Laboratory tests for evaluation of hypercortisolism

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Cushing’s syndrome

- ✓ Endogenous Cushing’s syndrome: an uncommon disorder
- ✓ The most common cause: iatrogenic from medically prescribed corticosteroids
- ✓ The biochemical hallmark of endogenous syndrome: excess cortisol production
- ✓ Signs and symptoms reflect prolonged and inappropriately high exposure of tissue to glucocorticoids
- ✓ Broad spectrum of clinical presentation

Classification of Causes of Cushing's syndrome

ACTH-Dependent Causes

Cushing disease (pituitary dependent)
Ectopic ACTH syndrome
Ectopic CRH syndrome
Macronodular adrenal hyperplasia
Iatrogenic (treatment with [1–24]ACTH)

ACTH-Independent Causes

Adrenal adenoma and carcinoma
Primary pigmented nodular adrenal hyperplasia and Carney syndrome
McCune-Albright syndrome
Aberrant receptor expression (gastric inhibitory polypeptide, interleukin-1β)
Iatrogenic (e.g., pharmacologic doses of prednisolone, dexamethasone)

Other Causes of Hypercortisolism (non-neoplastic)

Alcoholism
Depression
Obesity
Pregnancy

Features that best discriminate Cushing's syndrome

- ✓ Easy bruising
- ✓ Facial plethora
- ✓ Proximal myopathy (or proximal muscle weakness)
- ✓ Striae (especially if reddish purple and > 1 cm wide)
- ✓ In children, weight gain with decreasing growth velocity

- ➢ all are signs not symptoms
- ➢ most do not have a high sensitivity

Common features in the general population and/or less discriminatory

- **Symptoms**
  - ✓ depression
  - ✓ fatigue
  - ✓ weight gain
  - ✓ back pain
  - ✓ changes in appetite
  - ✓ decreased appetite
  - ✓ decreased concentration
  - ✓ decreased libido
  - ✓ impaired memory (especially short term)
  - ✓ insomnia
  - ✓ irritability
  - ✓ menstrual irregularity

- in children
  - ✓ slow growth

- **Signs**
  - ✓ buffalo hump
  - ✓ facial fullness
  - ✓ Obesity
  - ✓ supraclavicular fullness
  - ✓ thin skin
  - ✓ peripheral edema
  - ✓ acne
  - ✓ hirsutism or female balding
  - ✓ poor skin healing

- in children
  - ✓ abnormal genital virilization
  - ✓ short stature
  - ✓ pseudo-precocious puberty or delayed puberty

Overlapping conditions

- ✓ Hypertension
- ✓ Vertebral osteoporosis
- ✓ Type 2 diabetes

(Cushing's syndrome is more likely if onset of the feature is at a younger age.)

- ✓ Incidental adrenal mass
- ✓ Polycystic ovary syndrome
- ✓ Hypokalemia
- ✓ Kidney stones
- ✓ Unusual infections

For whom testing for Cushing's syndrome is recommended?

- ✓ Patients with unusual features for age (*e.g.* osteoporosis, hypertension)

(strong, low quality of evidence)

- ✓ Patients with multiple and progressive features, particularly those who are more predictive of Cushing's syndrome *(strong, low quality of evidence)*

- ✓ Children with decreasing height percentile and increasing weight *(strong, very low quality of evidence)*
- ✓ Patients with adrenal incidentaloma compatible with adenoma *(strong, very low quality of evidence)*

Investigation of patients suspected for Cushing Syndrome

- ✓ Does this patient have Cushing syndrome?
- ✓ If so, what is the cause?

Cushing's syndrome suspected

↓

Exclude exogenous glucocorticoid exposure

↓

Perform one of the following tests

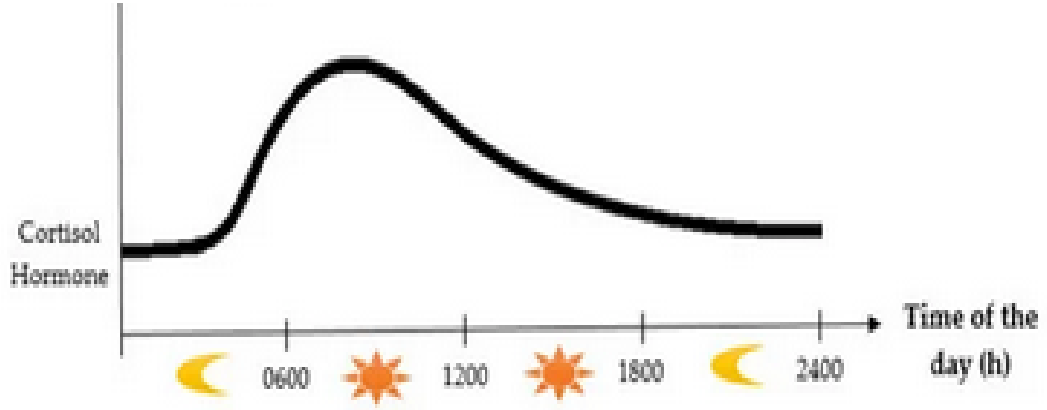
In the context of a high pretest probability of Cushing's syndrome, to accelerate diagnosis, the physician may request to perform 2 tests simultaneously.

