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Q&A

# A *Renin*-ssance in Primary Aldosteronism Testing: Obstacles and Opportunities for Screening, Diagnosis, and Management

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Primary aldosteronism (PA)[9] is a group of adrenal disorders characterized by autonomous production of aldosterone independent of angiotensin II (AngII) stimulation. Idiopathic adrenal hyperplasia is the most common cause followed by aldosterone-producing adenomas (APA or Conn syndrome), unilateral adrenal hyperplasia, adrenal carcinoma, and rare familial forms. Aldosterone excess results in sodium and water retention and potassium excretion, leading to volume expansion, concomitant hypertension, and variable degrees of hypokalemia. As the most common form of secondary hypertension, PA is recognized as an important public health concern. The diagnosis is infrequently considered despite widely available screening procedures. In addition to the hypertension, the negative effects of excess aldosterone are thought to be related to inflammation and fibrosis of various target organs. As a result, patients with PA are at increased risk of cardiovascular and chronic kidney disease compared with age-matched and blood pressure–matched patients with essential hypertension. For these reasons early identification and treatment are necessary to prevent morbidity and mortality associated with this curable form of chronic hypertension.

The diagnosis of PA relies on biochemical evidence of relative aldosterone excess and confirmation of abnormal aldosterone production using suppression testing. The identification of the PA subtype is primarily accomplished with adrenal venous sampling and imaging studies. The aldosterone-to-renin ratio (ARR) is generally considered the best first-line screening test for hypertensive patients in whom there is clinical suspicion of PA. Excessive and autonomous aldosterone secretion is characteristically accompanied by low or undetectable renin due to feedback inhibition from sodium excess and increased blood pressure. However, no consensus exists for an internationally recognized ARR cutoff and many challenges, from preanalytical to analytical to postanalytical, have hindered the development of definitive guidance on how to interpret the ARR in clinical practice.

In this Q&A, 4 experts from around the world discuss the state-of-the-art in screening and diagnosis of PA and the many challenges associated with laboratory testing.

***Estimates of prevalence suggest that PA is more common than once thought. What is known about the current prevalence, and why do you think historical estimates were misleading? Are certain populations at increased risk?***

**Daniel Holmes:** The largest studies have consistently determined the prevalence to lie between 5% and 15% in hypertensive populations. Historical estimates were misleading because it was assumed that hypokalemia was a sine qua non of the diagnosis. However, about half of patients with APA, and only 17% of those with idiopathic adrenal hyperplasia, are hypokalemic. The other contributing factor may be that many patients with PA do not have increases of aldosterone above the reference interval. Rather, they have increases relative to AngII stimulation,

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[9] Nonstandard abbreviations: PA, primary aldosteronism; AngII, angiotensin II; APA, aldosterone producing adenoma; ARR, aldosterone-to-renin ratio; PRA, plasma renin activity; DRC, direct renin concentration assay; SST, saline infusion suppression test; FST, fludrocortisone suppression test; ACTH, adrenocorticotropic hormone.

the surrogate measure of which is plasma renin activity (PRA). The Endocrine Society Guideline, in the US, recommends a targeted screening approach in subpopulations with hypertension having clinical features suggestive of PA, which are: 1) those with moderate to severe or resistant hypertension; 2) hypertension with spontaneous or diuretic-induced hypokalemia; 3) hypertension presenting with an adrenal incidentaloma; and 4) hypertension in the context of a family history of hypertension or early cerebrovascular accident. There is also evidence that PA is more likely to be found among those with obesity, diabetes, and obstructive sleep apnea.

**Michael Stowasser:** Throughout the 1970s to early 1990s, PA was considered a rare cause of hypertension and unworthy of consideration unless patients were hypokalemic. The two main factors that led to the demonstration that PA was much more common were: 1) the introduction of the ARR as a screening test, which proved to be more sensitive than aldosterone assessed separately with renin, and 2) the application of the ARR to a wider population to include normokalemic patients and those with milder forms of hypertension. Estimates of prevalence among all-comer hypertensive patients in most recent studies have been 5%–13%. Although PA is undoubtedly more common among hypokalemic hypertensive patients, this group represents only the "tip of the iceberg" (approximately 20% of patients), and only selecting this population for screening will miss most of the other patients with this condition. Other populations that appear to be at increased risk include hypertensive patients with obstructive sleep apnea, diabetic patients, and those with incidentally discovered adrenal lesions. A strong argument can also be made for early screening in all hypertensive patients. This claim is based on the fact that 1) PA is highly prevalent among hypertensive patients, 2) the ARR test is reasonably inexpensive, 3) surgical outcomes are better than nonspecific antihypertensive treatment, 4) early detection improves those outcomes, and 5) the ARR is most reliable if measured before patients go on antihypertensives.

