Chapter 7
Differential Diagnosis of Cushing's Syndrome

Bradley R. Javorsky, Ty B. Carroll, and James W. Findling

Abstract Determining the cause of spontaneous Cushing's syndrome is essential so that appropriate therapy can be recommended. Most patients (80%) have an ACTH-secreting neoplasm (pituitary or ectopic), while the rest have an adrenal-dependent (ACTH-independent) etiology. After hypercortisolism has been convincingly established, plasma adrenocorticotropic hormone (ACTH) levels are obtained to subdivide Cushing's syndrome into ACTH-dependent (>20 pg/ml) or ACTH-independent (<5 pg/ml) categories. Corticotropin-releasing hormone (CRH) stimulation testing can help to define these categories when ACTH levels are equivocal (5–20 pg/ml). Since clinical features are unreliable in distinguishing between subtypes of ACTH-dependent and ACTH-independent Cushing's syndrome, additional biochemical, radiologic, and angiographic tests are needed. Diagnostic accuracy of high-dose dexamethasone suppression testing and CRH stimulation testing are poor when trying to refine the diagnosis of ACTH-dependent Cushing's syndrome. Bilateral inferior petrosal sinus ACTH sampling with CRH stimulation has become the gold standard in this setting and should be used when magnetic resonance images of the pituitary do not reveal an unequivocal pituitary abnormality in a patient with clinical and biochemical findings consistent with ACTH-dependent Cushing's syndrome. In patients with ACTH-independent Cushing's syndrome, computed tomography of the adrenal glands is performed and will demonstrate either a single nodule (benign or malignant) or bilateral nodular hyperplasia caused by several unique pathophysiological mechanisms.

Keywords Adrenocorticotropic hormone • Hypercortisolism • Dexamethasone suppression testing • Corticotrophin-releasing hormone • Adrenal glands

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Introduction

During the past 30 years, the introduction of reliable plasma adrenocorticotropic hormone (ACTH) measurements, sensitive pituitary and adrenal imaging studies, and bilateral inferior petrosal sinus ACTH sampling with corticotropin-releasing hormone (CRH) stimulation (IPSS) have provided the diagnostic tools necessary to differentiate between the many causes of Cushing's syndrome [1]. The differential diagnostic testing for Cushing's syndrome should only be considered after the diagnosis of pathologic endogenous hypercortisolism has been well established. None of the differential diagnostic tests have any reasonable level of sensitivity or specificity for establishing the actual diagnosis of Cushing's syndrome [2]. For example, pituitary and adrenal imaging abnormalities are quite common in patients with no evidence of an endocrine disorder and plasma ACTH levels may even be elevated in some normal subjects due to the stress of venipuncture [3, 4]. Moreover, IPSS results in patients with Cushing's disease may overlap with those in normal subjects [5].

Etiologic Considerations (Table 7.1)

The majority (80%) of patients with spontaneous Cushing's syndrome have an ACTH-secreting neoplasm (ACTH-dependent Cushing's syndrome) either from a pituitary tumor (Cushing's disease) or a nonpituitary neoplasm (ectopic ACTH syndrome). Some patients with ectopic ACTH syndrome (e.g., bronchial carcinoids) may present with hypercortisolism several years before there is radiographic evidence of neoplasm (occult ectopic ACTH syndrome). These subtypes of ACTH-dependent Cushing's syndrome may be clinically and biochemically indistinguishable, and careful assessment of diagnostic studies and clinical expertise are required to differentiate them [6].

The most common cause of ACTH-independent Cushing's syndrome is prolonged exogenous glucocorticoid therapy. A very careful history is needed to be certain that patients with ACTH-independent Cushing's syndrome are not receiving

Table 7.1 Causes of Cushing's syndrome

ACTH-Dependent
ACTH-secreting pituitary tumor (Cushing's disease)
Ectopic ACTH secreting neoplasm
Ectopic CRH-secreting neoplasm (rare)
ACTH-Independent
Exogenous glucocorticoid therapy
Adrenal neoplasm (adenoma or carcinoma)
Bilateral nodular adrenal hyperplasia
Carney complex (protein kinase A mutation)
Aberrant adrenal receptors (e.g., GIP, vasopressin)
McCune–Albright syndrome (mutations of Gsα)

any type of exogenous corticosteroid treatment [7]. The majority of patients with adrenal-dependent (ACTH-independent) endogenous Cushing's syndrome have a solitary benign adrenocortical neoplasm, while a minority has adrenocortical carcinoma. In addition, there are many diverse causes of bilateral nodular hyperplasia associated with endogenous ACTH-independent hypercortisolism.

Differentiating ACTH-Dependent from ACTH-Independent Cushing's Syndrome

Plasma ACTH

The initial step in the differential diagnosis of spontaneous Cushing's syndrome is the measurement of plasma ACTH. The introduction of a sensitive and specific two-site immunometric assay for plasma ACTH has now provided clinicians with a reliable initial step in distinguishing patients with ACTH-independent Cushing's syndrome (subnormal ACTH) from those with ACTH-dependent Cushing's (normal or elevated plasma ACTH) [8]. If the morning plasma ACTH level is suppressed below the reference range (<5 pg/ml), patients should then undergo adrenal imaging by computed tomography (CT) scanning. By contrast, patients with plasma ACTH >20 pg/ml have an ACTH-secreting neoplasm. However, the recognition of mild (and often subclinical) Cushing's syndrome associated with incidentally discovered adrenal adenomas has complicated the differentiation between ACTH-dependent and ACTH-independent endogenous hypercortisolism. Many of these patients have intermittent and only modest increases in cortisol secretion and may not have full suppression of plasma ACTH. Some of these patients may have basal plasma ACTH levels between 5 and 20 pg/ml [9]. In these patients, a CRH stimulation test may be required for an accurate differential diagnosis.

