How to Escape from Primary Aldosteronism? News and Views on an Adrenal Disorder of Salt Retention

Introduction

»Salt over gold« – this old Slavic phrase and the custom to welcome guests with bread, salt, and water along with Biblical verses such as »You are the salt of the earth« (Matthew 5:13) shows the value that salt once had to man. While different evolutionary pressures helped to diversify adrenal steroid hormone biosynthesis and action and to establish sodium-retaining mechanisms, the human organism is less efficient in getting rid of salt. This is of particular importance in the age of manufactured nutrients, powdered ingredients, eatery in cafeterias, fast food restaurants or large-scale catering establishments, and overconsumption of salt. While hyperaldosteronism once may have helped to maintain normotremia and blood pressure, autonomous aldosterone secretion nowadays may become very dangerous and poses several threats to the organism. Indeed, it was shown that complications increase in mineralocorticoid excess models with the amount of dietary salt [1]. Whether or not high salt consumption suppresses renin and normal zona glomerulosa (zG) development and promotes expansion of mutated zG cells remains speculative.

However, primary aldosteronism is the main cause for secondary hypertension. Guidelines have been developed for a systematic approach to patients with autonomous aldosterone secretion [2–5]. This review highlights some aspects in the management of patients with PA and studies that may be of relevance.

Is There Escape from Aldosterone or Salt or how Many »A’s« are in the »RAA...S«?

The terms »electrocortin« and »mineralocorticoid« for aldosterone emphasize the sodium-handling character of this steroid hormone. Salt retention is achieved by active reabsorption of sodium in the collecting duct of the kidney, a site where free water cannot travel passively to follow the translocation of salt from urine into blood. Thus, aldosterone lowers urinary sodium concentration and sodium excretion through the kidneys. This effect of aldosterone and other mineralocorticoids, however, is visible for a short period of time when repeatedly administered to normal subjects. Therefore, an aldosterone escape mechanism had been proposed [5–9]. This idea seems to be supported by the fact that hypernatremia rarely develops in patients with PA. However, patients with PA do have an elevated total sodium content as compared to normal subjects and more water-free sodium was reported to be stored in the skin (reviewed by Tietze et al. [10]). In addition, sodium is tightly regulated in the plasma and a higher sodium retention activity is answered by further dilution of sodium in water, regulated mainly through arginine-vasopressin (AVP) and thirst. Several studies have shown that Brattleboro rats that do not synthesize active AVP and become hypernatremic following treatment with mineralocorticoids and salt but do not develop hypertension [11]. They only develop salt-sensitive hypertension when exogenous AVP is administered [12–14].
This strongly suggests that in salt-sensitive hypertension as in PA, mechanisms of water and volume regulation are involved, including AVP, aquaporin, atrial natriuretic peptide, and others (Fig. 1).

Thus, in primary aldosteronism, a state of hypervolemia develops to escape the salt load (Fig. 2), which promotes hypervolemic hypertension and distension of vessels along with inflammation although direct effects of aldosterone have also been noted [1, 15, 16]. Since hypervolemic hypertension causes hyperfiltration in the kidneys, the aldosterone-mediated retention of sodium is actually achieved against elevations in natriuretic peptides, higher total body sodium content next to a higher urinary filtration rate and polyuria in patients with PA. And with the progression of the disease, the exchange of potassium for sodium is also facilitated despite the development of hypokalemia.

All in all, there is no escape from aldosterone and the so-called «escape phenomenon»/«complete renal escape» expresses the end of adaption to and the start of maintenance of a new equilibrium rather than a mechanism of desensitization to aldosterone or a direct renal answer to lately established mineralocorticoid excess. However, at this new level of homeostasis, there are more molecules of sodium to be filtered and more molecules of sodium to be reclaimed and renin being lower while natriuretic peptides being higher [17], all of which leads the space to shrink for compensation of challenges to salt-water regulation, including saline loadings.

How to Diagnose Primary Aldosteronism?

