Genetics, prevalence, screening and confirmation of primary aldosteronism: a position statement and consensus of the Working Group on Endocrine Hypertension of The European Society of Hypertension

Genetics, prevalence, screening and confirmation of primary aldosteronism: a position statement and consensus of the Working Group on Endocrine Hypertension of the European Society of Hypertension#.

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Abstract

Autonomous aldosterone overproduction represents the underlying condition of 5-10% of patients with arterial hypertension and carries a significant burden of mortality and morbidity. The diagnostic algorithm for primary aldosteronism (PA) is sequentially based on hormonal tests (screening and confirmation tests), followed by lateralization studies (adrenal CT scanning and adrenal venous sampling) to distinguish between unilateral and bilateral disease. Despite the recommendations of the Endocrine Society guideline, PA is largely underdiagnosed and undertreated with high between-centre heterogeneity. Experts from the European Society of Hypertension have critically reviewed the available literature and prepared a consensus document comprising two articles to summarize current knowledge on the epidemiology, diagnosis, treatment and complications of PA. This position paper also discusses the next challenges and future directions of research in this field.

Condensed abstract

Primary aldosteronism is the most frequent form of endocrine hypertension and carries an important burden of mortality and morbidity. Despite the availability of the Endocrine Society guideline, PA is largely underdiagnosed and undertreated with high between-centre heterogeneity. This consensus document by the working group on Endocrine Hypertension of the European Society of Hypertension, aims to address the current state-of-the-art in epidemiology, genetics, diagnostic procedures, complications and treatment options for primary aldosteronism.

INTRODUCTION

Arterial hypertension represents the leading modifiable risk factor for cardiovascular disease, accounting for 10.4 million deaths globally and 218 million attributable disability-adjusted life-years in 2017 [1]. Over half a century, randomized controlled trials have illustrated the efficacy of blood pressure lowering in reducing the risk of major cardiovascular events, including coronary artery disease, stroke and heart failure [2,3]. Despite a substantial improvement in hypertension awareness, treatment and control since the 1980s, less than half of patients on medication have blood pressure values within the normal range [3,4]. The Lancet Commission on Hypertension recently highlighted that one of the major causes of poor blood pressure control is due to an absent or delayed diagnosis of secondary forms of hypertension [3].

Primary aldosteronism (PA) is widely recognized as the most common form of secondary hypertension [5,6]. Despite this, it remains underdiagnosed and undertreated [7] with an important burden of mortality and morbidity [8,9]. Beyond its classical actions in the epithelium of the distal nephron, colon and salivary glands, where it regulates fluid and electrolyte homeostasis, in the presence of excessive salt intake, aldosterone excess exerts deleterious effects in the vascular system and the kidney, promoting oxidative stress, inflammation and fibrosis, resulting in renal and cardiovascular injury [10].

The Endocrine Society clinical practice guideline for case detection, diagnosis and treatment of patients with PA [11] provides clinicians with the best available research evidence in the field and significantly contributes to improve the quality of care. Since the last update in 2016, clinical management of patients affected by PA has evolved further and important advances have been made in understanding the genetic determinants of PA.

However, the guideline is poorly applied, resulting in a low detection rate of the disease and there is a lack of standardisation of the diagnostic flow-chart. These shortfalls prevent patients from being diagnosed and successfully cured.

The working group on Endocrine Hypertension of the European Society of Hypertension prepared this consensus document to review the available knowledge on genetics, diagnosis, treatment and outcomes of PA and focuses on how to confront unresolved issues in the field.

Part I of the consensus focuses on genetics of sporadic and familial PA, on its relatively high prevalence in patients with hypertension and synthesises the current knowledge on the optimal approaches to diagnose PA, including screening and confirmation testing.

Part II of the consensus presents the most appropriate strategies for subtype differentiation, current treatment approaches, the most common associated cardiovascular and metabolic complications and the established method for evaluation of medical and post-surgical outcomes. We will also give a prospective look on the next challenges and future directions of research in this filed.

At the end of each section a statement summarizes the most important messages. An asterisk indicates the statements that require special attention from non-specialists (such as general practitioners).

