Diagnosis of Cushing's Disease

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KEYWORDS

• Cushing's disease • Ectopic ACTH syndrome • ACTH
• Bilateral inferior petrosal sinus sampling • Pituitary • MRI

KEY POINTS

- Cushing's disease (CD) is ~7 times more common than ectopic ACTH syndrome (EAS). Patients with ambiguous noninvasive test results should be definitively evaluated with bilateral inferior petrosal sinus sampling (IPSS) before EAS is diagnosed.
- Adrenal steroidal precursor levels and the 8 mg high-dose dexamethasone suppression test can help distinguish CD from adrenal Cushing's syndrome when ACTH levels are in a gray zone.
- Pituitary MRI may not reveal a lesion if a suboptimal imaging sequence is used; repeat imaging at a center specializing in pituitary disorders should be considered in patients with suspected CD without a pituitary lesion on initial imaging.
- Biochemical diagnostic testing, including assessments of ACTH-dependence, noninvasive dynamic tests and IPSS, must be performed during a period of confirmed hypercortisolism to avoid misleading results.
- The appropriate choice and interpretation of biochemical and radiologic diagnostic tests and the success of IPSS and transsphenoidal exploration require endocrinologists, radiologists and surgeons with extensive experience. Because of this, providers should have a low threshold to refer patients with suspected CD to a specialized center.

INTRODUCTION

Endogenous Cushing's syndrome (CS) is a rare disorder, affecting 2 to 8 per million people annually. The clinical presentation of CS reflects hypercortisolism that perturbs the normal physiology and regulation of the hypothalamic-pituitary-adrenal (HPA) axis. The diagnosis, cause, and effective treatment must be established correctly and quickly to mitigate the associated morbidity and excess mortality.

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Abbreviations	
ACTH	Adrenocorticotropin
CBG	corticosteroid-binding globulin
CD	Cushing's disease
CISS	constructive interference in steady state
CRH	corticotropin-releasing hormone
CS	Cushing's syndrome
DHEA	Dehydroepiandrosterone
EAS	ectopic ACTH syndrome
FLAIR	fluid-attenuated inversion recovery
FOV	field of view
HDDST	high-dose dexamethasone suppression test
HPA	hypothalamic-pituitary-adrenal
IPSS	inferior petrosal sinus sampling
LDDST	low-dose dexamethasone suppression test
NPV	negative predictive value
PPV	positive predictive value
SPGR	spoiled gradient echo
TSS	transsphenoidal surgery
UFC	urine free cortisol

Excess secretion of adrenocorticotropin (ACTH) from a pituitary corticotrope tumor (Cushing's disease, CD: 60%–70%) or from a nonpituitary, ectopic tumor (termed ectopic ACTH syndrome, EAS: 6%–10%) cause the majority of cases. A minority of cases (20%–30%) are caused by autonomous, non-ACTH mediated cortisol production by adrenal tissue.[1] Diagnostic testing to determine the etiology takes advantage of the unique pathophysiology of these entities.

It can be difficult to establish the presence of CS and its cause. In this article we will outline the best use of available diagnostic tests, the importance of optimized pituitary imaging and when to proceed with invasive biochemical testing, with an emphasis on the eventual diagnosis of CD.

DISCUSSION

Confirmation of Endogenous Hypercortisolism

After excluding exogenous glucocorticoids as the cause of CS, current guidelines for the diagnosis of endogenous hypercortisolism require a lack of normal cortisol suppression during a 1 mg low-dose dexamethasone suppression test (LDDST), loss of the normal sleep-entrained nadir of serum or salivary cortisol, and/or supraphysiologic excretion of urine free cortisol (UFC) over 24 hours. The diagnosis is established if two of the three screening tests meet criteria for an abnormal response.[2]

The choice of screening tests should be individualized to account for modality-specific pitfalls:

- LDDST relies on the measurement of total serum cortisol and appropriate exposure to dexamethasone. It is not recommended for pregnant women or patients taking oral estrogen (including estrogen-containing oral contraceptives). Oral estrogens can cause false positive (abnormally high) results through a first-pass hepatic effect that increases corticosteroid-binding globulin (CBG) production, and hence total serum cortisol levels. Similarly, in pregnancy, CBG is increased and cortisol suppression is blunted. Because dexamethasone is metabolized by CYP3A4, the LDDST may give false positive or negative results depending on the concomitant administration of drugs that stimulate or inhibit this enzyme complex. While a concurrent serum dexamethasone level at the time of cortisol

measurement can reveal this problem, additional testing with associated costs must then be undertaken.

