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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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 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, primary aldosteronism 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 constituting two articles to summarize current knowledge on the epidemiology, diagnosis, treatment, and complications of primary aldosteronism.

Keywords: aldosterone, primary aldosteronism, rennin, saline-loading test

Abbreviations: ACTH, adrenocorticotropic hormone; APA, aldosterone-producing adenoma; ARR, aldosterone-to-renin ratio; DRC, direct renin concentration; GRA, glucocorticoid remediable aldosteronism; LCMS/MS, liquid chromatography and tandem mass spectrometry; MRAs, mineralocorticoid receptor antagonists; OSA, obstructive sleep apnea; PAC, plasma aldosterone concentration; PASNA, primary aldosteronism with seizures and neurologic abnormalities; PRA, plasma renin activity; RAS, renin–angiotensin system

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 because of an

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absent or delayed diagnosis of secondary forms of hypertension [3].

Primary aldosteronism 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 primary aldosteronism [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 primary aldosteronism has evolved further and important advances have been made in understanding the genetic determinants of primary aldosteronism.

However, the guideline is poorly applied, resulting in a low detection rate of the disease, and there is a lack of standardization 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 primary aldosteronism and focuses on how to confront unresolved issues in the field.

Part I of the consensus focuses on genetics of sporadic and familial primary aldosteronism, on its relatively high prevalence in patients with hypertension and synthesizes the current knowledge on the optimal approaches to diagnose primary aldosteronism, 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 postsurgical outcomes. We will also give a prospective look on the next challenges and future directions of research in this field.

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

WHAT IS PRIMARY ALDOSTERONISM

Primary aldosteronism, 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 constitute 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 primary aldosteronism has given new insight into the molecular mechanisms underlying both sporadic and familial forms.

An extensive description of the genetics of primary aldosteronism, including the role of somatic mutations in the pathogenesis of sporadic primary aldosteronism is available in the supplemental file, http://links.lww.com/HJH/B363.

Germline mutations in familial primary aldosteronism

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

Familial hyperaldosteronism type I

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 PCR. 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 primary aldosteronism should be tested for FH-I when there is a family history of primary aldosteronism and/or early onset (<20 years) of the disease or in case of stroke at a young age [11].

Familial hyperaldosteronism type II

Familial hyperaldosteronism type II (FH-II) is an early onset form of primary aldosteronism caused by 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

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

Familial hyperaldosteronism type IV

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 primary aldosteronism has been described, named PASNA (primary aldosteronism with seizures and neurologic abnormalities) syndrome. It is a very rare condition, characterized by primary aldosteronism 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 primary aldosteronism remain unidentified. This observation raises the possibility that, given the high prevalence of sporadic primary aldosteronism in the general population with hypertension [5], some cases of apparently familial primary aldosteronism may represent coincidental sporadic forms within the same family.

Statement. *Considering the relatively low cost and noninvasive 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 primary aldosteronism (i.e. <20 years of age), irrespective of the severity of the clinical phenotype, and in patients with a family history of primary aldosteronism. 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 primary aldosteronism. Despite the possibility of coincidental occurrence of several sporadic cases in families with two or more affected patients, genetic testing should be offered.*

PREVALENCE OF PRIMARY ALDOSTERONISM

An expanded prevalence section is available in the supplemental file, http://links.lww.com/HJH/B363.

Primary aldosteronism has long been considered a rare condition [24]; however, compelling evidence indicates that primary aldosteronism is the most frequent form of secondary hypertension. Unilateral forms of primary aldosteronism (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, primary aldosteronism 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 primary aldosteronism, 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 primary aldosteronism [25,26] and proof of primary aldosteronism 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 as 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, that is, 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]. Primary aldosteronism 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 primary aldosteronism 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 primary aldosteronism should be implemented.

Screening for primary aldosteronism in subgroups of hypertensive patients

The Endocrine Society guideline experts recommended selection of patients with hypertension with a higher probability of primary aldosteronism based on their clinical or biochemical features (Table 1). The subgroups of patients with hypertension that may represent increased proportions of patients with primary aldosteronism are discussed further below (Fig. 1).

Therapy-resistant hypertension and severe hypertension

There is little doubt that full-blown primary aldosteronism 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 greater than 150/100 mmHg. It is well known that the prevalence of primary aldosteronism 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] primary aldosteronism 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 primary aldosteronism 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 as 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.

TABLE 1. Recommendations for primary aldosteronism screening in different categories of patients

Subgroup	Recommendation to screen for primary aldosteronism	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 years 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 less than 2.5 mmol/l [35]
Adrenal incidentaloma	Yes	Prevalence of PA in patients with adrenal incidentaloma is 1.6 to 4.33% [36,37][a]
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 at least 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

PA, primary aldosteronism.
[a]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.