WHAT IS PRIMARY ALDOSTERONISM

PA, also known as Conn syndrome, is a group of pathological conditions associated with an aldosterone secretion inappropriate for sodium intake, that is relatively autonomous from renin-angiotensin system activity and potassium levels. Aldosterone production is therefore relatively insensitive to manoeuvres, such as sodium loading, that should suppress its secretion. The high aldosterone production for sodium status is often associated with hypertension, cardiovascular and renal damage, and hypokalaemia. The most common subtypes comprise unilateral aldosterone-producing adenomas and bilateral hyperaldosteronism; however, a continuum may exist between clearly asymmetrical and bilateral aldosterone excess. Rare subtypes are familial forms and aldosterone-producing carcinoma.

GENETICS

The approach to human genetics has changed substantially in the past 15 years: the introduction of next-generation sequencing technologies created an unprecedented opportunity to discover germline and somatic disease-causing mutations. The application of next-generation sequencing to the field of PA has given new insight into the molecular mechanisms underlying both sporadic and familial forms.

An extensive description of the genetics of PA, including the role of somatic mutations in the pathogenesis of sporadic PA is available in the supplemental file.

Germline mutations in familial primary aldosteronism.

While the majority of PA cases are sporadic, up to 5% of patients may have a familial form of the disease [12]. Four forms of familial hyperaldosteronism (FH), with autosomal dominant transmission and with a known genetic alteration, have been reported so far.

Familial hyperaldosteronism type I (FH-I or glucocorticoid remediable aldosteronism, GRA) is the most common form of monogenic hypertension [13-15]. The diagnosis is based on the amplification of the chimeric *CYP11B1/CYP11B2* gene by long-range polymerase chain reaction. Therapeutically, low dose of dexamethasone (such as 0.125-0.25 mg) to suppress ACTH – alone or in the combination with mineralocorticoid receptor antagonists - is the mainstay of treatment [11]. Patients with PA should be tested for FH-I when there is a family history of PA and/or early onset (<20 years) of the disease or in case of stroke at a young age [11].

Familial hyperaldosteronism type II (FH-II) is an early onset form of PA due to germline mutations in the *CLCN2* gene, showing incomplete penetrance [16,17]. The diagnosis is made through sequencing of the *CLCN2* gene.

Familial hyperaldosteronism type III (FH-III) is a rare form of familial PA, due to germline mutations in the *KCNJ5* gene [14,18,19]. FH-III should be ruled out in all patients with very early onset PA [11]. Genetic testing is performed by direct *KCNJ5* sequencing.

Familial hyperaldosteronism type IV (FH-IV) is a rare disorder, caused by germline mutations in the *CACNA1H* gene [20-22]. The diagnosis is made by targeted sequencing of the gene.

A further genetic but not familial form of PA has been described, named PASNA (primary aldosteronism with seizures and neurologic abnormalities) syndrome. It is a very rare condition, characterized by PA and severe neurological impairment [23], reported so far in two paediatric patients. The genetic cause is a *de novo* gain of function mutation in the *CACNA1D* gene.

Despite major technological advances facilitating the discovery of disease-causing mutations, the underlying genetic alterations in most families with two or more members affected by PA remain unidentified. This observation raises the possibility that, given the high prevalence of sporadic PA in the general population with hypertension [5], some cases of apparently familial PA may represent coincidental sporadic forms within the same family.

***Statement**. Considering the relatively low cost and non-invasive nature of genetic testing and the unequivocal benefits of an early diagnosis of a familial disorder, we suggest that genetic testing should be performed in all patients with early onset PA (i.e. < 20 years of age), irrespective of the severity of the clinical phenotype, and in patients with a family history of PA. The genetic testing of the index patient should be followed by genetic counselling and careful evaluation of first-degree relatives with hypertension to diagnose or exclude PA. Despite the possibility of coincidental occurrence of several sporadic cases in families with two or more affected subjects, genetic testing should be offered.*

PREVALENCE OF PRIMARY ALDOSTERONISM

An expanded prevalence section is available in the supplemental file.