**Richard Auchus:** This is a trick question, because it depends on how PA is defined, particularly when not caused by an adrenal tumor. If strict criteria are used, such as a 24-h urine aldosterone >14 μg/day (>38.8 nmol/day) during the salt-loading confirmatory test or serum aldosterone >10 ng/dL (>277 pmol/L, using older RIA assays) after the saline infusion test, then the prevalence among hypertensive patients is approximately 5%–8%. The prevalence is up to 20% among those with resistant hypertension. Development of PA is a gradual process, and it is quite likely that lower amounts of autonomous aldosterone production can contribute to low-renin hypertension in some healthy individuals, depending on their genetic makeup and the sodium content of their diet.

***The ARR is established as the best front-line test to screen for PA. What makes the ARR superior to testing either serum aldosterone or renin separately?***

**Daniel Holmes:** When we think about endocrine disorders caused by autonomous hormone production, we often think of the end hormone concentration as being high-normal or overtly high while the stimulating hormone is low or undetectable. Graves disease is a good example of this: thyroxine is often overtly high and thyroid-stimulating hormone is low or undetectable. In the case of PA, this is often not the case. There are 2 reasons for this. First, the potassium wasting that frequently occurs with PA causes hypokalemia or low-normal potassium concentrations, which decreases aldosterone production. Second, patients are often receiving antihypertensives that lower aldosterone—specifically, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β blockers, and dihydropyridine calcium channel blockers. For this reason, aldosterone in isolation is not a very useful tool, since plasma concentrations are often well within the normal range and sometimes as low as 9 ng/dL (250 pmol/L) even in the absence of hypokalemia and antihypertensive use. Renin alone is not specific because it would fail to distinguish PA from other forms of low-renin hypertension such as severe Cushing syndrome, Liddle syndrome, and the hypertensive forms of congenital adrenal hyperplasia.

**Michael Stowasser:** Isolated measurement of plasma aldosterone lacks sensitivity for PA, since many patients exhibit concentrations that are within the wide normal range, even those with unilateral APA. Such "normal" concentrations could be viewed as "inappropriately normal" in the face of suppression of the renin-angiotensin-aldosterone system. While highly sensitive for PA, suppressed renin concentrations lack specificity. Suppressed renin can occur in treatment with β-blocking agents, clonidine, α-methyldopa, or nonsteroidal antiinflammatory agents, or with consumption of a high-salt diet, advancing age, chronic renal impairment, and a large list of other salt-dependent and low-renin forms of hypertension. In contrast to PA, aldosterone concentrations in

low-renin hypertension are chronically suppressed as a result of chronic suppression of renin/AngII.

**Richard Auchus:** Renin is critical for screening because the diagnosis can only be made when renin is below the normal range or undetectable. When renin is normal, aldosterone production can be considered physiologic. If renin is low, then any excess aldosterone is abnormal; the higher the serum aldosterone, the more likely the patient has PA. In fact, I never calculate an absolute ARR. I always interpret the 2 tests separately, especially in the context of serum potassium. This is because hypokalemia suppresses aldosterone production and often leads to falsely low serum aldosterone and, in turn, false-negative screens.

***There is considerable variability in cutoff values for the ARR. What has contributed to such variability? How could serum aldosterone thresholds be used to help improve interpretation of the ARR?***

**Etienne Cavalier:** This variability is the consequence of a lack of standardization between the analytical methods and between clinical laboratories. The ratio combines 2 analytical errors, which could confound the interpretation of the ARR. Furthermore, a cutoff value relies on the specific methods that were used to establish it, and, unfortunately, cutoff values are often derived without consideration of the effect of analytical methodologies and standardization. Until recently, aldosterone measurement was achieved via cumbersome RIA methods. Since then, automated immunoassays and LC-MS/MS methods have become available. While LC-MS/MS is undoubtedly more specific than immunoassay, a higher-order reference method as well as an International Standard Reference Material for aldosterone is still needed.