- Adequate UFC assessments require proper collection and normal renal filtration. UFC may be falsely negative (normal) when creatinine clearance is <60 mL/min, falsely positive (abnormally high) when daily fluid intake exceeds 4L, and either falsely positive or negative if the collection duration is more or less than 24 hours, respectively. UFC results are also affected by the ingestion of exogenous glucocorticoids. Hydrocortisone, the pharmaceutical generic name for cortisol, is detected as such and may elevate UFC results. By contrast, other exogenous glucocorticoids may suppress ACTH release and lead to a low UFC if measured by an assay that does not cross-react with the glucocorticoid.
- Salivary cortisol is entrained to sleep and relies on a regular sleep-wake cycle. False positives can occur in shift workers and during jet lag, or if samples are not collected at bedtime.[2] Contamination of the specimen with topical preparations containing hydrocortisone (i.e., cortisol), either in lip gloss or dermatologic creams/ointments, may also cause false positive results. In this setting, the concurrent measurement of salivary cortisone will provide an appropriately low or normal value rather than the high result expected if endogenous cortisol is truly elevated.[3]

The Challenge of Cyclical Cushing's Syndrome

It can be very difficult to establish the diagnosis in a patient with cyclical endogenous CS, which can occur with any tumoral etiology and is characterized by periods of hypercortisolism interspersed with periods of normal or even low cortisol.[4] As a result, screening tests may be normal or low during times of low disease activity. In patients for whom there is a high clinical suspicion, multiple UFC and/or bedtime salivary cortisol levels over a period of time (e.g. weekly) may be required. If patients have intermittent signs or symptoms consistent with hypercortisolism, such as hypertension or hyperglycemia, testing should target those intervals.

Second-Line Tests to Distinguish Between Non-neoplastic Hypercortisolism and Cushing's Syndrome

In some cases, hypercortisolism is secondary to nontumoral activation of the HPA axis due to, for example, severe obesity, uncontrolled diabetes mellitus, or alcoholism. A recent editorial evaluated the utility of the desmopressin stimulation test, dexamethasone-suppressed CRH test, and midnight serum or late-night salivary cortisol in this setting to distinguish or exclude CS. Although the Dex-CRH test had better sensitivity than the desmopressin test (91% vs 86%), it had a lower specificity (82% vs 90%). The performance of the late-night salivary cortisol test was similar to that of the desmopressin test, with a sensitivity of 80% and specificity of 90%. As CRH is no longer available, the desmopressin test and late-night salivary cortisol test represent reasonable alternatives. However, an important caveat is that neither test is performed identically at all sites, due to differences in salivary cortisol assays and different timing of blood draws, and criteria for interpretation of the desmopressin test.[5]

Diagnostic Strategies to Distinguish ACTH-Dependent and -Independent Causes of Cushing's Syndrome

Sustained hypercortisolism suppresses ACTH secretion from normal corticotropes. As a result, ACTH levels are low in primary adrenal causes. They are inappropriately normal or high in ACTH-dependent etiologies, which do not suppress ACTH levels in response to mild elevations in cortisol (**Fig. 1**). Thus, once CS is confirmed, a morning plasma ACTH level <10 pg/mL or >20 pg/mL provides high diagnostic accuracy for adrenal