Primary aldosteronism has long been considered a rare condition [24] however, compelling evidence indicates that PA is the most frequent form of secondary hypertension. Unilateral forms of PA (aldosterone-producing adenoma, APA, and unilateral hyperplasia) are effectively treated by

adrenalectomy, bilateral disease is treated by medical therapy based on mineralocorticoid receptor antagonists (MRAs) [11].

Currently, PA is most often diagnosed by following an algorithm advised by the Endocrine Society guideline task force, [11] based on selecting patients with a higher probability of PA, a screening test (aldosterone-to-renin ratio) and a confirmation test. However, it should be acknowledged that there is a continuum between low-renin primary (essential) hypertension and PA [25, 26] and proof of PA diagnosis is only obtained in patients who fulfill the criteria for biochemical cure after adrenalectomy for unilateral aldosterone overproduction [27]. Out of necessity we therefore depend on confirmatory test results for diagnosis. This group of tests, however, has drawbacks because the predictive properties depend on varying cut-off levels and, when results are indeterminate, are prone to subjective interpretation [11]. In addition to the bias introduced by the absence of well-established reference tests, prevalence studies also suffer from other sources of bias [28, 29].

Moreover, as for any disease condition, the prevalence depends on the population being examined, i.e. unselected hypertensive patients seen in general practice prevalence differ from those in referred patients with hypertension, with stage III and/or drug-resistant hypertension. These factors explain the high heterogeneity of prevalence estimates in different studies [28, 29] and why a recent systematic review, reported figures ranging from 3.2 to 12.7% in primary practice and from 1 to 30% in referral centers [28]. PA is an evolving condition starting with a normotensive phase [25] characterized by low renin and minimally elevated aldosterone levels progressing to arterial hypertension with a clear biochemical phenotype. The actual number of patients diagnosed with PA worldwide is likely nowhere near the expected number if all cases are diagnosed, indicating a huge and regrettable under diagnosis of a serious condition [7,30]. This raises the question if a systematic screening strategy for PA should be implemented.

Screening for PA in subgroups of hypertensive patients

The Endocrine Society guideline experts recommended selection of patients with hypertension with a higher probability of PA based on their clinical or biochemical features (Table 1). The subgroups

of patients with hypertension that may represent increased proportions of patients with PA are discussed further below (Figure 1).

Therapy-resistant hypertension and severe hypertension

There is little doubt that full-blown PA usually leads to severe hypertension in many cases, which is mostly characterized by either therapy resistance (blood pressure> 140/90 mmHg when on three antihypertensive drugs in adequate dosages, including a diuretic) or blood pressure >150/100 mmHg. It is well known that the prevalence of PA increases with the severity of hypertension [5,6,31] and can be as high as 20% in patients with resistant hypertension [32]. However, in patients with less severe hypertension [5] (or even normotension) [40] PA can also be present and when adopting the approach of subgroup screening these patients may be missed. Whether this leads to worse outcome for these patients is unknown. There are data indicating that the development of PA is gradual [25] and it might well be that patients with a mild phenotype may qualify for screening later in the course of the disease because their hypertension needs increased medication or hypokalemia sets in. This causes a delay and whether this delay leads to a worse cardiovascular prognosis is unknown.

Hypertension at younger age

Secondary hypertension is relatively more common in children and adolescents than in adults, but endocrine hypertension is thought to be an infrequent cause [41]. Although the idea that younger patients might derive more cardiovascular benefit from treatment for PA and therefore from diagnosis, the median age of patients with PA at the time of diagnosis is close to 50 years [5, 42]. The problem then is, where the cut-off level for age should be for screening? Young patients, for instance <40 years, with mild hypertension may have an early stage of PA and may not qualify for screening for other features. The benefit in terms of increased quality of life [43] can be considered at least as relevant to these patients as a better cardiovascular prognosis. There are no data to judge the trade-off between benefit of early diagnosis and the number of missed diagnoses but younger patients with severe PA will be identified by severity of their hypertension or hypokalemia anyway.

