

Consensus

SFE/SFHTA/AFCE Consensus on Primary Aldosteronism, part 2: First diagnostic steps

Consensus SFE/SFHTA/AFCE sur l'hyperaldostéronisme primaire, groupe 2 : premières étapes diagnostiques

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Abstract

In patients with suspected primary aldosteronism (PA), the first diagnostic step, screening, must have high sensitivity and negative predictive value. The aldosterone-to-renin ratio (ARR) is used because it has higher sensitivity and lower variability than other measures (serum potassium, plasma aldosterone, urinary aldosterone). ARR is calculated from the plasma aldosterone (PA) and plasma renin activity (PRA) or direct plasma renin (DR) values. These measurements must be taken under standard conditions: in the morning, more than 2 hours after awakening, in sitting position after 5 to 15 minutes, with normal dietary salt intake, normal serum potassium level and without antihypertensive drugs significantly interfering with the renin-angiotensin-aldosterone system. To rule out ARR elevation due to very low renin values, ARR screening is applied only if aldosterone is > 240 pmol/l (90 pg/ml); DR values < 5 mIU/l are assimilated to 5 mIU/l and PRA values < 0.2 ng/ml/h to 0.2 ng/ml/h. We propose threshold ARR values depending on the units used and a conversion factor (pg to mIU) for DR. If ARR exceeds threshold, PA should be suspected and exploration continued. If ARR is below threshold or if plasma aldosterone is < 240 pmol/l (90 pg/ml) on two measurements, diagnosis of PA is excluded.

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Keywords: Aldosterone-to-renin ratio (ARR); Screening; Primary aldosteronism; Standard conditions; Aldosterone; Renin

Résumé

Chez les patients suspects d'hyperaldostéronisme primaire (HAP), la première étape diagnostique, dite de dépistage, doit avoir une sensibilité et une valeur prédictive négative élevées. Le rapport aldostérone/rénine (RAR) est choisi car il présente une sensibilité meilleure et une variabilité moindre que les autres mesures (kaliémie, aldostéronémie, aldostéronurie). Le calcul du RAR est fait à partir de la mesure de l'aldostérone plasmatique (AP) et la mesure de la rénine : soit en activité (ARP), soit en mesure directe (RD). Ces mesures doivent être réalisées en conditions standardisées : le matin, plus de 2 heures après le lever, en position assise depuis 5 à 15 minutes, en régime normosodé, en normokaliémie et sans traitement interférant significativement avec le système rénine angiotensine. Pour éliminer les élévations du RAR liées essentiellement à des valeurs de rénine très basses, le calcul du RAR n'est appliqué que si l'aldostérone est > 240 pmol/L (90 pg/mL) et on majorera à 5 mU/L les valeurs de RD < 5 mU/L et à 0,2 ng/mL/h les valeurs d'ARP < 0,2 ng/mL/h. Il est alors proposé un seuil du RAR dont l'expression dépend des unités utilisées

Abbreviations: PA, Primary aldosteronism; ARR, Aldosterone-to-renin ratio; PRA, Plasma renin activity; DR, Direct renin; RAAS, Renin-angiotensin-aldosterone system; CEI, Converting enzyme inhibitor; NSAIDs, Non-steroidal anti-inflammatory drugs; SRI, Serotonin reuptake inhibitor; EP, Estrogen-progestin; RIA, Radioimmunoassay; LC-MS, Liquid chromatography-mass spectrometry; ARA-II, Angiotensin-II receptor antagonist.

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et, pour la mesure de la RD, du facteur de conversion (pg vs mUI). Si le RAR est supérieur à ce seuil, l'HAP est possible et les explorations devront être poursuivies. Si le RAR est en dessous de ce seuil ou si l'aldostérone plasmatique est, à deux reprises, en dessous de 240 pmol/L (90 pg/mL), le diagnostic d'HAP est exclu.

