. Patients who are thermodynamically unstable should be resuscitated with crystalloids and given dexamethasone prior to testing if the diagnosis of primary adrenal insufficiency is being considered.

PROCEDURE: An intravenous (IV) line is placed 30 minutes before the test for rapid phlebotomy and to eliminate a temporary rise in cortisol associated with a needle stick. The IV line is to be kept open with 0.9% sodium chloride (NaCl) at a rate of 50 ml/hr. Blood is drawn at 0 min for ACTH (2 ml in a lavender top tube on ice) and cortisol (2 ml in a red top tube). Cosyntropin, 0.25 mg is administered as an IV bolus over 2 minutes. The cosyntropin comes as a lyophilized powder which should be reconstituted with 1 ml of 0.9% NaCl. Thirty min after the injection, blood is obtained from the IV line (2 ml) for cortisol. The same is repeated at 60 min (2 ml) for cortisol.

SPECIAL CONSIDERATIONS: The test can be performed at any time of the day. If the patient is receiving hydrocortisone or cortisone acetate, the medication should be held for at least 12 hours prior to testing (if possible). Although the test can be

performed while the patient is receiving dexamethasone, there is some cross-reactivity in some assays and cortisol levels may not be accurate. Each laboratory should determine for itself, the effect of dexamethasone on their assay.

Patients with known sensitivity to cosyntropin or its preservatives should not have it administered. Oral estrogen use may result in elevation of the total serum cortisol level due to increased corticosteroid binding globulin (21). Patients with albumin <2.5 g/dL may also have a low cortisol level (21, 22).

CONTRAINDICATIONS: Hypersensitivity to cosyntropin or any component of the formulation.

WARNINGS / PRECAUTIONS: Use with caution in patients with pre-existing allergic disease or a history of allergic reactions to corticotropin. Class C in pregnancy.

ADVERSE REACTIONS 1% to 10%: Cardiovascular: Flushing. Central nervous system: Mild fever. Dermatologic: Pruritus. Gastrointestinal: Chronic pancreatitis. <1%: Hypersensitivity reactions

DRUG INTERACTIONS: Decreased effect: May decrease the effect of anticholinesterases in patients with myasthenia gravis; nondepolarizing neuromuscular blockers, phenytoin and barbiturates may decrease effect of cosyntropin

INTERPRETATION OF RESULTS: Baseline cortisol values <5 µg/dl and ACTH concentrations >100 pg/ml are usually diagnostic of primary adrenal insufficiency. The normal peak cortisol value post stimulation should be an increment no less than 7µg/dl. A peak stimulated cortisol value of >18 µg/dl at 30 min is considered normal. Since 37% of subjects had a peak response to cosyntropin at 30 min and 63% had a peak response at 60 min, both time points are analyzed in all patients and if either the 30 min or 60

min sample reaches the criteria as noted above, the test is considered normal (23). However, there is some suggestion that new generation cortisol assays may have different cutoff values, but these have not been verified (24).

Free cortisol, instead of total cortisol can be measured using a value of $>1.2 \mu\text{g/dl}$ at 30 or 60 min as a normal result. This can be indicated in patient with albumin levels $<2.5 \text{ g/dL}$ or those with low cortisol binding globulin.

Serum aldosterone can be measured in 0 min, 30 min and 60 min blood samples as ACTH stimulation of the adrenal cortex will also stimulate aldosterone. It has been suggested that a normal aldosterone response to ACTH in the presence of a suboptimal cortisol response is diagnostic of secondary adrenal insufficiency (25).

Low dose ACTH stimulation Test

WHEN TO USE THIS TEST: Patients with subtle signs of adrenal insufficiency or patients who have been treated with glucocorticoids in whom determination of adrenal reserve is necessary. Patients who have autoimmune disease and may have early adrenocortical insufficiency may be best assessed with this test.

PROCEDURE: An intravenous line is placed 30 minutes before the test for rapid phlebotomy and to eliminate a temporary rise in cortisol associated with a needle stick. The IV line is to be kept open with 0.9% NaCl at a rate of 50 ml/hr. Blood is drawn at 0 min for ACTH (2 ml in a lavender top tube on ice) and cortisol (2 ml in a red top tube).

Cosyntropin, 1 μg is administered as an IV bolus over 2 minutes. The injection material was prepared according to the method of Dickstein as follows: The cosyntropin was diluted with 50 ml of sterile saline to

a stock concentration of 5 $\mu\text{g/ml}$. Aliquots of 0.2 ml were added into sterile plastic tubes and kept at 4°C for a maximum of 4 months (26). Immediately prior to testing 0.8 ml of saline is added to the tube (final dilution 1 $\mu\text{g/ml}$) and 1 ml is injected into the patient. Thirty min after the injection blood is obtained from the IV line (2 ml) for cortisol. The same is repeated at 60 min (2 ml) for cortisol.

SPECIAL CONSIDERATIONS: Same as for high dose ACTH stimulation test, see above.

INTERPRETATION OF RESULTS: This test was originally developed to be more sensitive for diagnosing secondary adrenal insufficiency because it was more of a "physiologic" dose. It is much better at diagnosing secondary adrenal insufficiency than the high dose, although it is not at all recommended in acute or recent hypopituitarism when the intact adrenal glands can still respond normally to any dose of ACTH. Although probably not useful for the initial purpose of secondary adrenal insufficiency, it may be more sensitive at distinguishing milder forms of primary adrenal insufficiency (27). Furthermore, this low dose test was helpful in identifying mild adrenal suppression in asthmatic children being treated with inhaled steroids (28). As noted above, each laboratory should establish their normal values, however in general, a stimulated value at 30 or 60 min greater than 20 $\mu\text{g/dl}$ would be considered normal.

A meta-analysis of 30 studies enrolling 1209 adults and 228 children with secondary adrenal insufficiency, evaluating the diagnostic accuracy of high and low dose ACTH stimulation concluded that they have similar diagnostic accuracy. They are both adequate to rule in, but not rule out, secondary adrenal insufficiency (29).

