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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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1 **Abstract**

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

12

13 **Condensed abstract**

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

20

21

22 **INTRODUCTION**

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

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

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

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

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

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

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

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

59

60 **WHAT IS PRIMARY ALDOSTERONISM**

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

70

71 **GENETICS**

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

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

79 **Germline mutations in familial primary aldosteronism.**

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

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

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

93 Familial hyperaldosteronism type III (FH-III) is a rare form of familial PA, due to germline mutations
94 in the *KCNJ5* gene [14,18,19]. FH-III should be ruled out in all patients with very early onset PA
95 [11]. Genetic testing is performed by direct *KCNJ5* sequencing.

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

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

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

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

115

116 **PREVALENCE OF PRIMARY ALDOSTERONISM**

117 An expanded prevalence section is available in the supplemental file.

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

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

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

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

144 **Screening for PA in subgroups of hypertensive patients**

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

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

149 **Therapy-resistant hypertension and severe hypertension**

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

161 **Hypertension at younger age**

162 Secondary hypertension is relatively more common in children and adolescents than in adults, but
163 endocrine hypertension is thought to be an infrequent cause [41]. Although the idea that younger
164 patients might derive more cardiovascular benefit from treatment for PA and therefore from
165 diagnosis, the median age of patients with PA at the time of diagnosis is close to 50 years [5, 42]. The
166 problem then is, where the cut-off level for age should be for screening? Young patients, for instance
167 <40 years, with mild hypertension may have an early stage of PA and may not qualify for screening
168 for other features. The benefit in terms of increased quality of life [43] can be considered at least as
169 relevant to these patients as a better cardiovascular prognosis. There are no data to judge the trade-
170 off between benefit of early diagnosis and the number of missed diagnoses but younger patients with
171 severe PA will be identified by severity of their hypertension or hypokalemia anyway.

172 **Hypokalemia**

173 Current recommendations define the normal lower potassium limit from 3.5 to 3.8 mmol/L [44], with
174 < 3.5 mmol/L being the most widely adopted cut-off. However, an increased prevalence of PA was
175 observed in patients affected by arterial hypertension and serum K⁺ comprised between 3.5 and 3.7
176 mmol/L [35].

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

189 **Adrenal incidentaloma**

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

195 **Family history of PA or early stroke**

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

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

201 **Obstructive sleep apnea, metabolic syndrome and diabetes mellitus**

202 PA is associated with conditions where obesity is a common risk factor such as obstructive sleep
203 apnea (OSA), metabolic syndrome and diabetes mellitus [48]. Several studies reported a higher
204 prevalence of metabolic syndrome and insulin resistance/type 2 diabetes mellitus in patients with PA,
205 and various mechanisms involving the relevance of aldosterone excess in these conditions have been
206 proposed [49, 50]. However, it is still to be confirmed whether higher rates of cardiovascular events
207 reported in PA compared with essential hypertension, may be due to the increased prevalence of these
208 metabolic alterations. With respect to OSA, conclusive evidence for a causative relation is lacking. It
209 has also not been established if this subgroup is more likely to harbor an aldosterone-producing
210 adenoma. According to a single study conducted on 53 patients with OSA the prevalence of PA was
211 34%, however the small sample size and some potential selection bias may have affected the results
212 [51]. Despite limited available evidence, the 2016 Endocrine Society guideline recommends
213 screening for PA in all patients with hypertension and OSA (regardless of hypertension grade) [11].
214 In the recent cross-sectional multi-ethnic HYPNOS study, including 203 patients with OSA, the
215 prevalence of PA was found to be 8.9% [38], a figure not significantly different either from the
216 prevalence reported in the general population with hypertension [5] or in tertiary referral centres [6].
217 Notably, when considering only patients without other indications for PA screening (SBP above 150
218 mmHg, DBP above 100 mmHg or hypokalemia) the prevalence dropped to 1.5%, challenging the
219 current recommendation of the Endocrine Society guideline [38].

220 **Atrial fibrillation**

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

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

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

235

236 **DIAGNOSIS OF PRIMARY ALDOSTERONISM**

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

240 **Screening test**

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

244 **Plasma renin and aldosterone measurements**

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

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

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

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

256 **Plasma aldosterone to renin ratio (ARR)**

257 Hiramatsu et al. were the first to report the advantage of using the ARR for the diagnosis of PA in
258 1981 [56]. ARR has a better sensitivity than the measurement of plasma aldosterone, renin, and
259 potassium concentrations alone [11]. However, several methodological factors might affect the ARR
260 and undermine its diagnostic accuracy. First, due to the lack of accuracy of DRC measurements at
261 low concentrations, some authors recommend setting a minimum value for renin used to calculate the
262 ARR. Some studies have set this value for DRC at 5 mUI/L [54, 57]. Second, different cut-offs have
263 been proposed using different units of measurement for both renin and aldosterone concentrations.
264 Third, the method used to measure PAC may also have an impact on the ARR threshold. Indeed, the
265 aldosterone range using LC-MS/MS is usually 30% lower than measured with radioimmunoassay [54,
266 55] and adjustment of the current cut-offs for PA diagnostic testing is deemed necessary if PAC is
267 measured by LC-MS/MS.