CRH Stimulation Test

Reincke has reported the plasma ACTH response to human CRH (hCRH) in normal subjects compared to patients with subclinical Cushing's and those with overt adrenal Cushing's syndrome (Fig. 7.1). Patients with unequivocal ACTH-independent Cushing's syndrome will have basal plasma ACTH <5 pg/ml with minimal increase in plasma ACTH or cortisol after hCRH administration; however, subjects with subclinical Cushing's syndrome may have basal ACTH levels between 5 and 20 pg/ml and have a modest – albeit attenuated – plasma ACTH and cortisol increase following hCRH infusion. Unfortunately, there is limited data available on the normative ACTH response to CRH. Values will depend on the type of ACTH assay employed and the type of CRH (ovine or human) administered. In general,

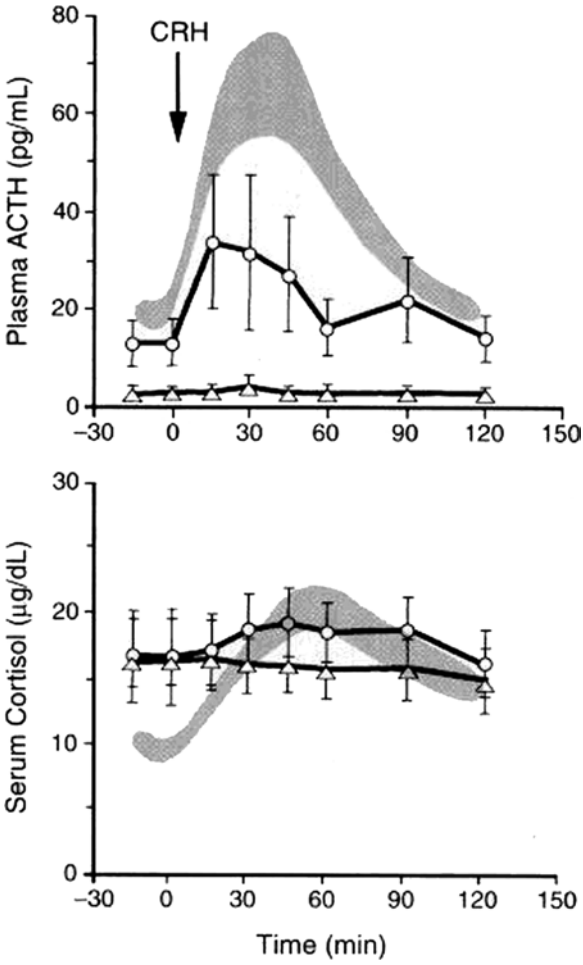

Fig. 7.1 Plasma ACTH and serum cortisol response to stimulation with human CRH (100 µg IV) in normal control subjects (mean ± SEM; shaded area), in six patients with subclinical Cushing's syndrome and in six patients with overt adrenal Cushing's syndrome. *Circles* preclinical Cushing's syndrome; *diamonds* adrenal Cushing's syndrome; *shaded areas* normal subjects (reprinted from Endocrinol Metab Clin North Am. Reincke, M, 2000:29:43–56 with permission from Elsevier)

patients with ACTH-independent Cushing's syndrome from mild hypercortisolism associated with nodular adrenal disease have peak plasma ACTH responses that are <30–40 pg/ml. By contrast, patients with ACTH-dependent Cushing's syndrome (usually due to Cushing's disease) have an exaggerated peripheral ACTH response to CRH as well as a significant increase in cortisol secretion.

It is not surprising that dexamethasone suppression testing (even high dose) does not reliably distinguish ACTH-dependent from ACTH-independent Cushing's syndrome [10]. This can be attributed to the fact that patients with predominantly adrenal-dependent Cushing's syndrome may have some degree of ACTH-dependency and suppression with high doses of dexamethasone may result in decreases but not complete suppression of cortisol secretion. However, as a rule, after either low-dose or high-dose dexamethasone suppression testing, plasma ACTH levels are usually undetectable in patients with adrenal-dependent Cushing's syndrome. And, as would be expected, the serum cortisol levels are not fully suppressed.

Imaging Studies

Imaging findings should not be used to distinguish ACTH-independent from ACTH-dependent Cushing's syndrome. Many patients with Cushing's disease will have CT evidence of nodular adrenal disease, presumably related to longstanding ACTH hypersecretion [11]. This nodular adrenal disease may be bilateral, but it is often unilateral. Patients with clinically significant Cushing's syndrome due to a cortisol-secreting adenoma (plasma ACTH <5 pg/ml) will usually have a small contralateral adrenal gland. However, the contralateral adrenal gland is usually normal in those subjects with subclinical adrenal-dependent Cushing's syndrome. Since small pituitary lesions are seen in as many as 10% of normal subjects, pituitary magnetic resonance imaging (MRI) alone should not be used to distinguish ACTH-dependent from ACTH-independent hypercortisolism. The differentiation of the subtype of Cushing's syndrome must be established biochemically.

ACTH-Dependent Cushing's Syndrome

Pituitary adenomas secreting ACTH (Cushing's disease) are responsible for at least 90% of cases of ACTH-dependent Cushing's syndrome. With the introduction of pituitary microsurgery in the 1970s as the treatment of choice for patients with Cushing's disease, it has become crucial to establish an accurate differential diagnosis in patients with ACTH-dependent Cushing's syndrome.

