

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Approach to the Patient on Antihypertensive Therapy: Screen for Primary Aldosteronism

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1888276> since 2023-01-29T16:59:03Z

Published version:

DOI:10.1210/clinem/dgac460

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Approach to the Patient on Anti-Hypertensive Therapy: Screen for Primary Aldosteronism.**

2 Paolo Mulatero^{1*}, Chiara Bertello¹, Franco Veglio¹, Silvia Monticone¹

3 ¹Division of Internal Medicine and Hypertension Unit, Department of Medical Sciences, University
4 of Torino, 10126, Torino, Italy (P.M., C.B., F.V., S.M.)

5 **Running title:** Screen for primary aldosteronism under treatment

6 **Key words:** aldosterone, primary aldosteronism, aldosterone to renin ratio, renin, secondary
7 hypertension

8 ***Correspondence to:** Paolo Mulatero, MD ORCID 0000-0002-5480-1116

9 Phone: +39.011.633.6959 / +39.011.633.6931

10 E-mail: paolo.mulatero@unito.it

11 **Word count:** 5096 (including references and figure legend)

12 **Grants or fellowships supporting the paper:** none

13 **Disclosure summary:** P.M. received fees for educational speeches from DIASORIN

14

15

16

17

18

19

20

21

22

23

24

25 **Abstract**

26 Primary aldosteronism is a condition that is still largely overlooked resulting in a significant burden
27 of mortality and morbidity. This is despite decades of clinical and translational research on the
28 deleterious effects of aldosterone on the cardiovascular system and the publication of several
29 guidelines and consensus on its diagnosis and treatment. One of the main reasons for the low rate
30 of testing is the difficulty of screening patients under anti-hypertensive therapy that potentially
31 interfere with aldosterone and renin levels and thus confound the interpretation of the aldosterone to
32 renin ratio, the accepted and conventionally used screening test. To avoid interference, usually the
33 therapies that affect the renin-angiotensin aldosterone system are withdrawn and substituted with non-
34 interfering medications. However, in many cases the screening test can be confidently interpreted
35 even when such therapies are not discontinued. In this review, we will evaluate the effects of anti-
36 hypertensive therapies on the screening test for primary aldosteronism and suggest a practical
37 approach for its interpretation.

38

39 **Background**

40 In recent decades, research in humans and animal models provided solid evidence that aldosterone
41 excess, in the presence of high sodium, causes adverse effects to the cardiovascular system (1,2,3), at
42 least partially independent from blood pressure levels. Patients with primary aldosteronism (PA)
43 display an increased prevalence of cardio- and cerebrovascular and renal complications compared
44 with patients with essential hypertension, even when they are matched for common risk factors
45 including blood pressure levels and duration of hypertension (4,5). PA is a frequent cause of
46 secondary hypertension and should be ruled out in most patients with hypertension since a specific
47 pharmacological treatment is available for patients with bilateral PA and potentially curative surgery
48 for unilateral forms (1,2). The prevalence of PA varies between around 5% in patients with
49 hypertension in primary care and around 10% in referral hypertension centres (6-8).

50 The Endocrine Society Guideline and a European Society of Hypertension (ESH) consensus suggest
51 screening patients with hypertension at a high risk of having PA (1,2) who represent more than 50%
52 of patients with hypertension. The ESH consensus suggests screening patients with blood pressure
53 values higher than 160/100 mmHg or resistant hypertension, patients with hypertension and
54 hypokalemia (either spontaneous or diuretic-induced), hypertension and incidentaloma, hypertension
55 and atrial fibrillation (not caused by an underlying cardiac disease), young patients with hypertension
56 (less than 40 years) and in cases of a family history of PA (1). The Endocrine Society Guideline also
57 suggests screening patients with hypertension and obstructive sleep apnea (2). By contrast, Japanese
58 guidelines and some experts suggest that all patients with hypertension should be screened, regardless
59 of pre-test probability (9-11). Despite these recommendations, in Europe and in the US (12-16), less
60 than 5% of patients with hypertension are tested and this disappointingly low screening rate has not
61 (13) or only marginally increased (14) compared with 20 years ago. Even when hypertension is
62 associated with severe hypokalemia (potassium levels less than 3.0 mEq/L), the rate of PA screening
63 is <4% (17). This is surprising since it has been shown that in patients with hypertension and severe
64 spontaneous hypokalemia the probability of PA is higher than 70% (18). The Lancet Commission
65 identified the lack or delayed diagnosis of secondary hypertension as one of the determining factors
66 of global insufficient blood pressure control (19). All guidelines suggest screening with the
67 aldosterone-to-renin ratio (ARR); renin can be measured as plasma renin activity (PRA) or as direct
68 renin concentration (DRC). The most frequently adopted cut-off for screening is 30 (ng/dL/ng/mL/h)
69 when PRA is measured (2), and around 2 (ng/dL/mU/L) when DRC is used (1). This is lower than
70 the 3.7 cut-off suggested by the ES Guideline; the cut-off suggested by the ESH consensus is based
71 on subsequent studies (20-22) that showed that the ARR displays the highest sensitivity between 1
72 and 2.7. Furthermore, a study in Chinese patients showed that using 3.7 instead of 2 as an ARR cut-
73 off would miss 18% of patients with PA (23). However, it should be underlined that the ARR should
74 be interpreted as a continuous rather than a categorical (yes/no) variable (24) and thus the higher the
75 ARR the higher the probability of having PA (1,2,24). In some florid cases of positive ARR, for

76 example, when renin is below the detection limit, aldosterone is above 20 ng/dL and concomitant
77 hypokalemia is present (potassium < 3.5 mEq/L), the diagnosis of PA is confirmed without the need
78 for suppression testing (1,2).

