Thieme

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

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**ABSTRACT**

The last years have seen substantial progress in primary aldosteronism (PA), which is the most common cause of secondary hypertension. Many programs have been established around the world to meet the needs in healthcare and the management of patients with PA according to published guidelines and clinical protocols. Systematic analysis of emerging data and meticulous scientific work have informed us on the molecular basis of the disease and helped to characterize hereditary forms of PA. Techniques have been developed to better diagnose PA and to establish genotype-phenotype relationships and their impact on hypertension. Studies have been undertaken to stratify patients for risk factors and to ensure quality of best medical treatment. This review focuses on some clinically relevant problems in characterizing autonomous aldosterone secretion and discusses testing and management strategies. Besides, this review puts the emphasis on some colorful studies not to pale soon beside an ever evolving painting background.

## 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 normonatremia 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.

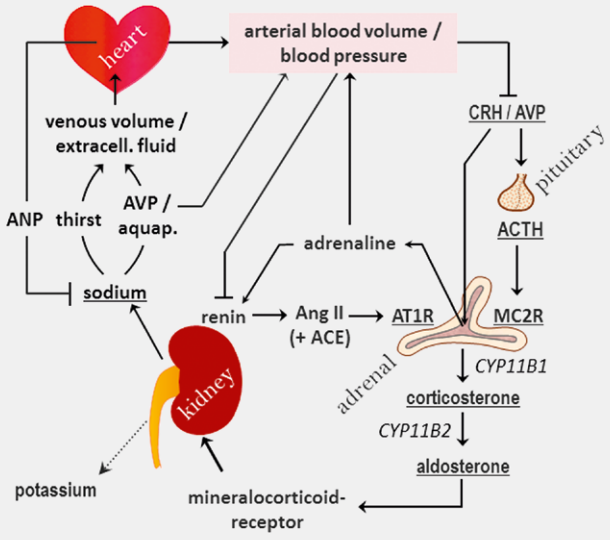
## Is There Escape from Aldosterone or Salt or how Many »A's« are in the »RAA...S«?

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.



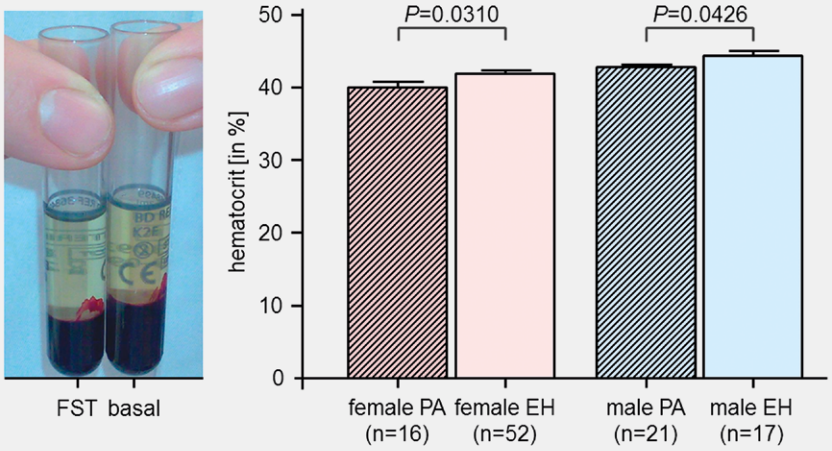

▶ **Fig. 1** This figure is a sketch of the renin-angiotensin-aldosterone system and includes the feedback loop through volume regulation. Aldosterone induces reabsorption of sodium in the kidney, which is diluted by water. Water is drunk or reclaimed with the help of vasopressin (AVP) and aquaporins. The volume enters the right heart which can release atrial natriuretic peptide (ANP) in case of hypervolemia. Venous volume translates to arterial volume in the absence of heart failure and is detected in the kidney or the carotid body. The latter is connected to the sympathetic nervous system. Hypervolemia/hypertension suppresses renin and adrenaline which actually would stimulate renin release by the kidneys. In the absence of renin, angiotensin is not formed from angiotensinogen and the angiotensin-converting enzyme (ACE) becomes passive within this system. If the receptor for angiotensin (AT1R) is not activated, aldosterone synthase (CYP11B2) will not be expressed. However, the hypothalamic pituitary adrenal axis still regulates adrenal steroidogenesis by corticotropin-releasing hormone (CRH), corticotropin (ACTH), activation of the ACTH receptor (MC2R) and expression of 11β-hydroxylase (CYP11B1) providing also mineralocorticoid active precursors of aldosterone. Accidentally, a number of names of the active participants in this physiologic system begin with the letter »A« (→ »How many A's are in the RAA…S?«).

## 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 straight forward 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 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




▶ **Fig. 2** The photograph insert shows 2 patients, one of which underwent a fludrocortisone suppression test (FST) and one of which came for a basal study. FST patients are easily recognized because of the volume expansion and drop in hematocrit values. The diagram shows the hematocrit values of female and male hypertensive patients with (PA) or without (EH) primary aldosteronism. Although differences between the single individuals are minor, a *t*-test showed significant lower hematocrit values for PA patients as a group in comparison to EH individuals.

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 disease-plagued patients therefore pay attention to. However, if antimineralocorticoid treatment is started in PA-affected patients, they feel relief from nocturia and polyuria and they are not as thirsty as compared to initial presentation.

A typical case history may include admissions for hypertensive crisis because in a state of hypervolemia, little additional vasoconstrictor activity has much effect on the rise in blood pressure and may mimic even pheochromocytoma.

In serious PA, symptoms of hypokalemia may be present and include muscle weakness or even cramps and extension spasms, which are promptly relieved upon potassium administration.