Current guidelines suggest a step-wise approach to establish the diagnosis and the source of autonomous aldosterone secretion and the protocols offer a straightforward clinical workup for the majority of patients [3, 4]. However, in a considerable portion, the diagnostic process leads through areas between proof and exclusion, areas of uncertainty, compromises between sensitivity and specificity and sometimes constitutes a challenge for doctors, patients, and unaffected hypertensive individuals [18]. There may be several underlying reasons for this, one of which is that the secretion of aldosterone depends on more factors than renin/angiotensin, such as ACTH and potassium as well as a number of peptides and physiological circumstances, including the circadian rhythm, menstrual cycle, and medication [19–25]. Other reasons may include the wide and changing landscape of assays and the problem that published cut-off values are readily adopted by laboratories although there is a substantial inter-assay variation [26]. Recent papers have put a main focus on testing in primary aldosteronism and addressed these and other aspects [3, 23, 27–33] and proposed ideas for possible solutions [34]. Ideally, antihypertensive medication should be switched to verapamil slow-release, hydralazine, prazosin, doxazosin, and terazosin [3]. However, the more independent and unopposed aldosterone secretion occurs causing «drug-resistant hypertension», the less does medication interfere with testing because renin and angiotensin are suppressed anyway [35–37]. The diagnostic procedures can take place provided that renin is low. It is even possible to have a patient undergo diagnostic tests when he is on antimineralocorticoid medication as long as it is not sufficient to cut aldosterone action and renin remains low [38]. Thus, the decision is up to the experienced clinician whether it is reasonable to change the drug regimen or he can read the test results obtained under complex medication. The less severe and complicated PA, the more time for the diagnostic process can be invested and the more room is left for switching medication. Some of the basic rules are listed in Table 1.

Of note, there seems to be a continuum of autonomous aldosterone secretion independent of renin, ranging from normotensive PA, over low-renin hypertension, normokalemic PA, hypokalemic PA, and clear-cut symptomatic primary aldosteronism due to an aldosterone-producing adenoma, APA [34, 39, 40]. Thus, the diagnostic tests have not been developed against the background of well-evaluated cardiovascular risk reduction following successful testing and treatment but to separate patients from more or less well-defined controls [34].

All in all, undergoing the diagnostic procedures may become both laborious and, nevertheless, rewarding for affected individuals since specific therapy of PA is associated with a better cardiovascular outcome of patients [41–43] and reduction of the inflammatory and pro-thrombotic state [44–46]. Recently, a consensus
for the definition of criteria for success of treatment has been reached (results not yet published).

If any, What Symptoms are Suspicious of PA?

Definite symptoms are usually found in patients with clear-cut aldosteronism. Mild symptoms, however, may be present in mild cases but easily explained in the context of other differential diagnoses. For this reason, the case finding process relies on hypertension and risk factors, followed by hormonal studies. This may also be the cause for a frequent downplay of symptoms that, however, may become very prominent to patients and help the physician to quickly judge over response to therapy. Since glycyrrhizic acid inhibits 11β-hydroxysteroid dehydrogenase activity offering cortisol less restricted access to the mineralocorticoid receptor, the case history should include a question on the consumption of licorice products, including certain sweets, cough pastilles or chewing tobacco. In addition, hypercortisolism can mimic and accompany primary aldosteronism making questions for possible medication and a physical examination mandatory [47–49].

It was reported that dogs develop a diabetes insipidus-like picture when salt-retaining steroids are administered [5], what also seems to be true for man in a later stage of PA [50]. Of note, even young affected individuals or patients with good cardiac function do sometimes complain of polyuria/nocturia. Also, a salty taste was noted when mineralocorticoids were administered [7]. Indeed, patients with severe PA describe the feeling of a dry mouth or being generally thirsty if they are on a western country salt diet. There is experience with a »paradoxical response« to potassium-sparing diuretics. A general side effect of diuretics is polyuria what is exchanged for sodium in the kidney and diluted by hypervolemia and because autonomous aldosterone secretion is detached from the external potassium balance, the development of hypokalemia in hypertensives is a specific characteristic for PA. However, laboratory results are also very much dependent on the habit of processing blood samples and the handling of the tourniquet, which should be released before the blood is actually drawn into the syringe – if possible. Also, serum potassium values are too insensitive to pick up milder forms of PA, »normokalemic variants«, which makes serum potassium alone an insufficient screening tool [3, 4, 32]. Nevertheless, demonstration of hypokalemia and renal potassium loss may be a valuable information during the screening process [32, 36] such as signs of metabolic alkalosis. This is of particular importance when RAAS-blocking agents are administered.

Screening Tests for PA

Patients with sustained blood pressure above 150/100 mm Hg, hypertension that is only controlled on 4 or more antihypertensive drugs, hypertension and spontaneous or diuretic-induced hypokalemia, hypertension and an incidentally discovered adrenal tumor, hypertension and sleep apnea, hypertension and a family history of early onset hypertension or cerebrovascular complication at an age younger than 40 years, and all hypertensive first-degree relatives of patients with PA must be screened for presence of PA [3].