79 One of the reasons for the low rate of PA screening is that clinicians are reluctant to leave the patient
80 untreated or to withdraw potentially interfering anti-hypertensive therapy for the time necessary for
81 screening and, when necessary, confirmation/exclusion testing. Furthermore, the patient is often
82 under treatment with medication that can interfere with the levels of aldosterone and renin, making
83 the interpretation of the screening test more difficult. However, in many cases, it is possible to
84 interpret ARR results even under anti-hypertensive treatment by taking into account the specific effect
85 of each interfering medication on the renin-angiotensin-aldosterone system (RAAS)(25-27).

86 Recently, a clinical score (STOP-PA score) and a machine learning algorithm have been shown to
87 efficiently predict the individual pre-test probability of having PA in patients with hypertension. This
88 can potentially reduce the number of patients for screening by 33% without missing patients with
89 unilateral PA (28).

90 We will discuss below some selected and illustrative cases in which the ARR is interpreted during
91 interfering anti-hypertensive therapy and clinical decisions are taken in accordance with the expected
92 effects of these drugs on the RAAS.

93 **Clinical case 1**

94 Patient 1 is a 50 year-old male under treatment with ramipril 10 mg and hydrochlorothiazide 25 mg,
95 blood pressure (BP) levels of 150/100 mm/Hg, potassium (K⁺) levels 3.4 mEq/L, left ventricular
96 hypertrophy at echocardiography, body mass index (BMI) 24 kg/m². The patient is then visited by a
97 hypertension specialist for a second opinion. The specialist prescribes an ARR test, but the patient is
98 reluctant to interrupt therapy and undergoes ARR testing without changing medications. DRC is 20

99 mU/L (PRA 1.6 ng/ml/h) and aldosterone 30 ng/dL, ARR-DRC = 1.5 (ARR-PRA = 18.7); urinary
100 sodium is 145 mEq/day.

101 The ARR test is negative, but not far from the cut-off for positivity. Notably, the patient is taking 2
102 medications that are potentially responsible for a false-negative ARR result (1,2,29,30). Both
103 angiotensin-converting enzyme inhibitors (ACE-Is) and diuretics increase DRC and PRA levels,
104 while diuretics increase and ACE-Is reduce aldosterone levels (Table 1). Therefore, we can
105 hypothesize that the substitution of hydrochlorothiazide with a calcium channel blocker (DHP-CCB),
106 such as amlodipine or verapamil, could result in a reduction of DRC and PRA and an increase of the
107 ARR above the cut-off for positivity (2 with DRC ng/dL/mu/L and 30 with PRA ng/dL/ng/mL/h).
108 Suspicion for PA in this patient is also raised by the diuretic-induced hypokalemia (which in turn can
109 reduce the ARR giving false negative results) (29). The risk of having PA for this patient according
110 to a recently validated pre-test clinical score is 41% (24% probability of unilateral PA) (28).
111 Repetition of screening under ramipril and amlodipine gave positive results (DRC 8 mU/L, PRA 0.6
112 ng/mL/h, aldosterone 26 ng/dL, ARR-DRC= 3.2 and ARR-PRA= 43.3, respectively); K⁺ levels were
113 now 3.7 mEq/L. After confirmation, CT scanning and adrenal vein sampling, the patient underwent
114 unilateral adrenalectomy with pathology showing an aldosterone-producing nodule (31) of 8 mm.
115 After surgery the patient had complete clinical and biochemical success (32), that is, PA was cured
116 and the patient became normotensive without therapy.

117 **Clinical case 2**

118 Patient 2 is a 55 year-old female under therapy with olmesartan 40 mg/amlodipine 10 mg, BP is
119 155/85 mmHg and K⁺ 4.4 mEq/L. DRC is 25 mU/L (PRA 2 ng/ml/h), aldosterone 9 ng/dL, ARR-
120 DRC= 0.36 (ARR-PRA= 4.5). This patient has a negative ARR. Theoretically, olmesartan can be
121 responsible for a false-negative ARR (1,2,25,28,32) (Table 1). However, aldosterone levels are lower
122 than the cut-off for a positive captopril-challenge test result and therefore, the probability that the
123 ARR becomes positive after olmesartan withdrawal is very low and a diagnosis of PA can be

124 excluded. The patient was tested again after substitution of the therapy with verapamil 240 mg and
125 doxazosin 4 mg per day: DRC was 16 mU/L, PRA 1.2 ng/ml/h aldosterone 13.5 ng/dL, ARR-DRC=
126 0.84 and ARR-PRA= 11.5.

127 **Clinical case 3**

128 Patient 3 is a 60 year-old male under therapy with atenolol 100 mg and nifedipine 60 mg. BP is 155/90
129 mmHg and K⁺4.6 mEq/L. DRC is 10 mU/L (PRA 0.7 ng/ml/h), aldosterone 13 ng/dL, ARR-DRC=
130 1.3 (ARR-PRA= 18.6).

131 This patient has a negative ARR under atenolol that can give a false-positive result (1,2,25,28,32,33)
132 (Table 1) and therefore, a diagnosis of PA is excluded.

133 **Clinical case 4**

134 Patient 4 is a 48 year-old male under therapy with chlortalidone 25 mg, valsartan 320 mg, amlodipine
135 10 mg. BP is 150/100 mmHg, K⁺ 3.3 mEq/L. DRC is 15 mU/L (PRA 1 ng/ml/h), aldosterone 30
136 ng/dL, ARR-DRC= 2 (ARR-PRA= 30).

137 In this case we have a borderline positive ARR under an angiotensin-II receptor blocker (ARB) and
138 a diuretic, which can both give false-negative results and hypokalemia that can result in the same
139 alteration (1,2,26,29,33) (Table 1). Therefore, we should consider the patients to be affected by PA
140 with a high probability and proceed with the diagnostic flow-chart (1,2). After adrenalectomy, the
141 final diagnosis was a unilateral aldosterone-producing adenoma (15 mm diameter) and the patient
142 displayed complete biochemical success and partial clinical success (normotension under amlodipine
143 5 mg per day) (32).

