

Biochemistry of Cushing Syndrome

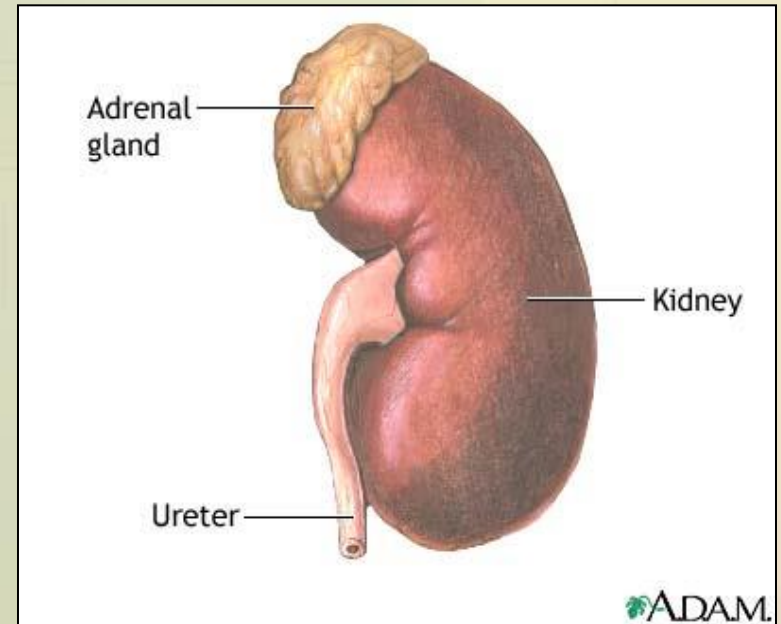
Endocrine Block

Objectives

- **To identify different causes of Cushing's syndrome**
- **To understand the diagnostic algorithm for Cushing's syndrome**
- **To understand the interpretation of laboratory and radiological tests of Cushing's syndrome**
- **To identify the importance of radiological investigations for diagnosis of Cushing's syndrome.**

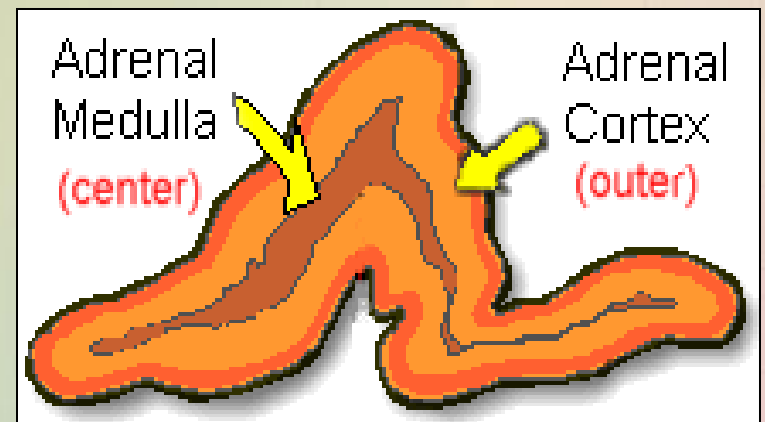
ANATOMICALLY:

- The adrenal gland is situated on the anteriosuperior aspect of the kidney



HISTOLOGICALLY:

- The adrenal gland consists of two distinct tissues of different embryological origin, the outer cortex and inner medulla.



The adrenal cortex comprises three zones based on cell type and function:

➤ Zona Glomerulosa

The outermost zone → **aldosterone** (the principal mineralocorticoid).