Tests that are not recommended for Cushing's syndrome

- ✓ Random serum cortisol or plasma ACTH levels
- ✓ Urinary 17-ketosteroids
- ✓ Insulin tolerance test
- ✓ Loperamide test
- ✓ Tests designed to determine the cause of Cushing's syndrome (e.g. pituitary and adrenal imaging, 8 mg DST).

Late night salivary cortisol

- ✓ In healthy stable conventional sleep-wake cycles, the level of serum cortisol:
  - ✓ begins to rise at 03:00–04:00 h
  - ✓ reaches a peak at 07:00–09:00 h
  - ✓ then falls for the rest of the day to very low levels unstressed and asleep at midnight.



- ✓ The loss of circadian rhythm with absence of a late-night cortisol nadir is a consistent biochemical abnormality in patients with Cushing's syndrome.

Late night salivary cortisol

- ✓ Equilibrium between biologically active free cortisol in the blood & cortisol in the saliva.
- ✓ Good correlation between salivary and simultaneous serum cortisol
- ✓ Not affected by the rate of saliva production.
- ✓ An increase in blood cortisol is reflected by a change in the salivary cortisol concentration within a few minutes.
- ✓ Absence of cortisol binding globulin (CBG) in saliva

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ORIGINAL ARTICLE

Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans

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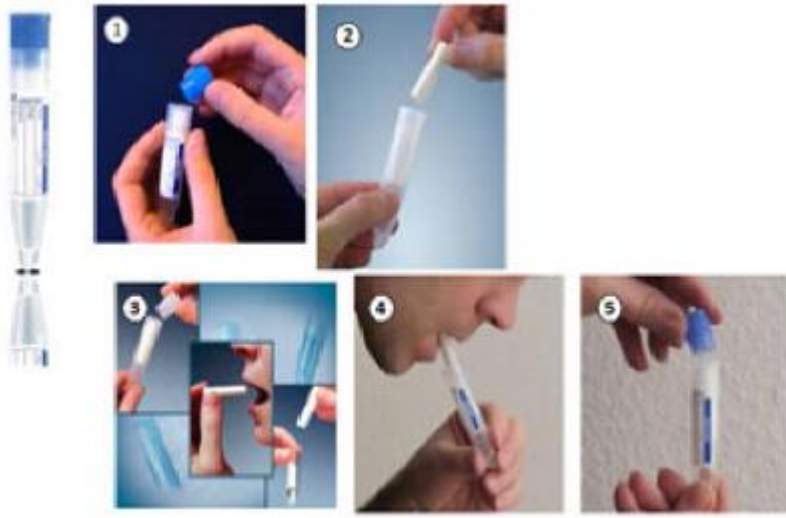
Diminished discriminating power in the elderly population

Variable	*N* (% total cohort)	Median (nmol/l)	LNSC range (nmol/l) [5%, 95%] values*	*P*-value†
Age 60 and over	95 (51)	2·7	1·1, 13·0	< 0·001
Age less than 60	92 (49)	1·9	0·7, 6·3	
Current diabetes mellitus	141 (75)	2·6	1·2, 9·7	< 0·001
Without diabetes mellitus	46 (25)	1·6	0·7, 4·7	

Remarks for late-night salivary cortisol

- ✓ It is suggested to collect a saliva sample on 2 separate evenings between 23:00 and 24:00 h.
- ✓ Collection methods:
  - ✓ passive drooling into a plastic tube
  - ✓ placing a cotton pledget (salivette) in the mouth and chewing for 1–3 min

- ✓ Samples collected using the device had lower cortisol than those collected by passive drooling, but they correlated better with total and free serum cortisol levels.
- ✓ Direct contamination of the device by steroid-containing lotion or oral gels may result in false-positive results.



- ✓ The sample is stable at room or refrigerator temperature for several weeks.

Remarks for late-night salivary cortisol

- ✓ It may not be appropriate for shift workers or those with variable bedtimes.
- ✓ It may be *transiently abnormal* in individuals crossing widely different time zones.

