Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Cortisol and Cushing's Syndrome

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Slide 2:
Cortisol is the body's primary glucocorticoid, and is synthesized in the zona fasciculata of the adrenal cortex. Like all adrenal corticosteroids, cortisol, the structure of which is shown in the upper right hand corner, is derived from enzymatic modifications of cholesterol.

Glucocorticoids primarily function to modulate metabolism by promoting formation and storage of glucose via stimulation of gluconeogenesis, glycogen formation, and lipolysis. Glucocorticoids also serve to suppress the immune system, and therefore are often used as therapeutic anti-inflammatory agents. Additionally, cortisol may be up-regulated in response to stresses such as acute trauma, pregnancy, alcoholism, obesity, and chronic illness.

Once in circulation, cortisol is mostly bound, primarily to cortisol-binding globulin, CBG (also known as transcortin or alpha 2-globulin), and, to a much lesser extent, albumin. Remember that since CBG and albumin concentrations can be affected by synthetic liver function, estrogens, certain drugs, and liver disease can all affect the amount of binding protein, though this has only a minimal effect on the relative amount of free cortisol. The free form of cortisol is the active form, and is also the form primarily filtered by the kidney for excretion. Since there is little extra unbound CBG available, elevations in cortisol will saturate CBG, thus producing an elevation in free cortisol. Because free cortisol is filtered into the urine (and can also diffuse into saliva), excess cortisol may be measured in urine or saliva.

Finally, cortisol is subject to marked diurnal variation: normally, cortisol is at its highest concentrations at 0800 hours, and falls by at least 50% by 2300 hours.

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Cortisol is regulated through feedback on the hypothalamic-pituitary-adrenal (HPA) axis. In the HPA system, various stimuli cause hypothalamic release of Corticotropin Releasing Hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the corticotropic cells of the anterior pituitary. ACTH, in turn, activates the adrenal zona fasiculata to make and release cortisol. Cortisol feeds-back on the hypothalamus and anterior pituitary to inhibit CRH and ACTH, respectively.

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Cortisol can be measured in serum, urine, and saliva. As a small molecule, cortisol and other corticosteroids can be easily identified by chromatographic techniques, such as gas or liquid chromatography coupled to a detector such as a tandem mass spectrometer. Such methods are generally very sensitive and specific and can be used to detect cortisol at very low concentrations.

More often, total cortisol is measured by immunoassay. Most major automated immunoassay platforms have reagents available to measure cortisol.

However, many cortisol immunoassays may cross-react with similar compounds, most commonly, prednisolone. Prednisolone is a metabolite of prednisone, so patients taking prednisone may have a falsely elevated cortisol when measured by affected immunoassays. If a patient needs a cortisol determination, and must be on an exogenous glucocorticoid, it is recommended to use another drug, such as dexamethasone, which does not cross-react.

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There are a few analytical considerations to bear in mind when measuring cortisol. Cortisol in serum, saliva, and urine is stable for up to a week when stored at 4 degrees Celsius and weeks to months when frozen. 24hr urine collections for cortisol are best collected with a preservative, most commonly 10 g boric acid.

Most assays for cortisol are not subject to interference from mild to moderate hemolysis, lipemia, and icterus.

The Institute for Reference Materials and Measurements and the International Federation of Clinical Chemistry have created a panel of cortisol reference samples against which assays may be standardized. Several, but not all, cortisol assays are standardized to these materials.

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Initially described in 1912 by Harvey Cushing, Cushing's syndrome is generally defined by excess glucocorticoids, primarily cortisol. Cushing's syndromes arise from a variety of states causing hyper-cortisolism. These include, but are not limited to: benign or malignant adrenal tumors, adrenal hyperplasia, pituitary adenoma, excess hypothalamic CRH release, and ectopic ACTH-secreting tumors, which are usually neuroendocrine in origin.

The term Cushing's disease specifically refers to hyper-cortisolism arising from a pituitary lesion, usually a pituitary adenoma, which releases excess ACTH, thereby causing elevated cortisol.