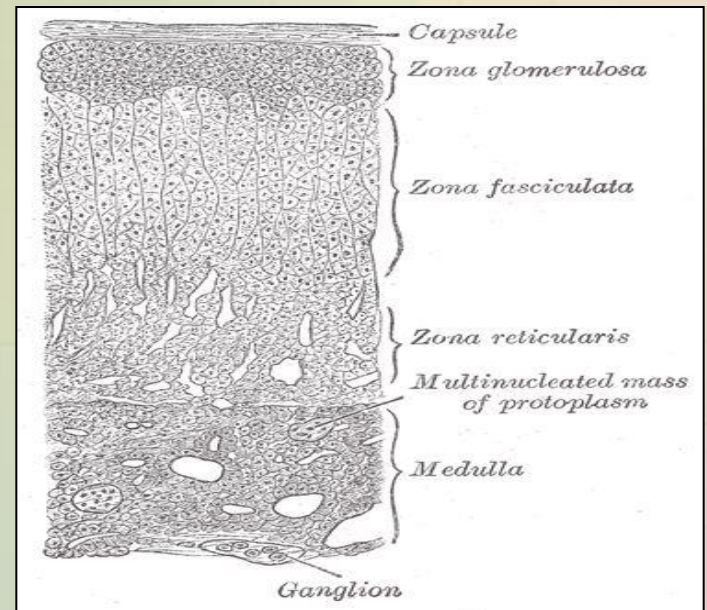
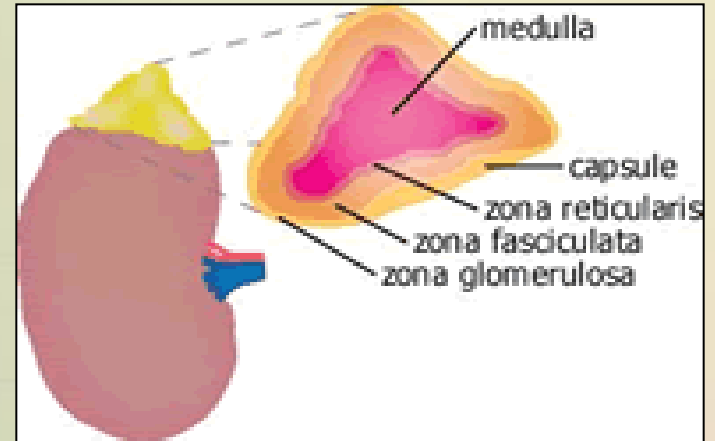
The deeper layers of the cortex:

➤ Zona Fasciculata

→ glucocorticoids – mainly **cortisol** (95%)

➤ Zona Reticularis

→ Sex hormones



Steroid Hormone Synthesis

Cholesterol (27C)



Pregnenolone (21C)



3- β -Hydroxysteroid dehydrogenase

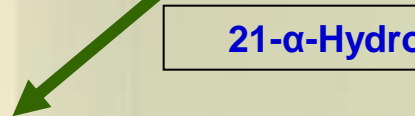
Progesterone (21C)



17- α -Hydroxylase

17- α -Hydroxyprogesterone (21C)

21- α -Hydroxylase



11-Deoxycorticosterone (21C)



Androstenedione (19C)

11-Deoxycortisol (21C)

11- β -Hydroxylase



Corticosterone



Aldosterone (21C)



Cortisol (21C)

Testosterone (19C)



Estradiol (18C)