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Mots clés : Rapport aldostérone/rénine ; RAR ; Dépistage ; Hyperaldostéronisme primaire ; Conditions standardisées ; Aldostérone ; Rénine

1. 1st diagnostic step in primary aldosteronism: rationale for aldosterone-renin ratio (ARR) as screening criterion

1.1. Aldosterone-renin ratio

After selecting a population for exploration for primary aldosteronism (PA) (see article 1) the first diagnostic step is screening. This requires a test with high sensitivity and negative predictive value, to confirm all situations liable to involve PA. In recent decades, several teams have sought an optimal criterion to screen for elevated aldosterone production and the corresponding inhibition of renin. The literature data of the last 20 years demonstrated that the aldosterone-renin ratio (ARR) is the parameter with the highest sensitivity (68–94%) for PA screening, compared to other biological variables: serum potassium, plasma aldosterone, or urinary aldosterone [1–6]. Its negative predictive value sometimes even approximates 100%, for diagnosis of Conn's adenoma [7]. Sensitivity, however, varies between reports, due to differences in ARR threshold values, assay methodology and sampling conditions. Nevertheless, ARR shows somewhat less variation related to environmental conditions (serum potassium, sodium load, age and posture) in a given patient than rival markers [8–10]. Correlations are excellent between measures taken in seated, prone and standing posture [11]. Reproducibility was also found to be acceptable in Rossi's 2010 study [9] of 1136 patients (plasma aldosterone/plasma renin activity [PRA]), although other studies reported variable reproducibility, even within PA populations [10]. Amar [12] reviewed the main studies reporting at least 1000 hypertension patients: 6.4 to 22.8% of patients showed ARR elevation, used as screening criterion (thresholds ranging from 20 to 40 ng/dl/ng.ml⁻¹.h⁻¹ after withdrawal of interfering drugs); ARR elevation was associated with actual PA in 5.9 to 11.3% of patients. Depending on the team, the gold standard against which ARR was compared was one of the following dynamic tests (thresholds varying according to team): intravenous sodium load (aldosterone > 5 ng/dl or > 10 ng/dl), oral sodium load (urinary aldosterone > 12 µg/d), fludrocortisone (aldosterone > 6 ng/dl or > 5 ng/dl), or oral captopril (positive if ARR > 30 ng/dl/ng.ml⁻¹.h⁻¹ 1 hour after 50 mg captopril) [13–19]. The drawback of ARR as criterion is that it is not yet perfectly standardized: values vary with assay technique, assay kit and unit used to express results, intra- and inter-patient variation coefficients vary, and environmental factors influence results: posture, medication, metabolic factors (serum potassium and sodium load), and factors related to comorbidity (kidney failure), age, or menstrual phase [20]. Finally, although

sensitive for a given threshold, several reports have shown that ARR lacks specificity as an isolated diagnostic criterion for PA.

1.2. 24-hour urinary ionogram

Urinary ionograms seem to have two useful features: to demonstrate that hypokalemia is due to renal leakage, and to determine sodium load. It is useful at screening to have urinary potassium and sodium values, to determine sodium load. We therefore recommend implementing not only a blood ionogram and ARR estimate at screening, but also a 24-hour urinary ionogram (urinary sodium, potassium and creatinine).

1.3. Urinary aldosterone

Urinary aldosterone assay is used by several teams for screening or to confirm diagnosis, and seems to be a better indicator than plasma aldosterone alone [21,22]. Sensitivity, however, is lower than ARR (cf. Section 1.1), and the assay is burdensome and sometimes unreliable: 24-hour collection, margin of error up to 50%. It is not used as a PA screening criterion. Some teams have studied the aldosterone/creatinine ratio in urine samples compared to 24 h urine collection; in 102 patients with PA, confirmed on IV sodium load test, a threshold aldosterone/creatinine ratio of > 3.0 ng/mg allowed a 90.6% specificity for the diagnosis of PA vs essential hypertension, with a sensitivity (51%) similar to the sensitivity of 24 h aldosteronuria (44%), which remain mediocre [23].

1.4. N-terminal probrain natriuretic peptide (NT-proBNP)

N-terminal probrain natriuretic peptide (NT-proBNP) may in the future prove to be a PA marker in association with ARR. It correlates positively with ARR, negatively with renin level, and may be an independent marker of positive response to the IV saline suppression test, identifying patients at high risk of confirmed PA [24,25]. This assay, however, could only contribute to diagnosis in the absence of pathologies tending to elevate NT-proBNP (heart or kidney failure).