## 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.

| Measure or circumstance | Reason |
|---|---|
| Respect circadian rhythm, diagnose in rest | Enhances signal-to-noise ratio |
| Avoid extra stimulation of ACTH on steroidogenesis when performing AVS | 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 |
| Avoid low potassium<br>substitute potassium with a goal to keep a serum concentration 4 mmol/l | Potassium stimulates aldosterone independently from renin/angiotensin, normative data are generated at normal potassium values |
| Pay special attention to following medication<br>CYP3A4-interfering drugs<br>glucocorticoids | <br>Prolong or shorten half-life of steroid hormones<br>interfere with adrenal hormone secretion |
| Antihypertensive and other medication<br>non-steroidal anti-inflammatory drugs, beta-blockers, clonidine-like substances<br>aliskiren<br>ACE inhibitors, angiotensin receptor blockers<br>sertraline, escitalopram<br>MR antagonists, potassium-sparing diuretics (less pronounced true also for other diuretics) | <br>Lower renin and aldosterone, increase screening sensitivity, lower specificity<br><br>Raises renin concentration, lowers renin activity<br>Raise renin and lower aldosterone, lower sensitivity, increase specificity<br>Raise renin, increase specificity, lower sensitivity<br>Cut aldosterone action and raise renin, thus stimulating aldosterone secretion also from normal *zG* cells |
| antimineralocorticoid progestins | Act as weak MR antagonists |
| Encourage high salt diet<br>urinary sodium >200 mmol/day | May suppress normal *zG* cells, enhances specificity of diagnostic tests |
| Check assay, use multiple tests, establish own cut-off values if possible | Substantial inter-assay variations |
| Heart failure * | Renin may not be suppressed because venous volume does not translate to arterial volume (**Fig. 1**) |
| Kidney damage * * | Renin may not be suppressed anymore [114] |
| Diagnose in early follicular phase | Progesterone acts as a weak MR antagonist, estrogens stimulate angiotensin |
| Renin should be low in all cases | Exceptions: heart failure, secondary kidney failure |

* 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

ures can be taken in order to enhance the performance of the ARR:

- since the low renin drives the ARR from a mathematical point of view, specificity (NOT sensitivity) increases very much if the absolute serum aldosterone value is also taken into consideration (▶ **Table 2**)
- for the same mathematical reason, accuracy of the ARR increases when aldosterone is squared or cubed (▶ **Table 2**)
- sensitivity and specificity of the ARR increases dramatically when it is multiplied by the SUSPPUP ratio (▶ **Table 2**), a parameter of aldosterone action which corrects for serum potassium and urinary electrolyte excretion (SUSPPUP = serum sodium/(serum potassium)$^2$: urinary sodium/urinary potassium) [53]
- determination of the ARR and aldosterone after overnight blockade with captopril, valsartan, and dexamethasone (▶ **Table 2**)
- the ARR can be combined with the body mass index and an estimation of the potassium clearance [54].

More discussion on screening tests and associated problems can be found in ▶ **Table 2** and elsewhere [3, 32].

## 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 5$^{th}$ day is when the state of aldosterone escape has been reached.

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

| Diagnostic test | Analyte(s) | Lower-Upper cut-off | Unit | References |
|---|---|---|---|---|
| **ARR** (aldosterone to renin-ratio)<br>Baseline | Aldosterone:renin concentration<br><br>Aldosterone:renin activity | <20–38 (75–144)<br><br><200 (750) | ng/l:ng/l (pmol/l: ng/l)<br><br>ng/l:ng/ml/hour (pmol/l: ng/ml/hour) | [3] |
| **A$^3$RR** (aldosterone$^3$ to renin-ratio)<br>Baseline | Aldosterone:renin activity<br><br>Aldosterone:renin concentration | 8 500 000<br><br>324 100 | ng/l:ng/ml/hour<br><br>ng/l : ng/l | [35]<br><br>[36] |
| **ARR and aldosterone**<br>Baseline | Aldosterone:renin activity **and**<br>Aldosterone | <20<br>150 (415) | ng/dl per ng/ml/hour<br>ng/l (pmol/l) | [35, 115] |
| **ARR or aldosterone after adrenal blockade**<br>50 mg captopril, 320 mg valsartan, and 2 mg dexamethasone at midnight | Aldosterone:renin concentration<br><br>Aldosterone | <3.2<br><br>30 (83) | ng/l per µU/ml<br><br>ng/l (pmol/l) | [116] |
| **ARR × SUSPPUP**<br>Baseline/24-h urine (or fasting urine) | Aldosterone; serum and urinary sodium, potassium (SUSPPUP) | <200 | l/mmol (ng/l:ng/l × l/mmol) | [36] |
| **Fludrocortisone suppression test (FST)**<br>4 days 0.1 mg of fludrocortisone every 6 h together with salt and potassium | Aldosterone (10 AM on day 5)<br><br>cortisol (8 AM and 10 AM on day 5) potassium everyday | <50–60 (140–165)<br><br>decrease on day 5<br>normal | ng/l (pmol/l) | [3] |
| **FST + DEX**<br>Similar to FST, in addition overnight 1 mg dexamethasone day 4 → 5 | Aldosterone,<br>cortisol;<br>potassium | <27 (74)<br><1.8 (50)<br>Normal | ng/l (pmol/l)<br>µg/dl (nmol/l) | [57] |
| **Sodium infusion test (SIT)**<br>2 l of 0.9 % saline solution i. v. over 4 h<br>Consider performing in sitting position | Aldosterone,<br><br>cortisol;<br>potassium | <50–100<br>(140–280)<br>drop<br>normal | ng/l (pmol/l) | [3] |
| **SIT + DEX**<br>Similar to SIT, in addition 1 mg dexamethasone the night before | Aldosterone,<br>cortisol;<br><br>potassium | <24 (66)<br><1.8 (50)<br><br>normal | ng/l (pmol/l)<br>µg/dl (nmol/l) | [59] |
| **Oral sodium load**<br>>6 g salt for 3 days<br>Consider combination with renin or SUSPPUP | 24-h urinary aldosterone<br>24-h urinary sodium | <10–12 (28–33)<br>>200 | µg (nmol) per 24 h<br>mmol per 24 h | [3]<br><br>[36] |
| CCT (25 mg of crushed captopril tablets) | Aldosterone (after 2 h)<br><br>ARR (after 2 h)<br><br>Renin (after 2 h) | Drop by 30 % or below 85–120<br>ARR remains elevated<br>no rise | ng/l | [3] |
| LCT (50 mg of crunched losartan tablets) | Aldosterone (after 2 h) and ARR<br>Renin (after 2 h) | Below 100 and 40<br>No rise | ng/l<br>ng/dl per ng/ml/hour | [27]<br>[117] |

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].

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 captopril (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 calmodulin kinase activity (aldosterone synthesis) and the control over adrenal steroidogenesis 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 calmodulin 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 *CTNNB1* 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* 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-calmodulin 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-channelopathy« 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 channelopathy (mainly mutated KCNJ5) [93]. This has consequences for the characterization of PA as unilateral or bilateral disease (see below) [93–95].