**Michael Stowasser:** Disparity between laboratory approaches is a major issue, with for example, some groups retaining RIAs for determining PRA, while many others are moving to LC-MS/MS or other automated immunometric methods of measuring direct renin concentration (DRC) using mass assays. The reporting of both aldosterone and renin in different units between laboratories has added to the complexity. Hopefully, this problem will diminish with time as more laboratories adopt the Système Internationale method of reporting aldosterone concentrations (as pmol/L), follow more uniform approaches to screening that are now widely circulated in published reviews and guidelines, and change to more reliable assay methodology. It has been shown that in the presence of extremely low renin concentrations (i.e., PRA $\leq$0.1 ng/mL · h or DRC $\leq$2 mU/L), the ARR may be increased even when plasma aldosterone is also very low (i.e., 110 pmol/L or 4 ng/dL) and clearly not consistent with PA. Some investigators have therefore suggested the inclusion of a minimum plasma aldosterone concentration of 15 ng/dL (415 pmol/L) within the screening criteria, but this approach would miss many cases because plasma aldosterone concentrations often fall below this cutoff. For this reason, our approach is to proceed with diagnostic workup for PA in all patients with increased ARR, except in those whose plasma aldosterone concentration is below the level used to define normal suppression during confirmatory fludrocortisone suppression testing (6 ng/dL or 165 pmol/L). In those patients, we will periodically repeat the ARR and consider further diagnostic workup.

**Daniel Holmes:** Published ARR screening thresholds vary up to 10-fold. While the variability in the ARR is attributable in part to cohort selection, screening protocols, confirmatory testing, and disease definition, the most underappreciated problem in clinical studies is an analytical one—both for aldosterone and renin. Considering renin in isolation, PRA results are affected by assay design (incubation time or buffering pH) and the analytical components (choice of antibody, analytical methodology, and calibrator). DRC assays have challenges, particularly in the low-renin state, due to both poor precision and correlation with the more established PRA. Cross-reactivity of DRC with catalytically active conformations of prorenin is also a concern because the concentration of prorenin, which is normally 10-fold higher than renin, goes up to 100-fold higher in low-renin states such as PA. Regarding aldosterone cutoff values, about one third of PA cases may have ambulatory aldosterone concentrations $<$15 ng/dL (415 pmol/L). Therefore, it is prudent to avoid definitive statements about the exact concentration below which PA will never be observed and to always interpret a result with clinical presentation in mind.

**Richard Auchus:** That is why I never calculate an ARR! At an ARR of 20 ng/dL:ng/mL · h (550 pmol/L:ng/mL · h using PRA) the sensitivity and specificity are both approximately 80%. As the cutoff value is set higher, the

specificity improves little, but the sensitivity plummets. My approach is to interpret renin and aldosterone separately. If renin is low (<1 ng/mL · h) and aldosterone relatively high [≥10 ng/dL (277 pmol/L)], I proceed to confirmatory testing. In fact, I will do confirmatory testing if aldosterone is 5–10 ng/dL (139–277 pmol/L) and my index of suspicion is high, such as in a young patient with hypokalemia. It is important to realize that these cutoff values are set for patients on no medications. When a patient is taking vasodilators and diuretics, a nonsuppressed PRA (i.e., 2–3 ng/mL · h) with an aldosterone >20 ng/dL (>550 pmol/L) is highly suspicious and warrants rescreening after withdrawing them.

***There are many preanalytical and analytical challenges to the measurement of aldosterone and renin. Briefly discuss which interferences you think are most important to consider, and what approaches should be used to minimize inaccurate results.***

**Michael Stowasser:** The major controllable confounders are posture, time of day, dietary sodium intake, plasma potassium, and medications, which all impact the renin angiotensin aldosterone system. Medications causing false positives include β-blockers, clonidine, methyldopa, and nonsteroidal antiinflammatory drugs. False negatives can occur with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, and dihydropyridine calcium blockers. Selective serotonin reuptake inhibitors lower the ARR, with the potential for false negatives. Renin inhibitors lower PRA but raise DRC, and so their effect on the ARR is method dependent. ARR values are higher in premenopausal women than in age-matched males, especially during the luteal phase of the menstrual cycle when false positives can occur if renin is measured by DRC (but not PRA). Estrogen-containing oral contraceptive agents may cause false-positive ARRs with DRC assays (rather than PRA). False positives can also occur in patients of advanced age and in patients with chronic kidney disease. To minimize inaccurate results, patients should be encouraged to liberalize sodium intake. Medications that significantly affect the ARR should be withdrawn at least 4 weeks for both potassium-sparing and potassium-wasting diuretics, and at least 2 weeks for other potentially interfering drugs. Where necessary to maintain hypertension control, other antihypertensive medications that have less significant effects on the ARR (α blockers or nondihydropyridine calcium channel blocker) are recommended. Blood collection timing is important and should be undertaken midmorning after the patient has been ambulant for at least 2 h and seated for 5–15 min.