Since potassium is exchanged for sodium in the kidney and diluted by hypervolemia and because autonomous aldosterone secretion is detached from the external potassium balance, the development of hypokalemia in hypertensives is a specific characteristic for PA. However, laboratory results are also very much dependent on the habit of processing blood samples and the handling of the tourniquet, which should be released before the blood is actually drawn into the syringe – if possible. Also, serum potassium values are too insensitive to pick up milder forms of PA, »normokalemic variants«, which makes serum potassium alone an insufficient screening tool [3, 4, 32]. Nevertheless, demonstration of hypokalemia and renal potassium loss may be a valuable information during the screening process [32, 36] such as signs of metabolic alkalosis. This is of particular importance when RAAS-blocking agents are administered.

Demonstration of an aldosterone concentration, which is inadequately high for renin suggests PA (▶Table 2). Mathematically, this is expressed by the aldosterone to renin ratio (ARR), which was introduced in 1981 and validated later [51, 52]. Certain simple meas-
Table 1: Rules to be heeded during the laboratory workup for primary aldosteronism together with explanations for these rules.

<table>
<thead>
<tr>
<th>Measure or circumstance</th>
<th>Reason</th>
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<tbody>
<tr>
<td>Respect circadian rhythm, diagnose in rest</td>
<td>Enhances signal-to-noise ratio</td>
</tr>
<tr>
<td>Avoid extra stimulation of ACTH on steroidogenesis when performing AVS</td>
<td>Cross-reactivity of immunoassays likely pick up also other steroid hormones with relative high concentrations in adrenal venous blood and decrease signal-to-noise ratio</td>
</tr>
<tr>
<td>Avoid low potassium substitute potassium with a goal to keep a serum concentration 4 mmol/l</td>
<td>Potassium stimulates aldosterone independently from renin/angiotensin, normative data are generated at normal potassium values</td>
</tr>
<tr>
<td>Pay special attention to following medication</td>
<td>Prolong or shorten half-life of steroid hormones interfere with adrenal hormone secretion</td>
</tr>
<tr>
<td>Antihypertensive and other medication</td>
<td>Lower renin and aldosterone, increase screening sensitivity, lower specificity</td>
</tr>
<tr>
<td>non-steroidal anti-inflammatory drugs, beta-blockers, clonidine-like substances aliskiren</td>
<td>Raises renin concentration, lowers renin activity</td>
</tr>
<tr>
<td>ACE inhibitors, angiotensin receptor blockers sertraline, escitalopram</td>
<td>Raise renin and lower aldosterone, lower sensitivity, increase specificity</td>
</tr>
<tr>
<td>MR antagonists, potassium-sparing diuretics (less pronounced true also for other diuretics)</td>
<td>Raise renin, increase specificity, lower sensitivity</td>
</tr>
<tr>
<td>antimineralocorticoid progestins</td>
<td>Cut aldosterone action and raise renin, thus stimulating aldosterone secretion also from normal zG cells</td>
</tr>
<tr>
<td>Encourage high salt diet urinary sodium &gt;200 mmol/day</td>
<td>Act as weak MR antagonists</td>
</tr>
<tr>
<td>Check assay, use multiple tests, establish own cut-off values if possible</td>
<td>May suppress normal zG cells, enhances specificity of diagnostic tests</td>
</tr>
<tr>
<td>Heart failure *</td>
<td>Renin may not be suppressed because venous volume does not translate to arterial volume (Fig. 1)</td>
</tr>
<tr>
<td>Kidney damage * *</td>
<td>Renin may not be suppressed anymore [114]</td>
</tr>
<tr>
<td>Diagnose in early follicular phase</td>
<td>Progesterone acts as a weak MR antagonist, estrogens stimulate angiotensin</td>
</tr>
<tr>
<td>Renin should be low in all cases</td>
<td>Exceptions: heart failure, secondary kidney failure</td>
</tr>
</tbody>
</table>

* The highest basal renin value in a patient in our cohort with proven unilateral PA who had a clear benefit from surgical intervention of a visible adenoma was around 9 ng/l!

** In most cases of co-existence of PA and renal artery stenosis, PA seems to be the leading entity promoting the suppression of renin

Confirming the Diagnosis of PA

Despite the fact that the outcome of a confirmation test may also be hampered by a number of uncertainties, each suspected case should undergo a confirmation test although the sensitivities and specificities range between 75 and 95 % for some tests only and do not necessarily exceed the accuracy rates of screening tests [3, 55]. While these tests are described in detail elsewhere [3, 30, 33], some advantages and disadvantages of tests are discussed below. For additional information, see Table 2.