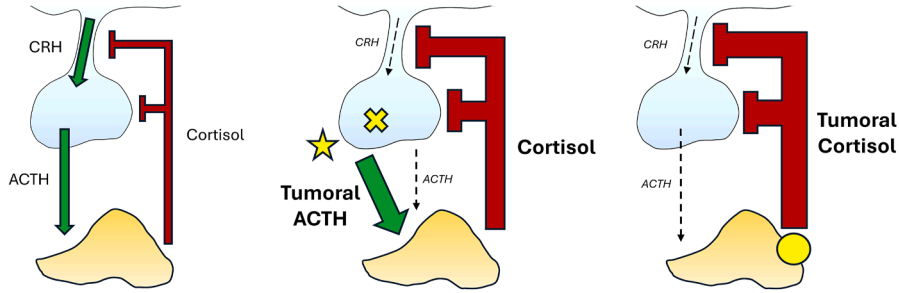

Fig. 1. The hypothalamic-pituitary-adrenal (HPA) axis is disrupted in Cushing's syndrome. In healthy people (*left*), the hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates adrenocorticotropin (ACTH) release from the anterior pituitary. In response, the adrenal cortex releases cortisol, which exerts negative feedback on both the hypothalamus and the pituitary. In ACTH-dependent forms of Cushing's syndrome (CS) (*center*), a tumor in the pituitary (Cushing's disease, tumor marked with an X) or elsewhere (Ectopic ACTH syndrome, tumor marked with a star) secretes excessive ACTH leading to high cortisol production and the inhibition of healthy CRH- and ACTH-releasing cells. In ACTH-independent CS (*right*), an adrenal tumor (*circle*) produces excessive amounts of cortisol, leading to low levels of CRH and ACTH.

disease or ACTH-dependent CS, respectively.[1] Importantly, ACTH-dependence must be assessed during sustained hypercortisolism. In cyclical CS, a period of low disease activity can produce a misleading normal or low ACTH level, depending on the degree of suppression of the normal corticotropes and the underlying etiology. Plasma ACTH values between 10 and 20 pg/mL constitute a diagnostic gray zone in which the cause of CS is almost always either CD or adrenal. (ACTH levels in EAS tend to be in the high-normal range or supraphysiologic). Additional tests that take advantage of the differential pathophysiology of ACTH and its action can be helpful.

Serum levels of dehydroepiandrosterone (DHEA), an adrenal androgen precursor that is largely dependent on ACTH for its production, and its sulfate ester DHEA-S, are often low-normal or subnormal (for sex and age) in ACTH-independent etiologies, and robust in the ACTH-dependent causes:

- In a study that compared fasting AM serum steroid profiles measured by mass spectrometry in patients with CD (n = 15) to those in patients with adrenal CS (n = 13), DHEA, DHEA-S, and androstenedione provided the most reliable discrimination between groups.
- DHEA performed best, with values <18% of the upper limit of the reference range for sex and age providing 100% diagnostic accuracy for adrenal CS.
- The optimal cut off for DHEA-S was <40% of the upper limit, which resulted in a diagnostic accuracy of 93%[6] to identify adrenal causes. It should be noted that these diagnostic cut offs may not perform equally well with all assays and that commonly used immunoassays are likely to provide more variable results than mass spectrometry.

The extent of cortisol suppression after dexamethasone, 8 mg, can also help to distinguish between an adrenal etiology and CD, since corticotrope tumors often retain some ability to suppress ACTH in response to glucocorticoid negative feedback, albeit at higher-than-normal doses:

- In one study of 30 patients with CD and 45 with adrenal CS, a high-dose dexamethasone suppression test (HDDST) consisting of a once daily dose of 8 mg

dexamethasone for 2 days suppressed AM serum cortisol by ≥50% in 87% of patients with CD vs 2% of patients with adrenal etiologies.
- Suppression of 24-hr urine free cortisol (UFC) on Day 2 provided the highest diagnostic accuracy, correctly identifying 73% of CD and 100% of adrenal CS using a criterion for CD of ≥90% reduction.[7]

Diagnostic Strategies to Distinguish Cushing's Disease from Ectopic ACTH Syndrome

Clinical and laboratory presentation

Once ACTH-dependence has been confirmed, the source of excess production must be determined. The initial reports of EAS suggested that these patients had a more explosive onset and rapid progression of hypercortisolism, with higher ACTH and UFC levels than patients with CD, and that EAS occurred most often in men with metastatic tumors.[8,9] We now recognize an extensive overlap between the clinical and laboratory presentations of these ACTH-dependent causes of CS, due to benign, indolent neuroendocrine tumors that cause the syndrome more often than widely metastatic disease.[10]

Thus, while the clinical and biochemical picture is not definitive, it can provide clues. EAS is more likely in patients who present with a rapid onset of symptoms, extremely high ACTH and UFC (>10 times normal), hypokalemia (due to the saturation of renal 11-beta-hydroxysteroid dehydrogenase 2, leaving more cortisol available to activate the mineralocorticoid receptor), and a normal pituitary MRI.[11,12] Patients with EAS with very elevated ACTH and cortisol levels are also more likely to present with hyperpigmentation, proximal muscle weakness and psychosis.[13]