Peripheral tissues

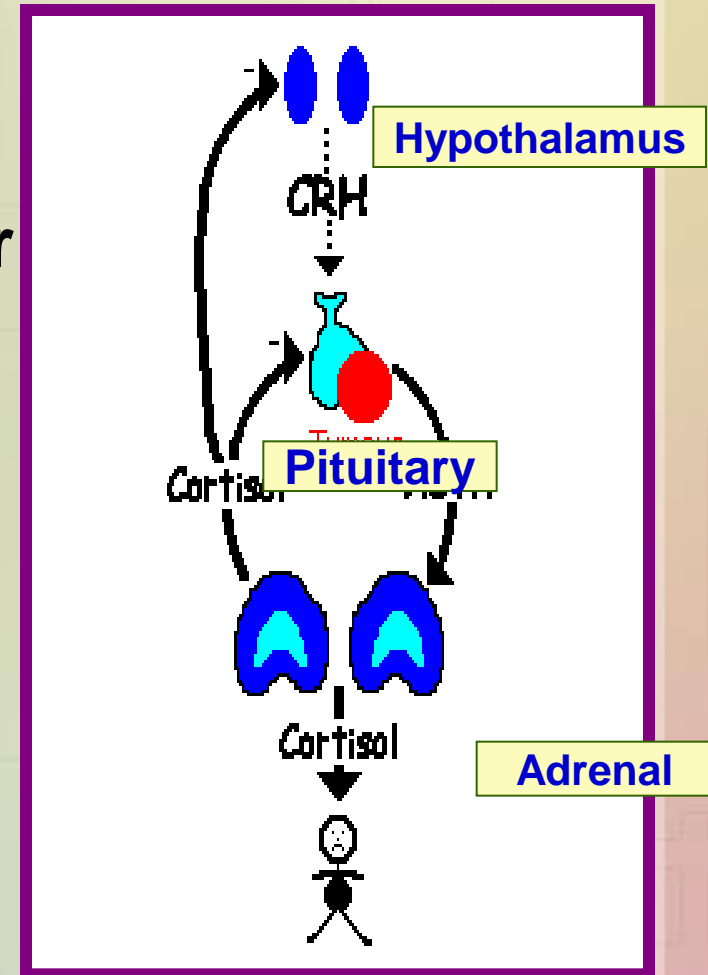
Hypothalamic-Pituitary-Adrenal (HPA) Axis

- The hypothalamus secretes **corticotropin-releasing hormone** (CRH) which **stimulates** the anterior pituitary gland to synthesis and release ACTH.
- **ACTH** acts on the zona fasciculata cells → release of glucocorticoids (**Cortisol**).

Regulation of ACTH and Cortisol Secretion:

1. Negative feedback control:

- ACTH release from the anterior pituitary is stimulated by hypothalamic secretion of corticotrophin releasing hormone (CRH).
- $CRH \rightarrow \uparrow ACTH \rightarrow \uparrow [Cortisol]$
- $\uparrow [Cortisol]$ or synthetic steroid suppress CRH & ACTH secretion



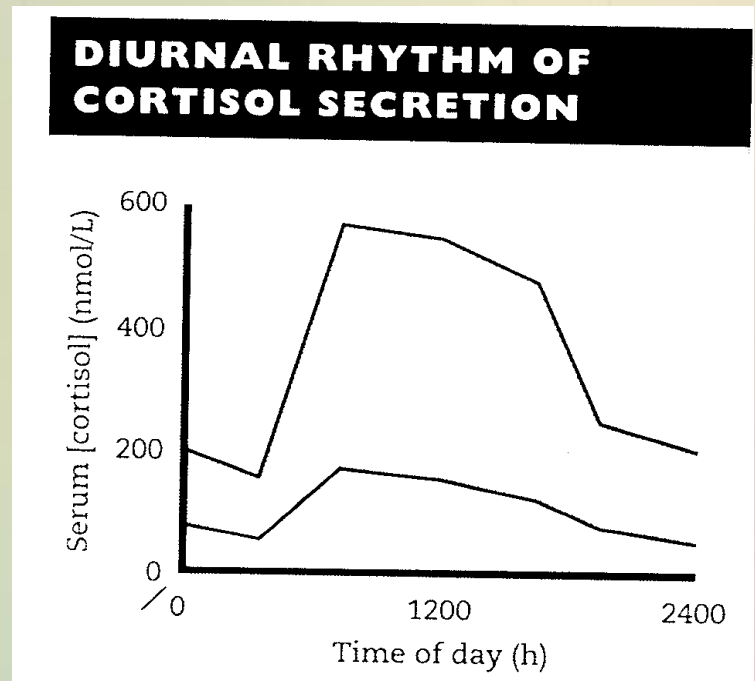
2. Stress (e.g. major surgery, emotional stress)

Stress → ↑↑ CRH & ACTH → ↑↑ Cortisol

3. The diurnal rhythm of plasma cortisol:

- Highest Cortisol level in the morning (8 - 9 AM).
- Lowest Cortisol level in the late afternoon and evening (8 - 9 PM).