Cushing's disease and ectopic ACTH syndrome may be clinically indistinguishable. Since the pretest probability of Cushing's disease is so high, any diagnostic test must have very good accuracy. Radiological studies and dynamic tests of steroid secretion are also often inconclusive or misleading in separating these disease entities [1–5]. Because of its high sensitivity and specificity, IPSS has become the test of choice in the differential evaluation of ACTH-dependent Cushing's syndrome.

Clinical Features

The clinical signs and symptoms of hypercortisolism may be similar in patients with Cushing's disease or ectopic ACTH syndrome. Early reports describing patients with the ectopic ACTH syndrome made note of features not typically seen in patients with Cushing's disease such as the absence of classic physical signs of Cushing's syndrome, weight loss, and hyperpigmentation [12, 13]. A more recent analysis of clinical features notes patients with ectopic ACTH syndrome as being significantly older, more likely to be male, having a shorter duration of clinical findings, more likely to have hypokalemia, and having higher 24-h urinary free cortisol and plasma ACTH levels [1, 14]. A logistic regression model using only these clinical and simple biochemical variables has an overall

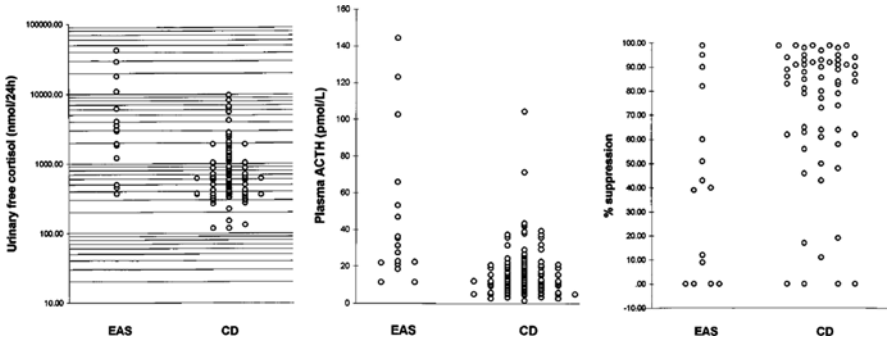

Fig. 7.2 Biochemical characteristics of patients with CD and EAS. *Left panel*, 24-h urinary free cortisol ($n=99$ CD; $n=15$ EAS); *middle panel*, plasma ACTH ($n=102$ CD; $n=17$ EAS); *right panel*, percent suppression with high-dose dexamethasone ($n=61$ CD; $n=15$ EAS). Used with permission from J Clin Endocrinol Metab. Aron et al. 1997:82:1780–5, Copyright 1997, The Endocrine Society

diagnostic accuracy of 91.2%; however, 26.7% of ectopic ACTH syndrome cases would be misdiagnosed using this model and a considerable amount of overlap is observed for cortisol and ACTH levels (Fig. 7.2) [15]. These findings underscore the need for additional diagnostic testing.

Biochemical Testing

Historically, clinicians have relied on provocative biochemical testing, in particular the high-dose dexamethasone suppression test (HDD) and CRH stimulation test, to distinguish Cushing's disease from ectopic ACTH production. These tests, however, have been associated with limited diagnostic accuracy [15–19].

Several variations of HDD have been developed in an attempt to differentiate Cushing's disease from ectopic ACTH syndrome. The idea behind the HDD is the concept that corticotroph cells, in contrast to ectopic ACTH-secreting tumors, retain a degree of sensitivity to glucocorticoid feedback. The original test designed by Liddle over 50 years ago, even before the ectopic ACTH syndrome had been described, used a protocol of 2 mg dexamethasone every 6 h for 48 h [20]. Urinary 17-hydroxysteroid was collected before and after dexamethasone administration. Suppression by 50% was felt to be consistent with Cushing's disease. This test has been modified in many ways since its introduction to improve accuracy and convenience. Urinary free cortisol levels have replaced measurement of 17-hydroxysteroid levels by most laboratories. Many investigators now use plasma cortisol levels (after 48 h of dexamethasone 2 mg every 6 h or after a single 8 mg evening dose of dexamethasone) rather than urinary measurements. Finally, protocols using 5–7 h continuous infusions of dexamethasone have been described [21, 22].

HDD test performance is suboptimal with a sensitivity of 79–85% and a specificity of 67–100% depending on the percent suppression of urinary or plasma cortisol levels used [23, 24] (Fig. 7.2). Variables that may account for low test performance include the importance of sample timing, incomplete urine collections, poor test adherence, and variable dexamethasone metabolism and absorption. Since the diagnostic accuracy of HDD is less than the pretest probability of Cushing's disease, HDD is no longer recommended for the differential diagnosis of ACTH-dependent Cushing's syndrome.

Almost since the isolation of CRH by Vale and colleagues in 1981, stimulation testing with this neuropeptide has been used in the differential diagnosis of ACTH-dependent Cushing's syndrome [22]. Orth et al. demonstrated that administration of CRH potently stimulates release of ACTH in normal subjects and patients with Cushing's disease [25]. In contrast to pituitary adenomas, nonpituitary ACTH-producing tumors respond poorly to CRH administration, presumably because of lower CRH receptor expression.

To distinguish between causes of ACTH-dependent Cushing syndrome, percent change between basal and peak ACTH or cortisol values are used after intravenous injection of CRH (1 μg/kg or 100 μg). Sensitivity and specificity of the CRH test depends on which criteria are chosen; ranges of 35–105% for the rise of ACTH above basal levels have been used resulting in sensitivities of 70–93% at high specificity. For cortisol, ranges of 14–50% have been used resulting in a sensitivity of only 50–91% [16, 26–28]. Furthermore, a combined analysis of all published series in a 1998 review by Newell-Price et al. indicated that 7–14% of all patients with Cushing's disease fail to respond to CRH if the best discriminating criteria are applied [28].