Currently, there is little difference in sex distribution in EAS, while CD is four times more common in women of reproductive age than men, with a median onset around 40 to 45 years of age.[1,13,14] After menopause, there is no sex difference in incidence.[15]

Pituitary MRI

A pituitary MRI should be performed as the first diagnostic test when trying to identify the source of excessive ACTH production. On precontrast imaging, pituitary adenomas are typically hypointense on T1-and hyperintense on T2-weighted sequences. Both contrast enhancement and washout are delayed in adenomas due to altered blood flow.[16] MRI of the pituitary gland is made difficult by its anatomy, being located near several interfaces of fluids (internal carotid artery, suprasellar cistern), air (sphenoid sinus), fat (cavernous sinus), and bone that can give rise to artifacts.[16] Additionally, nearly 50% of corticotrope adenomas are <5 mm in size.[17] In at least 20% of CD cases, no lesion is visible on MRI using a conventional 1.5T magnet, despite improvements in detection rates due to advancements in technology.[16,18] However, CD remains a likelier diagnosis than EAS even when no lesion is seen, since it is much more prevalent.

Standard pituitary MRI sequences include T1-weighted spin-echo (SE) or rapid acquisition with relaxation enhancement (RARE, e.g., fast SE [FSE] or turbo SE [TSE]) obtained pre- and post-intravenous gadolinium contrast in the coronal and sagittal planes, as well as a coronal T2-weighted FSE sequence with a section thickness ≤3 mm.[16–18] Compared with these standard sequences, the MRI sensitivity for corticotrope adenomas increases when using a 3D spoiled gradient echo (SPGR) sequence that allows for thinner slice thickness (**Fig. 2**), or dynamic imaging where data are acquired every 10 to 20 seconds over the first 1 to 2 minutes after contrast injection.[16–18] Other sequences that can be added to increase detection rates include 3D FSE (e.g., SPACE), contrast-enhanced fluid-attenuated inversion recovery (FLAIR),

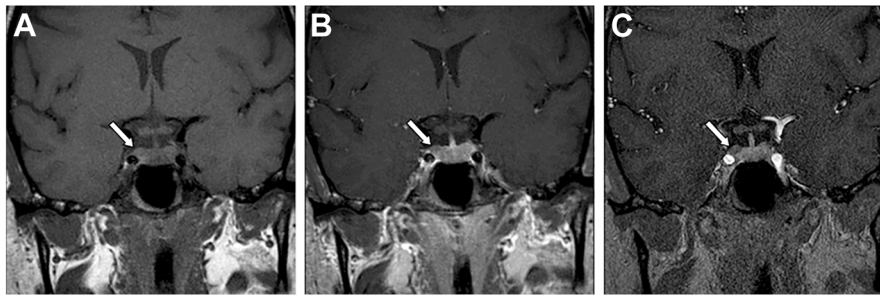

Fig. 2. Select MRI sequences increase the detection rates of corticotrope adenomas. In this patient with Cushing's disease, the right-sided pituitary microadenoma (*arrows*) was not apparent on standard T1-weighted precontrast images, slice thickness 2 mm (*A*). Some heterogeneity was seen in the tumor area on standard T1-weighted postcontrast images, slice thickness 2 mm (*B*). On T1-weighted, postcontrast imaging using a 3D spoiled gradient echo (SPGR) sequence with slice thickness 1 mm, the adenoma became apparent (*C*).

and constructive interference in steady state (CISS).[16,18] Sensitivity can also be improved with increased magnetic field strength (e.g. 3T), thinner sections, and a smaller field of view (FOV), all of which increase resolution. Using a lower dose of gadolinium contrast may also improve microadenoma detection by reducing the enhancement of the surrounding gland.[16,17] However, while these methods increase sensitivity, they also increases false positive rates, which may favor a stepwise approach to imaging.[18] Ultimately, the identification of a surgical target on imaging is important to increase the likelihood of tumor identification and resection at surgery, postoperative biochemical remission, and to reduce the likelihood of surgical complications.[16]

In cases with a negative initial pituitary MRI, considering the many intricacies involved in the choice and interpretation of MRI sequences and results, referral for repeat imaging at a center specializing in pituitary disorders could be considered. Other imaging methods combining PET technology with MRI have shown promise in improving detection rates (See Chapter 3).