2. Assay methods

2.1. Plasma aldosterone assay

Plasma aldosterone may be assayed:

R2.1: In suspected PA, the first diagnostic step should be to calculate the aldosterone-renin ratio (ARR) by simultaneous measurement of plasma aldosterone and either plasma renin activity (PRA) or direct renin (DR).

Measurements should if possible be taken under standard conditions:

- in the morning, more than 2 hours after awakening;
- after 5–15 min seated posture;
- under normal sodium diet (urinary sodium, 100–200 mmol/24 h)
- with normal serum potassium values
- antihypertensive drugs withdrawn for 2 weeks (or 6 weeks for mineralocorticoid receptor antagonists and renin inhibitors), except for long-action calcium inhibitors and alpha-blockers, which may be continued; amiloride may also be continued, up to 15 mg/day in case of hypokalemia uncorrected by potassium supplementation;
- estrogen-progestins withdrawn for 6 weeks.

(Strong; evidence: ++)

- by radioimmunoassay (RIA), the most common technique (5 methods in France: Beckman Coulter, Cisbio Bioassays, Diasorin, IDS, Siemens);
- by liquid chromatography-mass spectrometry (LC-MS), being developed in several laboratories for its greater precision and specificity [26–30]; several studies are underway to reassess ARR thresholds on this new technique.

Plasma aldosterone results are expressed either in mass (pg/ml or ng/dl) or molar units (pmol/l). There is an easy-to-use conversion factor for aldosterone, which has a molecular weight of 360: pmol/l value = $2.77 \times$ pg/ml or $27.7 \times$ ng/dl value.

2.2. Renin assay

Renin may be assayed in two ways:

- plasma renin activity (PRA) is assessed by RIA (3 suppliers on the French market: Cisbio Bioassay, Diasorin, Diasource). Results are expressed in either ng/ml/h or pmol/l/min (SI) (1 ng/ml/h = 12.8 pmol/l/min). It is a manual technique and time-consuming (24–48 h). Normal values are specified by the manufacturer;
- plasma renin concentration, also known as “Direct renin” concentration (DR), is now the most widely used renin assay in France. It may be performed by:
 - RIA (4 suppliers on the French market: Adaltis, Beckman, Cisbio Bioassay, DSL France),

- or by automated analyzer (1 supplier on the French market: Diasorin, Liaison or Liaison XL).

Normal values are specified by the manufacturer.

Most kits express DR results in mIU/l, and for each kit it is essential to know the equivalence in pg/ml (or ng/l). In some kits, this equals 1: i.e., 1 pg/ml = 1 mIU/l, and results are identical in the two systems of units. But this is not always the case: the Diasorin kit specifies a pg equivalent of 1.67×10^{-6} IU: i.e., 1 pg/ml (or ng/l) = 1.67 mIU/l, thus, to obtain a value in mIU/l the pg/ml (or ng/l) value has to be multiplied by 1.67.

PRA/DR conversion ratios vary between assay methods, which are constantly changing. Depending on the assay, extrapolation from PRA to DR and vice versa uses a conversion factor from PRA in ng/ml/h to irR in mIU/l (factor of 8.2 to convert DR into PRA, according to Funder [31]). However, the conversion factor varies according to assay kit and technique; a fixed relation between PRA and DR is probably only theoretic, ignoring individual adaptations of renin secretion to angiotensinogen concentration.