## Unilateral or Bilateral Disease?

Because of its spacial resolution, computed tomography is the method of choice for the detection of adrenal aldosterone-producing tumors [3]. However, the demonstration of an adrenal tumor is not proof that it is the (sole) source of excess aldosterone secretion and aldosteronomas may be too small to be picked up by imaging techniques. It was shown that sensitivity of conventional adrenal imaging is too poor for correctly identifying the character of 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:

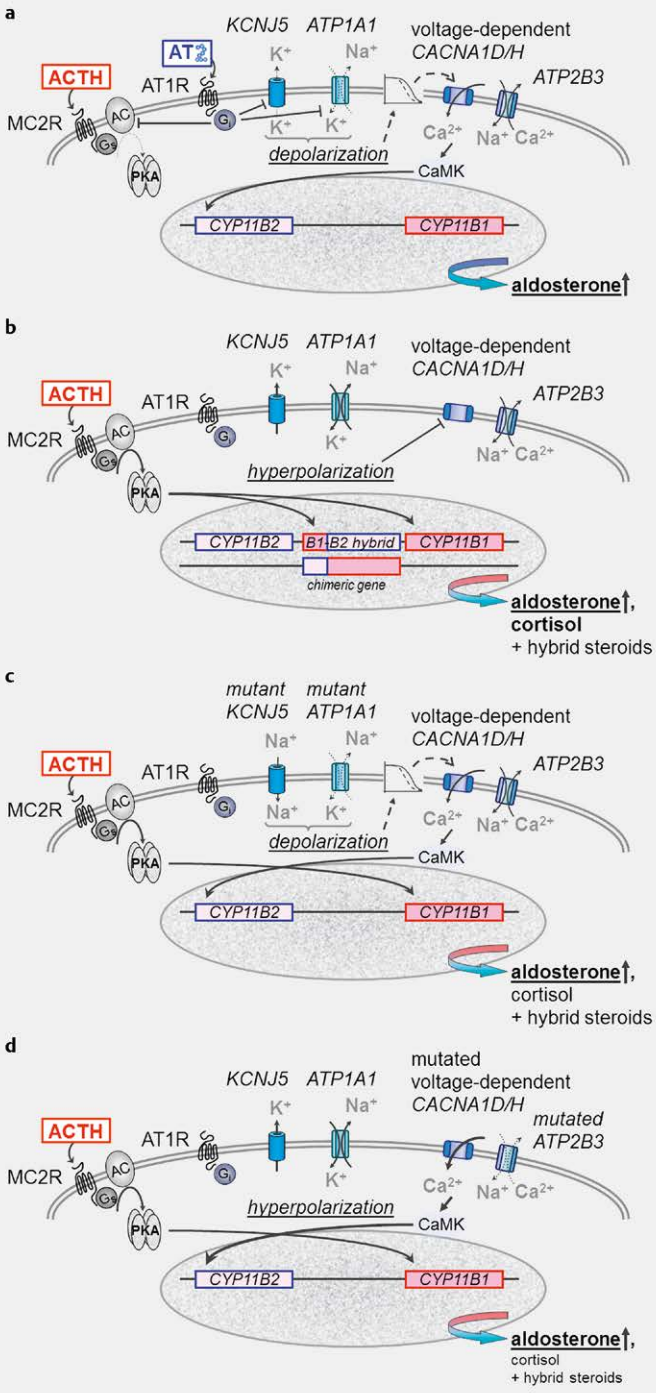
- young patients (< 35 years of age ) with hypokalemic PA and grossly elevated aldosterone [3]
- patients with PA and elevated aldosterone > 327 ng/l (> 900 pmol/l) [94]
- patients with PA and elevated plasma 18oxo-F > 6.1 ng/dl and excluded FHA [94]
- patients with PA, ARR > 40 $h^{-1}$ and urinary 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 lateralization 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 dehydroepiandrostenedione or/and 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 infusions 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,




▶ **Fig. 3** The sketch was further developed from Zennaro et al. and Korah et al. [79, 80, 85]. 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 has also 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.

▶ **Table 3** Screening criteria for familial forms of hyperaldosteronism (FHA) as suggested by Funder et al. [3] and Zennaro et al. [86].

| FHA type | Gene | Criteria for screening | Reference |
|---|---|---|---|
| **FHA 1** | Chimeric *CYP11B1/B2* | – onset of PA <20 years of age<br>– PA in a patient with family history of PA<br>– 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<br>– exclusion of FHA1, FHA3 and FHA4 | [126–127] |
| **FHA 3** | *KCNJ5* | – onset of confirmed PA <20 years<br>– resistant hypokalemic hypertension <20 years<br>– family history of PA <20 years<br>– 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<br>– 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]

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].

## Therapy: How to Escape from PA after Being Diagnosed?

Retroperitoneoscopic or laparoscopic adrenal surgery is recommended for unilateral disease and may have to be considered even in severe cases of bilateral or hereditary disease. Some clinicians send patients straight to adrenal surgery and others start patients on medication. The actual advantage of rapid surgery is that the aldosterone assay after intervention usually shows very or even undetectably low blood concentrations of aldosterone. This gives a quick feedback for the success of the diagnostic and therapeutic management during the past period. However, there may be need for mineralocorticoid substitution after operation (e. g., fludrocortisone at 0.05 mg per day for one month or more) if aldosterone autonomy was severe. Cases of hyperkalemia after surgery are well known and episodes of hypotension and pre-renal insufficiency were noted. This is of particular importance if patients are continued on other antihypertensive medication, including RAAS blocking agents (▶ **Table 1**).

If glucocorticoid co-secretion was also diagnosed, hydrocortisone should be administered after surgery with doses and time to be geared to the extent of the pre-surgically detected excess.

In cases of conservative management, patients should be educated about dietary salt and ways to escape salt overconsumption. For the vast majority of patients, however, it is easier to limit aldosterone secretion, prevent aldosterone action or counter the action of aldosterone than to cut salt ingestion to a very small, therapeutically relevant amount. Research is done in the field of aldosterone synthase inhibition and blockade of mutated channels. For now, antagonists of the mineralocorticoid receptor and the epithelial sodium channel [3, 111]. Spironolactone is the drug of choice. Since its additional antagonism against the androgen receptor and its agonism on the progesterone receptor low doses (e. g., 12.5, or 25 mg) are to be tried first. Combination therapy with amilorid (or triamterene), for example, in need of doses over 50 mg spironolactone, is also possible and may help to postpone the development of gynecomastia in men. These potassium-sparing diuretics are also an alternative to eplerenone in case when spironolactone is not tolerated.

While these and many more aspects are covered in detail elsewhere [111, 112] it is still disputed how to find an ideal drug regimen to counter fully aldosterone action without overtreatment. Since it is reported that reversal of hypertension is possible in less

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 kalemia, SUSPPUP 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.