Imaging Studies

Imaging of the pituitary to identify patients with corticotroph adenomas is limited by the frequency of incidental pituitary lesions in the normal population. Depending on the series, anywhere from 1.5% to 26.7% (the majority falling between 8% and 14%) of subjects at autopsy are found to have incidental pituitary adenomas [29]. Similarly, MRI detects focal areas of decreased signal intensity in the pituitary in 10–38% of normal volunteers [4, 30]. Conversely, despite dramatic improvements in the quality of pituitary imaging, from sellar polytomography to CT to MRI, approximately 20–50% of patients with pathologically confirmed Cushing's disease do not have tumors seen on even the most sensitive studies [31].

ACTH-secreting pituitary adenomas are typically hypodense on noncontrasted MRI. The administration of contrast (gadolinium-diethylenetriaminepentaacetic acid) may identify an additional population of tumors [32]. Other techniques have been introduced to improve the sensitivity of MRI such as dynamic contrast enhancement (where images are obtained within seconds of contrast injection to take advantage of different dynamics of contrast enhancement between normal

pituitary tissue and pituitary adenomas) and utilization of 1 mm spoiled gradient recalled acquisition in the steady-state sequences [31, 33]. These approaches have improved the sensitivity of detecting corticotroph adenomas, but at the expense of lower specificity.

In a study comparing MRI and IPSS, Kaskarelis et al. showed that the accuracy for detecting a pituitary source of ACTH was 50% for MRI and 88% for successful IPSS [34]. Of the 54 patients with confirmed final diagnoses, MRI resulted in 25 false negatives and 2 false positives, while IPSS had 2 false negatives and 3 false positives.

Inferior Petrosal Sinus ACTH Sampling

In light of the high pretest probability of Cushing's disease in patients with ACTH-dependent Cushing's syndrome, and the poor performance of biochemical testing and radiologic imaging, IPSS has emerged as the differential diagnostic test of choice. As previously noted, ACTH-dependent pathologic hypercortisolism must first be established before performing IPSS, as considerable overlap exists between patients with confirmed Cushing's disease and normal individuals or patients with "pseudo-Cushing" states [5].

Anterior pituitary hormones such as ACTH reach the systemic circulation via small hypophyseal and lateral adenohypophyseal veins that converge into the confluent pituitary veins, join the cavernous sinus on the same side, and empty into the inferior petrosal sinus. IPSS takes advantage of this anatomy to determine if there is a central to peripheral ACTH gradient. During the procedure, a skilled invasive radiologist catheterizes each inferior petrosal sinus and blood samples for ACTH are withdrawn in the basal state and then at 3, 5, and 10 min after intravenous administration of 1 μg/kg (or 100 μg) CRH from each inferior petrosal sinus and a peripheral vein (Fig. 7.3). Petrosal sinus to peripheral (IPS:P) ACTH ratios ≥2.0 at baseline or a peak ≥3.0 after CRH administration (at any of the time points) are diagnostic of Cushing's disease. Technical success rates are high (85–99%), and complications are uncommon, in institutions that perform IPSS regularly [35–38]. Minor groin hematomas occur 3–4% of the time, while more serious complications such as thromboembolism, venous subarachnoid hemorrhage, or brainstem injury have been rarely reported [35, 37, 39–41].

Anomalous venous drainage is well documented and may be responsible for false-negative IPSS results [42, 43]. In situations where the IPSS results suggest ectopic ACTH syndrome, measurement of prolactin in the inferior petrosal sinuses will help to validate the integrity of pituitary venous effluent. Normalization of the IPS:P ACTH ratio to the IPS:P prolactin ratio can increase the diagnostic accuracy of this test [44]. Normalized ratios greater than 0.8 are indicative of Cushing's disease, while ratios less than 0.6 are seen in patients with ectopic ACTH syndrome (Fig. 7.4). Therefore, we routinely store plasma samples during IPSS for possible measurement of prolactin in patients without a

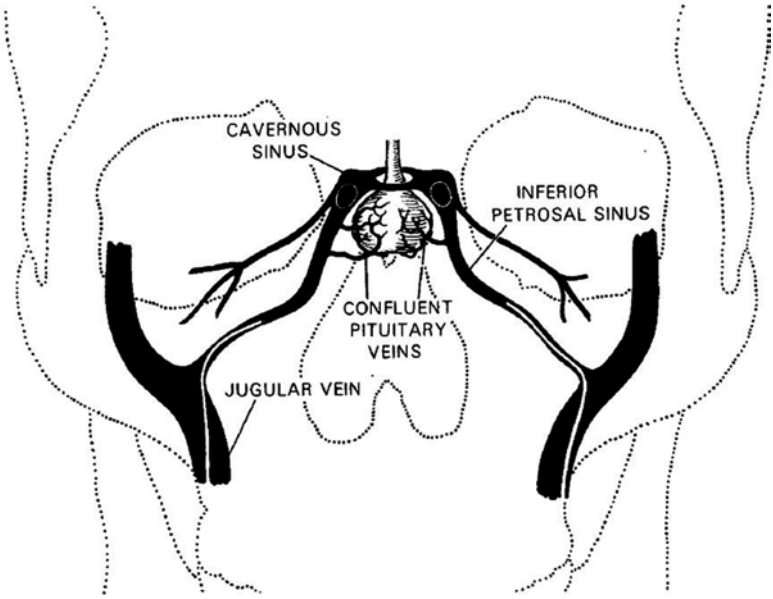

Fig. 7.3 Catheter placement for bilateral simultaneous blood sampling of the inferior petrosal sinuses. Confluent pituitary veins empty laterally into the cavernous sinuses, which drain into the inferior petrosal sinuses. Used with permission from N Engl J Med. Oldfield, et al. 1985;312:100–3. Copyright 1985, Massachusetts Medical Society, all rights reserved