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

273 However, in light of recent studies comparing the performances of PRA and DRC, we suggest that a
274 lower cut-off (between 1.12 and 2.7) [58-60] should be adopted with chemiluminescent methods.
275 Given the low correlation between PRA and DRC for PRA values < 1 ng/mL/h we discourage using
276 a direct conversion between DRC and PRA values. The most recent studies using LC-MS/MS as a
277 reference standard for aldosterone measurements, propose thresholds of 45 pmol/mU (aldosterone in
278 pmol/L and DRC in mUI/L, with a minimum set at 5 mUI/L; the threshold is 1.6 if aldosterone is
279 measured in ng/dL) [54] or a threshold of 55 pmol/mUI without a minimum for DRC [55].
280 Additionally, since with very low PRA or DRC levels the ARR might also be falsely elevated with
281 low plasma aldosterone levels, and thus it is important to include a minimum PAC for screening
282 criteria. Some authors suggest 15 ng/dL [61, 62], while others suggest that the PAC at the screening
283 test should not be lower than the cut-off used to define aldosterone suppression at the confirmatory
284 test [63]. Ideally, in the view of the very large variability of the different thresholds, each laboratory
285 should determine its own cut-off using the best available methods to measure renin and aldosterone
286 and avoid interfering drugs at the time of blood sampling collection (see below).

287 **Drug interference**

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

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

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

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

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

310 **Other conditions influencing renin and aldosterone determinations**

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

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

321

322 **Reproducibility of ARR measurements**

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

329

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

337

338 **Confirmatory/Exclusion tests**

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

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

349 evidence to recommend one test over the others; protocol, interpretation, advantages and drawbacks
350 of each test are detailed in supplemental table S2. As for screening, confirmatory testing requires
351 standardized conditions: potassium levels should be checked and hypokalemia corrected and
352 interfering antihypertensive drugs must be considered to avoid false positive or false negative results.
353 Over the last 20 years several studies attempted to compare the performances of two or more
354 confirmatory testing in the diagnosis of PA, however they suffer from several limitations, including
355 the retrospective nature, the different cut-offs adopted and, most importantly, the fact that often one
356 test was arbitrarily chosen as a reference standard over the others [11,76] with the exception of the
357 AQUARR Study [57]. A recent prospective study compared, with a robust methodology, the
358 performances of SIT and CCT, using the FST as reference [77]. A total of 236 patients (129 with an
359 $ARR > 3.7 \text{ ng} \cdot \text{dL}^{-1} / \text{mIU} \cdot \text{L}^{-1}$ and 107 with an $ARR < 3.7$) completed all three confirmatory test
360 procedures. Using post-test PAC to establish PA diagnosis, both SIT and CCT resulted as valid
361 alternatives to the cumbersome FST, while the areas under the receiver–operator characteristics
362 curves of the CCT fell significantly when considering the percentage of PAC reduction [77]. Similar
363 results were obtained by Meng et al in the Chinese population [78].
364 Stowasser et al. showed higher sensitivity of seated SIT (post-SIT plasma aldosterone concentration
365 cut-off 5.84 ng/dL) compared with recumbent SIT (post-SIT plasma aldosterone concentration, cut-
366 point: 3.82 ng/dl; 87% vs. 38%), and similar specificity (94% vs 94%) [79]. Of note, PAC after seated
367 SIT outperforms PAC post-CCT in predicting clinical outcomes after adrenalectomy in PA patients
368 [80].
369 Overall, seated SIT appears reliable and less complicated than FST and SLT. CCT may be a good
370 alternative in patients at risk of potential fluid overload, eg. patients with renal insufficiency or heart
371 failure [77, 79].
372 However, there is wide variability in both the choice of confirmatory test and in cut-off values
373 between referral centers, because of differences in patient characteristics and technical facilities. For

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

376

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

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11 **Figure legends**

12 **Figure 1.**

13 **Proposed diagnostic flow-chart for patients with PA.**

14 *For patients with PA and age < 35 years + aldosterone > 30 ng/dL + unilateral adenoma (> 10
15 mm) + normal contralateral adrenal at CT scan, adrenalectomy without AVS requirement has been
16 suggested [11], based on 3 studies [50-52]. However, some authors prefer to perform AVS in all
17 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

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

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	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

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

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