## References


[1] Catena C, Colussi G, Brosolo G, Novello M, Sechi LA. Aldosterone and left ventricular remodeling. Horm Metab Res 2015; 47: 981–986

[2] Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, Young WF Jr., Montori VM. Endocrine Society. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2008; 93: 3266–3281

[3] Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF Jr. The management of primary aldosteronism: case detection, diagnosis, and treatment: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2016; 101: 1889–1916

[4] Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, Tanabe A.Task force committee on primary aldosteronism, the japan endocrine society. guidelines for the diagnosis and treatment of primary aldosteronism – the japan endocrine society. 2009; Endocr J 2011; 58: 711–721

[5] Kuhlmann D, Ragan C, Ferrebee JW, Atchley DW, Loeb RF. Toxic effects of desoxycorticosterone esters in dogs. Science 1939; 90: 496–497

[6] Ragan C, Ferrebee JW, Phyfe P, Atchley DW, Loeb RF. Syndrome of polydipsia and polyuria induced in normal animals by desoxycorticosterone acetate. Am J Physiol 1940; 131: 73–78

[7] Zierler KL, Lilienthal JL Jr. Sodium loss in man induced by desoxycorticosterone acetate; study in a subject with myotonic dystrophy. Am J Med 1948; 4: 186–192

[8] August JT, Nelson DH, Thorn GW. Response of normal subjects to large amounts of aldosterone. J Clin Invest 1958; 37: 1549–1555

[9] Rovner DR, Conn JW, Knopf RF, Cohen EL, Hsueh MT. Nature of renal escape from the the sodium–retaining effect of aldosterone in primary aldosteronism and in normal subjects. J Clin Endocrinol Metab 1965; 25: 53–64

[10] Titze J, Rakova N, Kopp C, Dahlmann A, Jantsch J, Luft FC. Balancing wobbles in the body sodium. Nephrol Dial Transplant. 2016; 31: 1078–1081

[11] Intengan HD, Park JB, Schiffrin EL. Blood pressure and small arteries in DOCA-salt-treated genetically AVP-deficient rats: role of endothelin. Hypertension 1999; 34: 907–913

[12] Schmale H, Richter D. Single base deletion in the vasopressin gene is the cause of diabetes insipidus in Brattleboro rats. Nature 1984; 308: 705–709

[13] Zelena D, Barna I, Csabai K, Orlando GF, Makara GB, Engelmann M. Response of the adrenomedullary system to early postnatal stress in the Brattleboro rat. Ann N Y Acad Sci 2008; 1148: 456–561

[14] Gruber KA. The Brattleboro rat and salt-induced hypertension. Hypertension 1982; 4: 572–574

[15] Joffe HV, Adler GK. Effect of aldosterone and mineralocorticoid receptor blockade on vascular inflammation. Heart Fail Rev 2005; 10: 31–37

[16] Fiebeler A, Muller DN, Shagdarsuren E, Luft FC. Aldosterone, mineralocorticoid receptors, and ascular inflammation. Curr Opin Nephrol Hypertens 2007; 16: 134–142

[17] Douillard C, Houillier P, Nussberger J, Girerd X. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 2: First diagnostic steps. Ann Endocrinol 2016; 77: 192–201

[18] Burrello J, Monticone S, Tetti M, Rossato D, Versace K, Castellano I, Williams TA, Veglio F, Mulatero P. Subtype diagnosis of primary aldosteronism: Approach to different clinical scenarios. Horm Metab Res 2015; 47: 959–966

[19] Ehrhart-Bornstein M, Hinson JP, Bornstein SR, Scherbaum WA, Vinson GP. Intraadrenal interactions in the regulation of adrenocortical steroidogenesis. Endocr Rev 1998; 19: 101–143

[20] Willenberg HS, Schinner S, Ansurudeen I. New mechanisms to control aldosterone synthesis. Horm Metab Res 2008; 40: 435–441

[21] Adler GK, Bonyhay I, Curren V, Waring E, Freeman R. Hypoglycaemia increases aldosterone in a dose-dependent fashion. Diabet Med 2010; 27: 1250–1255

[22] Hattangady NG, Olala LO, Bollag WB, Rainey WE. Acute and chronic regulation of aldosterone production. Mol Cell Endocrinol 2012; 350: 151–162

[23] Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the aldosterone/renin ratio. Horm Metab Res 2012; 44: 170–176

[24] Beuschlein F. Regulation of aldosterone secretion: from physiology to disease. Eur J Endocrinol 2013; 168: R85–R93

[25] Ahmed AH, Gordon RD, Ward G, Wolley M, Kogovsek C, Stowasser M. Should aldosterone suppression tests be conducted during a particular phase of the menstrual cycle, and, if so, which phase? Results of a preliminary study. Clin Endocrinol 2015; 83: 203–207

[26] Schirpenbach C, Seiler L, Maser-Gluth C, Beuschlein F, Reincke M, Bidlingmaier M. Automated chemiluminescence-immunoassay for aldosterone during dynamic testing: comparison to radioimmunoassays with and without extraction steps. Clin Chem 2006; 52: 1749–1755

[27] Giacchetti G, Ronconi V, Lucarelli G, Boscaro M, Mantero F. Analysis of screening and confirmatory tests in the diagnosis of primary aldosteronism: need for a standardized protocol. J Hypertens 2006; 24: 737–745

[28] Alvarez-Madrazo S, Padmanabhan S, Mayosi BM, Watkins H, Avery P, Wallace AM, Fraser R, Davies E, Keavney B, Connell JM. Familial and phenotypic associations of the aldosterone Renin ratio. J Clin Endocrinol Metab 2009; 94: 4324–4333

[29] Mulatero P, Monticone S, Bertello C, Mengozzi G, Tizzani D, Iannaccone A, Veglio F. Confirmatory tests in the diagnosis of primary aldosteronism. Horm Metab Res 2010; 42: 406–410

[30] Haase M, Gruber M, Gao X, Vonend O, Willenberg HS. Confirmatory testing for primary aldosteronism. In: Hellman P. (ed.). Primary Aldosteronism – Molecular Genetics, Endocrinology and Translational Medicine. Berlin: Springer; 2014

[31] Sabbadin C, Fallo F. Hyperaldosteronism: screening and diagnostic tests. High Blood Press Cardiovasc Prev 2016; 23: 69–72

[32] Baguet JP, Steichen O, Mounier-Véhier C, Gosse P. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 1: Epidemiology of PA, who should be screened for sporadic PA? Ann Endocrinol 2016; 77: 187–191

[33] Reznik Y, Amar L, Tabarin A. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 3: Confirmatory testing. Ann Endocrinol 2016; 77: 202–207