144 **Clinical case 5**

145 Patient 5 is a 76 year-old male, with hypertension since he was 50 years' old, under therapy with
146 nebivolol 5 mg and chlortalidone 25 mg. BP is 160/70 mmHg, K⁺4.6 mEq/L, eGFR 45 ml/min/m^{1.73}.
147 The patient was referred to a cardiologist to switch interfering therapy to non-interfering medication.

148 This patient has a low probability of being affected by PA (normokalemia under full-dose diuretic).
149 Furthermore, the age, the long-standing hypertension and the associated organ damage (arterial
150 stiffness as shown by the high pulse pressure, and reduced kidney function) indicate a low probability
151 of complete clinical success after adrenal surgery (32). Elderly patients often display high ARR
152 levels, due to decreased renin levels that parallel a reduction in kidney function, and due to
153 aldosterone levels that remain unsuppressed by potassium that is less efficiently eliminated in case of
154 renal failure (Table 1) (1,2,29). Therefore, it is suggested not to proceed to ARR measurement and
155 treat the patient empirically.

156 **Medications and conditions affecting renin and aldosterone levels**

157 Most medications display an effect on the RAAS thereby potentially interfering with ARR
158 interpretation. The drugs with a neutral effect on the RAAS that therefore can be used during the
159 screening and confirmation of PA are alpha-blockers (doxazosin, prazosin, terazosin), non-
160 dihydropyridine calcium channel blockers (NDHP-CCB, verapamil and diltiazem) and moxonidine
161 (1,2,33,34). Also, DHP-CCB (such as amlodipine, lacidipine and lercanidipine) are used in most
162 centres to control blood pressure levels, although they can cause renin to increase due to sympathetic
163 activation and potentially a false negative ARR which, however, is observed in a very few cases
164 (2,29) (Table 1). Beta-blockers, clonidine and alpha-methyl dopa can cause false positive diagnoses,
165 especially if absolute levels of aldosterone are not taken into account (25,30,33,35). ACE-Is and
166 ARBs are potentially associated with a false-negative ARR by increasing DRC and PRA and reducing
167 aldosterone, and should be stopped whenever possible (1,2,30,33). However, as demonstrated by the
168 use of the captopril challenge test to confirm the diagnosis, aldosterone production in PA (especially
169 unilateral forms), is relatively independent from angiotensin-II stimulation and the ARR can be safely
170 interpreted and the florid forms of PA diagnosed even under treatment with ACE-I/ARB,. Recently,
171 it has been proposed a new screening test which uses the angiotensin-II over aldosterone ratio, both
172 measured by liquid chromatography and mass-spectrometry detection (36,37); this ratio seems to

173 provide results that are independent from the interference of ACE-Is treatment. Renin-inhibitors such
174 as aliskiren, reduce PRA and aldosterone and increase DRC, resulting in potential false-positive ARR
175 when renin is measured as PRA and false-negative when is measured as DRC. Thiazide and loop
176 (potassium-wasting) diuretics increase both renin and aldosterone by inducing volume depletion and
177 may result in false-negative ARR. This effect on ARR could be increased by the relative reduction of
178 aldosterone secretion due to a concomitant diuretic-induced hypokalemia (29) (Table 1).
179 Mineralocorticoid receptor antagonists (MRAs) block aldosterone effects and may determine false-
180 negative ARR (1,2,38): these drugs should be stopped for at least 6-8 weeks before measuring ARR.
181 However, in case of florid PA forms and low dose/short duration of MRA treatment, renin levels can
182 be still suppressed and the ARR diagnostic for PA (39-41). It is suggested to repeat the ARR after
183 MRA withdrawal when DRC or PRA levels are not suppressed and ARR negative. Similar effects on
184 the ARR can be observed by potassium-sparing diuretics such as amiloride and triamterene especially
185 when used at high dose (25).

186 Conditions in which PA is associated with activation of the RAAS may be associated with false-
187 negative screening tests: these include malignant or renovascular hypertension (29,42) and pregnancy
188 (43,44) (Table 1). Conditions associated with potentially false-positive ARR include elderly patients,
189 patients with familial hyperkalemic hypertension, women under estrogen-containing contraceptive
190 agents and patients treated with non-steroidal anti-inflammatory drugs (Table 1). Elderly patients
191 display a progressive reduction of renin levels that parallel the reduction of the kidney function; by
192 contrast, aldosterone production is maintained by the stimulus of potassium that is less efficiently
193 eliminated by the kidney (45). Familial hyperkalemic hypertension is a genetic condition
194 characterized by hypertension, hyperkalemia and hyperchloremic acidosis (45,46,47). In affected
195 patients, volume expansion determines suppression of renin levels whereas aldosterone levels are
196 variable but generally not suppressed because of the direct renin-independent stimulus of the
197 associated hyperkalemia on aldosterone secretion, resulting in an increased ARR. Women under

198 estrogen-containing pills may have a false-positive ARR when renin is measured as DRC (49,50). I
199 In women, the ARR can also result falsely elevated during the luteal phase of the menstrual cycle
200 (51). These effects are due to the negative feedback on renin levels determined by the stimulation of
201 angiotensinogen production by estrogens (30). Finally, non-steroidal anti-inflammatory drugs can
202 reduce renin more than aldosterone resulting in an increased ARR (29,52).

203 Ideally, ARR should be measured from blood samples collected in the morning after patients have
204 been out of bed for at least 2 hours and after they have been seated for few minutes (2).

