Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Primary Aldosteronism: Screening and Confirmatory Testing

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Slide 1:
Hello, my name is Jessica Colón-Franco. I am an Assistant Professor at the Medical College of Wisconsin and Medical Director of Chemistry at Dynacare Laboratories in Milwaukee, Wisconsin. Welcome to this Pearl of Laboratory Medicine on "Primary Aldosteronism: Screening and Confirmatory Testing."

Slide 2:
First, I would like to review the structure and function of the adrenal glands. The adrenal glands are pyramidal in shape and lie right above each kidney. Each one is ~2-3 cm wide, ~4-6 cm long, 1 cm thick, and weighs ~4 grams. As shown in the diagram, each gland consists of an encapsulated cortex and an inner medulla. The first layer of the cortex is the zona glomerulosa, followed by the zonas fasciculata and reticularis, the innermost zone. As listed in the table, the adrenal cortex synthesizes steroid hormones, whereas the medulla produces catecholamines. The zonas fasciculata and reticularis synthesize glucocorticoids and androgens. Aldosterone, the most potent mineralocorticoid, is made exclusively in the zona glomerulosa. Mineralocorticoids are steroids that regulate salt homeostasis and extracellular fluid volume. Other adrenocortical steroids also have mineralocorticoid activity, but with lesser degree of potency.

Deregulation in the synthesis of these hormones results in a number of clinical conditions. Diseases associated with excessive cortical hormone production include hyperaldosteronism and Cushing syndrome. On the other hand, congenital adrenal hyperplasia and Addison diasease result in deficiency of adrenal hormones. Excessive production of catecholamines is a feature of pheochromocytomas.

This talk will focus on hyperaldosteronism, specifically, its primary form, and commonly referred to as primary aldosteronism (PA).

Slide 3:
Aldosterone regulates reabsorption of sodium and water at the kidneys by promoting the synthesis of sodium and potassium channels on cells of the distal convoluted tubule (DTC). The main function of the DTC is to reabsorb sodium, with passive reabsorption of water. In exchange, potassium is excreted. The increase in water volume raises blood pressure.

Unlike most adrenal hormones regulated heavily through the hypothalamic-pituitary-adrenal axis, secretion of aldosterone depends minimally on the action of corticotropin-releasing hormone (CRH) and adrenocorticotropin hormone (ACTH). The main pathway of aldosterone secretion is the renin-angiotensin-aldosterone system. Potassium also regulates aldosterone by causing membrane depolarization and activation of signaling pathways that lead to aldosterone synthesis.

Slide 4:

In this slide, the steps of the renin-angiotensin-aldosterone system are represented. The system starts with renin production by the juxtaglomerular epithelial cells of the renal glomeruli. The stimuli for renin release are: a decrease in perfusion pressure to the juxtaglomerular apparatus and decline in sodium concentration. In circulation, renin hydrolyses angiotensinogen to produce a decapeptide called angiotensin I. Angiotensin-converting enzyme (ACE) rapidly converts angiotensin I to an octapeptide known as angiotensin II. Angiotensin II raises blood pressure through its vasoconstrictor actions and by releasing antidiuretic hormone. It also stimulates the cells of the zona glomerulosa to produce the enzyme aldosterone synthase and, consequently, aldosterone. Aldosterone then promotes sodium retention and increases blood pressure. When the renin-angiotensin system is suppressed, ACTH has a more prominent role in regulating aldosterone.

Slide 5:

PA is a group of disorders with inappropriately high production of aldosterone, which is autonomous and non-suppressible by sodium loading. The term primary indicates the hypersecretory defect is at the adrenal glands, as opposed to secondary in which the hyperaldosteronism is of extra-adrenal origin. PA was originally described in 1955 by Conn as a condition characterized by an elevated plasma concentration of aldosterone along with hypokalemia and hypertension. Using this classification, the prevalence was estimated to be <1% among hypertensives. However, recent studies indicate a much higher prevalence among patients with hypertension. The main causes of PA are bilateral adrenal hyperplasia and aldosterone producing adenomas, accounting for 65-70% and 30-35% of all PA cases, respectively.