pituitary ACTH gradient. False positive tests may be due to rare cases of CRH-secreting tumors or testing during a period of normocortisolemia in patients with intermittent ACTH secretion. To rule out the latter situation when interpreting IPSS results, we measure late night salivary cortisol levels prior to the procedure to confirm hypercortisolism.

Early series reported sensitivities and specificities of 100% [17, 36]. Subsequent studies have largely replicated these findings, reporting sensitivities ranging from 85% to 100% with specificities of 90–100% [17, 18, 23, 26, 42, 45–50]. A review of 21 studies found an overall sensitivity and specificity of 96 and 100%, respectively [51]. Lindsay and Nieman combined reports of 726 patients who had Cushing's disease and 112 who had ectopic ACTH syndrome, finding a diagnostic sensitivity and specificity for IPSS of 94% [2].

Use of desmopressin (a synthetic analog of vasopressin) during IPSS has also been investigated. Vasopressin receptors are present on corticotroph adenoma cells and only rarely on ectopic tumors producing ACTH. Furthermore, CRH is not available in all centers. In several studies using desmopressin, sensitivities were 92–95% with specificities of 100% [52]. When CRH and desmopressin were combined, sensitivity was 97–100% compared to 87% for CRH alone [53, 54]. Larger studies are needed to confirm these results.

When compared to other tests, IPSS is consistently found to be more accurate for separating pituitary-dependent ACTH excess from the ectopic ACTH syndrome.

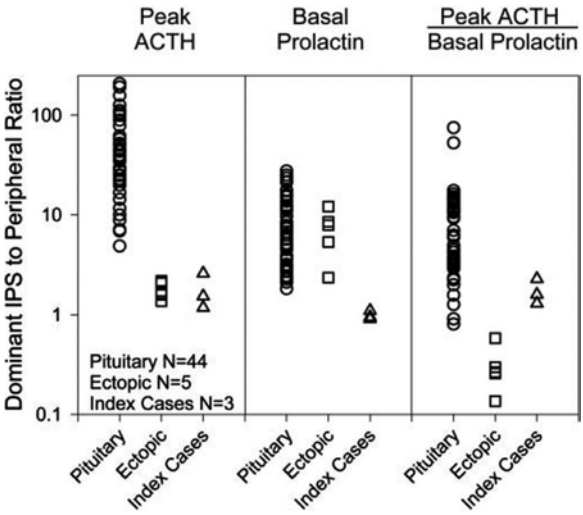

Fig. 7.4 IPS:P ratios for patients with proven Cushing's disease (pituitary; *circles*), ectopic ACTH syndrome (ectopic; *squares*), and the three index cases (*triangles*). The *left panel* is the post-CRH IPS to peripheral ACTH ratio. The *middle panel* is the basal (pre-CRH) IPS to peripheral prolactin ratio from the same sites as the ACTH ratios. The *right panel* is the *left panel* divided by the *middle panel* giving IPS:P ratios normalized to the ipsilateral prolactin ratio. Notice the overlap of the IPS-peripheral ACTH ratio for the ectopic ACTH and index cases, and also notice that the index cases had IPS-peripheral prolactin ratios that were lower than in pituitary and ectopic ACTH, and that normalizing the peak ACTH IPS:P ratio to the ipsilateral prolactin ratio led to results in index cases (with subsequently proven Cushing's disease) similar to prospectively proven pituitary Cushing's disease. Used with permission from J Clin Endocrinol Metab., Findling, et al. 2004;89:6005–9, Copyright 2004, The Endocrine Society

In a NIH series of patients with ectopic ACTH syndrome, 98% were found to have the expected findings with IPSS, while biochemical testing (2 day and overnight high-dose dexamethasone tests, and peripheral CRH administration) was at best 92% accurate [18]. Even more impressive differences between IPSS and high-dose dexamethasone or peripheral CRH testing were observed in a study by Wiggam et al. which compared IPSS (without CRH administration) to CRH testing and HDD in 44 patients with confirmed Cushing's disease and 1 patient with confirmed ectopic ACTH syndrome [19]. They showed that in patients with proven pituitary disease, only 48% met the criteria for HDD and 70% met the criteria for CRH testing (only 35% had a correct response to both tests). By contrast, IPSS successfully indicated Cushing's disease in 82% of cases. Presumably, if CRH had been administered during IPSS, the sensitivity would have been even higher.

Sampling at sites other than the inferior petrosal sinuses has been investigated in an effort to further improve diagnostic accuracy, increase availability, or further reduce the risk of complications. Compared to IPSS, jugular vein sampling has demonstrated a lower diagnostic sensitivity, suggesting the risks and costs of this procedure are not justified [17, 42, 55, 56]. Cavernous sinus sampling after CRH

stimulation has sensitivities and specificities similar to IPSS; however, it is technically more challenging and transient cranial nerve palsies have been reported with this procedure [37, 57].