Later in the discussion, we suggest further management based on initial pituitary MRI findings.

≥10 mm lesion on pituitary MRI: transsphenoidal surgery (TSS)

Pituitary lesions ("incidentalomas") up to 6 mm in diameter are seen on MRI in about 10% of the general population, consistent with the prevalence of microadenomas on autopsy in persons without suspected pituitary disease.[19,20] Among pituitary adenomas found incidentally on autopsy, almost all are microadenomas (<10 mm), and more than 90% are smaller than 5 mm.[21] Based on the high pre-test probability of CD compared to EAS, and the very low likelihood of an incidentally detected macroadenoma, patients with ACTH-dependent CS and pituitary lesions ≥10 mm can be referred for TSS without additional testing. (This recommendation only applies to the general population and not to patients prone to forming pituitary adenomas due to syndromic conditions, such as MEN-1).

≤6 mm lesion or no lesion on pituitary MRI: bilateral inferior petrosal sinus sampling

If MRI fails to detect a lesion or detects a lesion up to 6 mm in diameter - a common incidental finding - IPSS should be performed, as it is the gold standard test to discriminate between EAS and CD. As originally proposed, this test involves the cannulation

of each petrosal sinus and a peripheral vein, with the simultaneous collection of blood for ACTH measurement from each site, before and after the stimulation of ACTH secretion by corticotropin-releasing hormone (CRH).[22] The ratios of petrosal to peripheral ACTH levels at each timepoint are low in EAS and greater than 2 before, or 3 after, stimulation with CRH in CD. In expert hands, with the concurrent measurement of prolactin to confirm accurate sampling, IPSS is highly reliable for confirming CD using these thresholds, producing sensitivity (Se) 99%, specificity (Sp) 95%, positive predictive value (PPV) 98% and negative predictive value (NPV) 97%.[23] In the extremely rare case of ectopic CRH production (with or without concomitant ACTH production), IPSS may show a petrosal to peripheral ACTH gradient consistent with CD, leading to a low sensitivity for detecting EAS (~35%).[24] This is likely caused by the ectopic CRH stimulation of normal corticotropes so that they are not suppressed by cortisol feedback.

Lack of adequate catheterization and aberrant venous anatomy, especially atrophic vessels and arborization of the petrosal sinus, are associated with falsely negative IPSS results. Thus, in addition to prolactin measurement, petrosal sinus venography is done routinely to evaluate appropriate catheter placement and anatomy of the petrosal sinuses.[25]

Unfortunately, CRH has been taken off the market due to manufacturing issues, and the arginine vasopressin analog desmopressin is now used in the same manner. Initial data indicate that IPSS with desmopressin stimulation also produces a high Se (96%) for confirming CD, but more data are needed from patients with EAS to confirm specificity.[26]

While IPSS is highly accurate when trying to distinguish CD from EAS, it is less reliable for determining the intrapituitary location of an adenoma. In a systematic review of 461 patients with CD, IPSS achieved accurate lateralization in 69% of cases.[27] The suboptimal ability of IPSS to predict tumor location is especially important in cases of MRI-negative CD, where hemihypophysectomy is sometimes pursued, with the side for resection being chosen based on IPSS lateralization.

7 to 9 mm lesion on pituitary MRI: transsphenoidal surgery, inferior petrosal sinus sampling, or further non-invasive dynamic testing

If a 7 to 9 mm lesion is seen on pituitary MRI, IPSS may reasonably be deferred, as incidental lesions of this size are relatively uncommon. To increase diagnostic certainty before TSS, further noninvasive dynamic biochemical testing can be considered.[28] To be useful, these tests need to provide a very high PPV to enhance the high pre-test probability of CD. Importantly, most dynamic testing relies on suppression of normal corticotropes, and the possibility of cyclical CS should be excluded to avoid the misinterpretation of testing performed during a period of low disease activity.[10]

CRH or desmopressin stimulation test

Previously, serial measurements of ACTH and cortisol after CRH administration was the best available option. When interpreted with optimized response criteria, this test achieved Se 91%, Sp 92% and PPV 97%.[23] The desmopressin stimulation test is performed in a similar manner, with mixed performance data (overall performance from nine studies: Se 85%, Sp 62%) and lack of consensus on optimal cut offs.[29] Desmopressin is believed to act on aberrant V2 receptors or upregulated V3 receptors in corticotrope adenomas and does not trigger a response in most healthy individuals.[29,30] The desmopressin test can therefore be helpful to detect early postoperative recurrence or inactive phases of cyclic CD where hypercortisolism is not present, unlike the CRH test, which would trigger a response from healthy, unsuppressed corticotropes.[31,32]