2.3. Choice of assay technique between PRA and DR

Certain stimuli show different effects depending on whether PRA or DR is measured, and the choice may thus affect ARR values. The literature is currently richer concerning the ratio of aldosterone to PRA than to DR, especially as the latter came into use more recently [32]. However, this situation will be reversed now that DR is more commonly used; it is now the method of choice in community laboratories and in many hospital laboratories in France. Its main advantages lie in fast standardized analysis (30 min, versus 24–48 h for PRA), using an automated analyzer, and hence with lower production costs [33]. However, sensitivity is poorer at low values, and specificity may be vitiated by exogenous estrogen, reducing renin concentration with risk of false positive ARR; estrogen impregnation increases liver angiotensinogen production and thus angiotensin-II, inhibiting renin secretion by negative feedback with little change in enzymatic activity [8,34–36]. Some authors highlight the increased precision of assessment of an activity (PRA) that takes account of individual angiotensinogen concentration instead of direct renin (DR) measurement. However, considerations of time, cost and above all automated standardization have favored the development of DR measurement. Presently, except in case of estrogen-progestin impregnation, where PRA appears significantly less affected than DR [37], using PRA, although supported by powerful comparative trials, is not warranted. Moreover, a recent study by Glinicki, in 62 patients, showed very good correlation between ARR on PRA and DR, whether measured prone or standing [38].

In conclusion, ARR is presently most often assessed with renin measured by DR, being standardized, fast and thus more cost-effective than PRA. The latter, nevertheless, is still widely used, mainly in hospital laboratories, being more precise and less affected by estrogen impregnation.

3. What ARR threshold?

The question of the “right” ARR threshold for suspicion of PA was long troubled by intra- and inter-individual variation and lack of standard implementation conditions and assay technique [39–42]. The threshold should show high sensitivity and negative predictive value. A recent study confirmed the wide variation in PA screening by ARR between different centers in a single country [43]. The 2008 Endocrine Society expert group guidelines clarified this situation [31]. A literature analysis showed that thresholds varied according to team between 20 and 100 for plasma aldosterone expressed in ng/dl and PRA in ng/ml⁻¹h⁻¹, and between 68 and 338 for aldosterone expressed in pmol/l and PRA in pmol/l/min; but most of the studies concerned samples of less than 500 patients in varying postural conditions (prone, standing or seated) [5,44–47]. ARR threshold can be narrowed down to 20 to 40 if conditions (posture, time of day, assay technique) are more standardized. Improved sensitivity was further confirmed more recently, if assay is performed in the morning, seated, 2–4 hours after awakening [20]. In studies of at least 1000 patients ARR threshold (after withdrawal of interfering drugs) ranged between 20 and 40 ng/dl/ng.ml⁻¹.h⁻¹, or 554 and 1,108 pmol/l/ng.ml⁻¹.h⁻¹, although there were postural differences between studies [13–19]. Since then, other studies have supported these findings, with thresholds basically ranging between 30 and 35.9 ng/dl/ng.ml⁻¹.h⁻¹ [48]. In this context, the Endocrine Society PA screening guidelines recommended standardizing assay conditions: morning, seated for 5–15 min, more than 2 h after awakening [31]. However, the presentation of the thresholds in this consensus study (Table 5 of the publication) is confusing: there are three different thresholds for aldosterone in pg/ml, but only two in pmol/l, and conversion of these two gives none of the other three! Moreover, the conversion factor for DR from mIU/l to ng/l is 1.58, which is not applicable to all assays.

Rather than extrapolating from the various studies, some laboratories have developed their own ARR threshold to screen for or diagnose PA or Conn's adenoma. Such was the case of Massien-Simon's team in 1995 [49]. They compared 60 Conn's adenoma patients, 59 patients with essential hypertension, and 49 healthy controls. DR was expressed in pg/ml. The diagnostic threshold for Conn's adenoma (ROC curve) was studied. ARR assessed prone had a Youden index of 0.66 for a threshold at 23 (aldosterone in pg/ml, renin in pg/ml), which was better than in standing position (index, 0.57; threshold, 30). The threshold of 23 gave way in 2003 to a threshold of 64 (aldosterone in pmol/l, renin in mIU/l), as the laboratory in the Georges-Pompidou European Hospital in Paris found that the pg/mL values obtained with their IRMA kit and those from the automated analyzer in mIU/l were numerically the same: plasma aldosterone in ng/dl \times 27.7 = pmol/l value, and DR in pg/ml = mIU/l value (conversion factor = 1).

Subsequent studies confirmed this attitude: e.g., Ducher's team, with an optimal ARR threshold of 32 in standing position to diagnose Conn's adenoma [7].

Normal DR, PRA and aldosterone values should ideally be determined and/or checked by each laboratory, to have its own threshold; but this does not seem realistic in practice.