In addition to distinguishing between Cushing's disease and ectopic ACTH-secreting neoplasms, IPSS has been examined for its utility in lateralizing corticotroph adenomas within the pituitary. This would provide neurosurgeons with additional information when attempting to localize tumors too small to be seen on even the most sensitive imaging studies. Although theoretically possible in most individuals, lateralization data is confounded in persons with centrally located tumors, anomalous venous drainage patterns, or multifocal lesions [58]. Studies have reported variable rates of success, ranging from 50% to 100% [36, 59–61]. Lateralization data should, therefore, not replace a thorough surgical exploration of the entire pituitary gland.

Ectopic ACTH Syndrome

Approximately 10% of cases of endogenous ACTH-dependent Cushing's syndrome are secondary to the ectopic ACTH syndrome [13, 18, 62]. A wide variety of tumors have been reported to cause ectopic ACTH secretion. The most common of these tumors are neuroendocrine tumors of the lung; bronchial carcinoid tumors represent 25% of cases while small cell and adenocarcinomas of the lung together account for 20% [62]. The remaining half of ectopic ACTH syndrome is caused by tumors of the thymus, gastrointestinal tract, islet cell, pheochromocytoma, and medullary thyroid carcinomas (Fig. 7.5).

Localization of Ectopic ACTH-Secreting Neoplasms

As described above, only after a biochemical evaluation has confirmed ACTH-dependent hypercortisolism, and further differential diagnostic testing (i.e., IPSS) has suggested an ectopic source, should localization studies be undertaken. In 33–44% of cases, efforts to localize the source of ectopic ACTH-secretion are unsuccessful [63–65]. Initial imaging should begin with CT scan of the abdomen and chest. Additional imaging with MRI of the chest may provide localizing information in those patients without lesions seen on CT [66]. If no causative lesion is found using CT or MRI, somatostatin receptor scintigraphy may be useful; however, the relatively small size of the tumors is often at the limit of resolution for somatostatin receptor scintigraphy [51, 67]. Standard 18-fluorodeoxyglucose positron emission tomography scanning has not been found to be of additional benefit [65, 68]. Tumor markers including calcitonin, gastrin, glucagon, or somatostatin may be elevated in patients with ectopic ACTH syndrome but are rarely helpful in localization [67].

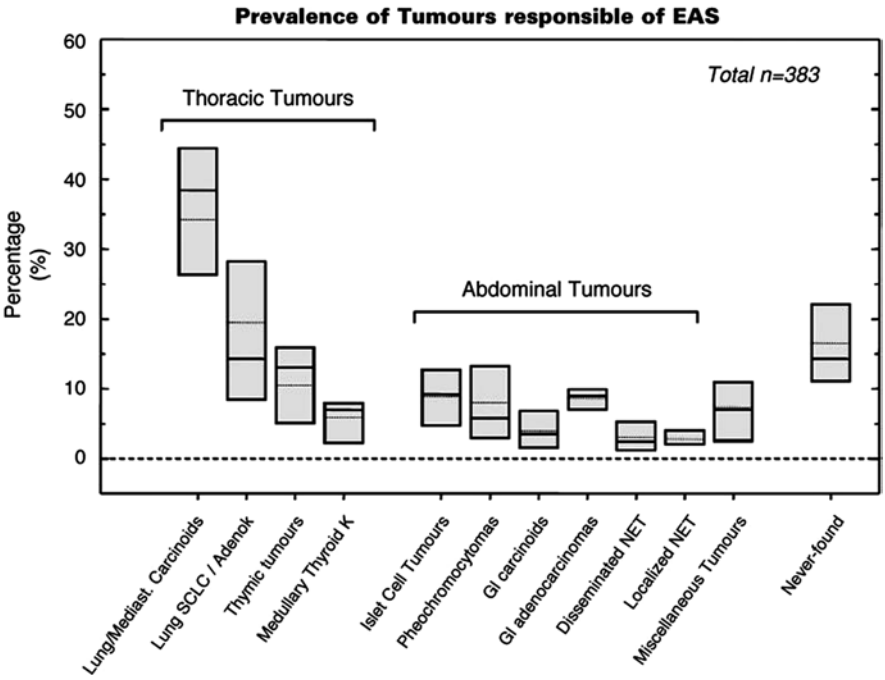

Fig. 7.5 Distribution of the most frequent source (>2%) of ectopic ACTH secretion in a group of 383 patients with EAS (used with permission from Isidori, AM and Lenzi, A. Arq Bras Endocrinol Metab 2007;51:1217–25)

ACTH-Independent Cushing's Syndrome

ACTH-independent hypercortisolism is caused by either exogenous steroid use or by autonomous adrenal production of cortisol. Distinguishing between these two etiologies is essential.

Exogenous Cushing's Syndrome

The exact prevalence of exogenous Cushing's syndrome is unknown. This is largely due to the lack of a uniformly accepted definition making estimates of prevalence difficult. Regardless, exogenous Cushing's syndrome is clearly much more common than all forms of endogenous hypercortisolism.

Exclusion of exogenous Cushing's syndrome should be the initial step in the investigation of all patients with suspected hypercortisolism [7]. Excluding exogenous

glucocorticoid use is often straightforward; however, determining a source of exogenous steroid use can be challenging as even the use of nonoral glucocorticoids can lead to Cushing's syndrome. Intra-articular, inhaled, nasal, topical, and even topical ocular preparations have all been reported to cause exogenous Cushing's syndrome [69–71]. Another group of patients in whom the diagnosis of exogenous Cushing's syndrome is more difficult to secure are those unknowingly exposed to glucocorticoids through drugs of abuse or herbal remedies [72, 73]. As such, a thorough review of any and all over-the-counter, herbal, and traditional medicines, as well as drugs of abuse, should be obtained.