**Daniel Holmes:** Aldosterone results produced by different immunoassays can vary 2-fold on the same samples. The origins of this bias are multifactorial. First, there is no international standardized reference material for aldosterone, meaning that all assays, whether commercial or lab developed, rely on gravimetric assignment. Second, though homogenous RIAs and automated homogenous chemiluminescent immunoassays have greatly simplified the analysis, they have introduced a problem of metabolite cross-reactivity previously mitigated by extraction steps. We have observed this to be particularly marked in the context of chronic renal impairment, where I would recommend that homogenous immunoassays for aldosterone not be used at all in favor of more specific LC-MS/MS methods. Furthermore, both PRA and DRC assays are vulnerable to spurious increases from prorenin cryoactivation. About 2% of prorenin is catalytically active but this fraction can be increased by the spontaneous and reversible unfolding of the prosegment, which exposes the active site and causes consumption of endogenous angiotensinogen leading to overestimation of renin activity and concentration. The unfolding of the prosegment is facilitated by refrigeration but can also occur at room temperature. We recommend rapid separation of EDTA plasma from cells, immediate freezing and storage at −20 °C, and rapid thawing at the time of analysis.

***PRA and DRC (mass assays) are both widely used methods for renin determination. In your opinion, what factors should laboratories consider when deciding which method to implement? Are there situations where one renin method would be better suited than the other?***

**Daniel Holmes:** Unfortunately, the decision to implement PRA vs DRC is often not a matter of choice for laboratorians and the endocrinologists/internists they serve. My experience is that the availability of suitable instrumentation has more bearing than do the preferences of interested parties. That being said, if there is a choice, PRA offers a long-established body of supporting literature, better low-end analytical sensitivity with longer incubation times, and the option to use LC-MS/MS requiring no proprietary reagents. However, PRA is more time-consuming and technically challenging.

**Michael Stowasser:** Concerns have been raised about the faster, more convenient methods of DRC using immunometric techniques and automated machinery, which have widely been adopted in large, busy laboratories in recent years. These include unreliability and poor reproducibility, particular at the low end of the reference range, which is particularly relevant to PA. Unlike PRA, DRC assays do not take into account circulating concentrations of endogenous substrate and are therefore affected by factors such as endogenous or exogenously administered estrogen. This has been demonstrated to

cause false-positive ARRs among women in the luteal phase of the menstrual cycle and in those receiving estrogen-containing oral contraceptives. For the PRA assay, analytical sensitivity can be improved by increasing the duration of the incubation step during which angiotensin I is generated (and directly measured) from cleavage of endogenous angiotensinogen by renin.

**Etienne Cavalier:** DRC and PRA assays provide different, but complementary, clinical information. Unspecialized laboratories should use immunoassays because, on the one hand, screening for PA must be available everywhere and, on the other hand, using immunoassays will help reduce lab-to-laboratory variability and improve turnaround times. PRA should be regarded as the reference method (preferably by LC-MS/MS) for renin measurement.

**Richard Auchus:** PRA is the gold standard method, but DRC has several advantages, primarily speed and automation. In the US, the DRC assay is not widely available. One challenge of the DRC method is that it is unclear where to set the cutoff for a "low" renin value. It is also well known that DRC is more vulnerable to false increases from estrogen therapy and renin inhibitor medications such as aliskerin.

***In the US, the Endocrine Society guidelines recommend that a positive ARR test should be followed up with 1 of 4 confirmatory tests: oral sodium loading, saline infusion (SST), fludrocortisone suppression (FST), or the captopril test. Which one is superior and what factors dictate the choice of one over the others? What tests are used to determine candidates for surgery?***

**Daniel Holmes:** The gold standard for the diagnosis of PA is the reversal of symptoms after therapeutic intervention: surgery for APA or administrate of mineralocorticoid receptor antagonist for bilateral IHA. For this reason "confirmatory" testing is not diagnostic in the strictest sense. Each test has advantages and disadvantages and it would be difficult to definitively assign one as best in all circumstances. Ultimately, it comes down to issues of safety and convenience vs test performance characteristics and available literature support. Our largest body of experience is with the SST and we have become comfortable with its strengths and weaknesses in the context of LC-MS/MS aldosterone analysis. But, the simplest test is the one you do not need to do at all, so it is worth mentioning that if the clinical presentation and screening biochemistry is pathognomonic of PA (e.g., hypertension, hypokalemia, aldosterone above the reference interval, and undetectable PRA), it is clinically reasonable to omit confirmatory testing and proceed to adrenal venous sampling for assessment of lateralization in patients who are potential surgical candidates.