Hypokalemia

Current recommendations define the normal lower potassium limit from 3.5 to 3.8 mmol/L [44], with < 3.5 mmol/L being the most widely adopted cut-off. However, an increased prevalence of PA was observed in patients affected by arterial hypertension and serum K^+ comprised between 3.5 and 3.7 mmol/L [35].

While a large number of studies investigated the prevalence of hypokalemia in patients with PA, surprisingly, the prevalence of PA in patients with hypertension and hypokalemia is unknown. Since increased aldosterone leads to potassium loss in the collecting ducts of the kidney, hypokalemia has long been considered an essential feature of PA [24]. However, hypokalemia develops only in a proportion of patients [5,6]. Nonetheless, if present and not explained by other causes, it mandates screening for PA. This applies to diuretic-induced hypokalemia as well, but debate exists whether the cut-off value for screening should be lower than for spontaneous hypokalemia (for instance, <3 mmol/L instead of <3.5 mmol/L). Although supportive data are lacking, many centers screen for PA in all patients with hypertension who develop potassium levels below the reference range, regardless of diuretic use. In light of recent advances on subclinical PA, future studies should evaluate the efficacy and cost-effectiveness of screening for PA in all patients with spontaneous hypokalemia, regardless of blood pressure values [45].

Adrenal incidentaloma

The prevalence of PA in patients with an adrenal incidentaloma (defined as adrenal mass detected on imaging performed for other reasons than suspected adrenal disease) is 1.6%-4.33% in two studies carried out in Italy and China, respectively [36, 37]. It must be acknowledged that the studies included both patients affected by arterial hypertension and normotensive subjects and the prevalence of PA is expected to significantly increase if considering only patients with BP ≥ 140/90 mmHg [11, 46].

Family history of PA or early stroke

Although monogenic forms of PA are very rare it could be worthwhile to screen for these, especially for glucocorticoid-remediable aldosteronism that is associated with hemorrhagic stroke at a young age [47]. It is likely however that this is warranted for PA at a young age and for first-degree family

members only. Since PA is so frequent, familial co-occurrence at an older age could also be a coincidence.

Obstructive sleep apnea, metabolic syndrome and diabetes mellitus

PA is associated with conditions where obesity is a common risk factor such as obstructive sleep apnea (OSA), metabolic syndrome and diabetes mellitus [48]. Several studies reported a higher prevalence of metabolic syndrome and insulin resistance/type 2 diabetes mellitus in patients with PA, and various mechanisms involving the relevance of aldosterone excess in these conditions have been proposed [49, 50]. However, it is still to be confirmed whether higher rates of cardiovascular events reported in PA compared with essential hypertension, may be due to the increased prevalence of these metabolic alterations. With respect to OSA, conclusive evidence for a causative relation is lacking. It has also not been established if this subgroup is more likely to harbor an aldosterone-producing adenoma. According to a single study conducted on 53 patients with OSA the prevalence of PA was 34%, however the small sample size and some potential selection bias may have affected the results [51]. Despite limited available evidence, the 2016 Endocrine Society guideline recommends screening for PA in all patients with hypertension and OSA (regardless of hypertension grade) [11]. In the recent cross-sectional multi-ethnic HYPNOS study, including 203 patients with OSA, the prevalence of PA was found to be 8.9% [38], a figure not significantly different either from the prevalence reported in the general population with hypertension [5] or in tertiary referral centres [6]. Notably, when considering only patients without other indications for PA screening (SBP above 150 mmHg, DBP above 100 mmHg or hypokalemia) the prevalence dropped to 1.5%, challenging the current recommendation of the Endocrine Society guideline [38].

Atrial fibrillation

It is now well established that atrial fibrillation is a complication of PA with an unusually high incidence [9]. It is therefore conceivable that in cohorts with lone atrial fibrillation and hypertension, where atrial fibrillation is ascribed to hypertension and hypokalemia attributed to diuretic use, the prevalence of PA can be particularly high [39]. This leads to the consideration of screening for PA in

patients with hypertension and atrial fibrillation unexplained by structural heart defects and/or other conditions known to cause the arrhythmia. This contention is also supported by the observation that identification of unilateral PA followed by surgery decreased incident atrial fibrillation during long-term follow-up [52].