Biochemical evaluation of these patients will show variable cortisol levels depending on the cross reactivity of the cortisol assay with the glucocorticoid to which the patient is exposed. Patients exposed to hydrocortisone will have high plasma cortisol levels, but other synthetic steroids (i.e., prednisone and dexamethasone) have much lower cross reactivity and can lead to low plasma cortisol levels. As a result, traditional testing for cortisol excess is not helpful in patients with exogenous Cushing's syndrome. The diagnosis is secured in a patient with clinical features of steroid excess and a history of exposure to supraphysiological doses of glucocorticoids. Complementary biochemical testing can reveal suppressed ACTH levels and often low dehydroepiandrosterone sulfate (DHEA-S) levels.

Adrenal-Dependent Cushing's Syndrome

Adrenal-dependent (ACTH-independent) Cushing's syndrome accounts for 20% of endogenous hypercortisolism. Solitary cortisol-secreting adrenal adenomas account for 90% of these cases [51]. Adrenal carcinoma, ACTH-independent macronodular adrenal hyperplasia (AIMAH), primary pigmented nodular adrenal disease, and McCune–Albright syndrome constitute the remaining 10% of cases [74–77] (Table 7.1).

Differentiating between the causes of adrenal-dependent Cushing's syndrome is based mainly on clinical grounds and imaging characteristics as very few biochemical differences exist between these etiologies.

Solitary Adrenal Adenoma

Incidental adrenal adenomas occur in 4% of patients undergoing abdominal imaging and 5–20% of these individuals have biochemical hypercortisolism [78–86]. Once endogenous ACTH-independent hypercortisolism has been confirmed, CT imaging of the adrenal glands should be obtained as it is a sensitive tool to detect cortisol-secreting neoplasms. These lesions are generally unilateral, rounded, well circumscribed and measure >1 cm in diameter. Importantly, these lesions are generally lipid rich and have a low density (<10 Hounsfield Units) on CT scanning. The contralateral adrenal gland is often small, but may be normal in size.

Adrenocortical Carcinoma

Adrenocortical carcinoma is a rare malignancy with only two new cases per million population per year. It occurs with a bimodal age distribution peaking before age 5 and from ages 50–60 [87]. Adrenocortical carcinoma is often hormonally active with hyperaldosteronism, virilization, and Cushing's syndrome occurring most commonly. The timing of onset for stigmata of Cushing's syndrome is a diagnostic clue as patients with adrenocortical carcinoma can develop symptoms rapidly. Refractory hypertension, hypokalemia, muscle wasting, and weight loss are other features that should raise the suspicion for adrenocortical carcinoma. The survival in patients with adrenocortical carcinoma is generally very poor, as most patients are diagnosed with stage III and IV disease with 5-year survival rates of 30% and 15% respectively [88].

The identification of a unilateral, irregular adrenal mass in a patient with ACTH-independent Cushing's syndrome is essentially diagnostic of adrenocortical carcinoma [89]. These tumors are generally large by the time of diagnosis, and CT scanning can often identify additional metastatic disease, assisting in staging and therapeutic decision making.

ACTH-Independent Macronodular Adrenal Hyperplasia

AIMAH is a rare form of bilateral nodular adrenal disease that results from aberrant hormone receptor signaling. The receptors identified to cause this condition are a diverse group of hormone receptors including receptors for gastric inhibitory polypeptide, β-adrenergic, vasopressin, serotonin, angiotensin II, luteinizing hormone/human chorionic gonadotropin, and leptin [90]. This process is thought to begin with binding of the normal ligand to its inappropriately expressed receptor resulting in stimulation of the adrenal glands via downstream signaling. This leads to growth of large monoclonal and polyclonal nodules in both adrenal glands with resultant overproduction and secretion of cortisol [91, 92].

AIMAH presents, on average, at a more advanced age than Cushing's syndrome caused by unilateral adrenal disease. Patients present at a mean age of 51 years of age with equal male: female distribution [93]. A diagnostic clue in patients with AIMAH is a greater than normal cortisol response to ACTH administration.

Abdominal imaging in these patients often reveals bilateral massively enlarged adrenal glands. There may be numerous nodules up to 4 cm in size, or the adrenal gland can appear diffusely enlarged [94]. On pathological examination, the adrenal cortex shows diffuse internodular hyperplasia which is in contrast to conditions such as McCune–Albright syndrome where the internodular cortex is atrophic [95, 96].

Primary Pigmented Nodular Adrenocortical Disease

PPNAD is another rare adrenal disease that results in hypercortisolism. In this condition, multiple small, often coalescent, adrenal nodules form and develop

autonomous function. The pathophysiology responsible for the nodule formation is not completely understood but appears to be due to mutations in the tumor suppressor protein kinase R1a subunit (PRKAR1A) [97]. Patients with Carney's complex, an autosomal dominant, inherited multiple neoplasia syndrome (that may present with acromegaly, calcifying Sertoli cells tumors, thyroid nodules, cutaneous and atrial myxomas, breast ductal adenomas, and psammomatous melanotic schwannomas, as well as PPNAD), account for 50% of patients with PPNAD with the remaining 50% being sporadic cases [97, 98].

PPNAD has a bimodal age distribution at presentation with most cases being diagnosed in the second and third decades of life [97]. Patients with PPNAD may not present with the typical stigmata of Cushing's syndrome. They are often lean and without central obesity. Common age-dependent features include short stature, osteoporosis, and severe muscle wasting [99]. A clinical feature that can help in diagnosis is that many patients with PPNAD have a paradoxical 50% or greater increase in cortisol production in 2-day-low and high-dose dexamethasone suppression testing [100].