We are here suggesting what seem to us to be the most appropriate threshold values, in the light of studies involving more than 1000 patients (notably, as regards the aldosterone-PRA ratio), or matching the values most widely adopted in our geographical territory (notably, as regards the aldosterone-DR ratio).

To avoid overestimating ARR (even in case of low plasma aldosterone), with consequent loss of specificity, several authors recommend using a minimum level for renin (5 mIU/l) or PRA (0.1 ng/ml⁻¹h⁻¹ or 0.2 ng/ml⁻¹h⁻¹, depending on the study) if biological results are below these thresholds or below detection threshold, as often happens in very elderly subjects [50,51].

Certain authors also recommend a minimum plasma aldosterone level of > 15 ng/dl (or 410 pmol/l) if ARR is to be used; in case of elevated ARR, diagnostic exploration should be pursued; however, certain teams argue that this could lead to missed diagnosis of some cases of confirmed PA: in a study of 125 patients operated on for Conn's adenoma, plasma aldosterone was < 15 ng/dl in 16% of cases and < 10 ng/dl in 4% [8].

Conversion factors:

- aldosterone: 1 pg/ml = 2.77 pmol/l, so that value in pmol/l = value in pg/ml \times 2.77; (value in pg/ml = value in pmol/l \times 0.36).

NB: value in pg/ml = value in ng/l = value in ng/dl \times 10;

- DR: with the Diasorin[®] kit, conversion factor C is 1.67: 1 pg/ml = 1.67 mIU/l so value in mIU/l = 1.67 \times value in pg/ml.

Obviously, the value in pg/ml = value in mIU/l divided by 1.67;

- PRA: ng/ml/h = 12.8 \times pmol/l/min.

4. Influence of serum potassium, sodium load, age posture, day-night cycle, renal function, menstrual cycle and medication on plasma aldosterone and ARR

4.1. Serum potassium

A normal serum potassium concentration is desirable, to avoid underestimating aldosterone production, as low concentrations inhibit aldosterone secretion [52] and may induce false negative PA screening [42,50,53,54].

4.2. Sodium load

Low-sodium diet elevates renin and plasma aldosterone levels and lowers ARR [55]: ARR screening sensitivity for PA is enhanced by freeing sodium intake, although there is a risk of false positives if sodium load is excessively high. Williams, however, contextualized the significant impact of sodium load on variation in renin and aldosterone secretion, in a study of essential hypertension or PA patients: low-sodium diet (< 10 mmol/day for 7 days) stimulated PRA and aldosterone production in essential hypertension, and also in PA, but with significantly lower PRA than in hypertension, with elevation showing only a statistical “tendency” toward elevation; there was conversely a tendency toward lower PRA associated with

R2.2: To eliminate ARR elevation basically due to a very low or sub-threshold renin concentration:

- a value of 5 mIU/l should be attributed to any direct renin result < 5 mIU/l;
- a value of $0.2 \text{ ng/ml}^{-1} \text{ h}^{-1}$ should be attributed to any plasma renin activity result $< 0.2 \text{ ng/ml}^{-1} \text{ h}^{-1}$.

The aldosterone/renin ratio (ARR) is expressed according to the measurement unit system used for plasma aldosterone, PRA or DR. The DR value should also take account of the pg to mIU conversion factor.

ARR then suggests a diagnosis of PA if above a threshold specific to the renin assay technique and measurement unit system:

- renin measured as DR in mIU/l:
 - 64 (plasma aldosterone in pmol/l and DR in mIU/l),
 - 23 (plasma aldosterone in pg/ml and DR in mIU/l);
- renin measured as DR in pg/ml, conversion factor $1 \text{ pg/ml} = C \text{ mIU/l}$:
 - $64 \times C$ (plasma aldosterone in pmol/l and DR in pg/ml),
 - $23 \times C$ (plasma aldosterone in pg/ml and DR in pg/ml);
- renin measured as PRA:
 - 300 (plasma aldosterone in pg/ml and PRA in ng/ml/h),
 - 830 (plasma aldosterone in pmol/l and PRA in ng/ml/h),
 - 25 (plasma aldosterone in pg/ml and PRA in pmol/l/min),
 - 70 (plasma aldosterone in pmol/l and PRA in pmol/l/min).