True Cushing's cases are fairly rare, with 2-3 cases occurring in every million persons (that's excluding cases arising from exogenous glucocorticoid use).

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Clinical signs and symptoms of Cushing's syndromes include:

- Body habitus changes such as weight gain, predominantly in the central or abdominal area, facial fullness (sometimes called moon facies), and increased size of the dorsocervical fat pad, or Buffalo Hump.
- Dermatological changes including thin skin, facial plethora, marked reddish-purple striae, easy bruising, acne, and poor wound healing
- Hormonal imbalance causing hirsutism, menstrual irregularities, and polycystic ovary syndrome
- Irregularities in glucose metabolism, such as glucose intolerance and type 2 diabetes.
- Fatigue is commonly seen, and weakness and/or wasting of proximal muscles is a strong indicator of Cushing's.
- Finally, psychiatric disturbances are often seen. These include depression, memory impairment, and decreases in concentration and libido.

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In 2008, the Endocrine Society published Clinical Practice Guidelines for the diagnosis of Cushing's syndrome. These guidelines are for the evaluation of individuals with clinical suspicion of Cushing's syndrome without exogenous glucocorticoid use. For the diagnosis of Cushing's syndrome, the guidelines recommend the use of two sequential first-line tests, which are the following: Urinary free cortisol, Dexamethasone suppression test, and/or a late-evening salivary cortisol.

Once Cushing's has been diagnosed, additional testing, both biochemical and imaging studies, can be performed to identify the source or cause of the elevated cortisol. All the tests shown here will now be discussed in further detail.

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Urinary free cortisol, or UFC, has been used for the diagnosis of Cushing's for over 40 years. Since the kidney filters free cortisol into the urine, a 24hr urine collection will reflect the total amount of free cortisol in circulation for a 24hr period. Thus, elevated UFC indicates Cushing's, or another state of stress, such as major illness or trauma, which physiologically elevates cortisol. The guidelines suggest that any UFC greater than the upper limit of normal be considered "Elevated".

Using the upper limit of normal as the cut-off, UFC may have false positives due to physiological hypercortisolism, as well as high fluid intake. Conversely, false-negatives may occur in patients with renal impairment, or in those with cyclic Cushing's syndrome. Therefore, it is recommended that there be at least two positive UFC determinations to proceed.

Finally, when collecting a 24hr urine for UFC, the sample should be collected with 10g boric acid to maintain acidic pH, and be kept refrigerated throughout the collection period.

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Since the diurnal variation of cortisol is often lost in Cushing's syndrome, measuring cortisol in the late evening, when it should be lowest, will readily demonstrate a loss of the cortisol nadir. Because unbound cortisol freely diffuses into saliva, salivary cortisol reflects the amount of free cortisol in circulation. Additionally, salivary cortisol is easily collected, and can be done by the patient at home, making late-evening collection convenient and feasible.

Studies have demonstrated that elevated salivary cortisol is highly sensitive and specific for Cushing's syndrome.

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In normal physiology, administration of an exogenous glucocorticoid, such as dexamethasone, will suppress adrenal cortisol secretion. However, in Cushing's patients, no matter what the cause, there should be a failure to suppress cortisol.

There are two dexamethasone suppression tests: low- and high-dose. The low-dose, or overnight suppression test, is the preferred screening test for Cushing's: In this test, 1 mg dexamethasone is administered at night, and serum cortisol is checked the following morning.

A normal response would be suppression of cortisol; a generally accepted cut-off is <5 ug/dL, although the cutoff may be lowered to 1.8 ug/dL to enhance sensitivity. Using a cut-off of 1.8 ug/dL, the low-dose DST demonstrates a high sensitivity of 95%, with a specificity of 80%.