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 primary aldosteronism, and therefore, from diagnosis, the median age of patients with primary aldosteronism 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 less than 40 years, with mild hypertension may have an early stage of primary aldosteronism and may not qualify for screening for other features. The benefit in terms of increased quality of life [43] can be

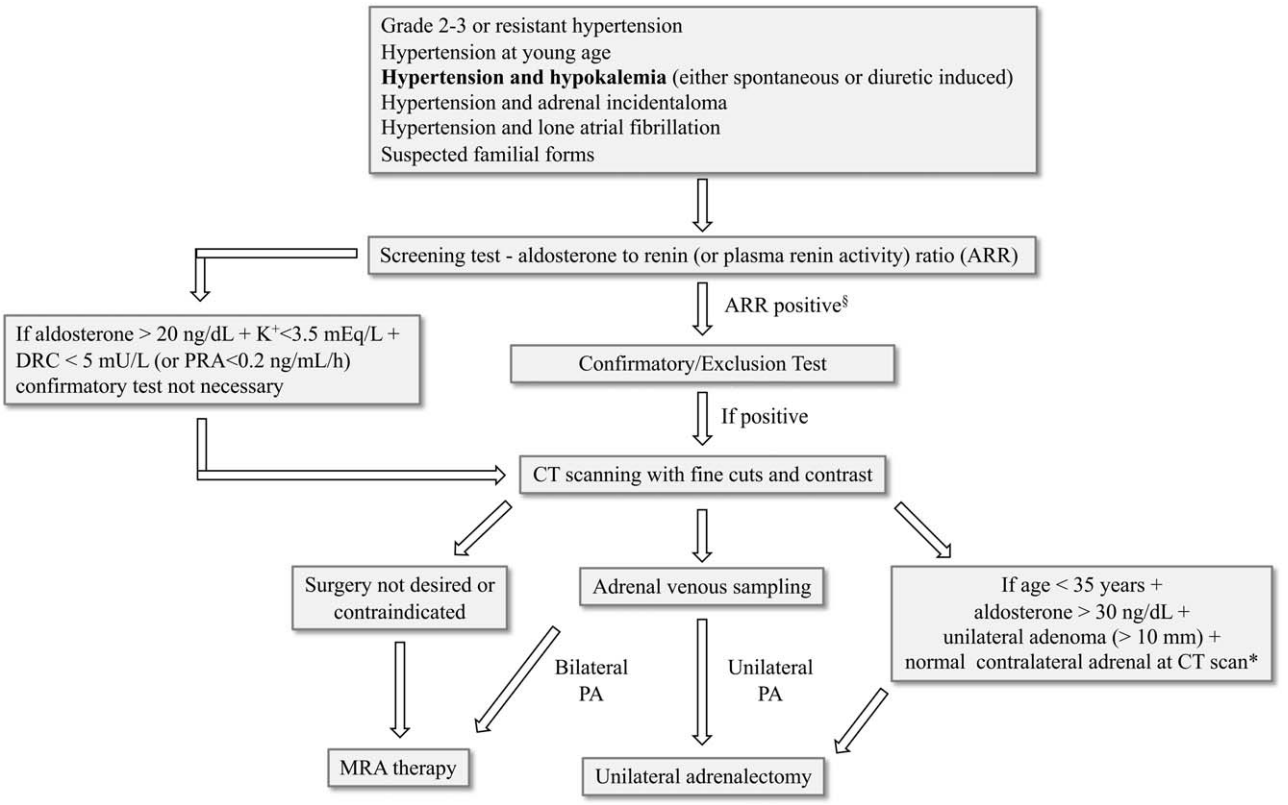

FIGURE 1 Proposed diagnostic flow-chart for patients with primary aldosteronism. *For patients with primary aldosteronism and aged less than 35 years with aldosterone greater than 30 ng/dl and unilateral adenoma (>10 mm) with normal contralateral adrenal at computed tomography (CT) scan, adrenalectomy without AVS requirement has been suggested [11], based on three studies [50–52]. However, some authors prefer to perform AVS in all patients.

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 primary aldosteronism 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 less than 3.5 mmol/l being the most widely adopted cut-off. However, an increased prevalence of primary aldosteronism was observed in patients affected by arterial hypertension and serum K^+ constituted between 3.5 and 3.7 mmol/l [35].

Although a large number of studies investigated the prevalence of hypokalemia in patients with primary aldosteronism, surprisingly, the prevalence of primary aldosteronism in patients with hypertension and hypokalemia is unknown. As increased aldosterone leads to potassium loss in the collecting ducts of the kidney, hypokalemia has long been considered an essential feature of primary aldosteronism [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 primary aldosteronism. 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 primary aldosteronism in all patients with hypertension who develop potassium levels below the reference range, regardless of diuretic use. In light of recent advances on subclinical primary aldosteronism, future studies should evaluate the efficacy and cost-effectiveness of screening for primary aldosteronism in all patients with spontaneous hypokalemia, regardless of blood pressure values [45].