- ✓ *Stress* immediately before the collection also may increase salivary cortisol.

- ✓ Smoking: higher late-night salivary cortisol in smokers compared to nonsmokers
  - ✓ *It is sensible to avoid cigarette smoking on the day of collection*

- ✓ Contamination with blood in the oral cavity & possibility of increase salivary cortisol level : *not established*

- ✓ Licorice & chewing tobacco (both contain the 11β-hydroxyl steroid dehydrogenase type 2 inhibitor glycyrrhizic acid): *a falsely elevated late-night salivary cortisol*

Urinary free cortisol (UFC)

- ✓ An integrated assessment of cortisol secretion over a 24-h period
- ✓ A measure of the cortisol that is not bound to CBG, which is filtered by the kidney unchanged.
- ✓ Importance of correct urine collection, assessed by creatinine measurement in the collected sample
- ✓ The criterion for a positive test: *assay-dependent upper limit of normal*
- ✓ Values *> 3 (4) times the upper limit of normal* are *diagnostic* of Cushing's syndrome.

Remarks for UFC

- ✓ Importance of *correct urine collection*, assessed by creatinine measurement in the collected sample
- ✓ *Storage* of the container in a cold temperature (preferably, refrigerator)
- ✓ Not to drink excessive amounts of fluid [*high fluid intake (5 liters/d) significantly increases UFC*].
- ✓ *Avoid the use of any glucocorticoid preparations*, including steroid-containing skin or hemorrhoid creams, during the collection.
- ✓ *At least two collections* should be performed because of *variability of UFC* levels in a patient with Cushing's syndrome, particularly in children (lower reproducibility)

Remarks for UFC

- ✓ Renal clearance of cortisol is dependent on normal kidney function
  - ✓ *unreliable* when *Cr clearance < 20 mL/min*
  - ✓ *reduced reliability* when *Cr clearance < 60 mL/min*

- ✓ Elevated UFC can be seen:
  - ✓ normal pregnancy
  - ✓ exercise/stress
  - ✓ polyuria
  - ✓ proteinuria
  - ✓ pseudo-Cushing's

- ✓ *Urinary tract infections may decrease UFC.*

Pseudo-Cushing's syndrome

- ✓ An entity characterized by excess activity of HPA axis but without true Cushing's syndrome.
- ✓ It has been described in:
  - ✓ *depression*
  - ✓ *anxiety disorders*
  - ✓ *alcoholism*
  - ✓ *poorly controlled diabetes*
  - ✓ *morbid obesity*

- ✓ Normalization of the HPA axis by resolution of the underlying problem

1- mg Dexamethasone suppression test

- ✓ A simple outpatient test
- ✓ 1mg dexamethasone is given between 23:00 and 24:00 h
- ✓ Sampling for cortisol measurement: 08:00-09:00 h the following morning.
- ✓ Up to 15% of patients with Cushing's disease show normal test result.
- ✓ Normal response:
  - ✓ *serum cortisol less than 1.8 μg*/dL (< 50 nmol/L)
    - ✓sensitivity > 95% , specificity 80%
  - ✓ *serum cortisol less than 5 μg/dL (< 140 nmol/L)*
    - ✓specificity > 95%

Recommendation of Endocrine Society Practice Guideline:
Cut off of 1.8 μg/dL to achieve high sensitivity

Remarks for 1- mg Dexamethasone suppression test

- ✓ Variable absorption and metabolism of dexamethasone
- ✓ Drug interference
- ✓ Inter-individual variation in Dexamethasone levels, even in healthy individuals on no medication
- ✓ Simultaneous measurement of both cortisol and dexamethasone for these tests to ensure adequate plasma dexamethasone concentrations [>5.6 nmol/L (0.22 > g/dl)].
  - ✓ to evaluate for false-positive and negative responses
  - ✓ a test with limited availability
  - ✓ not feasible

❖ ***Drugs that accelerate dex induction of CYP 3A4***

- ✓ Phenobarbital
- ✓ Phenytoin
- ✓ Carbamazepine
- ✓ Primidone
- ✓ Rifampin
- ✓ Rifapentine
- ✓ Ethosuximide
- ✓ Pioglitazone

❖ *Alcohol induce hepatic e*

➡ **False positive result**

❖ ***Drugs that impair dexamethasone metabolism by inhibition of CYP 3A4***

- ✓ Fluoxetine
- ✓ Diltiazem
- ✓ Cimetidine
- ✓ Aprepitant/fosaprepitant
- ✓ Itraconazole
- ✓ Ritonavir

➡ **False negative result**

48- h, 2 mg/d Dexamethasone suppression test

- ✓ An outpatient test
- ✓ Administration of dexamethasone 0.5 mg every 6 hours for 48 hours (09:00,15:00, 21:00, 03:00)
- ✓ Blood sampling for cortisol at 9 am on first day and again 48 hours later
- ✓ Cut off point:
  - ✓ normal response: less than <1.8 μg/dL (50 nmol/L)
  - ✓ 97% to 100% true-positive rate
  - ✓ false positive rate of less than 1%
- ✓ Some physicians prefer to use the 48-h, 2 mg/d low-dose DST as an initial test because of its improved specificity as compared with the 1-mg test.