The diurnal rhythm of cortisol secretion; the area between the curves represents values that lie within the reference range



Plasma [CBG] :

- In the circulation, glucocorticoids are mainly protein-bound (**about 90%**), chiefly to cortisol-binding globulin (CBG or transcortin).
 - **↑↑** in **pregnancy** and with estrogen treatment (e.g. oral contraceptives).
 - **↓↓** in **hypoproteinemic states** (e.g. nephrotic syndrome).
- The biologically active fraction of cortisol in plasma is **the free** (unbound) component.

Cortisol and ACTH measurements

Serum [cortisol] and plasma [ACTH]:

- Serum measurement is preferred for cortisol and Plasma for ACTH.
- Samples must be collected (**without venous stasis**) between 8 a.m. and 9 a.m. and between 10 p.m. and 12 p.m. because of the diurnal rhythm.
- Temporary ↑↑ in these hormones may be observed as a response to **emotional stress**.

Urinary cortisol excretion :

- Cortisol is removed from plasma by the liver → metabolically inactive compounds → excreted in urine mainly as conjugated metabolites (e.g. glucuronides).
- A small amount of cortisol is excreted unchanged in the urine (UFC).
- In normal individuals:
 - Urinary free cortisol (UFC) is < 250 nmol/24 h.
 - Cortisol / Creatinine ratio in an early morning specimen of urine is < 25 μmol cortisol / mol creatinine.

CAUSES OF ADRENOCORTICAL HYPERFUNCTION: CUSHING'S SYNDROME

- ACTH - dependent :

1. ↑ Pituitary ACTH **70%** (Cushing's disease).
2. Ectopic ACTH by neoplasms **10%**.

- ACTH - independent :

1. Adrenal tumor **20%** (adenoma or carcinoma)
2. Glucocorticoid therapy.

Causes of elevated serum cortisol concentrations:

1. Increased cortisol secretion:

- **Cushing's syndrome**
- **Exercise**
- **Stress, Anxiety, Depression**
- **Obesity**
- **Alcohol abuse**
- **Chronic renal failure**

2. Increased cortisol binding globulin (CBG):

- **Congenital**
- **Estrogen therapy**
- **Pregnancy**

Glucocorticoid functions

- Glucocorticoids have widespread metabolic effects on carbohydrate, fat and protein metabolism.
- Upon binding to its target, **CORTISOL** enhances metabolism in several ways:
 - **In the liver**, Cortisol is an insulin antagonist and has a weak mineralocorticoid action →
 - ↑↑ **Gluconeogenesis** → production of **glucose** from newly-released amino acids and **lipids**
 - ↑↑ **Amino acid uptake and degradation**
 - ↑↑ **Ketogenesis.**

- In the adipose tissue: Cortisol → ↑↑
Lipolysis through breakdown of fat .
- In the muscles: Cortisol → ↑↑ proteolysis
and amino acid release.
- Conserving glucose: by inhibiting uptake
into muscle and fat cells.

Cushing's Syndrome

Symptoms:

- **Weight gain:**
trunk and face with
sparing of the limbs
(**central obesity**)
- **Buffalo's hump.**
- **Moon face** →
- **Excessive sweating**



Symptoms (contd)

- **Atrophy of the skin and mucous membranes**
- **Purple striae on the trunk and legs**
- **Proximal muscle weakness (hips, shoulders)**
- **Hirsutism**
- **The excess cortisol may also affect other endocrine systems → ↓ libido, amenorrhoea and infertility**
- **Patients frequently suffer various psychological disturbances ranging from euphoria to frank psychosis.**

Signs:

- **Loss of diurnal rhythm of cortisol and ACTH.**
- **Hypertension (due to the aldosterone - like effects)**
- **Hyperglycemia or diabetes due to insulin resistance.**
- **Hypokalemic alkalosis**
- **↑ protein metabolism.**
- **Impaired immunity.**

Investigations of suspected adrenocortical hyperfunction

- A. Screening tests (out-patient):**
to assess the clinical diagnosis of adrenocortical hyperfunction.
- B. Confirmatory tests (in-patient):**
to confirm or exclude the provisional diagnosis
- C. Tests to determine the cause:** to ascertain:
 - (a) The site of the pathological lesion
(adrenal cortex, pituitary or elsewhere?)
 - (b) The nature of the pathological lesion.