[34] Funder JW. Primary aldosteronism: new answers, new questions. Horm Metab Res 2015; 47: 935–940

[35] Seiler L, Rump LC, Schulte-Mönting J, Slawik M, Borm K, Pavenstädt H, Beuschlein F, Reincke M. Diagnosis of primary aldosteronism: value of different screening parameters and influence of antihypertensive medication. Eur J Endocrinol 2004; 150: 329–337

[36] Balaş M, Zosin I, Maser-Gluth C, Hermsen D, Cupisti K, Schott M, Schinner S, Knoefel WT, Scherbaum WA, Willenberg HS. Indicators of mineralocorticoid excess in the evaluation of primary aldosteronism. Hypertens Res 2010; 33: 850–856

[37] Solar M, Malirova E, Ballon M, Pelouch R, Ceral J. Confirmatory testing in primary aldosteronism: extensive medication switching is not needed in all patients. Eur J Endocrinol 2012; 166: 679–686

[38] Haase M, Riester A, Kröpil P, Hahner S, Degenhart C, Willenberg HS, Reincke M. Outcome of adrenal vein sampling performed during concurrent mineralocorticoid receptor antagonist therapy. J Clin Endocrinol Metab 2014; 99: 4397–4402

[39] Grim CE. Evolution of diagnostic criteria for primary aldosteronism: why is it more common in "drug–resistant" hypertension today? Curr Hypertens Rep 2004; 6: 485–492

[40] Willenberg HS, Vonend O, Schott M, Gao X, Blondin D, Saleh A, Rump LC, Scherbaum WA. Comparison of the saline infusion test and the fludrocortisone suppression test in the diagnosis of primary aldosteronism. Horm Metab Res 2012; 44: 527–532

[41] Stowasser M, Gordon RD. Primary aldosteronism – careful investigation is essential and rewarding. Mol Cell Endocrinol 2004; 217: 33–39

[42] Catena C, Colussi G, Nadalini E, Chiuch A, Baroselli S, Lapenna R, Sechi LA. Cardiovascular outcomes in patients with primary aldosteronism after treatment. Arch Intern Med 2008; 168: 80–85

[43] Bauersachs J, Jaisser F, Toto R. Mineralocorticoid receptor activation and mineralocorticoid receptor antagonist treatment in cardiac and renal diseases. Hypertension 2015; 65: 257–263

[44] Funder JW. Aldosterone, mineralocorticoid receptors and vascular inflammation. Mol Cell Endocrinol 2004; 217: 263–269

[45] Gilbert KC, Brown NJ. Aldosterone and inflammation. Curr Opin Endocrinol Diabetes Obes 2010; 17: 199–204

[46] Petramala L, Iacobellis G, Carnevale R, Marinelli C, Zinnamosca L, Concistrè A, Galassi M, Iannucci G, Lucia P, Pignatelli P, Ciardi A, Violi F, De Toma G, Letizia C. Enhanced soluble serum CD40L and serum P-selectin levels in primary aldosteronism. Horm Metab Res 2016; 48: 440–445

[47] Findling JW, Raff H. Screening and diagnosis of Cushing's syndrome. Endocrinol Metab Clin North Am 2005; 34: 385–402

[48] Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2008; 93: 1526–1540

[49] Nieman LK. Cushing's syndrome: update on signs, symptoms and biochemical screening. Eur J Endocrinol 2015; 173: M33–M38

[50] Conn JW. Primary aldosteronism. J Lab Clin Med 1955; 45: 661–664

[51] Hiramatsu K, Yamada T, Yukimura Y, Komiya I, Ichikawa K, Ishihara M, Nagata H, Izumiyama T. A screening test to identify aldosterone–producing adenoma by measuring plasma renin activity. Results in hypertensive patients. Arch Intern Med 1981; 141: 1589–1593

[52] Weinberger MH, Fineberg NS. The diagnosis of primary aldosteronism and separation of two major subtypes. Arch Intern Med 1993; 153: 2125–2129

[53] Willenberg HS, Kolentini C, Quinkler M, Cupisti K, Krausch M, Schott M, Scherbaum WA. The serum sodium to urinary sodium to (serum potassium)2 to urinary potassium (SUSPPUP) ratio in patients with primary aldosteronism. Eur J Clin Invest 2009; 39: 43–50

[54] Kuo CC, Wu VC, Tsai CW, Huang KH, Wang SM, Li BC, Chang CC, Lu CC, Yang WS, Chao CT, Tsai IC, Lai CF, Lin WC, Wu MS, Lin YH, Lin CY, Chang HW, Wang WJ, Chiang WC, Kao TW, Chueh SC, Chu TS, Tsai TJ, Wu KD. TAIPAI Study Group. Combining body mass index and serum potassium to urine potassium clearance ratio is an alternative method to predict primary aldosteronism. Clin Chim Acta 2011; 412: 1637–1642

[55] Rossi GP, Belfiore A, Bernini G, Desideri G, Fabris B, Ferri C, Giacchetti G, Letizia C, Maccario M, Mallamaci F, Mannelli M, Montemurro D, Palumbo G, Rizzoni D, Rossi E, Semplicini A, Agabiti-Rosei E, Pessina AC, Mantero F.PAPY Study Investigators. Prospective evaluation of the saline infusion test for excluding primary aldosteronism due to aldosterone–producing adenoma. J Hypertens 2007; 25: 1433–1442

[56] Gordon RD. Mineralocorticoid hypertension. Lancet 1994; 344: 240–243

[57] Gouli A, Kaltsas G, Tzonou A, Markou A, Androulakis II, Ragkou D, Vamvakidis K, Zografos G, Kontogeorgos G, Chrousos GP, Piaditis G. High prevalence of autonomous aldosterone secretion among patients with essential hypertension. Eur J Clin Invest 2011; 41: 1227–1236

[58]Ahmed AH, Cowley D, Wolley M, Gordon RD, Xu S, Taylor PJ, Stowasser M. Seated saline suppression testing for the diagnosis of primary aldosteronism: a preliminary study. J Clin Endocrinol Metab 2014; 99: 2745–2753

[59] Pappa T, Papanastasiou L, Kaltsas G, Markou A, Tsounas P, Androulakis I, Tsiavos V, Zografos G, Vamvakidis K, Samara C, Piaditis G. Pattern of adrenal hormonal secretion in patients with adrenal adenomas: the relevance of aldosterone in arterial hypertension. J Clin Endocrinol Metab 2012; 97: E537–E545