Remarks for 48- h, 2 mg/d DST

- ✓ In cases of pseudo-Cushing,s syndrome, UFC is less useful as an initial test & the optimal test is the 48- h, 2 mg/d DST.

- ✓ In the case of alcoholism, at least 2 weeks of abstinence from alcohol are needed to reduce the false-positive rate

- ✓ The sensitivity & specificity varies using slightly different protocols & cut offs in different studies.

If initial tests: normal

❖ Subjects with high pretest probability

(patients with clinical features suggestive of Cushing's syndrome and adrenal incidentaloma or suspected cyclic hypercortisolism)

✓ further evaluation by an endocrinologist to confirm or exclude the diagnosis

(strong, low quality of evidence)

❖ In other individuals in whom Cushing's syndrome is very unlikely

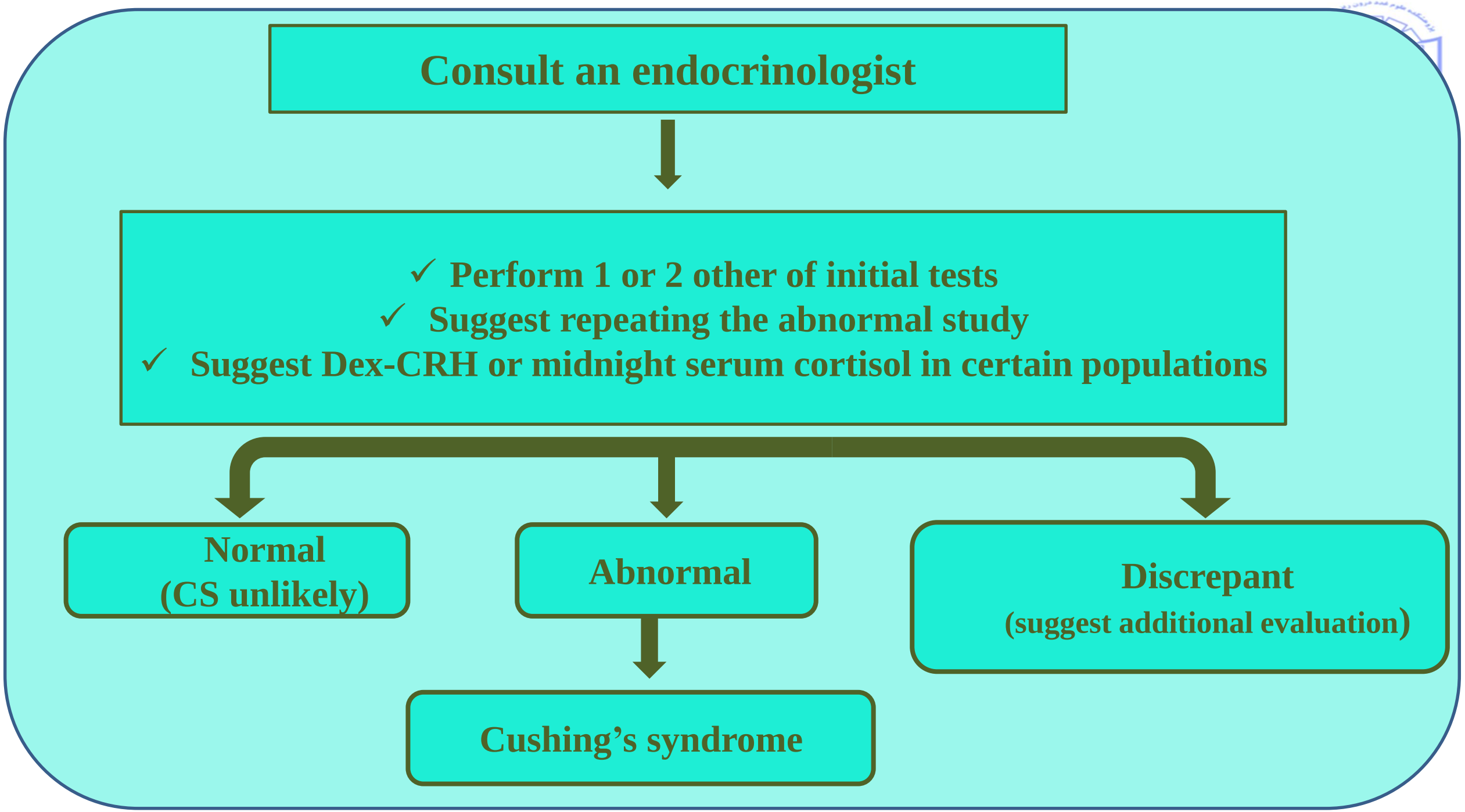
✓ re-evaluation in 6 months if signs or symptoms progress