205 **Suggested flow-chart in case of screening under anti-hypertensive therapy**

206 Considering the different effects of anti-hypertensive drugs on the RAAS, a pragmatic and operative
207 flow-chart can be designed to screen patients under potentially interfering therapies (Figure 1).
208 Patients under ACE-Is or ARBs, associated or not with CCBs, when they have a positive ARR should
209 undergo confirmatory testing since they have a high probability of PA (some authors could consider
210 PA as confirmed since one of the confirmatory tests, the captopril challenge test, uses an ACE-I). If
211 the ARR value is very low (for example $ARR < 10$ with PRA, measured in ng/mL/h, and < 0.5 with
212 DRC, measured in mU/L) and/or absolute aldosterone values are low (for example < 10 ng/dL or
213 below the value considered as cut-off for a confirmatory test) (23), the patient can be confidently
214 considered as not having PA. When ARR is negative but close to the cut-off (for example ARR
215 between 10 and 30 with PRA or between 0.5 and 2 with DRC) the ARR should be repeated after 2-3
216 weeks of withdrawal of the ACE-I/ARB that should be substituted with an alpha-blocker (such as
217 doxazosin) or moxonidine to maintain blood pressure control (Figure 1) (1,2).

218 A negative ARR under beta-blockers, with or without a CCB, can confidently exclude PA. If the
219 ARR result is positive, beta-blockers should be withdrawn (33,35) and if necessary, substituted with
220 a non-DHP-CCB that could be associated with doxazosin instead of giving also a DHP-CCB to the
221 patient (Figure 1).

222 CCBs and/or alpha-blockers do not affect the RAAS, hence it is possible to proceed with the
223 diagnostic flow-chart according with the ARR values, i.e. performance of confirmatory test or PA
224 exclusion (Figure 1) (1,2).

225 If the ARR result is positive under treatment with thiazide/loop diuretics or low-dose MRA with or
226 without ACE-I/ARBs and with or without CCBs, than the patient has a very high probability of
227 having PA and should proceed with the diagnostic work-up (1,2,25); on the contrary a negative ARR,
228 warrants to stop diuretics for 4 weeks (8 weeks for MRA especially if at high doses or for long
229 duration of time) and to repeat the ARR (diuretics should be substituted with other non-interfering
230 drugs) (figure 1). This is not the case if the ARR is low, but aldosterone is below the cut-off for a
231 confirmatory test (for example aldosterone < 10 ng/dL) or if renin levels are very high under low
232 doses of thiazide diuretic (for example DRC >30 mU/L or PRA > 3ng/mL/h under 12.5 mg of
233 hydrochlorothiazide).

234 When the patient is under treatment with both a beta-blocker and a thiazide diuretic the interpretation
235 of the ARR result is complex: ideally, the beta-blocker should be substituted with a non-DHP CCB
236 and the diuretic with doxazosin. However, the clinician may choose to only substitute the drug that
237 is supposed to have a higher impact on the ARR result, that is the beta-blocker in case of a positive
238 ARR and the diuretic in case of a negative ARR (Figure 1) and repeat the hormone measurements.

239 When patients with a high ARR undergo confirmatory testing, they should be treated with drugs with
240 minimal effects on the RAAS (1,2). In particular, drugs that increase aldosterone and renin levels
241 should be avoided since they can cause a false positive diagnosis of PA. Similarly, drugs that activate
242 RAAS should not be administered during adrenal vein sampling since they could stimulate
243 aldosterone production from the adrenal gland contralateral to an aldosterone-producing adenoma,
244 causing a false diagnosis of bilateral PA and exclude patients from curative surgery (1,2).

245 **Conclusions**

246 In conclusion, in many cases, when withdrawal of a drug or its substitution with another with a neutral
247 effect on the RAAS is considered unsafe or complex, ARR can be confidently interpreted in many
248 cases even when the patient is under treatment with two or more classes of anti-hypertensive drugs.
249 Therefore, this should not represent a major issue to discourage general practitioners or clinicians
250 from measuring the ARR in patients with hypertension that are candidates for PA screening.
251 Experimental and clinical findings demonstrated that diagnosis and targeted treatment of PA is of
252 fundamental importance and when it is not possible to switch therapy to drugs with minimal
253 interference with RAAS activity, it is highly suggested to screen the patients under their usual
254 medications, rather than not screening at all. Appropriate diagnosis and treatment of PA, either with
255 adrenalectomy for unilateral forms or with sufficient doses of MRAs for patients with bilateral forms,
256 determines a marked reduction of the risk of cardio- and cerebrovascular events (53,54). This
257 opportunity cannot be missed for the 5-6% of the patients with hypertension that are seen by general
258 practitioners or cardiologists if the reason is the difficulty of ARR interpretation under interfering
259 therapy.

260 **Data Availability Statement**

261 Data sharing is not applicable to this article as no datasets were generated or analyzed during the
262 current study

263 **References**

264 1) Mulatero P, Monticone S, Deinum J, Amar L, Prejbisz A, Zennaro MC, Beuschlein F, Rossi GP,
265 Nishikawa T, Morganti A, Seccia TM, Lin YH, Fallo F, Widimsky J. Genetics, prevalence, screening
266 and confirmation of primary aldosteronism: a position statement and consensus of the Working Group
267 on Endocrine Hypertension of The European Society of Hypertension. *J Hypertens.*
268 2020;38(10):1919-1928.

269 2) Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, Stowasser M, Young WF
270 Jr. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An
271 Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* 2016;101(5):1889-1916.

272 3) Buffolo F, Tetti M, Mulatero P, Monticone S. Aldosterone as a Mediator of Cardiovascular
273 Damage. *Hypertension.* 2022 Jun 29;101161HYPERTENSIONAHA12217964. doi:
274 10.1161/HYPERTENSIONAHA.122.17964. Online ahead of print.