SECONDARY ADRENAL INSUFFICIENCY (PITUITARY OR HYPOTHALAMIC)

Insulin Tolerance Testing (ITT)

WHEN TO USE THIS TEST: Patients in whom pituitary or hypothalamic disease may result in impaired corticotroph (or somatotroph) activity. Patients following pituitary surgery or pituitary radiation can be tested at any time, unlike the ACTH stimulation tests described above which are not useful in the acute setting. A random serum cortisol should be drawn prior to scheduling the test if the value is > 20 µg/dl, the test may not be necessary. This test, can be performed in the outpatient clinic, however while relatively safe it requires a trained endocrine registered nurse to be present with the patient during the course of the test.

PROCEDURE: A 50 ml vial of 50% Dextrose should be at the patient's bedside in a syringe ready for injection before beginning the procedure.

An intravenous line is placed 30 minutes before the test for rapid phlebotomy, to eliminate a temporary rise in cortisol associated with a needle stick, and in order to have IV access for 50% Dextrose in the event of severe hypoglycemia. The IV line is to be kept open with 0.9% NaCl at a rate of 50 ml/hr. Blood is drawn at 0' for cortisol (2 ml in a red top tube) and glucose (1 ml in a gray top tube). Blood glucose is also checked at the bedside with a glucose monitor.

Regular (short acting) insulin is administered as an IV bolus at a dose of 0.1 units/kg. Blood is sampled for cortisol and glucose as noted above at 10min, 15min, 30min, 45min, 60min, 90min and 120min. A bedside nurse should monitor the blood glucose more frequently if glucose drops below 60 mg/dl on the glucometer or if the patient complains of neuroglycopenic symptoms, such as fatigue, diaphoresis, hunger, lightheadedness, or nausea. The

test should continue until the blood glucose concentration drops below 40 mg/dl.

In patients with diabetes on insulin, consideration should be given that they may be insulin resistant. In which case, larger doses of insulin may be given. We usually begin with a single bolus of 0.1 U/kg and then re-bolus with insulin depending on the response to the initial dose (either give the same dose again if there was some response but insufficient, or double the dose if there was only minimal response to blood glucose, or give half the dose if the hypoglycemic response was close to the desired goal). This can be repeated several times until adequate hypoglycemia is reached.

Once the response goal of a glucose < 40 mg/dl is reached, patients can be fed a meal such as crackers and orange juice. Blood glucose should be checked at 5min, 10min and 15min post feeding. If there is no increase in glucose or a clinical response within 5min, intravenous glucose should be administered. If no response, then a repeat bolus of glucose is suggested. If no response or IV access is lost, glucagon 1 mg intramuscular can be given.

SPECIAL CONSIDERATIONS: The test can be performed at any time of the day, although due to the need for patients to be fasting it is most conveniently done in the morning. If the patient is receiving hydrocortisone or cortisone acetate, the medication should be held for at least 12 hours prior to testing (if possible). Unlike the ACTH stimulation tests, the ITT cannot be performed while the patient is receiving dexamethasone, due to suppression of the hypothalamic pathways necessary to respond to hypoglycemia.

In general ITT is not recommended in patients with uncontrolled seizure disorder or significant coronary artery disease.

In order to determine if the level of dysfunction is at the hypothalamus or at the pituitary this test is sometimes used in addition to the CRH stimulation test. When the ITT fails to stimulate cortisol, but the CRH test does stimulate it is likely that the patient is having hypothalamic dysfunction.

INTERPRETATION OF RESULTS: Serum cortisol should increase within 30 min of the hypoglycemic response to > 20 µg/dl. If the serum cortisol at baseline is 18 ug/dl the test may not be diagnostic. If the baseline serum cortisol is higher than 19 µg, adrenal insufficiency is unlikely. Although the response of cortisol is more reproducible than that of growth hormone in the ITT, intra-subject differences have been reported (20, 30).

Metyrapone Testing

WHEN TO USE THIS TEST: This test is perhaps the most sensitive to determine whether the HPA axis is intact. Although metyrapone is not generally available from your neighborhood pharmacy, it can be obtained by calling Novartis Pharmaceutical Corp. at 1-800-988-7768 on weekdays. Metyrapone blocks 11-β hydroxylase and results in the inhibition of conversion of 11-deoxycortisol to cortisol. Serum levels of cortisol decrease and concentration of 11-deoxycortisol increases, however 11-deoxycortisol does not down regulate ACTH. Therefore, in a normally functioning HPA axis there is an increase in 11-deoxycortisol. This metabolite can be directly measured in the serum or measured in the urine as 17-OH corticosteroids. This test can help differentiate primary adrenal deficiency from ACTH deficiency. It has a similar diagnostic performance to the ITT and it's a potential alternative when there is a contraindication to ITT.

PROCEDURE: For assessment of adrenal or pituitary insufficiency the test can be performed as an overnight test. Metyrapone is given orally (30 mg/kg body weight, or 2 grams for <70 kg, 2.5 grams for 70 to 90

kg, and 3 grams for >90 kg body weight) at midnight with a glass of milk or a small snack (24). Serum 11-deoxycortisol and cortisol are measured at 8 AM the next morning; it is also recommended to measure plasma ACTH levels (31).

SPECIAL CONSIDERATIONS: The concurrent use of glucocorticoids will interfere with the test. Any medications that the patient is taking which increase the P450 enzymes will increase the metabolism and clearance of metyrapone (such as rifampin, phenobarbital, and phenytoin) (32). Similarly, hypothyroidism or hyperthyroidism will affect clearance of metyrapone and the adrenal responsiveness. Therefore, thyroid function tests should be measured prior to performing this test. Measurement of 11-deoxycortisol, like cortisol itself is dependent on CBG and drugs such as estrogens and oral contraceptives will falsely increase the concentrations of 11-deoxycortisol (33).

PREGNANCY IMPLICATIONS - Use during pregnancy only if clearly needed. Subnormal response may occur in pregnant women and the fetal pituitary may be affected.