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Despite the original characterization of PA, only one-third of patients with PA are hypokalemic. In the setting of hypokalemia, urinary excretion of potassium may help distinguish renal from non-renal losses. For instance, daily excretion of potassium <30 mEq/day is appropriate with normal kidney function in attempt to reabsorb potassium. Urine excretion >30 mEq/day, suggests renal loss of potassium, which may occur in states of excess mineralocorticoid. Hypokalemia lacks specificity as it is also present in other aldosterone-related conditions and may be masked by pre-analytical interferences such as prolonged use of tourniquet or delayed separation from cells. It is now recognized that the majority of patients with PA are normokalemic at presentation. Therefore, the presence of hypokalemia should not be used as a requisite for pursuing screening tests for PA due to its low specificity, sensitivity, and positive predictive value for PA.

Slide 7:
In 2008, the Endocrine Society published clinical guidelines with a thorough algorithm for the diagnosis of PA. The guidelines recommend case detection in groups with high prevalence of PA. These groups include patients with moderate, severe, or drug-resistant hypertension, and patients with hypertension and one of the following: spontaneous or diuretic induced hypokalemia, adrenal incidentaloma, family history of early-onset hypertension, a cerebrovascular incident before 40 years of age, or first degree relative with PA. Detection of PA is important not only to target treatment, but also because patients with PA are more prone to cardiovascular events and end-organ damage than patients with essential hypertension. The absolute screening method recommended is the plasma aldosterone-renin ratio (ARR). Studies indicate superior clinical utility of the ARR compared to hypokalemia and aldosterone (low sensitivity) or renin (low specificity) in isolation.

Slide 8:
Various pre-analytical, analytical, and post-analytical considerations must be understood when interpreting the ARR.

First, let's discuss the pre-analytical aspects. The ARR results can be confounded by the intake of a variety of medications. The table lists general categories of some of these drugs, which can cause both false-positive and false-negative ARR results. Importantly, many of these are anti-hypertensive medications. The Endocrine Society guidelines for PA recommend withdrawing these drugs for weeks before the screening test and prescribing drugs with lesser effects on the ARR. This is not possible in most patients, and the risks may outweigh the benefits of a definite diagnosis. It is, therefore, important to understand how these drugs affect the ARR and interpret the results accordingly. Standardizing medications before testing may be necessary and it has been recommended by some groups, but more investigations are needed. Unfortunately, studies seldom describe the extent of the effects of the drugs on the ARR.

Other factors to consider are: age, gender, collection (time of day and posture), renal function, concentration of potassium, and the use of oral contraceptives. In elderly patients, renin levels fall and the ARR can be falsely increased. Aldosterone, similarly to ACTH, is subject to circadian variation with peak concentrations in the morning and the lowest at night. The samples for the ARR test should be preferably collected mid-morning from an upright position.

Finally, inadequate sample collection may lead to falsely decreased or elevated results, and the sampling and processing requirements may be method-dependent. For example, assays to measure direct renin concentrations are collected at room temperature, whereas renin activity assays require cold collection and processing and maintaining frozen until testing is performed. Prolonged cold storage promotes cryoactivation of prorenin to renin and falsely elevated renin activity. In summary, interpretation of the ARR involves good understanding of the pre-analytical requirements of the methodologies used, the patient history, and other pre-analytical variables that may confound the results.

Slide 9:

Until a few decades ago, GC-MS was considered the reference method but it was never widely adopted for routine analysis, mostly because sample preparation was laborious and complex. As an alternative for aldosterone measurement, most laboratories utilize in-house developed or commercially available radioimmunoassays (RIA). Needless to say, in-house developed assays are neither standardized nor harmonized among institutions and suffer from poor reproducibility. Recently, better performing non-isotopic automated immunoassays and tandem mass spectrometry methods for aldosterone have been developed and are becoming increasingly available. LC-MS/MS has been proposed as the reference method for aldosterone.