275 4) Monticone S, D'Ascenzo F, Moretti C, Williams TA, Veglio F, Gaita F, Mulatero P. Cardiovascular
276 events and target organ damage in primary aldosteronism compared with essential hypertension: a
277 systematic review and meta-analysis. *Lancet Diabetes Endocrinol.* 2018;6(1):41-50.

278 5) Monticone S, Sconfienza E, D'Ascenzo F, Buffolo F, Satoh F, Sechi LA, Veglio F, Mulatero P.
279 Renal damage in primary aldosteronism: a systematic review and meta-analysis. *J Hypertens.*
280 2020;38(1):3-12.

281 6) Monticone S, Burrello J, Tizzani D, Bertello C, Viola A, Buffolo F, Gabetti L, Mengozzi G,
282 Williams TA, Rabbia F, Veglio F, Mulatero P. Prevalence and Clinical Manifestations of Primary
283 Aldosteronism Encountered in Primary Care Practice. *J Am Coll Cardiol.* 2017;69(14):1811-1820.

284 7) Xu Z, Yang J, Hu J, Song Y, He W, Luo T, Cheng Q, Ma L, Luo R, Fuller PJ, Cai J, Li Q, Yang
285 S; Chongqing Primary Aldosteronism Study (CONPASS) Group. Primary Aldosteronism in Patients
286 in China With Recently Detected Hypertension. *J Am Coll Cardiol.* 2020;75(16):1913-1922.

287 8) Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, Ganzaroli C, Giacchetti G, Letizia
288 C, Maccario M, Mallamaci F, Mannelli M, Mattarello MJ, Moretti A, Palumbo G, Parenti G, Porteri
289 E, Semplicini A, Rizzoni D, Rossi E, Boscaro M, Pessina AC, Mantero F; PAPY Study Investigators.
290 A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am*
291 *Coll Cardiol.* 2006;48(11):2293-2300.

- 292 9) Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, Tamura N, Tanabe A; Task Force
293 Committee on Primary Aldosteronism, The Japan Endocrine Society. Guidelines for the diagnosis
294 and treatment of primary aldosteronism--the Japan Endocrine Society 2009. *Endocr J.*
295 2011;58(9):711-721.
- 296 10) Maiolino G, Calò LA, Rossi GP. The Time has Come for Systematic Screening for Primary
297 Aldosteronism in All Hypertensives. *J Am Coll Cardiol.* 2017;69(14):1821-1823.
- 298 11) Vaidya A, Carey RM. Evolution of the Primary Aldosteronism Syndrome: Updating the
299 Approach. *J Clin Endocrinol Metab.* 2020;105(12):3771-83.
- 300 12) Mulatero P, Monticone S, Burrello J, Veglio F, Williams TA, Funder J. Guidelines for primary
301 aldosteronism: uptake by primary care physicians in Europe. *J Hypertens.* 2016;34(11):2253-2257.
- 302 13) Cohen JB, Cohen DL, Herman DS, Leppert JT, Byrd JB, Bhalla V. Testing for Primary
303 Aldosteronism and Mineralocorticoid Receptor Antagonist Use Among U.S. Veterans : A
304 Retrospective Cohort Study. *Ann Intern Med.* 2021;174(3):289-297.
- 305 14) Gkaniatsa E, Ekerstad E, Gavric M, Muth A, Trimpou P, Olsson DS, Johannsson G, Ragnarsson
306 O. Increasing Incidence of Primary Aldosteronism in Western Sweden During 3 Decades - Yet An
307 Underdiagnosed Disorder. *J Clin Endocrinol Metab.* 2021;106(9):e3603-e3610.
- 308 15) Sivarajah M, Beninato T, Fahey TJ 3rd. Adherence to consensus guidelines for screening of
309 primary aldosteronism in an urban healthcare system. *Surgery.* 2020;167(1):211-215.
- 310 16) Liu YY, King J, Kline GA, Padwal RS, Pasiaka JL, Chen G, So B, Harvey A, Chin A, Leung
311 AA. Outcomes of a Specialized Clinic on Rates of Investigation and Treatment of Primary
312 Aldosteronism. *JAMA Surg.* 2021;156(6):541-549.

- 313 17) Hundemer GL, Imsirovic H, Vaidya A, Yozamp N, Goupil R, Madore F, Agharazii M, Knoll G,
314 Sood MM. Screening Rates for Primary Aldosteronism Among Individuals With Hypertension Plus
315 Hypokalemia: A Population-Based Retrospective Cohort Study. *Hypertension*. 2022;79(1):178-186.
- 316 18) Burrello J, Monticone S, Losano I, Cavaglià G, Buffolo F, Tetti M, Covella M, Rabbia F, Veglio
317 F, Pasini B, Williams TA, Mulatero P. Prevalence of Hypokalemia and Primary Aldosteronism in
318 5100 Patients Referred to a Tertiary Hypertension Unit. *Hypertension*. 2020;75(4):1025-1033.
- 319 19) Olsen MH, Angell SY, Asma S, Boutouyrie P, Burger D, Chirinos JA, Damasceno A, Delles C,
320 Gimenez-Roqueplo AP, Hering D, López-Jaramillo P, Martinez F, Perkovic V, Rietzschel ER,
321 Schillaci G, Schutte AE, Scuteri A, Sharman JE, Wachtell K, Wang JG. A call to action and a
322 lifecourse strategy to address the global burden of raised blood pressure on current and future
323 generations: the Lancet Commission on hypertension. *Lancet*. 2016;388(10060):2665-2712.
- 324 20) Burrello J, Monticone S, Buffolo F, Lucchiari M, Tetti M, Rabbia F, Mengozzi G, Williams TA,
325 Veglio F, Mulatero P. Diagnostic accuracy of aldosterone and renin measurement by
326 chemiluminescent immunoassay and radioimmunoassay in primary aldosteronism. *J Hypertens*.
327 2016;34(5):920-927.
- 328 21) Manolopoulou J, Fischer E, Dietz A, Diederich S, Holmes D, Junnila R, Grimminger P, Reincke
329 M, Morganti A, Bidlingmaier M. Clinical validation for the aldosterone-to-renin ratio and aldosterone
330 suppression testing using simultaneous fully automated chemiluminescence immunoassays. *J*
331 *Hypertens*. 2015;33(12):2500-11.
- 332 22) Rossi GP, Ceolotto G, Rossitto G, Seccia TM, Maiolino G, Berton C, Basso D, Plebani M.
333 Prospective validation of an automated chemiluminescence-based assay of renin and aldosterone for
334 the work-up of arterial hypertension. *Clin Chem Lab Med*. 2016;54(9):1441-50.
- 335 23) Song Y, Yang S, He W, Hu J, Cheng Q, Wang Y, Luo T, Ma L, Zhen Q, Zhang S, Mei M, Wang
336 Z, Qing H, Bruemmer D, Peng B, Li Q; Chongqing Primary Aldosteronism Study (CONPASS)