Clear-cut cases can be straightly offered subtype testing when the following criteria apply: spontaneous hypokalemia and inadequate high aldosterone (>200 ng/l, 555 pmol/l) in the absence of renin, which is below detection limits (e.g., <1.0 ng/l).

There is some minor evidence that the fludrocortisone-suppression test (FST) is more sensitive than the intravenous saline infusion test [40, 56]. Its performance may be enhanced when the influence of ACTH on aldosterone is blocked by low-dose dexamethasone the night before blood is drawn (Table 2) [57].

Of note, it was not shown, that the time of read-out on the 5th day is when the state of aldosterone escape has been reached.

More discussion on screening tests and associated problems can be found in Table 2 and elsewhere [3, 32].
Thus, the performance of the FST may be largely dependent on the amount of salt added. This also implies that clinical complications (e.g., heart failure) may become apparent when the test is actually over [40]. This test can also be combined with the determination of urinary aldosterone secretion over 24 h.

The saline infusion test with a rate of 500 ml per hour for 240 min leads to an unphysiological expansion of the intravascular volume. This may explain its limited power to differentiate between normokalemic primary aldosteronism and essential hypertension, its comparably low sensitivity and the wide range between exclusion and confirmation of PA [3, 26, 55]. However, this test enjoys a wide community for its ease of application. The Brisbane and the Munich groups see a benefit from performing this test in sitting position, others combine it with a low-dose dexamethasone suppression test (▶Table 2) [58, 59]. Further discussion can be found elsewhere [30, 33, 34].

▶Table 2 Diagnostic tests and suggested cut-off values for the analytes involved.

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Analyte(s)</th>
<th>Lower-Upper cut-off</th>
<th>Unit</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARR (aldosterone to renin-ratio) Baseline</td>
<td>Aldosterone:renin concentration</td>
<td>&lt;20–38 (75–144)</td>
<td>ng/l:ng/l (pmol/l:ng/l)</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td>Aldosterone:renin activity</td>
<td>&lt;200 (750)</td>
<td>ng/l:ng/ml/hour (pmol/l:ng/ml/hour)</td>
<td></td>
</tr>
<tr>
<td>A/RR (aldosterone(^3) to renin-ratio) Baseline</td>
<td>Aldosterone:renin activity</td>
<td>8 500 000</td>
<td>ng/l:ng/ml/hour</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td>Aldosterone:renin concentration</td>
<td>324 100</td>
<td>ng/l:ng/l</td>
<td>[36]</td>
</tr>
<tr>
<td>ARR and aldosterone Baseline</td>
<td>Aldosterone:renin activity and Aldosterone</td>
<td>&lt;20 150 (415)</td>
<td>ng/dl per ng/ml/hour ng/l (pmol/l)</td>
<td>[35, 115]</td>
</tr>
<tr>
<td>ARR or aldosterone after adrenal blockade 50 mg captopril, 320 mg valsartan, and 2 mg dexamethasone at midnight</td>
<td>Aldosterone:renin concentration</td>
<td>&lt;3.2 30 (83)</td>
<td>ng/l per μU/ml ng/l:ng/l (pmol/l)</td>
<td>[116]</td>
</tr>
<tr>
<td>ARR × SUSPPUP Baseline/24-h urine (or fasting urine)</td>
<td>Aldosterone; serum and urinary sodium, potassium (SUSPPUP)</td>
<td>&lt;200</td>
<td>l:mmol (ng/l:ng/l × l/mmol)</td>
<td>[36]</td>
</tr>
<tr>
<td>Fludrocortisone suppression test (FST) 4 days 0.1 mg of fludrocortisone every 6 h together with salt and potassium</td>
<td>Aldosterone (10 AM on day 5) cortisol (8 AM and 10 AM on day 5) potassium everyday</td>
<td>&lt;50–60 (140–165) decrease on day 5 normal</td>
<td>ng/l (pmol/l)</td>
<td>[3]</td>
</tr>
<tr>
<td>FST + DEX Similar to FST, in addition overnight 1 mg dexamethasone day 4 → 5</td>
<td>Aldosterone, cortisol; potassium</td>
<td>&lt;27 (74) &lt;1.8 (50) Normal</td>
<td>ng/l (pmol/l) μg/dl (nmol/l)</td>
<td>[57]</td>
</tr>
<tr>
<td>Sodium infusion test (SIT) 21 of 0.9 % saline solution i. v. over 4 h Consider performing in sitting position</td>
<td>Aldosterone, cortisol; potassium</td>
<td>&lt;50–100 (140–280) drop normal</td>
<td>ng/l (pmol/l)</td>
<td>[3]</td>
</tr>
<tr>
<td>SIT + DEX Similar to SIT, in addition 1 mg dexamethasone the night before</td>
<td>Aldosterone, cortisol; potassium</td>
<td>&lt;24 (66) &lt;1.8 (50) normal</td>
<td>ng/l (pmol/l) μg/dl (nmol/l)</td>
<td>[59]</td>
</tr>
<tr>
<td>Oral sodium load &gt; 6 g salt for 3 days Consider combination with renin or SUSPPUP</td>
<td>24-h urinary aldosterone 24-h urinary sodium</td>
<td>&lt;10–12 (28–33) &gt; 200</td>
<td>μg (nmol) per 24 h mmol per 24 h</td>
<td>[3] [36]</td>
</tr>
<tr>
<td>CCT (25 mg of crushed captopril tablets)</td>
<td>Aldosterone (after 2 h) Renin (after 2 h)</td>
<td>Drop by 30 % or below 85–120 ARR remains elevated no rise</td>
<td>ng/l</td>
<td>[3]</td>
</tr>
<tr>
<td>LCT (50 mg of crushed losartan tablets)</td>
<td>Aldosterone (after 2 h) and ARR Renin (after 2 h)</td>
<td>Below 100 and 40 No rise</td>
<td>ng/l ng/dl per ng/ml/hour</td>
<td>[27] [117]</td>
</tr>
</tbody>
</table>
The great advantage of aldosterone measurements in 24-h urine samples after oral sodium loading is that it can be easily combined with the determination of cortisol (and profiling of other steroids) and (nor)metanephrine during the investigation for secondary hypertension or an adrenal mass and even assessment of sodium excretion, creatinine clearance and albuminuria in an outpatient setting. Also, nowadays, salt consumption is rather high in many individuals and a high salt diet for a minimum of 3 days is easily achieved. However, also in these individuals, renin needs to be demonstrated as being low and there is considerable overlap between patients with essential hypertension and primary aldosteronism [60]. Therefore, it was suggested to increase the performance of urinary aldosterone estimation together with other indices of mineralocorticoid excess, such as the SUSPPUP ratio or renin [36].