(weak, low quality of evidence)

If initial tests: abnormal

❖ Other causes of hypercortisolism should be excluded.

Evaluation of the patient by an endocrinologist


Consult an endocrinologist
✓ Perform 1 or 2 other of initial tests
✓ Suggest repeating the abnormal study
✓ Suggest Dex-CRH or midnight serum cortisol in certain populations
Normal
(CS unlikely)
Abnormal
Discrepant
(suggest additional evaluation)
Cushing's syndrome

Remarks for initial testing

- ✓ The additional use of the *dexamethasone-CRH test* or the *midnight serum cortisol* test *in specific situations*.

- ✓ Further testing for Cushing's syndrome is not recommended in individuals with concordantly negative results on two different tests (except in patients suspected of having the very rare case of cyclical disease).

- ✓ Test to establish the cause of Cushing's syndrome is recommended for patients with concordantly positive results from

Dexamethasone-CRH test

- ✓ A combined test using dexamethasone & CRH
- ✓ It is developed to improve the sensitivity of the 48-h, 2 mg/d test
- ✓ First, 2 mg/d dexamethasone administrated for 48 hours
- ✓ then, administration of CRH(1μg/kg, IV) 2 h after the last dose of Dexamethasone.
- ✓ Sampling for cortisol measurement: 15 min later.
- ✓ Useful for equivocal UFC results
- ✓ In a small number of those with Cushing's disease, DST is normal, but if given CRH, they respond with an increase in ACTH and cortisol.

Midnight serum cortisol test

- ✓ Not as an initial testing for Cushing's syndrome
- ✓ Useful in specific situations
- ✓ May be assessed in the sleeping or awake state, using different diagnostic criteria.
  - ✓ *sleeping value : > 1.8 µg/dL*
  - ✓ *awake value > 7.5 µg/dL*
- ✓ Clinical utility:
  - ✓ In patients with a high clinical index of suspicion of Cushing's syndrome and who had normal UFC and full suppression on DST
  - ✓ In cases with a low clinical index of suspicion, such as in simple obesity, but lack of suppression on DST and mildly elevated UFC.
  - ✓ In the context of failure of suppression on DST due to anticonvulsant medication

Remarks for midnight serum cortisol test

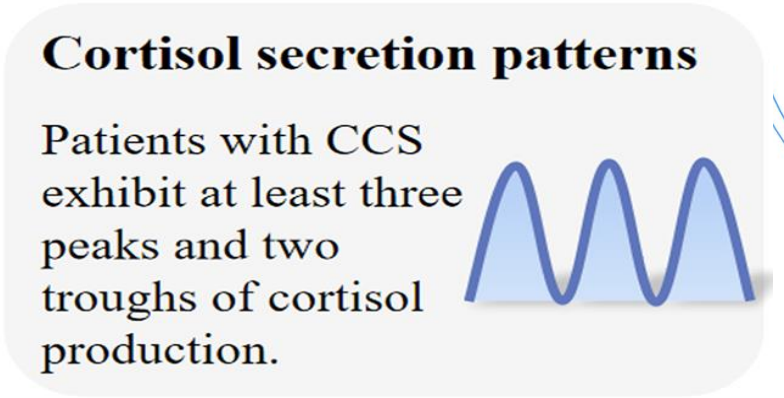
- ✓ Needs inpatient admission for a period of 48 h or longer to avoid false-positive responses

- ✓ Blood sampling:
  - ✓ The blood sample must be drawn within 5–10 min of waking the patient
  - ✓ through an indwelling line

- ✓ Young children may have their cortisol nadir earlier than midnight.