337 Group†. Confirmatory Tests for the Diagnosis of Primary Aldosteronism: A Prospective Diagnostic
338 Accuracy Study. *Hypertension*. 2018;71(1):118-124.

339 24) Maiolino G, Rossitto G, Bisogni V, Cesari M, Seccia TM, Plebani M, Rossi GP; PAPY Study
340 Investigators. Quantitative Value of Aldosterone-Renin Ratio for Detection of Aldosterone-
341 Producing Adenoma: The Aldosterone-Renin Ratio for Primary Aldosteronism (AQUARR) Study. *J*
342 *Am Heart Assoc*. 2017 21;6(5):e005574.

343 25) Young WF Jr. Diagnosis and treatment of primary aldosteronism: practical clinical perspectives.
344 *J Intern Med*. 2019;285(2):126-148.

345 26) Stowasser M, Ahmed A, Guo Z, Wolley M, Ungerer J, McWhinney B, Poglitsch M, Gordon R.
346 Can Screening and Confirmatory Testing in the Management of Patients with Primary Aldosteronism
347 be Improved? *Horm Metab Res*. 2017;49(12):915-921.

348 27) Reincke M, Bancos I, Mulatero P, Scholl UI, Stowasser M, Williams TA. Diagnosis and treatment
349 of primary aldosteronism. *Lancet Diabetes Endocrinol*. 2021;9(12):876-892.

350 28) Buffolo F, Burrello J, Burrello A, Heinrich D, Adolf C, Müller LM, Chen R, Forestiero V,
351 Sconfienza E, Tetti M, Veglio F, Williams TA, Mulatero P, Monticone S. Clinical Score and Machine
352 Learning-Based Model to Predict Diagnosis of Primary Aldosteronism in Arterial Hypertension.
353 *Hypertension*. 2021;78(5):1595-1604.

354 29) Young WF Jr, Calhoun DA, Lenders JWM, Stowasser M, Textor SC. Screening for Endocrine
355 Hypertension: An Endocrine Society Scientific Statement. *Endocr Rev*. 2017; 38 (2):103–122.

356 30) Stowasser M, Ahmed AH, Pimenta E, Taylor PJ, Gordon RD. Factors affecting the
357 aldosterone/renin ratio. *Horm Metab Res*. 2012;44(3):170-176.

358 31) Williams TA, Gomez-Sanchez CE, Rainey WE, Giordano TJ, Lam AK, Marker A, Mete O,
359 Yamazaki Y, Zerbini MCN, Beuschlein F, Satoh F, Burrello J, Schneider H, Lenders JWM, Mulatero

360 P, Castellano I, Knösel T, Papotti M, Saeger W, Sasano H, Reincke M. International Histopathology
361 Consensus for Unilateral Primary Aldosteronism. *J Clin Endocrinol Metab.* 2021;106(1):42-54.

362 32) Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, Satoh F, Amar
363 L, Quinkler M, Deinum J, Beuschlein F, Kitamoto KK, Pham U, Morimoto R, Umakoshi H, Prejbisz
364 A, Kocjan T, Naruse M, Stowasser M, Nishikawa T, Young WF Jr, Gomez-Sanchez CE, Funder JW,
365 Reincke M; Primary Aldosteronism Surgery Outcome (PASO) investigators. Outcomes after
366 adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures
367 and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol.* 2017;5(9):689-
368 699.

369 33) Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, Veglio F. Drug effects on
370 aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension.* 2002;40(6):897-902.

371 34) Ahmed AH, Gordon RD, Taylor P, Ward G, Pimenta E, Stowasser M. Effect of atenolol on
372 aldosterone/renin ratio calculated by both plasma Renin activity and direct Renin concentration in
373 healthy male volunteers. *J Clin Endocrinol Metab.* 2010;95(7):3201-3206.

374 35) Ahmed AH, Gordon RD, Ward G, Wolley M, McWhinney BC, Ungerer JP, Stowasser M. Effect
375 of Moxonidine on the Aldosterone/Renin Ratio in Healthy Male Volunteers. *J Clin Endocrinol Metab.*
376 2017;102(6):2039-2043.

377 36) Burrello J, Buffolo F, Domenig O, Tetti M, Pecori A, Monticone S, Poglitsch M, Mulatero P.
378 Renin-Angiotensin-Aldosterone System Triple-A Analysis for the Screening of Primary
379 Aldosteronism. *Hypertension.* 2020;75(1):163-172.