[60] Schirpenbach C, Seiler L, Maser-Gluth C, Rüdiger F, Nickel C, Beuschlein F, Reincke M. Confirmatory testing in normokalaemic primary aldosteronism: the value of the saline infusion test and urinary aldosterone metabolites. Eur J Endocrinol 2006; 154: 865–873

[61] Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L, Gomez-Sanchez CE, Veglio F, Young WF Jr.. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. J Clin Endocrinol Metab 2004; 89: 1045–1050

[62] Lau JH, Sze WC, Reznek RH, Matson M, Sahdev A, Carpenter R, Berney DM, Akker SA, Chew SL, Grossman AB, Monson JP, Drake WM. A prospective evaluation of postural stimulation testing, computed tomography and adrenal vein sampling in the differential diagnosis of primary aldosteronism. Clin Endocrinol 2012; 76: 182–188

[63] Weickert MO, Schöfl-Siegert B, Arafat AM, Pfeiffer AF, Möhlig M, Schöfl C. A reverse postural test as a screening tool for aldosterone-producing adenoma: a pilot study. Endocrine 2009; 36: 75–82

[64] Moors M, Williams TA, Deinum J, Eisenhofer G, Reincke M, Lenders JW. Steroid hormone production in patients with aldosterone producing adenomas. Horm Metab Res 2015; 47: 967–972

[65] Dringenberg T, Schwitalla M, Haase M, Scherbaum WA, Willenberg HS. Control of CYP11B2/CYP11B1 expression ratio and consequences for the zonation of the adrenal cortex. Horm Metab Res 2013; 45: 81–85

[66] Späth M, Korovkin S, Antke C, Anlauf M, Willenberg HS. Aldosterone and cortisol co-secreting adrenal tumors: the lost subtype of primary aldosteronism. Eur J Endocrinol 2011; 164: 447–455

[67] Scholl UI, Healy JM, Thiel A, Fonseca AL, Brown TC, Kunstman JW, Horne MJ, Dietrich D, Riemer J, Kücükköylü S, Reimer EN, Reis AC, Goh G, Kristiansen G, Mahajan A, Korah R, Lifton RP, Prasad ML, Carling T. Novel somatic mutations in primary hyperaldosteronism are related to the clinical, radiological and pathological phenotype. Clin Endocrinol 2015; 83: 779–789

[68] Azizan EA, Poulsen H, Tuluc P, Zhou J, Clausen MV, Lieb A, Maniero C, Garg S, Bochukova EG, Zhao W, Shaikh LH, Brighton CA, Teo AE, Davenport AP, Dekkers T, Tops B, Küsters B, Ceral J, Yeo GS, Neogi SG, McFarlane I, Rosenfeld N, Marass F, Hadfield J, Margas W, Chaggar K, Solar M, Deinum J, Dolphin AC, Farooqi IS, Striessnig J, Nissen P, Brown MJ. Somatic mutations in ATP1A1 and CACNA1D underlie a common subtype of adrenal hypertension. Nat Genet 2013; 45: 1055–1060

[69] Åkerström T, Willenberg HS, Cupisti K, Ip J, Backman S, Moser A, Maharjan R, Robinson B, Iwen KA, Dralle H, DVolpe C, Bäckdahl M, Botling J, Stålberg P, Westin G, Walz MK, Lehnert H, Sidhu S, Zedenius J, Björklund P, Hellman P. Novel somatic mutations and distinct molecular signature in aldosterone-producing adenomas. Endocr Relat Cancer 2015; 22: 735–744

[70] Monticone S, Castellano I, Versace K, Lucatello B, Veglio F, Gomez-Sanchez CE, Williams TA, Mulatero P. Immunohistochemical, genetic and clinical characterization of sporadic aldosterone–producing adenomas. Mol Cell Endocrinol 2015; 411: 146–154

[71] Geller DS, Zhang J, Wisgerhof MV, Shackleton C, Kashgarian M, Lifton RP. A novel form of human mendelian hypertension featuring nonglucocorticoid–remediable aldosteronism. J Clin Endocrinol Metab 2008; 93: 3117–3123

[72] Choi M, Scholl UI, Yue P, Björklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Patel A, Men CJ, Lolis E, Wisgerhof MV, Geller DS, Mane S, Hellman P, Westin G, Åkerström G, Wang W, Carling T, Lifton RP. K+ channel mutations in adrenal aldosterone–producing adenomas and hereditary hypertension. Science 2011; 331: 768–772

[73] Scholl UI, Goh G, Stölting G, de Oliveira RC, Choi M, Overton JD, Fonseca AL, Korah R, Starker LF, Kunstman JW, Prasad ML, Hartung EA, Mauras N, Benson MR, Brady T, Shapiro JR, Loring E, Nelson-Williams C, Libutti SK, Mane S, Hellman P, Westin G, Åkerström G, Björklund P, Carling T, Fahlke C, Hidalgo P, Lifton RP. Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism. Nat Genet 2013; 45: 1050–1054

[74] Scholl UI, Stölting G, Nelson-Williams C, Vichot AA, Choi M, Loring E, Prasad ML, Goh G, Carling T, Juhlin CC, Quack I, Rump LC, Thiel A, Lande M, Frazier BG, Rasoulpour M, Bowlin DL, Sethna CB, Trachtman H, Fahlke C, Lifton RP. Recurrent gain of function mutation in calcium channel CACNA1H causes early-onset hypertension with primary aldosteronism. Elife 2015; 4: e06315

[75] Scholl UI, Nelson-Williams C, Yue P, Grekin R, Wyatt RJ, Dillon MJ, Couch R, Hammer LK, Harley FL, Farhi A, Wang WH, Lifton RP. Hypertension with or without adrenal hyperplasia due to different inherited mutations in the potassium channel KCNJ5. Proc Natl Acad Sci USA 2012; 109: 2533–2538

[76] Zennaro MC, Fernandes-Rosa F, Boulkroun S, Jeunemaitre X. Bilateral idiopathic adrenal hyperplasia: genetics and beyond. Horm Metab Res 2015; 47: 947–952

[77] Schinner S, Willenberg HS, Schott M, Scherbaum WA. Pathophysiological aspects of Wnt-signaling in endocrine disease. Eur J Endocrinol 2009; 160: 731–737

[78] Bertagna X. Genetics of adrenal diseases in 2014: Genetics improves understanding of adrenocortical tumours. Nat Rev Endocrinol 2015; 11: 77–78

[79] Boulkroun S, Fernandes-Rosa FL, Zennaro MC. Molecular and cellular mechanisms of aldosterone producing adenoma development. Front Endocrinol 2015; 6: 95

[80] Zennaro MC, Rickard AJ, Boulkroun S. Genetics of mineralocorticoid excess: an update for clinicians. Eur J Endocrinol 2013; 169: R15–R25