LACTATION - Excretion in breast milk unknown/use caution

ADVERSE REACTIONS - Frequency not defined. Central nervous system: Headache, dizziness, sedation. Dermatologic: Allergic rash. Gastrointestinal: Nausea, vomiting, abdominal discomfort or pain. Hematologic: Rarely, decreased white blood cell count or bone marrow suppression.

INTERPRETATION OF RESULTS: 8 AM serum 11-deoxycortisol concentrations should be >7 µg/dL with serum cortisol less than 5 µg/dL (138 nmol/L), confirming adequate metyrapone blockade. The plasma ACTH concentration at 8 AM should exceed 75 pg/mL (17 pmol/L), confirming that any increases

in serum 11-deoxycortisol concentrations are ACTH-dependent, thereby separating primary from secondary adrenal insufficiency (34, 35).

Glucocorticoid Excess

DEXAMETHASONE SUPPRESSION TEST

Measurement of endogenous cortisol production in response to exogenous dexamethasone suppression was the first provocative test and still remains among the most useful tests used for the evaluation of excess cortisol. Dexamethasone, due to its high affinity to the glucocorticoid receptor is a potent inhibitor of ACTH synthesis and release. In addition, most of modern immunoassays for cortisol (both urine and serum) utilize an antibody that does not cross-react with

dexamethasone. Therefore, the combination of being able to use relatively low doses and at the same time not interfere with the measurement of cortisol make dexamethasone suppression useful for establishing the presence of a perturbation in the pituitary - adrenal axis and for diagnosing the etiology of hypercortisolism.

At least five different tests have been described using dexamethasone, which differ in the dose and timing of dexamethasone treatment and differ in whether there is measurement of urine or serum cortisol or 17-OH-corticosteroids (Table 1). Although the endocrine basis for the tests are in general the same, none are perfect. Confirming the diagnosis of patients with suspected hypercortisolism requires several tests for accurate diagnosis.

TABLE 1. Various Dexamethasone Suppression Tests				
Dex Supp Test	Dex Dose	Time of Admin	Normal Response	Sens/Spec
Low dose Oral/Night	1 mg	@23:00 x1	<1.8 mcg/dl or <5 mcg/dl	87% / 100%
High dose Oral/Night	8 mg	@23:00 x 1	<50% basal	92% / 100%
Low Dose 2day	0.5 mg	q 6h x 2 days	<10 µg/24h in urine	74-98%/69-100%
High Dose 2 day	2.0 mg	q 6h x 2 days	<50% basal	79% / 100%
Very High dose	8 mg	q 6h x 1 day	<50% basal	74% / 100%

Note: To assure patient compliance and determine whether there is abnormal metabolism of the dexamethasone, serum levels of dexamethasone can be measured. However, this is not a common diagnostic test. Testing can be done by specialized laboratories, such as Esoterix inc. CA. The principle of the assay is RIA after chromatographic sample separation and requires 1 ml of serum sample.

All these tests require significant patient participation as the patients are required to self-administer the dexamethasone at inconvenient hours of the day (11PM) or up to 4 times a day. Sampling requires either collection of urine for 24 hours or coming to the physician's office at 8 AM for multiple blood sampling. Drugs that induce hepatic cytochrome P-450 enzymes, such as barbiturates, phenytoin, rifampin, and aminoglutethimide, increase the metabolism of

dexamethasone and other steroids. Measurement of serum dexamethasone a few hours after the last dose will help determine if there is abnormal metabolism. All these caveats are in addition to the other problems associated with measurement of cortisol as noted above, including the variable diurnal variation as well as interference with concurrent administration of glucocorticoids, estrogen, or other medications that increase cortisol binding globulin.

A popular screening test for confirming hypercortisolism is the overnight 1 mg dexamethasone. A single dose of 1 mg is administered (or 0.3 mg/Kg for children (34) at 11PM and blood is obtained by 8 AM the following morning. The dexamethasone dose is given prior to the diurnal rise in endogenous ACTH release and therefore suppresses the early AM cortisol. A normal response would be a serum cortisol concentration of <1.8 mcg/dl, alternatively a cut point of < 5 µg/dl can be used which will yield more specificity with less sensitivity. If cortisol is >10 µg/dl the likelihood of hypercortisolism is high. The other dexamethasone suppression tests are reviewed in Table VIII. Patients with corticotroph macroadenomas or very active tumors, may have urine free cortisol in excess of 1000 µg/dl which will require higher doses of dexamethasone to confirm suppressibility and/or rule out ectopic ACTH production (36).

The two- day low dose dexamethasone suppression test can be used to differentiate Cushing's syndrome from pseudo-Cushing's which can present with many of the signs and symptoms associated with hypercortisolism in the setting of other clinical conditions such as depression, alcoholism, PCOS, obesity, and uncontrolled diabetes (37, 38). Dexamethasone 0.5 mg is delivered orally Q6 hours for 48 hours. Serum cortisol is measured 2 hours after the last dose and a cutoff level of <1.4 µg/dl is consistent with pseudo-Cushing's. Measurement of 24 hour urine excretion of 17-hydroxycorticosteroid and creatinine during the administration of dexamethasone starting at 1200h, has also been suggested with a cut point of 11 µmol/day or higher considered positive for Cushing's syndrome (39). This test however, can misclassify as many as 15% of patients with Cushing's syndrome and up to 15% of patients with pseudo Cushing's.

The overnight high dose dexamethasone suppression test can help differentiate Cushing's disease from

ectopic ACTH syndrome in patients with ACTH-dependent Cushing's syndrome. The basis for this differentiation is the fact that ACTH secretion in Cushing's disease is only relatively resistant to glucocorticoid negative feedback inhibition. Cortisol levels will not suppress normally with overnight 1 mg but will suppress with a higher dose of 8 mg of dexamethasone. Serum cortisol concentration at 8 AM is <5 µg/dL in most patients with Cushing's disease and is usually undetectable in normal individuals. A more than 50% decrease in cortisol on the day after taking 8 mg dexamethasone supports a diagnosis of Cushing's disease over ectopic ACTH production. In patients with non-ACTH dependent hypercortisolism, a lack of suppression of cortisol by more than 50% with a low normal ACTH level (5-20 pg/ml) suggests an adrenal etiology.