As the ARR is more specific when elevated despite ACE-inhibitor or AT1R-blocker therapy, the informative value of the ARR and blood aldosterone concentration after a captoril (CCT) or a losartan challenge (LCT) test reaches the standard of a confirmation test (Table 2) [3, 4, 30]. There are, however, various test modifications and different assay methodology because of which it is recommended to analyze own experience. Also, these tests may have a high rate of false positive or false negative results [29]. Nevertheless, their great advantage is that the state of hypervolemia is not deteriorated during the test.

The posture test can be used to study the extent of hypervolemia and renin suppression. However, the posture test does not actually distinguish bilateral from unilateral disease but rather less severe from more severe cases in whom aldosterone is not modulated by angiotensin anymore but solely dependent on precursor availability and ACTH action. This may explain the rather low sensitivity and specificity of the test [61, 62]. A better accuracy seems to be reached with a reversed version of the test [63]. The combination of the posture test together with 40 mg of furosemide is accepted by the Japanese Society of Endocrinology [4]. According to this guideline, the diagnosis of PA is made if plasma renin activity or renin concentrations remain below 2.0 ng/ml/h or 8.0 ng/l, respectively, 2 h after furosemide and standing (walking).

Co-Secretion of Glucocorticoids

Adrenal steroid biosynthesis is regulated by 2 main factors: ACTH and angiotensin II. These adrenotrophic peptides bind to their receptors, the ACTH (MC2R) receptor and the type 1 angiotensin II receptor (AT1R), initiating signaling through 2 pathways. The MC2R-cyclic AMP-protein kinase A-pathway results in 11β-hydroxylase (CYP11B1) expression and cortisol synthesis while the AT1R-calcium-calmodulin kinase-pathway leads to expression of aldosterone synthase (CYP11B2) and aldosterone secretion (Fig. 3). Since the glucocorticoid corticosterone is a precursor of aldosterone it is generated when aldosterone is to be produced (Fig. 1). It was shown to be significantly higher in patients with PA as compared to controls (for details, see Moors et al. [64]).