Statement*. *Available evidence indicates that PA is far more common than generally considered, and even if the real prevalence is not easily assessed, there is clearly a large gap between the number of patients diagnosed and the actual number of patients with PA. Screening categories of patients with hypertension advocated by the Endocrine Society guideline, with the exception of those with obstructive sleep apnea, and extending screening to patients with unexplained atrial fibrillation may help bridge this gap.*

DIAGNOSIS OF PRIMARY ALDOSTERONISM

According to the Endocrine Society guideline, the diagnosis of PA should follow a three-step approach in the vast majority of cases (Figure 1), comprising I) screening II) confirmation/exclusion testing and III) subtype diagnosis to distinguish unilateral from bilateral disease [11]

Screening test

The most reliable screening test for PA, which should be theoretically highly sensitive, is the calculation of the plasma aldosterone-to-renin ratio (ARR). However, many conditions influence the ARR thereby limiting its accuracy for the diagnosis of PA.

Plasma renin and aldosterone measurements

More detailed information on hormonal assays is provided in the supplemental file.

The most widely used method for measuring plasma renin is the direct renin concentration (DRC), even though the plasma renin activity (PRA) assay is still used in many centers.

For both DRC and PRA, careful precautions for collecting and processing blood samples at room temperature are essential to prevent inadvertent cryoactivation of plasma prorenin (inactive circulating renin) from a closed to an open conformation. This is particularly relevant in patients with low active renin values such as those with PA [53] in whom levels of inactive renin are particularly high.

Plasma aldosterone concentration (PAC) can be measured by radioimmunoassay, immunometric techniques or more recently by ultra-high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) [54, 55].

Plasma aldosterone to renin ratio (ARR)

Hiramatsu et al. were the first to report the advantage of using the ARR for the diagnosis of PA in 1981 [56]. ARR has a better sensitivity than the measurement of plasma aldosterone, renin, and potassium concentrations alone [11]. However, several methodological factors might affect the ARR and undermine its diagnostic accuracy. First, due to the lack of accuracy of DRC measurements at low concentrations, some authors recommend setting a minimum value for renin used to calculate the ARR. Some studies have set this value for DRC at 5 mUI/L [54, 57]. Second, different cut-offs have been proposed using different units of measurement for both renin and aldosterone concentrations. Third, the method used to measure PAC may also have an impact on the ARR threshold. Indeed, the aldosterone range using LC-MS/MS is usually 30% lower that measured with radioimmunoassay [54, 55] and adjustment of the current cut-offs for PA diagnostic testing is deemed necessary if PAC is measured by LC-MS/MS.

Given the heterogeneity of assay methods for measuring both PRA or DRC and aldosterone, various thresholds for ARR are used in different centers. As reported in Table 2, the most widely adopted cut-offs to define a positive ARR is 30, when aldosterone is measured in ng/dL and PRA in ng/mL/h, which should correspond to 3.7 if DRC is measured in mUI/L and a conversion factor of 8.2 is used, as suggested by the Endocrine Society guideline [11].

However, in light of recent studies comparing the performances of PRA and DRC, we suggest that a lower cut-off (between 1.12 and 2.7) [58-60] should be adopted with chemiluminescent methods. Given the low correlation between PRA and DRC for PRA values < 1 ng/mL/h we discourage using a direct conversion between DRC and PRA values. The most recent studies using LC-MS/MS as a reference standard for aldosterone measurements, propose thresholds of 45 pmol/mU (aldosterone in pmol/L and DRC in mUI/L, with a minimum set at 5 mUI/L; the threshold is 1.6 if aldosterone is measured in ng/dL) [54] or a threshold of 55 pmol/mUI without a minimum for DRC [55].

Additionally, since with very low PRA or DRC levels the ARR might also be falsely elevated with low plasma aldosterone levels, and thus it is important to include a minimum PAC for screening criteria. Some authors suggest 15 ng/dL [61, 62], while others suggest that the PAC at the screening test should not be lower that the cut-off used to define aldosterone suppression at the confirmatory test [63]. Ideally, in the view of the very large variability of the different thresholds, each laboratory should determine its own cut-off using the best available methods to measure renin and aldosterone and avoid interfering drugs at the time of blood sampling collection (see below).