CRH STIMULATION TEST

WHEN TO USE THIS TEST: This test is one of the most sensitive to determine if there is an abnormality in the HPA axis and for diagnosing the etiology of hypercortisolism in ACTH dependent Cushing's. Although CRH is expensive (\$300), when one considers the cost of multiple urine collections and analyses of cortisol as well as the cost of a single MRI of the pituitary (which generally exceeds \$1500), CRH is at least cost effective when one considers the overall expense in the evaluation of these patients.

PROCEDURE: An intravenous line is placed 30 min before the test for rapid phlebotomy and to eliminate a temporary rise in cortisol associated with a needle stick. Blood is drawn at -15' and 0' for cortisol and ACTH (2 ml in a lavender top tube on ice). CRH is then injected IV at a dose of 1 µg/Kg up to a maximum of 200 µg. Blood is obtained at 15, 30, 60, 90, 120, 180 and 210 min for cortisol and ACTH (2 ml in a lavender top tube on ice).

SPECIAL CONSIDERATIONS: The test can be performed at any time of the day, although the initial studies describing the test have been done in the morning.

Side effects: The patient may experience slight nausea, metallic taste, urgency to urinate, a change in blood pressure (either increase or decrease), a change in heart rate, headaches, abdominal discomfort, facial flushing, and lightheadedness. These side effects are mild and last for only few minutes. Category C in pregnancy.

INTERPRETATION OF RESULTS: The mean ACTH concentrations at 15 and 30 min after CRH should increase by at least 35% above the mean basal value at -15 and 0 min in patients with Cushing's disease, but not in patients with ectopic ACTH secretion. This measure gave the best sensitivity (93%) and specificity (100%) (40, 41). The best cortisol criterion was a mean increase at 30 and 45 min of 20% or more above mean basal values, which gave a sensitivity of 91% and a specificity of 88%. It should be noted that the criterion for Cushing's disease is based on the presence of hypercortisolism. The CRH test will not adequately differentiate subjects with pseudo-Cushing's and those with true pituitary dependent Cushing's disease.

CRH TEST WITH DEXAMETHASONE

WHEN TO USE THIS TEST: Several investigators have found that modifications of the CRH stimulation test can increase further the sensitivity and specificity in the diagnosis of the etiology of Cushing's disease. While the simultaneous use of vasopressin can augment the response to CRH, dexamethasone can be used to suppress all but pathologic responses to CRH stimulation [33]. Without dexamethasone the sensitivity and specificity of the CRH test is 65 and 100%, respectively, while with dexamethasone the CRH test is 100% sensitive and specific. This test is

also particularly useful to differentiate true Cushing's from pseudo-Cushing's state.

PROCEDURE: Dexamethasone, 0.5 mg is self-administered orally by the patient every 6 hours for 2 days, at 6 AM, 12 Noon, 6 PM and midnight. On the morning of the 3rd day an additional dose of dexamethasone is given at 6 AM. The patient arrives at the testing center by 8 AM and an intravenous line is placed 30 minutes before the test for rapid phlebotomy and to eliminate a temporary rise in cortisol associated with a needle stick. Blood is drawn at -15' and 0' for cortisol and ACTH (2 ml in a lavender top tube on ice). CRH is then injected IV at a dose of 1 µg/Kg up to a maximum of 200 µg. Blood is obtained at 15, 30 60, 90 120, 180 and 210 min for cortisol and ACTH (2 ml in a lavender top tube on ice).

SPECIAL CONSIDERATIONS: The test can be performed at any time of the day, although it is usually done in the morning.

Side effects that the patient may experience are: slight nausea, metallic taste, urgency to urinate, a change in blood pressure (either increase or decrease), a change in heart rate, headaches, abdominal discomfort, facial flushing, and lightheadedness. These side effects are mild and last for only few minutes.

Similar to the dexamethasone suppression test, the results should be interpreted with caution in patients taking estrogen therapy as they can present with falsely elevated cortisol levels due to an increase of cortisol-binding globulin. Drugs such as phenytoin, phenobarbitone, carbamazepine, rifampicin and alcohol induce hepatic enzymatic clearance of dexamethasone, mediated through CYP 3A4, thereby reducing the plasma concentration and may be associated with false positive results (42).

INTERPRETATION OF RESULTS: A normal response would be a plasma cortisol concentration less than 1.3 µg/dl measured 15 minutes after the administration of CRH. Values of cortisol greater than 1.3 µg/dl correctly identified all cases of Cushing's syndrome and all cases of pseudo-Cushing's states (100% specificity, sensitivity, and diagnostic accuracy). While this is a general recommendation, each laboratory should confirm based on the sensitivity of the respective cortisol assay. Furthermore, it is important to confirm the serum level of dexamethasone at the time of the blood draw to assure patient compliance with the dexamethasone regimen. Patients with ectopic ACTH production will have nonsuppressed cortisol and ACTH levels that are not stimulated by CRH.

DDAVP STIMULATION TEST

WHEN TO USE THIS TEST: This test can be used as part of the workup of ACTH dependent hypercortisolism. It can be used in addition to the CRH stimulation test as studies have shown that the combination of these two tests performs better than either of the tests separately. It can also be performed in lieu to the CRH test in situations in which CRH is not available. The aberrant expression of vasopressin V2 receptor in pituitary ACTH-secreting adenomas is the rationale for the use of the desmopressin test to differentiate corticotroph adenomas (which should respond to desmopressin injection) from ectopic ACTH secreting tumors or pseudo Cushing's (which should not respond)(43-45).