? Cushing



Low DST/UFC

Screening



Pseudo-Cushing

True Cushing



Confirmatory

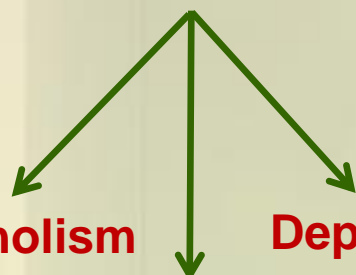
Insulin hypoglycemia



Normal response

No response

Cause



Alcoholism

Severe illness

Depression



ACTH/High DST



ACTH-dependent

Adrenal

CRH Test



Pituitary

Ectopic



MRI pituitary



CT chest



**ULS/CT
adrenals**

A. Screening tests:

Effective screening tests need to be sensitive but do not have to be highly specific.

It includes:

1. Low-dose dexamethasone (DXM) suppression test (DST):
(Overnight suppression test)

DXM → ↓ CRH → ↓ ACTH → ↓ cortisol

2. 24-hour urinary free cortisol

A. Screening tests:

1. Low-dose DST: (outpatient procedure)

Procedure:

1 mg DXM administered at 11-12 PM the night before attending the clinic.

serum cortisol is measured at 8-9 AM.

Result:

Cortisol < 50 nmol/L (suppression) → exclude hypercortisolnemia (Cushing Syndrome)

Precautions: Drugs that induce hepatic microsomal enzymes (**Phenobarbitone & phenytoin**) → ↑ DXM metabolism and ↓ DXM blood level to achieve CRH suppression (**false diagnosis of Cushing**)

A. Screening tests: Cont'D

2. 24- hour urinary free cortisol:

Result: Cortisol < 250 nmol/day → exclude Cushing Syndrome.

Disadvantage: incomplete collection of urine → a false-negative result

- **An alternative is to determine the urinary cortisol : creatinine ratio** on an early morning specimen

Interpretation of screening tests:

The screening tests serve to:

- distinguish simple non-endocrine obesity from obesity due to Cushing's syndrome.
- **Confirmatory tests (in-patient basis)** are required to rule out pseudo-Cushing's syndrome
- **Pseudo-Cushing's syndrome:**
 - Depressed or extremely anxious patients
 - Severe intercurrent illness
 - Alcoholism

B. Confirmatory tests: (Inpatient)

Insulin-induced hypoglycemia

Pseudo-Cushing patients show abnormal diurnal rhythm of S. cortisol, but, with Insulin-induced hypoglycemia → ↑ CRH, ACTH and cortisol blood levels

True Cushing patients:

No response to hypoglycemia

B. Confirmatory tests: ... Cont'd

Insulin-induced hypoglycemia:

- Hypoglycemia → ↑ CRH → ↑ ACTH → ↑ cortisol
- To test the integrity of the hypothalamic-pituitary-adrenal (HPA) axis.
- To distinguish true Cushing's syndrome from pseudo-Cushing's syndrome
- Contraindicated in: epilepsy or heart disease.

Insulin hypoglycemia test **Cont'd**

- **Procedure:**
- **Insulin I.V.** (0.15 U/kg) to lower blood glucose to 2.2 mmol/L or less .
- **Samples for simultaneous measurement of serum glucose and cortisol levels are taken basally (before insulin injection) and at 30, 45, 60 and 90 min after I.V. insulin injection.**
- **Failure to achieve a glucose level of 2.2 mmol/L invalidates the test and should be repeated with increment in step of 0.05U/kg.**

Insulin hypoglycemia test Cont'd

Interpretation of the results:

Normally:

- Basal serum cortisol: at least 145 nmol/L
- At 60 - 90 minutes: the level > 425 nmol/L

Patients with Cushing's syndrome:

- Whatever the cause, do not respond normally to insulin-induced hypoglycemia.
 - High basal serum cortisol than normal .
 - At 60 - 90 minutes: no increase in S. cortisol, despite the production of an adequate degree of hypoglycemia.