**Michael Stowasser:** We have found FST to be most sensitive and accurate but it requires hospital admission, is difficult to perform, and is relatively expensive. SST has the advantage of requiring only a brief outpatient visit. However, we have previously found recumbent SST to lack sensitivity, with many patients being missed because aldosterone concentrations are lower in the recumbent position. In a recently published pilot study, we showed that upright SST (performed in the seated position) correctly identified 23 of 24 patients with PA, while recumbent SST was positive in only 8. The oral sodium–loading suppression test, favored by the Mayo Clinic, appeared to be more sensitive than recumbent STT when we tested a modified version of it against FST. However, it has the potential to suffer from the usual issues related to obtaining accurate 24-h urine collections and the degree to which the measured aldosterone concentration truly reflects the rate of aldosterone synthesis due to cross-reactivity to conjugated forms found in urine. The captopril challenge test, while relatively convenient and easy to perform, is not as well validated and has been reported to be associated with a significant number of false positives and negatives.

**Etienne Cavalier:** The most reliable means of differentiating unilateral (curable by adrenalectomy) from bilateral (treated medically with drugs that reduce aldosterone activity) forms of PA is adrenal venous sampling of aldosterone and cortisol. Technical oversight is required to ensure correct sample labeling. A laboratory technologist should be present during the entire process. It is important to be aware that aldosterone and cortisol values may be very high, thus requiring multiple dilutions, which may lead to matrix effects.

***What are the major developments in our understanding of the genetic basis for PA, and how might this hold promise toward improvements in diagnosis and therapy?***

**Etienne Cavalier:** Familial PA type 1 (or glucocorticoid remediable PA), is the consequence of a chimeric gene occurring from an irregular crossover during meiosis causing expression of aldosterone synthase in response to adrenocorticotropic hormone (ACTH). Patients with this disease show severe hypertension and hemorrhagic stroke events at a young age. Treatment consists of glucocorticoid administration. Familial PA type 2 is characterized by various presentations among the same pedigree APA or hyperplasia. Although its transmission is autosomal dominant, no genes have been identified to date. Treatment of this subtype does not differ from classical

PA. The recent discovery a mutation in a gene [potassium inwardly-rectifying channel subfamily J member 5 (*KCNJ5*)[10]] coding for GIRK4, a potassium channel was shown to cause familial PA type 3. As a result, cellular membrane depolarization leads to aldosterone production, excretion and cellular growth, hyperplasia, and adenoma. In these cases, hypertension is severe with profound hypokalemia. Bilateral adrenalectomy is frequently necessary to control hypertension.

**Richard Auchus:** The identification of *KCNJ5* mutations in familial PA type 3 and in a high fraction of APAs is probably the most significant advance in the field in over a decade. This work then led to the discovery of various plasma membrane channel mutations [sodium/potassium-transporting ATPase subunit alpha-1 (*ATP1A1*), plasma membrane calcium-transporting ATPase 3 (*ATP2B3*), and voltage-dependent calcium channel type L alpha 1D subunit (*CACNA1D*)] in other APAs. These findings provide insight into the pathogenesis of idiopathic (bilateral) hyperaldosteronism, indicate the inherent heterogeneity of these tumors, and help identify potential therapeutic targets for medical management of PA. Several important questions remain unanswered. Are the molecular mechanisms of aldosterone production and tumor formation the same, different, or partially overlapping? Are these driver mutations sufficient to cause PA? Is there a preclinical state that can be detected early and treated to prevent the evolution to PA? We are likely to see many exciting developments along these lines in the next decade.

[10] Human genes: *KCNJ5,* potassium inwardly-rectifying channel subfamily J member 5; *ATP1A1*, sodium/potassium-transporting ATPase subunit alpha-1; *ATP2B3,* plasma membrane calcium-transporting ATPase 3; *CACNA1D,* voltage-dependent calcium channel type L alpha 1D subunit.

**Michael Stowasser:** The identification of these *KCNJ5*, *ATP1A1*, *ATP2B3*, and *CACNA1D* mutations has greatly enhanced knowledge regarding the roles of these channels in adrenal physiology and pathophysiology and has the potential to lead to new diagnostic and therapeutic strategies. However, the argument against genetic testing as a clinical tool is that these mutations are almost always somatic and only very rarely associated with the familial occurrence of PA. Their detection in removed tumors does not yet have obvious implications for differential management of patients with APA. This could change as more is discovered about those factors which predispose patients to somatic mutations and their associated phenotype.

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