- ✓ For children, pre-catheterization is essential

Cyclic Cushing's syndrome



- ✓ Not common
- ✓ Periodic and intermittent increases in cortisol levels, resulting in recurrent episodes of clinical symptoms
- ✓ The cortisol secretion cycle is unpredictable
- ✓ Laboratory tests often show negative results during the normal cortisol secretion period
- ✓ Pathogenesis of CCS remains uncertain, suggested potential mechanisms are:
  - ✓ hypothalamic factors
  - ✓ feedback mechanisms
  - ✓ tumor infarction

Remarks for all tests

- ✓ Endpoint of the all recommended tests: cortisol measurement
- ✓ Several sample collection methods
- ✓ Several assay methods with different accuracy
- ✓ Results near the cutoff value on a single measurement can be explained by assay variability.
- ✓ The expected salivary and serum concentrations in these tests are close to the functional limit of detection of the assays. So, selection of assays based on their performance at this low range is important.

Remarks for all tests

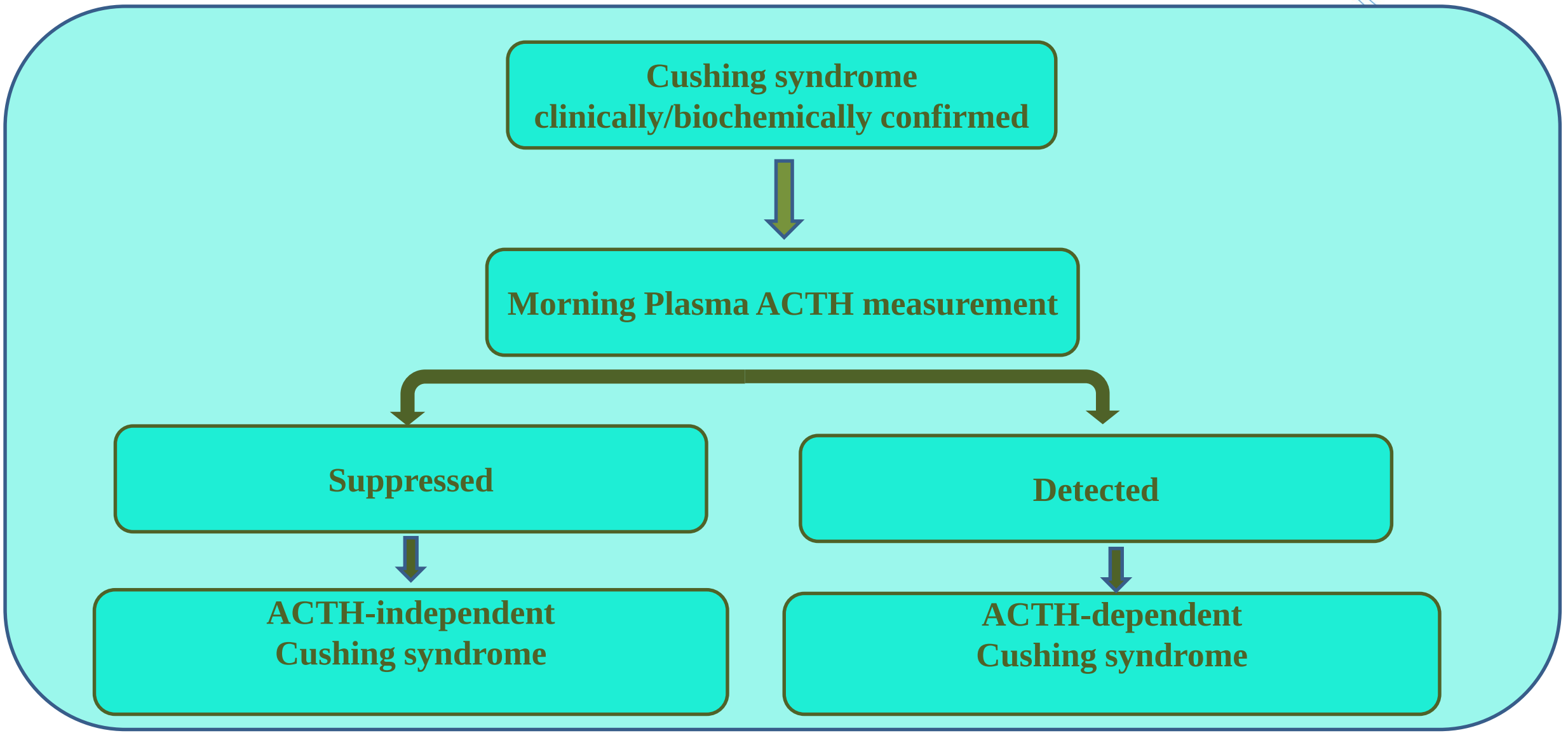
- ✓ Normal ranges: method-dependent, vary considerably
- ✓ Interpretation of results: considering the context of the appropriate normal range & cut off points recommended by the guidelines
- ✓ Assay methods:
  - ✓ antibody-based (RIA & ELISA) , affected by cross-reactivity with cortisol metabolites and synthetic glucocorticoids
  - ✓ structurally-based [HPLC and tandem mass spectrometry (LC-MS/MS)]
- ❖ *Upper limits of normal are much lower with HPLC or LC-MS/MS than in antibody-based assays.*
- ✓ Drug interference in the assay methods