C. Tests used to determine the cause of Cushing's syndrome:

- 1. To differentiate ACTH-dependant from ACTH-independent: Plasma ACTH (Diurnal rhythm)**
- 2. To distinguish between ACTH-dependent causes (Pituitary Vs Lung):**
 - a) High-dose DST.**
 - b) CRH stimulation test**
- 3. Radiological tests: MRI of pituitary and ultrasound or CT of adrenals**

1. Plasma [ACTH]:

Plasma [ACTH] should be measured on blood specimens collected at 8-9 a.m. and 8-9 p.m.

Plasma ACTH

Undetectable



Functional adrenal tumor → confirmed by an abdominal CT scan to detect an adrenal mass

↑↑ ACTH



Cushing's disease (pituitary-dependent)

↑↑↑↑ ACTH



Ectopic (non-endocrine) origin of ACTH

2 (a). High-dose DST:

It is used to distinguish Cushing's disease from ectopic ACTH secretion.

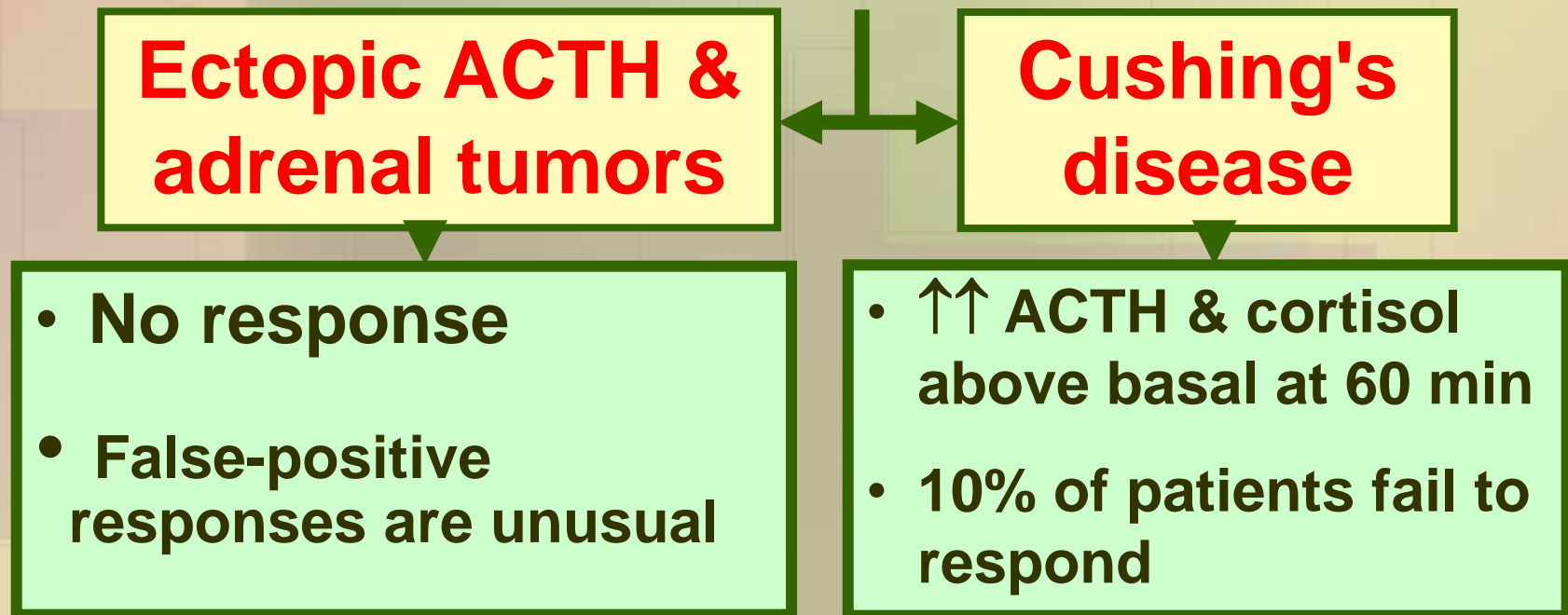
- **2 mg dexamethasone six-hourly for 48 hours to suppress cortisol secretion.**
- **Basal (pre-dexamethasone) serum cortisol or 24-hour urine free cortisol is compared with the results at the end of the 48-hour period.**