However, binding of angiotensin to AT1R leads to inhibition of the KCNJ5 potassium channel (resulting in depolarization and calcium influx) and to inhibition of cyclic AMP generation (thus disconnecting ACTH from its influence on cortisol synthesis). Therefore, the zG cell is able to specifically express CYP11B2 and secrete aldosterone in the presence of ACTH under normal circumstances. Following Dringenberg’s theory, this also implies that in the absence of angiotensin, for example, due to suppression of renin, or in a state of profound ACTH excess, the balance between protein kinase A activity (cortisol synthesis) and calcudolin kinase activity (aldosterone synthesis) and the control over adrenal steroi
dogenesis would be shifted towards ACTH [65]. When the influence of ACTH is maintained, cortisol can be made even when a mutated channel causes depolarization of the zG cell, calcium-dependent signaling and aldosterone synthesis (Fig. 3). Thus, the "mix" in signaling activity determines the amount of cortisol and hybrid steroids to be produced.

Therefore, mutations distal to the AT1R that cause mild hyperactivity in calcium signaling and a mild clinical picture of hyperaldosteronism would allow for more intracellular influence of ACTH and growth and a relevant cortisol co-secretion along with hybrid steroids. Alternatively, mutations distal to the AT1R that cause profound hyperactivity in calcudolin kinase activity utilize all steroid precursors for aldosterone synthesis causing clinically relevant hypertension and would be detected at a smaller size (yet some hybrid steroids can be made because of co-expression of 11β-hydroxylase and aldosterone synthase). Indeed, aldosteronomas with cortisol co-secretion seem to be larger than pure aldosterone-producing tumors [66]. Of note, mutant KCNJ5-affected aldosteronomas display a greater diameter on computed tomography than aldosteronomas with mutations in the CACNA1D, ATP1A1, ATP2B3, or CTNB1 genes [67]). Also, mutant KCNJ5-tumors exhibit more likely a "fasciculata"-like morphology in comparison to aldosteronomas with other mutations that appear more "glomerulosa"-like [67–70]. Interestingly, it was found that the expression of CYP11B2 is inversely correlated with nodule size but positively with aldosterone output and suppression of renin [70]. In addition, hereditary "mild" KCNJ5 channel mutations permit active calcium-calcudolin kinase and ACTH-CAMP-protein kinase A signaling in the absence of angiotensin and thus development of large adrenal tumors and generation of hybrid steroids, which was really observed [71, 72] while hereditary mutations in voltage-gated calcium channels (CACNA1D and CACNA1H) or "severe" aberrations in KCNJ5 are associated with small tumors or no hyperplasia [73, 74]. However, "very severe" mutations may become lethal for the affected adrenocortical cell thus preventing formation of hyperplastic or adenomatous tissue [75]. In addition, there are more genes to be associated with aldosterone production [76], and other "non-channelopathies" mutations are also known to be associated with aldosteronomas, including aberrations in the Wnt-β-catenin pathway [67, 77–82]. Of note, aldosteronomas are inhomogeneous tumors with variable degrees of CYP11B2 expression and underlying genetic changes [83]. In some cases of PA due to bilateral hyperplasia, more than one mutated channel was found [76, 84].

The knowledge on hereditary mutations expands the classification of familial forms of primary aldosteronism [3, 76, 85] and already led to recommendations for genetic counseling (Table 3) [3, 86]. The identification of specific genetic aberrations underlying the familial forms of PA obviated the need to perform specific biochemical function tests in order to establish the diagnosis of FHA.

However, the functional changes that result from mutations can be picked up by special assays and may become of diagnostic value
in a different context. While cortisol co-secretion is rare in patients with small aldosteronomas [3, 87] patients with PA and relatively large adrenal tumors (> 2 cm) or clinical signs of hypercortisolism [47] should undergo specific screening [48, 49].