Historically, some practitioners favor the high-dose dexamethasone suppression test, in which a total of 4 (or more) mg dexamethasone is administered over a 48hr period, with several serum cortisol measurements made. However, the Endocrine Society recommendations state that this test is no more useful for diagnosis of Cushing's than the low-dose DST.

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To summarize, criteria for the diagnosis of Cushing's Syndrome are duplicate 24hr urinary free cortisols (UFC) with results greater than the normal ranges of the assay, a low-dose dexamethasone suppression test without suppression, and/or two elevated late-night salivary cortisol determinations. If any of those three screening tests are abnormal, the patient should be re-evaluated to rule-out any physiological cause for elevated cortisol, such as pregnancy, obesity, or physical stress. In the absence of such a physiological explanation, additional diagnostic tests should be done or repeated. If these are repeatedly abnormal, a diagnosis of Cushing's syndrome can be made.

Once a diagnosis has been made, additional investigation may be done to identify the source of the defect.

With ever-improving imaging techniques, an MRI may be sufficient to identify the source of the defect in Cushing's syndrome—be it an adrenal, pituitary, or ectopic source. However, in the case of uninformative imaging results, additional laboratory evaluation can be beneficial.

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To identify the source of the defect, one may first check the concentration of ACTH: if ACTH is very low, or suppressed below the lower limit of normal, this suggests that the defect is adrenal in origin, and the high cortisol has inhibited release of ACTH from the pituitary. On the other hand, an elevated ACTH indicates that the defect lay in the pituitary or an ACTH-producing tumor.

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To further evaluate the source of defect in Cushing's, a CRH stimulation test may be employed, in which ovine or recombinant human CRH is infused, and ACTH and cortisol are measured at baseline, during, and after the infusion. In a normal response, ACTH and cortisol will rise and peak at 30 and 60 minutes, respectively. However, if the pituitary is the source of the defect, ACTH and cortisol, which are already elevated, respond with an insufficient elevation. Finally, if the defect is due to an ectopic ACTH-secreting tumor, there is no significant response of ACTH and cortisol to CRH.

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To further differentiate between a pituitary or ectopic tumor source of ACTH, a procedure known as bilateral inferior petrosal sinus sampling, or BIPSS, may be performed, which is the most direct manner by which to assess the status of the pituitary in relation to ACTH.

The right and left inferior petrosal venous sinuses directly drain blood from the left and right sides of the pituitary. Thus, ACTH release from each side of the pituitary can be evaluated independently, by inserting catheters via the jugular or femoral veins, directly into the right and left inferior petrosal sinuses. Blood samples can be obtained from each of the petrosal sinuses, as well as from a peripheral vein, and ACTH concentrations determined for each sample.

To overcome the pulsatile release of ACTH from the pituitary, CRH is administered to stimulate ACTH release. Interpretation is based upon comparing the ACTH values from central, or pituitary, to the peripheral samples, as well as the left-vs.-right inferior petrosal sinuses.

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Interpreting BIPSS results looks at the ratio between the central, or pituitary, results to the peripheral ACTH ratio. If the pituitary values are at least three-fold or higher than the peripheral ACTH, then the pituitary is the source of the ACTH. Conversely, if the ACTH is relatively higher in the peripheral samples, this is suggestive of an ectopic tumor source of ACTH, which should prompt an imaging-based search for the tumor.

If the pituitary is the source of the ACTH elevation, the pituitary tumor may be further localized to the left or right side of the pituitary, if one petrosal sinus has an ACTH concentration 1.4 times higher than the other. This information can be particularly helpful to supplement inconclusive imaging studies.

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To summarize:
Cortisol is the primary glucocorticoid in the body and acts to modulate metabolism and the immune system.

Cushing's syndrome is any disease state caused by elevations in cortisol, and presents with diverse signs and symptoms.

The Endocrine Society has published clear guidelines for the diagnosis of Cushing's syndrome.

Once a diagnosis of Cushing's has been established, additional laboratory and imaging tests may be performed to identify the source of the cortisol elevation.

Slide 18: References