2 (a). High-dose DST *Continued*

- **Suppression** is defined as a fall to less than 50 % of basal value.
- About 90 % of patients with Cushing's disease show suppression of cortisol output.
- In contrast, only 10% of patients with ectopic ACTH production (or with adrenal tumors) show suppression.

2 (b). CRH stimulation test:

Measures the ACTH and cortisol levels basally and 60 minutes after injection of 100 µg CRH.



In Cushing's disease: High-dose dexamethasone suppression test + the CRH test → 100 % specificity and sensitivity.

3. Radiological Investigations:

- **CT scanning of the adrenal glands/
Lungs**
- **MRI of the pituitary gland**

Other blood tests commonly performed for patients suspected to have Cushing's syndrome are:

- **Full blood count**
- **Blood glucose**
- **Blood electrolytes and pH**
- **Renal function tests**
- **Liver function tests**

? Cushing



Low DST/UFC



Pseudo-Cushing

True Cushing



Insulin hypoglycemia



Normal response

No response



ACTH/High DST



ACTH-dependent

Adrenal

Alcoholism

Depression

Severe illness

CRH Test



Pituitary

Ectopic



MRI pituitary



CT chest



**ULS/CT
adrenals**

Screening

Confirmatory

Cause

Adrenal Hyperfunction

Summary of Biochemical Tests

Test	Cushing's disease	Adrenal tumor	Ectopic ACTH secreting tumor
S. cortisol	↑	↑	↑
Low dose DST	Not suppressed	Not suppressed	Not suppressed
Urinary cortisol	↑	↑	↑
Diurnal rhythm	Lost	Lost	Lost
Insulin-induced hypoglycemia	No response	No response	No response
Plasma [ACTH]	Normal or ↑	Not detectable	↑ ↑ ↑
High dose DST	suppressed	Not suppressed	Not suppressed
CRH test	↑	No response	No response

Case study

58 years old man was admitted with weight loss and respiratory distress. He had increased pigmentation and BP was 140/80.

Lab tests

Urea	8.6	(2.5-7 mmol/L)
Sodium	144	(135-145 mmol/L)
Potassium	2.0	(3.5-4.5 mmol/L)
Cortisol	1650	(150-550 nmol/L)
Post overnight DMX	1530	(<50nmol/L)

Further investigation revealed the following

DMX suppression test	Basal	after 48 h 0.5 mg qid	after 48h 2.0 mg qid	
Serum cortisol	1350 8 am	1420 22.00 pm	1100	No suppression
Plasma ACTH (ng/L)	220	180		Ref. range: 7-51

CRH showed flat response for cortisol and ACTH

Take Home Message

- ACTH-dependent Cushing: due to pituitary causes (Cushing's disease) and due to ectopic production of ACTH.
- ACTH-independent Cushing: due to adrenal adenoma or carcinoma and due to steroid therapy (iatrogenic).
- Initial screening for Cushing by 24 h urine free cortisol or low-dose dexamethasone suppression test
- Confirmatory tests for Cushing by diurnal rhythm of plasma cortisol and insulin-induced hypoglycemia
- Tests to determine the cause of Cushing: Plasma ACTH, high-dose dexamethasone suppression test, CRH stimulation test and radiological investigations

References

- Lecture notes, Clinical Biochemistry, Wiley BlackWell, 9th edition, 2013, chapter 9, page 116-133.
- Clinical Chemistry, Principles, Procedures, Correlations, Lippincott Williams & Wilkins, 7th edition, 2013, chapter 21, page 453-471.
- Lippincott's Illustrated Reviews: Biochemistry 6th edition, Unit III, Chapter 18, Pages 219-244.