PROCEDURE: An intravenous line is placed 30 minutes before the test for rapid phlebotomy and to eliminate a temporary rise in cortisol associated with a needle stick. Blood is drawn at -15' and 0' for cortisol and ACTH (2 ml in a lavender top tube on ice). DDAVP is then injected IV at a dose of 5 to 10 ug. Blood is obtained at 15, 30, 60, 90, 120, 180 and 210 minutes

for cortisol and ACTH (2 ml in a lavender top tube on ice) (45).

INTERPRETATION OF RESULTS: No definitive cutoff values have been standardized for the interpretation of this test. The published established criteria for this test have generally been based on studies with small series of subjects. Malerbi et al. proposed a cortisol increase over baseline of 12% to be consistent with diagnosis of Cushing's disease (46). An absolute ACTH increase over baseline equal or greater than 6 pmol/L yielded higher sensitivity and specificity to differentiate Cushing's disease from pseudo Cushing's in a different study. Alternatively the criteria used for the CRH stimulation test can be used in the interpretation of the results (47).

INFERIOR PETROSAL SINUS SAMPLING (IPSS) WITH CRH STIMULATION

WHEN TO USE THIS TEST: Once the diagnosis of ACTH dependent Cushing's syndrome has been made based on endocrinologic testing, the next step in the evaluation of such patients should be an MRI of the pituitary to confirm the presence of a pituitary mass. Unfortunately, MRI imaging of the pituitary as a primary diagnostic tool is distinctly unhelpful due to the fact that 10% of all normal individuals may have slight abnormalities of their pituitary and that in many subjects with Cushing's disease, the tumor may be too small to be imaged with MRI scans. However, subjecting a patient to surgical pituitary exploration in the absence of a demonstrable mass is likely to result in an unsuccessful surgery. Furthermore, if previous dexamethasone and/or CRH testing is equivocal, then IPSS should be performed to further confirm the pituitary as the source of the ACTH (34). Although this test is less reliable in lateralizing the ACTH source (i.e., left versus right), than it is in confirming that the ACTH is central in origin, it can rule out ectopic ACTH production by a tumor (although ectopic CRH secreting tumors would be difficult to distinguish from

true Cushings' disease based on IPSS). Simultaneous measurement of prolactin in the central samples can normalize the data if there is any difference in the location of the catheters (48).

It is recommended that active hypercortisolism is confirmed by measuring a 24-hour UFC or overnight UFC the day preceding IPSS. Misleading results have been reported when this test is performed "out of cycle" in patients with cyclical Cushing's.

PROCEDURE: This test is done in conjunction with a skilled interventional neuroradiologist. It is important that the endocrinologist is personally present in the room during the procedure so that assurance can be made that the proper blood tests were drawn at the specified times. The patient is brought to the angiogram suite without sedation. A large bore IV line is placed in an antecubital fossa (to be certain there is access to peripheral blood sampling and CRH injection). Catheters (5 French) are placed in the femoral veins and threaded under fluoroscopic guidance to the inferior petrosal sinus. Injection of IV contrast confirms proper placement of the catheters.

Patients are on constant, pulse, blood pressure and oxygenation monitors during the course of the procedure. Test tubes are prechilled in ice and labeled so that during the rapid sampling period, blood can be placed in the tubes without delay.

It is recommended to routinely obtain 4 baseline measurements at -15, -10, -5 and at 0 minutes. This allows for practice allowing proper coordination between the radiologists drawing blood from the IPSS and the individual drawing blood from the brachial vein. Appropriate amounts of blood should be removed to discard the dead space of the catheter (this varies depending on the size of the catheter used). 2 ml of blood is obtained in lavender top vacutainer tubes on ice for measurement of cortisol

(on peripheral samples); ACTH and prolactin (on central samples).

At 0' CRH is then injected as described above for the peripheral CRH test. Alternatively, a combination of CRH and 10ug of desmopressin can be used, especially if the patient has had a negative response to a prior CRH test. If CRH is not available, IPSS can also be performed with desmopressin alone per the protocol described above. Blood is then sampled from both central and peripheral lines at 2', 5' 10' and 15'. After the 15' time point and right before the IPSS catheters are removed, repeat fluoroscopic localization of the catheters should be performed to confirm that there was no displacement during the sampling. However, sampling on peripheral blood may continue as described in the CRH test discussed above.

SPECIAL CONSIDERATIONS: The test can be performed at any time of the day, although it is usually done in the morning.

Side effects that the patient may experience are: slight nausea, metallic taste, urgency to urinate, a change in blood pressure (either increase or decrease), a change in heart rate, headaches, abdominal discomfort, facial flushing, and lightheadedness. These side effects are mild and last for only few minutes.

Patients greater than 300 pounds in weight may not be able to be supported by the standard fluoroscopic table. Furthermore, such large patients may have an abdominal pannus that precludes reasonable access to the femoral veins. In such instances the IPSS can be performed via catheters placed in the antecubital vein with the patient immobilized in the sitting position.

Strokes have been reported in the literature as a potential complication (36). To minimize this

possibility, it is recommended that the catheters remain in the petrosal sinus for no more than 30 min.

Freeze/thawing can decrease the ACTH concentration (see above); therefore, we recommend that the samples be brought to the endocrine lab and analyzed within 24 hours with the plasma separated and kept on ice during this time. If the analysis is not possible within 24 hours, the samples should be aliquoted and frozen to minimize the amount of freeze/thawing.

INTERPRETATION OF RESULTS: Plasma ACTH values are normalized to the prolactin value in order to correct for possible different localization of the catheters, or movement of the catheters during the study. The post CRH ACTH/Prolactin value of the central catheters should be >2.1 fold the ACTH/Prolactin value of the peripheral sample. In most cases of pituitary dependent Cushing's, the increase is > 5.0 -fold. A central to peripheral ACTH gradient higher or equal to 2 before CRH administration or higher or equal to 3, 10 min after CRH infusion is considered diagnostic of a pituitary source of ACTH (Cushing's disease). Lateralization would mean that the ratio of the left to right side is >2.0 . Frequently the ratio criteria can be met without the need for CRH stimulation, however, the diagnostic accuracy increases from 86% to 90% with CRH (37).