(Strong; evidence: ++)

R2.3: When ARR, measured under standard conditions, is below the thresholds specified in R2.2 and/or plasma aldosterone is below 240 pmol/l (90 pg/ml) on two measurements, PA is ruled out and no complementary exploration is warranted.

(Strong; evidence: ++)

on ARR, and the ARR threshold for PA screening should ideally be revised upward in over 50-year-olds [58].

4.4. Posture

Aldosterone and renin levels rise in upright stance under physiological adaptation:

- blood sequestration in the lower limbs and reduced renal perfusion associated with elevated sympathetic tonus by beta-adrenergic receptor stimulation combine to stimulate renin secretion (effect showing 2–4 hours after change in position);
- aldosterone elevation, caused by reduced liver clearance due to reduced hepatic blood flow (effect showing 1 hour after change in position).

Moreover, angiotensin-II-sensitive PA (50% of aldosterone-secreting adenomas and 70% of bilateral hyperplasias) shows standing levels more or less identical to those in non-angiotensin-II-sensitive PA, whereas prone levels are lower; this no doubt partly accounts for the better sensitivity found with upright posture [59].

4.5. Sample timing

Renin and aldosterone stimulation by adaptation to change in posture is stronger in the morning, and ARR is higher in the morning than in the afternoon or evening [60,61].

4.6. Renal function

Impaired renal function lowers renin concentration (reduced secretion with nephron loss, and water and salt retention, with a risk of false positive ARR, especially as plasma aldosterone levels are little changed, or even raised in case of hyperkalemia). By contrast, renal vascular lesions stimulate renin and aldosterone secretion, inducing secondary hyperaldosteronism.

4.7. Menstrual cycle

In a population of female subjects with hypertension and low renin concentration, significant elevation of aldosterone level, PRA and ARR was observed during the luteal phase, with positive correlation between aldosterone, PRA and progesterone [62]. Such cyclic variation in aldosterone levels was previously reported [63,64]. The natriuretic effect of progesterone by

sodium-rich diet ($> 200 \text{ mmol/day}$), although this was not significant in the PA population. ARR did not significantly vary in the PA population between sodium rich and poor diet; nor did 24-h urinary aldosterone [56]. Nevertheless, the statistical tendencies observed led the author to consider the specificity of ARR to be better with sodium-normal diet.

4.3. Age

Renin secretion decreases with age, probably in relation with progressive nephron loss [57]. This may lead to false positives

competition with aldosterone on the aldosterone receptor, reducing sodium load and consequently plasma level, explains the elevation in renin and aldosterone [34].

Progesterone may also stimulate aldosterone production directly [65]. More recently, Ahmed et al. reported a risk of false positive ARR elevation during the luteal phase in a control population (2 cases out of 19 women), exclusively on DR assay [66]. This was confirmed by a recent study by Fommei [67]. Results on fludrocortisone suppression test for suspected PA may also be affected by follicular versus luteal phase [68].

During pregnancy, renin and especially plasma aldosterone levels are increased.

In non-menopausal patients, assay should no doubt preferably be performed during the first part of the cycle if DR is being assessed.

4.8. Other physiological or pathological situations

Renin elevation (false negatives on ARR):

- pregnancy;
- renal artery stenosis;
- malignant hypertension.

Lowered renin (false positives on ARR):

- ethnicity (African);
- longstanding diabetes (dysautonomia).

Moreover, aldosterone is involved in visceral adipose metabolism; elevation seems to be implicated in onset of metabolic syndrome, notably by increasing insulin resistance. Several avenues of research are being pursued to better determine its role, especially in obese patients, and, conversely, the impact of obesity on aldosterone secretion. Insulin resistance associated with overweight enhances aldosterone production by the sympathetic stimulation of the zona glomerulosa.