Elevated levels of so called »hybrid steroids«, 18-oxocortisol (18oxo-F) or 18-hydroxycortisol (18OH-F), was first demonstrated in patients with aldosteronomas [88, 89]. Later, it was found that patients with PA in general have higher concentrations of 18OH-F and 18oxo-F in 24-h urine samples as compared to patients with essential hypertension [90]. They are believed to be synthesized from 11-deoxycortisol by aldosterone synthase but their regulation seems to be more dependent on ACTH than angiotensin II (for review Morra di Cella et al. [91]). However, the demonstration of grossly elevated hybrid steroids makes co-expression of aldosterone synthase and 11β-hydroxylase very likely and is consistent with type 1 or type 3 familial hyperaldosteronism [71], sporadic PA with cortisol co-secretion [92] or a sporadic aldosteronoma with a mild aldosterone excess [96]. Nevertheless, adrenal imaging should be performed in patients with PA for exclusion of an adrenal carcinoma, viewing vascular structures for adrenal venous sampling (AVS) and for the identification of an adrenal cortical tumor that has a very high likelihood to be an aldosteronoma. For the latter circumstance, the following additional criteria apply to avoid AVS:  

- young patients (< 35 years of age) with hypokalemic PA and grossly elevated aldosterone [3]
- patients with PA and elevated aldosterone > 327 ng/l (> 900 pmol/l) [94]
- patients with PA and elevated plasma 18oxo-F > 6.1 ng/dl and excluded FHA [94]
- patients with PA, ARR > 40 h⁻¹ and urinary 18oxoF > 510 μg/day and excluded FHA [90].

For all other patients with PA, it is advised to undergo adrenal vein sampling (AVS) when surgery is an option [3, 4, 97]. Usually, the concentrations of aldosterone and cortisol are measured whereby the step-up between the cortisol concentrations in a peripheral (e.g., femoral) and the adrenal veins are determined to calculate the selectivity index (SI = cortisol_adrenal_vein : cortisol_femoral_vein). If the SI is > 2 it means that the contamination of the adrenal outflow with venous blood of other origin is low enough to proceed with the study [3, 97]. Some groups perform a rapid measurement of cortisol to ensure correct placement of the catheter and for the decision whether or not to try cannulating the difficult right adrenal vein again [98–100]. For proof of asymmetric aldosterone secretion, the lateralization index (LI) is calculated (LI = aldosterone:cortisol concentrations of the right adrenal vein/aldosterone:cortisol concentrations of the left adrenal vein). The higher the LI is, the safer is the proof of localization to this side whereby a minimum of > 2–3 is necessary and a ratio > 4 is highly desirable [3, 97]. Ideally, both adrenal veins are cannulated simultaneously. If this technique is not available, sequential samplings seems possible when at both time points also peripheral blood for calculation of the SI is drawn and renin is low [38].

There are some variations in AVS [102]. Some groups have performed AVS with corticotropin infusion. In the SPARTACUS trial, an AVS-based decision was compared to computed tomography-based decision for surgery and the researchers did not find a difference in the clinical outcome of the patients [101]. Does this challenge the value of AVS for the definition of the source of excess aldosterone secretion? Certainly not, but it casts bright light on some of the shortcomings in the current management. First, cortisol is not the ideal parameter to judge successful AVS and not well suited as a denominator in the calculations. This is because it is bound to protein, buffered as cortisone and there are cross reactions with other adrenal steroid hormones with respect to immunoassays [103]. The determination of plasma metanephrine is much better suited to assess selectivity in AVS [104] and dehydroepiandrosterenedione or androstenedione are better denominators than cortisol in the assessment of lateralization when measured with state-of-the art methods [93, 95, 104]. Second, mutations that promote depolarization and calcium-mediated signaling in the adrenocortical cell lie distal to the action of the angiotensin receptor and disconnect aldosterone secretion from the control of renin and angiotensin (▶ Fig. 3). However, ACTH action still provides aldosterone precursors and increases aldosterone secretion, explaining its maintained circadian rhythm [105–107] and higher aldosterone values in AVS studies with vs. without ACTH infusion [95, 103, 104].

In the SPARTACUS trial, the usage of immunoassays and ACTH insuffations may have decreased the signal-to-noise ratios (▶ Table 1) necessitating very stringent cut-off values being employed as selectivity and lateralization indices and for the definition of improvement and cure after surgery [101]. This was conform with current recommendations [3, 97] and the calculation model for »daily defined doses« was the method available for the assessment of change in medication (definitions provided by the World Health Organization). However, since the judgement of the antihypertensive power of a drug is old and based on experience in essential hypertension it may not be meaningful in patients with PA. Possibly, both arms (AVS and computed tomography) provided a bias towards the identification of severe cases for surgical intervention and in that there was no difference between AVS-based or computed tomography-based management. This creates no discrepancy to the current guidelines [3].