Drug interference in test results

❖ ***Drugs that increase UFC results***

- ✓ Carbamazepine (increase if measured by HPLC)
- ✓ Fenofibrate (increase if measured by HPLC)
- ✓ Some synthetic glucocorticoids (immunoassays)
- ✓ Drugs that inhibit 11β-HSD2 (licorice, carbenoxolone)

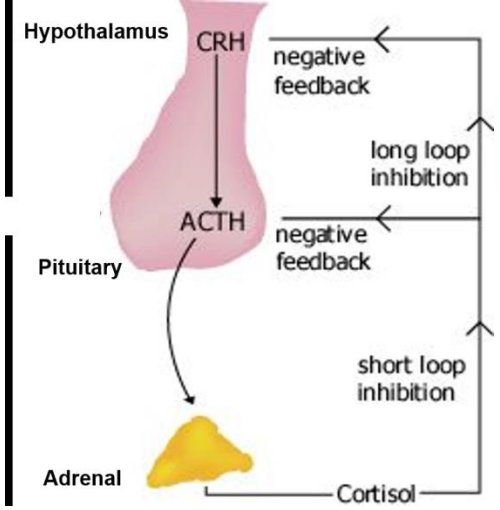
Determining the cause of Cushing Syndrome



Remarks for plasma ACTH

- ✓ Time of blood sampling: morning (08:00- 09:00)
- ✓ Assay method: two site sandwich immunoassay with *at least sensitivity of < 5 pg/mL*
- ✓ *At least 2 values* is recommended considering *episodic secretion*
- ✓ To avoid degradation of ACTH:
  - ✓ The blood sample should be taken in ice cold tubes
  - ✓ Plasma should be immediately separated (< 2 hours)
  - ✓ Plasma should be stored at −40°C
- ✓ Hemolysis & excessive tube EDTA can cause falsely low results

Remarks for plasma ACTH



- ✓ *Suppressed ACTH (< 10 pg/mL)* indicates an adrenal source of cortisol
- ✓ Cushing disease:
  - ✓ 50% of patients have a 9 am ACTH level within the normal reference range [9–52 pg/mL (2–11 pmol/L)]
  - ✓ in 50% of patients: modestly elevated ACTH level
  - ✓ Overlap values are seen in Cushing disease in 30% of cases
- ✓ *Ectopic ACTH syndrome*: *high ACTH level [usually (>90 pg/mL >20 pmol/L)]*
- ✓ *Adrenal tumors* (with clear clinical features): *ACTH is invariably undetectable* (<1 pmol/L)
- ✓ Macronodular adrenal hyperplasia: ACTH level is low-normal or intermittently detectable

Determining the cause of Cushing's Syndrome

ACTH-dependent Cushing's syndrome

↓

- ✓ **50% cortisol suppression following high- dose DST (2 mg 6 hourly for 48 hours)**
  AND
- ✓ **> 50% increased serum cortisol post CRH stimulation test**
  AND
- ✓ **Positive MRI scan of pituitary**