Adrenal incidentaloma

The prevalence of primary aldosteronism 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 primary aldosteronism is expected to significantly increase if considering only patients with BP at least 140/90 mmHg [11,46].

Family history of primary aldosteronism or early stroke

Although monogenic forms of primary aldosteronism 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 primary aldosteronism at a young age and for first-degree family members only. As primary aldosteronism is so frequent, familial co-occurrence at an older age could also be a coincidence.

Obstructive sleep apnea, metabolic syndrome, and diabetes mellitus

Primary aldosteronism 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 primary aldosteronism, 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 primary aldosteronism compared with essential hypertension, may be because of 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 primary aldosteronism 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 primary aldosteronism in all patients with hypertension and OSA (regardless of hypertension grade) [11]. In the recent cross-sectional multiethnic HYPNOS study, including 203 patients with OSA, the prevalence of primary aldosteronism 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 primary aldosteronism 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 primary aldosteronism 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 primary aldosteronism can be particularly high [39]. This leads to the consideration of screening for primary aldosteronism 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 primary aldosteronism followed by surgery decreased incident atrial fibrillation during long-term follow-up [52].

Statement*. *Available evidence indicates that primary aldosteronism 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 primary aldosteronism. 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 primary aldosteronism should follow a three-step approach in the vast majority of cases (Fig. 1), constituting: screening; confirmation/exclusion testing; and subtype diagnosis to distinguish unilateral from bilateral disease [11].

Screening test

The most reliable screening test for primary aldosteronism, 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 primary aldosteronism.

Plasma renin and aldosterone measurements

More detailed information on hormonal assays is provided in the supplemental file, http://links.lww.com/HJH/B363.

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 primary aldosteronism [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

Hiramatsu *et al.* [56] were the first to report the advantage of using the ARR for the diagnosis of primary aldosteronism in 1981. 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, because of 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 than that measured with radioimmunoassay [54,55] and adjustment of the current cut-offs for primary aldosteronism 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].

TABLE 2. Aldosterone-to-renin cut-off values, depending on assay

	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

Adopted conversion factor is: aldosterone 1 ng/dl = 27.7 pmol/l. DRC, direct renin concentration; PAC, plasma aldosterone concentration; PRA, plasma renin activity.

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 less than 1 ng/ml per/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, as with very low PRA or DRC levels, the ARR might also be falsely elevated with low plasma aldosterone levels, it is important to include a minimum PAC for screening criteria. Some authors suggest 15 ng/dl [61,62], whereas others suggest that the PAC at the screening test should not be lower than 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 antihypertensive drugs on the ARR is available in the supplemental file and Supplemental Table S1, http://links.lww.com/HJH/B363.

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-dose diuretics

except MRAs [11,62] (Supplemental Table S1, http://links.lww.com/HJH/B363).

The delay for withdrawal of the drugs is also heterogeneous ranging from 2 to 4 weeks for beta blockers, ACE inhibitors, ARB, dihydropyridine calcium channel blockers 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-dihydropyridine calcium channel blockers, hydralazine, α_1-antagonists, and moxonidine) [11,67]. The replacement of interfering drugs by noninterfering 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, http://links.lww.com/HJH/B363.

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 sex, race, and ethnicity is available in the supplemental file, http://links.lww.com/HJH/B363.

Reproducibility of aldosterone-to-renin 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 *et al.* report a good within-patient reproducibility of ARR in primary aldosteronism [71]. However, other studies have found that up to a 1/3 of patients with primary aldosteronism 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 primary aldosteronism. 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 primary aldosteronism diagnosis, one or more confirmatory tests should be performed to definitively demonstrate the nonsuppressibility of aldosterone production and to avoid an expensive, time-consuming, and invasive work-up (Fig. 1) [11,75]. It has been shown that the specificity of the ARR for primary aldosteronism diagnosis increases, and conversely the false positive rate decreases, with rising ARR values [57], and under predefined circumstances, that is, spontaneous hypokalemia together with PAC greater than 20 ng/dl and PRA (or DRC) below assay detection limits, patients may proceed directly to primary aldosteronism 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, http://links.lww.com/HJH/B363. 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 primary aldosteronism; 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/mIU/l and 107 with an ARR <3.7) completed all three confirmatory test procedures. Using posttest PAC to establish primary aldosteronism 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.* [78] in the Chinese population.

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 primary aldosteronism 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, for example, 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 [80,81].

Statement[*]. *Positive ARR screening for primary aldosteronism must be confirmed by one of four confirmatory tests. However, in patients with spontaneous hypokalemia, PAC greater than 20 ng/dl (550 pmol/l), and PRA (or DRC) below assay detection limits, the diagnosis of primary aldosteronism 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 percentage reductions.*

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Conflicts of interest

There are no conflicts of interest.

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