4.9. Medical treatment

4.9.1. Estrogen-progestins (EP)

Estrogen-based oral contraception can induce significant ARR elevation (false positive) if renin is assessed on DR rather than PRA [69,70]. Exogenous estrogen increases liver synthesis of the renin-angiotensin-aldosterone system (RAAS) substrate, angiotensinogen, then angiotensin and, by feedback, suppresses renin secretion (impacting DR, but hardly if at all PRA) [70] (cf. Section 2). Certain progestins can activate the renin-angiotensin system, others not [66]. One study showed greater PRA, DR and aldosterone elevation under drospirenone than gestodene or desogestrel. The risk of false positive ARR increases under EPs, and is greater for ARR assessed on DR than PRA, except in the case of drospirenone, a mineralocorticoid receptor antagonist, where the two are identical [37]. By contrast, ARR is not significantly affected by subcutaneous progestin implants such as etonogestrel, whether renin is measured by PRA or DR [66].

4.9.2. Other treatments

- Beta-blockers: reduced secretion of renin (principal effect) and plasma aldosterone (risk of false positive) [71].
- Converting enzyme inhibitor (CEI) and angiotensin-II receptor antagonist (ARA-II): renin elevation and lowered plasma aldosterone (risk of false negative) [72].
- Diuretics: renin elevation with all diuretics (including potassium-sparing diuretics: spironolactone, eplerenone, amiloride, triamterene), due to reduced plasma volume and sympathetic stimulation [73] and plasma aldosterone elevation (except with thiazides, with which plasma aldosterone level may remain low due to hypokalemia) (risk of false negative).
- Dihydropyridine calcium channel blockers: possible renin elevation by reflex sympathetic tonus due to fall in blood pressure and in aldosterone concentration due to defect in synthesis (the various steps of which are calcium-dependent) (risk of false negative) [74]. However, when the situation was stabilized under long-course treatment, Seifarth found no impact on ARR [75].
- Central antihypertensive drugs, such as alpha-2 agonists: reduced renin secretion by reduced sympathetic tonus, reduced plasma aldosterone (e.g., clonidine, methyldopa) (risk of false positive).
- Renin inhibitors: lowered renin level on PRA (risk of false positive) but elevated DR (risk of false negative), reduced plasma aldosterone [76].
- NSAIDs: reduced renin and plasma aldosterone levels due to water and salt retention and suppression of urinary prostaglandins, which usually stimulate renin secretion (risk of false positive) [77].
- Serotonin reuptake inhibitors (SRIs), such as sertraline and escitalopram: elevated plasma aldosterone and renin, on PRA and DR: lowered ARR (risk of false negative) [78].

For PA screening, it is usually recommended to avoid treatments interfering with the mineralocorticoid axis, to avoid increasing false negative ARR rates, vitiating screening performance (risk of false negative: CEI, ARA-II, diuretics, renin inhibitors for renin measured on DR). The issue is, however, controversial, as it is not always feasible in practice to withdraw medication safely, and several studies recommended screening without change of treatment. In Seiler's study [79], the risk was basically of false positives under beta-blockers, other drugs showing no significant impact; however, this study requires validation, due to the small series: 41 PA patients, with 3 to 15 per treatment group. The risk of false positives under beta-blockers was also reported elsewhere (principally affecting renin level) [80]. Browne's team even recommend interrupting beta-blockers for a while ahead of hormonal exploration, depending on the renin assay method: 3 weeks for PRA, 2 weeks for DR [81]. Mulatero [72] also studied the effects of certain drugs (amlodipine, atenolol, doxazosin, fosinopril and irbesartan), measuring ARR without and then with treatment in 230 hypertension patients selected for aldosterone-PRA ratio >50 associated with plasma aldosterone > 150 ng/dl, in 154 of whom

Table 1
Treatment impact on RAAS and treatment interruption interval ahead of exploration.

Medication	Aldosterone	Renin	ARR	Interruption interval (weeks)
Thiazide diuretics	→↑	↑↑	↓ (FN)	2
Loop diuretics	↑	↑↑	↓ (FN)	2
Mineralocorticoid receptor antagonists	↑	↑↑	↓ (FN)	6
CEIs and angiotensin receptor blockers (sartans)	↓	↑↑	↓ (FN)	2
Renin inhibitors	↓	↑ in DR ↓ in PRA	↓ (FN) ↑ (FP)	6
β-blockers	↓	↓↓	↑ (FP)	2
Central alpha-2 agonist (clonidine)	↓	↓↓	↑ (FP?)	2 (ideally)
NSAIDs	↓	↓↓	↑ (FP)	2 (ideally)
SRI	↑	↑	↓ (FN)	?