There are two categories of renin assays which measure plasma renin activity (PRA) or direct renin concentration (DRC). The PRA assay measures generation of angiotensin I from angiotensinogen, traditionally by RIA, and more recently by LC-MS/MS. The DRC measures the concentration of renin by immunoassay. DRC methods are not widely used yet and lack sensitivity at low concentrations. Indeed, correlation studies show good agreement between PRA and DRC but weaker at low renin concentrations.

Slide 10:

The method used to measure renin and aldosterone can impact the interpretation of the ARR. First, when interpreting the ARR, it is relevant to know which renin assay is used. There are ARR cut-offs specific for each type of renin assay. In the conventional unit system, the guidelines report most groups use ARR cut-offs between 20-40 for PRA and cut-offs 3.8-7.7 for direct methods. It is also of importance to use the correct units (conventional or SI system) and adequate conversion factors for each assay.

As new methodologies emerge for the measurement of renin and aldosterone, the applicability of ARR cut-offs for PA determined using “conventional methods” must be evaluated. Only a few studies to date have investigated this question. Likely, each institution will have to evaluate the literature and/or validate appropriate cut-offs for PA case detection for their methods.

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A limitation of only using an absolute ARR cut-off by itself manifests in cases of very low renin activity/concentration. In such cases, the ARR is elevated even when aldosterone is also low and not consistent with PA. For example, a patient with a PRA of 0.2 ng/dL and a low (normal) aldosterone concentration of 5 ng/dL has an ARR of 25. The low aldosterone suggests this is a false positive and the diagnosis of PA is unlikely. Undetectable PRA is often suggestive of “low renin essential hypertension.” Screening criteria including a concentration of aldosterone >15 ng/dL may overcome this limitation of the ARR. This additional criterion may exclude some cases, particularly of APA. Therefore, some groups will do further evaluations if the ARR is elevated regardless of the aldosterone concentration, others if the ARR is elevated and aldosterone is >10 ng/dL, and so on. Moreover, providing a definite aldosterone cut-off is difficult due to the high variability among laboratories. Before PA is completely ruled out due to a low aldosterone, it may be appropriate to review the patient’s medications and other pre-analytical factors that may lower aldosterone. In summary, opinions remain divided about the appropriate cut-off to use and whether including an elevated concentration of aldosterone in the criteria for a positive

screen outweighs the risk of missing APA cases. Alternatively, awareness of the rate of false positivity when renin is suppressed may be helpful for medical decisions in groups that avoid the use of an aldosterone cut-off in conjunction with the ARR.

Since this is a screening assay, the interpretative language itself should indicate that the results are not conclusive. The results of the ARR may be unlikely, borderline, or likely for PA. Confirmatory testing is highly recommended as follow up for borderline and likely results.

Slide 12:

As mentioned, an increased ARR is not by itself diagnostic of PA. Confirmatory testing may reduce the risk of patients with false positive results undergoing unnecessary invasive and costly procedures. Four tests are recognized by the Endocrine Society: oral sodium loading, saline infusion, fludrocortisone suppression, and captopril challenge. The underlying principle of these tests is to investigate if the aldosterone concentration is autonomous; in other words, if it is inappropriately elevated after increasing intravascular volume and it is non-suppressible. These scenarios are consistent with a diagnosis of PA. Although the fludrocortisone test may be considered to be the current gold standard, none of the four tests is clearly recommended over the other. All tests have appreciably advantages and disadvantages. The fludrocortisone suppression test is considered highly reliable but it is not widely used because it is complex, presents high risk of developing severe hypokalemia and hypertension, and requires staying at the hospital for several days. In Europe, the preferred test is the sodium infusion test. It is regarded as simple, reasonably accurate, inexpensive, and safe. The preferred confirmatory test in the US is the oral sodium loading test. The Endocrine Society guidelines list evidence-based cut-offs for interpreting each of these tests, but different institutions may choose to adopt their own. Ultimately, co-morbidities and possible risks for a given individual represent important considerations to select the appropriate test to use.