[81] Azizan EA, Brown MJ. Novel genetic determinants of adrenal aldosterone regulation. Curr Opin Endocrinol Diabetes Obes 2016; 23: 209–217

[82] Dutta RK, Söderkvist P, Gimm O. Genetics of primary hyperaldosteronism. Endocr Relat Cancer 2016; 23: R437–R454

[83]Nanba K, Chen AX, Omata K, Vinco M, Giordano TJ, Else T, Hammer GD, Tomlins SA, Rainey WE. Molecular heterogeneity in aldosterone-producing adenomas. J Clin Endocrinol Metab 2016; 101: 999–1007

[84] Dekkers T, ter Meer M, Lenders JW, Hermus AR, Schultze Kool L, Langenhuijsen JF, Nishimoto K, Ogishima T, Mukai K, Azizan EA, Tops B, Deinum J, Küsters B. Adrenal nodularity and somatic mutations in primary aldosteronism: one node is the culprit? J Clin Endocrinol Metab 2014; 99: E1341–E1351

[85] Korah HE, Scholl UI. An update on familial hyperaldosteronism. Horm Metab Res 2015; 47: 941–946

[86] Zennaro MC, Jeunemaitre X. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 5: Genetic diagnosis of primary aldosteronism. Ann Endocrinol 2016; 77: 214–219

[87] Fallo F, Bertello C, Tizzani D, Fassina A, Boulkroun S, Sonino N, Monticone S, Viola A, Veglio F, Mulatero P. Concurrent primary aldosteronism and subclinical cortisol hypersecretion: a prospective study. J Hypertens 2011; 29: 1773–1777

[88] Chu MD, Ulick S. Isolation and identification of 18-hydroxycortisol from the urine of patients with primary aldosteronism. J Biol Chem 1982; 257: 2218–2224

[89] Ulick S, Chu MD, Land M. Biosynthesis of 18-oxocortisol by aldosterone-producing adrenal tissue. J Biol Chem 1983; 258: 5498–5502

[90] Mulatero P, di Cella SM, Monticone S, Schiavone D, Manzo M, Mengozzi G, Rabbia F, Terzolo M, Gomez-Sanchez EP, Gomez-Sanchez CE, Veglio F. 18-Hydroxycorticosterone, 18-hydroxycortisol, and 18-oxocortisol in the diagnosis of primary aldosteronism and its subtypes. J Clin Endocrinol Metab 2012; 97: 881–889

[91] Morra di Cella S, Veglio F, Mulatero P, Christensen V, Aycock K, Zhu Z, Gomez-Sanchez EP, Gomez-Sanchez CE. A time-resolved fluoroimmunoassay for 18–oxocortisol and 18–hydroxycortisol. Development of a monoclonal antibody to 18–oxocortisol. J Steroid Biochem Mol Biol 2002; 82: 83–88

[92] Willenberg HS, Späth M, Maser-Gluth C, Engers R, Anlauf M, Dekomien G, Schott M, Schinner S, Cupisti K, Scherbaum WA. Sporadic solitary aldosterone and cortisol co-secreting adenomas – endocrine function tests, histological and genetic findings in a subtype of primary aldosteronism. Hypertens Res 2010; 33: 467–472

[93] Williams TA, Peitzsch M, Dietz AS, Dekkers T, Bidlingmaier M, Riester A, Treitl M, Rhayem Y, Beuschlein F, Lenders JW, Deinum J, Eisenhofer G, Reincke M. Genotype–specific steroid profiles associated with aldosterone–producing adenomas. Hypertension 2016; 67: 139–145

[94] Satoh F, Morimoto R, Ono Y, Iwakura Y, Omata K, Kudo M, Takase K, Seiji K, Sasamoto H, Honma S, Okuyama M, Yamashita K, Gomez-Sanchez CE, Rainey WE, Arai Y, Sasano H, Nakamura Y, Ito S. Measurement of peripheral plasma 18–oxocortisol can discriminate unilateral adenoma from bilateral diseases in patients with primary aldosteronism. Hypertension 2015; 65: 1096–1102

[95] Eisenhofer G, Dekkers T, Peitzsch M, Dietz AS, Bidlingmaier M, Treitl M, Williams TA, Bornstein SR, Haase M, Rump LC, Willenberg HS, Beuschlein F, Deinum J, Lenders JW, Reincke M. Mass spectrometry–based adrenal and peripheral venous steroid profiling for subtyping primary aldosteronism. Clin Chem 2016; 62: 514–524

[96] Kempers MJ, Lenders JW, van Outheusden L, van der Wilt GJ, Schultze Kool LJ, Hermus AR, Deinum J. Systematic: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. Ann Intern Med 2009; 151: 329–337

[97] Rossi GP, Auchus RJ, Brown M, Lenders JW, Naruse M, Plouin PF, Satoh F, Young WF Jr. An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. Hypertension 2014; 63: 151–160

[98] Mengozzi G, Rossato D, Bertello C, Garrone C, Milan A, Pagni R, Veglio F, Mulatero P. Rapid cortisol assay during adrenal vein sampling in patients with primary aldosteronism. Clin Chem 2007; 53: 1968–1971

[99] Auchus RJ, Michaelis C, Wians FH Jr, Dolmatch BL, Josephs SC, Trimmer CK, Anderson ME, Nwariaku FE. Rapid cortisol assays improve the success rate of adrenal vein sampling for primary aldosteronism. Ann Surg 2009; 249: 318–321

[100] Betz MJ, Degenhart C, Fischer E, Pallauf A, Brand V, Linsenmaier U, Beuschlein F, Bidlingmaier M, Reincke M. Adrenal vein sampling using rapid cortisol assays in primary aldosteronism is useful in centers with low success rates. Eur J Endocrinol 2011; 165: 301–306

[101] Dekkers T, Prejbisz A, Kool LJ, Groenewoud HJ, Velema M, Spiering W, Kołodziejczyk-Kruk S, Arntz M, Kądziela J, Langenhuijsen JF, Kerstens MN, van den Meiracker AH, van den Born BJ, Sweep FC, Hermus AR, Januszewicz A, Ligthart-Naber AF, Makai P, van der Wilt GJ, Lenders JW, Deinum J. SPARTACUS Investigators. Adrenal vein sampling versus CT scan to determine treatment in primary aldosteronism: an outcome-based randomised diagnostic trial. Lancet Diabetes Endocrinol 2016; 4: 739–746