380 37) Guo Z, Poglitsch M, Cowley D, Domenig O, McWhinney BC, Ungerer JPJ, Wolley M, Stowasser
381 M. Effects of Ramipril on the Aldosterone/Renin Ratio and the Aldosterone/Angiotensin II Ratio in
382 Patients With Primary Aldosteronism. *Hypertension.* 2020;76(2):488-496.

- 383 38) Pecori A, Buffolo F, Burrello J, Mengozzi G, Rumbolo F, Avataneo V, D'Avolio A, Rabbia F,
384 Bertello C, Veglio F, Mulatero P, Monticone S. Mineralocorticoid Receptor Antagonist Effect on
385 Aldosterone to Renin Ratio in Patients With Primary Aldosteronism. *J Clin Endocrinol Metab.*
386 2021;106(9):e3655-e3664.
- 387 39) Haase M, Riester A, Kröpil P, Hahner S, Degenhart C, Willenberg HS, Reincke M. Outcome of
388 adrenal vein sampling performed during concurrent mineralocorticoid receptor antagonist therapy. *J*
389 *Clin Endocrinol Metab.* 2014;99(12):4397-402.
- 390 40) Nanba AT, Wannachalee T, Shields JJ, Byrd JB, Rainey WE, Auchus RJ, Turcu AF. Adrenal
391 Vein Sampling Lateralization Despite Mineralocorticoid Receptor Antagonists Exposure in Primary
392 Aldosteronism. *J Clin Endocrinol Metab.* 2019;104(2):487-492.
- 393 41) Rossi GP, Ceolotto G, Rossitto G, Maiolino G, Cesari M, Seccia TM. Effects of Mineralocorticoid
394 and AT1 Receptor Antagonism on The Aldosterone-Renin Ratio In Primary Aldosteronism-the
395 EMIRA Study. *J Clin Endocrinol Metab.* 2020;105(6):dgaa080.
- 396 42) Pizzolo F, Pavan C, Guarini P, Trabetti E, Girelli D, Corrocher R, Olivieri O. Primary
397 hyperaldosteronism: a frequent cause of residual hypertension after successful endovascular
398 treatment of renal artery disease. *J Hypertens.* 2005;23(11):2041-7.
- 399 43) Monticone S, Auchus RJ, Rainey WE. Adrenal disorders in pregnancy. *Nat Rev Endocrinol.*
400 2012;8(11):668-78.
- 401 44) Forestiero V, Sconfienza E, Mulatero P, Monticone S. Primary aldosteronism in pregnancy.
402 *Rev Endocr Metab Disord.* 2022 May 10. doi: 10.1007/s11154-022-09729-6. Online ahead of print.
- 403 45) Mulatero P, Burrello J, Williams TA, Monticone S. Primary Aldosteronism in the Elderly. *J Clin*
404 *Endocrinol Metab.* 2020;105(7):dgaa206.

- 405 46) Pseudohypoaldosteronism type II: history, arguments, answers, and still some questions. Healy
406 JK. *Hypertension*. 2014;63(4):648-54.
- 407 47) Monticone S, Losano I, Tetti M, Buffolo F, Veglio F, Mulatero P. Diagnostic approach to low-
408 renin hypertension. *Clin Endocrinol (Oxf)*. 2018;89(4):385-396.
- 409 48) Hureaux M, Mazurkiewicz S, Boccio V, Vargas-Poussou R, Jeunemaitre X. The variety of genetic
410 defects explains the phenotypic heterogeneity of Familial Hyperkalemic Hypertension. *Kidney Int*
411 *Rep*. 2021;6(10):2639-2652.
- 412 49) Ahmed AH, Gordon RD, Taylor PJ, Ward G, Pimenta E, Stowasser M. Effect of contraceptives
413 on aldosterone/renin ratio may vary according to the components of contraceptive, renin assay
414 method, and possibly route of administration. *J Clin Endocrinol Metab*. 2011;96(6):1797-1804.
- 415 50) Ahmed AH, Gordon RD, Ward G, Wolley M, McWhinney BC, Ungerer JP, Stowasser M. Effect
416 of Combined Hormonal Replacement Therapy on the Aldosterone/Renin Ratio in Postmenopausal
417 Women. *J Clin Endocrinol Metab*. 2017;102(7):2329-2334.
- 418 51) Ahmed AH, Gordon RD, Taylor PJ, Ward G, Pimenta E, Stowasser M. Are women more at risk
419 of false-positive primary aldosteronism screening and unnecessary suppression testing than men? *J*
420 *Clin Endocrinol Metab*. 2011;96(2):E340-6.
- 421 52) Eriksson LO, Sturfelt G, Thysell H, Wollheim FA. Effects of sulindac and naproxen on
422 prostaglandin excretion in patients with impaired renal function and rheumatoid arthritis. *Am J Med*.
423 1990;89(3):313-21.
- 424 53) Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and
425 mortality in medically treated primary aldosteronism: a retrospective cohort study. *Lancet Diabetes*
426 *Endocrinol*. 2018;6(1):51-59.

427 54) Mulatero P, Sechi LA, Williams TA, Lenders JWM, Reincke M, Satoh F, Januszewicz A, Naruse
428 M, Doumas M, Veglio F, Wu VC, Widimsky J. Subtype diagnosis, treatment, complications and
429 outcomes of primary aldosteronism and future direction of research: a position statement and
430 consensus of the Working Group on Endocrine Hypertension of the European Society of
431 Hypertension. J Hypertens. 2020;38(10):1929-1936.

432

433 **Figure Legend**

434 **Legend to Figure 1. Suggested flow-chart for patients with hypertension under anti-**
435 **hypertensive treatment that should be screened for primary aldosteronism.**

436 Suggested practical approach to patients with hypertension and high risk of PA, which should be
437 screened with ARR but are under potentially interfering anti-hypertensive therapy. Boxes with a thick
438 outline indicate patients for whom a decision can be reached, either PA highly probable thus they
439 should undergo confirmatory testing and/or subtype diagnosis (grey boxes) or PA can be confidently
440 excluded and they should be considered as affected by essential hypertension (white boxes).