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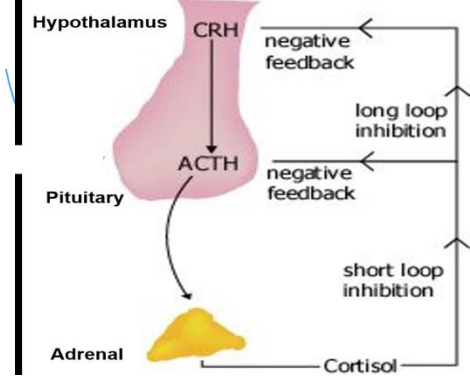
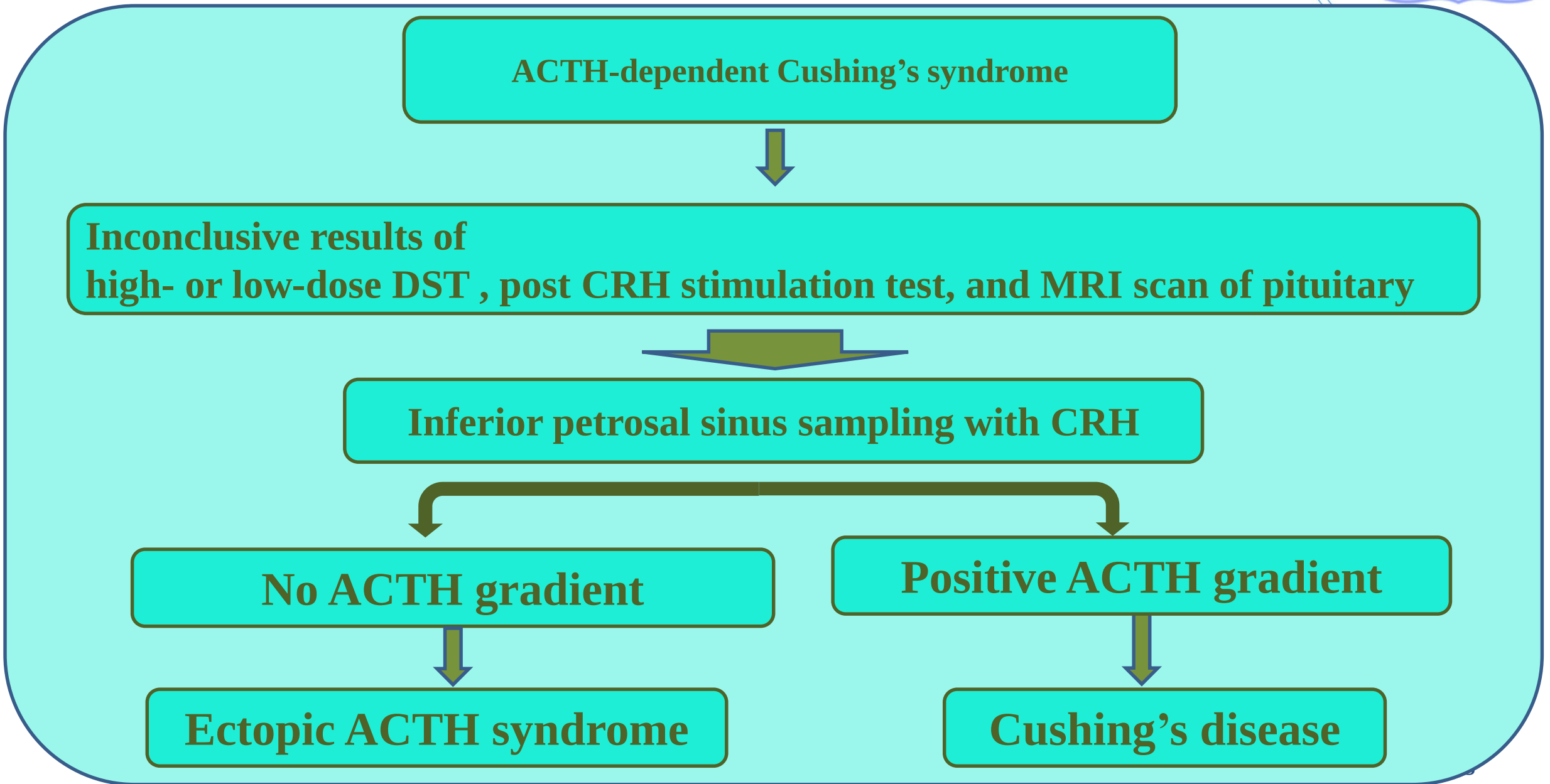
Cushing's disease

Remarks for high dose DST



- ✓ Administration of dexamethasone 2 mg every 6 hours for 48 hours
- ✓ Sampling: Blood or Urine for plasma cortisol or UFC (or both) at 0 and 48 hour
- ✓ Positive response: greater than 50% suppression
- ✓ The response is graded and is dependent on the original cortisol secretion rate, greater suppression in patients with lower basal cortisol values
- ✓ *Not added value, if there is greater than 50% fall in cortisol in low-dose DST*

Determining the cause of Cushing's Syndrome



In summary:

- ✓ Exclusion of exogenous glucocorticoid use
- ✓ Initial test: UFC, late night salivary cortisol 1 mg overnight DST
- ✓ Possible interfering conditions should be considered.
- ✓ Patients with an abnormal result are recommended to visit by an endocrinologist and undergo a second test, one of the above or, in some cases, a serum midnight cortisol.

Conclusions

- ✓ Patients with *concordant normal results* should not undergo further evaluation.
- ✓ Patients with *concordant abnormal results* should undergo testing for the cause of Cushing's syndrome (ACTH, high dose DST, …)
- ✓ Additional testing is recommended for patients with:
  - ✓ *discordant results*
  - ✓ *normal responses suspected of cyclic hypercortisolism*
  - ✓ *initially normal responses who accumulate additional features over time*
  - ✓ *whenever clinical impression & laboratory results are discordant*

Reference:

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Thank you for attention