The workup of ACTH dependent Cushing's to differentiate Cushing's disease from ectopic ACTH source can be quite challenging and often times requires combination of different dynamic testing in addition to imaging that often ultimately led to costly and invasive diagnostic procedures such as IPSS to be able to establish an accurate diagnosis. A retrospective study involving 167 patients with Cushing's disease and 27 patients with ectopic Cushing's found that using thresholds of a cortisol increase $>17\%$ with an ACTH increase $>37\%$ during CRH test and a cortisol increase $>18\%$ with an ACTH increase $>33\%$ during desmopressin test, the

combination of both tests gave 73% sensitivity and 98% PPV of Cushing's disease. The PPV was 100% in patients with positive response to both tests, with a negative pituitary MRI and whole-body CT scan. The NPV was 100% in patients with negative response to both tests, with negative pituitary MRI and positive whole body CT scan. This combination of dynamic tests with imaging studies is proposed as an accurate, cost-effective diagnostic strategy for the workup of ACTH dependent Cushing's that might minimize the need for IPSS which can be invasive, costly and unavailable in all institutions (43)

ADRENAL VEIN SAMPLING

WHEN TO USE THIS TEST: Patients diagnosed with ACTH independent Cushing's and found to have bilateral adrenal tumors on imaging pose a particular challenge to the clinician. The differential diagnosis in these cases includes unilateral cortisol secreting adenoma (or carcinoma) with contralateral non-functioning cortical adenoma, bilateral cortisol secreting adenomas, macronodular adrenal hyperplasia, and primary pigmented nodular adrenocortical disease. Adrenal vein sampling measuring cortisol can be very helpful in this scenario and give valuable information to elucidate the proper diagnosis and guide therapy.

PROCEDURE: This test is done in conjunction with a skilled interventional radiologist under sedation. The procedure is usually performed early morning after an overnight fast on the second day of either a low dose (0.5 mg orally every 6 hours) or high dose (2 mg orally every 6 hours) of dexamethasone administration. This eliminates the probability of endogenous ACTH secretion causing interference with the interpretation of autonomous adrenal gland cortisol secretion. The adrenal veins can be catheterized by the percutaneous femoral vein approach, the position of the catheter tip should be verified by venogram. Concentrations of cortisol and aldosterone should be

measured in blood obtained from both adrenal veins and the external iliac vein (for the detection of peripheral venous concentrations) (49).

SPECIAL CONSIDERATIONS: Potential complications include thrombosis with subsequent infarction or hemorrhage adrenal insufficiency and hypertensive crisis, however these are rare (48).

The aldosterone concentrations are usually much higher on the right adrenal vein compared to the left, this is presumably due to the anatomy differences and the catheter proximity to the right adrenal medulla. For this reason, although plasma epinephrine is measured to confirm success of adrenal vein catheterization, it cannot be used to correct for blood sample dilution between the 2 adrenal veins. There have been few case reports in which aldosterone has been used for side-to-side dilution differences, however whether it can be used for this purpose remains unclear (49, 50).

INTERPRETATION: Catheterization of each adrenal vein can be considered successful if plasma aldosterone concentration in the adrenal vein exceeds peripheral venous concentration by more than 100 pg/ml. An adrenal-to-peripheral venous cortisol gradient greater than 6.5 can be considered consistent with a cortisol secreting adenoma. Lateralization can be determined by measuring the side-to-side cortisol gradient (high-side to low-side). A ratio of 2.3 or greater is consistent with autonomous cortical secretion from predominately 1 adrenal gland (49).

IMAGING STUDIES IN THE HPA AXIS EVALUATION

The evaluation of the HPA axis function should always be approached through biochemical measurements. With few exceptions, imaging studies provide no information about hormonal function but can be very useful for the localization of tumors or lesions. Once a biochemical diagnosis of either deficiency or excess of

glucocorticoid production has been established, imaging studies can complement and assists the hormonal evaluation, providing valuable information about etiology, prognosis, and management.

Pituitary Imaging

In the vast majority of cases of ACTH dependent Cushing's syndrome (CS), the source of ACTH is in the pituitary (Cushing's Disease), so performing imaging studies of the pituitary gland in this scenario to try to localize a tumor is appropriate. However given the high incidence of non-functioning pituitary adenomas in the general population (up to 10%) (51) and the increasing sensitivity of the high resolution imaging modalities available that can lead to false positive results, it is important to perform a thorough dynamic testing evaluation of each case and consider inferior petrosal sinus sampling if appropriate (see section above), before committing a patient to pituitary surgery. Adrenocorticotrophic pituitary tumors represent about 10% of all pituitary tumors (52) and ACTH-secreting adenomas are most commonly microadenomas (<1cm). In cases of macroadenoma, assessment of extrasellar extension including chiasmatic compression and cavernous sinus involvement is imperative (53).

The other scenario in which pituitary imaging is indicated and can be useful in the evaluation of the HPA axis function, is in patients diagnosed with secondary adrenal insufficiency who have no history of recent exogenous glucocorticoid exposure or any other clear explanation for the clinical presentation. In these cases, a mass lesion disrupting the HPA function should be suspected, especially if the patient presents with deficiencies of other pituitary hormones and/or elevated prolactin, as isolated adrenal insufficiency from a non-functioning tumor affecting the pituitary is very rare.