Slide 13:

Once a diagnosis of PA is indicated by confirmatory tests, patients may benefit from subtype testing to understand the type of lesion, distinguish unilateral versus bilateral disease, and to guide treatment and management decisions. Initially, patients usually undergo adrenal imaging by CT scan. This is helpful in the identification of larger lesions, which may be carcinomas. However, this technique has low sensitivity and specificity and cannot differentiate BAH (bilateral adrenal hyperplasia) from APA (aldosterone producing adenomas). Most APAs are very small (microAPAs) and may go undetected by CT scan. If surgery to remove nodules is practicable and desired, the next step in subtype differentiation is adrenal vein sampling (AVS). AVS, an invasive procedure performed by an interventional radiologist, is the gold standard to differentiate unilateral from bilateral disease.

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During AVS, blood is obtained from both adrenal veins (AV) and the inferior vena cava, or peripheral samples. It is preferred to perform under continuous consyntropin (synthetic ACTH) infusion and to do sequential sampling. Intraoperative (IO) cortisol measurement enhances the accuracy of AV catheter placing and the success rate of the procedure. Elevated IO cortisol concentration in the AV samples

compared to the peripheral blood indicates successful cannulation. The AVS procedure followed (stimulated or unstimulated), the criteria for catheterization or the cut-offs for lateralizationare often study- and/or institution-dependent.

Usually, aldosterone is normalized to cortisol for both sides (left and right). This may correct possible dilutional effects if sampling was sub-optimal. Then, lateralization is assessed by calculating a side-to-side ratio of the normalized aldosterone. With continuous consytropin administration, the consensus cut-offs for lateralization are: >4 for unilateral excess (or an APA), <3 for bilateral excess (or BAH), and ratios >3 and <4 represent a grey zone, requiring additional tests and information to differentiate. Some groups rely on the concentration of aldosterone in the AV relative to the periphery. For example, a case with higher aldosterone concentrations in both AV than in the peripheral vein is interpreted as bilateral disease.

Patients with unilateral PA are candidates for unilateral laparoscopy. If the patient is unwilling or if clinically contraindicated, the alternative treatment is mineralocorticoid receptor (MR) antagonists such as spironolactone or eplerenone.

Slide 15:
This testing algorithm for PA summarizes the recommendations from the Endocrine Society. Severely hypertensive patients and those at high risk for PA should be screened for PA (even when normokalemic). The screening test of choice is the ARR. If the ARR result suggests that PA is likely, confirmatory testing using one of the four available tests should follow. The first step of subtype classification is imaging by CT scan, followed by AVS, particularly if surgery is desired. Finally, a diagnosis of unilateral or bilateral disease is important for proper case management and treatment.

Slide 16:
It is now recognized that PA is more prevalent than previously recognized. If identified, PA is a treatable and potentially curable form of hypertension. Practice guidelines for PA published in the last decade provide physicians and laboratorians specific information about screening and confirmatory testing for PA. Proper patient preparation, sample collection, and test interpretation are key to overcome the known limitations of the ARR. It is of great importance to interpret the ARR correctly to avoid confounding factors from masking PA in affected patients and also from falsely indicating a diagnosis of PA. This clarification may reduce unnecessary and costly follow-up testing associated with false positive ARR results.

Slide 17: References
I recommend consulting the references on this slide to obtain more information about detection, diagnosis, and treatment of PA.

Slide 18: Disclosures

Slide 19: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "Primary Aldosteronism: Screening and Confirmatory Testing." I am Jessica M. Colón-Franco.