PA was confirmed on sodium infusion test. ARR was reduced significantly under fosinopril, amlodipine and irbesartan and significantly increased under atenolol. An ARR threshold of > 50 ng/dl per ng/ml.h⁻¹ was associated in the PA population with a false negative risk of 23.5% for irbesartan (ARR -43%), corresponding to a drop in sensitivity from 100 to 76%. By contrast, there was no significant risk of false negatives under fosinopril (ARR -30%); only doxazosin and fosinopril showed no false negatives: ARR sensitivity fell from 100 to 98% under amlodipine and 10 to 76% under irbesartan. Spironolactone and other diuretics were not tested. In another recent study, of 235 hypertension patients, measuring ARR under treatment (except for amiloride and spironolactone), with a low threshold of 50 pmol/ng, the rate of confirmed PA was only 1.6%, due to extreme reduction in ARR under treatment (ARA-II, CEIs, thiazide diuretics) [82]. Recently, Jansen's team [83] reported 178 hypertension patients, 4 weeks after cessation of beta-blockers and potassium-sparing diuretics, in 27 of whom PA was confirmed on sodium infusion test. ARR was lower in the "classic treatment" group (calcium inhibitors, diuretics, CEIs, ARA-II, alpha-blockers) than in the "standardized treatment" group (calcium inhibitor + alpha-blocker). On ROC analysis, the optimal diagnostic threshold was 15.2 pmol/mIU for the "classic" group (sensitivity, 89%; specificity, 64%) and 40.2 pmol/mIU

for the "standardized" group (sensitivity, 89%; specificity, 64%). ARR showed identical diagnostic performance on ROC analysis in both groups, but with different thresholds, especially for screening purposes: for 95% sensitivity, as required for screening, the threshold should be 5.8 pmol/mIU for the "classic" group (sensitivity, 95%; specificity, 43%) and 16.3 pmol/mIU for the "standardized" group (sensitivity, 95%; specificity, 43%).

Thus, screening is feasible even under medication interfering with the RAAS, but the screening threshold has to be set according to the interfering drug; beta-blockers and potassium-sparing diuretics are an exception here, and need to be stopped before screening. Hitherto, most reference teams avoided assessing ARR under interfering drugs; the latest study suggests that treatment may be continued, with adaptation of the screening threshold, which should be lower in the case of most interfering drugs, except beta-blockers. Beta-blockers are sometimes maintained, notably in coronary patients; the risk of false positives is then high, but the patient will pass on to the diagnostic confirmation step, which will decide. Different authors recommend different interruption intervals [20,31,84].

Amiloride at < 15 mg/day has little impact on assay and can be maintained in case of poorly controlled hypokalemia with potassium supplementation [85].

Table 2
Main etiologies for false positive and false negative ARR.

ARR	False positive	False negative
Treatment	Beta-blockers Alpha-methyl DOPA Clonidine NSAIDs Renin inhibitors for renin on PRA Estrogen-progestins for renin on DR Estrogen-progestins if progestin is drospirenone	CEIs ARA-II Diurétiques (thiazide, loop, MR antagonists) SRI Renin inhibitors for renin on DR
Clinical situations	Excess sodium load Aging Kidney failure Luteal phase Longstanding diabetes (dysautonomia) Ethnicity (African)	Hypokalemia Low-sodium diet Pregnancy Renal artery stenosis Malignant hypertension

Treatment impact on the RAAS and treatment interruption intervals ahead of exploration are shown in [Table 1](#).

The main etiologies of false positive and false negative ARR are shown in [Table 2](#).

5. Conclusion

Aldosterone-renin ratio is the recommended screening method for primary aldosteronism; conditions of implementation and interpretation are laid out in R2.1, 2.2 and 2.3.

Disclosure of interest

The authors declare that they have no competing interest.

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