Drug interference

Medications used to treat patients with arterial hypertension usually interfere with the regulation of the renin-angiotensin system (RAS) and can therefore modify plasma concentrations of both renin and aldosterone and hence the ARR [64].

An extended analysis of the effects of anti-hypertensive drugs on the ARR is available in the supplemental file and supplemental table S1.

The interference of multiple drugs given in combination on the ARR is highly variable depending on the classes and doses of the drug combination [65]. In particular, MR antagonists and β-blockers might be associated with false negative and false positive results, making the ARR difficult to interpret. Ideally, it would be preferable to stop interfering drugs before measuring the ARR. However, in many cases ARR can be confidently interpreted considering the results in light of the

known effects of antihypertensive medications, even under ACE-inhibitors, angiotensin II receptor blockers and low-doses diuretics except MRAs [11, 62] (supplemental table S1).

The delay for withdrawal of the drugs is also heterogeneous ranging from 2 to 4 weeks for beta blockers, ACE inhibitors, ARB, dihydropyridines and diuretics and from 4 to 6 weeks for spironolactone or eplerenone [11, 66]. When the complete cessation of all antihypertensive medication is not feasible, the patient should be treated with medications that have only a minimal impact on ARR (non-DHP CCBs, hydralazine, α_1-antagonists and moxonidine) [11,67]. The replacement of interfering drugs by non-interfering ones according to a standardized protocol or even drug discontinuation did not confer any increased risk of acute cardiovascular events when performed in well-controlled settings in specialized hospitals and using home-BP monitoring [68]. However, precautions are mandatory in high risk patients [69]. Other drugs known to interfere with the RAS are listed in supplemental table S1.

Other conditions influencing renin and aldosterone determinations

It is well known that, under physiological conditions and "normal" RAAS regulation, a high sodium diet lowers renin more than aldosterone, potentially leading to false positive results. On the contrary, low sodium diet increases plasma renin and, to a lesser extent, aldosterone levels, leading to false negative ARR results and according to a recent study, increases the risk of misinterpreting milder cases of primary aldosteronism [70]. It is usually recommended to measure plasma renin and aldosterone on a free dietary salt intake [11] and verification of Na^+ intake at the time of ARR testing is worth consideration [70].

A diffuse evaluation of other factor acting on renin and aldosterone measurements, including timing of the blood withdrawal, posture and food intake, the influence of gender, race and ethnicity is available in the supplemental file.

Reproducibility of ARR measurements

Despite identical time of blood sampling during the day, posture and medication intake, there is a day-to-day variability in the ARR. Rossi and coworkers report a good within-patient reproducibility of ARR in PA [71]. However, other studies have found that up to a 1/3 of patients with PA had an ARR in the normal range at some timepoint during their diagnostic workup [72]. It is thus recommended that ARR is assessed at least twice in patients with a low renin profile [66, 73, 74] but this is not mandatory for patients with elevated renin levels.

Statement*. *The ARR should be used as a screening test for PA. Aldosterone and renin should be ideally assessed without any interfering drugs. If needed, verapamil, doxazosin and moxonidine can be used as substitutive medications in patients at high risk or with severe hypertension. When interfering medications cannot be withdrawn, ARR should still be performed and results interpreted considering the confounding effects of the medications. Hypokalemia should be corrected with oral potassium chloride, and sodium intake should be unrestricted. Blood should be collected during mid-morning in the seated position.*

Confirmatory/Exclusion tests

Given the low specificity of the ARR for PA diagnosis, one or more confirmatory tests should be performed to definitively demonstrate the non-suppressibility of aldosterone production and to avoid an expensive, time-consuming and invasive work-up (Figure 1) [11,75]. It has been shown that the specificity of the ARR for PA diagnosis increases, and conversely the false positive rate decreases, with rising ARR values [57], and under predefined circumstances, i.e. spontaneous hypokalemia together with PAC >20 ng/dL and PRA (or DRC) below assay detection limits, patients may proceed directly to PA subtyping [11].