The SPARTACUS trial, however, set the stages for a well-performed clinical study in the area of PA and will very much impact on future investigations in the field. And it underscores the need for improvements or even alternatives of AVS [101]. One such improvement may be the determination of hybrid steroids in adrenal outflows [90, 108] or even the simultaneous measurement of multiple adrenal steroid hormones, including aldosterone, 18-oxocortisol, 18-hydroxycortisol, 11-deoxycorticosterone, corticosterone,
Two main factors control adrenal steroidogenesis: corticotropin (ACTH) and angiotensin II (AT2). Panel a: The signaling pathway of ACTH through its receptor (MC2R), a stimulating G protein (Gs), adenylyl cyclase (AC), and protein kinase A (PKA) is silenced to a considerable degree in the presence of AT2 because AT2 receptors (AT1R) also associate to an inhibitory G protein (Gi). This principle also inhibits the actions of the GIRK4 potassium channel (product of the KCNJ5 gene) and a sodium-potassium-ATPase (ATP1A1), which leads to depolarization of the zona glomerulosa cell. As a consequence, voltage-gated calcium channels (CACNA1D/H) open and permit the influx of calcium, which is removed from the cell by an ATPase (ATP2B3). Via activation of the calmodulin kinase (CaMK) the aldosterone synthase (CYP11B2) is expressed while 11β-hydroxylase (the CYP11B1 gene product) is not. Panel b: In familial hyperaldosteronism type 1, ACTH also has access to CYP11B2 transcription because a fragment of the CYP11B1 gene has been transferred to the CYP11B2 gene, including the promoter region. Co-localization of CYP11B1 and CYP11B2 allows for generation of hybrid steroids. Panel c: Mutations in channels that are silenced by AT2 action may lead to AT2-independent depolarization and autonomous CaMK activity as well as aldosterone synthesis. The suppression of renin and AT2 frees the Gs activity of the ACTH receptor allowing ACTH-dependent expression of CYP11B1 at the same time. Panel d: Mutations in channels that are directly involved in increasing intracellular calcium promote CaMK activity and generation of aldosterone along with hybrid steroids when ACTH/MC2R signaling is active.
cortisol, and 21-deoxycortisol, which reflected the change in adrenal steroidogenesis biochemistry and allowed for diagnosis of an aldosteronoma [95] and to predict the phenotype of underlying mutations with remarkable accuracy [93]. It is to be studied whether this technique can be employed to avoid AVS in some of the cases (see above).

One good alternative may be functional imaging. The old method of iodocholesterol scintigraphy can be exchanged for 11C-metomidate-based PET-CT examinations, which are sufficiently sensitive to display even small aldosteronomas and are largely consistent with AVS results [109, 110].

<table>
<thead>
<tr>
<th>FHA type</th>
<th>Gene</th>
<th>Criteria for screening</th>
<th>Reference</th>
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| FHA 1    | Chimeric CYP11B1/B2 | – onset of PA < 20 years of age  
– PA in a patient with family history of PA  
– PA in a patient with family history of stroke < 40 years | [123–125] |
| FHA 2    | Gene locus 7p22 | – PA in a patient with family history of PA  
– exclusion of FHA1, FHA3 and FHA4 | [126–127] |
| FHA 3    | KCNJ5 | – onset of confirmed PA < 20 years  
– resistant hypokalemic hypertension < 20 years  
– family history of PA < 20 years  
– exclusion of FHA1 | [71–72] |
| FHA 4    | CACNA1H | – PA in a child < 10 years | [74] |
| PA       | CACNA1D | – PA in a child with neurologic abnormalities, incl. seizures | [68, 73] |
| MEN1     | Menin | – PA in a patient with primary hyperparathyroidism * and a second manifestation of MEN1  
– PA in a patient with primary hyperparathyroidism * and a family history of MEN1 | |

FHA: Familial hyperaldosteronism; PA: Primary aldosteronism.

* Secondary hyperparathyroidism seems to accompany PA and may develop into a form of tertiary hyperparathyroidism [117–122]
than 50% of patients through surgery [113] the height of blood pressure cannot be the only read-out for the assessment of cure or control of excess aldosterone secretion. Other parameters of PA or mineralocorticoid excess can be informative, including hyperkalemia, SUSSPPUP ratio, acid–base homeostasis, renin, and natriuretic peptides, whereby the impact on antihypertensive medication on the biochemical data has to be taken into account. Therefore, reversal of symptoms may also be worth to ask for. And even if this remains largely uninformative, this may at least be a tool for the clinician to promote drug adherence in a way it may have been useful in the beginning when the patient started the long way through the diagnostic procedures.

Conflict of Interest

The author declares no conflict of interest.

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