PITUITARY MRI

Magnetic resonance imaging (MRI) is the mainstay of pituitary assessment. MRI is more sensitive than computed tomography (CT) in detecting corticotroph adenomas, but still detects only about 50% of these tumors (54) and has a false positive rate of 12-19% (55, 56)]. Standard pituitary imaging protocols typically include thin-section (2 or 3 mm) of T1-weighted (w) spin echo sequences (SE) performed both in coronal and sagittal planes through the pituitary fossa, which are repeated after administration of intravenous gadolinium contrast medium, associated with a T2-weighted sequence in the coronal plane (57, 58). High spatial detail can be achieved by using thin slices, a fine matrix size and a small field of view focused on the pituitary (58). The classic MR features of a corticotroph adenoma include a less than 1 cm focal area of lesser enhancement on T1-w images following contrast administration, hyperintense or hypointense on T2-w images as compared with the normal pituitary gland, remodeling of the pituitary sella floor and deformity of the gland contour (59). Acquiring dynamic sequences in the first 1-2 minutes after contrast injection can increase the sensitivity (60), but this technique has not been unequivocally demonstrated to improve the usefulness of MR in Cushing's (61). The use of three-dimensional (3D) spoiled gradient recalled acquisition in the steady state (SPGR) sequence allows for superior soft tissue contrast compared to conventional spin echo sequences, this technique can be further optimized with thin-slice imaging (<1mm) (58). Compared to T1-w SE sequence, SPGR has been reported to increase sensitivity but also has a higher false positive rate (62, 63).

PITUITARY CT SCAN

Pituitary computed tomography (CT) scanning is less sensitive than MRI for the detection of pituitary adenomas (64) and it is usually reserved for those patients who cannot safely undergo brain MRI.

Acquisition of 1 mm (or less) axial sections through the pituitary fossa with coronal reconstructions can be helpful in the assessment of macroadenomas (57). It is also very helpful preoperatively in patients planned for transsphenoidal pituitary surgery to delineate the bony anatomy (65)

Adrenal Gland Imaging

There are a couple of scenarios in which adrenal gland imaging plays a role in the evaluation of the HPA axis. It is indicated and particularly important in the evaluation of patients diagnosed with ACTH independent CS, which is most commonly caused by adrenocortical adenomas or carcinomas and less frequently bilateral micronodular and macronodular hyperplasia. It can also be considered in cases of primary adrenal insufficiency. Tumors in the adrenal are fairly common in humans, they have been found to be present in 3% of autopsies performed in persons older than 50 years of age (66) and have been reported to be incidentally discovered in up to 5% of cross-sectional abdominal imaging carried out for unrelated problems (67). Most of these incidentally found adrenal tumors are nonfunctioning, 10 to 15% secrete excess amounts of hormones (68) of these, adrenocortical tumors are the most common. On the basis of imaging characteristics alone, no distinction can be made between a benign hyperfunctioning and a non-functioning adenoma, and this can only be differentiated based on clinical and biochemical diagnosis. Adrenal carcinoma represents <10% of adrenal tumors, 30 to 40% of these are hyperfunctioning in adults (69). There are multiple important imaging characteristics that can help differentiate benign adrenal adenomas from pheochromocytomas, adrenocortical carcinomas and metastasis, like percentage of lipid content, tumor size, homogeneity, border regularity, presence of calcifications, invasion of surrounding tissue, and lymph node enlargements (Table 2).

ADRENAL CT SCAN

Unenhanced thin-section CT scan followed by contrast-enhanced examination is the cornerstone of imaging of adrenal tumors. Unenhanced CT is important to provide density measurements of lesions (70). The rich intracytoplasmic fat in adenomas results in a low attenuation on nonenhanced CT. The Hounsfield (HU) scale is a semiquantitative method to measure radiograph attenuation. If an adrenal mass measures <10 HU on unenhanced CT, the likelihood that it is a benign adenoma is nearly 100% (71). However up to 30% of benign adenomas might not contain large amounts of lipid and present with higher HU on nonenhanced CT scan. This is when measuring the contrast washout on delayed images is very useful. Ten minutes after the administration of contrast, an absolute medium washout of more than 50% has been reported to be close to a 100% sensitive and specific for benign adenoma (72). Non-adenomas include metastases, pheochromocytomas and carcinomas.

Adrenal carcinomas usually appear as a unilateral mass, >4 cm in size with an inhomogeneous appearance due to necrosis, hemorrhage, fibrosis, and calcification. Careful assessment of the draining venous structures is essential on imaging, together with identification of direct infiltration of adjacent viscera (57).

ADRENAL MRI

When lesions cannot be characterized adequately with CT, MRI evaluation (with T1 and T2-weighted sequences, chemical shift and fat-suppression refinements) can be sought. Adrenal adenomas usually show low homogeneous signal on T1-weighted images and a signal intensity equivalent or higher than the liver on T2-weighted images. Chemical shift imaging will readily identify the lipid rich adenomas with signal loss on the out-of-phase

sequences (73). This loss of signal can be measured using the adreno-splenic-ratio (ASR) and the signal intensity index (SII). An ASR ratio of <70% has been shown to be highly specific for adenomas and has a 78% sensitivity. Using the SII, a minimum of 5% signal loss characterizes an adrenal adenoma with accuracy of 100% [61]. MRI can also be particularly useful to evaluate for local and distant invasion of adrenocortical carcinomas.

Primary pigmented nodular adrenocortical disease is a rare cause of Cushing's syndrome that has a female predilection and may be familial or associated with Carney complex. On imaging the adrenal glands may appear normal or minimally hyperplastic with multiple, usually <5 mm, unilateral or bilateral benign cortical nodules. The adrenal nodules are macroscopically pigmented; they demonstrate a lower T1 and T2 signal intensity on MRI compared to surrounding atrophic cortical tissue. When nodules are 1-2 cm in size, there might be atrophy of the intervening cortex, which helps distinguish this condition from ACTH-dependent hyperplasia (57).

Another rare cause of Cushing's syndrome is ACTH-independent macronodular adrenal hyperplasia, which has a male predilection. The imaging appearance of the adrenal glands is striking with massive bilateral adrenal enlargement, nodularity, and distortion of adrenal contour. Nodules can measure 1 to 5.5 cm. On MRI they are hypointense relative to liver on T1-w images and hyperintense or isointense in T2-w images. On chemical shift imaging, nodules lose signal intensity on out-of-phase due to their high lipid content (57).

OTHER ADRENAL IMAGING MODALITIES

Patients that harbor adrenal masses, which are not adequately characterized by CT or MRI, can be further evaluated with functional nuclear medicine modalities that include single photon emission computed

tomography (SPECT) scintigraphy with various radionuclide tracers, and positron emission tomography (PET) scintigraphy with various radionuclides. PET images provide a higher spatial resolution compared to SPECT (70).