Four testing procedures are currently recommended by the Endocrine Society guideline: fludrocortisone suppression test (FST), oral sodium loading test (SLT), saline infusion test (SIT) and captopril challenge test (CCT) [11]. To date, according to available literature, there is not enough

evidence to recommend one test over the others; protocol, interpretation, advantages and drawbacks of each test are detailed in supplemental table S2. As for screening, confirmatory testing requires standardized conditions: potassium levels should be checked and hypokalemia corrected and interfering antihypertensive drugs must be considered to avoid false positive or false negative results. Over the last 20 years several studies attempted to compare the performances of two or more confirmatory testing in the diagnosis of PA, however they suffer from several limitations, including the retrospective nature, the different cut-offs adopted and, most importantly, the fact that often one test was arbitrarily chosen as a reference standard over the others [11,76] with the exception of the AQUARR Study [57]. A recent prospective study compared, with a robust methodology, the performances of SIT and CCT, using the FST as reference [77]. A total of 236 patients (129 with an ARR>3.7 ng*dL^{-1}/mIU*L^{-1} and 107 with an ARR < 3.7) completed all three confirmatory test procedures. Using post-test PAC to establish PA diagnosis, both SIT and CCT resulted as valid alternatives to the cumbersome FST, while the areas under the receiver–operator characteristics curves of the CCT fell significantly when considering the percentage of PAC reduction [77]. Similar results were obtained by Meng et al in the Chinese population [78].

Stowasser et al. showed higher sensitivity of seated SIT (post-SIT plasma aldosterone concentration cut-off 5.84 ng/dL) compared with recumbent SIT (post-SIT plasma aldosterone concentration, cut-point: 3.82 ng/dl; 87% vs. 38%), and similar specificity (94% vs 94%) [79]. Of note, PAC after seated SIT outperforms PAC post-CCT in predicting clinical outcomes after adrenalectomy in PA patients [80].

Overall, seated SIT appears reliable and less complicated than FST and SLT. CCT may be a good alternative in patients at risk of potential fluid overload, eg. patients with renal insufficiency or heart failure [77, 79].

However, there is wide variability in both the choice of confirmatory test and in cut-off values between referral centers, because of differences in patient characteristics and technical facilities. For

example, the cut point of post seated SIT plasma aldosterone concentration ranges from 5 ng/dL [5] to 16 ng/dl [81, 82].

Statement*. *Positive ARR screening for PA must be confirmed by one of four confirmatory tests. However, in patients with 1) spontaneous hypokalemia 2) PAC >20 ng/dL (550 pmol/L) and 3) PRA (or DRC) below assay detection limits, the diagnosis of PA can be made on increased ARR alone. Seated saline infusion confirmatory testing may have the best trade-off between performance and limitations. In patients at risk of potential fluid overload, the captopril challenge test may be preferred. When captopril challenge testing is performed, the evaluation of absolute aldosterone levels is recommended over percent reductions.*

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Figure legends

Figure 1.

Proposed diagnostic flow-chart for patients with PA.