441 STOP-PA: Score To Predict Primary Aldosteronism; ML: machine learning; PA: primary
442 aldosteronism; ARR: aldosterone renin ratio; PRA: plasma renin activity; ACE: angiotensin-
443 converting enzyme; ARB: angiotensin II receptor blockers; DRC: direct renin concentration; DHP-
444 CCB: di-hydropyridin calcium channel blocker; MRA: mineralocorticoid receptor antagonist.

445

446

447

448 **Table 1. Drugs and conditions that interfere with the interpretation of the ARR**

FALSE POSITIVE SCREENING TEST	FALSE NEGATIVE SCREENING TEST
-------------------------------	-------------------------------

Anti-Hypertensive Drugs that Frequently Cause False-positive ARR*				Anti-Hypertensive Drugs that Frequently Cause False-negative ARR*			
	Renin	Aldo	ARR		Renin	Aldo	ARR
Beta-Blockers	↓↓	↓	↑	MRA's and ENaC blockers	↑↑	↑	↓
Clonidine/Alpha-Methyl Dopa	↓↓	↓	↑	Thiazides and Loop Diuretics	↑↑	↑	↓
Aliskiren#	↓↓	↓	↑	Anti-Hypertensive Drugs that May Cause False-negative ARR			
Other Conditions				ACE-Is, ARBs and Aliskiren§	↑	↓	↓
Advancing age/reduced renal function	↓↓	↓	↑	Other Conditions			
FHH	↓↓	↓↔	↑	Hypokalemia	↔	↓	↓
Women under estrogen contraceptive agents§	↓	↑	↑	Concomitant Malignant or RVH	↑↑	↑	↓
Anti-inflammatory drugs	↓↓	↓	↑	Pregnancy	↑↑	↑	↓

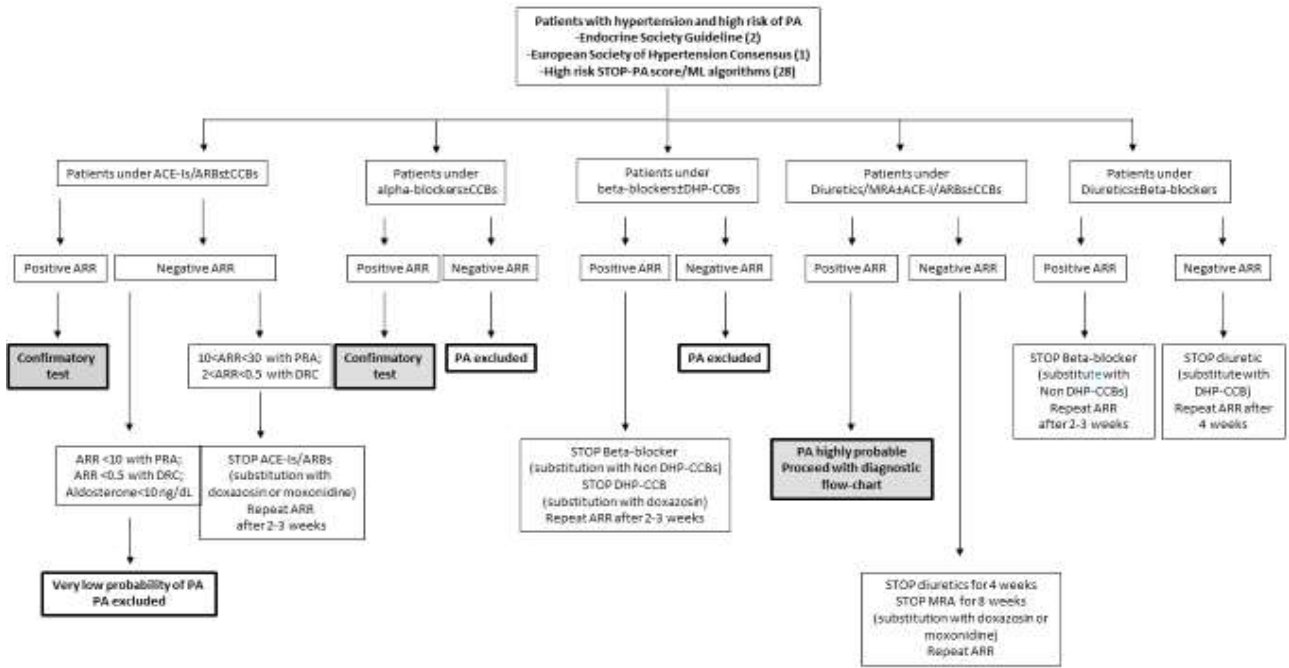
449

450 ARR: aldosterone renin ratio; ENaC: epithelial sodium channel; PRA: plasma renin activity; ACE-
451 Is: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers; DRC: direct
452 renin concentration; Aldo: aldosterone; FHH: familial hyperkalemic hypertension; MRA:
453 mineralocorticoid receptor antagonist; RVH: renovascular hypertension; # when renin is measured
454 as PRA; §when renin is measured as DRC) *ARR is intended false-positive (or false negative)
455 when interfering drugs determine an increase (or a decrease) of the ARR above (or below) the cut-
456 off for positive (or negative) test, for example 30 when renin is measured as PRA in ng/mL/h and
457 aldosterone in ng/dL or 2 when renin is measured as DRC in mU/L and aldosterone in ng/dL. In
458 many centres a minimum aldosterone level is required to consider the ARR as positive, that is > 10
459 ng/dL in our unit (>15 ng/dL or higher than the cut-off of the confirmatory tests in others)(1,2,24).

460

461

462



463

464

465