8 mg overnight high-dose dexamethasone suppression test

The 8 mg overnight HDDST is currently the most often used option in clinical practice. Unlike the 1 mg test, which uses an absolute cortisol criterion of 1.8 μg/dL (50 nmol/L), the 8 mg test assesses the relative degree of cortisol suppression. Using the most common response criterion, a 50% reduction in AM serum cortisol from the day before dexamethasone administration to the morning after, provides Se 79% and Sp 77% for CD. Specificity can be increased with an optimized response criterion (−69% suppression), increasing the PPV from 92% to 96%. A negative HDDST is not helpful, with an NPV around 50%.[23]

Combined dynamic testing

Combinations of the noninvasive tests, if responses are positive and concordant, can further improve diagnostic accuracy and provide a stronger argument for forgoing IPSS and proceeding to surgery. Positive results of both the desmopressin stimulation test and HDDST identify 66% to 68% of patients with CD with a PPV of 98%, based on retrospective data.[33,34] Should CRH become available again, similar positive results

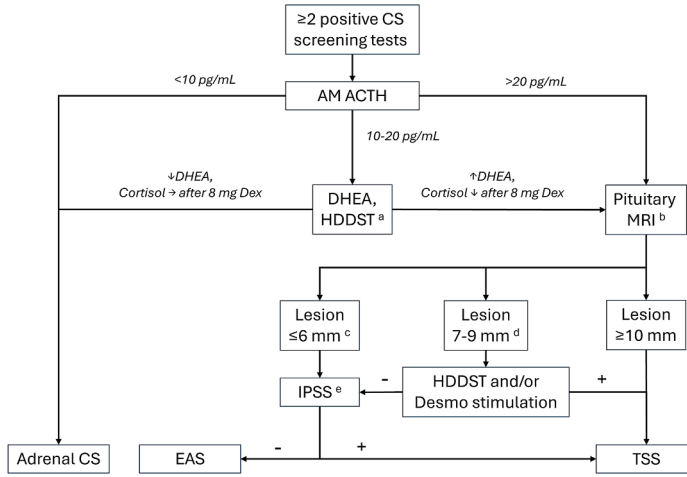

Fig. 3. Diagnostic algorithm for the etiology of CS. All biochemical testing should occur during periods of confirmed hypercortisolemia. Patients with suspected CD are ideally evaluated at a specialized center with optimized MRI protocols, access to IPSS and an experienced pituitary surgeon. [a]Fasting AM DHEA <18% or DHEA-S <40% of the assay's ULN for sex and age, and a lack of ≥50% suppression of AM serum cortisol after 8 mg Dex are highly specific indicators of adrenal CS. [b]If the initial pituitary MRI is negative, repeat imaging at a specialized pituitary center should be considered before deciding on further steps. [c]In cases of pituitary lesions ≤6 mm where IPSS is not available, noninvasive dynamic testing with HDDST and/or Desmo stimulation, ideally both, is a second-line option. Concordant positive results signify a high likelihood of CD. [d]In cases of pituitary lesions 7 to 9 mm, confirmation with IPSS or directly proceeding to TSS are reasonable options as well. [e]A central-to-peripheral ACTH ratio >2 before, or >3 after, Desmo signifies a positive IPSS result. If IPSS indicates CD despite a lack of a visible pituitary lesion, a repeat MRI should be considered before exploratory TSS to try identifying a surgical target. ACTH, adrenocorticotropin; CD, Cushing's disease; CS, Cushing's syndrome; Desmo, desmopressin; Dex, dexamethasone; DHEA, dehydroepiandrosterone; DHEA-S; dehydroepiandrosterone sulfate; EAS, ectopic ACTH syndrome; HDDST, high-dose dexamethasone suppression test; IPSS, bilateral inferior petrosal sinus sampling; TSS, transsphenoidal surgery; ULN, upper limit of normal.

on a CRH stimulation test and HDDST provide Se 65% to 76% and PPV 99%.[23,35] Concordant negative results are less helpful, with NPV 70% for desmopressin + HDDST and 84% to 89% for CRH + HDDST.[23,33,35]

A suggested diagnostic algorithm for CS is summarized in Fig. 3.