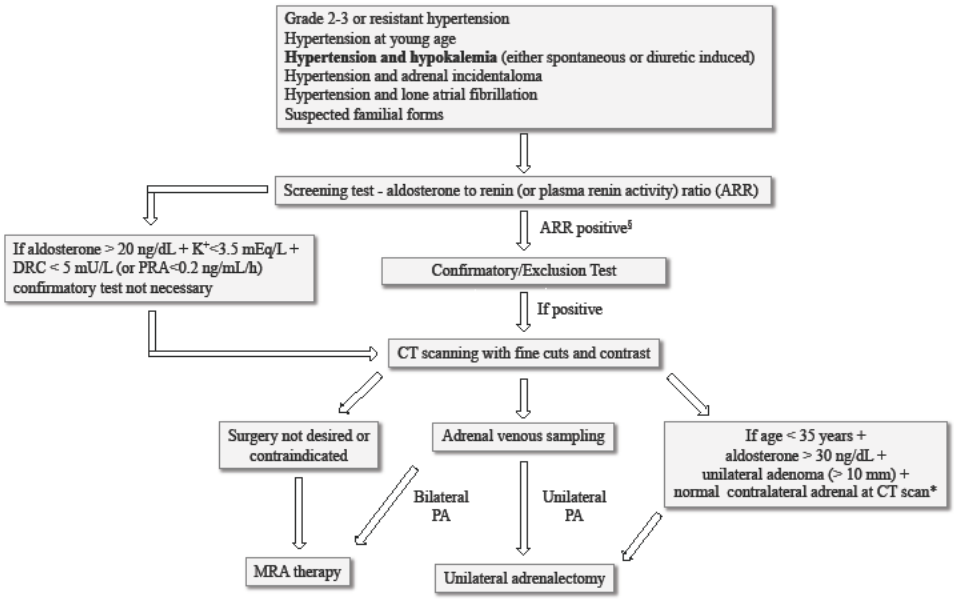
*For patients with PA and age < 35 years + aldosterone > 30 ng/dL + unilateral adenoma (> 10 mm) + normal contralateral adrenal at CT scan, adrenalectomy without AVS requirement has been suggested [11], based on 3 studies [50-52]. However, some authors prefer to perform AVS in all patients.

Subgroup	Recommendation to screen for PA	Comment
Therapy-resistant hypertension/grade 3 hypertension	Yes	Prevalence of PA increases with the severity of hypertension [5,6,31,32]
Hypertension at young age (< 40 year old)	Probably, may require lower cut-offs	No data to confirm high prevalence/benefit in young patients with hypertension [33,34]
Hypokalemia	Yes	PA prevalence in patients affected by hypertension and serum K^+<3.7 mmol/L is 28.1% and rises up to 88.5% in patients with spontaneous hypokalemia of <2.5 mmol/L [35]
Adrenal incidentaloma	Yes	Prevalence of PA in patients with adrenal incidentaloma is 1.6%-4.33% [36, 37]*
Family history of PA/early stroke	Yes	Only in young, first-degree relatives with hypertension
Obstructive sleep apnea, obesity	No	The vast majority of patients with PA are tested for blood pressure levels grade ≥ 2 or hypokalemia [38]
Atrial fibrillation	Yes	If unexplained by structural heart disease and other conditions like hyperthyroidism [39]
Grade 2 hypertension	Yes	Especially if treatment response is poor; Prevalence of PA increases with the severity of hypertension [5,6,31]
Grade 1 hypertension	Doubtful	Balance between costs and benefits should be considered

Table 1. Recommendations for PA screening in different categories of patients. * It must be acknowledged that the prevalence is calculated including also patients not affected by arterial hypertension and it is expected to double if considering only patients affected by arterial hypertension.

	PRA (ng/ml/h)	DRC (mU/L)
PAC (ng/dL)	20	1.3
	30	2
	40	2.7
PAC (pmol/L)	550	36
	830	55
	1100	74

Table 2. ARR cut-off values, depending on assay. Adopted conversion factor is: aldosterone 1 ng/dL = 27.7 pmol/L.


Grade 2-3 or resistant hypertension
Hypertension at young age
Hypertension and hypokalemia (either spontaneous or diuretic induced)
Hypertension and adrenal incidentaloma
Hypertension and lone atrial fibrillation
Suspected familial forms
Screening test - aldosterone to renin (or plasma renin activity) ratio (ARR)
ARR positive§
If aldosterone > 20 ng/dL + K+<3.5 mEq/L + DRC < 5 mU/L (or PRA<0.2 ng/mL/h) confirmatory test not necessary
Confirmatory/Exclusion Test
If positive
CT scanning with fine cuts and contrast
Surgery not desired or contraindicated
Adrenal venous sampling
If age < 35 years + aldosterone > 30 ng/dL + unilateral adenoma (> 10 mm) + normal contralateral adrenal at CT scan*
Bilateral PA
Unilateral PA
MRA therapy
Unilateral adrenalectomy

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