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

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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 consensuses 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33 therapies that affect the renin-angiotensin aldosterone system are withdrawn and substituted with non-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/ μ U/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).

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

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

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

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

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

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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.

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

FALSE POSITIVE SCREENING TEST	FALSE NEGATIVE SCREENING TEST
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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	↓↓	↓	↑	MRAs 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	↑↑	↑	↓

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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).

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