SUMMARY

Cushing's disease (CD) is the most common cause of endogenous Cushing's syndrome (CS). After hypercortisolism has been established on ≥2 screening tests, ACTH-dependence is assessed with an AM plasma level. If this level is indeterminate (10–20 pg/mL [2.2–4.4 pmol/L]), low AM levels of DHEA and DHEA-S, and a lack of cortisol suppression on a high-dose dexamethasone suppression test (HDDST) can be helpful to point toward an adrenal etiology. Once ACTH-dependence has been proven, a more severe clinical and biochemical picture raises the suspicion of ectopic ACTH syndrome (EAS). However, the converse is not true, as milder and more indolent presentations are common in both CD and EAS.

A normal pituitary MRI is found in a substantial portion of patients with CD, which remains a likelier diagnosis than EAS even in this situation, due to its higher incidence. However, not all pituitary MRI sequences produce equivalent results. Thus, unless CD has been confidently excluded by bilateral inferior petrosal sinus sampling (IPSS), referral to a center specializing in pituitary disorders should be considered to ensure that optimal imaging techniques are used. Identification of a surgical target before transsphenoidal surgery (TSS) improves the rate of biochemical remission and reduces surgical complications.

Large pituitary lesions on MRI are unlikely to be incidental. Patients with ACTH-dependent CS and lesions ≥10 mm can proceed directly to TSS. For those with lesions 7 to 9 mm, positive dynamic testing with HDDST and the desmopressin stimulation test strengthens the argument to proceed with TSS. For patients with a negative MRI or lesions ≤6 mm, IPSS with desmopressin stimulation and concurrent prolactin sampling to ensure adequate cannulation should be performed. If IPSS is not available, concordant positive results on the two currently available dynamic tests can provide a strong argument for proceeding with TSS in cases of diagnostic uncertainty. IPSS, noninvasive dynamic tests and assessments of ACTH-dependence should only be performed during periods of confirmed hypercortisolism, as results during disease inactivity can be misleading.

Due to the complexities of diagnostic testing, differences in MRI technique, the limited availability of IPSS, and the eventual need for an experienced pituitary surgeon to maximize chances of a curative resection, providers should have a low threshold to refer patients with suspected CD to a center specializing in pituitary disorders.

CLINICS CARE POINTS

- The choice of screening tests for Cushing's syndrome (CS) should take patient factors into account, such as renal function, regularity of sleep-wake cycle, and use of oral contraceptives or medications that affect CYP3A4.
- Biochemical testing to identify the etiology of CS should take place during periods of confirmed hypercortisolism to avoid misleading results from disease inactivity.
- ACTH-dependence is not established by an AM ACTH of 10 to 20 pg/mL. In these cases, androgen precursor (e.g., DHEA) levels below the age- and sex-adjusted reference range and

a lack of significant cortisol suppression after high-dose dexamethasone support an adrenal etiology rather than Cushing's disease (CD).

- The likelihood of ectopic ACTH syndrome (EAS) increases with severe hypercortisolism, but an indolent clinical picture is not reliable for diagnostic purposes, as there is a large overlap in presentations.
- All pituitary MRIs are not equally sensitive, and cases of CD with negative MRI findings are not uncommon. In cases of negative imaging, repeat imaging at a center specializing in pituitary disorders with optimized imaging techniques should be considered, since CD remains a likelier diagnosis than EAS even in this scenario, due to its higher prevalence.
- Pituitary incidentalomas $\leq$6 mm in size are common. Incidentally found macroadenomas ($\geq$10 mm) are rare. The size of pituitary lesions in patients with ACTH-dependent CS therefore guide further management:
  - Lesion $\geq$10 mm: Transsphenoidal surgery (TSS)
  - Lesion 7 to 9 mm: TSS or further noninvasive dynamic testing
  - Lesion $\leq$6 mm or no lesion: bilateral inferior petrosal sampling (IPSS)
- Currently available noninvasive tests (8 mg dexamethasone suppression, desmopressin stimulation) are not helpful when negative, but provide a strong argument to proceed directly to TSS when concordant positive results are seen along with a pituitary lesion of any size.

DISCLOSURES

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