Imaging reveals diverse findings. Some patients can have frankly enlarged adrenals bilaterally, while other can have essentially normal appearing glands on CT. Another well-known finding is a "string of beads" appearance of the adrenals. When examined pathologically there are darkly pigmented nodules ranging in size from microscopic to 1 cm. The intervening adrenal cortex is generally atrophic, which is in contrast to AIMAH [101].

McCune–Albright Syndrome

McCune–Albright syndrome, the result of a mutation in the α subunit of the stimulatory guanine nucleotide-binding protein (Gsα), is a familial form of adrenal-dependent Cushing's syndrome. This mutation leads to constitutive activation of the cyclic AMP pathway resulting in excessive cortisol secretion.

Patients with McCune–Albright syndrome generally present at a very young age with other manifestations such as polyostotic fibrous dysplasia, café au lait spots, and other autonomous endocrine hyperfunctions (i.e., premature puberty in girls and growth hormone excess).

The mutation in Gsα leads to a genetic mosaic with adrenal nodules containing the mutation. The adrenal cortex between nodules is atrophic and does not contain the Gsα mutation.

Subclinical Cushing's Syndrome

An important caveat to the differential diagnosis of adrenal-dependent Cushing's syndrome is patients with mild or subclinical Cushing's syndrome. Although there

is no consensus upon diagnostic criteria for mild cortisol excess, most agree that inappropriate and autonomous cortisol secretion in a patient with an incidentally discovered adrenal nodule, is consistent with subclinical Cushing's syndrome. These individuals often lack the classic stigmata of Cushing's syndrome, and as such any patient with an incidentally discovered adrenal nodule should be evaluated for autonomous cortisol secretion. Urine free cortisol is a poorly sensitive tool for this evaluation and is positive in only 32% of patients with subclinical Cushing's syndrome [80]. There are conflicting data regarding the diagnostic performance of late-night salivary for subclinical Cushing's syndrome [102, 103]. The 1-mg overnight dexamethasone suppression test and suppression of morning ACTH appear to perform best in the diagnosis of mild cortisol excess [80].

The complete clinical implications of subclinical Cushing's syndrome have not been fully elucidated. However, patients with mild cortisol excess have been found to have lower bone density and a greater risk of fracture [104]. These patients also have higher rates of obesity, glucose intolerance and metabolic syndrome. Additionally, small studies have shown a benefit in these metabolic findings after adrenalectomy [105].

Algorithm for Cushing's Syndrome Differential Diagnosis (Fig. 7.6)

After hypercortisolism has been convincingly demonstrated, plasma ACTH levels should be obtained to distinguish between ACTH-dependent and ACTH-independent Cushing's syndrome. A plasma ACTH level ≥20 pg/ml indicates an ACTH-dependent source. In this circumstance, a dedicated MRI of the sella should be obtained to look for a pituitary adenoma. Generally speaking, patients with clinical and biochemical findings consistent with Cushing's disease, and an unequivocal mass in the pituitary, can be directly referred to an experienced neurosurgeon for consideration of transsphenoidal tumor resection. However, if there is an equivocal or absent pituitary lesion on imaging, or the patient has atypical clinical features, it is recommended that patients be referred to a specialized center for IPSS. Patients found to have a pituitary source of excess ACTH should then be referred to a neurosurgeon. If there is no central to peripheral gradient on IPSS, a search for an ectopic source of ACTH should be pursued.

An ACTH level ≤5 pg/ml on initial testing is indicative of ACTH-independent hypercortisolism. The next step is to obtain an adrenal CT for differential diagnosis of adrenal-dependent Cushing's syndrome.

The most challenging diagnostic scenario occurs when ACTH levels are between 5 and 20 pg/ml. Patients with ACTH levels in this range should be evaluated by an experienced clinician familiar with the differential diagnosis of Cushing's syndrome. CRH stimulation is generally the next step in the diagnostic algorithm.

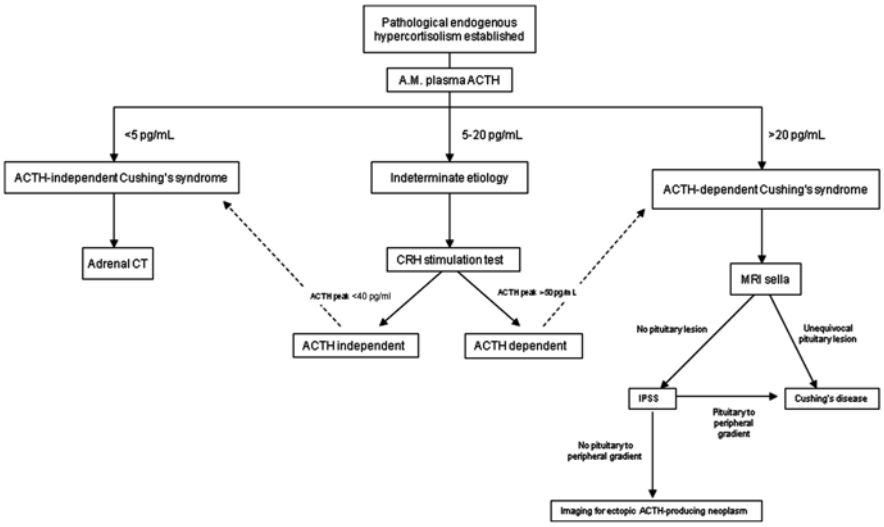

Fig. 7.6 Algorithm for Cushing's syndrome differential diagnosis

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