[102] Blondin D, Quack I, Haase M, Kücükköylü S, Willenberg HS. Indication and technical aspects of adrenal blood sampling. Rofo 2015; 187: 19–28

[103] Dekkers T, Deinum J, Schultzekool LJ, Blondin D, Vonend O, Hermus AR, Peitzsch M, Rump LC, Antoch G, Sweep FC, Bornstein SR, Lenders JW, Willenberg HS, Eisenhofer G. Plasma metanephrine for assessing the selectivity of adrenal venous sampling. Hypertension 2013; 62: 1152–1157

[104] Peitzsch M, Dekkers T, Haase M, Sweep FC, Quack I, Antoch G, Siegert G, Lenders JW, Deinum J, Willenberg HS, Eisenhofer G. An LC-MS/MS method for steroid profiling during adrenal venous sampling for investigation of primary aldosteronism. J Steroid Biochem Mol Biol 2015; 145: 75–84

[105] Kem DC, Weinberger MH, Gomez-Sanchez C, Kramer NJ, Lerman R, Furuyama S, Nugent CA. Circadian rhythm of plasma aldosterone concentration in patients with primary aldosteronism. J Clin Invest 1973; 52: 2272–2277

[106] Sonoyama T, Sone M, Tamura N, Honda K, Taura D, Kojima K, Fukuda Y, Kanamoto N, Miura M, Yasoda A, Arai H, Itoh H, Nakao K. Role of endogenous ACTH on circadian aldosterone rhythm in patients with primary aldosteronism. Endocr Connect 2014; 3: 173–179

[107] Lichtenauer U, Gerum S, Asbach E, Manolopoulou J, Fourkiotis V, Quinkler M, Bidlingmaier M, Reincke M. The clinical value of salivary aldosterone in diagnosis and follow–up of Primary aldosteronism. Horm Metab Res 2016; 48: 638–643

[108] Nakamura Y, Satoh F, Morimoto R, Kudo M, Takase K, Gomez-Sanchez CE.18-Oxocortisol measurement in adrenal vein sampling as a biomarker for subclassifying primary aldosteronism. J Clin Endocrinol Metab 2011; 96: E1272–E1278

[109] Hennings J, Sundin A, Hägg A, Hellman P. 11C-metomidate positron emission tomography after dexamethasone suppression for detection of small adrenocortical adenomas in primary aldosteronism. Langenbecks Arch Surg 2010; 395: 963–967

[110] Burton TJ, Mackenzie IS, Balan K, Koo B, Bird N, Soloviev DV, Azizan EA, Aigbirhio F, Gurnell M, Brown MJ. Evaluation of the sensitivity and specificity of (11)C-metomidate positron emission tomography (PET)-CT for lateralizing aldosterone secretion by Conn's adenomas. J Clin Endocrinol Metab 2012; 97: 100–109

[111] Pechère-Bertschi A, Herpin D, Lefebvre H. SFE/SFHTA/AFCE consensus on primary aldosteronism, part 7: Medical treatment of primary aldosteronism. Ann Endocrinol 2016; 77: 226–234

[112] Sechi LA, Colussi GL, Novello M, Uzzau A, Catena C. Mineralocorticoid receptor antagonists and clinical outcomes in primary aldosteronism: as good as surgery? Horm Metab Res 2015; 47: 1000–1006

[113] Steichen O, Zinzindohoué F, Plouin P-F, Amar L. Outcomes of adrenalectomy in patients with unilateral primary aldosteronism: a review. Horm Metab Res 2012; 44: 221–227

[114] Oelkers W, Diederich S, Bähr V. Primary hyperaldosteronism without suppressed renin due to secondary hypertensive kidney damage. J Clin Endocrinol Metab 2000; 85: 3266–3270

[115] Young WF. Primary aldosteronism: renaissance of a syndrome. Clin Endocrinol 2007; 66: 607–618

[116] Tsiavos V, Markou A, Papanastasiou L, Kounadi T, Androulakis II, Voulgaris N, Zachaki A, Kassi E, Kaltsas G, Chrousos GP, Piaditis GP. A new highly sensitive and specific overnight combined screening and diagnostic test for primary aldosteronism. Eur J Endocrinol 2016; 175: 21–28

[117] Wu VC, Chang HW, Liu KL, Lin YH, Chueh SC, Lin WC, Ho YL, Huang JW, Chiang CK, Yang SY, Chen YM, Wang SM, Huang KH, Hsieh BS, Wu KD. TAIPAI Study Group. Primary aldosteronism: diagnostic accuracy of the losartan and captopril tests. Am J Hypertens 2009; 22: 821–827

[118] Asbach E, Bekeran M, Reincke M. Parathyroid gland function in primary aldosteronism. Horm Metab Res 2015; 47: 994–999

[119] Ferriss JB, Brown JJ, Cumming AM, Fraser R, Lever AF, Peacock M, Robertson JI. Primary hyperparathyroidism associated with primary hyperaldosteronism. Acta Endocrinol 1983; 103: 365–370

[120] Herd GW. A case of primary hyperparathyroidism, primary hyperaldosteronism and Cushing's disease. Acta Endocrinol 1984; 107: 371–374

[121] Maniero C, Fassina A, Guzzardo V, Lenzini L, Amadori G, Pelizzo MR, Gomez-Sanchez C, Rossi GP. Primary hyperparathyroidism with concurrent primary aldosteronism. Hypertension 2011; 58: 341–346

[122] Concistré A, Petramala L, Zinnamosca L, Settevendemmie A, Corpaci F, Marinelli C, Tonnarini GF, D'Ermo G, De Toma G, Letizia C. Primary aldosteronism with concurrent primary hyperparathyroidism: clinical case load in a single centre. Eur Rev Med Pharmacol Sci 2015; 19: 971–976

[123] Sutherland DJ, Ruse JL, Laidlaw JC. Hypertension, increased aldosterone secretion and low plasma renin activity relieved by dexamethasone. Can Med Assoc J 1966; 95: 1109–1119

[124] New MI, Peterson RE. A new form of congenital adrenal hyperplasia. J Clin Endocrinol Metab 1967; 27: 300–305

[125] Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, Lalouel JM. A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. Nature 1992; 355: 262–265

[126] Stowasser M, Gordon RD, Tunny TJ, Klemm SA, Finn WL, Krek AL. Familial hyperaldosteronism type II: five families with a new variety of primary aldosteronism. Clin Exp Pharmacol Physiol 1992; 19: 319–322

[127] Lafferty AR, Torpy DJ, Stowasser M, Taymans SE, Lin JP, Huggard P, Gordon RD, Stratakis CA. A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). J Med Genet 2000; 37: 831–835