PET scan with either Fluorodeoxyglucose (FDG) or ^{11}C -metomidate (MTO) can be useful in selected cases to differentiate benign adrenal adenomas from adrenocortical carcinomas. An elevated uptake on the FDG scan correlates with high metabolic activity and raises the suspicion for malignancy (74) with high sensitivity and specificity (75). Limitations of this technique include physiological excretion of FDG into renal inflammatory system and high metabolic uptake in inflammatory and infectious processes as well as in benign pheochromocytomas, leading to false positive results (64). Metomidate is an inhibitor of 11 beta-hydroxylase (CYP11B1) and aldosterone synthetase (CYP11B2), and based on this property its use can help differentiate tumors of adrenocortical origin from non-cortical lesions. Originally developed as a PET imaging agent radiolabeled with ^{11}C , more recently it has been labeled with ^{18}F and ^{123}I , allowing SPECT and SPECT/CT imaging (76).

Integrated or “fused” PET-CT imaging allows to combine CT attenuation measurements with the intensity of FDG uptake, as described by the standardized uptake value (SUV), improving the performance of either imaging technique alone (77).

Scintigraphy with Iodine-131-Iodomethyl-19-norcholesterol (NP 59) is a functional nuclear medicine imaging modality that can be used to differentiate adrenal cortical adenomas from carcinomas. This is a labeled cholesterol analogue that specifically binds to low-density lipoproteins and after receptor-mediated uptake it is stored in the adrenocortical cells (70). NP 59 uptake is regulated by ACTH and suppressed by dexamethasone, concentrating in hyperfunctioning cortisol and

aldosterone secreting adenomas and showing low uptake in adrenocortical carcinomas because of the inefficient concentration of radiotracer by malignant tissue (78).

Other Imaging Modalities for Ectopic Cushing’s

Patients diagnosed with ACTH dependent Cushing’s whose biochemical dynamic tests suggests an ectopic source, pose a special challenge to the clinician. In 12 to 20% of these patients, the source remains undiscovered despite repeated biochemical and radiological investigations (55).

In the setting of ectopic ACTH production, imaging studies play a crucial role in trying to identify the source of the tumor causing the disease and guide management and prognosis. The optimal imaging study to detect these tumors has not been defined. CT, MRI, PET scan, ^{111}In -pentetreotide (OCT) scintigraphy at conventional or higher radionuclide doses, as well as newer molecular imaging techniques like $^{131}\text{I}/^{123}\text{I}$ -metaiodobenzylguanidine (MIBG), ^{18}F -fluoro-2-deoxyglucose-positron emission tomography (FDG-PET), ^{18}F -fluorodopa-PET (F-DOPA-PET), ^{68}Ga -DOTATATE-PET/CT or ^{68}Ga -DOTATOC-PET/CT scan (^{68}Ga Gallium-SSTR-PET/CT) are complementary and have been shown to be useful in different scenarios with variable sensitivity and specificity (79-83). For the most part at least two different imaging modalities are needed to establish a diagnosis and sometimes, repeated imaging over several months is required to identify the source. The choice of imaging modalities is guided by the sensitivity of the procedure balanced with the risk of false-positive findings (72).

A good approach is to start by obtaining images of the chest, since most ACTH-secreting tumors are located in this area. The most common causes are bronchial carcinoid tumors and small cell lung cancer. Other sources of excess ACTH production include

neuroendocrine tumors of the thymus, bowel and pancreas, medullary carcinoma of the thyroid, pheochromocytomas, and mesotheliomas.

CT of the chest, abdomen and pelvis with intravenous contrast medium injection is the most commonly used initial imaging test performed and is very useful in many cases. In patients with equivocal CT imaging findings, MRI can be useful, particularly for tumors within the abdomen. It is recommended to follow CT and MRI imaging with a functional imaging modality, being OCT scintigraphy the most widely used. Functional imaging reduces false-positive results because it relies on the specific properties of tumor

cells, not just their anatomic characteristics. However, tumors lacking the relevant receptor can have false negative results (83). Site-specific differences occur and different imaging modalities might have higher sensitivity and specificity depending on this. A recent systematic review showed that FDG-PET can be very sensitive in the detection of neuroendocrine tumors with high proliferation index, particularly in the pancreas. This review also showed that ⁶⁸Gallium-SSTR-PET/CT had a 100% sensitivity but this is an imaging technique that has limited availability and was only performed in a minority of the patients in their series (80).

Table 2. Imaging Characteristics of Adrenal Tumors				
Characteristic	Adenoma	Carcinoma	Pheochromocytoma	Metastasis
Size	<4 cm	>4 cm	Variable	>4 cm
Shape	Round	Irregular	Round	Irregular
Border	Smooth	Irregular	Well delineated	Irregular
Laterality	Unilateral	Unilateral	May be bilateral or unilateral	May be bilateral
Appearance	Round, homogeneous	Inhomogeneous with central necrosis. May have calcifications	Cystic and hemorrhagic changes.	Inhomogeneous
Vascularity	Normal	Increased	Increased	Increased
Growth rate	Slow (1 cm/year)	Fast (>2 cm/year)	Slow (0.5-1 cm/year)	Variable/Fast
Lipid content	Lipid rich or poor	Lipid poor	Lipid poor	Lipid poor
CT attenuation	<10 HU unenhanced. >50% absolute washout.	>20 HU unenhanced. <50% absolute washout.	>20 HU unenhanced. <50% absolute washout.	>20 HU unenhanced. <50% absolute washout.
MRI	Isointense with liver in T1 and T2-w. Chemical shift	Hypointense compared to liver on T1-w High to intermediate signal on T2-w	High signal intensity on T2-w	Hypointense compared to liver on T1-w High to intermediate signal on T2-w
FDG-PET-CT	Low SUV	High SUV	Variable SUV	High SUV
Other		Evidence of invasion or metastasis		History of prior cancer

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